

FLAVOR CHEMISTRY OF LEMON-LIME CARBONATED BEVERAGES

BY

BETHANY J. HAUSCH

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Food Science and Human Nutrition
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Advisor:

Professor Keith R. Cadwallader

ABSTRACT

The most potent aroma-active components of Sprite[®] (SP), Sierra Mist[®] (SM), and 7UP[®] (7UP) were identified. Aroma extracts were prepared by liquid-liquid continuous extraction/solvent-assisted flavor evaporation (LLCE/SAFE). Twenty eight compounds were detected by gas chromatography-olfactometry (GCO) with linalool (floral, lavender), octanal (pungent orange) and 2,3-dehydro-1,8-cineole (minty) determined to be predominant aroma compounds based on their high flavor dilution (FD) factors by aroma extract dilution analysis (AEDA). The data indicate that lemon-lime is composed of a small number of compounds (22 at the most in SM) and only a subset of these may be important since many compounds were only detected at low FD factors.

Predominant aroma compounds in three commercial brands of lemon-lime carbonated beverages were quantified using static headspace solid phase microextraction (SPME) combined with stable isotope dilution assays (SIDA). The compounds chosen for quantification were 2,3-dehydro-1,8-cineole, 1,8-cineole, octanal, nonanal, decanal, linalool, borneol, isoborneol, neral, geranial, nerol, geraniol and *p*-cresol. Benzoic acid was quantified separately by HPLC using an external standardization method. Concentrations of the all compounds, except neral, differed between at least two brands. Concentrations of 1,8-cineole, octanal, nonanal, decanal, linalool, isoborneol, geraniol and benzoic acid differed among all brands. In contrast to FD factors, the calculated odor-activity values (OAVs) indicated that decanal was the most potent aroma compound, followed by octanal and dehydrocineole; with linalool and nonanal being moderately important to the aroma of lemon-lime carbonated beverages. Possible errors in the determination of threshold values and the nature of GCO analysis preclude the

results of the two methodologies from matching exactly. The results demonstrate that lemon-lime carbonated beverages share many of the same compounds but the relative abundance of these compounds varies by brand. Recommendations for further research include conducting sensory model studies based on the quantification data and determining compound thresholds in a carbonated matrix.

I lay my trophy at the feet of Jesus,
without whom this work would not have been possible.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Dr. Keith Cadwallader for his partnership in this project, for his invaluable advice and “pearls of wisdom” that extend beyond research, and for allowing me the privilege of being his student.

I would like to thank my mom and dad for their love and life-long support of me pursuing my dreams, for their encouragement, and for helping me through the difficult moments of graduate school.

Thank you to my friends and roommate for their encouragement and support throughout this project.

Thanks to Dr. Hun Kim for sharing his expertise with me, his steadfast patience and encouragement. Many thanks to my labmates as well for their encouragement, understanding, and examples of hard work.

There are also many wonderful science teachers from my academic career who deserve recognition. They have nourished my curiosity and let me ask endless questions. In particular, special thanks to Mr. Bob Tadsen for helping me fall in love with chemistry and recommending that I take some chemistry classes in college, “sooner rather than later.” I’m so glad I did.

Finally, thank you to Drs. Soo-Yeun Lee and M. Susan Brewer for their recommendations and willingness to serve on my committee.

TABLE OF CONTENTS

LIST OF FIGURES.....viii

LIST OF TABLES.....ix

CHAPTER 1: INTRODUCTION.....1

CHAPTER 2: REVIEW OF THE LITERATURE.....7

**CHAPTER 3: IDENTIFICATION OF AROMA-ACTIVE COMPONENTS OF
THREE COMMERCIAL BRANDS OF LEMON-LIME CARBONATED
BEVERAGES.....23**

**CHAPTER 4: QUANTIFICATION OF KEY AROMA-ACTIVE COMPONENTS
OF THREE COMMERCIAL BRANDS OF LEMON-LIME CARBONATED
BEVERAGES.....55**

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS.....99

**APPENDIX A: SPECTRA FOR UNLABELED AND ISOTOPE STANDARDS
USED FOR DETERMINING RESPONSE FACTORS; ADDITIONAL
COMPOUND INFORMATION; ISOTOPE CALIBRATION CURVES.....103**

APPENDIX B: MASS SPECTRA FOR SELECTED SYNTHESIS INTERMEDIATE COMPOUNDS.....	130
APPENDIX C: QUANTIFICATION CONCENTRATION DATA & CALCULATION OF <i>Q</i> VALUES.....	138
AUTHOR'S BIOGRAPHY.....	142

LIST OF FIGURES

2.1	Timeline of key developments in the history of carbonated beverages.....	18
2.2	Overview of the acid-catalyzed reactions occurring when citrus oils are exposed to an acidic environment.....	19
2.3	Simplified schematic of the acid-catalyzed rearrangement of limonene.....	20
3.1	Total ion GC-MS chromatograms for aroma extracts prepared from commercial lemon- lime carbonated beverages.....	42
3.2	Structures of the aroma-active compounds identified in three commercial brands of lemon-lime carbonated beverages.....	43
3.3	Proposed mechanism for conversion of citral into <i>p</i> -cresol.....	45
3.4	Proposed mechanism for rearrangement of α - or β -pinene to borneol.....	46
4.1	Structures for stable isotopic labeled standards used for quantification of key aroma components of lemon-lime carbonated beverages.....	85
4.2	Scheme for synthesis of $^2\text{H}_3$ -isoborneol and $^2\text{H}_3$ -borneol.....	86
4.3	Mass Spectra (EI) for (a) linalool and (b) d_2 -linalool.....	87
4.4	Calibration curve for $^2\text{H}_2$ -linalool.....	88

LIST OF TABLES

3.1	Predominant aroma-active components of three commercial brands of lemon-lime carbonated beverages.....	47
3.2	Orthonasal odor detection thresholds of selected aroma compounds identified in commercial brands of lemon-lime carbonated beverages.....	49
4.1	Response factors (EI-MS) of key aroma compounds quantified in three commercial brands of lemon-lime carbonated beverages.....	89
4.2	Raw data used to calculate the EI-MS response factor for ² H ₂ -linalool.....	90
4.3	Remaining beverage after decarbonation of three commercial brands of lemon-lime carbonated beverage.....	91
4.4	Raw data for the determination of linalool concentration.....	92
4.5	Concentrations for key aroma components of three commercial brands of lemon-lime carbonated beverages.....	93
4.6	Benzoic acid content of three commercial brands of lemon-lime carbonated beverages.....	94
4.7	Odor activity values for key flavor compounds in three commercial brands of lemon-lime carbonated beverages.....	95
4.8	Titrateable acidity (TA) for three commercial brands of lemon-lime carbonated beverages.....	96
4.9	pH for three commercial brands of lemon-lime carbonated beverages.....	96
4.10	Percent soluble solids (°Brix) content for three commercial brands of lemon-lime carbonated beverages.....	96

CHAPTER 1

INTRODUCTION

Today's beverage market is immense and varied. While flavored carbonated beverages may have been a treat reserved for special occasions only a generation or two ago, they are now ubiquitous and consumed by nearly everyone. This is seen from the global consumption of carbonated beverages, which was 185 million liters in 2001 (1). The estimated carbonated soft drink consumption in the United States in 2008 was 760 eight-ounce servings per capita (2). Although the volume of carbonated beverages consumed has declined in recent years, it is clear that these drinks continue to be very popular refreshments (2).

The use of flavorings in beverages dates back as early as the 1660s with the consumption of lemonade and orangeade (3). Although colas were the top ranked drinks in 2008 by market share, lemon-lime flavored beverages are still very popular, with Sprite (Coca-Cola Company, Atlanta, GA) taking the seventh largest market share in 2008 (2). Despite this tremendous market importance, details regarding the chemistry of lemon-lime flavor are not available. The absence of this information is likely an attempt of the beverage companies to prevent others from producing imitations. While the main constituents of carbonated beverages are clearly labeled on the packaging, the source and composition of the flavoring is not so clear. A review of the literature on essential oils indicates that certain lemon and lime oils are important to the food industry including the oils from *Aloysia citriodora* Palau (lemon verbena) (4) and *Citrus aurantifolia* (Christm.) Swingle (lime) (5). Much work has been done to identify and quantify the composition

of various oils for the purposes of detecting oil adulteration (6) or determining the antimicrobial activity of the oil (7). It is also well known that terpenes, the largest class of compounds in lemon oils, are unstable in acidic environments and undergo rearrangements (8-12). It is precisely for this reason that lemon-lime flavor is an intriguing area of study.

The acidic environment in lemon-lime beverages comes from two sources: citric acid (with a small contribution from ascorbic acid in some brands) and dissolved carbon dioxide. According to the literature, 0.03-0.05% (m/v) is the typical acid level in carbonated beverages and 0.3-0.6% (m/v) is typical for carbon dioxide (13). Acid-catalyzed reactions of terpenes can occur at even mildly acidic pH ($\text{pH} < \sim 6$) (8). The reported pH values for the three lemon-lime carbonated beverages studied range from 3.11 (SM) to 3.35 (SP) (14). Numerous citrus varieties exist and there is subsequently variation in the most abundant compounds in citrus. However, limonene is most abundant in lime oils on a percent by weight basis (4, 8, 15). Neral and geranial (citral isomers) have been found to be very abundant in some varieties of lemon verbena (4). In flavor studies, neral and geranial - in addition to linalool - were found to have the highest flavor dilution (FD) factors in fresh lemon oil (12). However, storage studies of fresh lemon oil at 37 °C for 30 days, showed that the FD factors of neral and geranial, which had FD factors of 64 initially, declined to less than 1 after storage (12). Limonene is also unstable in acidic environments, with some rearrangement to terpineols and terpinolene within four hours at 75 °C (0.073N sulfuric acid in 95% acetone/water) (8). The dynamic nature of terpenes in acid makes lemon lime flavor a complex system to study. Although acid-catalyzed reactions have been extensively studied, particularly in the case of citral, it

is interesting to look at a real food system and find out which flavor compounds are important, for example in lemon-lime carbonated beverages. The main motivation for the present research is simply to learn about a popular beverage system in order to understand it better. Due to the large commercial value of lemon-lime carbonated drinks, this research has great potential to benefit the beverage industry. When a customer pops the tab on their favorite lemon-lime beverage, the product they drink is distinctly different from that which was manufactured at the plant. Our ultimate goal is to understand the changes that take place as a result of the dynamic nature of the complex lemon lime beverage system. The snapshot of the flavor a customer experiences when they actually consume the product in their hand is what this research aspires to capture. The hypotheses of this research is: 1) there is a unique set of odorants responsible for lemon-lime flavor and 2) this set of odorants transcends commercially available lemon-lime carbonated beverage products.

In order to conduct this research, three commercial lemon-lime brands were chosen. Prior to analysis, the samples were decarbonated and the volatile compounds separated from the other matrix components. The decarbonation was accomplished during continuous liquid-liquid extraction, which is a mild technique that would limit the loss of volatiles and also yield a solvent (ether) extract of the volatile components of the beverage. The ether extract was separated from any extracted non-volatile constituents using solvent-assisted flavor evaporation (SAFE). Analysis of the aroma extract obtained after SAFE enabled the first objective of this research to be accomplished: identification of the aroma-active compounds in lemon-lime flavor by gas chromatography mass spectrometry (GC-MS) and gas chromatography-olfactometry (GCO). The second

objective was to quantify the aroma-active compounds. The identification and quantification of these odorants allowed differences between the brands to be elucidated.

REFERENCES

1. Shachman, M. *The soft drinks companion: a technical handbook for the beverage industry*; CRC Press: Boca Raton, FL, 2005; pp. 1-261.
2. Sicher, J., Ed. Special Issue: Top-10 CSD results for 2008. *Beverage Digest*, **2009**, 54.
3. Hargitt, B. Introduction. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D. P., Ashurst, P. R., Eds.; Blackwell Publishing Ltd: Oxford, UK, 2006; pp. 1-14.
4. Lira, P.; van Baren, C. M.; Retta, D.; Bandoni, A. L.; Gil, A.; Gattuso, M.; Gattuso, S. Characterization of lemon verbena (*Aloysia citriodora* Palau) from Argentina by the essential oil. *J. Essent. Oil Res.* **2008**, *20*, 350-353.
5. Patil, J. R.; Jayaprakasha, G. K.; Chidambara Murthy, K. N.; Tichy, S. E.; Chetti, M. B.; Patil, B. S. Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of *Citrus aurantifolia*. *Food Chem.* **2009**, *114*, 1351-1358.
6. Lifshitz, A.; Stepak, Y.; Basker, H. B. Characterization of Israel lemon oil and detection of its adulteration. *J. Food Sci.* **1969**, *34*, 254-257.
7. Thavanapong, N.; Wetwitayaklung, P.; Charoenteeraboon, J. Comparison of essential oils compositions of *Citrus maxima* Merr. peel obtained by cold press and vacuum steam distillation methods and of its peel and flower extract obtained by supercritical carbon dioxide extraction method and their antimicrobial activity. *J. Essent. Oil Res.* **2010**, *22*, 71-77.
8. Clark, B. C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-flavors in foods and beverages*; Charalambous, G., Ed.; Elsevier Science Publishers, 1992; pp. 229-285.
9. Baxter, R.L.; Laurie, W.A.; McHale, D. Transformations of monoterpenoids in aqueous acids - reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron* **1978**, *34*, 2195-2199.
10. Ueno, T.; Masuda, H.; Ho, C. Formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677-5684.
11. Peacock, V. E.; Kuneman, D. W. Inhibition of the formation of *α*-*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.
12. Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil citric-acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
13. Taylor, B. Ingredients and formulation of carbonated soft drinks. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D. P., Ashurst, P.R., Eds.; Blackwell Publishing Ltd: Oxford, UK, 2006; pp. 48-86.

14. Kappes, S. M. Ph.D. thesis. University of Illinois, Urbana, ILLINOIS, 2006.
15. Chisholm, M. G.; Wilson, M. A.; Gaskey, G. M. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). *Flavour Frag. J.* **2003**, *18*, 106-115.

CHAPTER 2

REVIEW OF THE LITERATURE

Early history of beverage flavorings and the development of carbonation

The use of flavorings in beverages partially owes its beginning to unsafe water prior to the 1900s, as observed by outbreaks of cholera, dysentery and other waterborne illnesses (1). The result was the development of ‘small beers’ - beverages that were boiled and flavored with ingredients such as herbs and/or slightly fermented (1). Other early flavored beverages were barley waters (1320), lemonade (1663) and orangeade (1660s) (1). Carbonated beverages, on the other hand, first came from natural sources, specifically effervescent mineral springs and yeast fermentation. Records of champagne date back to 1693 at the latest (1). Early scientific investigations of carbon dioxide in water date back to 1741 when Brownrigg produced carbonated water using bicarbonate salts, and by the late the 1760s and 1770s, scientists had developed ways of dissolving carbon dioxide in water under pressure (1). The first commercial manufacture of carbonated water occurred in the late 1770s when Thomas Henry, a chemist and apothecary in Manchester, England, designed an apparatus that could carbonate up to 12 gallons per batch and sold the product in corked glass bottles (1, 2). Henry’s design was an improvement on a previously apparatus designed by Dr. John Mervin Nooth. Nicholas Paul is the first person credited with the use of a high pressure gas pump, which allowed his mineral water to contain several volumes of carbon dioxide (1).

The purpose or intended use of carbonated water changed about the same time new carbonation technologies were being developed. Initially, artificially carbonated mineral water, which was called “soda water” was used for medicinal purposes and was

required to contain sodium bicarbonate (2). Soda water was distinguished from plain carbonated water, with the later described as “aqua acidi carbonici”(2). The transition of “soda water” from serving a medicinal purpose to one of refreshment resulted in the elimination of soda and the addition of flavorings (2). The early flavorings used in carbonated water for refreshment were largely from fruit and included: sarsaparilla, lemon, pineapple, orange, strawberry, vanilla, peach, grape, almond, ginger and cloves, among others (2).

The earliest manufacturers of carbonated beverages were Dr Pepper, which began operations in ca. 1888, and the Coca-Cola Company, incorporated in 1892. A few years later (ca. 1896) Pepsi-Cola was established, although its name came later (1901) (1). Figure 2.1 highlights some of the key advances in the use of flavoring in beverages, the development of carbonation and the establishment of beverage manufacturers.

Methods for the procurement of essential oils

The methodology used to obtain citrus essential oils to create flavorings deserves some attention. The outer layer of the fruit peel, called the flavedo, contains the oil glands and pigments (3). The oil glands in a mature orange number between 8,000-12,000 (4). A variety of techniques are employed for releasing the oil from the flavedo, specifically distillation and expression/cold pressing are used (5). A brief description of these traditional methods will be presented below. Several researchers have explored various extraction methods to obtain shorter extraction times, reduce or eliminate the use of organic solvents (5) and/or protect the integrity of the oil (5). This latter reason is particularly important to this discussion because the processing technique influences the

chemical composition (6). Many of the compounds in the oil react under acidic conditions, which the fruit juice itself provides since it is composed of mainly citric acid and water (6). Some of the other methods employed to extract the essential oil are microwave 'dry' distillation (5) and supercritical carbon dioxide extraction (7).

In the expression method, the first step is to mechanically rupture the fruit oil glands using a screw press, thus exposing the oil to the juice (3, 6). The press has perforated walls which allow everything except the peel to leave the press (6). The oil is collected by a stream of water, which results in the formation of an oil-water emulsion consisting of only 0.5-2% oil (3). The emulsion undergoes two centrifugation steps, the first concentrates the oil to 70-90% and the second centrifuge step concentrates the oil up to 99% (3). Nonvolatile impurities of the oil such as cuticle wax can be removed by chilling (winterizing) the oil, causing the impurities to precipitate (3). Clark and Chamblee described two methods for producing expressed oil, calling oil produced using the method just described type A expressed oil (6). In the case of type B expressed oil, an extractor/roller machine tears or ruptures the skin of the fruit to release the oil (6). Water is used to carry away the oil and the mixture goes to a finisher to remove particulate material (6). The mixture is then centrifuged to separate the oil from the water. Type B expressed oil has the advantage that the oil does not come in contact with the fruit juice (6).

On the other hand, in the distillation method, the fruit is mechanically crushed and the resulting oil-water emulsion is steam distilled in batches for 8-10 h at pH 2-2.5 and 96-98°C (6). The steam causes the essential oil components to vaporize. As the steam

and essential oil components condense upon cooling their differing densities cause them to separate (5).

As expected, these processes give oils of different compositions. Although there are differences between type A and B expressed lime oil, the differences are more striking between distilled and expressed oils. During the distillation process, the concentrations of the bicyclic monoterpene hydrocarbons α -thujene, sabinene and β -pinene decrease dramatically (6). Many more monocyclic C₁₀ hydrocarbons are found in distilled lime oil and the content of terpene alcohols also substantially increases (6). In addition, some new alcohols are formed (6). Differences among expression, hydrodistillation and microwave 'dry' distillation methods used for the production of lemon oil were studied (5). The microwave 'dry' distillation method gave higher amounts of oxygenated compounds and lower amounts of monoterpene hydrocarbons, as compared to the hydrodistillation and expression methods (5). The researchers did not provide statistics for the comparison of the three methods making only guarded conclusions possible; however, noticeable differences in composition existed between hydrodistillation and expression methods for the oxygenated monoterpenes in lemon oils. Specifically, the percentages of terpin-4-ol, α -terpineol and neral were higher in the hydrodistilled oil compared to the expressed oil (9).

Composition of lemon and lime oils

There are hundreds of named cultivars of citrus fruit (3) and their production in 2006 was over 95 million tons (8). Oranges are the most commonly produced citrus fruit, accounting for over 60% of the total world production (3). Lemon flavor follows orange

as the most popular type of citrus flavor (3, 9). Lemon oil is used in the flavor and fragrance industries (10). The importance of lemon oil is apparent by the need to characterize its volatile constituents to prevent adulteration (3, 11). Citrus essential oils contain over one hundred volatile compounds (12, 13) and limonene is one of the most abundant volatile constituents (9). The abundance of the the citral isomers (i.e., geranial and neral) is a common measure of the quality of lemon oil (9, 10). These aldehydes have also been recognized as important to fresh lemon oil by sensory-directed flavor studies (13).

Moshonas and Shaw conducted an early study on aqueous lemon and lime juice essences obtained from the condensation of vapors from the first stage of a commercial juice evaporator (14). Alcohols and aldehydes comprised the largest compound classes in the lemon and lime extracts. Lemon and lime share many of the same compounds including linalool, nerol, geranial, neral, perillaldehyde and limonene (14).

A study conducted approximately 10 years later, also published by Shaw and others, focused on mandarin and grapefruit flavor. They found hydrogen sulfide in the headspace above most types of citrus juices, including lemon and lime (15). However, hydrogen sulfide content was low in lemons and limes compared to oranges and grapefruit; in the latter two fruits hydrogen sulfide may play a role in the overall aroma and flavor of the fresh juice (15).

The aroma-active compounds were identified and compared in lime peel oil obtained via extraction and distillation (16). The two varieties of lime studied were *Citrus aurantifolia* Swingle (key or Mexican lime) and *Citrus latifolia* Tanaka (Persian or Tahiti lime). Oils from both varieties are commonly used in the flavor industry, with key

lime oil preferred for use in beverages (16). Results of CharmAnalysis revealed that the compounds with the seven highest odor spectrum values (OSVs, normalized Charm values) in lime oil prepared by both methods were the same: geranial, perilla aldehyde, nonanal, linalool, nerol, citronellol and neral (16). Straight chain saturated (i.e., normal) aldehydes from C₆ to C₁₈, minus C₇ and C₁₇, in addition the geranial, neral and citronellal, made up about 66% of the total volatile content of the extracted lime oil (16).

Lemon verbena (*Aloysia citriodora* Palau) oil is important to the flavor and fragrance industry due to its lemony profile and its heavy use by the soft drink industry (10). A study was done to characterize typical lemon verbena from Argentina for quality control purposes. The researchers studied 27 samples of South American lemon verbena oil, 23 from of Argentina, two from Paraguay and two from Chile (10). Most of the Argentine samples and the two samples from Paraguay had similar volatile profiles (10). Twenty seven compounds, accounting for about 90% of the total oil content, were identified in the typical lemon verbena oils studied (10). The isomers of citral (i.e., geranial and neral) were the most abundant volatile constituents of *Aloysia citriodora*, comprising 29.0% and 20.0% of the composition, respectively (10). Caryophyllene oxide (11.1%) and limonene (10.3%) were the next most abundant compounds. Other compounds contributing to at least 1% of the composition were: geranyl acetate (3.9%), neryl acetate (3.0%), *ar*-curcumene (2.6%), spathulenol (2.6%), α -muurolol (1.7%) and nerol (1.1%) (10). The samples originating from Chile were higher in sabinene and 1,8-cineole than the typical oils described earlier (10). It should be noted that the most abundant compounds are not necessarily the most important contributors to the flavor, although quantification of the compounds present is useful for quality control purposes.

Acid-catalyzed rearrangements of terpenes

A rich amount of organic chemistry takes place under aqueous acidic conditions in citrus oils. Figure 2 give an overview of the acid-catalyzed rearrangements that occur and shows the complexity of the terpene chemistry. A single compound such as limonene can be the starting point of a whole series of reactions. Additionally, a single compound may be derived from several sources. For example, α -terpienol can be formed from either limonene, α - or β -pinene or terpinolene.

Limonene emerged as an important aroma-active compound in sensory-directed research of fresh lemon oil (17) and also accounts for a large portion of lemon oil (6, 16). In distilled lime oil, 39-47% w/w of the oil is limonene, and cold-pressed lime oil contains 50% w/w limonene (16). Furthermore, orange peel oil contains about 80% limonene (18). The limonene rearrangement products may actually be more important than limonene itself: an aqueous limonene emulsion that had been stored for 15 days at pH 2.8 and 25-30 °C was significantly preferred to a limonene control that had been stored at 5 °C (6).

The main acid catalyzed hydration products of limonene in 95% acetone/water at 75 °C are α -terpineol, β -terpineol and terpinolene (6). The reaction scheme with intermediates is shown in Figure 2.3. The double bond in β -terpineol reacts to form the secondary products *trans*- and *cis*-1,8 terpin (6). Terpeneols and terpins contributed about 97% of the volatile products when a dilute aqueous emulsion of limonene was allowed to react (60 ppm, pH 2.8) (6). Under harsher conditions, carbocation C reacts to form terpinen-4-ol, which can react further to give 1,4-cineole and 1,4-terpin (6).

Another study on the degradation of lemon oil found that elevated temperatures and low pH promote the decomposition of citral (19). When beverages containing citral were stored at 45 °C, only trace amounts of citral remained after 40 hours of storage (19). Less degradation occurred at 4° C, where after 20 days (480 hours) of storage 30% of the original citral remained (19). The ultimate goal of the Freeburg et al. study was to determine the importance of citral degradation products to off-flavors in lemon-flavored beverages in acidic environments. Aromagrams from gas chromatography-olfactometry (GCO) and sensory analysis found that citral degradation is not a significant factor for off-flavor development (19). The aromagrams for citral-containing versus citral-free aged beverages were very similar, differing by only two odorants, in addition to citral. The other two citral degradation products, *p*-mentha-1,5-dien-8-ol and *p*- mentha-1(7),2-dien-8-ol, did not contribute to the aromagram (19). This data shows that citral degradation products contribute little to off-flavors in lemon flavor and the loss of citral may be more important than its degradation products (19).

Overview of selected methodology

Solvent-assisted flavor evaporation (SAFE): The lemon-lime carbonated beverages were extracted using liquid-liquid continuous extraction, followed by SAFE. The aroma extract isolation method requires careful consideration due to the bias inherent in any method chosen. SAFE was developed in the late 1990's by Engel et. al (20). The precursor to this technique is high vacuum transfer (HVT) which has several drawbacks, including the partial condensation of higher boiling point aroma compounds on the tubing of the apparatus prior to reaching the cold traps (20). SAFE was determined to be

superior in the recovery of selected compounds, as compared to HVT. SAFE was shown to give higher percent yields of a series of *n*-alkanes. Although both methods showed a decrease in percent yield as *n*-alkane chain length increased (with subsequent increase in boiling point), SAFE gave significantly higher yields for each *n*-alkane. Further, SAFE gave higher yields when distilling polar compounds, such as vanillin, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) and 3-methylbutanoic acid, compared to HVT (20). Other research comparing the isolation methods of SAFE, steam distillation under reduced pressure coupled with continuous liquid-liquid extraction (DRP-LLE), and high-flow dynamic headspace sampling (DHS) showed that SAFE was able to better recover certain volatiles that the other methods could not recover or poorly recovered, especially very polar compounds (21). SAFE was able to recover more of the aromatic and aliphatic constituents than the other methods but performed the worst of the three methods at recovering terpenoids (21). Other advantages of SAFE, although less important for the research discussed here, is that SAFE can be applied to high-fat (50%) matrices and to some foods without prior extraction (milk, beer, fruit pulps) (20). The high recovery of volatile and semi-volatile constituents makes SAFE an excellent choice for aroma extraction.

Stable isotope dilution assay (SIDA): One potential problem in determining the concentration of selected aroma compounds in a mixture is extraction bias, as previously mentioned. The technique chosen for analysis will unfortunately favor some compounds over others, i.e., in a headspace technique the more volatile components may be detected more easily and an erroneous conclusion would be made that these highly volatile

compounds are more abundant than other, less volatile compounds. Using stable isotopes for quantification, so called stable isotope dilution assay (SIDA), can overcome the problem of extraction bias. This is a widely accepted technique for quantification, as seen by its common use in recent years (22-25). A stable isotope is a compound identical to the target compound, except that it contains (or is labeled with) a known number of deuterium (^2H) or carbon-13 (^{13}C) atoms. A stable isotope has a mass spectrum that contains one or several m/z ions that differ from the target or unlabeled compound. However, despite this difference in spectra, the target compound and its isotope have many other properties that are the same, such as volatility and odor. In SIDA, a known amount of the stable isotope is spiked into the sample prior to extraction and GC-MS analysis. The peak areas of the isotope and target compound are then compared; this information coupled with the amount of isotope added to the sample and the MS response factor (which relates the MS of isotope to the MS of the unlabelled compound) allows for the determination of the amount of target compound that was initially present in the sample. Therefore, even if the target compound is poorly extracted by the chosen isolation method, the use of a stable isotope standard will correct for this since the isotope is extracted to the same extent as the target compound.

Solid Phase Microextraction (SPME): SPME is a widely used extraction technique owing to its speed and simplicity. However, the use of this technique requires rigorous calibration to achieve accuracy. SPME is subject to sampling bias and will favor extraction of nonpolar compounds. Roberts et. al determined that sensitivity is better for nonpolar compounds, which are easily detected at ppb levels, whereas polar compounds

at the ppm level may be more difficult to detect (26). This means that important aroma compounds like 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and vanillin may not be detected by SPME because these highly polar compounds are normally present in the ppb or low ppm range (26). Additionally, some compounds analyzed by SPME may exceed their linear range, meaning that the concentration of a compounds in the headspace is not proportional to its concentration bound to the fiber (26). Another potential source of error in SPME results from compounds competing for adsorption by the fiber, which was observed in a study where the addition of a nonpolar compound caused a decrease in absorption by other compounds having polar functional groups (26). The use of stable isotopes as internal standards (i.e., SIDA) solves the above problems. As already mentioned, the target analyte will be extracted to the same extent as its isotope analogue. As a result, the distinct advantages of SPME can be utilized, namely that large numbers of sample can be analyzed because virtually no sample preparation is necessary. The time for sample extraction via the SPME fiber is dramatically reduced compared to the time required for solvent extractions. SPME is a very powerful and useful tool, provided that its limitations are recognized and steps, such as application of SIDA, are taken to minimize them.

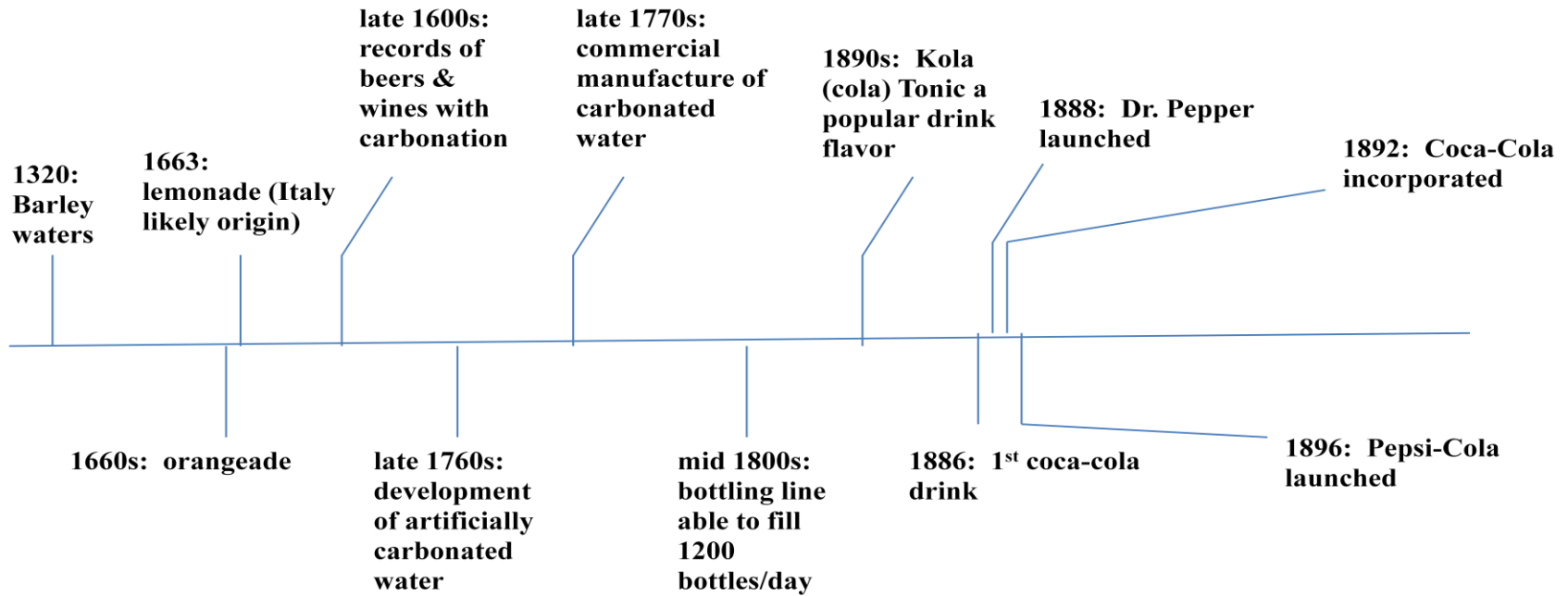


Figure 2.1 Timeline of key developments in the history of carbonated beverages (based on information in ref (1))

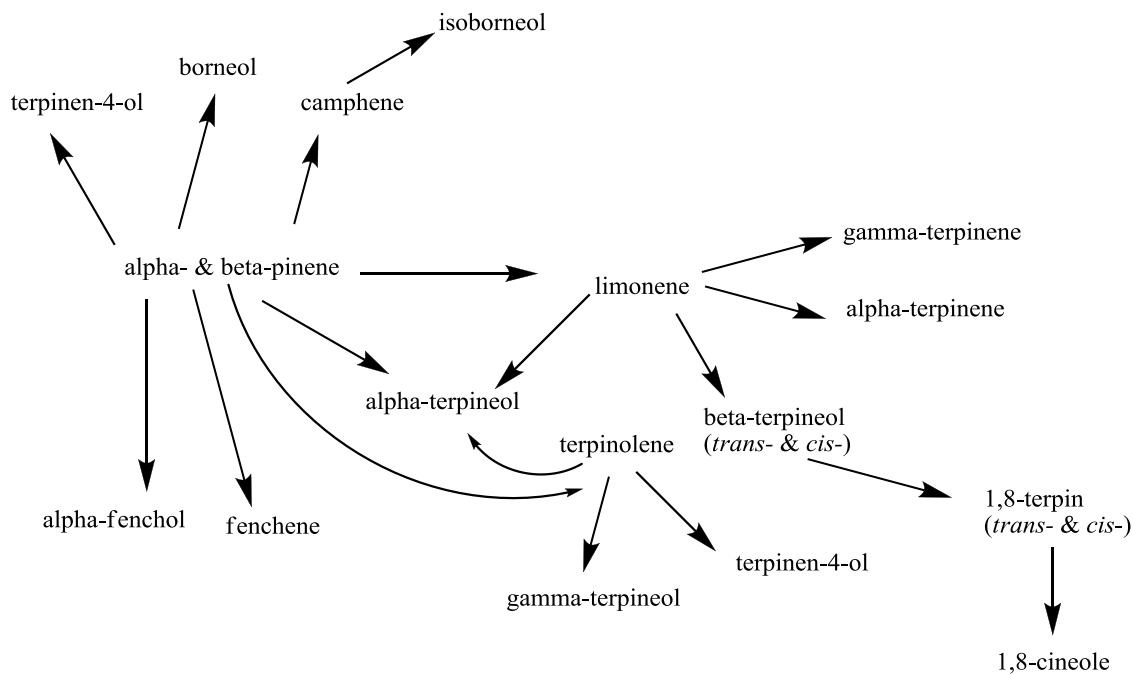


Figure 2.2 Overview of the acid-catalyzed reactions occurring when citrus oils are exposed to an acidic environment (based on information in ref (6))

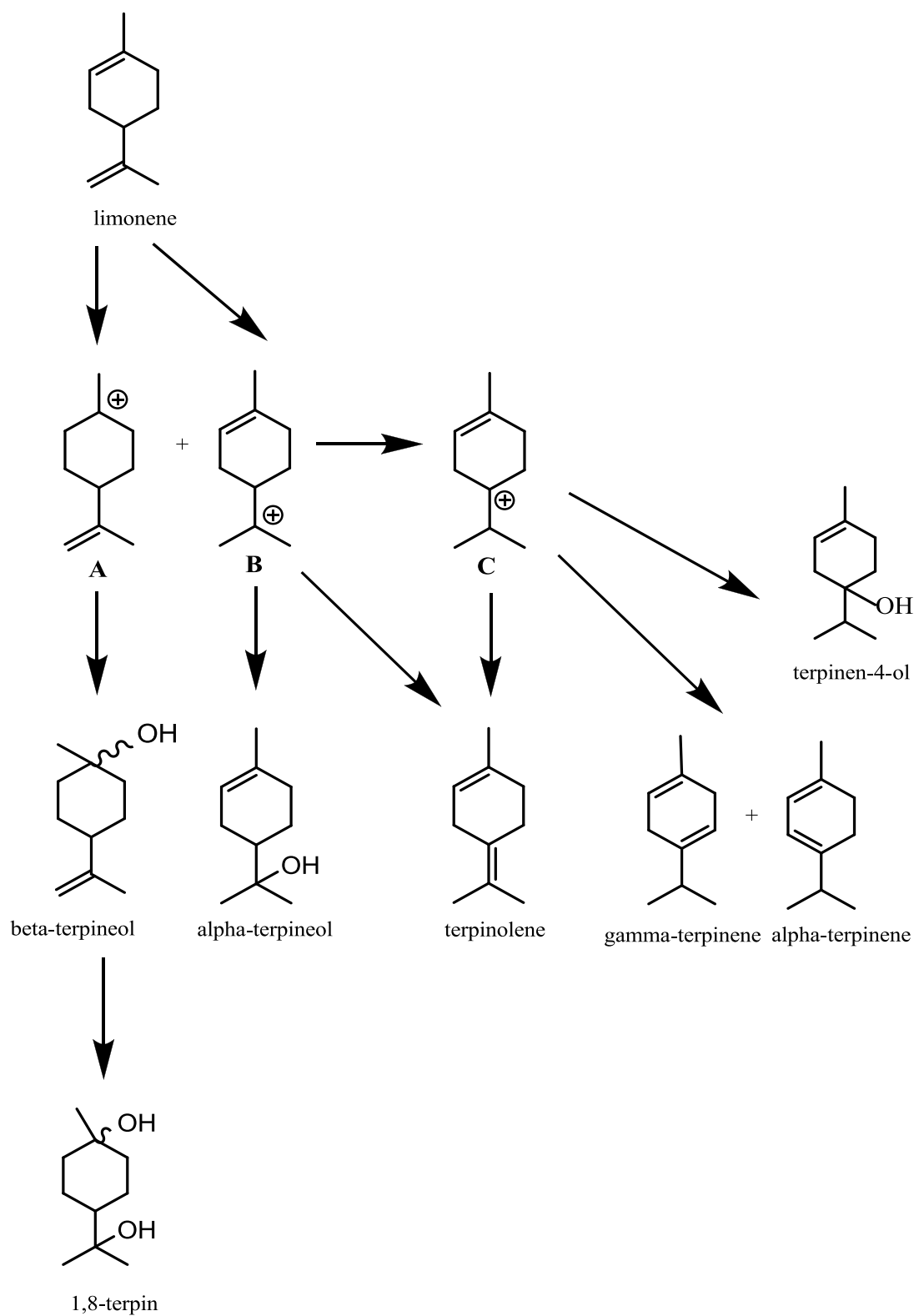


Figure 2.3 Simplified schematic of the acid-catalyzed rearrangement of limonene, (from (6))(A, B, C are carbocation intermediates and C is only formed under harsh distillation conditions).

REFERENCES

1. Hargitt, B. Introduction. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D. P., Ashurst, P. R., Eds.; Blackwell Publishing Professional: Oxford, UK, 2006; pp. 1-14.
2. Riley, J. J. *A History of the American Soft Drink Industry*, American Bottlers of Carbonated Beverages: Washington, DC, 1958; pp. 1-302.
3. Rouseff, R.; Perez-Cacho, P. R. Citrus flavour. In *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*; Berger, R.G., Ed.; Springer: Berlin, Germany, 2007; pp. 117-134.
4. Knight, T. G.; Klieber, A.; Sedgley, M. The relationship between oil gland and fruit development in Washington Navel orange (*Citrus sinensis* L. Osbeck). *Ann. Bot. - London* **2001**, *88*, 1039-1047.
5. Ferhat, M. A.; Meklati, B. Y.; Chemat, F. Comparison of different isolation methods of essential oil from *Citrus* fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. *Flavour Frag. J.* **2007**, *22*, 494-504.
6. Clark, B. C., Jr.; Chamblee, T. S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-flavors in foods and beverages*; Charalambous, G., Ed.; Elsevier Science Publishers, 1992; pp. 229-285.
7. Thavanapong, N.; Wetwitayaklung, P.; Charoenteeraboon, J. Comparison of essential oils compositions of *Citrus maxima* Merr. peel obtained by cold press and vacuum steam distillation methods and of its peel and flower extract obtained by supercritical carbon dioxide extraction method and their antimicrobial activity. *J. Essent. Oil Res.* **2010**, *22*, 71-77.
8. Barboni, T.; Luro, F.; Chiaramonti, N.; Desjobert, J.-M. ; Muselli, ; Costa, J. Volatile composition of hybrids *Citrus* juices by headspace solid-phase micro extraction/gas chromatography/mass spectrometry. *Food Chem.* **2009**, *116*, 382.
9. Veriotti, T.; Sacks, R. High-speed GC and GC/time-of-flight MS of lemon and lime oil samples. *Anal. Chem.* **2001**, *73*, 4395-4402.
10. Lira, P.; van Baren, C. M.; Retta, D.; Bandoni, A. L.; Gil, A.; Gattuso, M.; Gattuso, S. Characterization of lemon verbena (*Aloysia citriodora* Palau) from Argentina by the essential oil. *J. Essent. Oil Res.* **2008**, *20*, 350-353.
11. Lifshitz, A. Stepak, Y.; Basker, H. Characterization of Isreal lemon oil and detection of its adulteration. *J. Food Sci.* **1969**, *34*, 254-257.
12. Mondello, L.; Casilli, A.; Tranchida, P. Q.; Dugo, P.; Dugo, G. Comprehensive two-dimensional GC for the analysis of citrus essential oils. *Flavour Fragr. J.* **2005**, *20*, 136-140.
13. Schieberle, P.; Grosch, W. Potent odorants resulting from the peroxidation of lemon oil. *Z Lebensm Unters Forsch* **1989**, *189*, 26-31.

14. Moshonas, M. G.; Shaw, P. E. Analysis of flavor constituents from lemon and lime essence. *J. Agr. Food Chem.* **1972**, *20*, 1029-1030.
15. Berry, R. E.; Shaw, P. E.; Tatum, J. H.; Wilson, C. W., III. Citrus oil flavor and composition studies. *Food Technol.-Chicago.* **1983**, 88-91.
16. Chisholm, M. G.; Wilson, M. A.; Gaskey, G. M. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). *Flavour Frag. J.* **2003**, *18*, 106-115.
17. Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil citric-acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
18. Sell, C. S. *A Fragrant Introduction to Terpenoid Chemistry*, The Royal Society of Chemistry: Cambridge, UK, 2003; pp. 43-63.
19. Freeburg, E. J.; Mistry, B. S.; Reineccius, G. A.; Scire, J. Stability of citral-containing and citralless lemon oils in flavor emulsions and beverages. *Perfumer and Flavorist* **1994**, *19*, 23-32.
20. Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237-241.
21. Park, B.; Lee, K.; Takeoka, G. Comparison of three sample preparation methods on the recovery of volatiles from taheebo (*Tabebuia impetiginosa* Martius ex DC). *Flavour Frag. J.* **2004**, *19*, 287-292.
22. Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862-2866.
23. Semmelroch, P.; Grosch, W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537-543.
24. Lorenzo, G.; Morales, F. Quantitation of acrylamide in potato chips by hplc-ms. *Agro. Food Industry Hi. Tech.* **2010**, *21*, 22.
25. Roland, A.; Schneider, R.; Le Guerneve, C.; Razungles, A.; Cavelier, F. Identification and quantification by LC-MS/MS of a new precursor of 3-mercaptohexan-1-ol (3MH) using stable isotope dilution assay: Elements for understanding the 3MH production in wine. *Food Chem.* **2010**, *121*, 847-855.
26. Roberts, D.; Pollien, P.; Milo, C. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.* **2000**, *48*, 2430-2437.

CHAPTER 3

IDENTIFICATION OF AROMA-ACTIVE COMPONENTS OF THREE COMMERCIAL BRANDS OF LEMON-LIME CARBONATED BEVERAGES

ABSTRACT

The most potent aroma-active components of Sprite[®] (SP), Sierra Mist[®] (SM), and 7UP[®] (7UP) were identified. Aroma extracts were prepared by liquid-liquid continuous extraction/solvent-assisted flavor evaporation (LLCE/SAFE). Twenty eight compounds were detected by GCO with linalool (floral, lavender), octanal (pungent orange) and 2,3-dehydro-1,8-cineole (minty) determined to be predominant aroma compounds based on their high flavor dilution (FD) factors by aroma extract dilution analysis (AEDA). Other important aroma-active compounds in at least one brand included nonanal (orange), decanal (cilantro), borneol (camphorous), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (burnt sugar), *p*-cresol (stable, dung), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (curry), benzoic acid (sweet, candy), and an unknown (fresh, melony). Although many compounds are common to the three brands, the relative importance of the aroma-active compounds is brand dependent. The data indicate that lemon-lime is composed of a small number of compounds (22 at the most in SM) and only a subset of these may be important since many compounds were only detected at low FD factors.

INTRODUCTION

7UP[®] (7UP), originally a caramel colored carbonated beverage with the name Bib-Label Lithiated Lemon-Lime Soda, was created in 1929 and was the third best-

selling carbonated beverage in the world in the late 1940s (1). In fact, 7UP's success was so great that the company founder changed the Corporation's name from The Howdy Corporation to The Seven-Up Company (1). According to Coca-Cola's web site, Sprite[®] (SP) is the leading lemon-lime carbonated beverage in the world and the 4th ranked carbonated beverage overall in the world, being sold in over 190 countries (2). SP has been sold since 1961 (2). Sierra Mist[®] (SM) is a relative newcomer to the lemon-lime market, created by PepsiCo, Inc. in 2000, although the brand Mountain Dew[®] has been on the market since 1948, even before PepsiCo, Inc. existed (PepsiCo, Inc. was formed as a result of the merger of Pepsi-Cola and Frito-Lay in 1965) (3). In the 1940's Mountain Dew was first bottled as a personal mixer for hard-liquor and the flavor was described as "similar to 'lemon-lime soda'" (4). Interestingly, the beverage was dubbed Mountain Dew after Tennessee Mountain Moonshine (4). The flavor of Mountain Dew was not a subject of the present study and the beverage differs from the other three brands mentioned above in that it contains caffeine, whereas the other three are caffeine free (5-7), making SP, SM, and 7UP more equal competitors for comparison purposes.

The glimpse at the history of these three lemon-lime brands shows their immense popularity and even the global acceptance of lemon-lime as a carbonated beverage flavor. The central hypothesis of the present study is that there are particular compounds common to each brand, perhaps necessary for what is generally recognized as "lemon-lime" flavor. However, at the same time, each brand has unique compounds and the relative importance of compounds is brand dependent.

Food and beverage flavors often contain hundreds of compounds and the concentrations of these compounds, as well as how they interact with each other, creates

the unique sensory impressions that the human nose can almost instantaneously recognize (8). Flavor compounds are generally present at very low levels (ppm to ppt range) and normally a group of compounds, each at a particular concentration, is necessary for a particular odor, as opposed to a single “flavor impact compound” (8). Due to the extremely large number of compounds that are possible in an aroma extract, there is a need to order or categorize the compounds into levels of importance. The gas chromatographic (GC) peak area or abundance of a compound may seem at first to be effective at determining compound importance. However, the compound’s threshold also provides key information regarding the compound’s importance to the flavor. Aroma extract dilution analysis (AEDA) is one of several sensory-directed methods available for determining compound importance (CharmAnalysis is another common method). The use of AEDA can lead to the identification of compounds that may be overlooked if only the GC-MS chromatogram was examined because aroma-active compounds are usually not the major food volatiles (9). AEDA serves an effective screening tool for the important compounds in an aroma extract and compounds with high and medium dilution factors become the focus of compound identification (9). Some recent examples of previous studies employing GCO and AEDA included the identification of compounds producing off-flavors in raw coffee beans (10) and determination of the key aroma compounds in a black tea infusion (11). The latter study used AEDA to compare the aroma-active compounds in the tea leaves and hot water tea infusions; this work was followed with quantification using stable isotope dilution assays (11).

The main objectives of the present research were to: 1) identify the key aroma-impact compounds in three commercial lemon-lime carbonated beverages; 2) determine

the relative importance of these compounds based on their flavor dilution factors; and 3) compare aroma-active constituents across brands.

METHODS

Materials

Sprite (Coca-Cola Company, Atlanta, GA) (expiration September 21, 2009 HOB; 0652 4), Sierra Mist (PepsiCo, Inc., Purchase, NY) (expiration June 15, 2009; 1914QY011293), and 7UP (Dr Pepper/Seven Up, Inc., Plano, TX) (Lot 81001A3CK066) were purchased in January 2009 at a local grocer (Urbana, IL).

Chemicals

Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO). Nerol and geraniol were obtained from Bedoukian Research, Inc. (Danbury, CT) and acetic acid was obtained from Fisher Scientific (Fair Lawn, New Jersey). Deodorized distilled water was prepared by boiling glass-distilled deionized water in an open flask until its volume was reduced by one forth. The compound *trans*-4,5-epoxy-(*E*)-2-decenal was synthesized as previously described (12). The compound 2,3-dihydro-5-hydroxy-6-methyl-4(*H*)-pyran-4-one (dihydromaltol) was synthesized using the method described by Mills (13). The synthesis of 2,3-dehydro-1,8-cineole is described in chapter 4.

Preparation of Aroma Extracts

Volatile components were isolated by liquid-liquid continuous extraction (LLCE). This method allowed for simultaneous decarbonation of the beverage during extraction,

thus minimizing volatile losses that might have occurred if the product had undergone a decarbonation step prior to extraction. For each extraction, 500 mL of a carbonated beverage plus 100 mL of deodorized deionized-distilled water and 20 μ L of an internal standard solution (417 μ g/mL of 6-undecanone in methanol) were placed in the LLCE apparatus (Z101567, Sigma-Aldrich Co.). The device was connected to 250-mL round bottom flask containing the extraction solvent (200 mL of diethyl ether), which was subsequently refluxed by heating the flask in a 47 °C water bath. The condenser of the LLCE apparatus was held at 5 °C. Extractions were carried out for 18 h at room temperature (~ 23 °C) with constant stirring of the sample chamber using a magnetic stir bar. After extraction, the ether layers were recovered, concentrated to 50 mL using a Vigreux column at 43 °C, and then subjected to solvent-assisted flavor evaporation (SAFE) for 2 h according to method described by Rotsachakul et al. (14). After SAFE, the aroma extract was further concentrated to 10 mL using a Vigreux column at 43 °C. The aroma extract was dried over anhydrous sodium sulfate and then concentrated to 500 μ L using a gentle stream of nitrogen. Each aroma extract was stored in a 2 mL vial equipped with a PTFE-lined screw cap at -70 °C until analysis.

Gas Chromatography-Olfactometry (GCO) and Aroma Extract Dilution Analysis (AEDA)

The relative potency of individual odorants was determined using AEDA according to the method previously described (15). This method is based on the concept that the relative potency of a compound in an aroma extract depends on both its concentration and its odor detection threshold. In AEDA, the concentrated extract

undergoes serial dilution. In each successive dilution, fewer compounds are detected by GCO. The highest dilution at which a compound can be detected corresponds to its flavor dilution (FD) factor. A dilution factor of three was used in the present study. Therefore, if a compound could be detected in original aroma extract, the 1:3 dilution and the 1:9 dilution, but not in the 1:27 dilution, it would have an FD factor of 9. Compounds with the high FD factors are ranked higher in importance than those with lower FD factors.

Serial dilutions (1:3, v/v) of each aroma extract were prepared in diethyl ether. Each dilution was stored in a 2 mL vial equipped with a PTFE-lined screw cap at -70 °C until analysis. The GCO system consisted of a 6890 GC (Agilent Technologies Inc., Palo Alto, CA) equipped with a FID, an on-column injector (+3 °C temperature tracking mode) and an olfactory detection port (DATU Technology Transfer, Geneva, NY). Dilutions and concentrated extracts were analyzed on polar capillary column (RTX-Wax, 15 m × 0.32 mm i.d.; 0.5 μm film; Restek, Bellefonte, PA). Concentrated aroma extracts were also analyzed using a nonpolar capillary column (RTX-5MS, 15 m × 0.32 mm i.d.; 0.5 μm film; Restek) for calculation of retention indices (RI). For GCO, column effluent was split 1:5 between the FID and olfactory detection port using deactivated fused silica tubing (1 m × 0.25 mm i.d.; Restek), with both detector temperatures held at 250 °C. The GC oven temperature was programmed from 40 to 225 °C at a rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. Helium was used as a carrier gas at a constant flow rate of 2.2 mL/min. Other conditions of GCO have been previously described (16).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS system consisted of a 6890 GC/5973N MSD (Agilent Technologies Inc.) equipped with cool on-column injector (+3 °C temperature tracking mode). Separations were performed on 1 µL injections of each extract using either a polar capillary column (RTX-Wax, 30 m × 0.25 mm i.d.; 0.5 µm film; Restek) or a nonpolar column (RTX-5SLIM, 30 m × 0.25 mm i.d.; 0.5 µm film; Restek). The oven temperature was programmed from 35 to 225 °C at a rate of 4 °C/min with initial and final hold times of 5 and 20 min, respectively. Helium was used as carrier gas at a constant rate of 1.0 mL/min. The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35-300 amu; electron multiplier voltage (Autotune + 200 V); scan rate, 5.27 scans/s.

Compound Identification

Compound identification was based on matching retention indices (on two different GC column phases), odor descriptions and mass spectra of unknowns with those of authentic standards. Tentative identifications were based on one or more, but not all, of the above criteria. A homologous series of *n*-alkanes was used for the determination of retention indices according to the method of van Den Dool and Kratz (17).

RESULTS & DISCUSSION

Total ion (GC-MS) chromatograms of aroma extracts prepared from the three brands of lemon-lime carbonated beverages are presented in Figure 3.1. To enable side-by-side comparisons the scale for each chromatogram was adjusted so that the maximum abundance of the chromatogram is 3.2×10^7 and the first 55 minutes of the run is

displayed. It is immediately evident that the brands varied in their volatile compositions. For example, SP and SM both contained appreciable amounts of benzoic acid, which was not present in 7UP. This compound is added as preservative in SP and SM, but is not an ingredient of 7UP, as indicated on the ingredient labels (5-7). Furthermore, it is clear that α -terpineol was in much greater abundance in SP and 7UP than in SM. This brief and cursory comparison of the lemon-lime volatile constituents demonstrates that there are distinct differences among brands that likely account for the differences in their perceived flavors.

Potent Odorants in Lemon-Lime Carbonated Beverages

Predominant aroma-active compounds in the three brands of lemon-lime carbonated beverages were identified by means of gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA). A combined total of 28 aroma-active compounds were detected in the three brands (Table 3.1). Twenty-two aroma compounds were detected in SM, with 20 being detected in both SP and 7UP. The corresponding structures for these compounds are found in Figure 3.2.

The identification data for the aroma-active compounds in SP, SM, and 7UP showed that linalool (no. **10**) was the single most potent aroma compound in lemon-lime carbonated beverages and was separated from the second most potent aroma compound in each brand by at least one flavor dilution (FD) factor (FD factors of 243, 729, and 2187 in SP, SM, and 7UP, respectively). In SP, the second most potent aroma compounds were 2,3-dehydro-1,8-cineole (dehydrocineole, no. **2**), octanal (no. **4**), borneol (no. **14**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF, no. **23**) and *p*-cresol

(no. **24**) (FD factors of 81). In SM, octanal was the second most important aroma-active compound (FD factor of 243). Similar to SP, 7UP's second most important aroma-active compounds were dehydrocineole, octanal and *p*-cresol (FD factors of 81). Benzoic acid (no. **28**) had moderately high FD factors in SP and SM, but was not detected in 7UP. Odorants with low FD factors in all three brands included isoborneol (no. **12**), nerol (no. **16**), geraniol (no. **18**), sotolon (no. **26**) and an unknown compound (no. **27**). The data indicate that lemon-lime is composed of a small number of compounds (22 at the most, in SM) and only a subset of these may be important since many compounds were only detected at the 1:3 dilution or below.

Linalool is hypothesized to be necessary for a recognizable lemon-lime flavor, based on the high FD factors observed for linalool in all three brands of lemon-lime carbonated beverages. Omission studies excluding linalool from a lemon-lime model would be necessary to test this hypothesis.

Origins and Properties of Specific Odorants

It should stand out from Table 3.1 that the lemon-lime carbonated beverages contain several groups of compounds with similar or nearly identical odor properties and which also elute at similar retention indices (on the polar GC column). Specifically, these include 2,3-dehydro-1,8-cineole (no. **2**) and 1,8-cineole (no. **3**) with piney and eucalyptus notes; isoborneol (no. **12**) and borneol (no. **14**) with camphorous notes; neral (no. **13**), geraniol (no. **15**), nerol (no. **16**) and geraniol (no. **18**) with lemony/lemon cleaner notes. Additionally, there were four compounds with burnt sugar notes [an unknown (no. **9**), dihydromaltol (no. **19**), maltol (no. **20**), and HDMF (no. **23**)]. Three

aliphatic, straight chain (*n*-) aldehydes, octanal (no. **4**), nonanal (no. **5**) and decanal (no. **8**), with similar pungent citrusy notes were also detected. The compounds in each group are structurally similar to one another. The presence of two or more structurally similar compounds makes unambiguous compound identification more challenging due to the close or overlapping retention times during GCO. It is also interesting to consider that similar compounds and isomers are often present in an aroma extract. This is a strong indication that compound reactions and rearrangements have occurred, which may be particularly important in the present “acidic” system. Although the aforementioned groups of compounds are similar with respect to their odor properties, one should not forget that even compounds that are very structurally similar, e.g., enantiomers, can sometimes have distinctly different odor properties (18). The classic example is carvone: (+)-carvone has a caraway-like odor, while (-)-carvone is spearmint-like (18). As seen in Figure 3.2, isoborneol (no. **12**) and borneol (no. **14**) differ only in the orientation of the hydroxy group; in this case no. **12** is the *exo*-isomer and no. **14** is *endo*-isomer. Neral (no. **13**) and geranial (no. **15**) are *cis* and *trans* isomers of citral, respectively. Furthermore, nerol (no. **16**) and geraniol (no. **18**) are the respective alcohols of these two aldehydes. Some of the compounds are present in the original flavoring (derived from lemon oil), such as the citral isomers, but others are acid-catalyzed rearrangement products of the numerous terpene and terpenoid constituents of the flavoring. Therefore, it is intuitive that many compounds should be structurally similar.

The compounds found in lemon-lime carbonated beverages generally come from three sources: 1) they may be naturally present in the lemon oil-based flavoring (e.g. citral isomers), 2) they may be formed as a result of acid-catalyzed rearrangement or 3)

they may come from some other source, such as being intentionally added to the beverage by the manufacturer (e.g. benzoic acid).

Across all three brands under consideration in the present study, linalool (no. **10**) was determined to be most potent, having the highest FD factor (243, 729, and 2187 in SP, SM and 7UP, respectively). Linalool is commonly found in lemon and lime oils (19-23), as well as other citrus oils (24, 25). Linalool has a fresh, floral and lavender-like odor. The odor detection threshold for linalool is 6 ppb (26) (Table 3.2). Linalool has a chiral center at carbon 3; it is interesting to note that (*R*)-(-)-linalool has an odor threshold about 80 times lower than the (*S*)-(+)-enantiomer (27). Therefore, it is important to consider the enantiomeric distribution of linalool in lemon oil, where the (*R*)-enantiomer usually exists in enantiomeric excess (ranging from 4.6 to 28.3%), but experiences a large amount of seasonal variation (21, 22).

In addition to being naturally present in lemon and lime oils, linalool can be produced by structural rearrangements of other compounds (e.g. geraniol and nerol) frequently found in lemon/lime oils (28). A stability study was performed in an acidic medium (pH 2.9) to obtain an idea of how quickly geraniol and nerol rearrange to form linalool. It was found that after one hour at 25 °C linalool was present at a detectable level in the case of geraniol degradation and after 2 hours in the case of nerol degradation (29). Furthermore, after 8 hours, 2% and 1% of the geraniol and nerol, respectively, had been converted to linalool (29). While it is clear that linalool is formed relatively quickly from nerol and geraniol under acidic conditions, it should also be noted that linalool itself is not stable in an acidic environment. In this same stability study (at pH 2.9), linalool was transformed into α -terpineol after only 2 hours (29). Similarly, Baxter et al. found

that linalool was the major component in solution after storing geraniol for 10 days in aqueous 0.025 M citric acid solution at 24 °C (42.4% of initial geraniol had been converted to linalool) (30). After 20 days, the concentration of linalool had decreased from day 10 (42.4% to 35.0%) and the concentration of α -terpineol had more than doubled from day 10 to 20 (13.8% to 29.9%) (30).

As expected, the citral isomers, geranial (no. **14**) and neral (no. **12**), were among the aroma-active components in the lime-lime carbonated beverages. The levels of these isomers can be quite high in lemon oil, e.g., 29.0% geranial and 20.0% neral were reported in lemon verbena oil (23). The presence of high levels of citral can be used as measure of essential oil quality (23, 31). Compounds nos. **12** and **14** have lemon and lemon cleaner-like aromas. Yet, although these aldehydes are clearly important in lemon and lime oils, they did not emerge as predominant aroma components in lemon-lime carbonated beverages, as indicated by their relatively low FD factors. The conspicuous absence of neral and geranial as potent aroma compounds can be explained by their tendency to readily undergo acid-catalyzed rearrangements.

A study which examined the peroxidation of lemon oil exposed to oxygen and light at neutral pH showed that the concentrations of geranial and neral, among other compounds, declined to the point where they made a negligible odor contribution to the lemon oil flavor after only five days (32). Many new odorants were formed after peroxidation of the oil, with carvone, *p*-methylacetophenone, 4-acetyl-1-methyl-1-cyclohexene and *p*-cresol (no. **24**) emerging as the most potent aroma constituents of the abused oil (32). Although this study considered peroxidation of lemon oil rather than acid-catalyzed reactions, it is informative to see which off-flavors were formed. Of

greatest interest is *p*-cresol, which in the present study was an important aroma-active compound in SP and 7UP as demonstrated by its high FD factors of 81 in both products.

A study that followed the degradation of citral over two weeks in an acidic buffer solution (pH 3.0; stored in the dark at 40 °C) demonstrated that nearly all the geranial and neral was transformed into other compounds in only three days (33). The main compounds formed were *p*-cymene, *p*-mentha-1,5-dien-8-ol, *p*-mentha-1(7),2-dien-8-ol, *trans*-*p*-menth-2-ene-1,8-diol and *cis*-*p*-menth-2-ene-1,8-diol (33). An even more interesting finding was that all of these compounds, except for *p*-cymene, began to decline as soon as the citral was depleted (33). It should be noted that *p*-cresol is not formed directly from citral. Instead, the above study indicated that *p*-cresol is formed from the intermediate products, meaning *p*-cresol is a secondary degradation product from citral. The concentration of *p*-cresol increased steadily over the two week study period, which further support this proposition (33). Another one of the oxidation products formed was *p*-methylactophenone (33). Both *p*-methylactophenone and *p*-cresol were important odorants derived from citral in the aforementioned study of the peroxidation of lemon oil (32).

The proposed mechanism for the conversion of citral to *p*-cresol is presented in Figure 3.3. The autoxidation step of *p*-mentha-1,4(8),5-triene requires direct reaction with molecular oxygen (34). However, in the commercial production of carbonated beverages the product is de-aerated prior to carbonation to minimize oxidative deterioration of the product and to eliminate false readings of carbon dioxide level due to the partial pressure contribution made by air (35). The target concentration of air in carbonated beverages is below 0.5 ppm (35). Although there appears to be a discrepancy

between the mechanism of formation for *p*-cresol and the actual beverage environment, it is important to note that *p*-cresol is present at low levels. Furthermore, *p*-cresol has a low threshold value of 55 ppb (36, 37). Therefore, 0.5 ppm of oxygen might be adequate for the reaction to occur to a sufficient extent. In a study that examined the degradation of citral in a carbonated beverage model, the authors concluded that some of the oxidation products formed (α -*p*-dimethylstyrene and *p*-cymen-8-ol) resulted from the reaction of *p*-mentha-1,5-dien-8-ol and *p*-mentha-1(7),2-dien-8-ol with dissolved oxygen (38). *p*-Cresol may react in the same way but these researchers did not report on *p*-cresol. In the years following that study, the importance of low threshold compounds such as *p*-cresol and *p*-methylacetophenone was revealed (34, 39). This is certainly an area where there is room for further exploration and understanding. It would be informative to conduct an experiment on terpene and terpenoid degradation in a carbonated beverage model in which the amount of oxygen present is varied so that the effect of oxygen concentration on *p*-cresol content can be properly assessed. Additionally, the role of carbon dioxide, if any, in this reaction should be explored.

As mentioned above, there were three aliphatic straight-chain (*n*-) aldehydes present in the lemon lime carbonated beverages, namely octanal (no. **4**), nonanal (no. **5**) and decanal (no. **8**). These compounds are present in the original citrus oil used to produce the lemon-lime flavorings (40-42). Based on results of AEDA, octanal is the second most potent odorant in SM with an FD of 243. It is also an important odorant in SP and 7UP (FD factors of 81 in SP and 7UP). The odor properties of *n*-aldehydes change slightly as a function of an increase in chain length. All three aldehydes are pungent, with octanal and nonanal producing an orange-like character and decanal

eliciting a cilantro note. Some work has been done to establish whether carbon chain length affects the odor property of *n*-aldehydes. In general, subjects were able to distinguish between pairs of *n*-aldehydes of differing chain length; however, octanal could not be distinguished from nonanal, nor could nonanal be distinguished from decanal (43). Based on the above observations and the results of AEDA it is clear that collectively, but not necessarily individually, the *n*-aldehydes are important to the overall aroma of lemon-lime carbonated beverages .

An unknown compound (no. 7) with a retention index (RI) of 1451 (wax) and contributing a fresh, melony aroma was detected at FD factors of 27 in SM and 9 in both SP and 7UP. It is hypothesized that this compound is a low threshold nonenal isomer. A number of the unsaturated C₉ aldehydes have green, cucumber-like (*cis*-3- and *cis*-4-nonenal) or melon-like odors (*cis*-5-, *trans*-6-, *cis*-6-, *trans*-7- and *cis*-7-nonenal) (44). Some of these compounds have extremely low odor detection threshold values in the low ppb range, such as *cis*-4-nonenal with an odor threshold of 80 ppb and *trans*-6-nonenal with an odor threshold of 4.6 ppb (both thresholds determined in paraffin oil) (44). In the present study no mass spectral data was available for this compound since it was below the GC-MS detection limits. This further supports the hypothesis that the unknown has a very low threshold. In the identification work done up until this point, two unsaturated nonenal isomers were eliminated, specifically *cis*-6-nonenal or 8-nonenal, as neither compound had the same odor properties or RI values when compared to the unknown. Further investigation of the other possible isomers of nonenal could lead to the identification this compound.

Another important potent odorant identified in lemon-lime carbonated beverages was 2,3-dehydro-1,8-cineole (dehydrocineole, no. **2**). This minty, eucalyptus smelling compound had an FD factor of 81 in all three products. Dehydrocineole has been reported as a degradation product of citral by Clark and Chamblee (28), as well as by Peacock and Kuneman (38) the latter who evaluated citral degradation in a model beverage system. Peacock and Kuneman (1985) reported dehydrocineole as a degradation product of *p*-mentha-1,5-dien-8-ol and *p*-mentha-1(7),2-dien-8-ol, which were also shown to be the precursors to *p*-cresol (38). Dehydrocineole has been reported in numerous essential oils, including lemon grass (45), lime (46), licorice root (47) and garden lovage (48), among others. This compound may be present in both the essential oil used for making the lemon-lime flavorings, as well as formed from citral in the acidic environment of the carbonated beverage. Despite its existence in numerous essential oils, dehydrocineole has received little attention in the literature.

Borneol (no. **14**) was reported as a constituent of citrus oils, including citron (41) and lemon and lime oils (40). Additionally, borneol is known to be an acid-catalyzed rearrangement product of α - and β -pinene (28). Under acidic conditions the double bond of pinene is protonated to give a carbocation, which then undergoes a Wagner-Meerwin rearrangement to form an isobornyl cation (28, 49). Figure 3.4 shows a scheme for this rearrangement. Further rearrangement of the isobornyl cation and deprotonation leads to the formation of camphene (49). Isoborneol is possibly a hydration product of camphene (28).

4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF, no. **23**) is considered to be moderately important in all lemon-lime carbonated beverages, with FD factors of 81, 9

and 27 in SP, SM and 7UP, respectively. The orthonasal odor detection threshold for HDMF has been reported to be in the ppb range, which explains why frequently a peak cannot be found for this compound by GC-MS using the scan acquisition mode (50). HDMF's threshold is pH dependent and increases as pH decreases. At pH 3, most relevant for lemon-lime carbonated beverages, the threshold is 21 ppb (50). The odor quality of HDMF is concentration dependent, being ripe strawberry-like at low concentrations and caramel- or burnt pineapple-like at higher concentrations (51).

HDMF has been identified in numerous fruits including pineapple (52), mango (53) and strawberry (54). Furanones are formed largely via Maillard reactions (51). Therefore, it would be reasonable to expect this compound to be formed from the sugar in the carbonated beverage. However, research conducted by Haleva-Toledo et al. indicated that this is not necessarily the case. The objective of their study was to identify sugars and amino acids that could account for the formation of HDMF in citrus juices. Their data showed that HDMF was only formed in the presence of rhamnose and arginine under acidic conditions. HDMF could only be formed from glucose and fructose at higher pH values, 6.0-8.0 (51). These pH values are much higher than what is found in carbonated beverages, e.g. the decarbonated lemon-lime beverages have mean pH values ranging from 3.11 (SM) to 3.35 (SP) (55). All three brands under consideration in the present study are formulated with high fructose corn syrup (HFCS), which is composed of fructose and glucose as the main constituents, with small amounts of glucose polymers, such as maltose (di-glucose) and maltotriose (triglucose) present (56). The alternatives, therefore, are that HDMF comes from the lemon oil or comes from the HFCS (HDMF present before addition to carbonated beverage). HDMF has been

identified in both clementine (57) and grapefruit (58) oils. In both of these oils HDMF was tentatively identified based on its odor descriptor and retention indices, since these researchers were unable to confirm its identity with mass spectral data.

Benzoic acid produced an enormous peak in the chromatograms of SP and SM (Figure 3.1). This compound is added in the form of sodium benzoate to SP and as potassium benzoate to SM, with the explanation “to protect taste” or “preserves freshness” provided on the label (6, 7). The salt form of benzoic acid is naturally found in cranberries, prunes, plums, cinnamon, ripe cloves and most berries (59, 60). According to Title 21 of the US Code of Federal Regulations, the maximum amount of benzoic acid allowed in foods is 0.1% (1984). In Europe, the allowed level is somewhat lower at 0.015% (150 mg/L) (59).

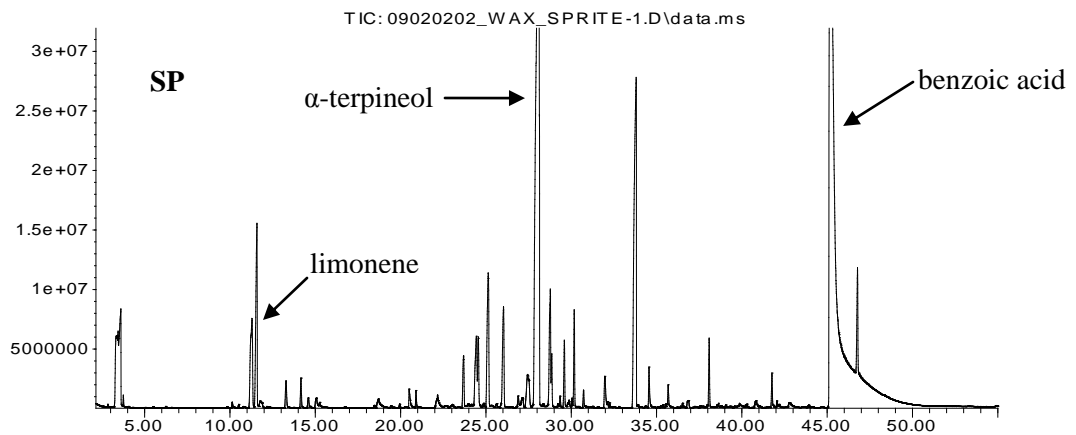
CONCLUSIONS

Based on results of AEDA, linalool was the predominant aroma compound in all three brands, followed by octanal and dehydrocineole. Other compounds with moderately high FD factors in all brands were HDMF, *p*-cresol (except SM, FD <3), nonanal (no. 5), decanal, an unknown compound (no. 7) and borneol. Benzoic acid had moderately high FD factors in SP and SM but was not detected in 7UP.

Areas of further research include identification of the currently unknown compounds, with the compound (no. 7) eluting at the RI of 1451 (wax), described as fresh, melony, being of particular interest due to its moderate importance. At this time, some compounds are considered as tentatively identified and additional work may provide data that would allow the compounds to be categorized as positively identified.

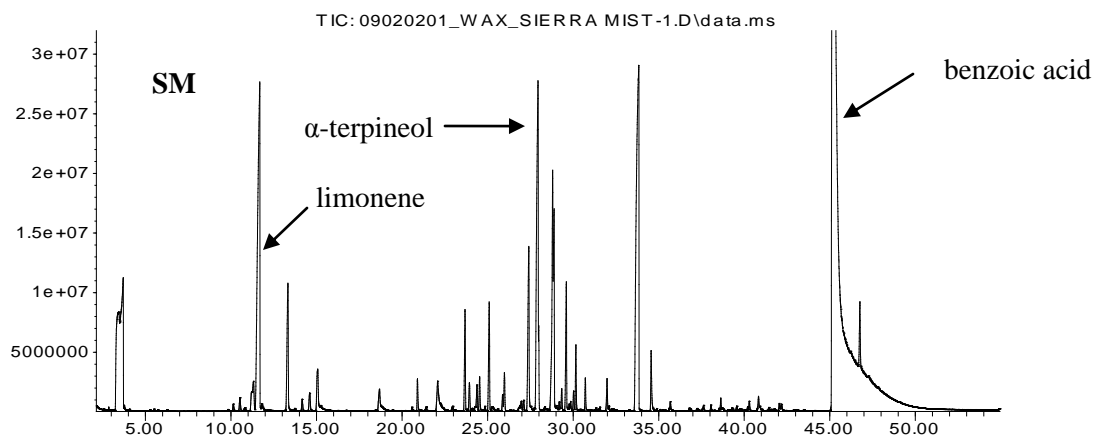
For example, HDMF is important to lemon-lime flavor but mass spectral data cannot be found to confirm its existence. By extracting and concentrating a larger initial volume of lemon-lime carbonated beverage, coupled with the use of SIM mode on the GC-MS, it may be possible to obtain mass spectral evidence for this compound, which would also enable the quantification of HDMF.

Abundance



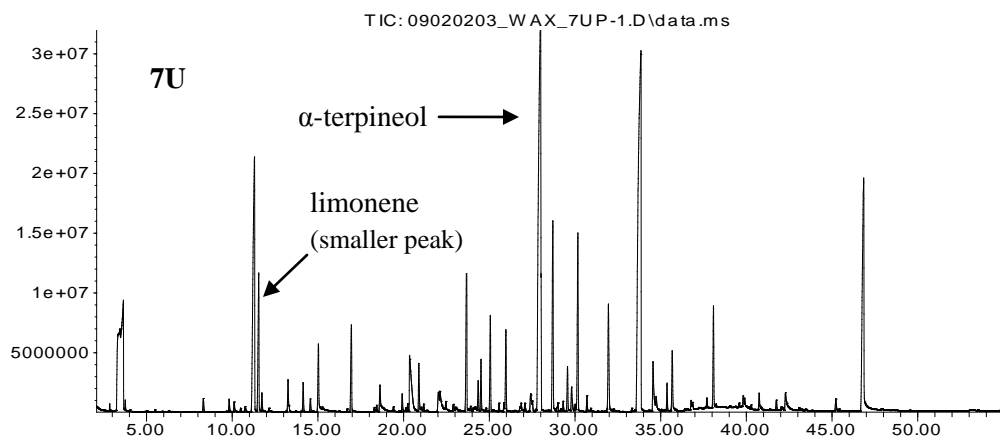
Time-->

Abundance



Time-->

Abundance



Time-->

Figure 3.1 Total ion GC-MS chromatograms for aroma extracts prepared from commercial lemon-lime carbonated beverages (SM, Sierra Mist; SP, Sprite).

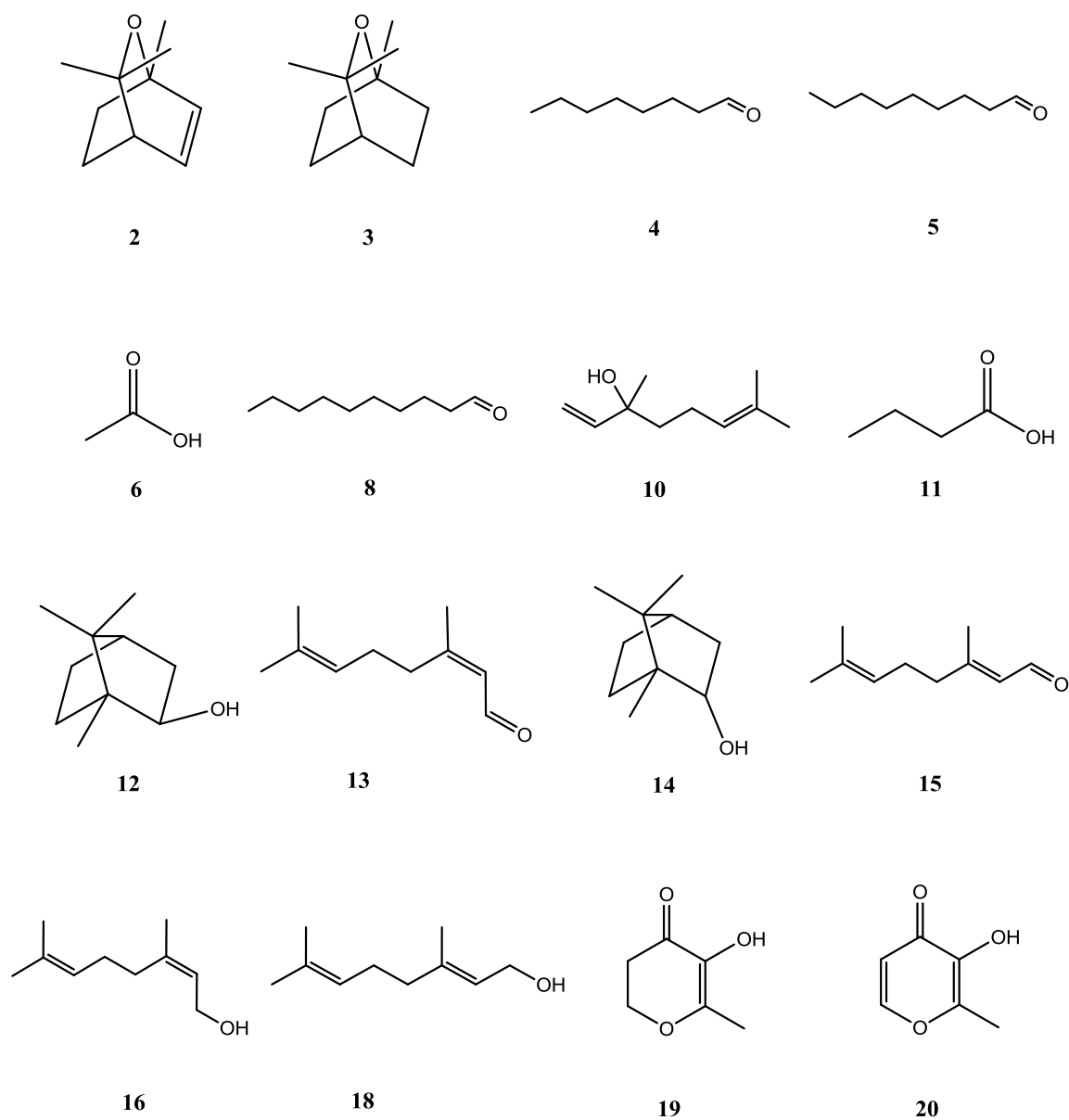
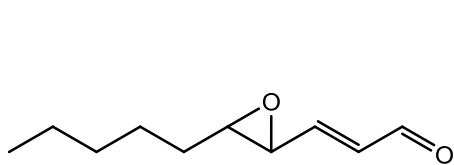
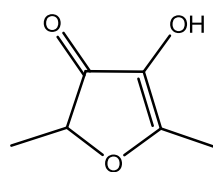


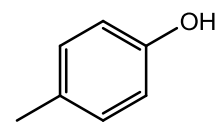
Figure 3.2 Structures of the aroma-active compounds identified in three commercial brands of lemon-lime carbonated beverages (Compound numbers correspond to those in Tables 3.1 and 3.2). *Figure 3.2 is continued on page 41.*



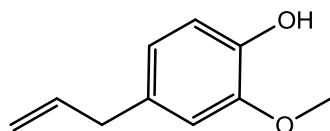
22



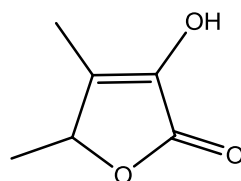
23



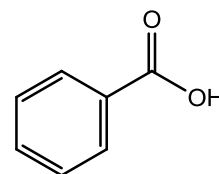
24



25



26



28

Figure 3.2 (cont.)

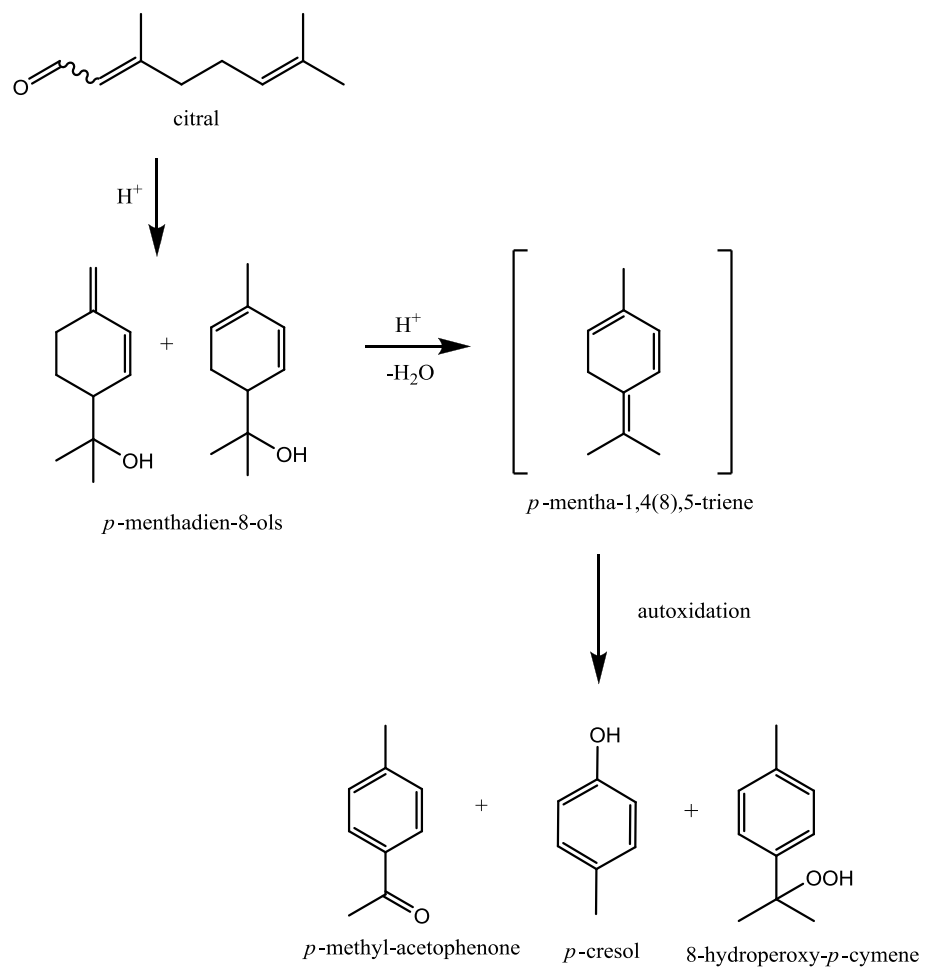


Figure 3.3 Proposed mechanism for conversion of citral into *p*-cresol (Adapted from (34)).

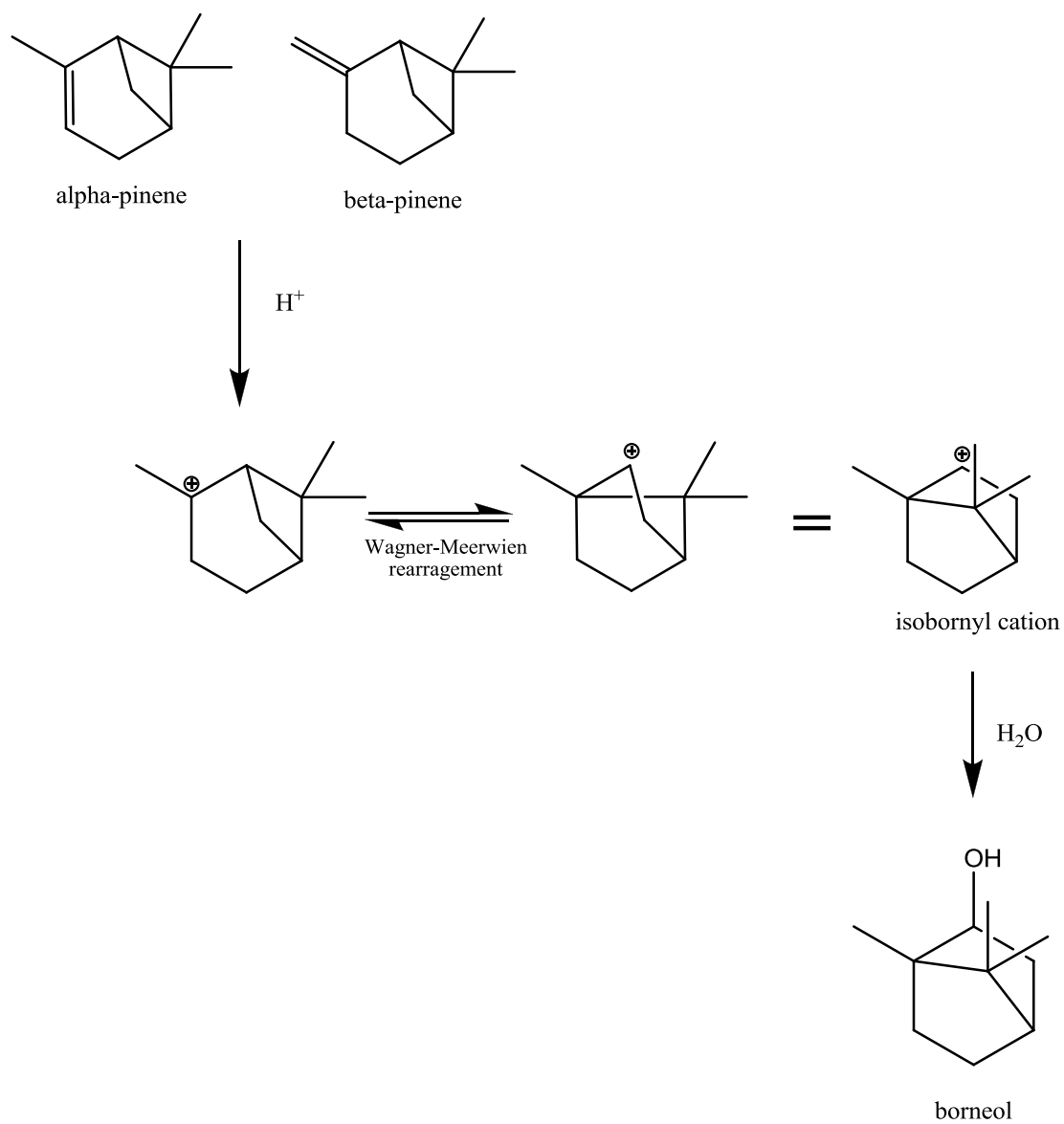


Figure 3.4 Proposed mechanism for rearrangement of α - or β -pinene to borneol (Based on (28, 49)).

Table 3.1 Predominant aroma-active components of three commercial brands of lemon-lime carbonated beverages

No. ^a	Compound	RI ^b		Odor description ^c	FD factor ^d		
		WAX	RTX-5		SP	SM	7UP
1	unknown	1155	-- ^e	piney, terpiney	3	-	-
2	2,3-dehydro-1,8-cineole ^f	1196	985	minty, pine needles	81	81	81
3	1,8-cineole ^f	1211	1024	minty/eucalyptus	-	3	-
4	octanal ^f	1297	1002	pungent orange	81	243	81
5	nonanal ^f	1406	1103	orange, sweet, pungent	3	27	9
6	acetic acid ^g	1450	--	vinegar	-	-	9
7	unknown	1451	--	fresh, melony	9	27	9
8	decanal ^f	1511	1205	pungent, green, cilantro	9	27	9
9	unknown	1534	--	stale, burnt sugar	3	-	9
10	linalool ^f	1545	1099	floral, lavender	243	729	2187
11	butanoic acid ^h	1638	--	cheesy	<3	-	3
12	isoborneol ^f	1692	1160	camphorous	9	<3	<3
13	neral ^f	1701	1240	lemony	3	3	-
14	borneol ^f	1725	1168	earthy, camphorous	81	3	3
15	geranial ^f	1753	1271	lemon oil, pledge	3	9	-
16	nerol ^f	1771	1229	lemon cleaner	3	<3	3
17	unknown	1812	--	sweet, fruity	-	-	3
18	geraniol ^f	1854	1254	lemon cleaner	9	9	9
19	2,3-dihydro-5-hydroxy-6-methyl-4(<i>H</i>)-pyran-4-one (dihydromaltol) ^h	1884	--	burnt sugar	-	3	-
20	3-hydroxy-2-methyl-4(<i>H</i>)-pyran-4-one (maltol) ^h	2000	--	burnt sugar	-	-	9

Table 3.1 (continued)

21	unknown	2026	--	inky, phenolic	-	<3	3
22	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal ^{<i>i</i>}	2029	1381	unripe	-	3	-
23	4-hydroxy-2,5-dimethyl-3(<i>2H</i>)-furanone (HDMF) ^{<i>i</i>}	2046	1088	burnt sugar	81	9	27
24	4-methylphenol (<i>p</i> -cresol) ^{<i>f</i>}	2096	1083	dung, stable	81	<3	81
25	eugenol ^{<i>h</i>}	2148	--	cloves	-	3	-
26	3-hydroxy-4,5-dimethyl-2(<i>5H</i>)-furanone (sotolon) ^{<i>i</i>}	2225	1111	curry	3	<3	27
27	unknown	2346	--	waxy, liver-like	3	<3	<3
28	benzoic acid ^{<i>f</i>}	2449	1306	sweet, candy	27	81	-

^{*a*} Peak number corresponds to those in Table 3.2 and Figure 3.2. ^{*b*} Retention index determined from GCO data. ^{*c*} Odor property as perceived during GCO. ^{*d*} Flavor dilution factor determined on RTX-Wax column (SM, Sierra Mist; SP, Sprite). ^{*e*} Odor not detected. ^{*f*} Compound positively identified based on odor property, mass spectral data, and RIs on both RTX-Wax and RTX-5 columns. ^{*g*} Compound tentatively identified based on odor property, mass spectral data, and RI on RTX-Wax column. ^{*h*} Compound tentatively identified bases on odor property on RTX-Wax column. ^{*i*} Compound tentatively identified based on odor property and RI on RTX-Wax column and RTX-5 columns.

Table 3.2 Orthonasal odor detection thresholds of selected aroma compounds identified in three commercial brands of lemon-lime carbonated beverages

No. ^a	Compound	Threshold ^b	
		(µg/L in water)	(µg/L in water, pH 3)
2	2,3-dehydro-1,8-cineole	17 ^{c,f}	
3	1,8-cineole	1.3 ^{c,g}	
4	octanal	0.7 ^{c,h}	
5	nonanal	1 ^{c,h}	
8	decanal	0.1 ^{c,h}	
10	linalool	6 ^{c,g}	
12	isoborneol	2.5-16 ^{d,i}	
13	neral	30 ^{d,j}	
14	borneol	140 ^{e,k}	
15	geraniol	32 ^{c,l}	
16	nerol	300 ^{d,m}	
18	geraniol	40 ^{c,g}	
23	HDMF	60 ^{c,n}	21 ^{c,o}
24	<i>p</i> -cresol	55 ^{c,p}	47 ^{c,f}
28	sodium benzoate (benzoic acid)	-- ^q	1 900 000 ^{c,f}

^a Numbers correspond to those in Table 3.1 and Figure 3.2. ^b Odor (orthonasal) detection threshold determined in water. ^c Threshold determined using polyethylene or PTFE sniff bottles. ^d Methodology for determining threshold not available/provided. ^e Threshold determined using sniffing cups.

^f Determined in this lab, as described in Chapter 4. ^g ref (26): Buttery, R.G.; Ling, L.C.; Light, D.M. 1987 ^href (61): Guadagni, D.G; Buttery, R.G.; Okano, S., 1963 ⁱref (62): Burdock, G.A. 2004. ^jref (63): Buttery R.G. 1993 ^k ref (40): Tamura, H.; Yang, R.-H.; Sugisawa, H., 1993 ^lref (64): Buttery, R.G. et. al, 1971 ^m ref (65): Rychlik, M.; Schieberle, P.; Grosch, W., 1998. ⁿref (66): Buttery, R.G; Ling, L. C. 1998 ^oref (50): Buttery, R.G.; Takeoka, G.R.; Ling, L.C., 1995 ^pref (37): Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C., 1988 ^qNot available.

REFERENCES

1. Dr Pepper/Seven Up, Inc. About: Get to know us a little better. <http://www.7up.com/page/about/> (accessed August 9,2010).
2. The Coca-Cola Company. Product descriptions. <http://www.virtualvender.coca-cola.com/ft/index.jsp> (accessed August 9, 2010).
3. PepsiCo, Inc. Our history. <http://www.pepsico.com/Company/Our-History.html> (accessed August 9, 2010).
4. PepsiCo, Inc. The history of Dew: 1940's. <http://mountaindew.com/#/products/timeline.php> (accessed August 9, 2010).
5. Dr Pepper/Seven Up, Inc. 7UP: Ingredients. <http://www.7up.com/products/> (accessed August 9, 2010).
6. PepsiCo, Inc. Nutrition and ingredient information. http://www.pepsiproductfacts.com/infobyproduct.php?brand_fam_id=1049&brand_id=1000&product=Mountain%20Dew&or=md (accessed August 9, 2010).
7. The Coca-Cola Company. Nutrition connection. <http://productnutrition.thecocacola.com/products/sprite#ingredients> (accessed August 9, 2010).
8. Werkhoff, P.; Brennecke, S.; Bretschneider, W.; Bertram, H.-J. Modern Methods for Isolating and Quantifying Volatile Flavor and Fragrance Compounds. In *Flavor, Fragrance, and Odor Analysis*; Marsili, R., Ed.; Marcel Dekker, Inc.: New York, 2002; pp 139-204.
9. Blank, I. Gas chromatography-olfactometry in food aroma analysis. In *Flavor, Fragrance, and Odor Analysis*; Marsili, R., Ed.; Marcel Dekker, Inc.: New York, 2002; pp. 297-331.
10. Scheidig, C.; Czerny, M.; Schieberle, P. Changes in key odorants of raw coffee beans during storage under defined conditions. *J. Agric. Food Chem.* **2007**, *55*, 5768-5775.
11. Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916-924.
12. Lin, J.; Fay, L. B.; Welte, D. H.; Blank, I. Synthesis of *trans*-4,5-epoxy-(*E*)-2-decenal and its deuterated analog used for the development of a sensitive and selective quantification method based on isotope dilution assay with negative ion chemical ionization. *Lipids* **1999**, *34*, 1117-1126.
13. Mills, F. D. Synthesis of acetoxydihydromaltol acetate and dihydromaltol. *Carbohydr. Res.* **1972**, *23*, 433-436.
14. Rotsatchakul, P.; Chaiseri, S.; Cadwallader, K. R. Identification of characteristic aroma components of Thai fried chili paste. *J. Agric. Food Chem.* **2008**, *56*, 528-536.

15. Grosch, W. Detection of potent odorants in food by aroma extraction dilution analysis. *Trends Food Sci. Technol.* **1993**, *17*, 142-144.
16. Zhou, Q.; Wintersteen, C. L.; Cadwallader, K. R. Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. *J. Agric. Food Chem.* **2002**, *50*, 2016-2021.
17. van Den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr. A* **1963**, *11*, 463-471.
18. Teranishi, R.; Buttery, R. G.; Guadagni, D. G. Odor quality and chemical structure in fruit and vegetable flavors. *Ann. NY Acad. Sci.* **1974**, *237*, 209-216.
19. Veriotti, T.; Sacks, R. High-speed GC and GC/time-of-flight MS of lemon and lime oil samples. *Anal. Chem.* **2001**, *73*, 4395-4402.
20. Chisholm, M. G.; Wilson, M. A.; Gaskey, G. M. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). *Flavour Frag. J.* **2003**, *18*, 106-115.
21. Bicchi, C.; D'Amato, A.; Manzin, V.; Galli, A.; Galli, M. Cyclodextrin derivatives in the gas chromatographic separation of racemic mixtures of volatile compounds: VII. The use of 2,6-di-O-methyl-3-O-pentyl- β -cyclocextrin diluted in phases with different polarity in the separation of racemates in complex-mixtures. *J. Chromatogr. A* **1994**, *666*, 137-146.
22. Mondello, L.; Catalfamo, M.; Cotroneo, A.; Dugo, G.; McNair, H. Multidimensional capillary GC-GC for the analysis of real complex samples: Part IV. Enantiomeric distribution of monoterpene hydrocarbons and monoterpene alcohols of lemon oils. *J. High Res. Chromatog.* **1999**, *22*, 350-356.
23. Lira, P.; van Baren, C. M.; Retta, D.; Bandoni, A. L.; Gil, A.; Gattuso, M.; Gattuso, S. Characterization of lemon verbena (*Aloysia citriodora* Palau) from Argentina by the essential oil. *J. Essent. Oil Res.* **2008**, *20*, 350-353.
24. Song, H. S.; Phi, N. T. L.; Sawamura, M. Volatile profile of the peel oil of Kiyomi (*Citrus unshiu* Marcov. x *C. sinensis* Osbeck), a new hybrid sweet citrus fruit. *J. Essent. Oil Res.* **2009**, *21*, 26-30.
25. Choi, H.-S.; Kondo, Y.; Sawamura, M. Characterization of the odor-active volatiles in citrus Hyuganatsu (*Citrus tamurana* Hort. ex Tanaka). *J. Agric. Food Chem.* **2001**, *49*, 2404-2408.
26. Buttery, R. G.; Ling, L. C.; Light, D. M. Tomato leaf volatile aroma components. *J. Agric. Food Chem.* **1987**, *35*, 1039-1042.
27. Steinhaus, M.; Fritsch, H.; Schieberle, P. Quantitation of (R)- and (S)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100-7105.

28. Clark, B. C., Jr.; Chamblee, T. S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-flavors in foods and beverages*; Charalambous, G., Ed.; Elsevier Science Publishers, 1992; pp. 229-285.
29. Pedersen, D.; Capone, D.; Skouroumounis, G.; Pollnitz, A.; Sefton, M. Quantitative analysis of geraniol, nerol, linalool, and alpha-terpineol in wine. *Anal. Bioanal. Chem.* **2003**, *375*, 517-522.
30. Baxter, R. L.; Laurie, W. A.; McHale, D. Transformations of monoterpenoids in aqueous acids - reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron* **1978**, *34*, 2195-2199.
31. Vekiari, S. A.; Protopapadakis, E. E.; Papadopoulou, P.; Papanicolaou, D.; Panou, C.; Vamvakias, M. Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *J. Agric. Food Chem.* **2002**, *50*, 147-153.
32. Schieberle, P.; Grosch, W. Potent odorants resulting from the peroxidation of lemon oil. *Z Lebensm Unters Forsch* **1989**, *189*, 26-31.
33. Ueno, T.; Masuda, H.; Ho, C. Formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677-5684.
34. Ueno, T.; Kiyohara, S.; Ho, C.; Masuda, H. Potent inhibitory effects of black tea theaflavins on off-odor formation from citral. *J. Agric. Food Chem.* **2006**, *54*, 3055-3061.
35. Steen, D. P. Carbon dioxide, carbonation and the principles of filling technology. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P. and Ashurst, P.R., Eds.; Blackwell Publishing Ltd: Oxford, 2006; pp. 112-143.
36. Karagul-Yuceer, Y.; Vlahovich, K. N.; Drake, M.; Cadwallader, K. R. Characteristic aroma components of rennet casein. *J. Agric. Food Chem.* **2003**, *51*, 6797-6801.
37. Buttery, R. G.; Turnbaugh, J. B.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006-1009.
38. Peacock, V. E.; Kuneman, D. W. Inhibition of the formation of α -*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.
39. Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil citric-acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
40. Tamura, H.; Yang, R.-H.; Sugisawa, H. Aroma profiles of peel oils of acid citrus. In *Bioactive volatile compounds from plants*; Teranishi, R.; Buttery, R.G.; Sugisawa, H., Eds.; ACS Symposium Series 525; American Chemical Society: Washington, DC, 1993; pp. 121-136.
41. Gabriele, B.; Fazio, A.; Dugo, P.; Costa, R.; Mondello, L. Essential oil composition of *Citrus medica* L. Cv. Diamante (Diamante citron) determined after using different extraction methods. *J. Sep. Sci.* **2009**, *32*, 99-108.

42. Miyazawa, N.; Tomita, N.; Kurobayashi, Y.; Nakanishi, A.; Ohkubo, Y.; Maeda, T.; Fujita, A. Novel character impact compounds in Yuzu (*Citrus junos* Sieb. ex Tanaka) peel oil. *J. Agric. Food Chem.* **2009**, *57*, 1990-1996.
43. Laska, M.; Teubner, P. Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes. *Chem. Senses* **1999**, *24*, 263-270.
44. Meijboom, P.W.; Jongenotter, G.A. Flavor preceptibility of straight chain, unsaturated aldehydes as a function of double-bond position and geometry. *JAOCS* **1981**, *58*, 680-682.
45. Leimann, F. V.; Goncalves, O. H.; Machado, R. A. F.; Bolzan, A. Antimicrobial activity of microencapsulated lemongrass essential oil and the effect of experimental parameters on microcapsules size and morphology. *Mat. Sci. Eng.C - Bio. S.* **2009**, *29*, 430-436.
46. Patil, J. R.; Jayaprakasha, G. K.; Chidambara Murthy, K. N.; Tichy, S. E.; Chetti, M. B.; Patil, B. S. Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of *Citrus aurantifolia*. *Food Chem.* **2009**, *114*, 1351-1358.
47. Tanaka, A.; Horiuchi, M.; Umamo, K.; Shibamoto, T. Antioxidant and anti-inflammatory activities of water distillate and its dichloromethane extract from licorice root (*Glycyrrhiza uralensis*) and chemical composition of dichloromethane extract. *J. Sci. Food Agric.* **2008**, *88*, 1158-1165.
48. Raal, A.; Arak, E.; Orav, A.; Kailas, T.; Muurisepp, M. Composition of the essential oil of *Levisticum officinale* W.D.J. Koch from some European countries. *J. Essent. Oil Res.* **2008**, *20*, 318-322.
49. Ebmeyer, F. Theoretical investigations towards an understanding of the α -pinene/camphene rearrangement. *J. Mol. Struct. - Theochem.* **2002**, *582*, 251-255.
50. Buttery, R. G.; Takeoka, G. R.; Ling, L. C. Furanol: odor threshold and importance to tomato aroma. *J. Agric. Food Chem.* **1995**, *43*, 1638-1640.
51. Haleva-Toledo, E.; Naim, M.; Zehavi, U.; Rouseff, R. L. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone formation in buffers and model solutions of citrus juice. *J. Agric. Food Chem.* **1997**, *45*, 1314-1319.
52. Rodin, J. O.; Himel, C. M.; Silverstein, R. M.; Leeper, R. W.; Gortner, W. A. Volatile flavor and aroma components of pineapple.: I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Food Sci.* **1965**, *30*, 280-285.
53. Wilson, C. W., III; Shaw, P. E.; Knight, R. J., Jr. Importance of some lactones and 2,5-dimethyl-4-hydroxy-3(2H)-furanone to mango (*Mangifera indica* L.) aroma. *J. Agric. Food Chem.* **1990**, *38*, 1556-1559.
54. Pickenhagen, W.; Velluz, A.; Passerat, J.-P.; Ohloff, G. Estimation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (FURANEOL[®]) in cultivated and wild strawberries, pineapples and mangos. *J. Agric. Food Chem.* **1981**, *32*, 1132-1134.

55. Kappes, S. M. Ph.D. thesis. University of Illinois, Urbana, ILLINOIS, 2006.
56. White, J. S. Straight talk about high-fructose corn syrup: what it is and what it ain't. *Am. J. Clin. Nutr.* **2008**, *88*, 1716S-1721S.
57. Chisholm, M. G.; Jell, J. A.; Cass, D. M., Jr. Characterization of the major odorants found in the peel oil of *Citrus reticulata* Blanco cv. Clementine using gas chromatography-olfactometry. *Flavour Frag. J.* **2003**, *18*, 275-281.
58. Lin, J.; Rouseff, R. L. Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time-intensity GC-olfactometry and GC-MS. *Flavour Frag. J.* **2001**, *16*, 457-463.
59. Taylor, B. Ingredients and formulation of carbonated soft drinks. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing Ltd: Oxford, UK, 2006; pp. 48-86.
60. U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm> (accessed May 2010), Sec. 184.1021 Benzoic acid.
61. Guadagni, D.G.; Buttery, R.G.; Okano, S. Odour thresholds of some organic compounds associated with food flavours. *J. Sci. Food Agric.* **1963**, *14*, 761-765.
62. Burdock, G.A. *Fenaroli's Handbook of Flavor Ingredients*, 5; CRC Press: Boca Raton, 2005; p. 913.
63. Buttery, R.G. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In *Flavor Science: Sensible Principles and Techniques*; Acree, T.E., Teranishi, R., Eds.; American Chemical Society: Washington, DC, 1993; pp. 259-286.
64. Buttery, R.G.; Seifert, R.M.; Guadagni, D.G.; Ling, L.C. Characterization of additional volatile components of tomato. *J. Agric. Food Chem.* **1971**, *19*, 524-529.
65. Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*. Deutsche Forschungsanstalt fuer Lebensmittelchemie and Institut fuer Lebensmittelchemie der Technischen Universitaet Muenchen: Garching, Germany, 1998; p. 63.
66. Buttery, R. G.; Ling, L. C. Additional studies on flavor components of corn tortilla chips. *J. Agric. Food Chem.* **1998**, *46*, 2764-2769.

CHAPTER 4

**QUANTIFICATION OF KEY AROMA-ACTIVE COMPONENTS OF THREE
COMMERCIAL BRANDS OF LEMON-LIME CARBONATED BEVERAGES**

ABSTRACT

Predominant aroma compounds in three commercial brands of lemon-lime carbonated beverages – Sprite[®] (SP), Sierra Mist[®] (SM), and 7UP[®] (7UP) – were quantified using headspace solid phase microextraction (SPME) combined with stable isotope dilution assays (SIDA). The compounds chosen for quantification were based on results from Chapter 3 and included 2,3-dehydro-1,8-cineole (dehydrocineole), 1,8-cineole, octanal, nonanal, decanal, linalool, borneol, isoborneol, neral, geranial, nerol, geraniol and *p*-cresol. Benzoic acid was quantified separately by HPLC using an external standardization method. Four replicate analyses were conducted for each brand by analysis of different cans from the same production lot. Concentrations of the all compounds, except neral, differed between at least two brands. Concentrations of 1,8-cineole, octanal, nonanal, decanal, linalool, isoborneol, geraniol and benzoic acid differed among all brands. Benzoic acid was the most abundant compound in SP and SM, but was not detected in 7UP. Dehydrocineole was the most abundant compound in 7UP and the second most abundant compound in SP and SM. In contrast to FD factors reported earlier (Chapter 3), the calculated odor-activity values (OAVs) indicated that decanal was the most potent aroma compound, followed by octanal and dehydrocineole; with linalool and nonanal being moderately important to the aroma of lemon-lime carbonated beverages. The results demonstrate that lemon-lime carbonated beverages share many of

the same compounds but the relative abundance of these compounds varies by brand. Additionally, differences between at least two brands were observed for titratable acidity, pH and percent soluble solids (°Brix).

INTRODUCTION

After successful identification of the potent aroma components in lemon-lime carbonated beverages (Chapter 3), the next logical step in the present research was to determine the exact concentrations of these compounds. Such data can reveal similarities and differences among brands from an analytical perspective. Furthermore, quantification data coupled with odor detection threshold data allow for the determination of OAVs, which can be used to compare the relative aroma intensities among compounds. Quantification data also lay the foundation for further studies with model aroma systems, which can show whether the complete aroma system, including the influence of the matrix, has been adequately captured by the analytical results.

The identification of key aroma-active compounds in the previous chapter showed that linalool was the predominant aroma compound in all three brands, followed by octanal and dehydrocineole. Other compounds with moderately high FD factors in all brands were HDMF, *p*-cresol (except SM, FD <3), nonanal, decanal, an unknown compound (no. 7) and borneol. Also, benzoic acid had moderately high FD factors in SP and SM but was not detected in 7UP. Critiques of the GCO method in the literature expound on its ability to distinguish important aroma-active compounds from other volatiles with little or no aroma impact (1). However, GCO and AEDA are considered to give an approximation of aroma potency, with bias resulting from the aroma extraction

step and the assessor (1). Further, GCO does not take the matrix effects of a food into account, which could lead to the overestimation of a compound's importance (2).

Therefore, the analytical evaluation of the aroma-active compounds in a food by quantification is a valuable next step in understanding a food system.

In the present study, thirteen aroma compounds - 2,3-dehydro-1,8-cineole, 1,8-cineole, octanal, nonanal, decanal, linalool, isoborneol, neral, borneol, geranial, geraniol, *p*-cresol and benzoic acid - were selected for quantification. These compounds were all found to be aroma-active by GCO and present at a high enough concentrations to produce a GC-MS peak.

MATERIALS AND METHODS

Materials

Sprite (Coca-Cola Company, Atlanta, GA) (expiration February 07 11 PAC; 2A 1616 or 2B 1617), Sierra Mist (PepsiCo, Inc., Purchase, NY) (expiration October 11 10; 0606QY051103 or 0607QY051103), and 7UP (Dr Pepper/Seven Up, Inc., Plano, TX) (D0116 1620 OT1 or D0116 1622 OT1) were purchased in June 2010 at a local grocer (Urbana, IL).

Chemicals

Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO) for unlabeled standards. Nerol and geraniol were obtained from Bedoukian Research, Inc. (Danbury, CT). The purities of these compounds were determined by neat injection (hot split, 250 °C) on a nonpolar column (DB-5) using a gas chromatograph (GC) equipped with a flame ionization detector. Compound purities are

presented in Appendix A. All compound purities were above 90%, except in the case of neral and geranial. These isomers were present as a mixture (35.8% neral and 59.9% geranial) and the combined purity of these isomers was 95.7%.

Stable isotopes: sources and synthesis methods

Except in the case of 4-($^2\text{H}_3$ -methyl)-phenol all stable isotopes were synthesized. The novel synthetic routes are described below or a reference is given for compounds for which the synthetic method is published. Figure 4.1 shows the structure and positions of the deuterium atoms for each isotope standard. The purities of these compounds were determined by neat injection (hot split, 250 °C) on a nonpolar column (DB-5) using a gas chromatograph (GC) equipped with a flame ionization detector. Compound purities are presented in Appendix A. In most cases the purities were over 90%. The exceptions were compounds that existed as a pair of isomers: $^2\text{H}_3$ -isoborneol and $^2\text{H}_3$ -borneol (48.3 % $^2\text{H}_3$ -isoborneol and 47.0 % $^2\text{H}_3$ -borneol) having a combined purity of 95.3%; $^2\text{H}_6$ -(Z)-3,7-dimethyl-2,6-octadienal ($^2\text{H}_6$ -neral) and $^2\text{H}_6$ -(E)-3,7-dimethyl-2,6-octadienal ($^2\text{H}_6$ -geranial) (21.8% $^2\text{H}_6$ -neral and 52.3% $^2\text{H}_6$ -geranial having a combined purity of 74.10%; and $^2\text{H}_6$ -(Z)-3,7-dimethyl-2,6-octadien-1-ol ($^2\text{H}_6$ -nerol) and $^2\text{H}_6$ -(E)-3,7-dimethyl-2,6-octadien-1-ol ($^2\text{H}_6$ -geraniol) (32.8% $^2\text{H}_6$ -nerol and 59.3% $^2\text{H}_6$ -geraniol having a combined purity of 92.1%.

Synthesis of 2,3-dehydro-1,8-cineole (no. 2) and ($^2\text{H}_3$)-2,3-dehydro-1,8-cineole (no. I-2)

Ayorinde et. al demonstrated the synthesis of 2,3-dehydro-1,8-cineole (dehydrocineole) from α -terpineol in a one flask procedure at -78°C (3). The main

limitations of that method were low product yield and the formation of a large number of product contaminants resulting from side reactions with the oxidizing agent, hydrogen peroxide. Therefore, in the present study the synthesis described by Bugarčić et al. (4) for 1,8-cineole was adapted for the synthesis of dehydrocineole. For this synthesis α -terpineol in the presence of pyridine was reacted with phenylselenyl chloride at room temperature to form a stable cyclic ether intermediate. The phenylselenyl ether was purified and then reacted with 3% H₂O₂ in THF at 0 °C to obtain the target compound in high yield and purity. The procedure below describes the detailed synthesis of the ²H₃-dehydrocineole (no. **I-2**). The unlabelled dehydrocineole (no. **2**) was prepared from unlabeled α -terpineol using an identical procedure.

²H₃- α -terpineol. ²H₃- α -Terpineol was synthesized via a Grignard reaction of ²H₃-methylolithium with 1-acetyl-4-methylcyclohexene. For the synthesis of 1-acetyl-4-methylcyclohexene, isoprene (0.68 g, 10 mmol) was combined with 3-butene-2-one (0.71 g, 10 mmol) in dichloromethane (20 mL). The solution was cooled to -78 °C and then, while stirring, AlCl₃ (0.13 g, 1 mmol) was added in one portion. The solution was warmed to room and stirred at that temperature for 3 h. The reaction mixture was washed with 50 mL of an aqueous 10% (w/v) Na₂SO₄ solution and the organic layer was dried over anhydrous Na₂SO₄. Yield of the target compound after vacuum distillation and removal of the solvent was 1.02 g (73%). MS (EI) data: see Appendix B.

For the synthesis of ²H₃- α -terpineol, a solution of ²H₃-methylolithium [complexed with lithium iodide; 0.5M solution in diethyl ether] (20 mL, 10 mmol) was added to a dry three-neck 100-mL round bottom flask equipped with a mechanical stirrer and rubber septa (with nitrogen purge and vent needles). The flask was cooled in an ice-water bath

(~ 0 °C). The 1-acetyl-4-methylcyclohexene (1.02 g; 7.3 mmol, dissolved in 5 mL of ether) was added (slowly) via syringe needle. The solution was stirred for 2 h (at ~ 0 °C) and then 20 mL of an aqueous saturated NH₄Cl solution (aqueous) was added (dropwise) to the flask, followed by the addition of 20 mL of H₂O to dissolve the precipitate. The reaction mixture was extracted with ether (3 x 20 mL) and the ether phase was washed with an aqueous saturated NaCl solution (2 x 20 mL). Most of the ether was distilled off using a Vigreux column (43 °C) and then the product was purified by flash chromatography (silica gel) using a 20% ether in pentane as mobile phase. Final yield of the purified product was 0.93 g (81%). MS (EI) data: see Appendix B.

²H₃-2,3-dehydro-1,8-cineole (no. **I-2**). ²H₃-α-Terpineol (0.760 g, 4.84 mmol) was combined with pyridine (0.40 mL, 4.93 mmol) in dichloromethane (25 mL) with stirring. Phenylselenyl chloride (1.05 g, 5.42 mmol) was added with stirring at room temperature and allowed to react for 15 minutes. The resulting mixture was a medium yellow color. Product was washed sequentially with of aqueous 1 M HCl, saturated aqueous NaHCO₃, and brine (25 mL each). The cyclic ether intermediate was dried over sodium sulfate and concentrated (~1.5 g crude). Diphenyl-diselenyl impurities were removed by flash chromatography (silica gel) using 10% pentane in dichloromethane as mobile phase. MS (EI) data for both unlabeled phenylselenyl ether and ²H₃-phenylselenyl ether are in Appendix B.

The resulting purified ²H₃-phenylselenyl ether intermediate (0.702 g, 2.25 mmol) was combined with tetrahydrofuran (~8 mL) and cooled in an ice-water bath. To the stirred solution, 3% H₂O₂ (~3.8 mL, ~3.4 mmol) was added dropwise. The reaction mixture was allowed reach room temperature. After 18 h, diethyl ether (20 mL) was

added and the mixture washed 2x sequentially with distilled water and saturated brine. The product was dried over anhydrous sodium sulfate (~0.33 g crude). The product was later purified by high vacuum distillation. MS (EI) data: see Appendix A.

Synthesis of $^2\text{H}_3$ -1,8-cineole (I-3)

The $^2\text{H}_3$ -1,8-cineole isotope was synthesized starting from $^2\text{H}_3$ - α -terpineol according to the procedure described by Bugarčić, et al. (4) for unlabeled 1,8-cineole. MS (EI) data: see Appendix A.

Synthesis of [3,3,4,4- $^2\text{H}_4$]-octanal (no. I-4), [3,3,4,4- $^2\text{H}_4$]-nonanal (no. I-5) and [3,3,4,4- $^2\text{H}_4$]-decanal (no. I-8).

The deuterated aldehydes were synthesized by following a previous published procedure described for the synthesis of [5,6- $^2\text{H}_2$]-hexanal (5). The synthesis of [3,3,4,4- $^2\text{H}_2$]-octanal is provided here as an example. Yields, purities and MS(EI) spectra for the other two aldehydes (no. I-5 and I-8) are provided in Appendix A.

[3,3,4,4- $^2\text{H}_4$]-octan-1-ol. Wilkinson's catalyst [Chlorotri(triphenylphosphine)rhodium(I), 0.15 g, 15 wt% of the alkynol] plus 1.0 g of 3-octyn-1-ol (7.9 mmol) and 5 mL of methanol- ^2H were placed in a pressure reactor (equipped with stir bar and rubber septum). The reactor was flushed for 5 min with deuterium gas (UHP grade 99.995%, isotopic enrichment 99.7%, Matheson Tri-Gas, Parsippany, NJ) using a needle that was placed below the solution. Pressure was maintained at 40 psi and reaction progress was monitored periodically by GC-MS. Once the reaction was complete the spent catalyst was removed by centrifugation and the target

compound isolated by vacuum distillation (0.79 g, 5.9 mmol, 75% yield). MS (EI) data: see Appendix B.

[3,3,4,4-²H₄]-octanal. The above alcohol was oxidized to the corresponding aldehyde using a published procedure (6). In one portion, *[3,3,4,4-²H₄]-octan-1-ol* (0.41 g; 3 mmol, in 2 mL of dichloromethane) was added to a 10 mL suspension of pyridinium chlorochromate (PCC, 1.1 g, 0.005 mol, in dichloromethane). The mixture was stirred at room temperature for 1.5 h and then 20 mL of ether was added and the supernatant decanted. The residue was extracted with ether (3 x 10 mL) until the black gum became granular in consistency. The ether extract was filtered through a 10 g bed of Florisil and most solvent was removed by distillation using a Vigreux column (43 °C). Yield of the target compound after vacuum distillation and removal of the solvent was 0.18 g (27%) with a purity of 92.2% (GC-FID). MS (EI) data: see Appendix A.

[3,3,4,4-²H₄]-nonan-1-ol (4.7 mmol; 66 % yield) MS (EI) data: see Appendix B.

[3,3,4,4-²H₄]-nonanal (no. **I-5**) [2.0 mmol; 66 % yield, purity 93.6% (GC-FID)]

MS (EI) data: see Appendix A.

[3,3,4,4-²H₄]-decan-1-ol (5.0 mmol; 77 % yield) MS (EI) data: see Appendix B.

[3,3,4,4-²H₄]-decanal (no. **I-8**) [1.9 mmol; 63 % yield, purity 95.2% (GC-FID)]

MS (EI) data: see Appendix A.

Synthesis of [1,2-²H₂]-3,7-dimethyl-1,6-octadien-3-ol (linalool) (no. I-10)

The [²H₂]-linalool was synthesized according to the procedure described by Steinhaus, et al. (7). MS (EI) data: see Appendix A.

Synthesis of $^2\text{H}_3$ -isoborneol (no. I-12) and $^2\text{H}_3$ -borneol (no. I4)

The borneol isomers were synthesized in a novel six step procedure beginning with ketopinic acid. A somewhat similar procedure was used by Havens and Meloan (8) for synthesis of [9,9,9- $^2\text{H}_3$]-bornylacetate and [9,9,9- $^2\text{H}_3$]-isobornylacetate from *trans*-isoketopinic acid.

Ketopinic acid acid was converted to a methyl ester via Fisher esterification. The two carbonyl groups in methyl ketopinate were reduced to alcohols via LiAlD_4 , introducing three or five deuterium atoms onto the compound. Then this alcohol was reacted with toluene-*p*-sulphonyl chloride, followed by reduction with LiAlD_4 , forming a $^2\text{H}_{4-5}$ -borneol. The $^2\text{H}_{4-5}$ -borneol is unstable (i.e., some of the deuteriums are readily exchangeable), making it necessary to oxidize the borneol isomers to $^2\text{H}_3$ -camphor. $^2\text{H}_3$ -Isoborneol and $^2\text{H}_3$ -borneol are finally formed through a reduction of $^2\text{H}_3$ -camphor with borane-THF complex. The synthesis scheme is detailed in Figure 4.2.

Ketopinic acid (**A**) (1.06 g, 5.82 mmol), methanol (~21 g, 640 mmol), and concentrated sulfuric acid (0.26g) were added to a 50-mL screw top test tube and incubated at 65°C overnight (9). After cooling, the mixture was neutralized with aqueous sodium bicarbonate solution and the excess methanol was evaporated in the hood. The resulting methyl ketopinate (**B**) was brought up in ether and washed 2x with aqueous saturated sodium chloride, followed by drying over sodium sulfate. The solvent was evaporated to yield methyl ketopinate (0.618 g, 3.15 mmol, 54.1% yield). MS (EI): see Appendix B.

Lithium aluminum deuteride (0.210 g, 4.99 mmol, 1.5 mol excess) was weighed into a dry 50 mL centrifuge tube equipped with a magnetic stir bar, following some of the

recommendations of Vogel (10). Diethyl ether (20 mL) was added to the tube and then the reaction mixture was cooled in an ice-water bath with stirring under a nitrogen gas purge. Methyl ketopinate (0.618 g, 3.15 mmol) in ether (5 mL) was added dropwise to the stirred tube, while purging. (< 1-2 min). The sealed reaction mixture was removed from the ice-water bath after the addition of the ester was complete and the reaction was allowed to proceed for 2 h at room temperature with stirring to yield $^2\text{H}_{3-5}$ -1-(hydroxymethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-ol (**C**). After the reaction reached completion, as determined by GC-MS, the tube was cooled in an ice-water bath and excess lithium aluminum deuteride was quenched with deuterium oxide (2 mL), followed by water (10 mL). The solution was acidified to <pH 2 using aqueous 4 N H_2SO_4 . The ether layer was recovered and the aqueous layer was extracted with ether (2 x 20 mL). The pooled ether extracts were washed with aqueous saturated sodium chloride solution and dried over sodium sulfate and concentrated under a gentle stream of nitrogen. Evaporation of the solvent yielded ~0.62 g crude material (containing some unreacted methyl ketopinate). MS (EI): see Appendix B.

The synthesis of $^2\text{H}_{3-4}$ -(2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methyl 4-methylbenzenesulfonate (**D**) was accomplished using a method with some similarities to Vogel (pyridine method) (11). **C** (~0.62 g crude) was dissolved in dichloromethane (5 mL) and cooled in an ice-water bath. The test tube was equipped with a magnetic stir bar. Pyridine was added (0.510 mL, 6.30 mmol), followed by the addition of toluene-*p*-sulphonyl chloride (0.905 g, 4.7 mmol) in small portions with vigorous stirring. The reaction was checked by GC-MS and additional pyridine (0.260 mL, 3.21 mol) and toluene-*p*-sulphonyl chloride (0.449 g, 2.36 mol) was added after approximately 48 h to

promote the complete reaction of the starting material. The reaction was stopped after an additional 24 h. For work-up, ether (10 mL) and water (2.5 mL) were added to the reaction mixture and then the organic layer was washed with aqueous 2 M HCl and then aqueous 5% sodium bicarbonate. The final product, **D** (~1.1 g crude), was dried over sodium sulfate. MS (EI): see Appendix B.

The reduction of **D** to form a mixture of $^2\text{H}_{4-5}$ -borneol and $^2\text{H}_{4-5}$ -isoborneol (**E**) was achieved in a similar fashion to the reduction of methyl ketopinate (synthesis step 2). Lithium aluminum deuteride (0.15 g, 3.6 mmol, ~1 molar equiv.) was added to a dry flask, followed by the addition of ether (20 mL). Ether was determined as a superior solvent for the reduction of alkyl tosylates (12). **D** (~1.1 g crude) in ether (5 mL) was added dropwise while purging with nitrogen gas. The reaction proceeded over several days and additional portions of lithium aluminum deuteride were added. Work-up was the same as previously described. ($^2\text{H}_{4-5}$ -borneol/ $^2\text{H}_{4-5}$ -isoborneol = ~0.51g crude). MS (EI): see Appendix B.

The oxidation of $^2\text{H}_{4-5}$ -borneol and $^2\text{H}_{4-5}$ -isoborneol to $^2\text{H}_3$ -camphor (**F**) was achieved via reaction with pyridinium chlorochromate (PCC) according to Corey and Suggs (6). A suspension (10 mL) of PCC (1.12 g; 5.19 mmol in anhydrous CH_2Cl_2) was added to a 40-mL vial. **E** (~0.51g crude in 2 mL of CH_2Cl_2) was added to the mixture in one portion and stirred. At the end of 1.5 h, ether (20 mL) was added and the supernatant was decanted. The residue was extracted with ether (3 x 10 mL) until the black gum became granular. The ether extract was filtered through a bed of Florisil (10 g) and the solvent removed by Vigreux distillation (47 °C). The crude $^2\text{H}_3$ -camphor product (~0.32

g crude) was purified using 5% ether in pentane as mobile phase and a 30 g silica gel column. MS (EI): see Appendix B.

The final step of the synthesis was to obtain a mixture of $^2\text{H}_3$ -isborneol (**G**) and $^2\text{H}_3$ -borneol (**H**) by reduction of $^2\text{H}_3$ -camphor with 1.0 M borane-tetrahydrofuran complex (0.839 mL, 0.839 mmol, 1.3 mol excess). Previously, Andrews and Crawford demonstrated that ammonia borane reacts with camphor to produce a 49:51 ratio of isborneol to borneol (13), and Havens and Meloan reduced camphor with borane-*t*-butylamine to a 3:2 ratio of isborneol to borneol (8). The borane-THF complex was added into a dry 25 mL centrifuge tube, equipped with a magnetic stir bar, followed by the addition of THF (2 mL). This mixture was purged with nitrogen gas and cooled in an ice-water bath while stirring, before adding **F** (106 mg, 0.682 mmol) in 1-2 mL THF dropwise to a stirred tube, while purging. (< 1-2 min) After addition was complete, the tube was removed from the ice-water bath and sealed, with periodic venting. At the completion of the reaction, as determined by GC-MS, the reaction was worked up as previously described for the lithium aluminum deuteride reductions. The ratio $^2\text{H}_3$ -isborneol to $^2\text{H}_3$ -borneol obtained was 51:49 (GC-FID). MS (EI) data: see Appendix A.

*[3,3,3,7,7,7- $^2\text{H}_6$]-(*Z*)-3,7-dimethyl-2,6-octadien-1-ol (no. **I-16**, $^2\text{H}_6$ -nerol) and*
*[3,3,3,7,7,7- $^2\text{H}_6$]-(*E*)-3,7-dimethyl-2,6-octadien-1-ol (no. **I-18**, $^2\text{H}_6$ -geraniol)*

Compounds nos. **I-16** and **I-18** were synthesized by following closely the procedure described for the synthesis of geraniol-7- ^{14}C , except that [$^2\text{H}_6$]-acetone was substituted for acetone-2- ^{14}C (14). Purification was accomplished using a two step mobile phase of 10% ether in pentane, followed by 20% ether in pentane on a silica gel

column. The ratio of no. **I-16** versus **I-18** was 34:66 (GC-FID). MS (EI) data: see Appendix A (target compounds) and B (intermediates).

[3,3,3,7,7,7-²H₆](Z)-3,7-dimethyl-2,6-octadienal (²H₆-neral) and [3,3,3,7,7,7-²H₆](E)-3,7-dimethyl-2,6-octadienal (²H₆-geranial)

After purification of the ²H₆-nerol and ²H₆-geraniol, the alcohols were oxidized using the Dess-Martin periodinane. The procedure followed was similar to method B described by Meyer and Schreiber, except that wet dichloromethane was prepared with 2 μL H₂O/1 mL CH₂Cl₂ as opposed to 1 μL H₂O/1 mL CH₂Cl₂ (15). MS (EI) data: see Appendix A.

4-(²H₃-methyl)phenol

The 4-(²H₃-methyl)-phenol isotope was purchased from C/D/N Isotopes Inc. (Quebec, Canada).

Response factors

For most compounds, the unlabeled compound and its isotope do not completely resolve. As a result, one (or more) unique ions are selected for integration of the unlabeled compound and the stable isotope. In order to compensate for differences in instrument sensitivity to these ions, a response factor was calculated for each compound/isotope ion pair. The response factor for a compound is found by plotting the peak area ratio of the selected ion of the unlabeled compound and its stable isotope against the mass ratio of the unlabeled compound and its stable isotope for multiple mass

ratios. The response factor is defined as the inverse of the slope. Five mass ratios were chosen to create a calibration curve: 10:1; 5:1, 1:1, 1:5; 1:10.

SPME

In Chapter 3, compound identification was done using cool on-column injection, the most non-destructive and unbiased injection technique available. However, use of on-column injection requires considerable sample work-up before obtaining a suitable extract for analysis. SPME offers the advantage of extraction of the volatiles by a fiber, eliminating sample preparation, and subsequently making it practical to conduct many more sample replications. SPME was further suitable for lemon-lime carbonated beverages because most compounds of interest could be detected using this method.

Sample preparation

Lemon-lime carbonated beverages were obtained from a local grocer (Urbana, IL), packaged in aluminum cans. All replications came from the same or sequential lots. The samples were stored at room temperature. The SPME vials and caps used were new to prevent any potential contamination from previous samples or soap. Magnetic stir bars and sodium chloride were baked (~200 °C) overnight prior to use. All SPME vials (20 mL) were marked with a line to indicate the 5 mL level, and immediately after opening the can of carbonated beverage, the sample was poured into the vial to the 5 mL line and capped. The exact weight of the sample was then recorded (by weight difference). The carbonated beverage was poured directly from the can into the vial, in order to minimize volatile and carbonation loss. After initially pouring the carbonated beverage into the

vial, sample would be added or poured out if necessary to obtain 5 ± 0.35 grams. The stable isotope solutions were spiked through the septum using a 10 microliter syringe. The isotopes were spiked individually and after addition was complete, the vial was stirred to evenly distribute the isotope standards. At this point, the vial was quickly uncapped, 1.0 g pre-weighted sodium chloride was added, and the vial was recapped with a new cap. New caps were put on the vial in order to prevent volatile loss through the needle holes during the tray wait time and sample incubation time during SPME analysis.

SPME conditions

Pre-incubation time: 15.0 min; incubation temperature: 40 °C; pre-incubation agitator speed: 250 rpm; agitator on time: 5 s; agitator off time: 2 s; vial penetration 22 mm; vial fiber exposure: 12 mm; extraction time: 10.0 min; injection needle penetration: 43 mm; injection fiber penetration: 22 mm; desorption time: 25.00 min. The SPME fiber used was a 50/30 μm DVB/CarboxenTM/PDMS StableFlexTM, obtained from Supelco (Bellefonte, PA).

GC-MS conditions

Splitless injection (260 °C; 4 min valve delay). Compound separation was achieved with a polar column (RTX-Wax, 30 m \times 0.25 mm i.d.; 0.25 μm film; Restek, Bellefonte, PA). GC oven conditions were: 35 °C initial temperature, held for 5.00 min; 4.0 °C/min ramp to 225 °C, held for 20 min (total run time = 72.50 min). The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization

energy, 70 eV; mass range, 35-300 amu; electron multiplier voltage (Autotune + 200 V); scan rate, 5.27 scans/s.

Determination of carbonation level in SPME vials

SPME vials (with cap) were weighed using an analytical balance. One can of each lemon-lime carbonated beverage was opened and the carbonated beverage was poured into the SPME vial to the line marked for five milliliters, the same as the procedure used for filling the vials with lemon-lime carbonated beverages for analysis by SPME. Four replications (same can) of each brand was poured into the vials, the vials were capped, and then reweighed. The vials were stored loosely capped in the refrigerator for two weeks to decarbonate. To ensure complete decarbonation, the lemon-lime beverages were uncapped and sonicated for 30 minutes prior to reweighing the capped vials. The correction factor for converting carbonated weight to a decarbonated basis was found by dividing the weight of the decarbonated beverage by the weight of the initial “just opened” carbonated beverage. The correction factor was an average of four replications for each brand.

HPLC determination of benzoic acid

High performance liquid-chromatography (HPLC) was performed using a Hewlett Packard Series 1050 HPLC to analyze the benzoic acid content of each brand of lemon-lime carbonated beverage. A Waters Nova-Pak C18 60 A, 4 μm , 150 x 3.9 mm analytical column was used without a guard column. The mobile phase was 80/20 1 M acetic acid/methanol run isocratically at 1.0 mL per minute. The UV detector was set at 254

nm. Samples were decarbonated prior to injection by sonicating the carbonated beverage in an uncapped vial for 30 minutes (16). Twenty microliters of decarbonated beverage was injected using a 20 microliter fixed volume loop. The pressure was 2120 psi. A five point external calibration curve was run on each day that analyses were conducted. Samples were decarbonated prior to injection by sonicating the carbonated beverage in an uncapped vial for 30 minutes (16). Four replications (replications from different cans; same cans as used for SPME analysis) were made for each brand and two injections into the HPLC were made for each replication. Benzoic acid concentrations were calculated by the instrument in milligrams per liter (ppm).

Titrateable Acidity

Titrateable acidity was determined by the general procedure described by Sadler and Murphy (17). The sodium hydroxide titrant was standardized using potassium hydrogen phthalate (KHP; EM Science, Gibbstown, New Jersey) (3 replications) and found to have a concentration of 0.02604 ± 0.0001 M. A 20 milliliter aliquot of beverage (decarbonated as previously described) was titrated to the phenolphthalein endpoint. Four replications (replications from different cans; same cans as used for SPME analysis) were made for each brand.

pH

The pH of the beverages (decarbonated as previously described) was read using an Accumet pH meter model AB15 (Fisher Scientific, Fair Lawn, New Jersey). The pH probe was standardized using a three point calibration with 4, 7, and 10 pH buffer

solutions (Fisher). Four replications (replications from different cans; same cans as used for SPME analysis) were made for each brand.

Total soluble solids (°Brix)

A Bellingham & Stanley Ltd. RFM 390 Refractometer (Tunbridge Wells, England) was used to determine °Brix at 21 °C (controlled using a recirculating water bath). Distilled water and a fresh 50.36% (w/w) sucrose solution were used to calibrate the instrument. Four replicate measurements were made (replications from different cans; same cans as used for SPME analysis).

Threshold determination

The thresholds of 2,3-dehydro-1,8-cineole (dehydrocineole), *p*-cresol and sodium benzoate were determined using the general procedure described by Watcharananun et al. (18). Dehydrocineole, synthesized as previously described in this chapter, was further purified by flash chromatography (silica gel) using 5% ether in pentane to a purity of 99.1% (GC-FID). *p*-Cresol (99.9%, GC-FID) and sodium benzoate (99.9%, manufacturer label) were obtained from Aldrich (Milwaukee, WI). ASTM procedure E679-91 (19) was used to determine orthonasal odor detection thresholds in odor-free water (dehydrocineole) or citrate buffer (pH 3, 1.3% citric acid) (*p*-cresol and sodium benzoate). Stock solutions of dehydrocineole and *p*-cresol were prepared in methanol and dissolved in the matrix. Sodium benzoate was dissolved directly in the citrate buffer. The solutions were presented to the panelists using 125-mL PTFE squeeze bottles. Panelists (12 for dehydrocineole, 15 for *p*-cresol and 9 for sodium benzoate) were given each concentration (1:3 dilution series) along with two matrix blanks containing the same

volume of methanol used in preparing the sample solutions. Six concentrations of the compounds were presented in ascending order. The individual best estimate threshold was calculated as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response using the criteria previously described (19). The group best estimate threshold (BET) was calculated as the geometric mean of the individual BETs.

RESULTS & DISCUSSION

Sample calculations

The response factors of the compounds chosen for quantification are presented in Table 4.1. As this table demonstrates, many of the response factors could not be accurately approximated by assuming that the unlabeled standard and its corresponding isotope have equal responses to the MS detector. Therefore, determination of response factors was a necessary part of quantification. The R^2 values are all acceptable, ranging from 0.985-1.000.

The step by step logic involved in calculating a response factor will be discussed for linalool, followed by a description of how the concentration of linalool in the carbonated beverages was determined. The spectra, calibration curves, compound purities, and additional information relevant to determination of response factors can be found for all compounds in Appendix A.

Figure 4.3 shows the mass spectra for the unlabeled and deuterium labeled linalool. The first step in calculating a response factor, and one which requires careful consideration, is to select which ion or ions to use for relating the abundance of the unlabeled compound against the labeled compound. Ideally, the selected ions will: 1) be

present in only the spectrum of the unlabeled standard/isotope, 2) not be present in the spectrum of another compound in the sample that coelutes with the target compound and 3) be at least moderately abundant. Sometimes it is not possible to find an ion unique to the unlabeled standard/isotope. This is not a problem when: 1) the unlabeled standard and isotope have well-resolved peaks or 2) the contribution the ion's peak area from the other compound is so small that it can be considered negligible. For an example of this second point, suppose the ion pair selected for quantification for compound A is ion 125 in the unlabeled standard and ion 128 in the isotope. The isotope of compound A fragments in such a way that it also has an ion at m/z 125 but it is so small that the ion is only visible when the spectrum is zoomed in, showing that ion 125 has an abundance of only around 50. In this case, it would be acceptable to use the ion pair 125 and 128. Using an ion that the unlabeled compound and isotope share is certainly a choice that needs to be made with caution but is sometimes unavoidable. When the spectra of linalool and $^2\text{H}_2$ -linalool are compared (Figure 4.3), it is clear that many of the isotope fragments are increased by two m/z units. However, some of these fragments would be poor choices for quantification, such as ions 71 (unlabeled) and 73 (isotope) because the isotope has a peak for ion 71 as well. In the present study the ions selected were 121 and 123 for the unlabeled standard and isotope, respectively, because there is no or only slight contribution from ion 123 in the unlabeled standard's spectrum and little contribution from ion 121 in the isotope's spectrum.

Now that ions have been selected, they can be extracted from the total ion chromatogram using ChemStation software and the peak areas integrated. The areas of these ions are recorded and the ratio of the unlabeled standard ion area to isotope ion area

is taken. The next step is to relate the peak area ratio of the ions to the mass ratio of the compounds. Table 4.2 details the volumes and concentrations used to make the calibration solutions. The mass of the unlabeled standard/isotope in solution is found by multiplying the concentration of the unlabeled standard/isotope by the volume added to the solution. The mass ratio of the unlabeled standard and its isotope is then taken. After the mass and area ratios have been determined for all calibration solutions, the data is plotted as seen in Figure 4.4 to give a linear relationship between the area ratio and the mass ratio of the unlabeled standard against its isotope. The slope for the calibration curve of $^2\text{H}_2$ -linalool is 0.990 and the response factor is the reciprocal of the slope, which is 1.01. After the response factors are determined for all the compounds being quantified, the task of determining compound concentrations in the carbonated beverages can begin.

Compounds are always in a pursuit of equilibrium, with carbon dioxide being no exception. The carbonated beverages cannot be analyzed at the full carbonation level because it is impossible to sample the carbonated beverage in the can without changing the carbonation level. Therefore, the samples were analyzed at a just-out-of-the-can carbonation level but the concentrations of target compounds were calculated on a corrected decarbonated beverage basis. Table 4.3 shows the average remaining beverage (mass percent) after decarbonation. Since the mass percent of remaining beverage presented in this table does not relate to the overall carbonation level of the beverage, it should not be compared with carbonation levels others have reported in lemon-lime carbonated beverages. However, these data allow for very good estimates of the actual amounts of decarbonated beverage in the SPME vials used for analysis. The carbonation

lost upon opening the can is compensated for by using the percent weight of remaining beverage.

Table 4.4 details the next steps in reaching a concentration value for linalool in each sample. Columns **B** and **C** show the area for the selected ions found by extracting ions 121 and 123 from the chromatogram and integrating the resulting peaks. Column **F** shows the amount of isotope that was spiked in the beverage under analysis. This mass was found by multiplying the concentration of the isotope solution by the volume spiked. The amount of target compound in the actual sample vial (**G**) can be determined by multiplying the peak area ratio of ion 121/ion 123 by the amount of isotope spiked and by the response factor. The mass of the carbonated beverage weighed out was multiplied by a correction factor to give the mass of decarbonated beverage in column **J**. Finally, the concentration of linalool is given as nanograms per gram of decarbonated beverage (column **K**). Now that the meticulous work of calculating compound concentrations is complete and the method for doing this has been elucidated, quantification data for all the important aroma components of lemon-lime carbonated beverages can be completed and the aroma profile as a whole can be examined.

Concentrations for Selected Aroma Compounds

The quantitative data for 12 key aroma-active components of the lemon-lime carbonated beverages are presented in Tables 4.5 and 4.6. ANOVA (single factor) was run on all the quantitative data to determine if statistical differences existed among brands. Fisher's LSD was used to determine which brands were statistically different when ANOVA yielded F values above F critical. All analyses were conducted on four

replications of each carbonated beverage except where otherwise noted. The *q* test was used to eliminate data that would not normally be found 95% of the time (20, 21).

Appendix C shows the raw data and *Q* values used to evaluate the data.

Statistical variation exists between at least two brands for all aroma compounds analyzed, except for neral, which did not differ among all three brands. The compounds 1,8-cineole, octanal, nonanal, decanal, linalool, isoborneol and geraniol differed among all brands. Large variations existed among brands for some compounds. For example, much more 2,3-dehydro-1,8-cineole (dehydrocineole) was present in SM and 7UP than in SP: e.g., 2380-2890 and 2490-3010 ng/g of dehydrocineole was present in SM and 7UP, respectively, versus 1360-1740 ng/g in SP (Table 4.5). With the exception of benzoic acid, the amount of dehydrocineole present was also an order of magnitude higher than the amount of any other compound present. The three brands differed in their 1,8-cineole content. SP has more 1,8-cineole than either SM or 7UP: e.g. 28.3-31.1 ng/g in SP versus 16.9-17.9 and 15.2-15.7 ng/g of 1,8-cineole in SM and 7UP, respectively.

The aldehydes octanal, nonanal and decanal also differed across all three brands. There was more octanal in all three brands than either of the other two aldehydes. This was expected based on the FD factors of the three aldehydes (Table 3.1), as octanal had higher FD factors than nonanal and decanal. The difference in octanal concentration is particularly salient between SP and SM; SP contained between 155-181 ng/g octanal, whereas SM contained 411-487 ng/g. The octanal concentration in 7UP (332-362 ng/g) was closer to that of SM than SP. Due to both its relatively high FD factor in all brands and distinct brand differences in octanal concentration, it is likely that octanal is one of the main distinguishing compounds among the brands.

Linalool, which was previously determined by AEDA (chapter 3) to be the most potent aroma compound based on its high FD factor, also differed among all three brands, although the magnitude of this difference among brands was not as great as observed with octanal. At the 95% confidence interval, SP contained between 202-239 ng/g, SM contained 277-314 ng/g and 7UP contained 353-403 ng/g linalool (Table 4.5). Linalool was the third most abundant compound in lemon-lime carbonated beverages, following only dehydrocineole and benzoic acid in abundance.

The borneol isomers were also distinguishing compounds among the three brands. SM and 7UP contained similar concentrations of borneol. SP, however, contained 3-4 times more borneol than SM and 7UP. Borneol was present at a higher level than isoborneol, which agrees with the slightly higher FD factors for borneol as compared to isoborneol (Chapter 3).

The quantitative data makes it quite clear that very little citral (neral and geranial) remains in the lemon-lime carbonated beverages. In all brands, the concentration for both isomers was below 15 ng/g. Although there were some statistical variations among brands for geranial – no variation was observed among brands for neral – these compounds likely do not play an important role in the overall aroma of lemon-lime carbonated beverages due to their low abundance. This result is most intriguing given the importance of citral in fresh lemon oil (22).

The alcohol analogues of citral were more abundant than citral itself. The nerol content of 7UP was higher than SP and SM, which contained about the same amount of nerol. 7UP also contained much more geraniol than either SP and SM.

p-Cresol was present at low levels in all three brands, with 7UP having statistically more *p*-cresol than the other two brands. There was considerable error in the determination of *p*-cresol content, which can be largely attributed to its small peak size and the variation in the integration of the peak area. Larger RSDs were also observed for other compounds present at very low levels, such as neral and geraniol.

Odor-Activity Values

Before considering the odor-activity value (OAV) data, a few words of caution are in order. The first is in regard to the generalization of the quantitative data. For consistency sake, all the replications were conducted using different cans within the same lot. The same lot was used because significant lot to lot variation is possible and it was first necessary to capture the aroma composition of a single lot to get a handle on the composition of each brand. Unfortunately, the scope of this research did not allow within-city or cross-country lot-to-lot variation in aroma composition to be studied. Therefore, although it is a fair generalization to say that SP, SM, and 7UP have significant aroma component variations, it must be remembered that this is based only on one lot of each brand and there is no way to conclude that each lot is truly representative of a particular brand. Yet, before one takes this data too lightly, it should be pointed out that the GCO data culminating in FD factors for compounds by brand were determined for beverages from different lots that the beverages used for quantification. Reasonable agreement in trends is seen from these two data sets, which allows for some generalization to be made in good faith.

The second word of caution is in regards to the OAVs that will be discussed next. As seen in Table 3.2, the threshold values used to calculate OAVs were determined by several different research groups, and in some cases, by different methods. The use of PFTE (i.e., Teflon) sniff bottles is the superior method for determining thresholds, as demonstrated by Guadagni in 1963 (23). Guadagni's study showed that the use of sniff bottles produces lower thresholds and less variation between replications of threshold determinations (23). Since the same research group has not determined thresholds for all the aroma compounds of interest in the present study, some other possibly less reliable thresholds are used instead. However, before saying that a compound is not important because it has a low OAV, it must be considered how the compound's threshold was determined: the threshold used to calculate the OAV may be erroneously high, making the compound seem less important. Specifically, borneol may have a reported threshold slightly higher than what it should be, since the reported threshold was determined using sniff cups (24). The methodology used for determining the threshold of isoborneol, nerol and nerol was not reported in the respective sources providing these thresholds. Therefore, the reliability of these thresholds may be questioned because the methodology used in their determination cannot be evaluated.

According to Table 4.7, decanal and octanal were predominant aroma active compounds in lemon-lime carbonated beverages. The OAV for decanal in SM and 7UP was extremely high, being 1910 in SM and 1330 in 7UP. In SP, the OAV for decanal was 613. The OAVs for octanal ranged from 240 (SP) to 641 (SM). Dehydrocineole is also among the most potent odorants with the third highest OAV across the three brands. Linalool, which was expected to be a key aroma compound based on its overall highest

FD factor (chapter 3), appeared to make only a moderate contribution when its OAVs were considered. The OAVs for linalool were between 37 (SP) and 63 (7UP). According to OAVs, linalool and nonanal appear to be of approximately equal importance. There are two plausible explanations for these observed differences in odor potency for linalool as determined by FD factor and OAV. First, there is error originating from the thresholds. As already discussed, the method used to determine a compound's threshold affects the threshold value (23). Therefore, an erroneously high threshold would cause the compound to seem less potent than it actually is, compared to the other compounds with accurately determined thresholds. The odor thresholds of the *n*-aldehydes were determined by Guadagni et al. (23) and the same research group, using the same methodology, albeit 20 years later, determined linalool's threshold (25). As a result, it is unlikely that the threshold determination methodology accounts for linalool's unexpectedly low OAVs. A second reason for differences in odor potency, as determined by FD factor and OAV, result from differences in analyzing the flavor. When the aroma extract was injected into the GCO, the compounds were completely volatilized and the GCO assessor detected compounds by sniffing a stream of air exiting the sniff port. This means that FD factors depend on each compound's odor threshold in air. On the other hand, calculated OAVs depend on each compound's threshold in water/solution. (Thresholds in water/solution should be used because when the food is smelled or consumed, the perceived flavor depends on matrix effects, ie the matrix will affect the aroma release.) A compound's threshold may be different in water and in air, as observed with linalool. The threshold of linalool in air is 0.4-0.8 ng/L (ppt) (26), whereas linalool's threshold in water is 6 µg/L (ppb) (25), indicating that the matrix has a

tremendous impact on a compound's threshold. Therefore, the FD factor of a compound may overestimate its importance due to the fact that it is being detected in air. The complex nature of compounds and differences in methodology for the two techniques prevent FD factors and OAVs from reaching absolute agreement. However, when a compound is determined to be potent by both methods the researcher can be confident that it is a key aroma-impact compound.

According to the OAVs, the other moderately important compounds were 1,8-cineole, isoborneol and geraniol, which all had OAVs greater than unity across all three brands. OAVs confirmed that the citral isomers were only minor aroma constituents. *p*-Cresol also appeared to be an insignificant aroma component.

Benzoic acid content was also a distinguishing factor among the three brands based on FD factor (Table 4.6). As expected 7UP was found to have no benzoic acid by HPLC, since the label did not include any type of benzoate as an ingredient. SM contained more benzoic acid than SP; however, the high threshold (1900 ppm) of sodium benzoate (exists as benzoic acid in the pH 3 solution) causes SP and SM to have an OAV of < 1 for benzoic acid.

Matrix components

Analysis of the decarbonated lemon-lime carbonated beverage matrix for titratable acidity (Table 4.8), pH (Table 4.9) and percent soluble solids (w/w, °Brix) (Table 4.10) showed that these constituents were present at different levels between at least two brands. The three brands differed in their citric acid (%TA) content. SM was the most acidic, containing 0.1467 % citric acid, and SP was the least, containing only

0.1208% citric acid. SM was also found to have the lowest pH. Furthermore, the percent soluble solids contents of SP and SM differed from 7UP. SP and SM contained 10.41 and 10.40% (w/w) soluble solids ($^{\circ}$ Brix), respectively, and 7UP contained 10.51% (w/w) soluble solids. Due to the very small RSD for the determination of percent soluble solids, although 7UP differed statistically from SP and SM, this very slight difference may not be of any practical significance.

CONCLUSIONS

This chapter highlighted the quantitative differences among aroma components of SP, SM and 7UP. Except for neral, differences in concentration were observed between at least two brands for all aroma compounds quantified. Benzoic acid was the most abundant aroma compound in SP and SM, and was not detected in 7UP. 2,3-Dehydro-1,8-cineole (dehydrocineole) was the most abundant aroma component in 7UP and second most abundant, after benzoic acid, in SP and SM. Based on their overall high odor activity values (OAVs), decanal, octanal and dehydrocineole emerged as the most potent aroma compounds in all three brands of lemon-lime carbonated beverages. Linalool and nonanal were found to be moderately important based on the OAV concept. Despite the abundance of benzoic acid, the high threshold of this compound results in OAVs < 1 for benzoic acid in SP and SM.

The next step in this research would be to conduct model studies, using ranking tests, based on the quantification data. If the model of a particular brand adequately captures its flavor, panelists would confuse the model and the actual commercial lemon-lime carbonated beverage (after decarbonation) when comparing it the reference

(commercial lemon-lime carbonated beverage after decarbonation). It is hypothesized that the three commercial lemon-lime brands are distinguishably different and each model (if it is adequate) would be ranked as more similar to its respective brand than the other models. However, if the models were found to be distinguishable from the three commercial brands of lemon-lime carbonated beverages, it may be necessary to quantify abundant compounds with no or little aroma activity, e.g., α -terpineol and limonene, which may affect the partitioning of the aroma active compounds in the headspace.

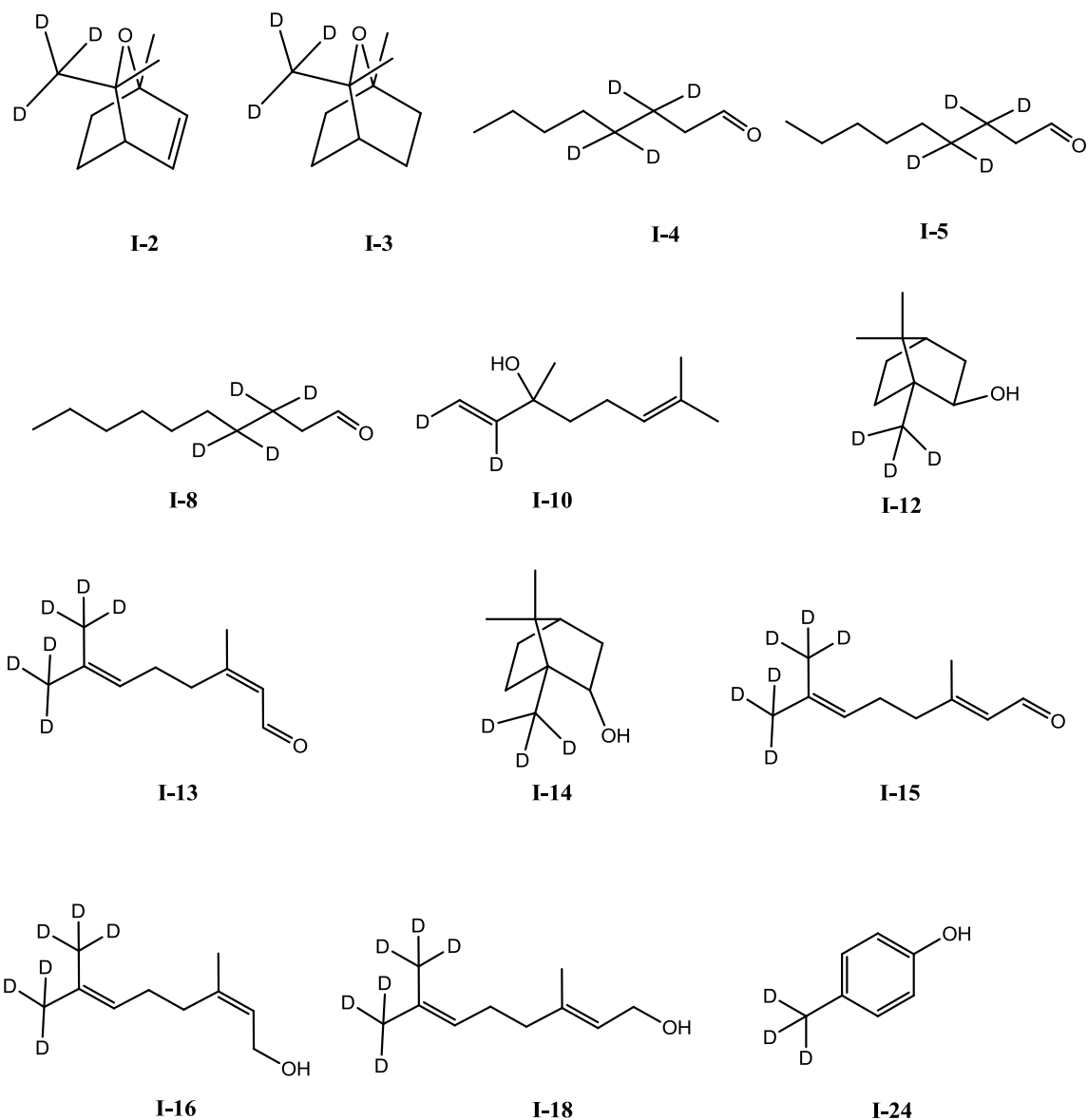


Figure 4.1 Structures for stable isotopic labeled standards used for the quantification of key aroma components of lemon-lime carbonated beverages. (**I** denotes that the compound is an isotope; compound numbers correspond to those in Table 4.1.)

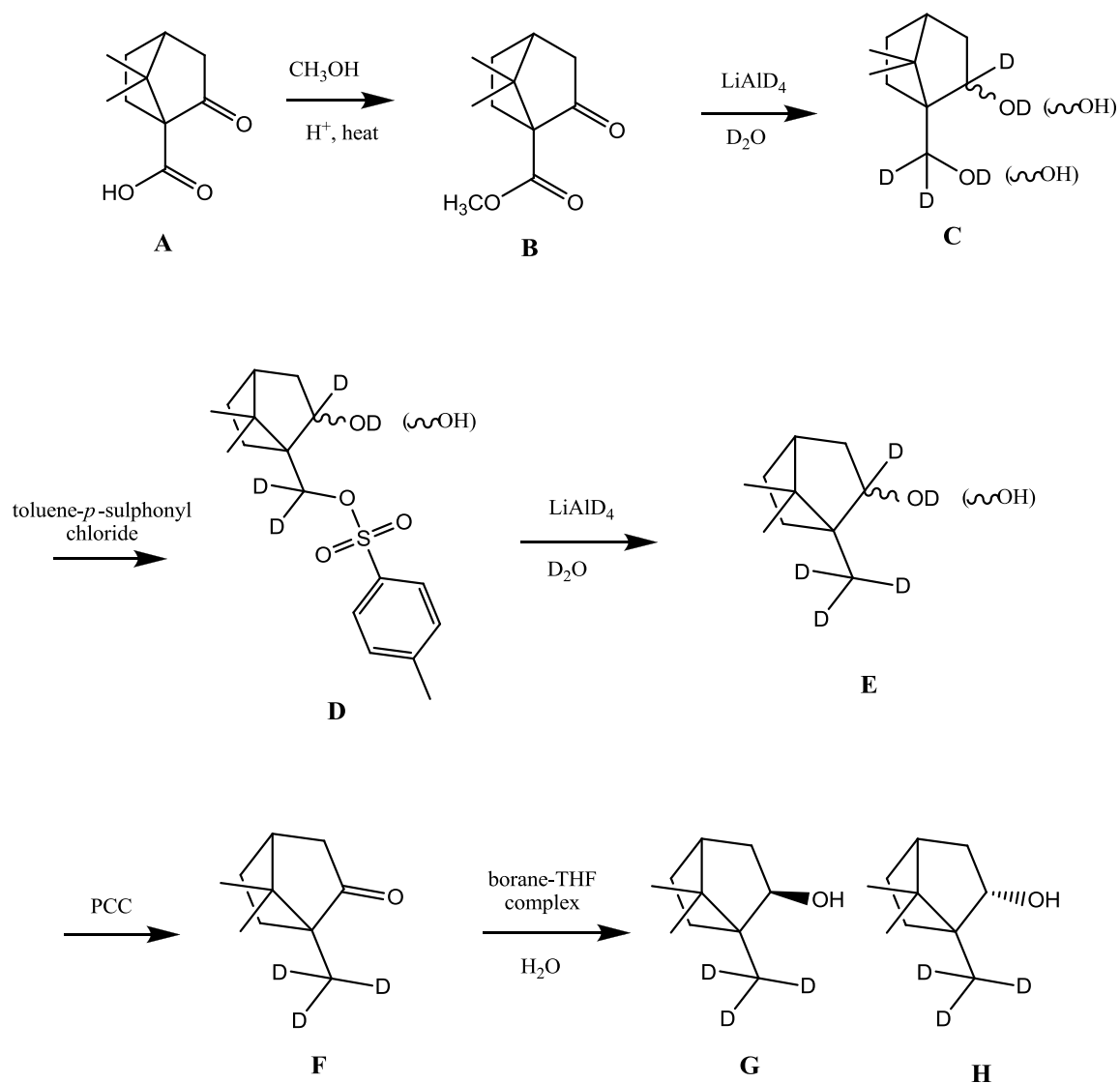
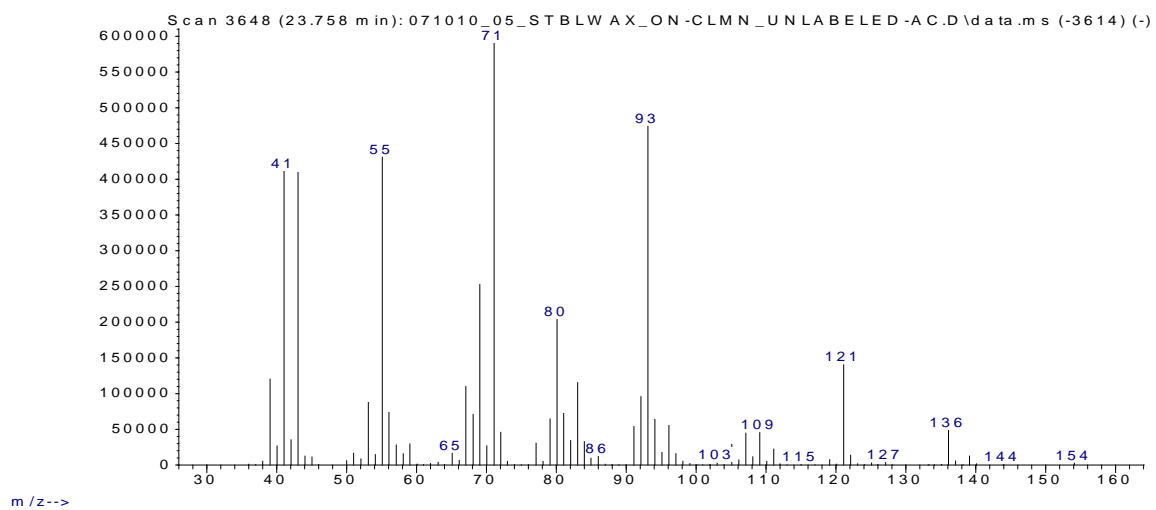


Figure 4.2 Scheme for synthesis of $^2\text{H}_3$ -isoborneol (**G**, no. **I-12**) and $^2\text{H}_3$ -borneol (**H**, no. **I-14**). [ketopinonic acid (**A**), methyl ketopinonic acid (**B**), $^2\text{H}_{3.5}$ -1-(hydroxymethyl)-7,7-dimethylbicyclo-[2.2.1]-heptan-2-ol (**C**), $^2\text{H}_{3.4}$ -(2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methyl-4-methylbenzenesulfonate (**D**), $^2\text{H}_{4.5}$ -isoborneol and $^2\text{H}_{4.5}$ -borneol (**E**) and $^2\text{H}_3$ -camphor (**F**)].

(a)

Abundance



(b)

Abundance

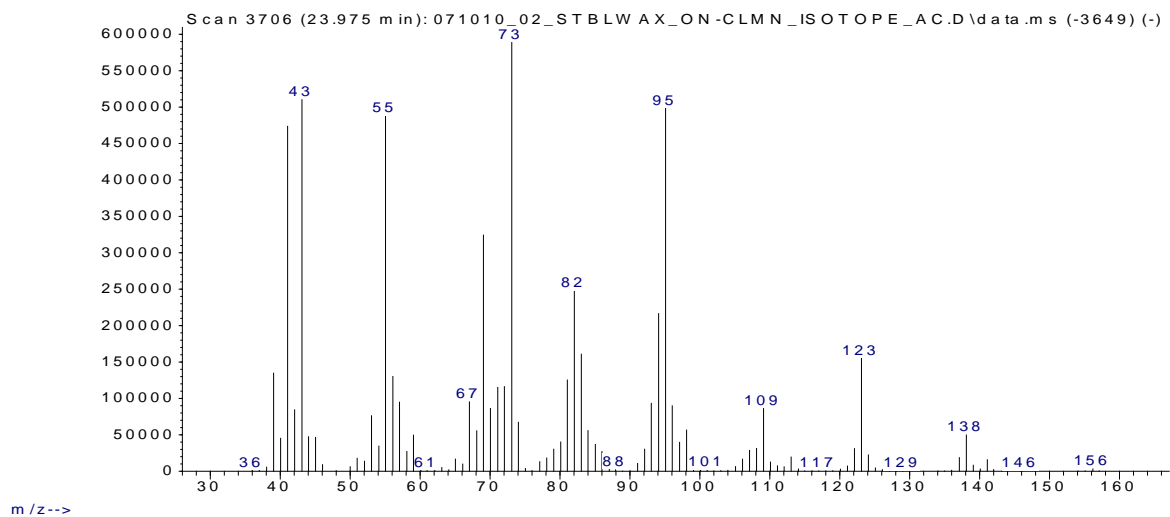


Figure 4.3 Mass Spectra (EI) for (a) linalool and (b) $^2\text{H}_2$ -linalool

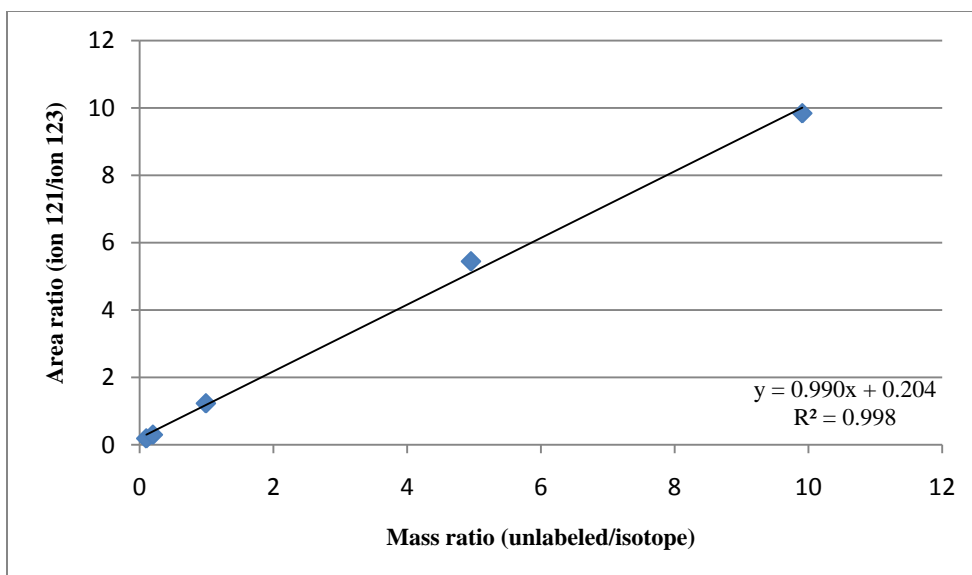


Figure 4.4 Calibration curve for $^2\text{H}_2$ -linalool

Table 4.1 Response factors (EI-MS) of key aroma compounds quantified in three commercial brands of lemon-lime carbonated beverages

No.	Unlabeled standard	Isotope standard	Unlabeled ion	Isotope ion	Response factor (isotope) ^a	R ²
2	2,3-dehydro-1,8-cineole	[10,10,10- ² H ₃]-2,3-dehydro-1,8-cineole (I-2)	124	127	1.19	1.000
3	1,3,3-trimethyl- 2-oxabicyclo[2,2,2]octane (1,8-cineole)	[10,10,10- ² H ₃]- 1,3,3-trimethyl- 2-oxabicyclo[2,2,2]-octane (I-3)	154	157	0.971	0.999
4	octanal	[3,3,4,4 ² H ₄]-octanal (I-4)	110	114	0.487	1.000
5	nonanal	[3,3,4,4 ² H ₄]-nonanal (I-5)	114	116	0.437	0.985
8	decanal	[3,3,4,4- ² H ₄]-decanal (I-8)	128	130	1.92	1.000
10	3,7-dimethyl-1,6-octadien-3-ol (linalool)	[1,2- ² H ₂]-3,7-dimethyl-1,6-octadien-3-ol (I-10)	121	123	1.01	0.998
12	<i>exo</i> -1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (isborneol)	[10,10,10- ² H ₃]- <i>exo</i> -1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (I-12)	110	113	1.07	0.993
13	(<i>Z</i>)-3,7-dimethyl-2,6-octadienal (neral)	[8,8,8,10,10,10- ² H ₆]-(<i>Z</i>)-3,7-dimethyl-2,6-octadienal (I-13)	69	75	0.684	1.000
14	<i>endo</i> -1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (borneol)	[10,10,10- ² H ₃]- <i>endo</i> -1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (I-14)	110	113	0.833	0.996
15	(<i>E</i>)-3,7-dimethyl-2,6-octadienal (geranial)	[8,8,8,10,10,10- ² H ₆]-(<i>E</i>)-3,7-dimethyl-2,6-octadienal (I-15)	69	75	0.688	1.000
16	(<i>Z</i>)-3,7-dimethyl-2,6-octadien-1-ol (nerol)	[8,8,8,10,10,10- ² H ₆]-(<i>Z</i>)-3,7-dimethyl-2,6-octadien-1-ol (I-16)	69	75	0.682	0.998
18	(<i>E</i>)-3,7-dimethyl-2,6-octadien-1-ol (geraniol)	[8,8,8,10,10,10- ² H ₆]-(<i>E</i>)-3,7-dimethyl-2,6-octadien-1-ol (I-18)	69	75	0.961	1.000
24	4-methylphenol (<i>p</i> -cresol)	4-(² H ₃ -methyl)phenol (I-24)	108	111	1.27	1.000

^a Determined using a Stabilwax column.

Table 4.2 Raw data used to calculate the EI-MS response factor for $^2\text{H}_2$ -linalool

Target mass ratio (unlabeled: isotope)	Conc. unlabeled (mg/mL)	Volume unlabeled ^{a, c} (μL)	Conc. isotope (mg/mL)	Volume isotope ^{b, c} (μL)	Mass unlabeled (mg)	Mass isotope (mg)	Mass ratio	Area ion 121	Area ion 123	Area ratio (ion 121/ion 123)
1 to 20	1.09	10	1.10	200	0.0109	0.22	0.0495	1678640	14151632	0.119
1 to 10	1.09	10	1.10	100	0.0109	0.11	0.0991	1625499	8575252	0.190
1 to 5	1.09	10	1.10	50	0.0109	0.055	0.198	1352649	4516109	0.300
1 to 1	1.09	10	1.10	10	0.0109	0.011	0.991	1101168	894700	1.23
5 to 1	1.09	50	1.10	10	0.0545	0.011	4.95	5447514	1000378	5.45
10 to 1	1.09	100	1.10	10	0.109	0.011	9.91	11593225	1178142	9.84
20 to 1	1.09	200	1.10	10	0.218	0.011	19.8	21867311	1446552	15.1

^a Concentration of unlabeled stock solution = 1.09 mg/mL. ^b Concentration of stock isotope solution = 1.1 mg/mL. ^c The volume of unlabeled and isotope standards appearing in the same row were added to the same vial and then diluted to one milliliter. One microliter of the final solution was analyzed by GC-MS (see methods section for details).

Table 4.3 Remaining beverage after decarbonation of three commercial brands of lemon-lime carbonated beverage

Brand	Average remaining beverage (mass percent) *	RSD
Sprite	97.4 ± 1.55	1.59
Sierra Mist	97.8 ± 1.50	1.54
7-Up	99.0 ± 0.18	0.18

* Average of four replications

Table 4.4 Raw data for the determination of linalool concentration

Sample ^a	Area (ion 121)	Area (ion 123)	Ion Ratio (121/ 123)	Response factor	Amt isotope ^b (ng)	Mass of target compound ^c (ng)	Mass of soda (g)	Correction factor ^e	Mass of decarbonated soda ^d (g)	Cncn ^f (ng/g)
SP, R1	5964171	5869442	1.016139	1.01	1100	1129	4.98	0.97388	4.85	233
SP, R2	5370210	5511097	0.974436	1.01	1100	1083	4.99	0.97388	4.86	223
SP, R3	5279446	5320814	0.992225	1.01	1100	1102	5.12	0.97388	4.99	221
SP, R4	6759873	7455507	0.906695	1.01	1100	1007	5.05	0.97388	4.92	205
SM, R1	6730273	4841519	1.390116	1.01	1100	1544	5.05	0.97753	4.94	313
SM, R2	8523723	6733114	1.265941	1.01	1100	1406	4.95	0.97753	4.84	291
SM, R3	8145781	6380934	1.276581	1.01	1100	1418	5.06	0.97753	4.95	287
SM, R4	8166131	6135262	1.331016	1.01	1100	1479	5.18	0.97753	5.06	292
7UP, R1	9126230	4945739	1.845271	1.01	1100	2050	5.21	0.98968	5.16	398
7UP, R2	11881241	7332571	1.620338	1.01	1100	1800	5.04	0.98968	4.99	361
7UP, R3	10900996	6554039	1.663249	1.01	1100	1848	5.04	0.98968	4.99	370
7UP, R4	10461699	6097588	1.715711	1.01	1100	1906	5.04	0.98968	4.99	382
A	B	C	D	E	F	G	H	I	J	K

^a SP = Sprite, SM = Sierra Mist, 7UP = 7-Up, R = replication number. ^b Two microliters of a 0.55 mg/mL solution added to beverage sample. ^c Amount target compound obtained = [ratio (ion 121/ion 123)] x [response factor]x[amt isotope]. ^d Mass decarbonated soda = [mass soda]x[correction factor]. ^e Correction factor, see Table 4.3. ^f Concentration = [amt target compound]/[mass decarbonated soda].

Table 4.5 Concentrations for key aroma components of three commercial brands of lemon-lime carbonated beverages

No.	Compound	Sprite			Sierra Mist			7-Up		
		Average cncn (ng/g)	RSD* (%)	95% confidence interval (ng/g)	Average cncn (ng/g)	RSD (%)	95% confidence interval (ng/g)	Average cncn (ng/g)	RSD (%)	95% confidence interval (ng/g)
2	2,3-dehydro-1,8-cineole	1550 ^a	7.78	1360-1740	2630 ^b	6.05	2380-2890	2750 ^{#,b}	3.84	2490-3010
3	1,8-cineole	29.7 ^a	3.01	28.3-31.1	17.4 ^b	1.88	16.9-17.9	15.5 ^{#,c}	0.65	15.2-15.7
4	octanal	168 ^a	4.78	155-181	449 ^b	5.29	411-487	347 ^c	2.76	332-362
5	nonanal	33.5 ^a	3.22	31.8-35.2	54.3 ^b	0.75	53.7-55.0	42.1 ^c	9.78	35.5-48.6
8	decanal	61.3 ^a	6.67	54.8-67.8	191 ^b	2.73	183-199	133 ^c	5.65	121-145
10	linalool	220 ^a	5.25	202-239	296 ^b	3.97	277-314	378 ^c	4.19	353-403
12	isoborneol	32.5 ^a	5.23	29.8-35.3	16.0 ^b	6.44	14.4-17.7	12.2 ^c	7.39	10.8-13.6
13	neral	3.58 ^a	19.7	2.46-4.70	3.83 ^a	19.3	2.65-5.01	4.59 ^a	14.2	3.56-5.63
14	borneol	201 ^a	3.75	189-213	53.8 ^b	1.46	52.6-55.1	58.6 ^b	6.96	52.1-65.1
15	geranial	5.64 ^a	25.4	3.36-7.91	6.58 ^a	18.5	4.65-8.52	10.0 ^b	24.5	6.11-13.9
16	nerol	32.9 ^a	6.59	29.5-36.4	35.0 ^a	9.38	29.8-40.2	49.5 ^b	8.10	43.2-55.9
18	geraniol	47.5 ^a	10.0	39.9-55.0	67.5 ^b	7.63	59.3-75.7	119 ^c	7.17	105-132
24	<i>p</i> -cresol	10.9 ^a	14.7	8.37-13.5	14.0 ^a	26.1	8.18-19.8	20.2 ^b	19.4	13.9-26.4

* Percent relative standard deviation. # Average of three replications, all other concentrations reported are the average of four replications.

^{a-c} Different letters in the same row denote statistical difference, as determined by Fisher's LSD ($\alpha = 0.05$).

Table 4.6 Benzoic acid content of three commercial brands of lemon-lime carbonated beverages

Brand	Cncn [#] (ppm)	RSD [*] (%)	95% confidence interval (ppm)
Sprite	152 ^a	1.574	148 - 156
Sierra Mist	199 ^b	2.199	192 - 206
7-Up	0.0 ^c	0	- -

[#] Average (expressed on a decarbonated basis) of four replications (from different cans).

Within each replication two analyses of each decarbonated beverage were made and these data were averaged before the overall average was calculated. ^{*} Percent relative standard deviation.

^{a-c} Different letters in the same column denote statistical differences, as determined by Fisher's LSD ($\alpha = 0.05$).

Table 4.7 Odor activity values for key flavor compounds in three commercial brands of lemon-lime carbonated beverages

No.	Compound	Threshold (ppb, in water ^a or in aqueous pH 3 soln ^b) ^c	Cncn (ppb) ^d			OAV ^e		
			SP	SM	7UP	SP	SM	7UP
2	2,3-dehydro-1,8-cineole	17 ^a	1550	2630	2750 ^f	91	155	162
3	1,8-cineole	1.3 ^a	29.7	17.4	15.5 ^f	23	13	12
4	octanal	0.7 ^a	168	449	347	240	641	496
5	nonanal	1 ^a	33.5	54.3	42.1	34	54	42
8	decanal	0.1 ^a	61.3	191	133	613	1910	1330
10	linalool	6 ^a	220	296	378	37	49	63
12	isoborneol	2.5 ^a	32.5	16.0	12.2	13	6	5
13	neral	30 ^a	3.58	3.83	4.59	< 1	< 1	< 1
14	borneol	140 ^a	201	53.8	58.6	1	< 1	< 1
15	geranial	32 ^a	5.64	6.58	10.0	< 1	< 1	< 1
16	nerol	300 ^a	32.9	35.0	49.5	< 1	< 1	< 1
18	geraniol	40 ^a	47.5	67.5	119	1	2	3
24	<i>p</i> -cresol	47 ^b	10.9	14.0	20.2	< 1	< 1	< 1
28	benzoic acid	1 900 000 ^b	152000	199000	0.0	< 1	< 1	0

^a Odor (orthonasal) detection threshold in water (µg/L or ppb). ^b Odor (orthonasal) detection threshold in pH 3 solution (µg/L or ppb).

^c Threshold references can be found in Table 3.2 footnotes. ^d Concentrations are reported on a decarbonated basis ^e Odor-activity value = [average concentration, ppb]/[threshold, ppb]. ^f Average of three replications, all other concentrations reported are the average of four replications.

Table 4.8 Titratable acidity (TA) for three commercial brands of lemon-lime carbonated beverages

Brand	TA(g/100 mL) [#]	RSD [*] (%)	95% confidence interval
Sprite	0.1208 ^a	1.3	0.1183-0.1234
Sierra Mist	0.1467 ^b	1.5	0.1432-0.1501
7-Up	0.1290 ^c	0.74	0.1275-0.1305

[#] Percent (w/v) titratable acidity (after decarbonation) expressed on a citric acid basis. Average of four replications. ^{*} Percent relative standard deviation. ^{a-c} Different letters in the same column denote statistical difference, as determined by Fisher's LSD ($\alpha = 0.05$).

Table 4.9 pH for three commercial brands of lemon-lime carbonated beverages

Brand	pH [#]	RSD [*]	95% confidence interval
Sprite	3.29 ^a	0.72	3.25-3.32
Sierra Mist	3.09 ^b	1.10	3.03-3.14
7-Up	3.23 ^c	1.15	3.17-3.28

[#] pH (after decarbonation). Average of four replications. ^{*} Percent relative standard deviation. ^{a-c} Different letters in the same column denote statistical difference, as determined by Fisher's LSD ($\alpha = 0.05$).

Table 4.10 Percent soluble solids (°Brix) content for three commercial brands of lemon-lime carbonated beverages

Brand	Percent soluble Solids (°Brix) [#]	RSD [*]	95% confidence interval
Sprite	10.41 ^a	0.24	10.37-10.45
Sierra Mist	10.40 ^a	0.096	10.38-10.41
7-Up	10.51 ^b	0.091	10.50-10.53

[#] Average of four replications (after decarbonation). ^{*} Percent relative standard deviation. ^{a-b} Different letters in the same column denote statistical difference, as determined by Fisher's LSD ($\alpha = 0.05$).

REFERENCES

1. Blank, I. Gas chromatography-olfactometry in food aroma analysis. In *Flavor, Fragrance, and Odor Analysis*; Marsili, R., Ed.; Marcel Dekker, Inc.: New York, 2002; pp. 297-331.
2. Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916-924.
3. Ayorinde, F. O.; Wheeler, J. W.; Duffield, R. M. Synthesis of dehydrocineole, a new monoterpene from the acarid mite, *Caloglyphus rodriguezii* (Arachnida: Acari). *Tetrahedron Lett.* **1984**, *25*, 3525-3528.
4. Bugarcic, Z.; Dunkic, J.; Mojsilovic, B. A simple, convenient and expeditious approach to cineol. *Heteroatom Chem.* **2004**, *15*, 468-470.
5. Lin, J.; Welti, D. H.; Vera, F. A.; Fay, L. B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999**, *47*, 2813-2821.
6. Corey, E. J.; Suggs, J. W. Pyridium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, *31*, 2647-2650.
7. Steinhaus, M.; Fritsch, H.; Schieberle, P. Quantitation of (R)- and (S)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100-7105.
8. Havens, B. R.; Meloan, C. E. The application of deuterated sex pheromone mimics of the American cockroach (*Periplaneta americana*, L.), to the study of Wright's vibrational theory of olfaction. In *Food Flavors: Generation, Analysis and Process Influence*; Charalambous, G., Ed.; Elsevier Science B.V., 1995; Vol.37, pp. 497-524.
9. Vogel, A. I. Experiment 6.163 Methyl Benzoate. In *Vogel's Textbook of Practical Organic Chemistry*; 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, P. W. G., Tatchell, A. R., Eds.; Longman Group UK Ltd.: Harlow, England, 1989; pp. 1077-1079.
10. Vogel, A. I. Experiment 5.38 Hexane-1,6-diol. In *Vogel's Textbook of Practical Organic Chemistry*; 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, P. W. G. and Tatchell, A. R., Eds.; Longman Group UK Limited: Harlow, England, 1989; pp. 530-531.
11. Vogel, A. I. Experiment 6.46 Butyl toluene-*p*-sulphonate, In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, P. W. G. and Tatchell, A. R., Eds.; Longman Group UK Limited: Harlow, England, 1989; pp. 886-887.
12. Krishnamurthy, S. Remarkable solvent control of functional-group selectivity in complex metal hydride reductions. *J. Org. Chem.* **1980**, *45*, 2550-2551.
13. Andrew, G. C.; Crawford, T. C. Synthetic utility of amine borane reagents in the reduction of aldehydes and ketones. *Tetrahedron Lett.* **1980**, *21*, 693-696.

14. Durst, H. D.; Leete, E. Synthesis of geraniol-7-¹⁴C. *J. Labelled Compd.* **1971**, *7*, 52-55.
15. Meyer, S. D.; Schreiber, S. L. Acceleration of the Dess-Martin oxidation by water. *J. Org. Chem.* **1994**, *59*, 7549-7552.
16. Health Canada. Determination of saccharin, sodium benzoate and caffeine in carbonated beverages by HPLC. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/chem/lpfc-102_01-eng.php (accessed August 11, 2010).
17. Sadler, G. D.; Murphy, P. A. pH and Titratable Acidity. In *Food Analysis*, 3rd ed.; Nielsen, S.S., Ed.; Springer: New York, 2003; pp. 207-225.
18. Watcharananun, W. Cadwallader, K. R.; Huangrak, K.; Kim, H.; Lorjaroenphon, Y. Identification of predominant odorants in Thai desserts flavored by smoking with "Tian Op," a traditional Thai scented candle. *J. Agric. Food Chem.* **2009**, *57*, 996-1005.
19. ASTM. *Standard Practice E 679-91, Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits*; American Society for Testing and Materials: Philadelphia, PA, 1992.
20. Dean, R. B.; Dixon, W. J. Simplified statistics for small numbers of observations. *Anal. Chem.* **1951**, *23*, 636-638.
21. Rorabacher, D. B. Statistical treatment for rejection of deviant values: critical values of Dixon's "Q" parameter and related subrange ratios at the 95% confidence level. *Anal. Chem.* **1991**, *63*, 139-146.
22. Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil citric-acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
23. Guadagni, D. G.; Buttery, R. G.; Okano, S. Odour thresholds of some organic compounds associated with food flavours. *J. Sci. Food Agric.* **1963**, *14*, 761-765.
24. Tamura, H.; Yang, R.-H.; Sugisawa, H. Aroma profiles of peel oils of acid citrus. In *Bioactive volatile compounds from plants*; Teranishi, R.; Buttery, R.G.; Sugisawa, H., Eds.; ACS Symposium Series 525; American Chemical Society: Washington, DC, 1993; pp. 121-136.
25. Buttery, R. G.; Ling, L. C.; Light, D. M. Tomato leaf volatile aroma components. *J. Agric. Food Chem.* **1987**, *35*, 1039-1042.
26. Blank, I.; Fischer, K.-H.; Grosch, W. Intensive neutral odourants of linden honey: differences from honeys of other botanical origin. *Z Lebensm Unters Forsch* **1989**, *189*, 426-433.

CHAPTER 5

CONCLUSIONS & RECOMMENDATIONS

Three popular brands of lemon-lime carbonated beverages were studied to determine the potent odorants responsible for typical “lemon-lime” flavor. Compound identification was accomplished by gas chromatography-olfactometry (GCO) and GC-mass spectrometry (MS) analysis of aroma extracts prepared by liquid-liquid continuous extraction-solvent assisted flavor evaporation (LLCE-SAFE). Results indicated that lemon-lime flavor is composed of a mixture of compounds 1) found naturally in citrus oils, 2) formed via acid catalyzed rearrangement of citrus oil components and 3) compounds added directly into the beverage by the manufacturer. A combined total of 28 compounds were detected by GCO, with linalool, octanal and 2,3-dehydro-1,8-cineole (dehydrocineole) emerging as the most potent aroma compounds in all three brands according to their highest flavor-dilution (FD)-factors. Other moderately important compounds, depending on the brand, included borneol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, *p*-cresol and benzoic acid. Quantification data showed that benzoic acid is the most abundant compound in Sprite[®] (SP) and Sierra Mist[®] (SM), with dehydrocineole being the most abundant in 7UP[®] (7UP) and the second most abundant in SP and SM. In contrast to FD factors, OAVs indicated that decanal was the most potent aroma compound, followed by octanal and dehydrocineole; with linalool and nonanal being moderately important to the flavor. SP, SM, and 7U differed statistically for concentrations of 12 compounds, with neral being the only compound for which the three brands did not differ ($\alpha=0.05$). Each brand differed from the other two brands for

concentrations of 1,8-cineole, octanal, nonanal, decanal, linalool, isoborneol, geraniol and benzoic acid. The data demonstrate that lemon-lime carbonated beverages share many of the same compounds but the distribution of these compounds varies by brand.

This work lays the foundation for several more paths of research. Little research exists on the effect of carbonation on aroma release (1) or taste (2) and to date no thresholds have been published for compounds in a carbonated matrix. This would be a technically challenging project to undertake due to the difficulty in creating a leak proof carbonating system, but the resulting data would be extremely interesting. In addition to the threshold work, model studies should be preformed. The first preliminary ranking test would ask panelists to compare the three lemon-lime beverage brands (after decarbonation) to a reference (one of the three brands) and ask them to rank the lemon-lemon samples in similarity to the reference. Calculation of an R-index would then indicate whether the three brands are distinguishable. Based on the quantification data and informal tests with members of the lab, it is hypothesized that people would be able to discern one brand from another. If this was found to be true experimentally by R-index, then a model could then be created for each lemon-lime beverage brand and more ranking tests would be conducted to determine whether the models were able to capture the unique aspects of each brand.

Additionally, more work could be done on identification. Some compounds remain unknown or tentatively identified. This work would be particularly important if the models are inadequate representations of the lemon-lime beverages. The unknowns of greatest interest are the compound described as fresh, melony with an RI of 1451 (wax) and the compound described as stale, burnt sugar eluting at 1534 (wax).

The final recommendation proposed here is to conduct basic research on how compound interactions affect flavor. Although humans are good at describing the attributes of complex flavors, they are quite poor at being able to identify the individual compounds that compose this flavor when the mixture exceeds four compounds (3, 4). It would be interesting to know how compound omissions affect the perception of lemon-lime aroma models. Such information may lead to further insights into the most important aroma components of lemon-lime carbonated beverages.

REFERENCES

1. Pozo-Bayon, M. A.; Santos, M.; Martin-Alvarez, P. J.; Reineccius, G. Influence of carbonation on aroma release from liquid systems using an artificial throat and a proton transfer reaction-mass spectrometric technique (PTR-MS). *Flavour Frag. J.* **2009**, *24*, 226-233.
2. Cowart, B. J. The addition of CO₂ to traditional taste solutions alters taste quality. *Chem. Senses* **1998**, *23*, 397-402.
3. Laing, D. G. Perceptual odour interactions and objective mixture analysis. *Food Qual. Prefer.* **1994**, *5*, 75-80.
4. Laing, D. G.; Francis, G.W. The capacity of humans to identify odors in mixtures. *Physiol. Behav.* **1989**, *46*, 809-814.

APPENDIX A

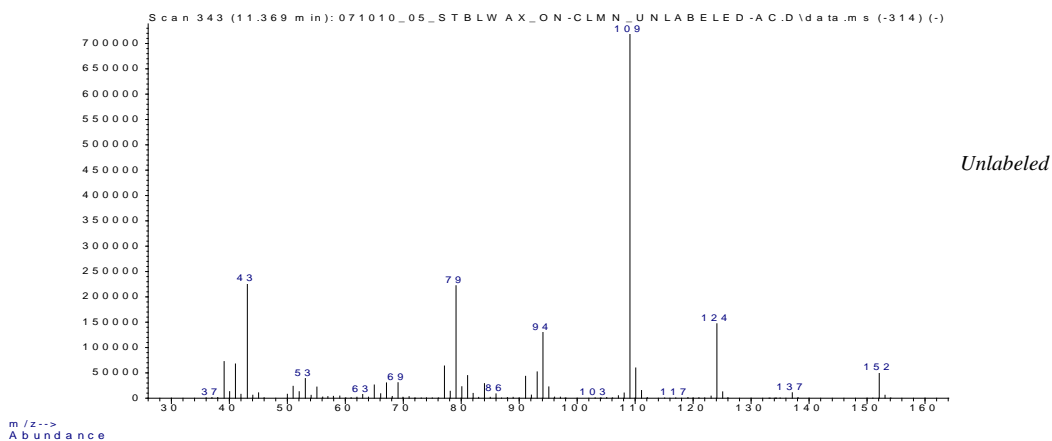
**SPECTRA FOR UNLABELED AND ISOTOPE STANDARDS USED FOR
DETERMINING RESPONSE FACTORS; ADDITIONAL COMPOUND
INFORMATION; ISOTOPE CALIBRATION CURVES**

Response Factor of d₃-2,3-Dehydro-1,8-cineole (July 2010)

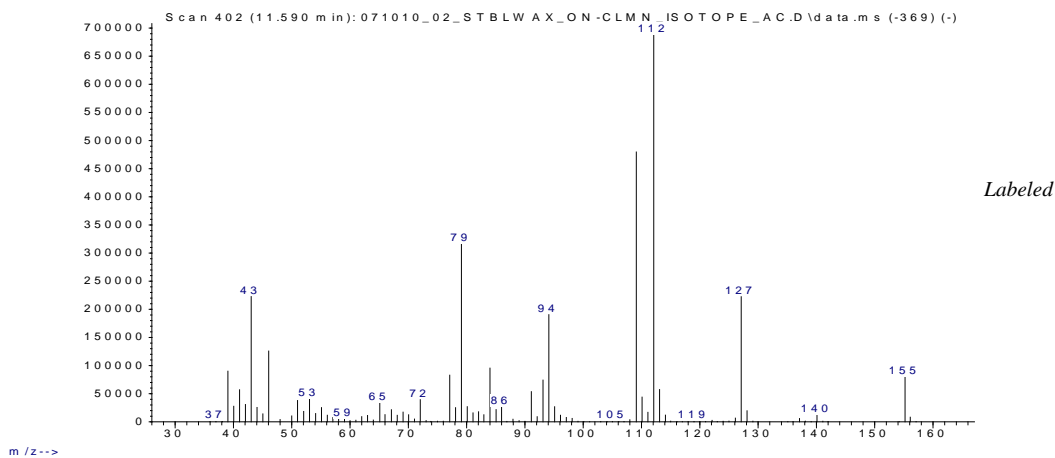
Standard:	Isotope (² H ₃)-2,3-dehydro-1,8-cineole	Unlabeled 2,3-dehydro-1,8-cineole
CAS:		92760-25-3
Mfg/Reference:	synthesized	synthesized
No.; Catalog #; Batch#/Lot#:		
% Purity (by GC-FID)	91.72%	90.203%

Spectra:

Abundance



m/z-->
Abundance



m/z-->

Methods

Matrix: dichloromethane

Extraction: none

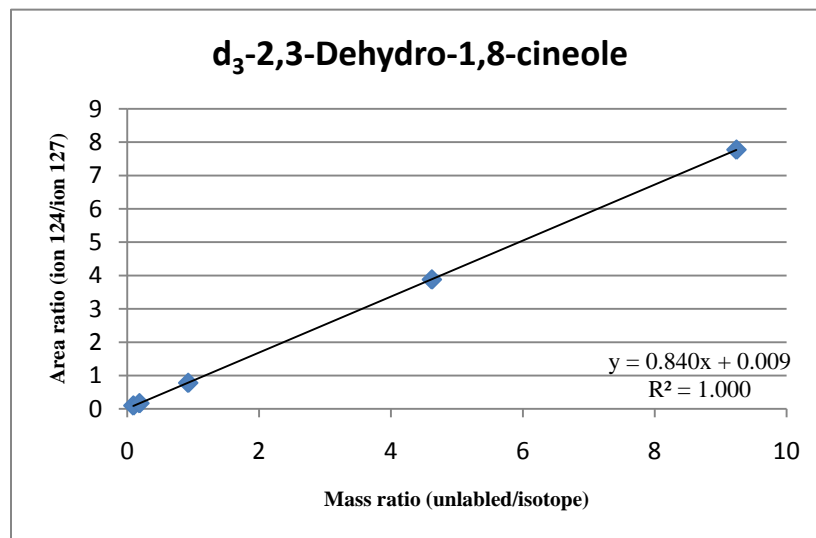
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 124 (unlabeled) 127 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0924	1737439	17918537	070910_06_STBLWAX_ON-CLMN_C_1-10.D
0.1848	1712509	10262034	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
0.9238	1788915	2301234	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
4.6190	8898288	2294344	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
9.2381	18284986	2352411	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



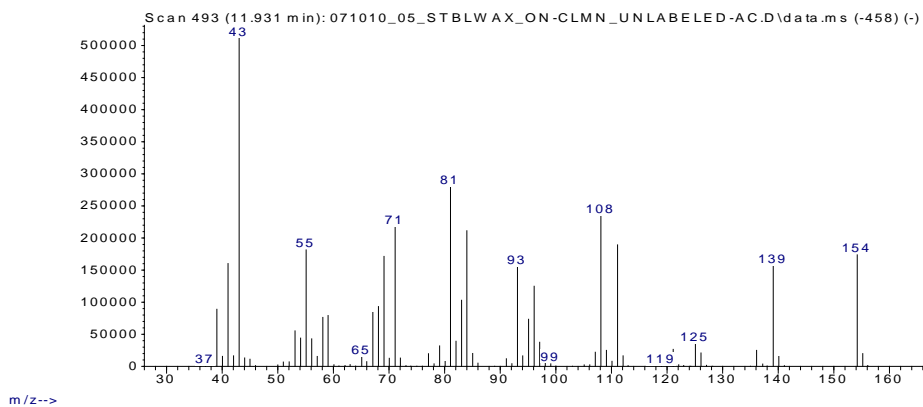
slope = 0.840
response factor = 1.19

Response Factor of d₃-1,8-cineole (July 2010)

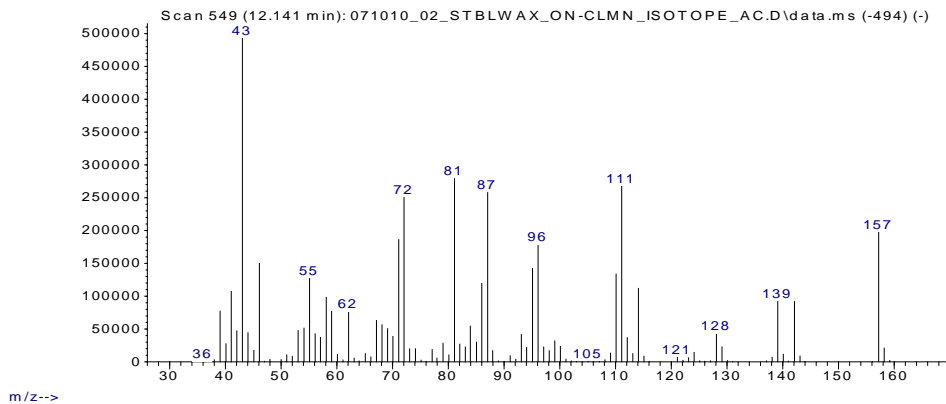
Standard:	Isotope	Unlabeled
CAS:	(² H ₃)-1,8-cineole	1,8-cineole
Mfg/Reference:	synthesized	470-82-6
No.; Catalog #; Batch#/Lot#:		Aldrich
% Purity (by GC-FID)	96.075%	597; C8,060-1; 06501DP
		98.331%

Spectra:

Abundance



Abundance



Methods

Matrix: dichloromethane

Extraction: none

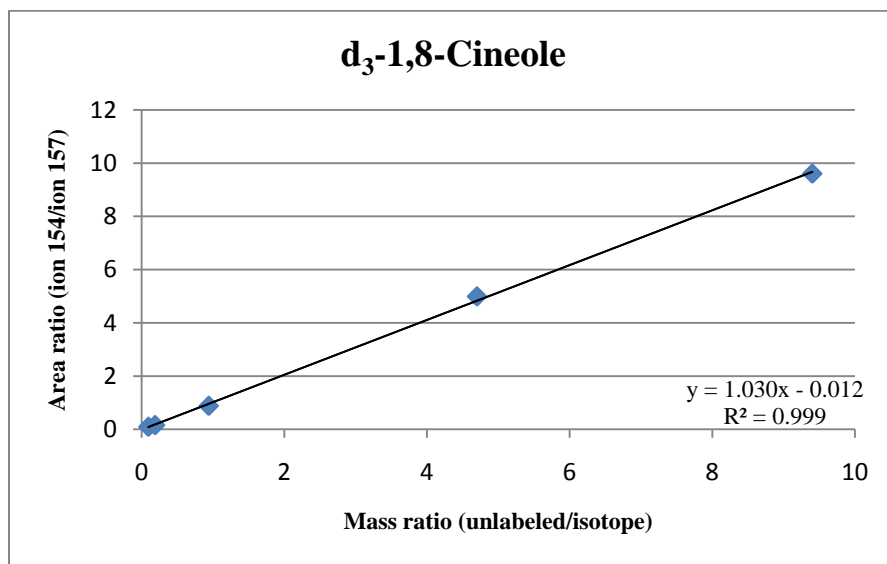
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 154 (unlabeled) 157 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0940	2101359	21953571	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.1881	1798331	11371678	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
0.9403	1838392	2083359	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
4.7015	10074889	2018348	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
9.4030	21594049	2248491	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D

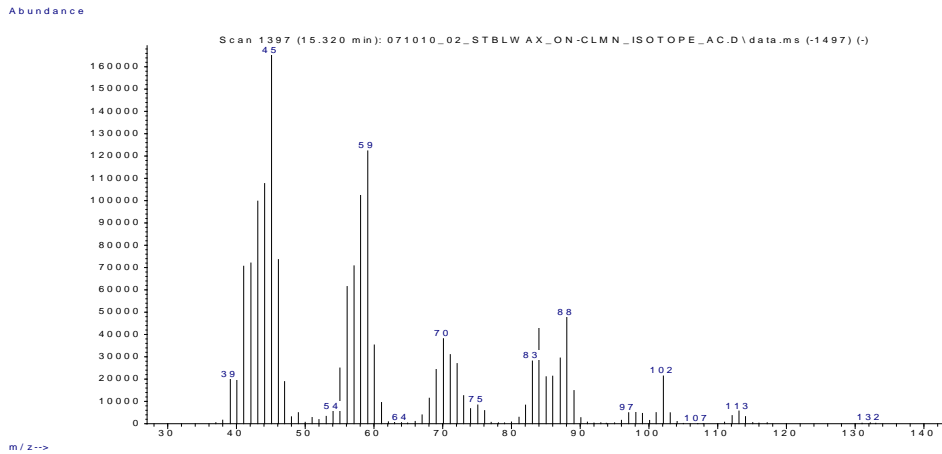
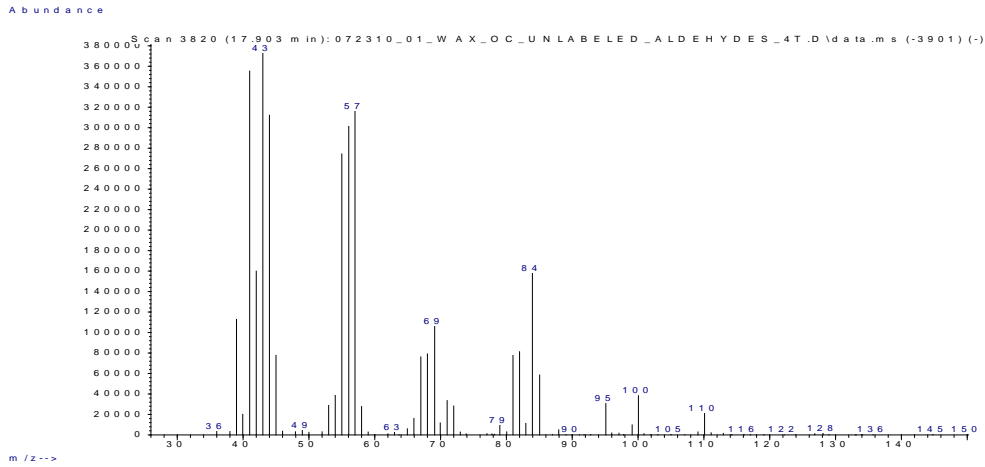


slope = 1.030
response factor = 0.971

Response Factor of d₄-Octanal (July 2010)

	Isotope	Unlabeled
Standard:	[² H ₄ -3,3,4,4]-octanal	octanal
CAS:		124-13-0
Mfg/Reference:	synthesized	Aldrich
No.; Catalog #; Batch#/Lot#:		265; O-560-8
% Purity (by GC-FID)	92.215%	95.823%

Spectra:



Methods

Matrix: dichloromethane

Extraction: none

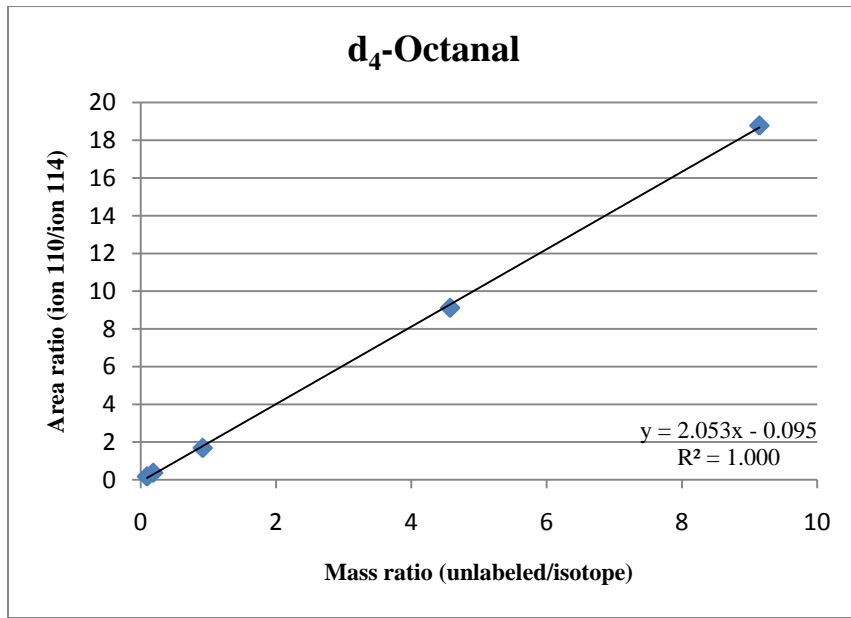
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 110 (unlabeled) 114 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0915	31470	178143	071210_07_STBLWAX_ON-CLMN_ALDEHYDES-4T_10-1.D
0.1829	69242	186247	071210_06_STBLWAX_ON-CLMN_ALDEHYDES-4T_5-1.D
0.9145	93140	55133	071210_05_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-1.D
4.5726	201392	22091	071010_07_STBLWAX_ON-CLMN_ALDEHYDES_4T_1-5.D
9.1453	444102	23652	071010_04_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-10.D

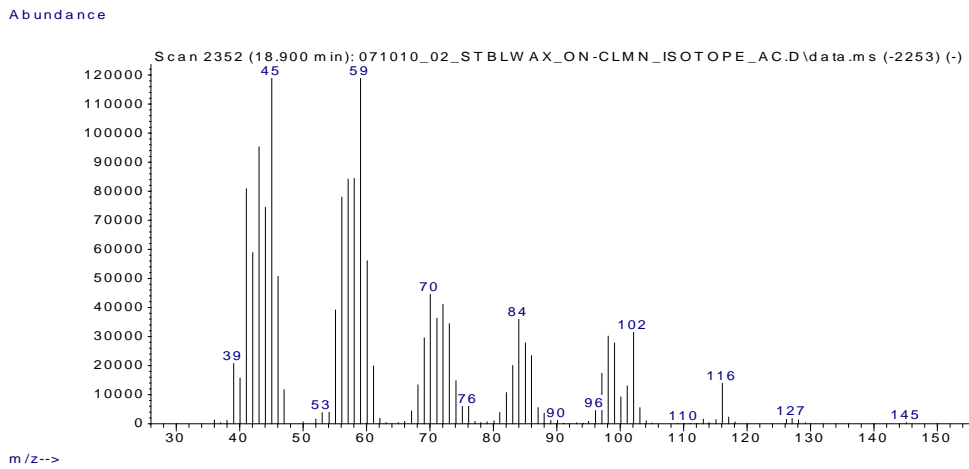
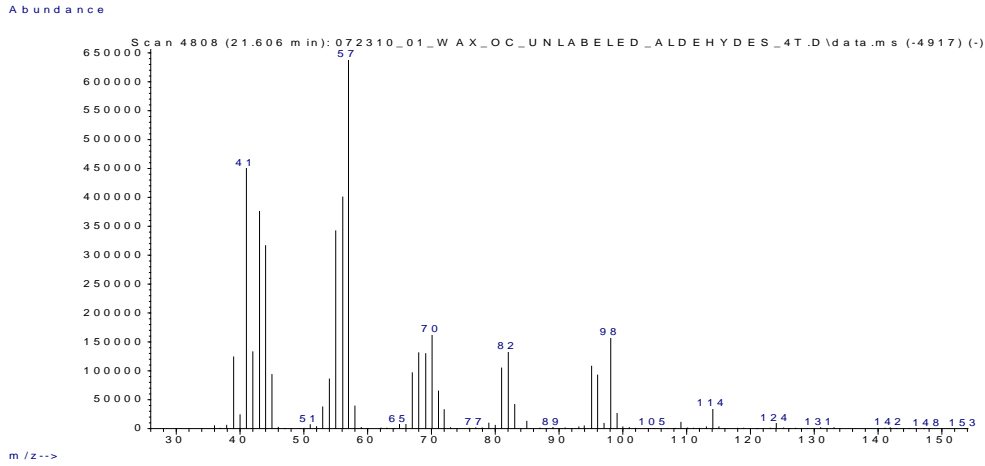


slope = 2.05
response factor = 0.487

Response Factor of d₄-Nonanal (July 2010)

	Isotope	Unlabeled
Standard:	[² H ₄ -3,3,4,4]-nonanal	nonanal
CAS:		124-19-6
Mfg/Reference:	synthesized	Aldrich
No.; Catalog #; Batch#/Lot#:		75; N3080-3
% Purity (by GC-FID)	93.550%	90.954%

Spectra:



Methods

Matrix: dichloromethane

Extraction: none

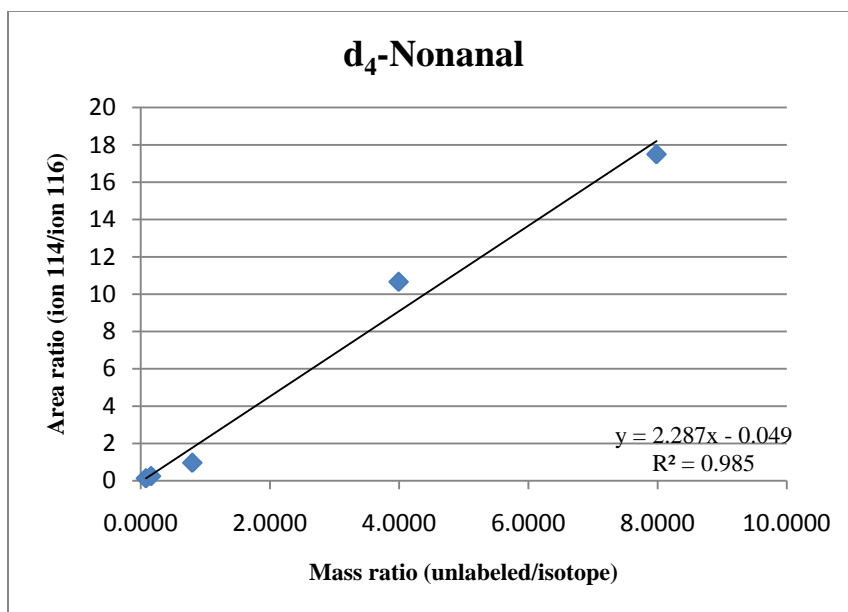
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 114 (unlabeled) 116 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0798	187908	1388491	071210_07_STBLWAX_ON-CLMN_ALDEHYDES-4T_10-1.D
0.1596	360852	1436449	071210_06_STBLWAX_ON-CLMN_ALDEHYDES-4T_5-1.D
0.7982	386979	400424	071210_05_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-1.D
3.9912	1043851	97920	071010_07_STBLWAX_ON-CLMN_ALDEHYDES_4T_1-5.D
7.9825	2227001	127279	071010_04_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-10.D



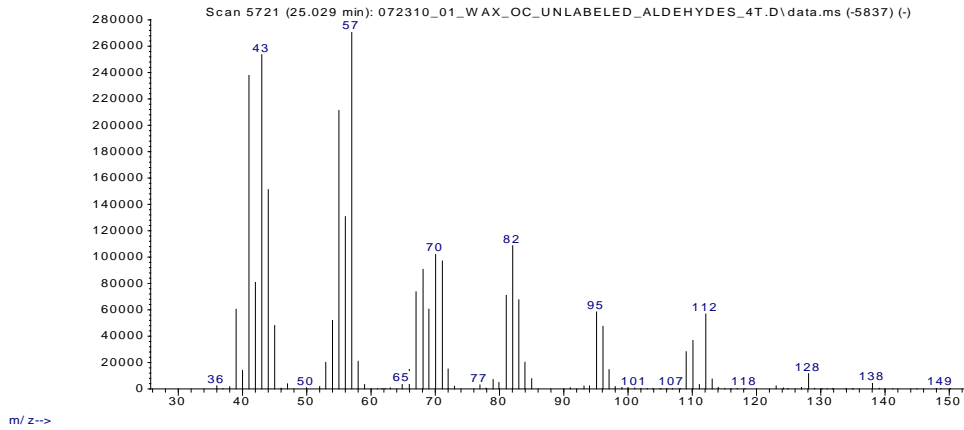
slope = 2.29
response factor = 0.437

Response Factor of d₄-Decanal (July 2010)

Standard:	Isotope	Unlabeled
CAS:	[² H ₄ -3,3,4,4]-decanal	decanal
Mfg/Reference:	synthesized	112-31-2
No.; Catalog #; Batch#/Lot#:		Aldrich
% Purity (by GC-FID)	95.179	60; 12577-6
		96.364

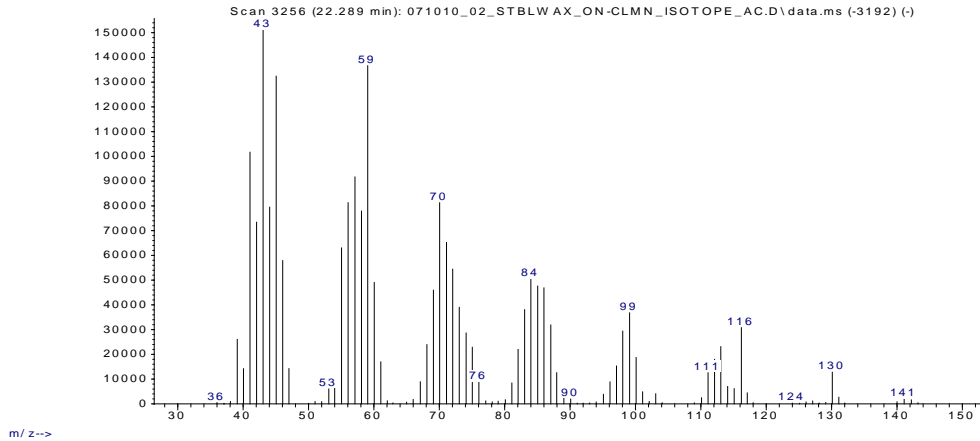
Spectra:

Abundance



Unlabeled

Abundance



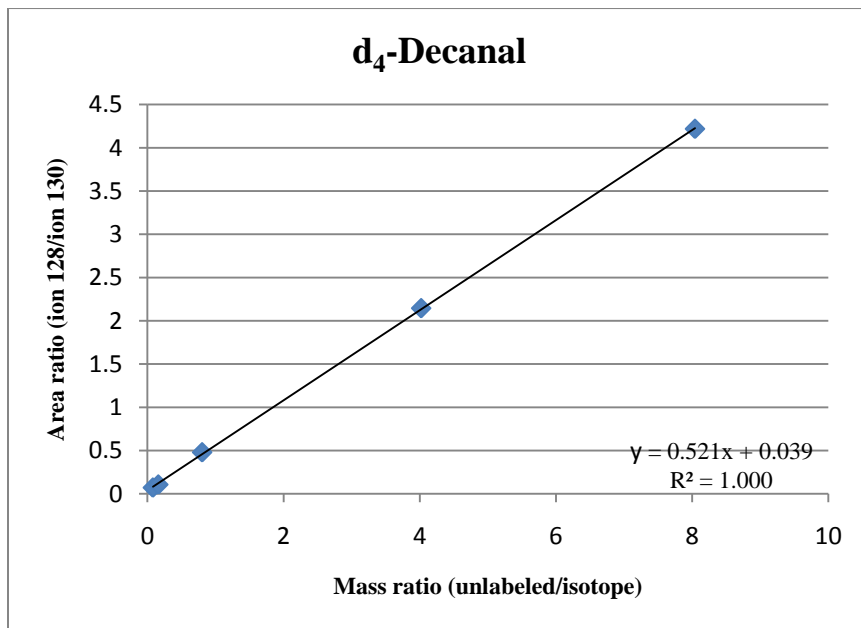
Labeled

Methods

Matrix: dichloromethane
Extraction: none
Injection: cool on-column
column: Stabilwax
(chromatograms: "Soda-response factor data" folder)

Select ion: 128 (unlabeled) 130 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0804	90730	1269145	071210_07_STBLWAX_ON-CLMN_ALDEHYDES-4T_10-1.D
0.1608	136146	1272680	071210_06_STBLWAX_ON-CLMN_ALDEHYDES-4T_5-1.D
0.8041	165732	345552	071210_05_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-1.D
4.0207	403103	187881	071010_07_STBLWAX_ON-CLMN_ALDEHYDES_4T_1-5.D
8.0413	876146	207661	071010_04_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-10.D



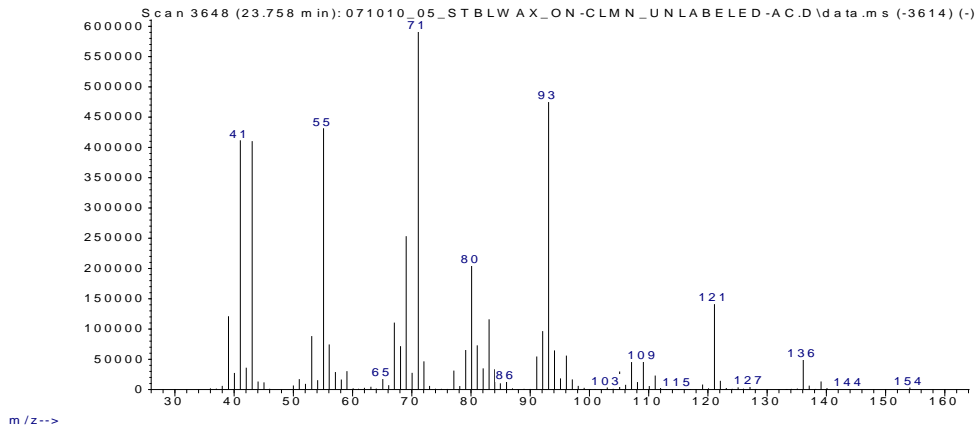
slope = 0.521
response factor = 1.92

Response Factor of d₂-Linalool (July 2010)

	Isotope	Unlabeled
Standard:	(² H ₂)-3,7-dimethyl-1,6-octadien-3-ol	(-)-3,7-dimethyl-1,6-octadien-3-
ol (linalool)		
CAS:		126-91-0
Mfg/Reference:		Fluka
No.; Catalog #; Batch#/Lot#:		360 (-); 62139; 1345240
51308329		
% Purity (by GC-FID)	92.95%	96.923%

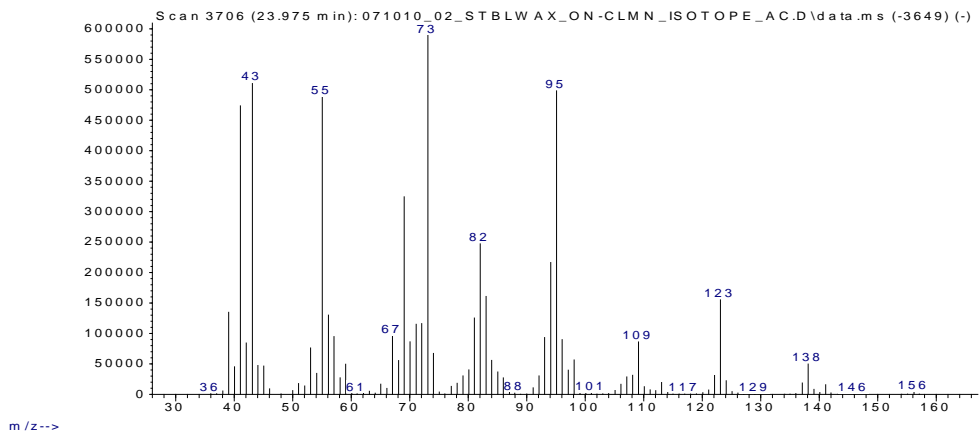
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

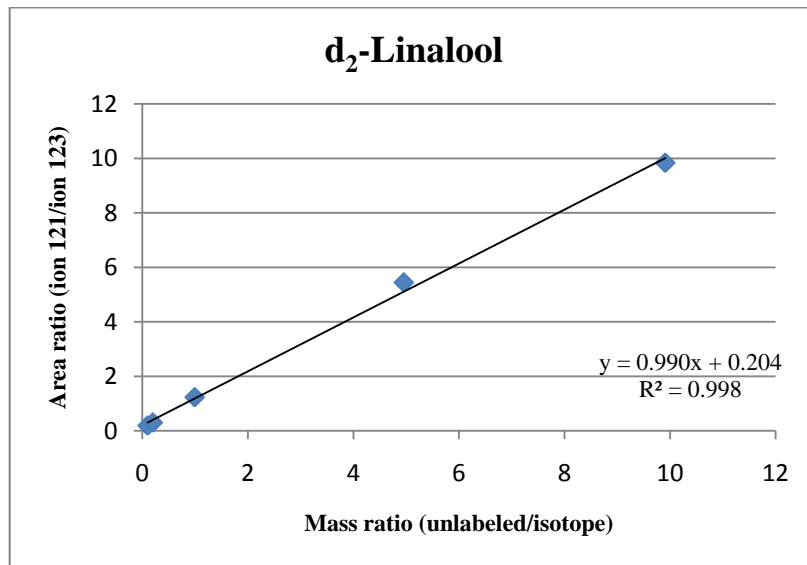
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 121 (unlabeled) 123 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0991	1625499	8575252	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.1982	1352649	4516109	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
0.9909	1101168	894700	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
4.9545	5447514	1000378	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
9.9091	11593225	1178142	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



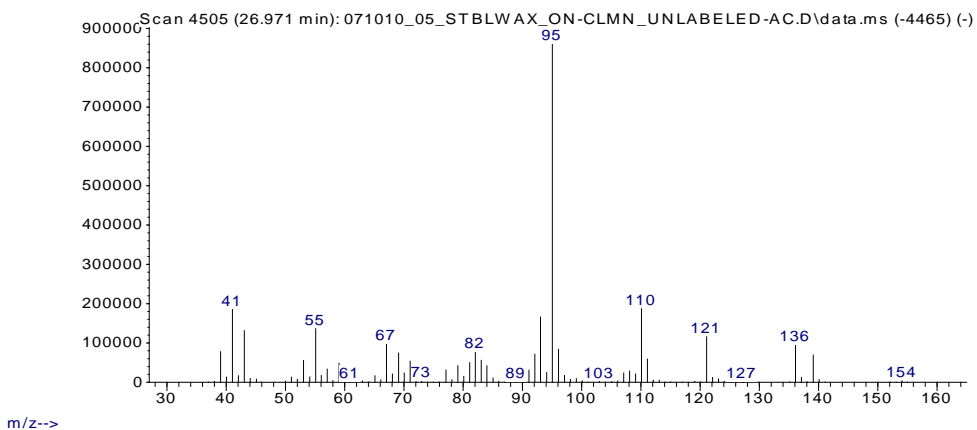
slope = 0.990
response factor = 1.01

Response Factor of d₃-Isoborneol (July 2010)

	Isotope	Unlabeled
Standard:	(² H ₃)-isoborneol	DL-isoborneol
CAS:		124-76-5
Mfg/Reference:		Aldrich
No.; Catalog #; Batch#/Lot#:		26; I-1,390-1; 06321EP
% Purity (by GC-FID)	48.323% (mix with d ₃ -borneol)	96.390%

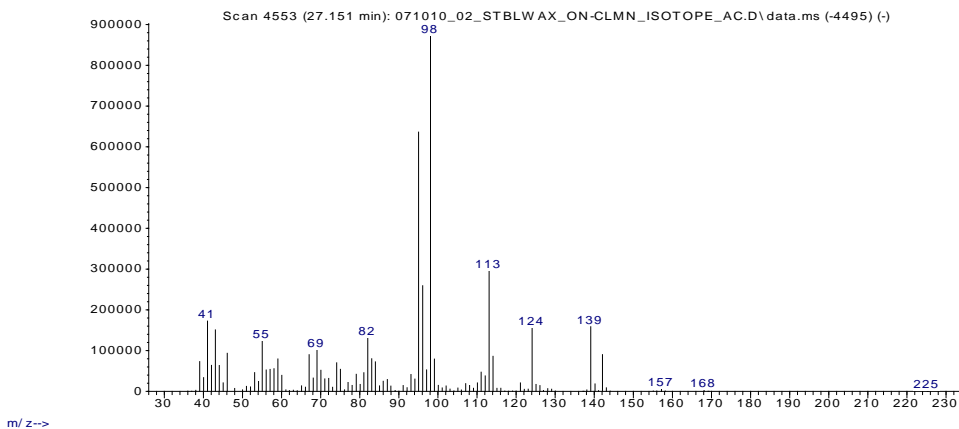
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

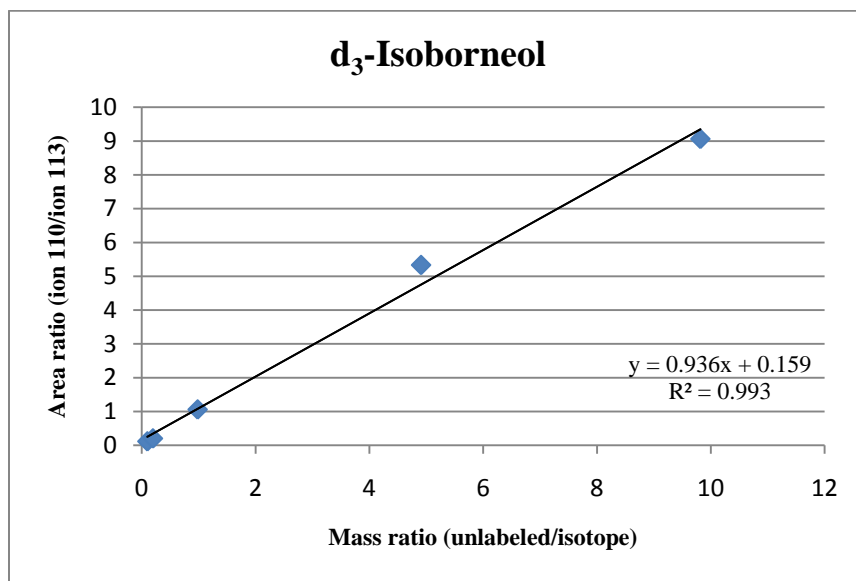
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 110 (unlabeled) 113 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0982	2045721	17819175	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.1963	1944578	9643533	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
0.9817	1987936	1878132	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
4.9083	9993096	1873777	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
9.8165	20417336	2252754	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



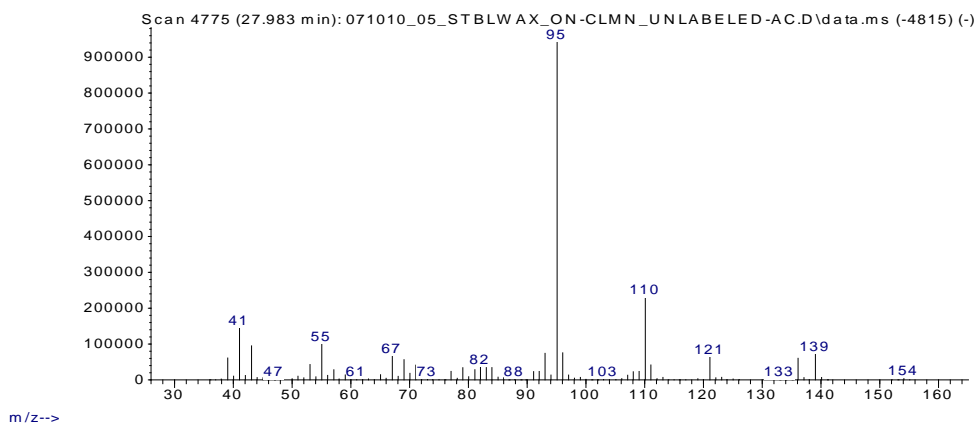
slope = 0.936
response factor = 1.07

Response Factor of d₃-Borneol (July 2010)

	Isotope	Unlabeled
Standard:	(² H ₃)- borneol	(1S)-(-)-endo-borneol
CAS:		464-45-9
Mfg/Reference:	synthesized	Aldrich
No.; Catalog #; Batch#/Lot#:		472; 25192-5
% Purity (by GC-FID)	46.986% (mix with d ₃ -isoborneol)	98.509%

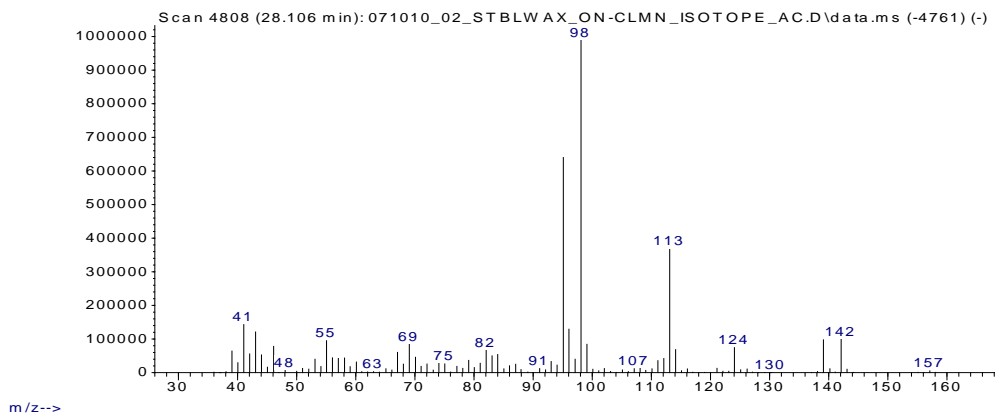
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

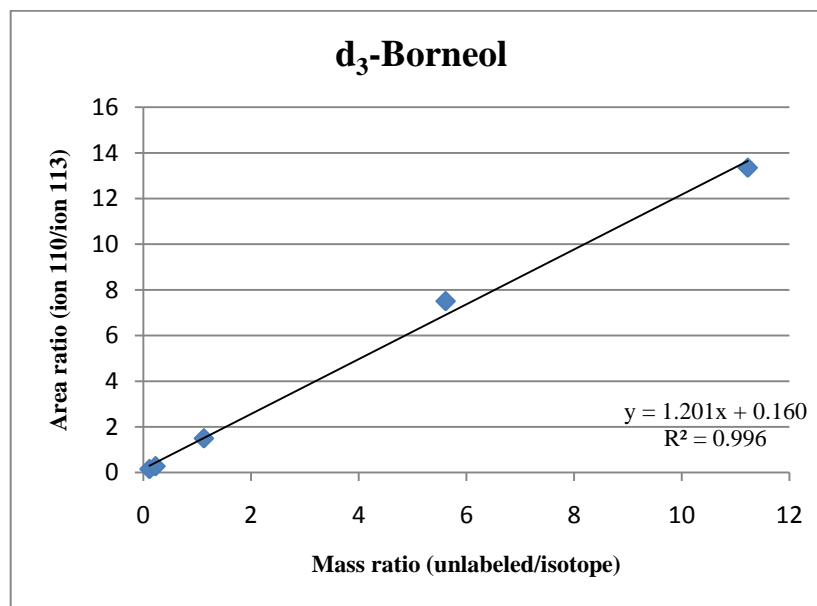
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 110 (unlabeled) 113 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.112264151	3413413	27.683	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.224528302	3331163	27.804	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
1.122641509	3449370	27.949	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
5.613207547	17525618	27.799	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
11.22641509	35777367	27.754	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



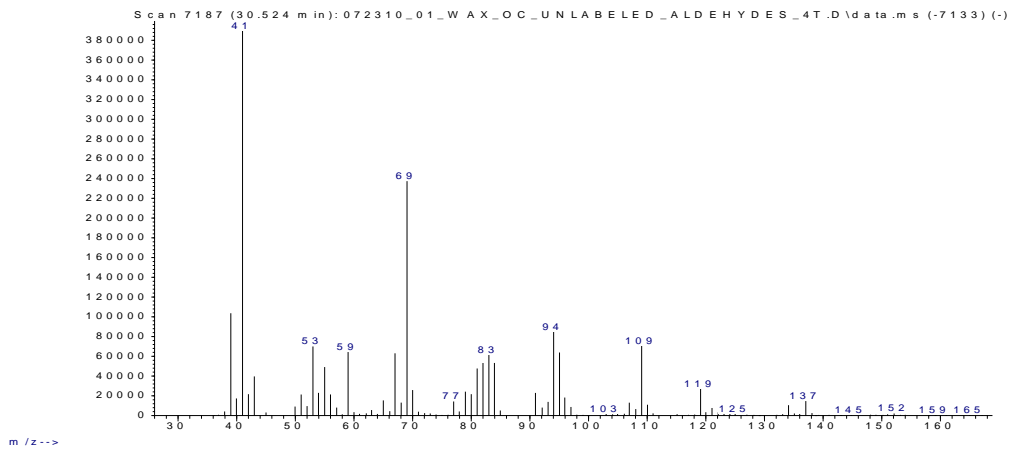
slope = 1.20
response factor = 0.833

Response Factor of d₆-Neral (July 2010)

	Isotope	Unlabeled
Standard: (neral)	(² H ₆)-(Z)-3,7-dimethyl-2,6-octadienal	(Z)-3,7-dimethyl-2,6-octadienal
CAS: (citral)		106-26-3 (neral); 5392-40-5
Mfg/Reference:	synthesized	Aldrich
No.; Catalog #; Batch#/Lot#:		605; C8,300-7; 00703BW
% Purity (by GC-FID)	21.83% (mix with d ₆ -geranial)	35.767% (mix with geranial)

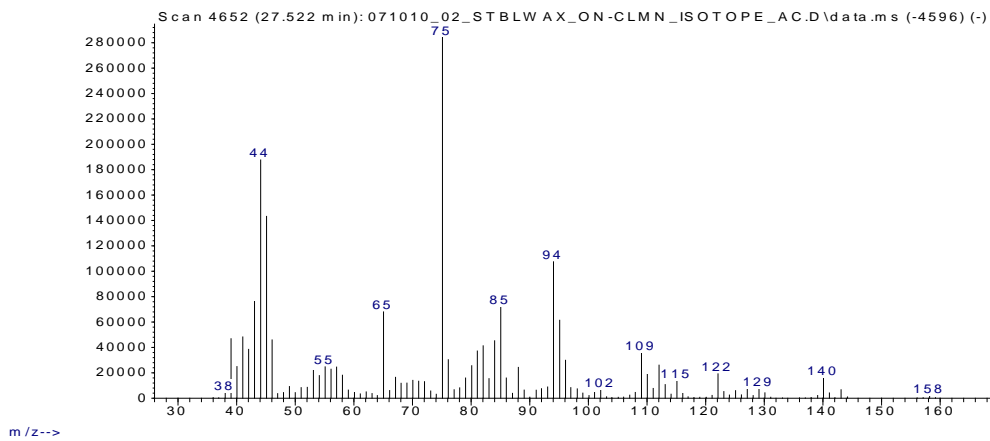
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

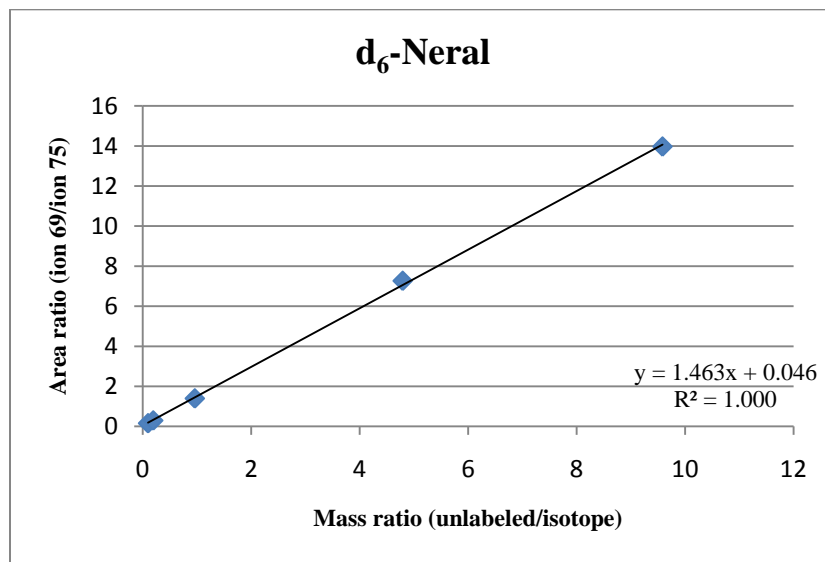
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 69 (unlabeled) 75 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0958	1412402	8976703	071210_07_STBLWAX_ON-CLMN_ALDEHYDES-4T_10-1.D
0.1917	2549347	8631331	071210_06_STBLWAX_ON-CLMN_ALDEHYDES-4T_5-1.D
0.9583	3284792	2351227	071210_05_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-1.D
4.7917	9800005	1348323	071010_07_STBLWAX_ON-CLMN_ALDEHYDES_4T_1-5.D
9.5833	21051555	1506730	071010_04_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-10.D



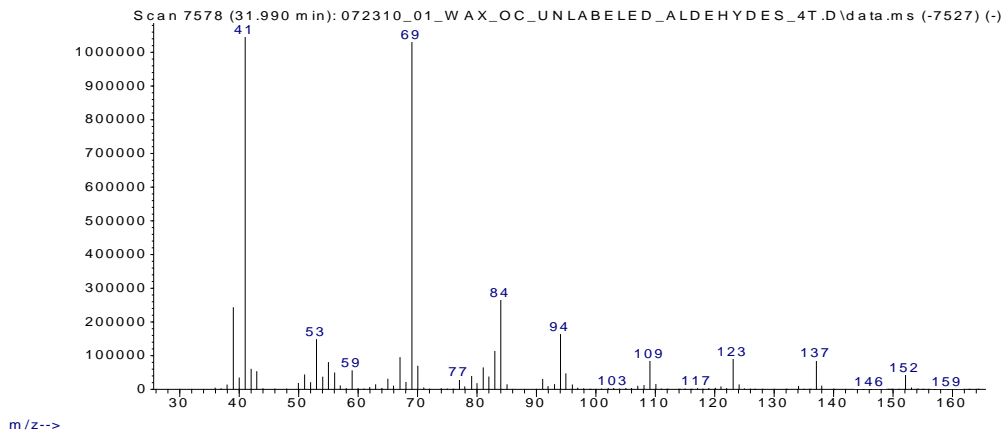
slope = 1.463
response factor = 0.684

Response Factor of d₆-Geranial (July 2010)

	Isotope	Unlabeled
Standard: (geranial)	(² H ₆)-(E)-3,7-dimethyl-2,6-octadienal	(E)-3,7-dimethyl-2,6-octadienal
CAS: (citral)		141-27-5 (geranial); 5392-40-5
Mfg/Reference:	synthesized	Aldrich
No.; Catalog #; Batch#/Lot#:		605; C8,300-7; 00703BW
% Purity (by GC-FID)	52.27% (mix with d ₆ -neral)	59.935% (mix with neral)

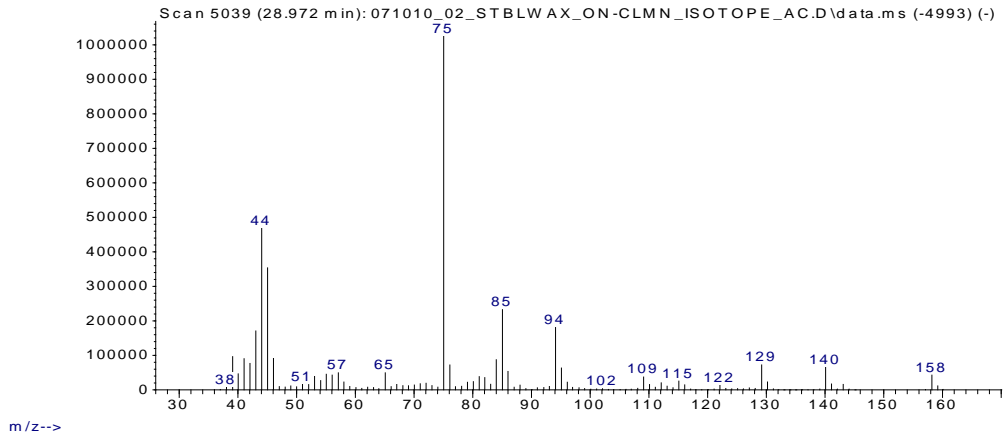
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

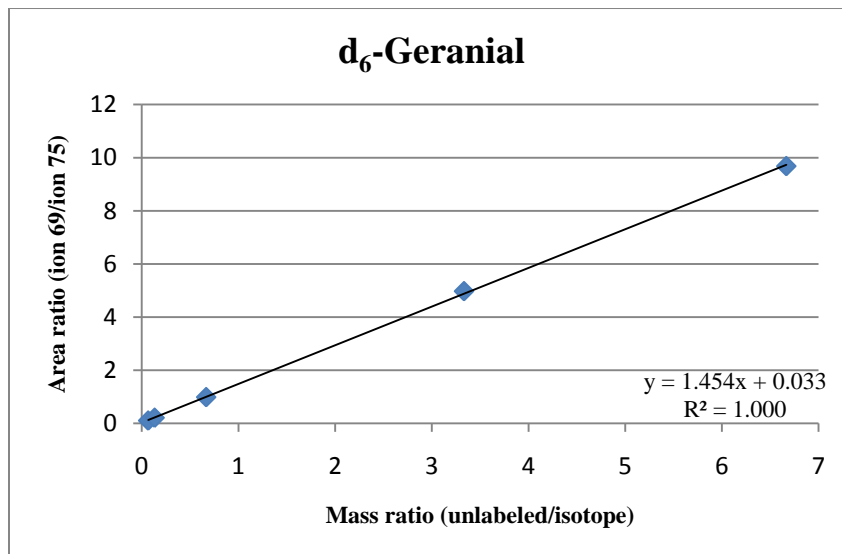
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 69 (unlabeled) 75 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.0667	4089829	38074022	071210_07_STBLWAX_ON-CLMN_ALDEHYDES-4T_10-1.D
0.1333	8155251	38132268	071210_06_STBLWAX_ON-CLMN_ALDEHYDES-4T_5-1.D
0.6667	10427762	10539905	071210_05_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-1.D
3.3333	30402183	6108175	071010_07_STBLWAX_ON-CLMN_ALDEHYDES_4T_1-5.D
6.6667	62527408	6456860	071010_04_STBLWAX_ON-CLMN_ALDEHYDES-4T_1-10.D



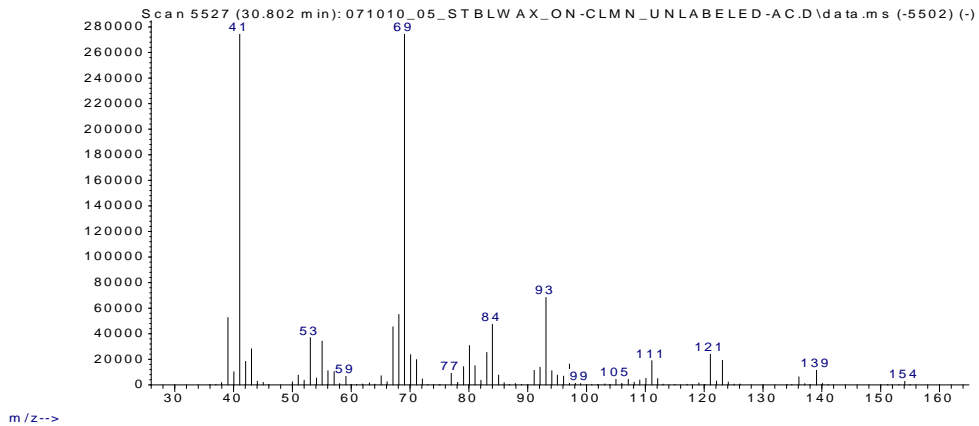
slope = 1.45
response factor = 0.688

Response Factor of d₆-nerol (July 2010)

	Isotope	Unlabeled
Standard: ol (nerol)	(² H ₆)-(Z)-3,7-dimethyl-2,6-octadien-1-ol	(Z)-3,7-dimethyl-2,6-octadien-1-
CAS:		106-25-2
Mfg/Reference:	synthesized	Bedoukian Research, Inc.
No.; Catalog #; Batch#/Lot#:		868; 700; 2008129-0017 (bri)
% Purity (by GC-FID)	32.773% (mix with d ₆ -geraniol)	94.703%

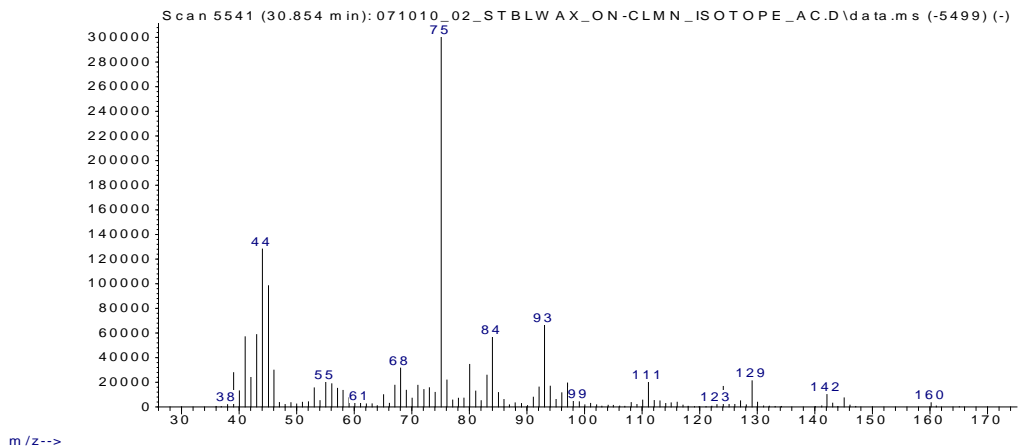
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

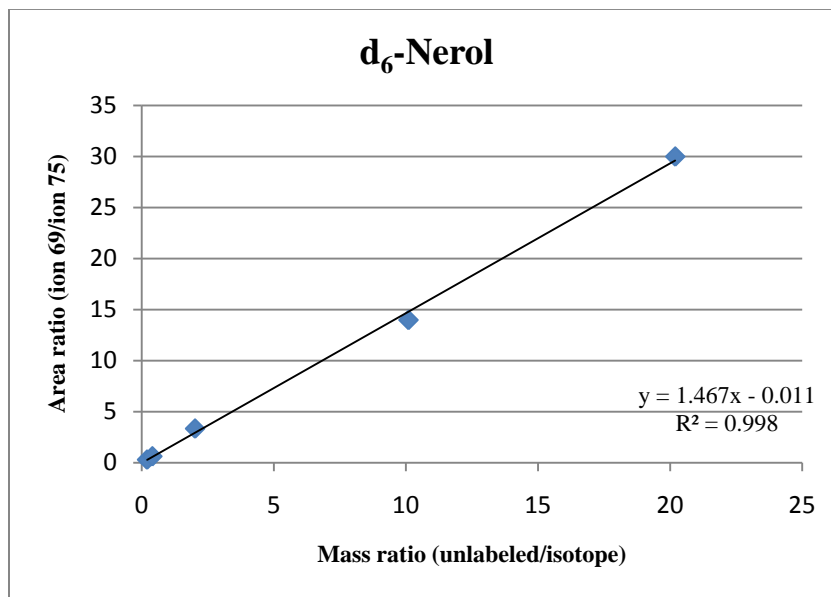
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 69 (unlabeled) 75 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.202	3900890	12982540	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.404	5646433	8988367	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
2.019	7014853	2097811	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
10.096	32932145	2355468	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
20.192	69911362	2330952	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



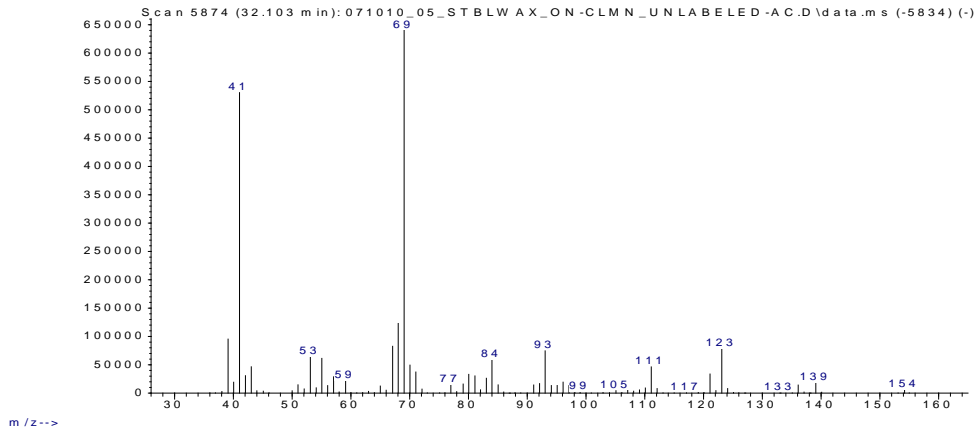
slope = 1.467
response factor = 0.682

Response Factor of d₆-geraniol (July 2010)

	Isotope	Unlabeled
Standard: ol (geraniol)	(² H ₆)-(E)-3,7-dimethyl-2,6-octadien-1-ol	(E)-3,7-dimethyl-2,6-octadien-1-
CAS:		106-24-1
Mfg/Reference:	synthesized	Bedoukian Research, Inc.
No.; Catalog #; Batch#/Lot#:		870; 710; 94262
% Purity (by GC-FID)	59.306% (mix with d ₆ -nerol)	96.665%

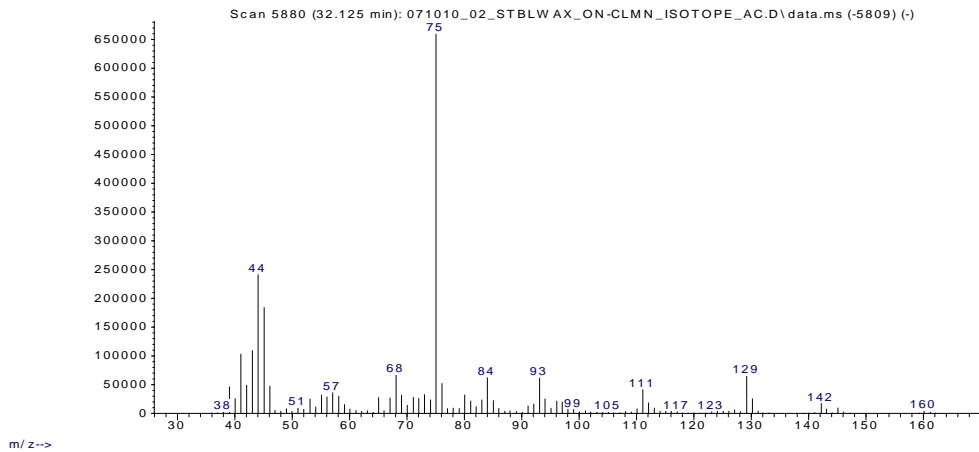
Spectra:

Abundance



Unlabeled

Abundance



Labeled

Methods

Matrix: dichloromethane

Extraction: none

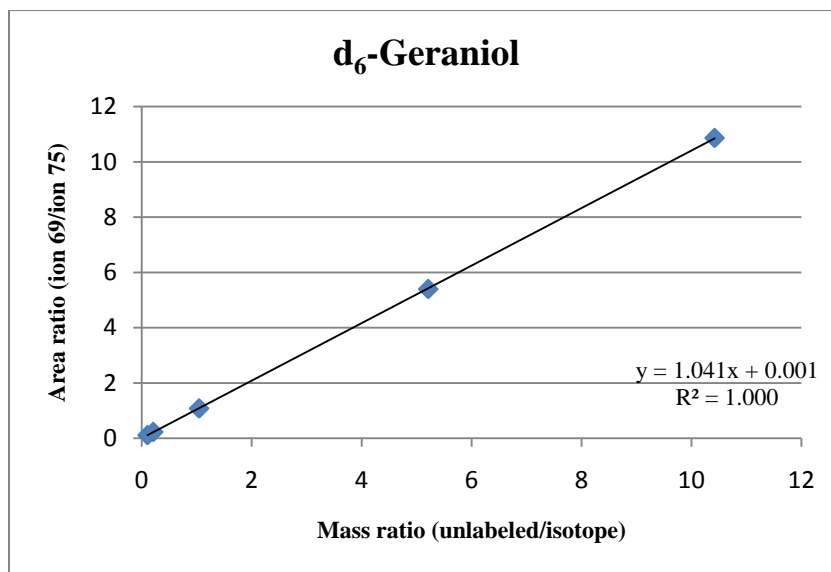
Injection: cool on-column

column: Stabilwax

(chromatograms: "Soda-response factor data" folder)

Select ion: 69 (unlabeled) 75 (isotope)

Mass ratio	Area unlabeled	Area isotope	Filename
0.104	4728131	40717872	070910_05_STBLWAX_ON-CLMN_A12_1-10.D
0.208	5960920	25883757	070910_04_STBLWAX_ON-CLMN_MIX_AC_1-5.D
1.042	6973439	6450666	070910_03_STBLWAX_ON-CLMN_MIX_AC_1-1.D
5.211	31767961	5882475	070910_02_STBLWAX_ON-CLMN_MIX_AC_5-1.D
10.421	72789943	6699265	070910_01_STBLWAX_ON-CLMN_MIX_AC_10-1.D



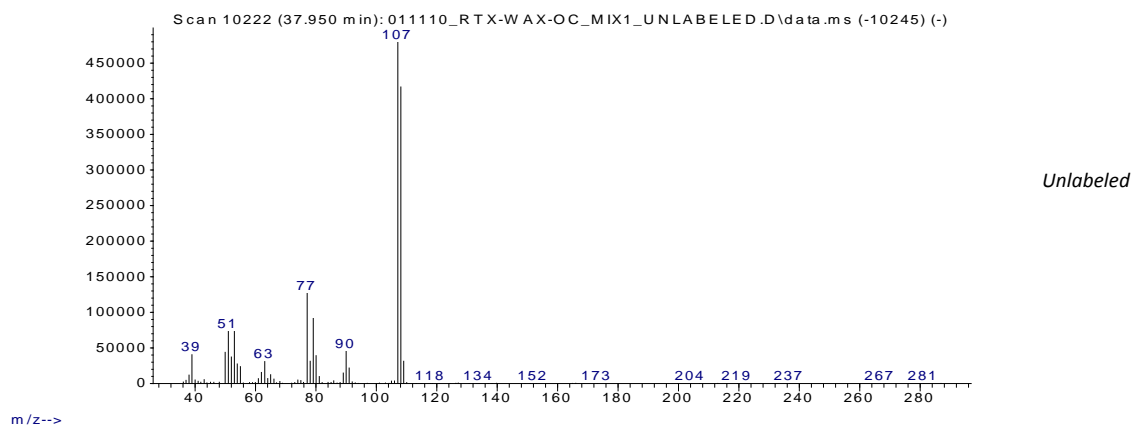
slope = 1.041
response factor = 0.961

Response Factor of d₃-*p*-Cresol (Jan 2010), compiled by Jacob Lahne

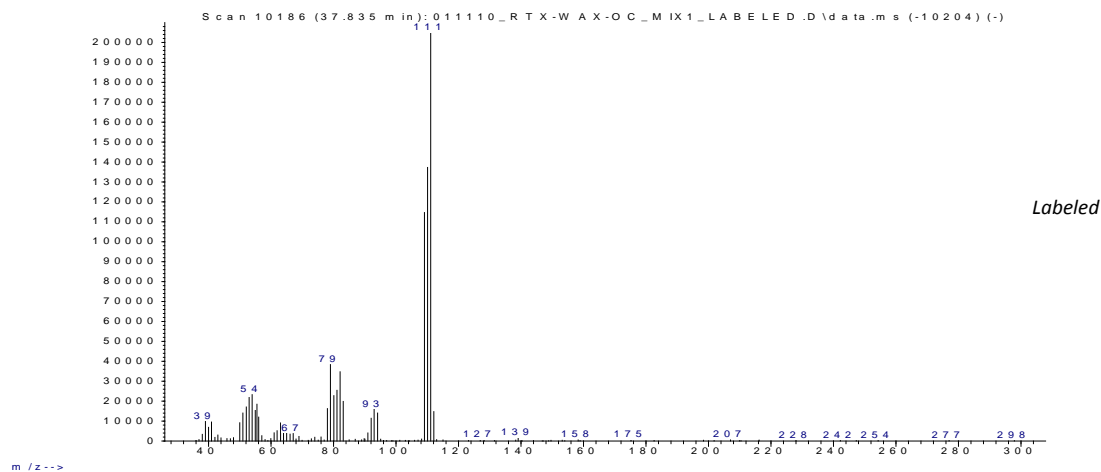
	Isotope	Unlabeled
Standard:	4-(² H ₃ -methyl)-phenol	4-methylphenol (<i>p</i> -cresol)
CAS:	108561-00-8	106-44-5
Mfg/Reference:	CDN (Quebec, Canada)	Aldrich (Milwaukee, WI)
No.; Catalog #; Batch#/Lot#:	ISO-5; D-5638; R653P1	425; C8,575-1; 09410PI
% Purity (by GC-FID)	99.3%	99.9%

Spectra:

Abundance



Abundance



Methods

Matrix: dichloromethane

Extraction: none

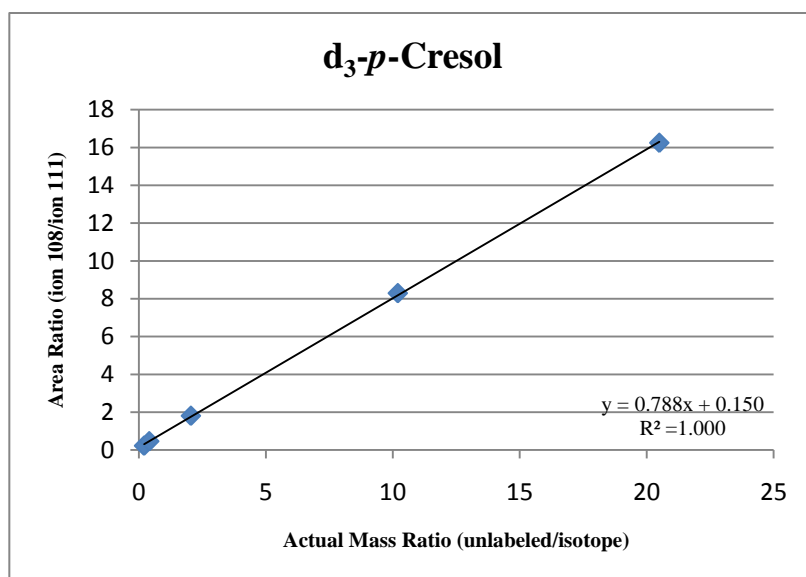
Injection: cool on-column

column: RTX-Wax

(chromatograms: "Calibration" folder)

Select ion: 108 (unlabeled) 111 (isotope)

Mass ratio	Area unlabeled	Area isotope
0.205	21481512	94929880
0.41	23059432	49813489
2.05	22186772	12258698
10.2	106319570	12816042
20.5	177588483	10928800

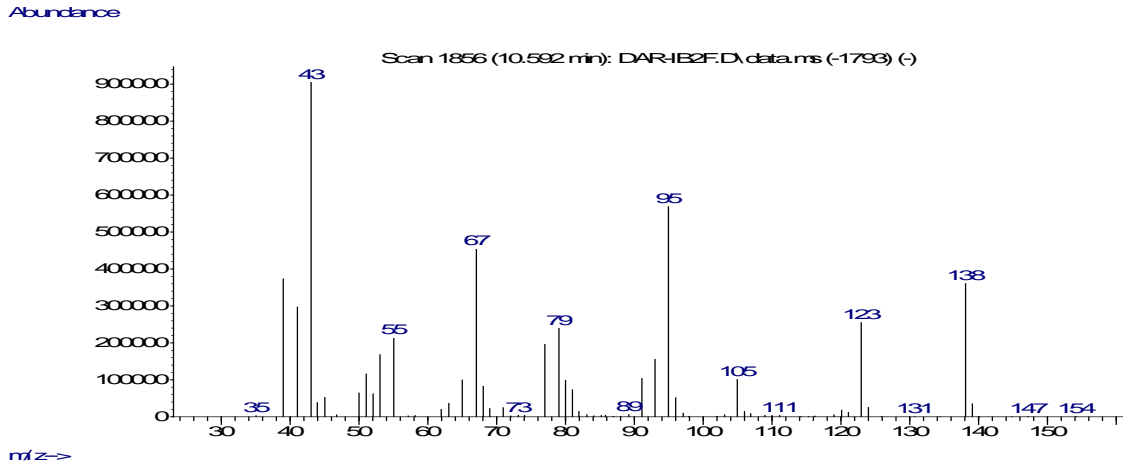


slope = 0.788
response factor = 1.27

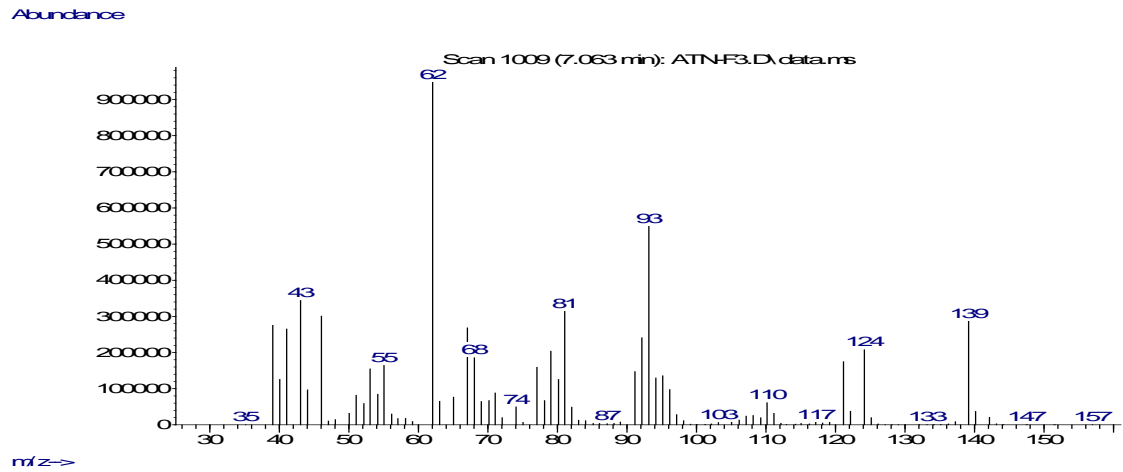
APPENDIX B

MASS SPECTRA FOR SELECTED SYNTHESIS INTERMEDIATE COMPOUNDS

Mass Spectrum (EI) of 1-acetyl-4-methylcyclohexene

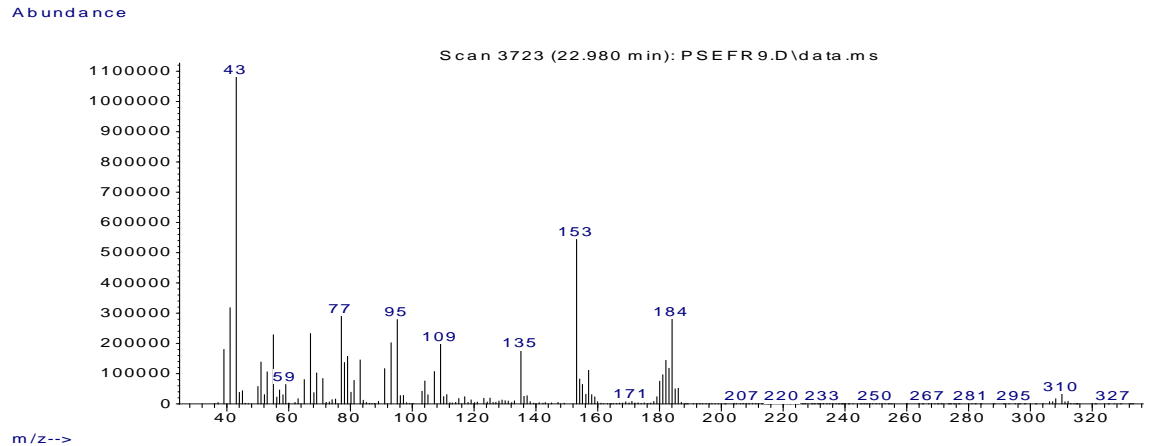


Mass Spectrum (EI) of [9,9,9-²H₃]- α -terpineol



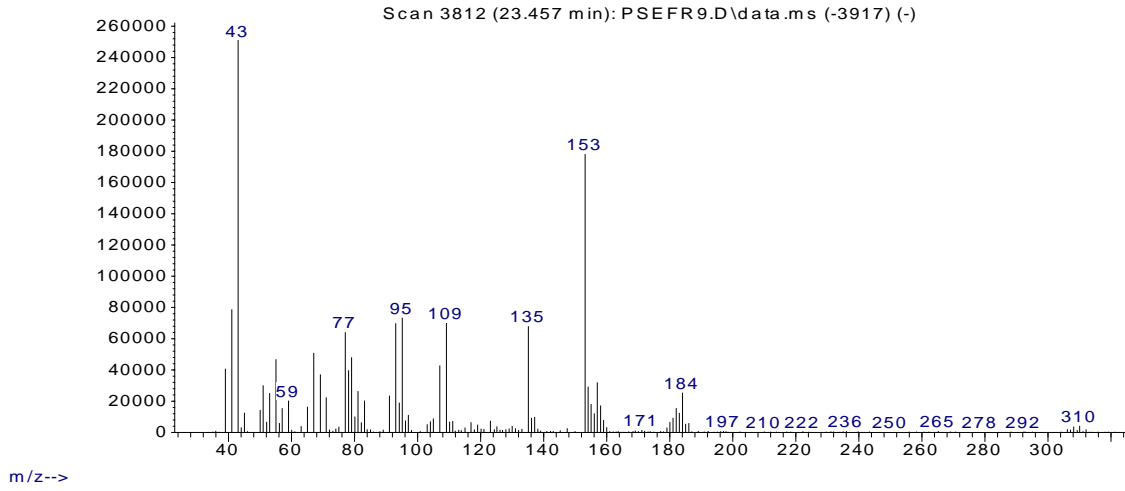
Mass Spectrum (EI) of phenylselenenyl ether

Isomer 1

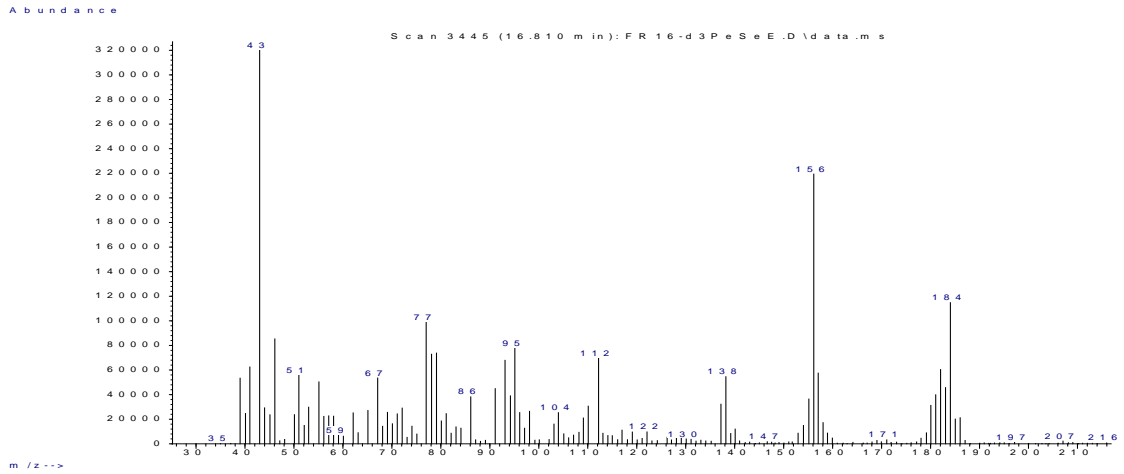


Isomer 2

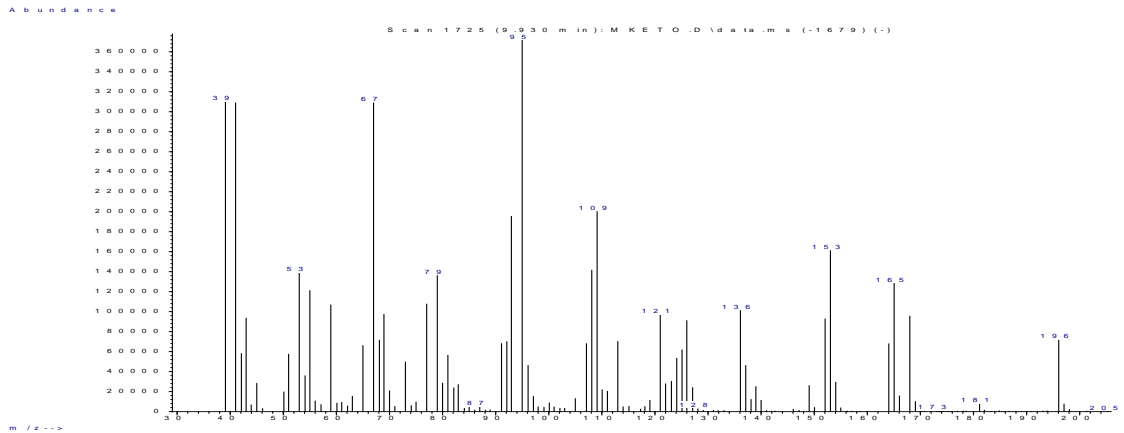
Abundance



Mass Spectrum (EI) of $^2\text{H}_3$ -phenylselenyl ether



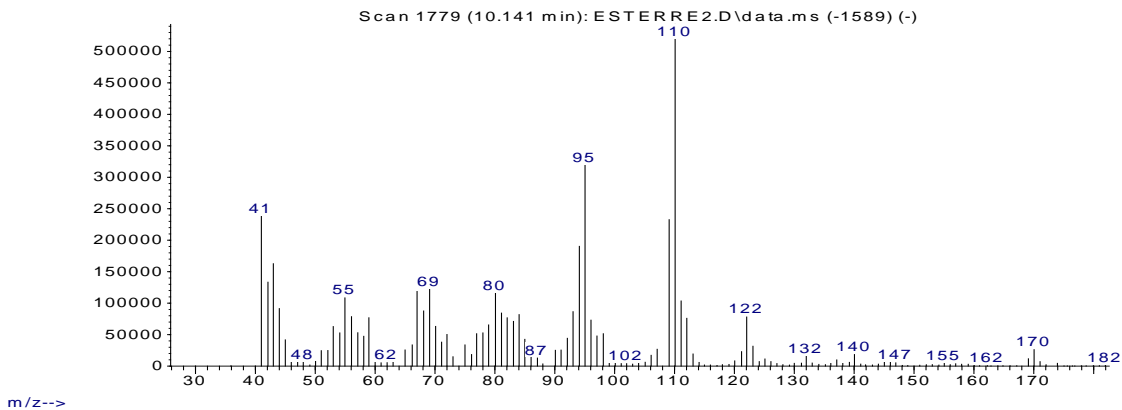
Mass Spectrum (EI) of methyl ketopinate



Mass Spectrum (EI) of $^2\text{H}_{3,5}$ -1-(hydroxymethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-ol

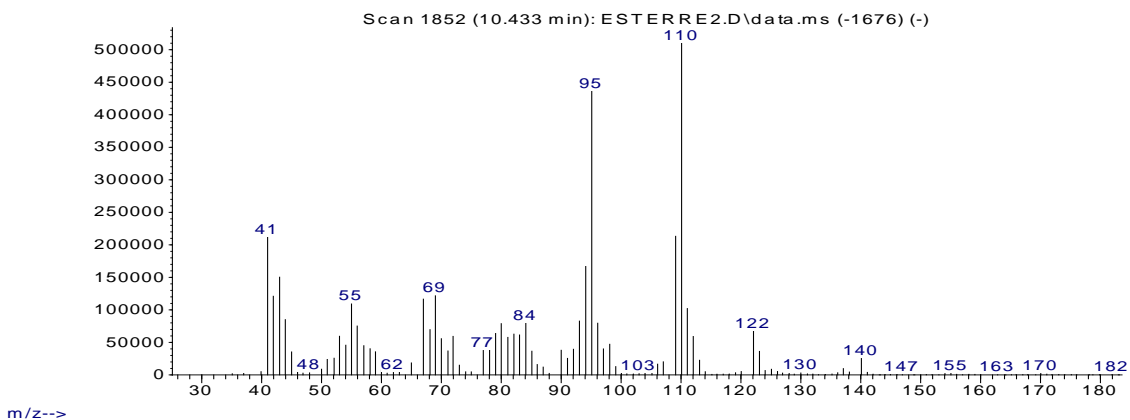
Isomer 1

Abundance



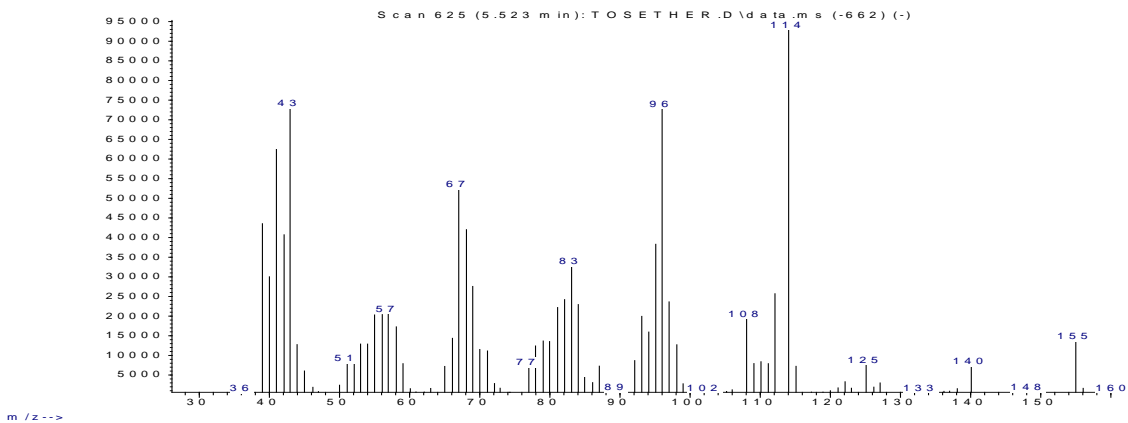
Isomer 2

Abundance



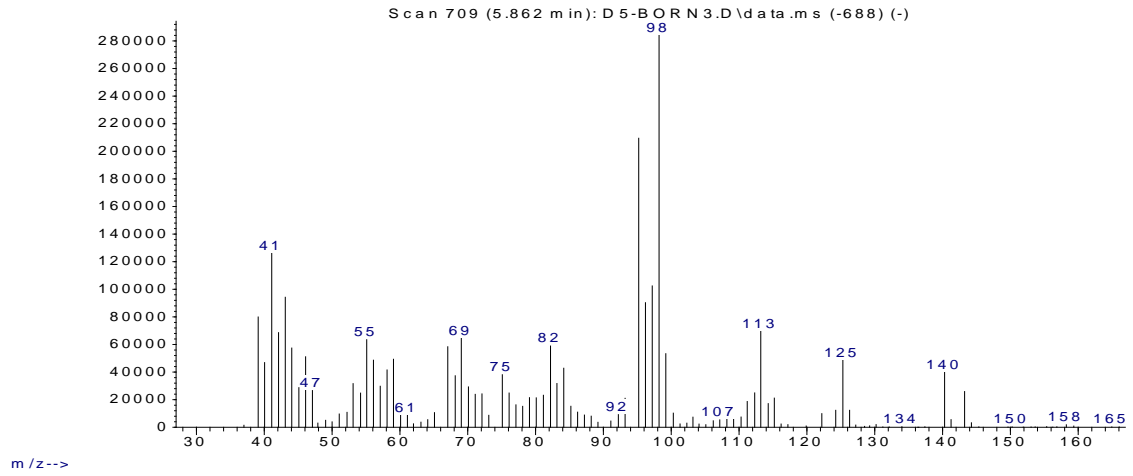
Mass Spectrum (EI) of $^2\text{H}_{3,4}$ -(2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methyl 4-methylbenzenesulfonate

Abundance



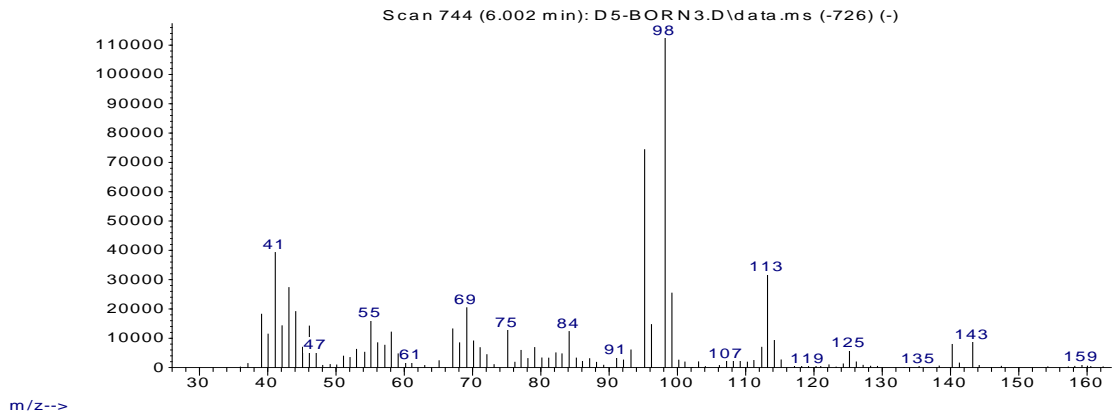
Mass Spectrum (EI) of $^2\text{H}_4$ -isoborneol

Abundance



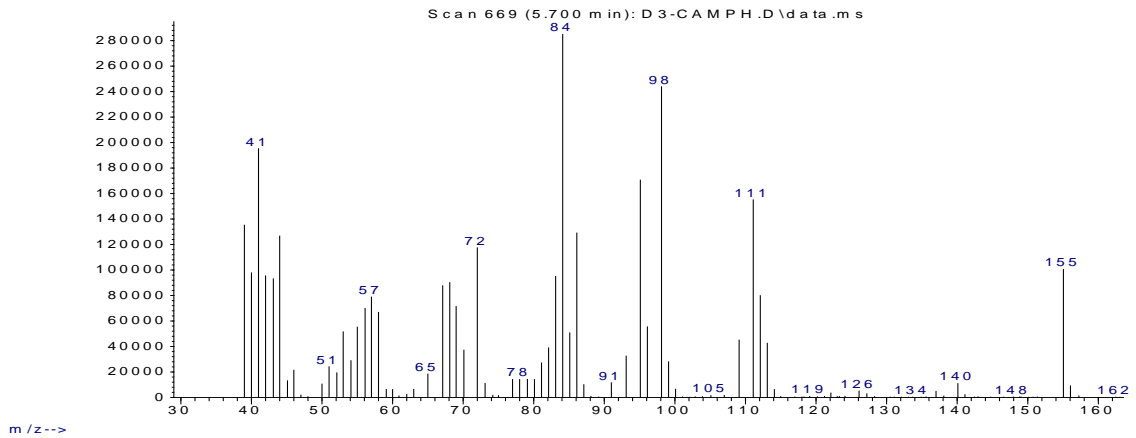
Mass Spectrum (EI) of $^2\text{H}_4$ -borneol

Abundance

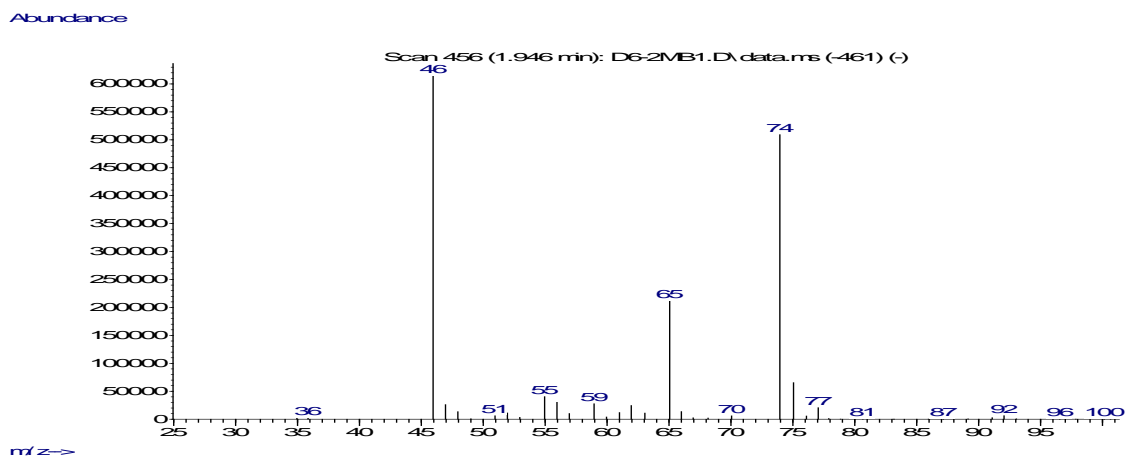


Mass Spectrum (EI) of $^2\text{H}_3$ -camphor

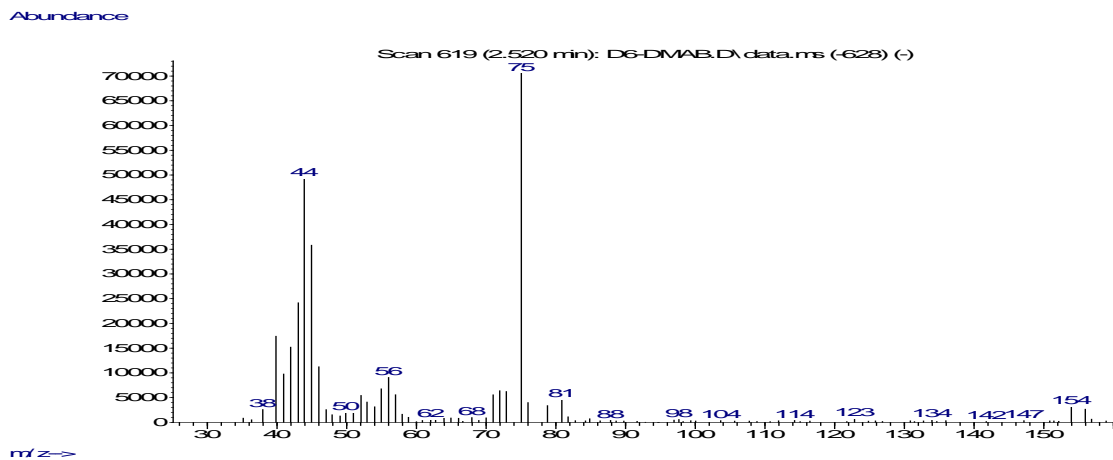
Abundance



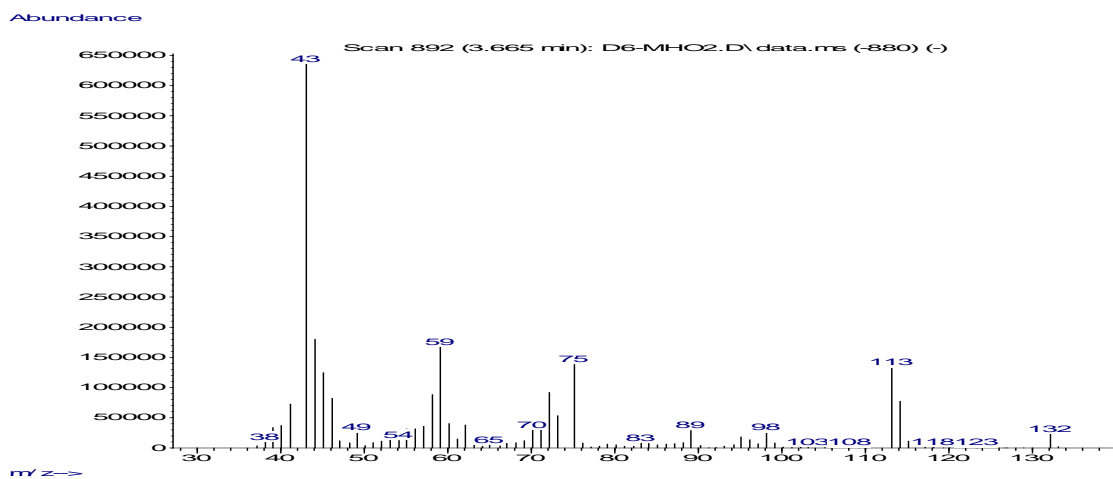
Mass Spectrum (EI) of [1,1,1, 5,5,5-²H₆]-2-methyl-3-buten-2-ol



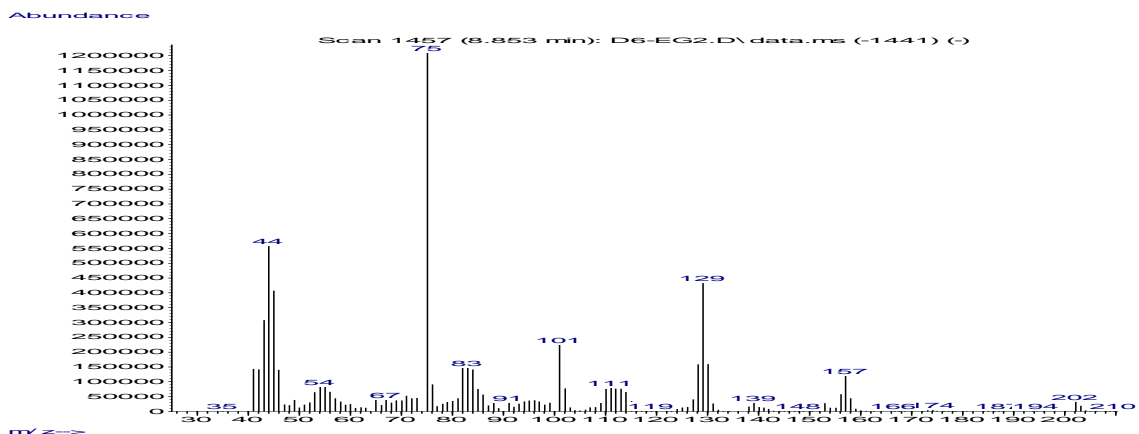
Mass Spectrum (EI) of [4,4,4,5,5,5-²H₆]-3,3-dimethylallylbromide



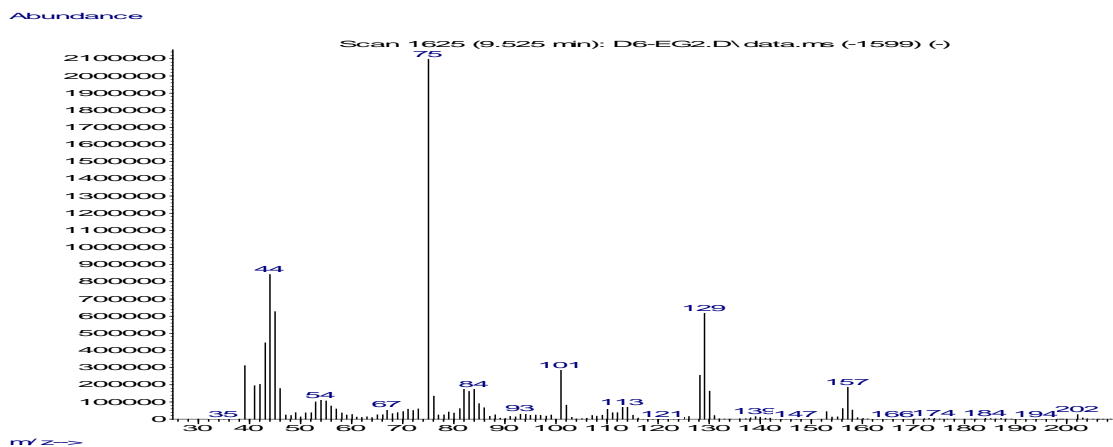
Mass Spectrum (EI) of [7,7,7,8,8,8-²H₆]-6-methyl-5-hepten-2-one



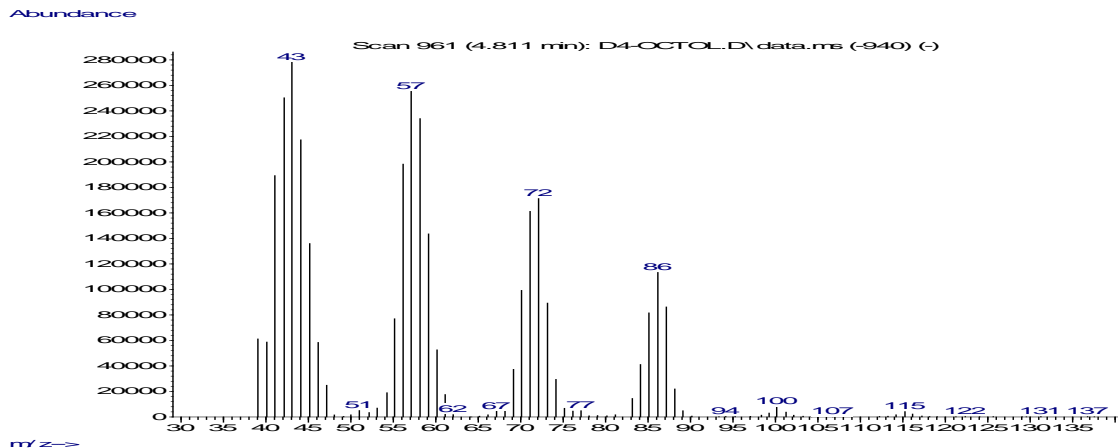
Mass Spectrum (EI) of [8,8,8,10,10,10-²H₆]-(*Z*)-3,7-dimethyl-2,6-octadienoic acid, ethyl ester



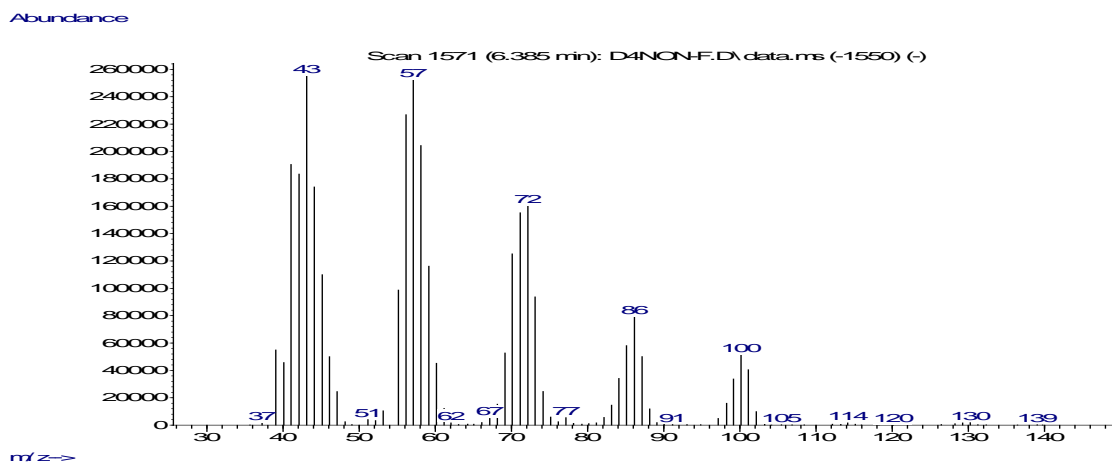
Mass Spectrum (EI) of [8,8,8,10,10,10-²H₆]-(*E*)-3,7-dimethyl-2,6-octadienoic acid, ethyl ester



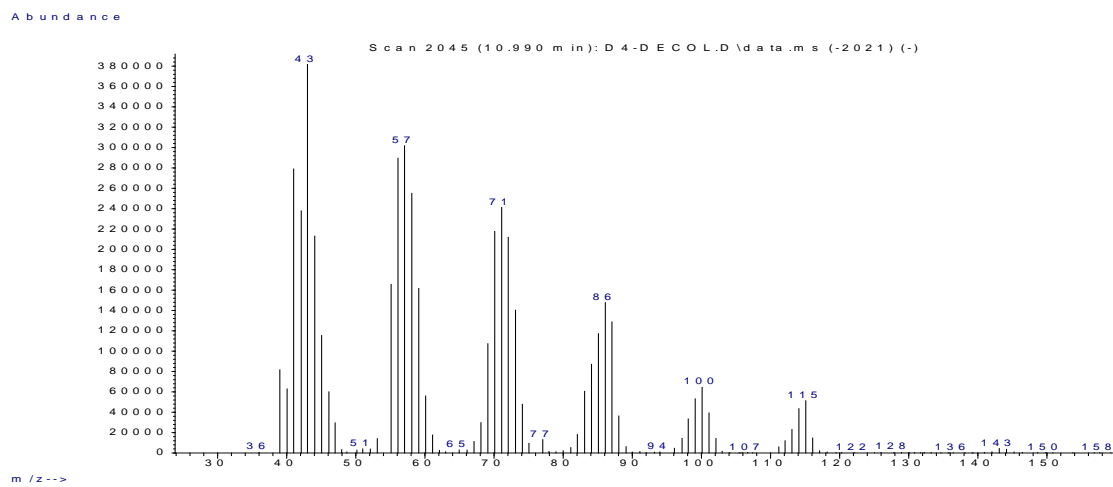
Mass Spectrum (EI) of [3,3,4,4-²H₄]-octan-1-ol



Mass Spectrum (EI) of [3,3,4,4-²H₄]-nonan-1-ol



Mass Spectrum (EI) of [3,3,4,4-²H₄]-decan-1-ol



APPENDIX C

QUANTIFICATION CONCENTRATION DATA & CALCULATION OF Q VALUES

Note 1: SP, Sprite; SM, Sierra Mist

Note 2: All concentrations are given in ng/g

Note 3: For n=4 observations, q critical is: (see references 20-21 in Chapter 4 for the below Q critical values and more information about the q test; Q_{95} was used)

$$Q_{90}=0.765$$

$$Q_{95}=0.829$$

$$Q_{99}=0.926$$

Raw concentration (ng/g) data for Sprite, used in the q test

Compound	SP, R1	SP, R2	SP, R3	SP, R4	w	highest value - closest neighbor	Q	closest value - lowest value	Q
2,3-dehydro-1,8-cineole	1571.83	1582.03	1664.40	1379.70	284.70	82.37	0.29	192.13	0.67
1,8-cineole	29.78	29.74	28.56	30.74	2.19	0.96	0.44	1.18	0.54
octanal	161.56	163.44	167.23	179.41	17.84	12.17	0.68	1.87	0.11
nonanal	32.28	33.54	34.87	33.17	2.59	1.34	0.52	0.89	0.34
decanal	64.15	60.13	55.96	64.78	8.81	0.62	0.07	4.16	0.47
linalool	232.77	222.77	221.08	204.82	27.95	10.00	0.36	16.26	0.58
isoborneol	33.82	32.06	33.95	30.35	3.61	0.13	0.04	1.71	0.47
neral	4.25	3.70	3.80	2.59	1.66	0.45	0.27	1.11	0.67
borneol	192.80	201.79	200.00	211.13	18.33	9.34	0.51	7.21	0.39
geranial	6.69	5.78	6.51	3.58	3.11	0.17	0.06	2.20	0.71
nerol	32.60	33.25	35.58	30.30	5.28	2.33	0.44	2.30	0.44
geraniol	45.79	50.68	51.83	41.52	10.31	1.15	0.11	4.27	0.41
<i>p</i> -cresol	12.68	10.31	9.00	11.64	3.68	1.04	0.28	1.31	0.36

Raw concentration (ng/g) data for Sierra Mist, used in the q test

Compound	SM, R1	SM, R2	SM, R3	SM, R4	w	highest value - closest neighbor	Q	closest value - lowest value	Q
2,3-dehydro-1,8-cineole	2853.84	2530.70	2503.01	2642.19	350.83	211.65	0.60	27.69	0.08
1,8-cineole	17.17	17.87	17.41	17.19	0.71	0.46	0.66	0.02	0.03
octanal	414.65	459.21	452.52	468.90	54.25	9.69	0.18	37.87	0.70
nonanal	54.37	53.71	54.57	54.58	0.87	0.01	0.01	0.66	0.76
decanal	189.71	198.64	187.55	187.86	11.09	8.93	0.81	0.31	0.03
linalool	312.86	290.66	286.74	292.04	26.12	20.82	0.80	3.93	0.15
isoborneol	14.60	16.17	17.08	16.25	2.47	0.83	0.34	1.57	0.63
neral	2.96	3.48	4.47	4.43	1.51	0.04	0.03	0.52	0.34
borneol	54.32	54.44	52.72	53.82	1.73	0.13	0.07	1.10	0.64
geranial	4.93	6.57	7.01	7.82	2.88	0.80	0.28	1.64	0.57
nerol	39.35	35.68	32.87	32.10	7.25	3.67	0.51	0.77	0.11
geraniol	74.60	67.63	62.52	65.39	12.08	6.98	0.58	2.87	0.24
<i>p</i> -cresol	18.20	9.27	14.30	14.23	8.93	3.90	0.44	4.96	0.56

Raw concentration (ng/g) data for 7UP, used in the q test

Compound	7UP, R1	7UP, R2	7UP, R3	7UP, R4	w	highest value - closest neighbor	Q	closest value - lowest value	Q
2,3-dehydro-1,8-cineole	1514.17	2700.31	2868.81	2674.87	1354.645	168.50	0.12	1160.70	0.86
1,8-cineole	13.47	15.54	15.56	15.38	2.09	0.02	0.01	1.91	0.91
octanal	342.47	339.32	360.93	345.88	21.61	15.05	0.70	3.15	0.15
nonanal	36.01	45.16	43.51	43.63	9.15	1.53	0.17	7.50	0.82
decanal	124.67	142.77	132.20	131.12	18.11	10.57	0.58	6.45	0.36
linalool	397.60	360.91	370.46	382.15	36.69	15.45	0.42	9.56	0.26
isoborneol	11.00	13.15	12.50	12.09	2.14	0.65	0.30	1.09	0.51
neral	3.75	4.51	5.32	4.80	1.56	0.52	0.33	0.75	0.48
borneol	64.23	58.03	57.63	54.48	9.75	6.20	0.64	3.15	0.32
geranial	7.01	9.35	12.80	10.86	5.80	1.94	0.33	2.34	0.40
nerol	53.52	46.05	46.12	52.47	7.47	1.05	0.14	0.07	0.01
geraniol	130.18	116.02	119.40	109.85	20.34	10.78	0.53	6.17	0.30
<i>p</i> -cresol	25.72	17.34	20.02	17.53	8.38	5.70	0.68	0.19	0.02

AUTHOR'S BIOGRAPHY

Bethany Jean Hausch was born on April 4, 1986 in Toledo, OH. She grew up in Northwest Ohio. Bethany attended Bowling Green State University (Bowling Green, OH) and earned her Bachelor's degree in Chemistry, with minors in Psychology and General Science. After graduating from BGSU in May 2008, she moved to Urbana, IL to pursue her Master's degree in Food Science at the University of Illinois. Bethany expects to receive Master of Science degree in December 2010. In August 2010, Bethany will start work at Kerry Ingredients & Flavours (Beloit, WI) in the dairy division.