PROFILE OF MISSOURI NORTON WINE AROMA USING SOLID PHASE MICROEXTRACTION OF HEADSPACE, GAS CHROMATOGRAPHY – OLFACTOMETRY, MASS SPECTROMETRY

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by
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The undersigned, appointed by the dean of the Graduate School, have examined thesis entitled

PROFILE OF MISSOURI NORTON WINE AROMA USING SOLID PHASE MICROEXTRACTION OF HEADSPACE, GAS CHROMATOGRAPHY – OLFACTOMETRY, MASS SPECTROMETRY.

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a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Ten Norton wines from across the state of Missouri were analyzed using gas chromatography/mass spectrometry/olfactometry (GC/MS/O) in order to catalog common volatile compounds. Extraction of volatile compounds was performed using headspace solid-phase microextraction (HS-SPME) and identified by a trained panel. The samples were then diluted to determine the most important odor active compounds, resulting in thirty one compounds responsible for the nine most common descriptors of diluted Norton samples. Positive identification was confirmed with Kovat's Retention Indices (RI) using C_5-C_{27} standards. In total 119 volatile compounds were identified, 39 of which had previously reported RI values. This research aims to provide the basis for further investigation into important odorants and characteristic aromas of Norton produced in Missouri.

CHAPTER 1

INTRODUCTION

The aroma of a wine is a critically important factor for both the winemaker and the consumer. The complexity and depth of an aroma can entice consumers, while certain compounds can alert a winemaker to trouble in the wine. Indeed it is possible to get a clear picture of the quality of a wine and its origins from the aroma alone. Distinct characteristics of a region and its grapes can differentiate wines created only a few miles apart. Missouri has developed a unique style of wine from the premium red wine grape, Norton.

Experienced wine drinkers have clear expectations of a wine's aroma based on its origin, and new analyses have enabled objective determination of these aromas. For example, Bordeaux often exhibits green pepper aromas from isobutyl methoxypyrazine and Sauvignon Blanc from Marlborough demands attention with boxwood and tropical fruit aromatics from thiol compounds. Beyond regional differences in the grapes, stylistic choices made by a winemaker can have a profound effect on the final wine. The type of yeast used, how long a wine is aged sur lees (on yeast), the age and type of oak barrels, and malo lactic fermentation are all decisions the winemaker makes in order to produce a desired style. While many compounds have been found in different wines, unique compounds and differing concentrations impact the aroma. No aroma analysis has been performed on the makeup of Missouri Norton; there is no knowledge on how the grape and winemaking style creates Norton's unique aroma.

Microbial contamination is avoided as much as possible during the winemaking process, but an infection can change the wine more profoundly than any conscience

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choice. *Acetobacter* can contaminate grapes and wine to produce vinegar, and fungi can produce trichloroanisole (cork taint) in bottles. Such contamination leaves a sensory trademark on a wine, and also a chemical fingerprint. It is possible to determine the exact cause of a problem with a wine, a maybe identify potential defects before they contribute to a fault in the wine.

While there are many factors which can affect a single wine, there are defining characteristics in regional wines due to similar grapes, climate, and a general style which has proven to be successful. Missouri's wine industry has almost two centuries of history, including a peak as the second largest wine producing state (Stonebridge Research Group 2010), however Prohibition cut the line between modern production and Missouri wine's early days. Beyond the 14 year ban on wine production starting in 1920, the industry lost most of its wineries and did not begin rebuilding until the 1970's. The reestablishment of Missouri wine has occurred at a brisk pace, led by the state's flagship wine, Norton and its close cousin Cynthiana.

As consumption of Missouri wine increases, the demand for a complex and quality dry (no perceivable sweetness) red wine has also gone up. Norton (Cynthiana) is known as a native American grape, and while it is grown successfully in other states like Virginia and Arkansas, there is a unique Norton style from Missouri. This style has been evolving since the resurgence of the industry in the 1970's and consumers have begun to expect a rich, dry red wine with strong notes of blackberry and spice. While much is known about the aroma chemical makeup of popular "old world" varieties such as Merlot and Cabernet Sauvignon, very little is known about the profile of Norton. A unique parentage and the Missouri climate may create unknown compounds in Norton wine.

While there are many factors effecting wine aroma, a profile of typical compounds and those most active in the aroma can be established to objectively define Norton's character. Descriptive analysis involves panelists smelling and discussing a wine's aroma, a practice which requires a large number of trained judges to participate in dozens of sessions. This is limiting because the inherent bias and the difficulty of expressing an exact smell to others. Training may mitigate some communication issues, however the time required training panelists and carrying out analysis is extensive. The matter is further complicated by the nature of wine aroma; a smell may be created by a mixture of multiple aroma compounds, something that a human nose could not determine. However, gas chromatography can separate the odor compounds for single identification by both a human nose and a mass spectrometer. Volatile compounds in wine are classified into three groups; primary aroma refers to compounds imparted by the fruit, secondary to the compounds produced by yeast and fermentation, and tertiary the compounds formed during aging. GC/O/MS will also allow the identification of tertiary aroma characteristics, such as oak barrel aromas, and of faults like 4-ethyl phenol (a "barnyard" aroma produced by contamination yeast). In addition, the chemical identification of an aroma compound can provide insight into its origin, based on the class of chemical.

Similar research has been done with Chardonnay, Riesling, Vidal blanc, Gewürztraminer, Schreube, Pinot Noir, Merlot, Cabernet Sauvignon, Tempranillo, Rioja, Grenache, and Champagne (Aznar 2001). The goal of this research is to find the first comprehensive list of Norton wine aroma compounds, and identify the most potent contributors. Most wines sold under the "Norton" or "Cynthiana" label are varietal, are

dry, and have characteristic dark fruit and spice aromas. While body and style can be comparable to other varieties, Norton is susceptible to the high acid/high pH phenomenon and displays truly unique aromas. Norton is typically aged in oak barrels, under five years old and a majority American oak. While a wine's aroma is a result of a complex system, this research aims to identify both familiar and new volatile compounds which contribute to Norton's aroma.

CHAPTER 2

LITERATURE REVIEW

2.1 ECONOMIC IMPACT OF NORTON WINE

The Missouri wine industry has an estimated total economic impact of 1.6 billion

dollars and pays over 175 million dollars in local, state, and federal taxes (Stonebridge

Research Group 2010). For the period 2005-2010, the amount of wineries in the state of

Missouri nearly doubled from 50 to 97. Despite the economic downturn of 2008 and a

drop in winery tourism, total wine production rose 16% between 2008 and 2009. Over the

same period California, New York, and Oregon all saw decline.

The area devoted to wine grape production increased by 400 acres over the same

period. Norton acreage increased over 44% between 2005 and 2009, and reached nearly

20% of total wine grape acreage. As Missouri's premium red wine, Norton grapes and

wine command prices higher than most varieties. Sixty-two wineries in the state produce

Norton wine.

2.2 HISTORY OF NORTON WINE

There remains controversy over the exact origins of Norton wine, but Ambers and

Ambers (2004) describe the history of Norton grape in literature. The first mention of the

Norton grape was by William Prince in 1830 in his book *Treatise on the Vine*, in which

he gives Dr. Daniel Norton's description of the "Norton" grape. The book describes

Norton as being raised from the seed of the variety Bland, which had flowered near the

varieties Meunier and Miller's Burgundy in Dr. Norton's vineyard near Richmond,

Virginia. Prince describes the Norton vine's appearance and foliage as resembling Miller's Burgundy and conjectures that Norton is a hybrid of Bland and Miller's Burgundy (Prince 1830). However, Norton so closely resembles *Vitis aestivalis*, a grape species native to Virginia, that it is believed that Bland was cross pollinated with a wild grape rather than the Miller's Burgundy Prince described.

Controversy arises from the story of a wild grape being discovered by Dr. F.A. Lemosq on Cedar Island in the James River in 1835 (Bush and Son 1883). Dr. Daniel Norton dug up this wild grape, and according to Bush and Son, recommended it for wine production. Ambers and Ambers (2004) cite Prince "as close to a primary source as we may ever get" and pursue the origin of Norton as a product of open pollination on Dr. Norton's farm, rather than a seedling gathered from Cedar Island.

Further confusion regarding Norton is due to its mother vine, Bland, which no longer exists. Bland was described as having a slightly foxy (sweet musky) character, and bearing a resemblance to Chasselas, a white *vinifera* variety (Ambers and Ambers 2004). This indicates that Bland is a hybrid of *labrusca* x *vinifera,* which would explain why Norton can have a mild foxiness and why self pollinated Norton seedlings often exhibit white or pale fruit indicating a white grape ancestor.

During the 1880's, Norton was described as "the great and leading variety for red wine…" (Bush and Son 1883). Norton was spread as far west as Missouri and Arkansas, and even to some areas in France. When Prohibition was passed, most of the Norton vineyards where ripped out, and Norton wine was lost for almost fifty years. In 1965, Stone Hill Winery in Hermann, Missouri began propagating Norton vines from a

surviving planting. This began Norton's resurgence to the mainstream, and it quickly

spread across the state and returned to its home in Virginia (Cattell 2010).

2.3 CHARACTERISTICS OF NORTON GRAPEVINE

Norton was first described as having "strong and vigorous shoots, and of red

colour," and as a vine that could withstand harsh winters "never failing to produce fruit"

(Prince, 1830). It was described as not requiring pruning and growing rapidly, so that it

was even recommended for decorative purposes. The fruit is described as almost black

and ripening in September, but will continue accumulating sugar through October. Prince

described Norton's clusters as eight to nine inches long weighing a quarter of a pound,

with berries low in juice. He compared Norton's violet color and taste to Tinto Madeira.

In the 1880's Norton's clusters are still described as long and compact, with small

dark fruit which ripen in October (Bush and Son 1883). The Norton vine was described

as vigorous and hearty, with hard wood, long canes, and productive when "well

established." Bush and Son also mention the difficulty of propagating Norton and its

resistance to *Phylloxera*. If Norton fails to fully ripen, it is susceptible to winter damage

like other vines, so early claims that Norton does not require pruning were over

exaggerated. Despite its vigor Norton has a relatively low yield, around 3.5 tons per acre

in Missouri (Smith and Becker 2000). Low yields can "concentrate" the flavor in juice, a

phenomenon observed in Norton by Bush and Son where it was grown on rocky hillsides.

2.4 CHARACTERISTICS OF NORTON WINE

Appellation America held a "Discovery Panel" in 2005 consisting of five Missouri wine experts in an effort to define the characteristics of a signature Missouri Norton. Typical Missouri Norton wines are fermented between 70-80° Fahrenheit without stems, malolactic fermented, and barrel aged. Norton is characterized by high titratable acidity with high malic acid; however, this doesn't result in low pH. This phenomenon is due to the high levels of potassium in Norton, which contribute positive ions in solutions like an acid but do not lower pH. Tannin additions are common in order increase mouthfeel and stabilize color. Alcohol ranges from 12-14%, often as a result of chapitilization and pH ranges from 3.6 to 4. Nortons often have high acid, and overwhelmingly have a high fruity character. Typical fruits described are berries and dark fruit. Oak is characteristic in Norton wines, usually manifesting as vanilla.

Floral notes such as rose petal can be found in Missouri's Nortons, a character not associated with Nortons from other areas. A less desirable aroma of nuttiness also appears sometimes in Norton wines. Consistently there is a vegetative aroma found in Missouri Norton, the discovery panel described it as dill, which can add depth to balanced wine. Other common tasting notes are earthiness and minerality.

2.5 GAS CHROMATOGRAPHY OLFACTOMETRY OF WINE

2.5.1 Principle of GC-O

Volatile compounds which make up aroma are critical to human sensory perception. The strong link between smell and taste has allowed humans to evaluate food prior to consumption. While this link probably developed in humans as a safety mechanism, modern people still depend on aroma for determining taste and quality. Smell can evoke strong memories and emotions in addition to physiological responses, which has led to preoccupation with food. This is demonstrated nowhere more than in wine.

From this ancient obsession humans have created a vast array of styles, laws, evaluating groups, sections of supermarkets, and entire stores dedicated to wine. While humans have been evaluating wine for eons, advances in technology have allowed a more objective measure of smell. Chromatography refers to the separation of a mixture of compounds into its individual parts. Gas chromatography uses the unique polarity and size of compounds to separate them by heating a mixture and carrying it through a column. A GC column is a long, small diameter tube coated with a non polar substance. Depending on the chemistry of a compound, the amount of time it takes for compounds to travel through the column differs. Non polar compounds interact more with the column coating and take longer to elute than a polar compound which is repelled by the coating. This separation allows identification of each constituent individually. Gas

chromatography can show the specific volatile compounds in aroma; however, it is important to understand how compounds are perceived in order to understand their impact on aroma. By separating an aroma into its constituents, it is possible to harness the power of the human sense of smell in a concentrated form.

How a compound smells and its sensory threshold varies widely from person to person. Sommeliers are trained for years to identify aromas in wine, and some people simply cannot detect minute aromas in wine. Finding a specific smell in the complex of wine aroma is like searching for a needle in a haystack, however even people who aren't supertasters have a powerful ability to identify single odors. By separating the compounds using gas chromatography, a person is able to smell one compound at a time without being distracted by another more powerful aroma. Imagine someone handing you a haystack one stalk at a time, and your job was to determine if you had been handed a piece of dried grass or a needle. Gas chromatography olfactometry (GC-O) accomplishes this powerful coupling by splitting the eluents leaving the column so simultaneously chemical identification by MS and sensory evaluation by the human nose are performed.

2.5.2 GC-O Determination of Compound Characteristics

Originally GC-O was used to determine if a single compound in a sample had a perceivable odor, however it has evolved to evaluate multiple compounds and their impact in a sample (Blank and Marsili 2002). Volatile compounds can have differing odor intensities, while others have no odor at all. In this research method the result is the identification of contributing volatiles, based on a recorded smell corresponding to a

compound's identification, as well as an initial measurement of intensity. By diluting a sample, some compounds drop to levels below the human threshold, leaving only more intense volatiles to be recorded by smell.

GC-O often involves the panelist recording the intensity of the smell; however, the purpose of this research is to catalog the contributors to Norton wine. There is no previous research identifying the compounds in Norton wine, so it is premature to focus on any single compound. When focusing on a single compound, it is possible to predict fairly accurately the elution time of the desired compound, where after a panelist can provide a detailed description and intensity. When focusing on the entirety of an aroma, it is more prudent to record a more basic description which will allow analysis of which compounds are contributing rather than what their contribution is exactly. In either analysis, the GC method is critical to the human data collection. The compounds need to be separated by a sufficient amount of time for the panelist to make a record, however it is possible for the human nose to tire which decreases the quality of the data. An issue of profiling an entire aroma is that smells can be short and close together, which is why training is needed. Some odors are so strongly associated that a good description can be given easily, however a trained panelist will be able to classify a fleeting aroma effectively. While the exact aroma may not be determined, it is possible to associate a compound with a more general descriptor such as "fruity." General descriptors can show which compounds have an aroma, and classify the aroma. Future researchers may search for a more specific "blackberry" aroma and these descriptors can steer them towards a concise list of compounds. Specific identification of an aroma impact of a compound

requires threshold and intensity analysis; however, an initial catalogue and classification of important odorants will provide a starting point for those analyses.

2.5.3 Advantages of GC-O

Gas chromatography-mass spectrometry (GC-MS) provides accurate chemical identification of aroma compounds, but the nose is important for interpreting a compound's impact. Two compounds which are structurally very different may smell the same, while closely related compounds can have two distinct aromas. There are extensive catalogues of aroma compounds, but the complexity of wine aroma results in numerous unique compounds. Previously identified compounds are common in Norton aroma, but a vast majority of the compounds have had little research performed on them. By the nature of their precursors and formation, many compounds are similar to those which have been previously identified. The final constitutions of a wine's aroma can be influenced by where and how it was produced. Wines have some characteristic compounds. The aim of this research is to identify the compounds which are important to Missouri Norton, so compounds unique to one region or winery which have a large impact on a single wine are not the focus. In order to find only the major odorants which affect Missouri Norton as a whole, analysis will only be performed on compounds found in five or more samples.

Each aroma compound has an aroma threshold and intensity. This information can be used to identify the impact of a certain compound. Before such analysis can be

performed, the compound must be extracted then diluted successively. First it is important to determine which compounds have a smell and when they elute so extraction can be concentrated. By diluting the samples in this research, compounds warranting further analysis can be determined. Aroma is a complex system of volatile compounds, so the knowledge of compounds which have a higher threshold is also necessary. It may be a strong aroma in conjunction with weaker aromas which creates a certain odor in a wine. In addition, compounds which do not have an associated smell may also play a part in the aroma complex. It is therefore important to pair the data of active odor compounds found by a human nose with chemical identification of volatile compounds.

2.5.4 GC-O Hardware

In order to smell the eluents from the GC column, the flow is split to a chemical detector (MS) and a sniffer port. The sniffer port protrudes from the GC oven at the end of the column and is fitted with a nose cone. The gas used to carry compounds through the GC is stripped of water and baked. Sniffing dry air for forty minutes can dry out the nasal cavity and impact the sense of smell. In order to increase smelling accuracy and comfort, a humidifier is used to add moisture to the air as it approaches the nosecone. It is introduced after the split as not to interfere with chemical identification. The line protruding from the GC is made of a flexible tube, which allows easy adjustment. Paired with a secondary computer screen displaying time and real time MS peaks, the sniffer set up allows each panelist to sit comfortably during the run.

2.5.5 GC-O Methodologies

Two common GC-O methodologies are direct intensity and dilution to threshold. In direct intensity a trained panel is asked to rate the intensity of a compound as it elutes. This method requires a lot of training; however, a relatively small trained panel is able to accurately determine the aroma profile of a substance quickly. The dilution to threshold method involves a series of increasing diluted samples in order to find the dilution factor and threshold of a compound. The dilution continues until the compound is no longer detectable by the human nose. Dilution to threshold requires more panelists (at least seven), and must take into account for physiological differences in the panelists. A hybrid of these two methods would allow for identification of odor active compounds, while also providing insight into which compounds have the lowest threshold. It is possible to train a panel to accurately detect a certain compound among many, but it is not feasible to train a panel to detect every odor in a wine. Especially troublesome for training is that the aromas of Norton have never been profiled by GC-O and it would be impossible to train for unforeseen compounds. It would be possible to run headspace analysis on Norton with GC-MS and identify aroma compounds for GC-O analysis, but with the capability to link GC-O with MS identification, odor and chemical data can be collected simultaneously. While such identification does not provide intensity and threshold for any single compound, it can eliminate many of the extraneous compounds which would be shown by using only GC-MS. The same technique can then be applied to a diluted sample in order to narrow the list of odor active compounds even further. Rather than a physiological measurement of compounds, the hybrid method filters the MS identification data.

2.5.6 Olfactometry Data Collection, Panel Selection, and Training

How GC-O data is recorded is important for accuracy of the results. For an untrained panelist, employing a button with voice recognition software can be effective. The panelist is asked to press a button when they perceive an odor, which records the time and activates a microphone to record the descriptor. A trained panel which is familiar with the equipment may choose to simply watch a timer and record the descriptor by hand. A trained staff will be trained on a list of certain aromas, however due to the unknowns in Norton wine the panel should be free to introduce novel descriptors. This is especially important with compounds such as isobornyl propionate which may smell like fruit to one panelist and turpentine to another. In order to ensure accuracy, the panelist must be concentrated on the GC-O instrument. While it is not always feasible to remove any chance of outside odors, the lab should take every effort to reduce ambient interfering smells. This requires that no one in the lab wear perfume or cologne, drink coffee, or work with aromatic chemicals. All panelists were non-smokers. Reducing interference is also assisted by the use of a nose cone which fits tightly to the panelist's nose.

Common GC-O analysis involves training a panel to accurately recognize one or a few aromas. This allows the determination of a flavor dilution factor for a certain compound through a series of dilutions. This method of training is only effective in studying one or a few compounds, rather than the aroma of an entire wine. Without such training it is not possible to definitively link a compound to a certain smell and determine its intensity; however, training for a single aroma of Norton wine is impossible due to the lack of previous research. In order to effectively filter the volatile compounds determined

by the GC-MS, a panel using GC-O can provide insight into the odor active compounds. This approach can be enhanced by diluting samples in order to determine the most important odor contributors. A panel trained to quickly classify aromas into categories will provide insight into all the aroma compounds in a wine.

2.5.7 Panelist Bias and Sensitivity

Because GC-O is a sensory based analysis, bias is inevitable. The panelists may learn to expect a certain smell based on time, while some compounds may be missed due to exhaling a breath. One advantage of identifying so many compounds is that it becomes hard for a panelist to remember specific times for smells. Some aromas will be found in every wine, but with a trained panel this can be advantageous. Aromas consistently identified with the same descriptors can help build a picture of Norton's aroma. In addition, aromas are somewhat grouped based on retention times which can help narrow the decision tree for the panelist. In order to fight expectations, diluted samples which have much longer blocks without smells are inserted randomly into the testing order. With no previous GC-O research performed on Norton, it is impossible for the panel to have expectations of aromas. It is important to train the panel in order to assure honest recording, as unexpected aromas will be present. In addition to consistent descriptors, the panel must also understand wine aroma. Knowing that an aroma in the glass is made up of multiple compounds reduces the anticipation for a "characteristic" aroma.

When determining the threshold and intensity of a compound, it is important to take into account individual sensitivity. However when searching for odor active

compounds in a sample, the panelists are only asked to determine the presence of a smell. While some compounds may be missed by one panelist, there is a low chance that all three panelists will be anosmic to the same compound. Determining sensitivity requires diluting a standard to each individual threshold; however, the aroma compounds of Norton are unknown. Even with volatile analysis, many of the compounds which would be present in the MS data are not odor active and/or do not have available standards.

2.5.8 GC-O Sample Extraction

Extracting a sample for GC-O can have an impact on the results. Some volatiles are bound to compounds in the wine, while others are so highly volatile they are hard to collect. GC-O is commonly performed on aroma extracts of a substance, one obtained either by distillation or solvent extraction. This presents a problem for results because these methods extract all of the volatile compounds which may not have the same proportions as the original aroma. Headspace extraction collects the sample from the air above a sample in an equilibrated chamber, a better representation of the true aroma. An issue with headspace extraction is that the concentration of compounds is much smaller. Solid-phase micro-extraction (SPME) can be used to concentrate the volatiles from the headspace and maintain the aroma profile.

2.6 HEADSPACE SAMPLING USING SOLID-PHASE MICROEXTRACTION

Solid-phase microextraction provides many benefits when coupled with gas chromatography and gas chromatography/olfactometry. Often, GC-O is performed with aroma extracts of samples, a method which concentrates aroma compounds but adds a time consuming step and may betray the original composition of the aroma. Another advantage of sampling headspace with SPME is the possibility to utilize different fiber thicknesses in place of sample dilution. The ability to utilize different fibers promises quicker dilution analysis of Norton wine aroma, which may expedite the process of identifying key compounds and their impact. Dilution to threshold analysis using different fiber thickness requires multiple fibers, the ranges of which are limited on the market. Other approaches to "in place" dilution are changing the length of fiber exposed to the headspace and altering the split ratio of the carrier gas to the MS and the nosepiece (Deibler and others 1999). These methods are most useful when analyzing a single compound, but a wider ranged analysis still benefits from the time saved by not requiring aroma extraction. Diluting samples using SPME manipulation requires a lengthy process of optimization, and for a single dilution it is more time efficient to dilute the samples directly. The type of fiber used during extraction has an impact on the measurement of a compound, however divinylbenzene/carboxen/polidimethylsiloxane fibers are most commonly used effectively in alcoholic liquid analysis (Fang and Qian 2005).

SPME is more effective than static headspace sampling, which may not be sensitive enough for the detection of some volatile compounds (Miller and Stewart 1998). The identification of unique aroma compounds can be useful in identifying key characteristics of an aroma, allowing objective aroma differentiation of an apricot, for

example (Guillota and Peytavi, 2006). It is also possible to trace the origin of a sample with its unique aroma compounds (Bicchi and others 1997). This objective analysis of unique aroma compounds has the potential to chemically define *terroir* (the impact of how and where a wine was made) and differentiate Missouri Norton wine on the market.

PROBLEM STATEMENT: Results of this research will provide initial insight into specific compounds and their impact in Norton aroma. Norton is the flagship wine of Missouri, where it has developed a distinct style as a premium red wine. Aroma analysis has been used to identify defining characteristics of other prominent wine regions, but never on Missouri Norton.

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLE SELECTION

Ten samples were selected from across the state of Missouri, ranging in years between 2003 and 2008 (Table 1). Only one sample from 2007 was selected due to the abnormal season, a result of a late spring hard freeze which caused massive primary bud mortality. The goal was to examine "typical" Norton wines, however some of the older bottles may be impossible to locate in the future and comparison to an atypical Norton (e.g. 2007 vintage) may provide insight for future research. Each sample was analyzed in duplicate, as well as each 1:10 dilution of the samples. All samples had no perceivable faults or flaws, as determined by the enology staff of the Institute for Continental Climate Viticulture and Enology.

Table 1. Norton samples, year, and origin.

No.	Sample Name	Winery	Year	Location
1	Norton "Claret"	Les Bourgeois	2003	Rocheport, Missouri
2	Norton "Claret"	Les Bourgeois	2008	Rocheport, Missouri
3	Cynthiana	Baltimore Bend	2005	Waverly, Missouri
4	Cynthiana	Baltimore Bend	2006	Waverly, Missouri
5	Norton	Adam Puchta	2005	Hermann, Missouri
6	Norton Reserve	Adam Puchta	2008	Hermann, Missouri
7	Norton	Augusta	2008	Augusta, Missouri
8	Norton	Stone Hill	2007	Hermann, Missouri
9	Norton	St. James	2006	St. James, Missouri
10	Norton	St. James	2008	St. James, Missouri

3.2 SAMPLE PREPARATION

An 8 ml aliquot of wine was transferred to a 20 ml glass headspace sample vial with 3 g of NaCl. Samples were stored in a dark refrigerator at 40°F prior to analysis. Diluted wine was prepared for analysis in the same manner. Diluted samples were prepared by mixing the wine sample in a 1:10 ratio in model wine. The model wine was a 13% ethanol solution containing 8 g/L tartaric acid. The pH of the model wine was adjusted to that of the sample prior to dilution.

3.3 HS-SPME

Polydimethylsiloxane (PDMS), 100 μm thickness, 24 gauge, of SPME (Headspace Solid-Phase Micro Extraction) fibers was used. The samples of wines were warmed to 40°C for 10 min before exposing the SPME fiber to the headspace. Headspace extraction times of 30 min with continuous stirring at 500 rpm were analyzed.

3.4 GC-MS ANALYSIS

A PAL System autosampler mounted to a Varian 431-gas chromatograph paired with a Varian 220-mass selective detector constituted the analytical system. The software used was MSD ChemStation. SPME injections were splitless at 240°C for 1 min during which time thermal desorption of analytes from the fiber occurred. Following SPME desorption for 20 minutes a DB-Wax column (30 m × 0.25 mm I.D., 0.25 μm film thickness) was used for all analyses. Helium carrier gas was used with a total flow of 1 ml min−1. The oven parameters were as follows: initial temperature was 40°C held for

4.0 min, followed by an increase to 110°C at a rate of 5°C min−1, and a final increase to 220°C at a rate of 2°C min−1. The oven was then held at 220°C for 20 min before returning to the initial temperature. The total cycle time was 70 min. The MS detector was operated in the scan mode (mass range 45–650) and the transfer line to the MS system was maintained at 250°C.

3.5 GC-O ANALYSIS

A SGE Olfactometry Detector Outlet (ODO II) system was facilitated through a detector transfer tube of Varian 431-gas chromatograph. The capillary column outlet was connected to a line of humidified air. Components were separated in a DB-WAX column and passed through the transfer tube to the panelist. Column oven temperature was programmed from 40 to 220°C at 5°C/min with a 4 min hold. Helium was used as carrier gas at a flow rate of 2.5 mL/min. The injector and detector temperature was 240°C. Retention times and verbal descriptors were recorded to permit aroma descriptors to be coupled with computerized aroma time-intensity plots. Three trained assessors evaluated the sample in duplicate. Average intensity was calculated for each odorant detected. Identification of the aroma-active components was based on the combination of sensory descriptors, standardized retention indices, and identification confirmed by GC-MS.

3.6 PANELIST TRAINING

Three panelists were selected to perform olfactory analysis on Norton wine. The panel's age and gender are as follows; 22 male, 23 male, 30 female. All panelists were involved with the Institute for Continental Climate Viticulture and Enology's wine

analysis laboratory. Prior to analysis, the panelists practiced blind identification of wine

aroma's using Le Nez Du Vin® master kit. To facilitate novel compound identification,

the panel had access to Ann Noble's Wine Aroma Wheel® during practice and analysis.

The panel was also trained with neutral wines spiked with common aromas: banana,

green pepper, anise, bay leaves, and oak. Spiked wine was used to test classification skills

with subtle aromas. Training using the GC-O hardware consisted of preliminary testing

during the development of the method. Seating arrangements and data recording was

established during the preliminary testing period.

3.7 DATA HANDLING

The purpose of this study was to identify key aroma compounds of Missouri

Norton wine. Compounds were identified by matching MS data to the NIST library.

Many aroma compounds are unique to a single wine and are not the focus. Only

compounds which were present in at least five samples were analyzed and presented from

the non diluted samples. The nature of diluting samples resulted in fewer compounds

available for identification. As such, all compounds found in diluted wine samples were

analyzed for their potential as key odorants. The large number of compounds and aromas

present in the non diluted wine samples made it impossible to definitively link

compounds to an aroma, but olfactometry data will be collected to provide insight for

future research. MS data of non diluted samples also provided novel identification of

aroma compounds along with previously identified compounds for comparison.

The reduced number of compounds and aromas in diluted wine samples provided

better connections between compounds and specific aromas. Identification of previously

known compounds were confirmed by using Kovats retention indices (RI). The use of RI

allowed correction for any shift in retention time due to the unique method employed in

this research by analyzing the shift of elution times of a standard set of chemicals

compared to other methods. Future research concentrating on a single compound of

interest will have a specialized GC method, but RI can be compared for positive

identification.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PREVIOUSLY IDENTIFIED VOLATILE COMPOUNDS

Analysis of non diluted wine samples revealed 86 volatile compounds with odor active potential, 27 of which were previously identified, which were present in at least five samples. Kovats Retention Index analysis of the 27 previously identified compounds revealed that 25 compounds had a calculated RI within 2.7% of RI's found in literature. (+)-Spathulenol and E-Whiskey Lactone showed a 5.86% and 10.74% error, respectively. Both compounds were identified with over 25% probability by MS according to NIST; however, the difference in RI suggests misidentification.

Table 2. Retention Indices of Previously Identified Volatile Compounds Found in Non-Diluted Norton Headspace Using DB-Wax.

No.	Identity	CAS	RI	RI (*literature*)	Aroma (*literature*)
1	1-Butanol [21]	71-36-3	1108	1138	Fruity, Medicinal, Cheesy
2	Isoamyl Acetate [21, 10, 16]	123-92-2	1097	1118, 1132, 1147	Banana, Fruity, Pear
3	(+)-Spathulenol [*, 12]	6750-60-3	2011	2129	Fruity, Herbaceous, Herbal
4	1-Hexanol [^, 21, 10]	111-27-3	1353	1351, 1354, 1360	Resin, Flower, Green
5	1-Pentanol [33]	71-41-0	1214	1244	Fruity, Green, Pungent
6	4-methyl-1-Pentanol [29, 33]	626-89-1	1353	1301, 1360	Oily green-fruity, Herbaceous, Yeasty
7	2-methyl-1-Propanol [10, 16, 21]	78-83-1	1098	1085, 1108, 1125	Glue, Alcohol, Leek, Licorice
8	E-whiskey lactone [*, 2]	39212-23-2	2215	1977	Flower, coconut
9	2-Propenoic acid, 3-phenyl-, ethyl ester [10]	103-36-6	2145	2149	Honey, Cinnamon, Flowery, Fruity
10	3-Hexen-1-ol, (Z)- [28, 16, 10]	928-96-1	1382	1401	Green grasslike, Leafy
11	Butanedioic acid, diethyl ester [29, 14]	123-25-1	1684	1690, 1705	Fabric, Fruity, Watermelon, Flower, Sweat
12	Butanoic acid, 2-methyl-, ethyl ester [16, 10]	7452-79-1	1029	1056	Fruity, Strawberry, Blackberry, Green apple
13	Butanoic acid, 3-methyl-, ethyl ester [10, 35]	108-64-5	1066	1070	Cashew, Fruity, Anise, Sweet fruit, Apple
14	Butanoic acid, ethyl ester [16, 10, 27]	105-54-4	1030	1036	Fruity, Banana, Strawberry, Bubblegum
15	Decanoic acid, ethyl ester [27]	110-38-3	1643	1630	Grape, Fruity
16	Ethyl 9-decenoate [29]	67233-91-4	1693	1694	n/a
17	Eugenol [35]	97-53-0	2183	2186	Clove, Honey, Balsamic
18	Hexanoic acid, ethyl ester [16, 21, 27]	123-66-0	1226	1244	Fruity, Strawberry, Anise, Wine gum
19	1,1,6-trimethyl-1,2-dihydronaphthalene[38]	30364-38-6	1755	1712	Petrol, Kerosene

Table 2. *Continued*

No.	Identity	CAS	RI	RI (*literature*)	Aroma (*literature*)
20	Octanoic acid, ethyl ester [27]	106-32-1	1442	1446	Fruity, Floral, Green leafy, Menthol, Anise
21	Octanoic acid, methyl ester [36]	111-11-5	1389	1378	Fruity, Green
22	4-ethylguaiacol [14, 10, 16]	2785-89-9	2279	2048	Clove-like, Phenolic, Flowery
23	Phenylethyl Alcohol [14, 10, 16]	60-12-8	1921	1940	Honey-like, Yeast-like, Floral, Spicy
24	Propanoic acid,2-hydroxy-, ethyl ester [29]	97-64-3	1341	1353	Ethereal-buttery
25	Propanoic acid,2-hydroxy-,methyl ester [33]	2155-30-8	1308	1314	n/a
26	Propanoic acid, 2-methyl-, ethyl ester [21, 10]	97-62-1	956	955	Fruity, Strawberry
27	E-beta-damascenone [16, 18, 10]	23726-93-4	1832	1832	Honey, Fruity, Apple, Tobacco

* Calculated RI lies outside of 5% of RI reported in literature

^ Aroma descriptors given by Flavornet.org

[2]*Aznar and Lopez 2001,* [10] *Choi 2003,* [12]*Cullere and Escudero 2004,* [14]*Escudero and Etievant 1999,* [16]*Ferriera and Aznar 2001,*

[18]*Hognadottir and Rouseff 2003,* [21]*Lee and Noble 2003,* [27]*Qian and Reineccius 2003,* [29]*Selli and Cabaroglu 2004,*

[33]*Umano and Nakahara 1999,* [35]*Valim and Rouseff 2003,* [36]*Varming and Petersen 2004,* [38]*Winterhalter 1991.*

When analyzing samples, many fruity aromas were recorded in every sample between 4 and 8 minutes of each run. When comparing the compounds identified during this time, none met the five sample criteria. This suggests that low weight esters which impart a fruity aroma to Norton wine are prevalent; however, these compounds are unique to each specific wine. As such, there is no fruity aroma from these compounds which characterizes Norton. While fruitiness is a main descriptor of Norton aroma, aromas may be specific to one region, vineyard, year, or yeast strain.

1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) was very prevalent in the samples, and has been previously identified as the compound responsible for "petrol" aromas in aged German Reisling. "Tar" is a common descriptor in Norton aroma and TDN may play a role in the aroma complex. Two β-damascenone isomers were found commonly in Missouri Norton. β-damascenone has been shown to have a ripe fruit, honey aroma (Suzuki and others 2001). More importantly it has been shown to play a role in enhancing fruity aromas via masking of vegetal aromas (Pineau 2007). β-damascenone's low odor threshold may suggest an important role in Norton aroma even at low concentrations.

A potential of GC-O analysis of wine aroma is the identification of wine faults. *Brettanomyces* contamination of wine is a common problem and is often cited as the source of 4-ethylphenol, which imparts a barnyard and medicinal aroma. Usually 4-EP contamination is found in conjunction with 4-ethylguiacol, which imparts a spicy clove note. While 4-EP was not found in the samples, 4-EG was very common. The presence of 4-EG may be caused by the use of American oak barrels for wine aging (Cerdán 2002). Aging in American oak barrels is common in Missouri, while not exclusive, due to

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Missouri's prominence in American cooperage. Along with 4-EG, whiskey and oak

lactones were commonly found in Missouri Norton, which have a similar aroma profile.

4.2 NOVEL VOLATILE AROMA COMPOUNDS IN NORTON WINE

Fifty-seven volatile compounds were identified in Missouri Norton which were

previously unidentified using a DB-WAX column (Table 3). No RI's were available for

these compounds. Some of these compounds may have never been previously identified

in wine. Using non diluted samples eliminates the ability to positively link these

compounds with an aroma, but they may play a role in key aroma characteristics.

Table 3. Previously Uncalculated Retention Indices of Volatile Compounds from Norton using DB-Wax column.

No.	Compound	CAS	RI
1	(-)-Spathulenol	77171-55-2	1784
2	L-lactic acid	79-33-4	1345
3	(S)-3,4-Dimethylpentanol	-	1359
4	4-Pentyl-2-valeryl-1,3-cyclopentanedione	69796-08-3	2070
5	10,12-Tricosadiynoic acid, methyl ester	-	1752
6	Acetic acid 2,6-dimethyl-1-cyclohexenyl ester	6203-89-0	1941
7	2-Hexyl Decanol	2425-77-6	2207
8	2-Ethyl-1-Hexanol	104-76-7	1485
9	3,4,4a,5,6,7-Hexahydro-1,1,4a-trimethyl-2(1H)-naphthalenone	4668-61-5	1525
10	(Z)-oak lactone	55013-32-6	2135
11	Methyl (2E,4E)-7-hydroxy-2,4-octadienoate	69734-24-3	2167

Table 3. *Continued*

No.	Compound	CAS	RI
12	2-Allyl-1,4-dimethoxy-3-methyl-benzene	-	1729
13	beta-damascenone	23696-85-7	1832
14	2-Furanmethanol, 5-ethenyltetrahydro-.al	5989-33-3	1457
15	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6	14049-11-7	1771
16	2-Propanol, 1-methoxy-	107-98-2	1164
17	2-Propanone, 1-(3,5,5-trimethyl-2-cycloh	16695-72-0	1727
18	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohe	4951-40-0	1711
19	3-Hydroxybutyric acid	300-85-6	1193
20	3-Nonenoic acid, ethyl ester	91213-30-8	1632
21	4,4-Dimethyl-3-(3-methylbut-3-enylidene)	79718-83-5	1790
22	6-Hexadecen-4-yne, (E)-	74744-52-8	2012
23	7-Methoxybenzofuran-2-carboxylic acid	4790-79-8	1731
24	Acetic acid, 2-phenylethyl ester	103-45-7	2039
25	Benzeneacetic acid, ethyl ester	101-97-3	1786
26	1-Methyl-9-(1-methylethylidene)bicyclo[3.3.1]nonan-2-one	56630-95-6	1533
27	Butanoic acid, pentyl ester	540-18-1	1210
28	Butyl caprylate	589-75-3	1549
29	Butylated Hydroxytoluene	128-37-0	2137
30	Carbamic acid, methyl ester	598-55-0	1450
31	Cedran-diol, 8S,14-	62600-05-9	1877
32	cis-3-Methyl-4-octanolide	39638-67-0	2208
33	Decanoic acid, methyl ester	110-42-9	1770
34	Dodecanoic acid, ethyl ester	106-33-2	1852
35	Ethanedioic acid, bis(3-methylbutyl) est	2051-00-5	1201
36	Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0	15120-94-2	1371
37	Ethyl 9-hexadecenoate	54546-22-4	2292
38	Ethyl trans-4-decenoate	76649-16-6	1698

Table 3. *Continued*

No.	Compound	CAS	No.
39	Furan, 2,2'-[oxybis(methylene)]bis-	4437-22-3	1281
40	Hexadecanoic acid, ethyl ester	628-97-7	2259
41	Ionone	8013-90-9	1538
42	Isoaromadendrene epoxide	-	1811
43	Isopentyl hexanoate	2198-61-0	1456
44	Nonanoic acid, 5-methyl-, ethyl ester	116530-40-6	1648
45	Nonanoic acid, ethyl ester	123-29-5	1511
46	Octane, 3-methyl-6-methylene-	74630-07-2	1386
47	Octanoic acid, 3-methylbutyl ester	2035-99-6	1666
48	Oxalic acid	144-62-7	1687
49	Pentadecanoic acid, 3-methylbutyl ester	2306-91-4	1870
50	Phenol, 2,4,6-tris(1-methylethyl)-	2934-07-8	1927
51	Phenol, 2-methoxy-3-(2-propenyl)-	1941-12-4	2167
52	Phenol, 3-ethyl-	620-17-7	2419
53	Propane, 1-methoxy-2-methyl-	625-44-5	1165
54	Propanoic acid, 2-hydroxy-, ethyl ester,	687-47-8	1512
55	Santalol, cis,.alpha.-	19903-72-1	1815
56	Succinic acid, ethyl 3-methylbutyl ester	-	1906
57	Tetradecanoic acid, ethyl ester	124-06-1	2055

Among the compounds in Table 3, spathulenol was identified. Spathulenol is a component of some floral and wood shrub essential oils (Martelli and others 1985). Also found was santalol, a critical component of the oil of sandalwood (Hongratanaworakit 2004). In addition to β-damascenone, another C-13 Isoprenoid, Ionone, was identified commonly in Missouri Norton wine. Ionone is a component of rose oil and is an important chemical used in the perfume industry.

4.3 POTENTIALLY ODOR ACTIVE VOLATILES OF DILUTED NORTON SAMPLES.

Diluted samples contain less volatile compounds in headspace, which results in less interference during MS identification. The result is 33 compounds identified in diluted samples which were not identified in non diluted samples. Seventy-five compounds were identified as potential aroma contributing in diluted wine samples, 31 of which showed odor activity (Table 4). Of the 31 compounds associated with a GC-O descriptor, 12 were previously identified and 15 were found in the non diluted wine samples. All RI's of diluted wine samples were within 5% of RI reported in literature, except 2,3-Butanediol (Table 4). The same was true for RI's compared to non dilute samples, except for 3-ethylphenol, butylated hydroxytoluene, and 1-methoxy-2-propanol. The difference in RI's between diluted and non diluted samples is most likely due to MS interference in non diluted sample analysis.

Table 4. Potentially Important Odor Active Compounds Detectable at 1:10 Dilution.

No	Name	CAS	RI^C	RIND	RI^L	Aroma	Aroma (*pherobase.com*)
1	10-Nonadecanone	504-57-4	2161	-	-	Dental office, light floral, spicy	-
2	1-Butanol, 3-methyl-	123-51-3	1208	-	1206	Yeasty, dough, wet dog, coffee	Pungent, Balsamic, Alcohol, Malty
3	1-Butanol, 3-methyl-, acetate	123-92-2	1130	1097	1118	Banana	Banana, Fruity, Pear odor
4	2(1H)-Naphthalenone, 3,4,4a,5,6,7,8,8a.a	28684-99-3	1852	-	-	floral, berry, peachy	-
5	3,4,4a,5,6,7-Hexahydro-1,1,4a-trimethyl-2(1H)-naphthalenone	4668-61-5	1537	1525	-	Detergent, floral	-
6	2,3-Butanediol	513-85-9	1026	-	1523	Fruity, strawberry, alcohol	Fruity
7	2-Propanol, 1-methoxy-	107-98-2	949	1164	-	Strawberry, fruity, cherry	-
8	Geranylacetone	689-67-8	1867	-	-	blueberry, rasberry, candy	Magnolia, Green
9	Nerylacetone	3879-26-3	1871	-	-	Koolaid, grape jelly	-
10	6-Propenylbicyclo[3.1.0]hexan-2-one	75283-46-4	1804	-	-	smoke	-
11	8-Pentadecanone	818-23-5	1951	-	-	floral	-
12	Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	1071	1029	1069	Strawberry, nail polish	Fruity, Strawberry, Blackberry, Green apple
13	ethyl isovalerate	108-64-5	1083	-	1082	tropical fruit, fake grape	Cashew, Fruity, Anise, Sweet fruit, Apple
14	Butanoic acid, ethyl ester	105-54-4	1050	-	1047	ether, fruity	Fruity, Banana, Strawberry, Bubblegum
15	Butylated Hydroxytoluene	128-37-0	1924	2137	-	floral	-
16	Dodecanoic acid, ethyl ester	106-33-2	1856	1852	-	potporri, floral, berry	Mango-like

Table 4. *Continued*

No	Compound	CAS	RI^C	RIND	RI^L	Aroma	Aroma (*Literature*)
17	diisopentyl oxalate	2051-00-5	1207	1201	-	coffee	-
18	Ethyl Acetate	141-78-6	829	-	885	fruity	Caramel, Solvent-like, Fruity, Buttery
19	difurfuryl ether	4437-22-3	1290	-	-	Ether, nail polish	-
20	Hexanoic acid, ethyl ester	123-66-0	1230	1226	1229	licorice candy, berry	Fruity, Strawberry, Anise
21	Ionone	8013-90-9	1536	1538	-	floral	Wood, violet
22	Isopropyl Alcohol	67-63-0	983	-	917	peach, fruit, vanilla	Ethereal, Alcohol
23	Methanecarbothiolic acid	507-09-5	834	-	-	buttery, burnt	-
24	Octane, 3-methyl-6-methylene-	74630-07-2	1206	1386	-	Dough, wet dog, yeasty	-
25	Octanoic acid, ethyl ester	106-32-1	1439	1442	1444	Fresh cut grass, woody, floral, glue	Floral, Green leafy, Menthol, Anise
26	Ethyl pivaloylacetate	17094-34-7	1082	-	-	Fruity, ether	-
27	Phenol, 2,4,6-tris(1-methylethyl)-	2934-07-8	1927	1927	-	Floral	-
28	Phenol, 3-ethyl-	620-17-7	2192	2419	-	Ginger bread, clove	-
29	Phenylethyl Alcohol	60-12-8	1922	1921	1931	floral	Honey, Sweet, Yeast, Floral
30	Propanoic acid, 2-methyl-, 1-(1,1-dimeth	74381-40-1	1888	-	-	Floral, spicy, berry	-
31	Tetradecanoic acid, ethyl ester	124-06-1	2055	2055	-	Basil, floral, spicy	-

RI ^{C}- Calculated Retention Index

RI ND- Calculated Retention Index from Non Dilute Samples

RI ^{L}- Retention Index from Literature

Aroma descriptors for diluted samples were consistently grouped and associated with volatile compounds. There was an average of 9 aroma descriptors for diluted wine samples, resulting in accurate aroma description. Prevalent were aromas of ethereal, strawberry, blackberry, yeast/dough, grass, cedar, and clove. Floral aromas were also common but there was no consistent detailed description of the aroma.

4.4 NON ODOR ACTIVE VOLATILES FROM DILUTED NORTON

There were 46 compounds found in diluted Norton headspace that appeared to have no activity at a 1:10 dilution. They may contribute and be identifiable at lower dilutions, and among them are volatiles of interest. Carophyllene, limonene, and linalool were all identified in diluted samples but were not associated with an aroma. Carophyllene is a constituent of clove oil and spice notes that are common in Norton wine. Limonene has a citrus aroma not typically associated with Norton. Linalool is associated with a floral, slightly spicy, aroma and is a terpene associated with other wines, especially muscat.

Table 5. Volatile compounds below detection at 1:10 dilution.

Compound	CAS	RI^C	RIND	RI^L
L-Lactic Acid	79-33-4	1346	1345	-
(S)-3,4-Dimethylpentanol		1358	1359	-
1-Butanol	71-36-3	1110	1108	1138
1-Heptanol, 2-propyl-	10042-59-8	1397	-	-
1-Hexanol	111-27-3	1358	1353	1351
1-Octanol, 2,7-dimethyl-	15250-22-3	1361	-	-
1-Octanol, 2-butyl-	3913-02-8	1359	-	-
1-Pentanol	71-41-0	1202	1214	1244
1-Propanol, 2-methyl-	78-83-1	1110	1098	1,085
2-Allyl-1,4-dimethoxy-3-methyl-benzene		1733	1729	-
(Z)-linalool oxide	5989-33-3	1444	1457	-
2H-1-Benzopyran,3,5,8,8a-tetrahydro-2,5,5,-8a-tetramethyl-	72468-40-7	1734	-	-
2-Hexanol, 3-methyl-	2313-65-7	1122	-	-
2-Hexyl-1-octanol	19780-79-1	1366	-	-
4,4-Dimethyl-3-(3-methylbut-3-enylidene)	79718-83-5	1796	1790	-
5,6-Epoxy-2,2-dimethyl-3-heptyne	212687-69-9	1805	-	-
6,7-Dimethyl-3H-isobenzofuran-1-one	343852-50-6	1878	-	-
7-Methoxybenzofuran-2-carboxylic acid	4790-79-8	1734	1731	-
Benzeneacetic acid, ethyl ester	101-97-3	1794	1786	-
1-Methyl-9-(1-methylethylidene)bicyclo[3.3.1]nonan-2-one	56630-95-6	1537	1533	
diethyl succinate	123-25-1	1685	-	1690
Carbamic acid, methyl ester	598-55-0	1414	1450	
Caryophyllene	87-44-5	1607	-	1608
l-Limonene	5989-54-8	1194	-	-
Decanoic acid, ethyl ester	110-38-3	1647	1643	1630
Decanoic acid, methyl ester	110-42-9	1598	1770	-
Ethane, 1,1,1-trimethoxy-	1445-45-0	818	-	-

Table 5. *Continued*

Compound	CAS	RI^C	RIND	RI^L
Ethanedial, dioxime	557-30-2	860	-	-
Ethyl 9-decenoate	67233-91-4	1698	-	1694
Ethyl hydrogen oxalate	617-37-8	1682	-	-
Ethyl trans-4-decenoate	76649-16-6	1698	1698	-
Hexadecanoic acid, ethyl ester	628-97-7	2260	2259	-
Isopentyl hexanoate	2198-61-0	1459	1456	-
1,1,6-trimethyl-1,2-dihydronaphthalene	30364-38-6	1756	1755	1712
Nonanoic acid, 5-methyl-, ethyl ester	116530-40-6	1639	1648	-
Octanoic acid, 3-methylbutyl ester	2035-99-6	1666	1666	-
Octanoic acid, methyl ester	111-11-5	1392	1389	1378
Oxalic acid	144-62-7	1646	1687	-
Oxirane, (1-methylbutyl)-	53229-39-3	1353	-	-
Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	2316	-	-
Ethyl lactate	97-64-3	1346	1341	1353
Propanoic acid, 2-hydroxy-, ethyl ester,	687-47-8	1345	1512	-
Propanoic acid, 2-hydroxy-, methyl ester	2155-30-8	1346	1308	1314
Succinic acid, ethyl 3-methylbutyl ester		1916	1906	-

RI ^{C}- Calculated Retention Index

RI ND- Calculated Retention Index from Non Dilute Samples

RI ^{L}- Retention Index from Literature

4.5 RELEVANCE OF RESULTS

Positively identified compounds can provide insight into Norton's relation to other wines. In addition, research done on compounds in common can provide insight into the sources of such compounds and their impact in Norton. The results support that the compounds found to be odor active in the diluted samples have the largest impact on Norton's aroma. The catalogue of compounds may yield important chemicals which are part of an aroma complex, or may contain an important odorant which requires further dilution trials to characterize. For the most part, the RI's support the identification of compounds, and further comparison of RI with compounds with discrepancies can determine the source of the difference. HS-SPME showed its usefulness in analyzing Norton aroma compounds, and the narrow grouping of aroma descriptors supports the GC method for GC-O analysis of diluted (or perhaps fractioned) samples.

CHAPTER 5

CONCLUSION AND FUTURE RESEARCH

5.1 CONCLUSION

The 31 compounds identified in the diluted samples represent the most important odorants of Missouri Norton wine. These compounds should be the first concentration of continuing research due to their importance, and because the list contains previously identified and novel compounds. Research seeking the explanation of aromas can use the most odor active compounds as well as the extensive catalogue of aroma compounds to determine the makeup of characteristic Norton aromas. The presence of some essential oils is unique to Norton, while there are many compounds Norton shares with other wines. The unique heritage of Norton wine has created a distinct wine, with definitive relationships to its modern cousins. The Kovats retention indices provided clear confirmation of the majority of chemicals, and discrepancies may be the result of misidentification or a lack of research into little known compounds. In addition, RI's given for all the compounds will be critical in future analysis of Norton wine.

5.2 FUTURE RESEARCH

As previously stated, the goal of this research was to catalogue Norton volatile compounds and provide initial insight into odor activity. Now that it is possible to choose a compound to research, the most likely next step in research is quantification. A larger sample of Norton wines can be used to determine which compounds commonly show up in the largest concentration, and this information can be paired with dilution to threshold and direct intensity GC-O methods. Further narrowing of the important odor active compounds can define the unique characteristics of Missouri Norton. The Missouri wine industry can sell Missouri Norton as a distinct product with defined qualities.

Understanding the source of important aroma compounds can help increase consistency of Norton produced in the state. It is important to deliver a consistent product to consumers in order to raise the quality of the industry, and objective analysis can provide not only grading but potential tools for winemakers. Balancing Norton's fruity and spicy notes can be challenging in less than ideal years, but information on how the wine is impacted by climate and winemaking can provide a winemaker with tools early in the process to produce quality wine. In addition some of these compounds may be impacted by specific viticultural practices which can potentially increase desired volatile compounds and mitigate undesired aromas.

Specific questions raised by this research are the lack of universal low weight esters. These compounds may be most impacted by *terroir*, and further investigation of regions and wineries can differentiate the regions in Missouri. Missouri regions can be defined through their wine rather than location for the first time. In conjunction with

differentiation of wine regions, it may also be possible to track the source of wines through identifying compounds.

Two compounds of interest are limonene and β-damascenone. Limonene is a well researched compound, but does not appear to be a main odorant in Norton. Its impact may be unique to Norton as a red wine, reacting in conjunction with other aroma compounds. β-damascenone has the potential to be an important tool for winemakers. If it is possible to increase β-damascenone concentration in wine, the winemaker can mask vegetal aromas and increase fruitiness in cold or damaged years (Pinneau 2007). It is important for Norton to maintain fruitiness due to the palates of the majority of consumers, in the same way Cabernet Sauvignon from California tends to be more fruit forward than its Bordeaux counterparts. The challenges of Missouri's climate could be mitigated somewhat by the use of β-damascenone to increase fruitiness.

One descriptor which was vague during this research is "floral." The descriptor is valuable as insight; however it does not provide a characteristic aroma. This may be due to the various sources of floral notes in Norton wine. The descriptor was very common and should be further investigated to determine the important aroma compounds and how they translate into a unique characteristic of Norton wine. Ionone, butylated hydroxytoluene, and 8-Pentadecanone all have a connection with a vague "floral" descriptor. β-damascenone may also have an important role in floral notes in Missouri Norton.

Speaking with Missouri winemakers, a common discovery is the development of flavor during Norton ripening. Rather than depending only on acid and sugar levels in the

grapes to determine harvest date, many winemakers sample juice for a characteristic "tomato juice" aroma present in under ripe Norton. Searching for the compound responsible can provide another objective analysis for determining ripeness rather than depending on informal sensory analysis. An identification of the compound may also provide tools in the future to mitigate the flavor in juice/wine that was forced to be harvested early.

The potential for specific chemical manipulation of wine to increase quality is currently unknown. It may provide a diverse bag of winemaking tools which can raise Missouri's wine industry back to its historic levels. This initial insight into Norton's aroma will provide the basis for future enological research in the state of Missouri. Missouri's lack of research into its state grape does have a positive side. Results from other regions in the world can provide a concentrated research effort which can bring Missouri up to comparable research levels quickly.

REFERENCES

Ambers R, Ambers, C. 2004. Dr. Daniel Norborne Norton and the Origin of the Norton Grape. American Wine Society 36(3):77-87.

Aznar M, Lopez, R., Cacho, J., and Ferreira, V. 2001. Identification and quantification of impact odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. Journal of Agricultural and Food Chemistry 49:2924-2929.

Bicchi CP, Panero OM, Pellegrino GM, and Vanni AC. 1997. Characterization of Roasted Coffee and Coffee Beverages by Solid Phase Microextraction−Gas Chromatography and Principal Component Analysis. Journal of Agricultural and Food Chemistry 45(12):4680-4686.

Blank I, Marsili, R. 2002. Flavor, Fragrance and Odor Analysis. New York.

Bush and Son. 1883. Illustrated Descriptive Catalogue of American Grape Vines, A grape grower's manual. Bushberg, Missouri.

Cattell H. 2010. Norton -- The Wild Vine. Wines and Vines. San Rafeal, California: Wine Communications Group.

Cerdán T. 2002. Volatile composition of aged wine in used barrels of French oak and of American oak Food Research International 35(7):603-610.

Choi H-S. 2003. Character impact odorants of citrus hallabong [(C. unshiu Marcov x C. sinensis Osbeck) x C. reticulata Blanco] cold-pressed peel oil. Journal of Agricultural and Food Chemistry 51:2687-2692.

Cullere L, Escudero, A., Cacho, J., and Ferreira, V. 2004. Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. Journal of Agricultural and Food Chemistry 52:1653-1660.

Deibler KD, Acree, T.E., Lavin, E.H. 1999. Solid phase microextraction application in gas chromatography/olfactometry dilution analysis. Journal of Agricultural and Food Chemistry 47:1616-1618.

Escudero A, and Etievant, P. 1999. Effect of antioxidants on the flavor characteristics and the gas chromatography/olfactometry profiles of champagne extracts. Journal of Agricultural and Food Chemistry 47:3303-3308.

Fang Y, Qian, M. 2005. Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). Flavour Fragr J 20:22-29.

Ferreira V, Aznar, M., Lopez, R., and Cacho, J. 2001. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. Journal of Agricultural and Food Chemistry 49:4818-4824.

Guillota S, Peytavi, L. 2006. Aroma characterization of various apricot varieties using headspace–solid phase microextraction combined with gas chromatography–mass spectrometry and gas chromatography–olfactometry. Food Chem 96(2):147-155.

Hognadottir A, and Rouseff, R.L. 2003. Identification of aroma active compounds in orange essence oil using gas chromatography - olfactometry and gas chromatography - mass spectrometry. J Chromatogr A 998:201-211.

Hongratanaworakit T. 2004. Evaluation of the effects of East Indian sandalwood oil and alpha-santalol on humans after transdermal absorption. Planta Med 70(1):3-7.

Lee S-J, Noble, A.C. 2003. Characterization of odor-active compounds in Californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry. Journal of Agricultural and Food Chemistry 51:8036-8044.

Martelli A, Frattini C, and Chialva F. 1985. Unusual essential oils with aromatic

   properties—I. Volatile components of Stevia rebaudiana bertoni. Flavour and

   Fragrance Journal 1(1):3-7.

Miller ME, and Stuart JD. 1998. Comparison of Gas-Sampled and SPME-Sampled Static

   Headspace for the Determination of Volatile Flavor Components. Analytical

   Chemistry 71(1):23-27.

Pineau B, Barbe JC, Van Leeuwen C, and Dubourdieu D. 2007. Which impact for β-

   Damascenone on Red Wines Aroma? Journal of Agricultural and Food Chemistry

   55(10):4103-4108

Prince WR. 1830. A Treatise on the Vine; Embracing its History from the Earliest Ages

   to the Present Day, with Descriptions of Above Two Hundred Foreign, and Eighty

   American Varieties; Together with a Complete Dissertation on the Establishment,

   Culture, and Management. New York: T. & J. Swords.

Qian M, and Reineccius, G. 2003. Potent aroma compounds in Parmigiano Reggiano

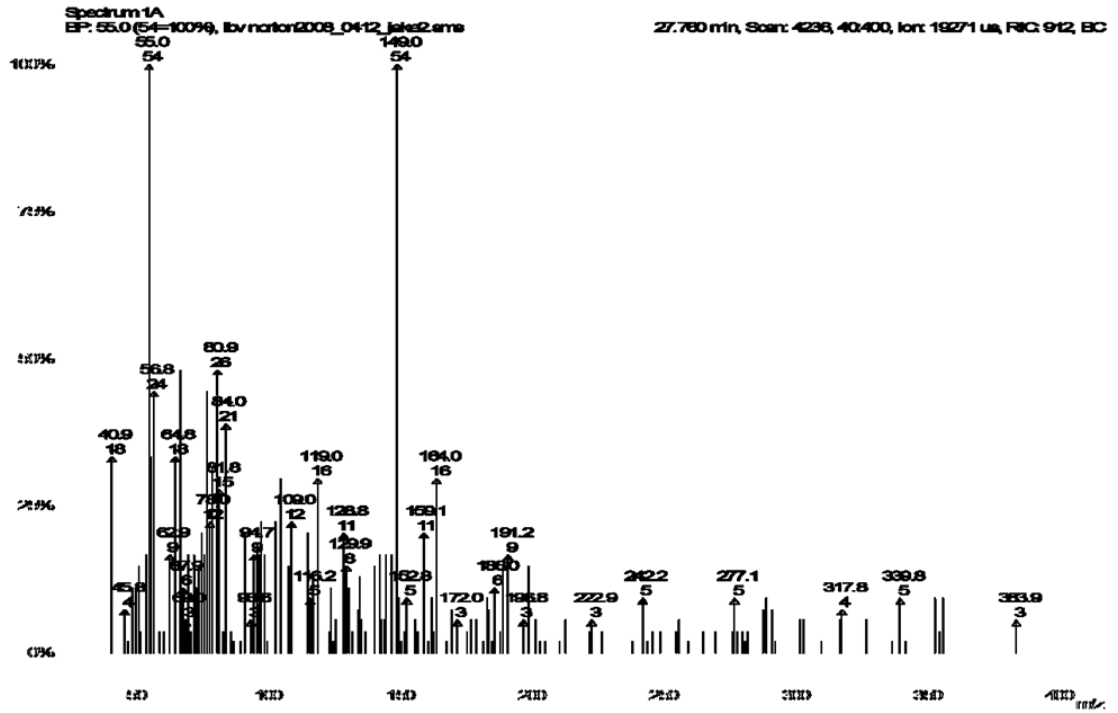   cheese studied using a dynamic headspace (purge-trap) method. Flavour Fragr J

   18:252-259.

Selli S, Cabaroglu, T., Canbas, A., Erten, H.,Nurgel, C., Lepoutre, J.P., and Gunata, Z. 2004. Volatile composition of red wine from cv. Kalecik Karasi grown in central Anatolia. Food Chem 85:207-213.

Smith GS, Becker, S.A. 2000. Crop Profile for Grapes in Missouri.

Stonebridge Research Group. 2010. The Economic Impact of Wine and Grapes in Missouri 2010. In: Board MGaW, editor. Jefferson City, Missouri.

Suzuki M, Matsumoto S, Fleischmann H-P, Shimada H, Yamano Y, Ito M, and Watanabe N. 2001. Identification of Beta-Damascenone Progenitors and Their Biogenesis in Rose Flowers "Rosa damascena". Carotenoid-Derived Aroma Compounds: American Chemical Society. p 89-101.

Umano K, Nakahara, K., Shoji, A., and Shibamoto, T. 1999. Aroma chemicals isolated and identified from leaves of Aloe arborescens Mill. var. natalensis Berger. Journal of Agricultural and Food Chemistry 47:3702-3705.

Valim MF, Rouseff, R.L., and Lin, J. 2003. Gas chromatographic-olfactometric aharacterization of aroma compounds in two types of cashew apple nectar. Journal of Agricultural and Food Chemistry 51:1010-1015.

Varming C, Petersen, M.A., and Poll, L. 2004. Comparison of isolation methods for the

    determination of important aroma compounds in blackcurrant (Ribes nigrum L.)

    juice, using nasal impact frequency profiling. Journal of Agricultural and Food

    Chemistry 52:1647-1652.

Winterhalter P. 1991. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) formation in wine.

    1. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-

    2,8-diol rationalizing the origin of TDN and related C13 norisoprenoids in

    Riesling wine. Journal of Agricultural and Food Chemistry 39(10):1825-1829.
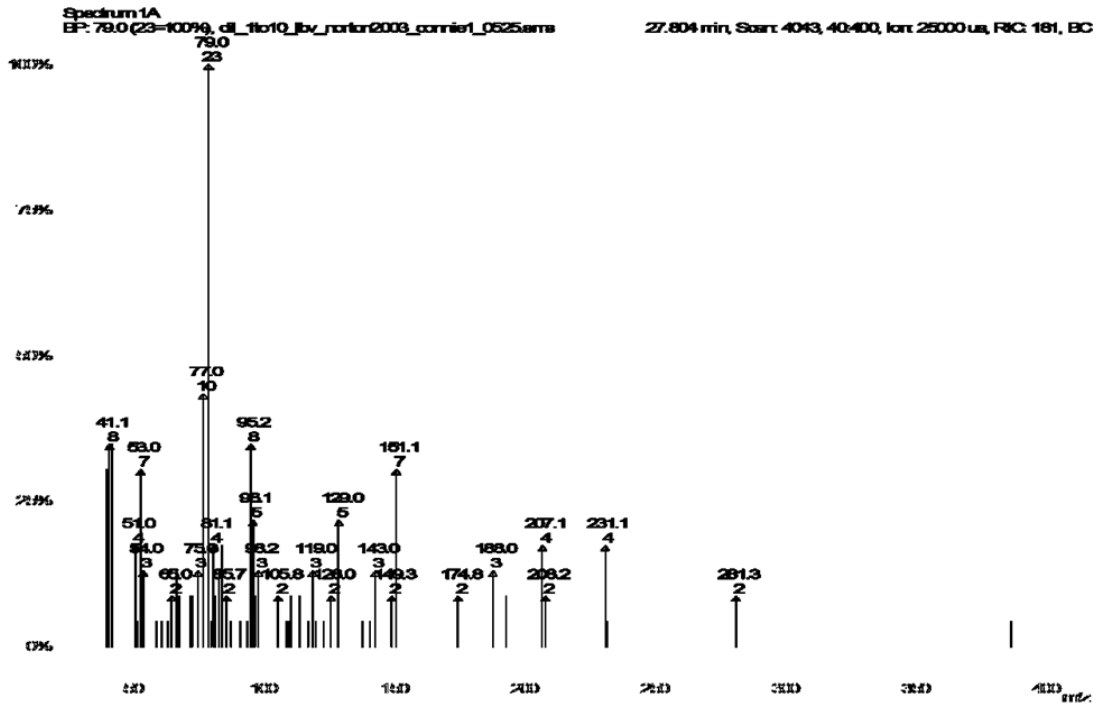
SUPPLEMENTAL MATERIAL

Supplemental Figure 1: Octanoic Acid Ethyl Ester Sprectrum

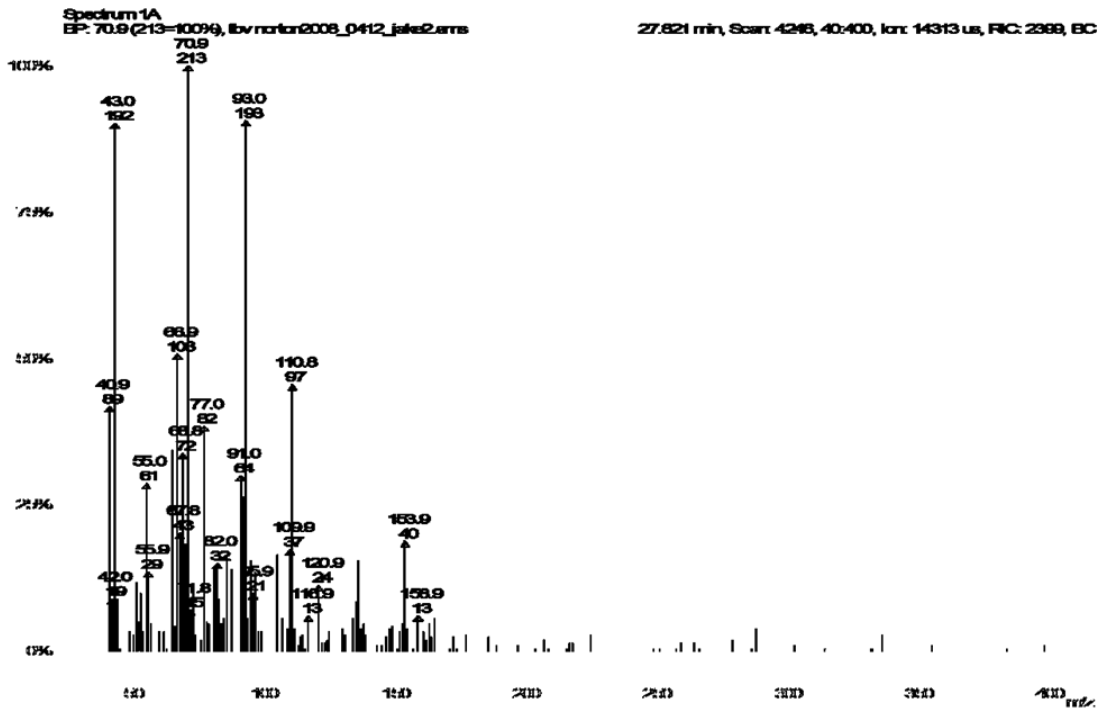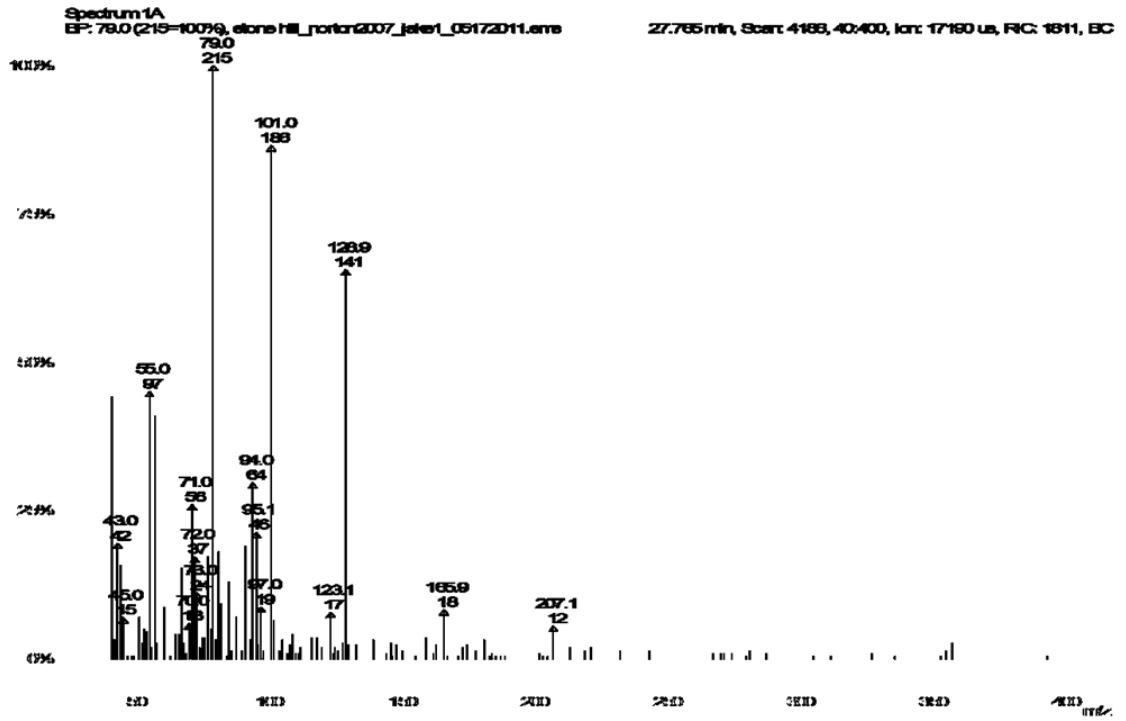**Supplemental Figure 2:** Spathulenol Spectrum
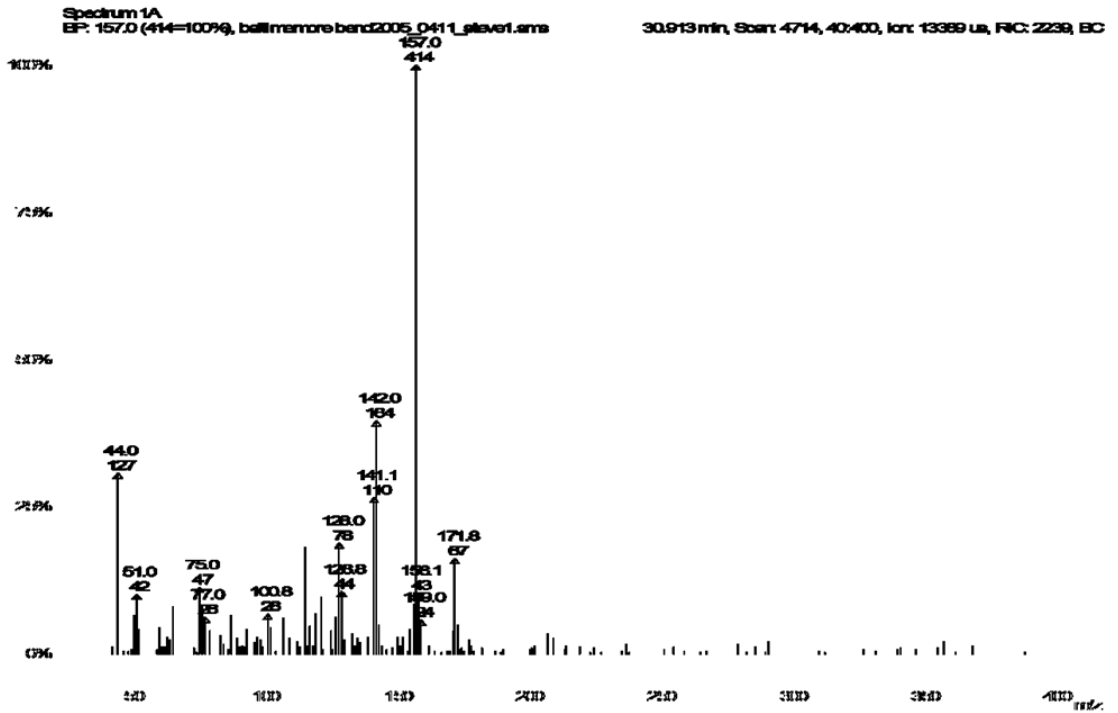
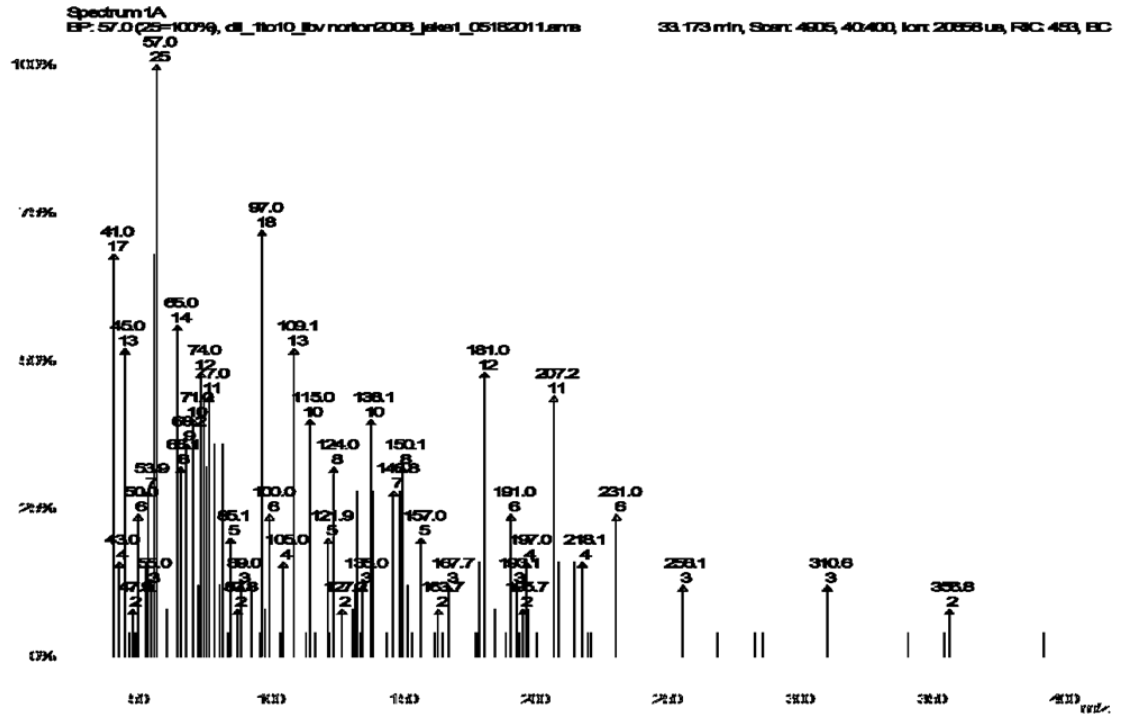**Supplemental Figure 3:** Dodecanoic Acid Ethyl Ester Spectrum

Supplemental Figure 4: Propanoic Acid, 2-methyl-,(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester spectrum
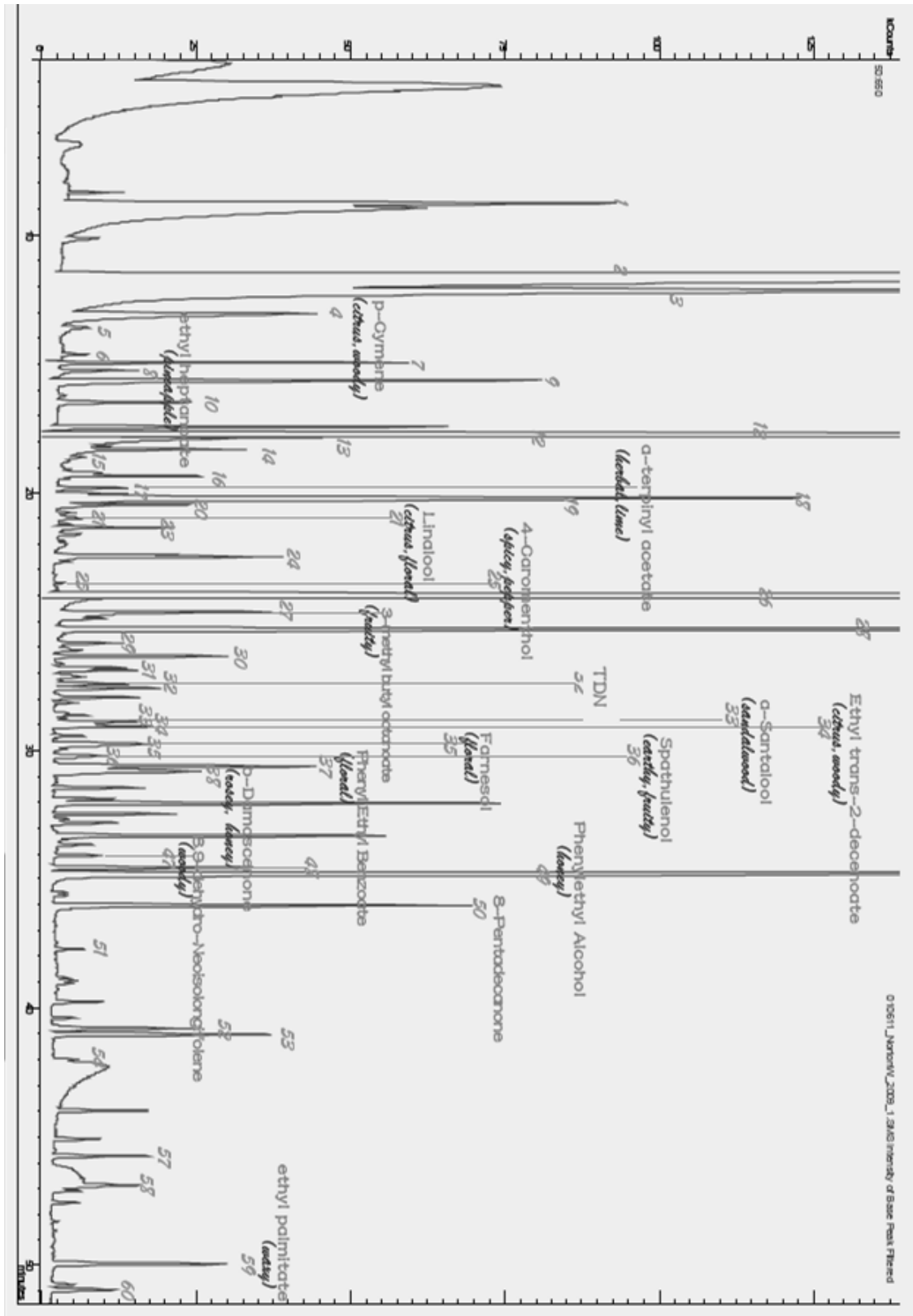
Supplemental Figure 5: Decanoic Acid Ethyl Ester

Supplemental Figure 6: Phenylethyl Acetate Spectrum

Supplemental Figure 7: Example Norton Chromatogram

Appendix Table 1: Compounds Identified in Non-Dilute Samples

Name	CAS	RI
Ethane, 1,1,1-trimethoxy-	1445-45-0	752
Ethyl Acetate	141-78-6	797
Ethane, 1,1-diethoxy-	105-57-7	798
Ethanol, 2-(1-methylethoxy)-	109-59-1	833
2-Propanol, 1-methoxy-	107-98-2	851
Propanoic acid, 2-oxo-	127-17-3	862
2,3-Butanediol	513-85-9	866
Propanoic acid, 2-methyl-, ethyl ester	97-62-1	881
Isopropyl Alcohol	67-63-0	939
Propane, 1-methoxy-2-methyl-	625-44-5	940
Ethanol, 2-methoxy-	109-86-4	990
Propanoic acid, 2-hydroxy-, methyl ester	2155-30-8	1002
Butanoic acid, ethyl ester	105-54-4	1013
3-Hydroxybutyric acid	300-85-6	1014
Oxalic acid	144-62-7	1014
Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	1029
Acetic acid, 2-methylpropyl ester	110-19-0	1033
Carbamic acid, methyl ester	598-55-0	1036
Formic acid, 1-methylethyl ester	625-55-8	1040
1-Propanol	71-23-8	1041
Butanoic acid, 3-methyl-, ethyl ester	108-64-5	1067
Pentanoic acid, 4,4-dimethyl-3-oxo-, eth	17094-34-7	1084
1-Butanol, 3-methyl-, acetate	123-92-2	1097
1-Propanol, 2-methyl-	78-83-1	1098
1-Butanol	71-36-3	1108
Acetic acid, butoxyhydroxy-, butyl ester	68575-73-5	1113
2,3-Butanediol, [S-(R*,R*)]-	19132-06-0	1165
1-Pentanol	71-41-0	1178
1-Butanol, 3-methyl-	123-51-3	1178
Hexanoic acid, ethyl ester	123-66-0	1193
Butanoic acid, pentyl ester	540-18-1	1206
(S)-(+)-1,2-Propanediol	4254-15-3	1209
Butanoic acid, 3-hydroxy-, ethyl ester	5405-41-4	1222
1-Hepten-4-ol	3521-91-3	1280
Hexadecanoic acid, ethyl ester	628-97-7	1288
Hex-4-enoic acid, ethyl ester (other iso		1296
Heptanoic acid, ethyl ester	106-30-9	1296
Decanoic acid, ethyl ester	110-38-3	1306
Propanoic acid, 2-hydroxy-, ethyl ester	97-64-3	1308
1,2-Propadiene-1,3-dione	504-64-3	1318
1-Hexanol	111-27-3	1320

Appendix Table 1: *Continued*

1-Pentanol, 4-methyl-	626-89-1	1330
Propanoic acid, 2-hydroxy-, ethyl ester,	687-47-8	1345
Octanoic acid, methyl ester	111-11-5	1355
(S)-3,4-Dimethylpentanol		1356
Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0	15120-94-2	1357
trans-1,10-Dimethyl-trans-9-decalinol		1358
3-Hexen-1-ol, (Z)-	928-96-1	1378
Octanoic acid, ethyl ester	106-32-1	1406
Ethanediamide	471-46-5	1408
Carboisopropoxy isopropoxy sulfide		1418
7,7a-Dimethyl-3a,4,5,7a-tetrahydro-3H-be		1419
Isopentyl hexanoate	2198-61-0	1425
.alpha.-Methyl-.alpha.-[4-methyl-3-pente		1437
Cyclohexanol, 2-methyl-3-(1-methyletheny	54845-29-3	1441
2-Furanmethanol, 5-ethenyltetrahydro-.al	5989-33-3	1447
2-Pentanone, 5-(2-methylenecyclohexyl)-,	22629-28-3	1455
Acetic acid	64-19-7	1457
1-Hexanol, 2-ethyl-	104-76-7	1458
E-3-Pentadecen-2-ol		1491
2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahy	4668-61-5	1496
1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(		1497
Bicyclo[3.3.1]nonan-2-one, 1-methyl-9-(1	56630-95-6	1500
Nonanoic acid, ethyl ester	123-29-5	1503
Octanoic acid, 4-methyl-, ethyl ester, (	54831-51-5	1506
Eicosanoic acid, ethyl ester	18281-05-5	1507
Geranyl vinyl ether		1512
9-Octadecenoic acid (Z)-, hexyl ester	20290-84-0	1518
Ionone	8013-90-9	1524
Butyl caprylate	589-75-3	1542
.alpha.-Caryophyllene	6753-98-6	1551
1-Octanol	111-87-5	1561
Benzene, 1,2,4-trimethoxy-5-(1-propenyl)	5273-86-9	1576
1-Octanol, 2-butyl-	3913-02-8	1585
Decanoic acid, methyl ester	110-42-9	1598
3-Nonenoic acid, ethyl ester	91213-30-8	1622
Octanoic acid, 3-methylbutyl ester	2035-99-6	1628
Nonanoic acid, 5-methyl-, ethyl ester	116530-40-6	1646
Butanedioic acid, diethyl ester	123-25-1	1647
3,3-Dimethyl-4-methylamino-butan-2-one	123528-99-4	1658
Octadecanoic acid, ethyl ester	111-61-5	1661
Ethyl 9-decenoate	67233-91-4	1662
Dimethyl-(6-methyl-2-thioxo-[1,3,2]oxath	139575-19-2	1663

Appendix Table 1: *Continued*

3-Phorbinepropanoic acid, 9-ethenyl-14-e	25145-41-9	1664
Oleic Acid	112-80-1	1675
Ethyl trans-4-decenoate	76649-16-6	1687
7-Methoxybenzofuran-2-carboxylic acid	4790-79-8	1688
2-Allyl-1,4-dimethoxy-3-methyl-benzene		1689
Aromadendrene, dehydro-		1693
2-Propanone, 1-(3,5,5-trimethyl-2-cycloh	16695-72-0	1696
2-Propenal, 3-(2,6,6-trimethyl-1-cyclohe	4951-40-0	1697
Santalol, E-cis,epi-.beta.-	14490-17-6	1705
Naphthalene, 1,2-dihydro-1,1,6-trimethyl	30364-38-6	1713
3-Cyclohexene-1-acetaldehyde, .alpha.,4-	29548-14-9	1723
cis-7-Decen-1-al	21661-97-2	1724
Farnesol	4602-84-0	1726
Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-		1730
1H-Indene,2,3-dihydro-2,2-dimethyl-	20836-11-7	1730
2H-1-Benzopyran, 3,5,8,8a-tetrahydro-2,5	72468-40-7	1734
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro	473-13-2	1736
Methyl (Z)-5,11,14,17-eicosatetraenoate	59149-01-8	1736
10,12-Tricosadiynoic acid, methyl ester		1748
(-)-Spathulenol	77171-55-2	1753
2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6	14049-11-7	1757
Benzeneacetic acid, ethyl ester	101-97-3	1758
1H-Cycloprop[e]azulen-7-ol, decahydro-1,	6750-60-3	1780
Acetic acid, 2-phenylethyl ester	103-45-7	1787
6-Propenylbicyclo[3.1.0]hexan-2-one	75283-46-4	1789
Bicyclo[4.1.0]hept-2-ene	2566-57-6	1790
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cy	23726-93-4	1792
Cyclohexanol, 1,3,3-trimethyl-2-(3-methy	69296-93-1	1795
4-Isopropenyl-4,7-dimethyl-1-oxaspiro[2.		1797
5-Hydroxy-2,3,3-trimethyl-2-(3-methyl-bu		1799
Isoaromadendrene epoxide		1799
3-(Benzylthio)propene	6937-97-9	1799
5,6-Epoxy-2,2-dimethyl-3-heptyne	212687-69-9	1799
Oxirane, 2-(hexyn-1-yl)-3-methoxymethyle		1804
Nonanoic acid, 9-oxo-, ethyl ester	3433-16-7	1805
Oxazolidine, 2-(1,1,4,8-tetramethylnona-	331275-31-1	1805
5-Sec-butylpyrogallol	56707-65-4	1806
Succinic acid, butyl ethyl ester		1806
3-Cyclohexene-1-methanol	1679-51-2	1809
3-(2-Methoxymethoxyethylidene)-2,2-dimet		1811
Santalol, cis,.alpha.-	19903-72-1	1813
Dodecanoic acid, ethyl ester	106-33-2	1815

Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9		1823
3-Cyclopenten-1-one, 2-hydroxy-3-(3-meth	69745-70-6	1824
3-Isopropyl-6a,10b-dimethyl-8-phenethyld		1829
Bicyclo[3.3.0]octan-2-one, 7-neopentylid		1833
1,3-Cyclopentanedione, 2-(1-oxopentyl)-4	69796-08-3	1834
2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.1	121841-67-6	1834
2(1H)-Naphthalenone, 3,4,4a,5,6,7,8,8a.a	28684-99-3	1834
Pentadecanoic acid, 3-methylbutyl ester	2306-91-4	1835
Dehydroxy-isocalamendiol		1853
Serverogenin acetate		1867
Succinic acid, ethyl 3-methylbutyl ester		1872
Cedran-diol, 8S,14-	62600-05-9	1876
Butylated Hydroxytoluene	128-37-0	1880
3,4-Heptadien-2-one, 3-cyclopentyl-6-hyd	63922-48-5	1880
Phenylethyl Alcohol	60-12-8	1881
E-11-Hexadecenoic acid, ethyl ester		1886
1H-Indole, 2-(1,1-dimethylethyl)-	1805-65-8	1899
1H-Indole, 2,3-dihydro-1,3,3-trimethyl-2	118-12-7	1901
Benzyl alcohol, .alpha.-isobutyl-2,4,5-t	10425-87-3	1902
3,5,7-Nonatrien-2-one, 8-methyl-7-(1-met	70372-94-0	1909
dl-Isocitric acid lactone	4702-32-3	1915
2,5-Furandione, 3-methyl-4-propyl-	16493-20-2	1922
cis-3-Methyl-4-octanolide	39638-67-0	1924
1-(4-Acetamidoanilino)-3,7-dimethylbenzo	299200-97-8	1925
Phenol, 2,4,6-tris(1-methylethyl)-	2934-07-8	1925
(7,7-Dimethyl-1-oxo-2,3,4,5,6,7-hexahydr	55085-50-2	1929
9-Hexadecenoic acid, phenylmethyl ester,	77509-01-4	1937
Aromadendrene oxide-(2)		1937
1-Cyclohexen-1-ol, 2,6-dimethyl-, acetat	6203-89-0	1937
4,4-Dimethyl-cyclohex-2-en-1-ol		1941
2-Acetylcyclopentanone	1670-46-8	1942
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	21835-01-8	1942
2,4-Octadienoic acid, 7-hydroxy-, methyl	69734-24-3	1943
10-Methyl-8-tetradecen-1-ol acetate		1945
1-Hydroxy-6-(3-isopropenyl-cycloprop-1-e		1950
6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8	86917-79-5	1951
2(3H)-Furanone, 5-butyldihydro-4-methyl-	55013-32-6	1974
cis-4-Hydroxy-3-methylundecanoic acid la	148806-09-1	1974
Tetracontane, 3,5,24-trimethyl-	55162-61-3	1977
2(1H)-Naphthalenone, 7-ethynyl-4a,5,6,7,	55220-87-6	1989
Naphthalene, 1,7-dimethyl-	575-37-1	1996
1,3,7,11-Cyclotetradecatetraene, 2-methy	61141-96-6	1996

Appendix Table 1: *Continued*

Naphthalene, 1,4-dimethyl-	571-58-4	1996
2-[3-Carbethoxypropionamide]-3,4-dicarbe	78994-93-1	2004
6-Hexadecen-4-yne, (E)-	74744-52-8	2006
Octadecane, 6-methyl-	10544-96-4	2009
Tetradecanoic acid, ethyl ester	124-06-1	2014
Naphthalene, 2,3-dimethyl-	581-40-8	2016
Nerolidyl acetate	2306-78-7	2036
Caryophyllene	87-44-5	2055
Aromadendrene	109119-91-7	2055
Hydrastininic acid	490-25-5	2057
4'-Ethoxy-2'-hydroxyoctadecanophenone	22198-51-2	2063
Octanoic Acid	124-07-2	2077
1,5-Cyclododecanediol, diacetate, cis-	2938-57-0	2078
4-Pentenoic acid, 2,2-diethyl-3-oxo-5-ph	337503-48-7	2092
10,11-Dimethyl-tricyclo[4.3.1.1(2,5)]und	174226-41-6	2099
Succinic acid, ethyl octyl ester		2108
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,	82304-66-3	2111
4'-Ethoxy-2'-hydroxyoctanophenone	22198-47-6	2117
Phenol, 4,6-di(1,1-dimethylethyl)-2-meth	616-55-7	2124
Eugenol	97-53-0	2128
2-Propenoic acid, 3-phenyl-, ethyl ester	103-36-6	2129
Phenol, 2-methoxy-3-(2-propenyl)-	1941-12-4	2129
Cyclohexanecarboxaldehyde, 3,3-dimethyl-	65080-66-2	2162
Phenol, 2-methoxy-6-(2-propenyl)-	579-60-2	2163
1-Decanol, 2-hexyl-	2425-77-6	2184
Phenol, 2-ethyl-	90-00-6	2189
Phenol, 3-ethyl-	620-17-7	2189
1-Naphthalenol, 1,2,3,4-tetrahydro-2,5,8	55591-08-7	2257
Phenol, 4-ethyl-2-methoxy-	2785-89-9	2267
Ethyl 9-hexadecenoate	54546-22-4	2273