

2014-1516 End of Year Report

## 1. Summary

**Title:** Chiral Terpenes - Quantitation, Threshold Determination and Sensory Impact on Aromatic White Wines

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The specific accomplishment of the last year for this project was the development of a quantitative method using MDGC to measure chiral terpenes in white wine. Specifically within this method it was necessary to synthesize new isotopes to ensure that measurement of specific compounds were accurate. Currently available isotopes varied in their chemical structure compared to the compounds of interests that made accuracy problematic. Two deuterated isotopes, d2-limonene and d2- $\alpha$ -pinene were successfully synthesized. The method was successfully developed and measurement of the following chiral compounds was achieved;  $\alpha$ -terpineol,  $\alpha$ -pinene, cis-rose oxide, limonene, linalool and linalool oxide. Separation for citronellol was problematic and has been removed for this method, as additional sample preparation may be required for measurement of this compound. Additionally wines from around the world have been collected and this study will have chemical analysis of a large range of Riesling and Pinot gris wines from the around the world, information that has not been done. The scale of the comparison of white varieties is also much larger than has previously been conducted, producing some ground breaking information for the wine industry and field of aroma and flavor chemistry.

**2. Report.** This is an end of year report for a continuing project that commenced in September 2013.

**3. Project title & UGMVE number:** Chiral Terpenes - Quantitation, Threshold Determination and Sensory Impact on Aromatic White Wines, 2014-1516

**4. Principal Investigator:** Elizabeth Tomasino, Ph.D. Oregon Wine Research Institute, Department of Food Science & Technology, Oregon State University, 100 Wiegand Hall, (541)-737-4866, [elizabeth.tomasino@oregonstate.edu](mailto:elizabeth.tomasino@oregonstate.edu)

**5. Objectives and experiments conducted to meet stated objectives:**

*Objective 1* – Development of methodology using multi-dimensional gas chromatography-mass spectrometry to measure chiral terpenes (limonene, linalool, citronellol,  $\alpha$ -terpineol and  $\alpha$ -pinene) in aromatic white wines.

Method to measure chiral terpenes in aromatic white wine has been completed. Method includes measurement of  $\alpha$ -terpineol,  $\alpha$ -pinene, cis-rose oxide, limonene, linalool and linalool oxide.

*Objective 1a* – Measurement of chiral terpenes in Riesling and Pinot gris wines from different region of origins.

Measurement of chiral terpenes of Riesling and Pinot gris wines is presently on-going

*Objective 1b* – Measurement of chiral terpenes in different aromatic white wine varieties

Measurement of chiral terpenes is ongoing.

*Objective 2*

Once quantitation of enantiomers is achieved the impact of the enantiomers will be investigated through a series of sensory tests.

This objective is slated to begin during Fall 2014.

*Objective 3*

Besides the importance of perception threshold the impact of each chiral terpene on wine aroma will be assessed.

This is not scheduled for 2014.

## 6. Summary of Major research Accomplishments and Results by Objective

### Objective 1

As part of the MDGC method we have achieved successful synthesis of two deuterated internal standards; d2-limonene and d2- $\alpha$ -pinene. The choice of internal standards is important for quantitative chemical analysis in order to obtain accurate measurements. In previous work the principal investigator has found that using internal standards that closely match the structure of the target compound(s) producing a more accurate and true measurement. A lack of appropriate internal standard has made measurement of limonene problematic. Deuterium replaces hydrogen atoms in chemical structures creating an isotope that has the same structure as the internal standard (figure 1) but is heavier and has a slightly different mass to charge profile (figure 2).

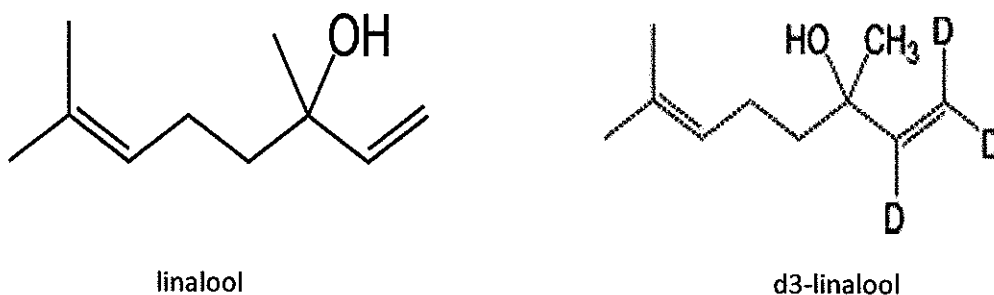


Figure 1 – Structures of linalool and d3-linalool, molecular weight 154.25 g/mol and 157.27 g/mol respectively.

Basics method parameters are detailed below (*method will be submitted for publication in fall 2014, please keep the method confidential until paper is available*)

### *HS-SPME-GC-MS Analysis of Wines*

#### Internal standard

Stock standard solutions of all chemicals were prepared in 14% (v/v) aqueous ethanol. A composite standard was created using the stock solutions. This composite contained all the non-deuterated reference compounds. A separate internal standard was prepared from the stock solutions containing the isotopically labelled (deuterated) compounds. Sub-samples of the composite standard solution were then placed in 2-mL amber vials and stored at -20°C until use. Each

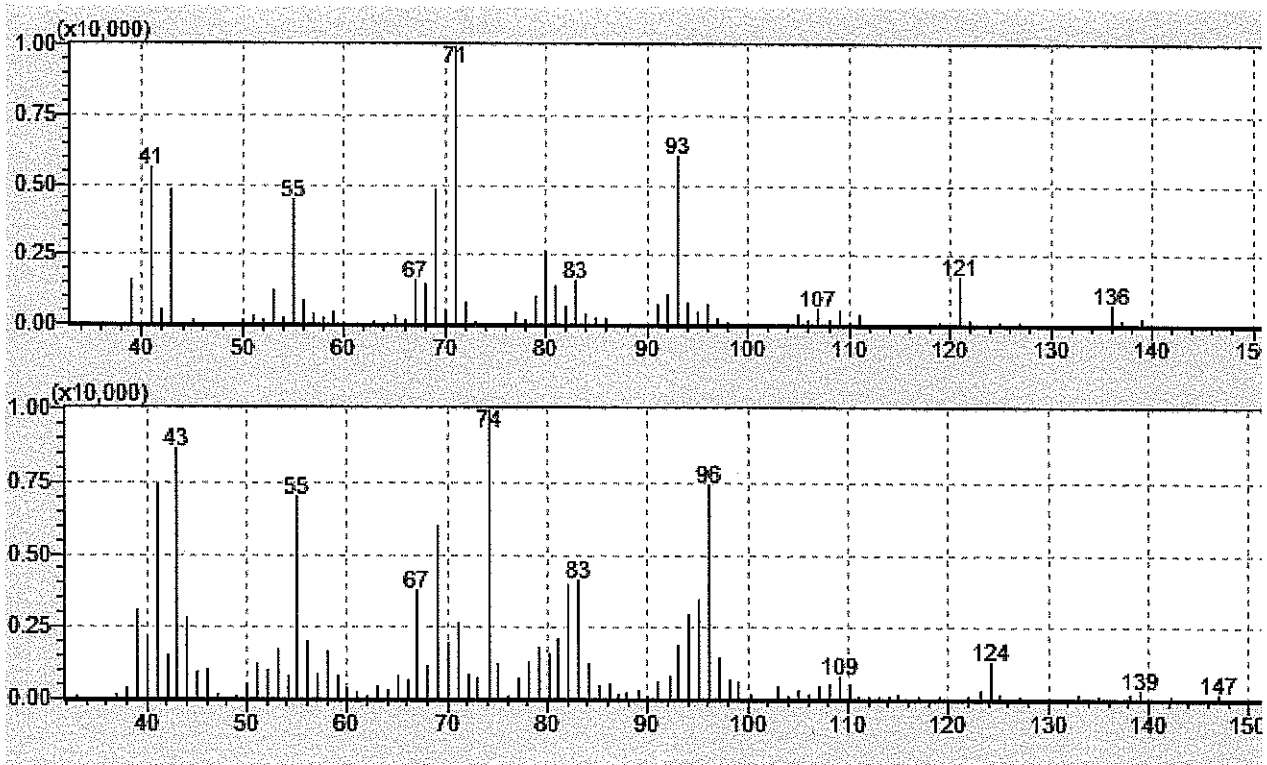


Figure 2 – Mass spectrum for linalool (top) and d3-linalool (bottom). Ions that are different in d3-linalool include 74, 96 and 124.

subsample was only used once and any excess was discarded. To prepare working standards for each analysis run, one of the composite standard sub-samples was thawed to room temperature and the required volumes needed to prepare each working standard (0 to 0.90 mL) plus the required volume (0.90 to 0 mL) of “white wine” (dearomatized white wine) were pipetted together with 8.06 mL of acidified (pH 3.5) deionised water into 20-mL amber glass, screw cap vials, 22.5 x 75.5 mm, followed by 40  $\mu$ L of the composite isotopically-labelled internal standard solution and 4.5 g sodium chloride. For analysis, frozen wine samples were brought to room temperature, diluted 10-fold with deionised water and spiked with 40  $\mu$ L of composite internal standard mix.

### *SPME Fiber and Conditioning*

A 2 cm long Stableflex DVB/CAR/PDMS combination SPME fiber (p/n 57348-U, 50/30  $\mu\text{m}$  thickness, 24 gauge) was selected for this work (Supelco Bellefonte, PA, USA). This type of fiber has been shown previously to be suitable for most VOC analyses in wine (Carrillo, et al. 2006, Ferreira and de Pinho 2003). Prior to use the SPME fiber was conditioned at 250°C in the injection port for 1 hour. Immediately prior to each sample analysis the SPME fiber was further conditioned in helium for 10 min at 250°C in a fiber conditioning station attached to the Combi-Pal auto-sampler used with the Shimadzu GC-MS instrument.

### *Chiral Terpene Method*

Samples were run on a MDGC, GC201 plus connected to a Shimadzu GC-MS-QP2010 gas chromatograph–mass spectrometer (Shimadzu Scientific Instruments, USA) equipped with a CTC Combi-Pal autosampler (CTC-Analytics AG, Switzerland). The first GC oven contained a Rtx-wax column (30m x 0.25mm ID x 0.5 $\mu\text{m}$  film thickness, polyethylene glycol, Restek). The second GC oven contained a Rt®-bDEXsm column (30m x 0.25mm ID x 0.25  $\mu\text{m}$  film thickness, 2,3-di-O-methyl-6-O-*tert*-butyl dimethylsilyl beta cyclodextrin added into 14% cyanopropylphenyl/86% dimethyl polysiloxane, Restek) connected in sequence to a Rt®-bDEXse column (30 m x 0.25 mm ID x 0.25  $\mu\text{m}$  film thickness, (2,3-di-O-ethyl-6-O-*tert*-butyl dimethylsilyl beta cyclodextrin added into 14% cyanopropylphenyl/86% dimethyl polysiloxane, Restek). The Rt®-bSEXsm and Rt®-bSEXse are columns designed specifically for chiral separation of aroma compounds. Prepared diluted samples (10-fold) were incubated and agitated for 10 minutes at 60°C and exposed to the SPME fiber at 60°C for 60 minutes. The SPME fiber was then desorbed in the GC injection port at 250°C for 10 minutes.

Both GC's used helium as the carrier gas. The first GC was set at a constant pressure of 304.7 kPa. The GC oven temperature was held at 65°C for 3 minutes then ramped to 145°C at 4°C min<sup>-1</sup> and then held at this temperature for 10 minutes, ramped to 230°C at 15 min<sup>-1</sup> and held for 15 minutes. Switching pressure was 255.0 kPa with switching windows from 6-6.5min, 9.9-11.25min, 11.5-13.75min, 18.05-18.5min, 18.8-19.5min, 21.25-22.5min, 26.25-27.75min and

29.5-31.25min. The second GC was run with a constant linear velocity of 25.5 cm/sec. The GC oven temperature was held at 40°C for 10min then ramped to 125°C at 3°C min<sup>-1</sup> and then held at this temperature for 15 minutes, then ramped to 184°C at 3°C min<sup>-1</sup> and held at this temperature for 10min, ramped to 230°C at 3°C min<sup>-1</sup> and held at this temperature for 10min. The interface and MS source temperatures were set at 230°C and 200°C respectively, with the MS source operated in electron impact (EI) mode at an ionization energy of 70 eV. The MS acquisition mode was set to full scan for all compounds. The NIST11 (National Institute of Standards and Technology) mass spectral library and reference standards composed of pure compounds were used to confirm the identities for all standards used.

#### *Objective 1a*

Analysis of chiral terpenes of Pinot gris and Riesling wines is ongoing. Currently we have collected half of the wines being analyzed in the study (in total we anticipate upwards of 200 wines). For the investigation of region of origin on chiral terpene content in Riesling and Pinot gris wines we have wines from the following locations; Australia (Margaret River, Eden Valley, Barossa Valley, Hunter Valley), Canada (mainly the Niagra region), New York (Finger Lakes), Washington, Oregon (Willamette Valley, Umpqua Valley, Columbia River, Applegate Valley, Rogue Valley), New Zealand, France (Alsace), Austria, Germany, California, Michigan and New Jersey. About half of the wines have been analyzed and statistical analysis has not yet been conducted, so we refrain from making any general remarks about chiral terpenes and region of origin at this time.

#### *Objective 1b*

As with objective 1a about half the wines have been analyzed to date, with the rest of the analysis to be completed over the next 2 months. White varieties being investigated include, Riesling, Pinot gris, Pinot grigio, Chardonnay, Pinot blanc, Sauvignon blanc, Muscat, Traminette, Seyval Blanc, Gewurztraminer, Arneis and Vermentino. At this date we can make some general remarks on the type of chiral terpenes in aromatic white wine varieties. (+) and (-) –  $\alpha$ -pinene were only found at trace levels in some of the Rieslings, at approximate 1:1 ratios. It was not found in any of the other white wines measured to date. (+) and (-)-cis rose oxide was

found in Gewurztraminer and Traminette wines. As agreement with previous work the dominant form of the terpene was (+)-cis-rose oxide (cis-(4s,2R)-rose oxide), although some differences in the ratio of enantiomers was found among the Gewurztraminer wines tested. (+) and (-)-limonene were found to vary in the wines, with higher concentrations found in dryer styles of wine (low residual sugar). The ratio of enantiomers varied based on wine varietal with Gewurztraminer wines containing about twice the amount of (-)-limonene than (+)-limonene. Pinot gris was found to contain the opposite ration with more (+)-limonene. Riesling, Muscat and Traminette contained very low levels of limonene. Of the four linalool oxide enantiomers measured there were various ratios in the different wines and within the same varietals. However in general dry Rieslings only contained the furanoid form of linalool oxide and gewürztraminer and Muscat wines contained high levels of all four enantiomers. The ratio of alpha-terpineol enantiomers were also found to vary between the wine sin each varietal. Previous work has shown that sugar content may be linked to terpene concentration, as these compounds are bound to glycosides in grapes. We are also measuring residual sugar in the wines to determine if this may be a driving force off enantiomers in the wines. Again full results will be presented in the next report when all wines have been analyzed.

## **7. Outside Presentation of Research**

None to this date

## **8. Research Success Statements**

This research project will provide important information based on the highly “aromatic” compounds that are drivers of aromatic white wine styles. Specifically it has the potential to provide markers for terroir and will also provide accurate information on the “aroma active” concentrations in wines. Much of this information is currently based on thresholds measurements that were not conducted in wine, and have been found to be unrepresentative of wines. All of this information provides excellent information to the grape and wine industry where consistent quality is a goal, a difficult prospect with ever changing climate.

**9. Funds Status:** Only partial funds were awarded for 2013-2014. These were used to support a graduate student that was hired to complete this work. Additional funds for chemical compounds were used from the PI's start-up funds (unavailable after June 2014). All wines in the project were generously donated from the producers.