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Chemo-diversity of chiral monoterpenes in different styles of Riesling wine from different regions

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ABSTRACT

Monoterpenes are important characteristic compounds for aromatic white wines, including Riesling, but their enantiomer composition has been little explored in wine. Enantiomers can differ depending on region and style, as they are sensitive to environmental factors, and thus could be used for wine authentication. Thirteen monoterpenes were quantified by HS-SPME-MDGC-MS in fifty-four commercial Riesling wines from three wine styles (dry, medium dry and medium sweet) and four well-established wine-growing regions in Germany, France (Alsace) and the USA (New York and Oregon). Significant differences were found for nine out of the 13 enantiomers among different regions and eight enantiomers among styles. X-Y scatterplots of enantiomer pair concentrations, with excellent fitted lines, implies low variation of enantiomeric ratios from each region. The study suggests that wines from different regions and styles were differentiated by chiral monoterpenes. Chiral monoterpenes analyses could provide supporting information in Riesling wine authentication by offering an objective measure of flavour quality, as these compounds are key compounds for Riesling aroma and flavor.

KEYWORDS: SPME-MDGC-MS, enantiomer fraction, white wine, discriminant analysis
INTRODUCTION

Riesling is one of the world’s most widely-planted white grape varieties, with a total of more than 60,000 hectares worldwide since 2015 (German Wine Institute, 2019). The first documentation of Riesling cultivation was around 1350 AD in the Rhine Valley (Liu et al., 2008; Sechrist, 2012). The Mosel and Rhine rivers of Germany and the Alsace region of France are considered to be the world’s top Riesling regions (Jacobson, 2006). Riesling is also grown in new world regions, including New Zealand, Australia, and New York and Oregon in the United States, as well as other locations since 1955 (Sechrist, 2012).

Riesling is the result of a cross of Heunisch (the dominant cultivar) and Vitis sylvestris (wild cultivar) (Anhalt et al., 2011). Riesling wines are very diverse, as the composition of grapes can be altered by environmental characteristics and viticultural practices (Kozina et al., 2008; Myers et al., 2013; Zanzotti et al., 2021). The sensitivity of the grapes to environmental factors (e.g., the climate and soil in which the vines are grown) results in distinctly different flavours in the wines. It has been reported that Riesling wines from Ontario, Canada, showed distinctly different sensory profiles based on two terroirs: ‘bench’ and ‘plains’. Wines from ‘bench’ had greater lemon/lime aroma than those from ‘plains’ (Douglas et al., 2001). Riesling wines can also be characterised according to viticulturalpractices, specifically relating to residual sugar and ripeness at harvest; e.g. dry, medium dry, medium sweet or sweet wines, and the wines from the German Prädikatswein classifications (Sweet, 2009; Krebiehl, 2019).

There has been growing interest in monoterpane compounds due to the important impact of these compounds on varietal distinctiveness (Peña et al., 2005). Additionally, understanding grape-derived wine aroma compounds (e.g., monoterpenes) has been a keystone in understanding wine flavour chemistry for many years (Ribéreau-Gayon et al., 1975); for example, monoterpenol alcohols can add subtle floral and citrus aromas to Riesling wines (Peña et al., 2005). cis-Rose oxide, citronellol, linalool and α-terpineol have been reported to be important monoterpenes in Riesling wines that underwent more than three years of aging (Black et al., 2015). Simpson and Miller (1983) found linalool, hotrienol and α-terpineol to be major monoterpenes in young Riesling wines, with concentrations decreasing with age (Simpson and Miller, 1983; Strauss et al., 1986).

Enantiomer differentiation is of interest, because enantiomers display different aroma descriptors and sensory detection thresholds (Bentley, 2006). Enantiomeric composition has been used to assess information about foods by geographic origin (Ebeler et al., 2001; Marchelli et al., 1996) and the authenticity of fruit beverages (Ebeler, 2007; Ruiz del Castillo et al., 2003). Region of origin is an important factor for many wine consumers. In Alsace, wine quality categories on the label are determined by a geographic classification system and are used to ensure wine quality (Fischer et al., 1999).

As the adulteration of wine becomes more and more sophisticated, there is a need for accurate methods for wine characterisation to prevent this, and to ensure that wines are from the stated geographical origins or countries (Cordella et al., 2002; González and Peña-Méndez, 2000; Kallio et al., 1915; Liu et al., 2008; Versari et al., 2014).

Rapp et al. (1978) have shown that the ratios of the various monoterpenes can be used to not only distinguish one cultivar from another but also to differentiate cultivars according to region or origin. Such ‘terpene profiles’ are useful for the separation of Riesling wines from other so-called Riesling wines (e.g., Welsch Riesling, Kap Riesling and Emerald Riesling). Rapp (1998) reported a significant differentiation between Riesling and Welsch Riesling from different growing regions (Austria, Italy and Yugoslavia) based on an analysis of their terpene profiles; specifically, significantly higher concentrations of select ed monoterpen compounds (e.g., linalool, trans-linalool oxide and α-terpineol) were present in true Riesling wines compared to Welsch Riesling. The objective of this study was to determine the effect of regions and styles on chiral monoterpine profiles and enantiomer fractions of Riesling wines to determine whether monoterpane enantiomers can be used for wine authentication.

MATERIALS AND METHODS

1. Chemicals

Standards of S-(-)limonene (≥ 99.0 %), R-(+)limonene (≥ 99.0 %), furanoid linalool oxide (≥ 97.0 %), and R-(+)linalool (≥ 98.0 %) from Sigma Chemical Co. (St. Louis, MO, USA) were used to check the elution order of the isomers. Linalool (≥ 97.0 %), R-(+)α-terpineol (≥ 97.0 %) and R-(+)β-citronellol (98.0 %) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). S-(+)α-Terpineol (96.0 %) was obtained from BOC sciences (Ramsey Road, Shirley, NY, USA) and nerol oxide (99.0 %) from ALFA chemistry (Waverly Avenue, Holtsville, NY, USA). The isotopic standards d3-(-)α-terpineol and d3-(+)-linalool (≥ 99.4 %) were purchased from CDN Isotopes (Pointe-Claire, QC, Canada), while d3-(-)limonene was synthesised as described in Song et al. (2015). Milli-Q water was obtained from a Millipore Continental water system (EMD-Millipore, Billerica, MA, USA). HPLC grade ethanol came from Pharmco-AAPER (Vancouver, WA, USA). Sodium chloride (> 99 %) was supplied by J.T. Baker (Avantor® Performance Materials, PA, USA).

2. Sample Preparation and Headspace Solid Phase MicroExtraction Multidimensional gas Chromatography Mass Spectrometry (HS-SPME-MDGC-MS)

The wine sample preparation and quantification method by HS-SPME-MDGC-MS for S-(-)limonene, R-(+)limonene, (2R, 5R)-(+)-trans-linalool oxide, (2R, 5S)-(-)-cis-linalool oxide, (2S, 5S)-(-)-trans-linalool oxide, (2S, 5R)-(+)-cis-linalool oxide, R-(+)linalool, S-(+)linalool, S-(-)α-terpineol, R-(+)α-terpineol and R-(+)β-citronellol can be found in Song et al. (2015).
HS-SPME was conducted using a Shimadzu AOC-5000 plus autosampler fitted with a stack cooler. A three-phase StableFlex SPME fiberTM (50/30 μm DVB/CAR/PDMS, 2 cm, 24 Ga, Supelco®) was used for SPME. The heart-cut-MDGC-MS analyses were performed using a Shimadzu GC-2010 QCMS with a heart cutting dean switch (Shimadzu, USA). The first column was a Rtx-wax column (30m, 0.2 mm ID, 0.5 μm film thickness, Restek Corportation, Bellefonte, PA, USA) and the second GC contained two columns connected in sequence: an Rt®-βDEXsm and an Rt®βDEXxe (60 m, 0.25 mm ID, 0.25μm film thickness, Restek Corportation). The isomers were quantified using the standard curves and the stable isotope dilution analysis method (Siebert et al., 2005). All of the wines were run in three groups based on their styles (dry, medium dry and medium sweet) according to the wine label or available information for instrument analysis. Each wine was analysed in triplicate: three samples per bottle of wine. The standard curves for each wine style were run in the de-aromatized corresponding wine matrices respectively in order to minimise the matrix effects, and they were run in each batch to minimise any SPME fiber effects (Song et al., 2015). Additional details of the method and analysis parameters can be found in Song et al. (2015).

3. Wines

Fifty-four commercial Riesling wines (2012 vintage) with three wine styles, dry, medium dry and medium sweet, were randomly selected from five well-established regions: old world producers from Germany (DE), France (FR; Alsace), and new world producers from the United States (NY; Finger lakes region and OR; Willamette valley AVA) (Table 1). Sample sizes were not well-balanced in terms of styles as differences were found between the style stated on the label and the actual style based on residual sugar measurements. To avoid matrix effects, five different styles were run in each batch to minimise any matrix effects (Song et al., 2015). Moreover, medium sweet wine could not be obtained from Alsace during the course of this study. Alsace typically produces many dry style Rieslings and researchers had difficulty obtaining medium-sweet French wines in the USA. Wine style was categorised according to the Regulation of the European Community no 753/2002 (Commission Regulation, 2002), with revisions based on the measured residual sugar content. Wine can be considered as dry, medium dry or medium sweet when they have the following residual sugar contents: less than 4 g/L, between 7 g/L and 12 g/L and between 12 g/L and 45 g/L respectively. One bottle of each wine was purchased in April 2014. We chose to study as many different individual wines as the funding would allow, rather than multiple bottles of the same wine. Upon arrival, the wines were stored in the OSU wine cellar at 8 °C for 1 month. The bottles were then opened, and samples of the wine were poured into three 40 mL amber vials (Supelco®, Bellefonte, PA, USA) and three 50 mL centrifuge tubes (VWR International Corp. Visalia, CA, USA) and then stored at -20 °C until analysis.

### Table 1. Distribution of Riesling wines across region of origin, vintage and wine style.

<table>
<thead>
<tr>
<th>Region *</th>
<th>Vintage</th>
<th>Dry</th>
<th>Medium dry</th>
<th>Medium sweet</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR</td>
<td>2012</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>2012</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>NY, USA</td>
<td>2012</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>OR, USA</td>
<td>2012</td>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>


4. Statistical Analysis

General linear models (GLM) were used to study the effect of region and wine style on chiral monoterpene profiles or enantiomer fraction of Riesling wines. Statistics were carried out using IBM® SPSS® statistic 20 (SPSS Inc., Chicago, IL). Linear discriminant analysis (DA) was carried out using XLSTAT 2014. 6. 01 software (Addinsoft, New York, USA).

## Results

1. Separation, Identification and Quantification of Chiral Monoterpenes in Riesling Wines

Thirteen chiral monoterpenes were investigated in 54 bottles of Riesling wines from four regions and three wine styles (Figure 1). Significant differences were found on chiral monoterpenes between regions and styles (Table 2). However, no interaction effect of region and style was found. (+)-Limonene, (-)-limonene, (+)-α-terpineol and (+)-α-terpineol were not found to be important for regional differentiation. German wines contained higher concentrations of (+)-linalool, (-)-linalool and R-(+)-β-citronellol. The wines from New York and Alsace had very similar chiral monoterpene profiles composed primarily of linalool and nerol oxides and R- (+)-β-citronellol. Oregon wines contained high concentrations of all oxide isomers.

The significant differences found between the three styles from all the regions were due to all monoterpene enantiomer concentrations, except (+)-limonene, (+)-α-terpineol and (2S, 5R)-(+) cis-linalool oxide. In the considered wine from the 2012 vintage, medium dry wines contained the highest concentrations of isomers compared to the other two styles in Rieslings from France, Germany and New York. Medium sweet Riesling from Oregon had the highest concentrations of the three wine styles investigated. In addition, within the French wines from Alsace, the concentrations of linalool oxide enantiomers were significantly higher in the medium dry wines compared to the dry wines, and monoterpene alcohols had low concentrations in the medium dry wines. In the German wine samples from the 2012 vintage, there were no significant differences for any of the isomer concentrations among the three styles.

However, medium dry wines had the highest concentrations for most of the isomers, except for (2R, 5S)-(+) cis-linalool oxide, (2S, 5R)-(+) cis-linalool oxide and R-(+)-β-citronellol.
<table>
<thead>
<tr>
<th>Isomers</th>
<th>FR</th>
<th>DE</th>
<th>NY</th>
<th>OR</th>
<th>F value (significance)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Medium dry</td>
<td>Medium dry</td>
<td>Medium sweet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>Medium dry</td>
<td>Medium sweet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>Medium dry</td>
<td>Medium sweet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>Medium dry</td>
<td>Medium sweet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-(-)limonene</td>
<td>1.42±0.32</td>
<td>1.50±0.51</td>
<td>1.72±0.07</td>
<td>2.58±0.64</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>R(+)-limonene</td>
<td>0.78±0.50</td>
<td>0.74±0.28</td>
<td>0.81±0.19</td>
<td>1.55±0.04</td>
<td>0.37±0.10</td>
</tr>
<tr>
<td>Limonene EF*</td>
<td>0.65±0.08</td>
<td>0.68±0.19</td>
<td>0.68±0.23</td>
<td>0.67±0.32</td>
<td>0.72ab±0.29</td>
</tr>
<tr>
<td>(2R,5R)+-trans-linalool oxide</td>
<td>35.26±3.55</td>
<td>62.93b±5.09</td>
<td>28.91±2.64</td>
<td>38.89±5.51</td>
<td>28.56±3.11</td>
</tr>
<tr>
<td>(2S,5S)-trans-linalool oxide</td>
<td>6.68±1.06</td>
<td>15.56b±2.49</td>
<td>3.62±1.04</td>
<td>5.00±1.14</td>
<td>4.27±0.79</td>
</tr>
<tr>
<td>trans-linalool oxide EF*</td>
<td>0.82±0.02</td>
<td>0.80±0.01</td>
<td>0.86±0.03</td>
<td>0.91±0.02</td>
<td>0.86±0.05</td>
</tr>
<tr>
<td>(2R,5S)+-cis-linalool oxide</td>
<td>10.06±2.11</td>
<td>23.55b±4.56</td>
<td>19.37±6.35</td>
<td>9.75±1.01</td>
<td>9.08±2.59</td>
</tr>
<tr>
<td>(2R,5S)-cis-linalool oxide</td>
<td>16.06±2.48</td>
<td>39.05b±7.46</td>
<td>14.06±1.07</td>
<td>11.85±2.52</td>
<td>8.62±1.82</td>
</tr>
<tr>
<td>cis-linalool oxide EF*</td>
<td>0.63±0.01</td>
<td>0.62±0.00</td>
<td>0.41±0.04</td>
<td>0.47±0.05</td>
<td>0.50±0.02</td>
</tr>
<tr>
<td>R(+)-nerol oxide</td>
<td>20.85±4.55</td>
<td>32.41±2.64</td>
<td>11.96±2.00</td>
<td>16.31±1.71</td>
<td>11.33±1.45</td>
</tr>
<tr>
<td>R(+)-nerol oxide</td>
<td>22.58±6.05</td>
<td>36.00±5.19</td>
<td>12.69±1.89</td>
<td>16.84±2.83</td>
<td>12.61±1.82</td>
</tr>
<tr>
<td>Nerol oxide EF*</td>
<td>0.47±0.05</td>
<td>0.47±0.02</td>
<td>0.48±0.02</td>
<td>0.48±0.04</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>R(+)-terpinol</td>
<td>5.34±1.84</td>
<td>11.9±0.34</td>
<td>16.75±4.21</td>
<td>30.11±3.35</td>
<td>5.19±0.98</td>
</tr>
<tr>
<td>S-(-)-terpinol</td>
<td>4.86±0.47</td>
<td>1.15±0.26</td>
<td>15.32±2.39</td>
<td>27.25±6.08</td>
<td>7.80±1.18</td>
</tr>
<tr>
<td>Linalool oxide EF*</td>
<td>0.44±0.04</td>
<td>0.39±0.03</td>
<td>0.52±0.01</td>
<td>0.46±0.00</td>
<td>0.50±0.02</td>
</tr>
<tr>
<td>S-(-)-terpineol</td>
<td>26.98±4.70</td>
<td>24.69±4.29</td>
<td>34.30±6.41</td>
<td>45.61±6.84</td>
<td>20.91±2.30</td>
</tr>
<tr>
<td>R(+)-terpineol</td>
<td>22.64±6.40</td>
<td>21.00±5.89</td>
<td>24.92±7.12</td>
<td>38.75±6.57</td>
<td>14.73±2.33</td>
</tr>
<tr>
<td>terpineol EF*</td>
<td>0.55±0.11</td>
<td>0.54±0.05</td>
<td>0.59±0.04</td>
<td>0.60±0.02</td>
<td>0.60±0.01</td>
</tr>
<tr>
<td>R(+)-citronellol</td>
<td>0.39±0.08</td>
<td>0.42±0.12</td>
<td>2.02±0.29</td>
<td>1.46±0.31</td>
<td>0.01±0.00</td>
</tr>
</tbody>
</table>

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Tukey HSD letters are compared across the row for each isomer.

* Significant difference level: * p < 0.05, ** p < 0.01, *** p < 0.001.

Enantiomeric fractions are calculated as follows: S-(-)limonene/total, (2R, 5R)+-trans-linalool oxide/total trans, (2R, 5S)-cis-linalool oxide/total cis, S-(-)nerol oxide/total, R(+)-linalool/total, S-(-)-terpinol/total.
In terms of linalool enantiomers in New York wines, significant differences were found between the medium dry wines and the dry and medium sweet wines. All the different styles of wines from Oregon showed significant differences for all oxide isomers and $R\cdot(+)-\alpha$-terpineol in terms of their concentration. The medium sweet wines had the highest concentrations of oxide isomers and $R\cdot(+)-\alpha$-terpineol, while the dry wines had the highest concentration of the other isomers.

The distribution of chiral monoterpenes as affected by region and style can be visualised in a DA plot. DA was performed on isomer concentrations (mean of the three replicates) from all samples. Ninety-four percent of the variation was obtained in the first two discriminant functions for the regions, with $F_1$ and $F_2$ contributing 67% and 27% respectively (Figure 2). The Alsace and New York wines were separated from the other wines along the $F_1$ axis. The New York wines were characterised by (+)-limonene, (-)-limonene, (+)-$\alpha$-terpineol and (-)-$\alpha$-terpineol compounds. The German wines had high negative scores along the $F_2$ axis, characterised by (+)-linalool, (-)-linalool and $R\cdot(+)-\beta$-citronellol isomers, whereas the Oregon wines on the opposite side of the $F_2$ axis were characterised by all of the oxides, especially (+)-linalool oxides.

Likewise, two statistically significant discriminant functions were obtained for the styles, with $F_1$ and $F_2$ contributing 72% and 28% respectively (Figure 2). All three styles were significantly separated from each other into three of the four quadrants of the DA. The medium dry wines were on the positive side of $F_1$, separated from the other two styles and characterised by the majority of the isomers. The dry and medium sweet wines were separated by the $F_2$ axis and characterised by lower $R\cdot(+)-\beta$-citronellol concentration in the medium sweet wines. $\alpha$-Terpineol enantiomers were not an important variable for differentiating wines by style.

2. Quantification of Enantiomer Fractions in Riesling Wines by Region and Style

Enantiomer fractions (EF) were calculated by dividing the concentration of the first eluting enantiomer in the chromatogram by the total enantiomer concentration of each monoterpene (Harner et al., 2000). The effect of region, style and interaction of region × style on enantiomer fractions was analysed by a general linear model. Significant differences were found between the regions, but not in terms of style and interaction effect (Table 2). For example in New York and Oregon wines, $(2R, 5S)$-(-)-cis-linalool oxide EF and $R\cdot(-)$-linalool EF were significantly higher in the medium dry New York wines, $(2R, 5R)$-(-)-trans-linalool oxide EF and $S\cdot(-)$-terpineol EF had the highest values in the medium dry Oregon wines. The $S\cdot(-)$-nerol oxide and $R\cdot(-)$-linalool concentrations observed from all regions and styles were lower or equal to the corresponding enantiomers (EFs ≤ 0.50). Likewise, $(2R, 5S)$-(-)-cis-linalool oxide was lower or equal to the enantiomer pair in the German wines.
The differences in enantiomer fractions between regions can be seen vividly in the X-Y scatterplots of six enantiomer pairs (Figure 3). The fitted lines with slope and R² were plotted for all the wines from the same region. The slope was used to compare the similarity of enantiomer fractions in the different regions (there were no significant differences in styles). As can be seen in Figure 4.3, the slopes for the four regions in (2R, 5R)(+)-trans-linalool oxide and (2R, 5S)(-)-cis-linalool oxide pairs were quite different. The larger slope from Alsace wines compared to the German wines implied that the latter had greater (2R, 5R)(+)-trans-linalool oxide EF. The results from the GLM analysis showed that there were significant differences for S-(−)-limonene EF and S-(−)-α-terpineol EF between regions. All of the regions contained similar S-(−)-nerol oxide and R-(−)-linalool EFs.

The coefficient of determination, R², indicates the variation of enantiomeric ratios in each region (higher R² values indicate less variations). Most of the regions showed high R² values in the enantiomer pairs, with R² values greater than 0.8, except for (2R, 5R)(+)-trans-linalool oxide pair and (2R, 5S)(-)-cis-linalool oxide pair. Low R² values were found for (2R, 5R)(+)-trans-linalool oxide pair for the Alsace, New York and Oregon wines and (2R, 5S)(-)-cis-linalool oxide pair for France, Germany and New York; this was due to one data point in the fitted line that deviated from the others in each region. This implies that there was only one wine in each region that had significantly different EFs for these two pairs (data now shown). However, (2R, 5R)(+)-trans-linalool oxide EF for the German wines did vary. The wines from New York and Germany showed lower R² values (lower than 0.68) in these two pairs, but higher R² values (greater than 0.97) in the other pairs. Less variation was found among regions and styles in terms of enantiomer fractions compared to differentiation in terms of chiral monoterpene content (Figure 2 & 4).

Two statistically significant discriminant functions were obtained for all the regions, with F1 and F2 contributing 87% and 11% respectively. The German wines were separated from the others along the F1 axis. S-(−)-limonene, S-(−)-α-terpineol, (2R, 5R)(+)-trans-linalool oxide and R-(−)-linalool EFs had positive loadings along F1 (Figure 4.4), indicating greater amounts of these enantiomer fractions in the German wines. Alsace, New York and Oregon had similar EFs, characterised by (2R, 5S)(-)-cis-linalool oxide EF. The opposite position of (2R, 5R)(+)-trans-linalool oxide EF and (2R, 5S)(-)-cis-linalool oxide EF on the plot indicated that these two EFs were important variables in the classification of regions. No significant differences were found in the three styles in terms of EFs.

**FIGURE 2.** Linear discriminant plot on concentration of chiral monoterpene contents of Riesling wines in regions and styles.

Panel A, regions scores were represented by centroids surrounded by 95% confidence regions (the solid circle). Panel B, all isomers vectors were shown based on regions. Panel C, wine styles scores were represented by centroids surrounded by 95% confident regions. Panel D, all isomer vectors were shown based on wine style.
The amount of each chiral monoterpene across all Rieslings has been determined in previous work. Webster et al. (1993) reported that linalool oxides were the dominant monoterpenes in Riesling wines from 1986, 1987 and 1988. In the present study, (2R, 5R)-(−)-trans-linalool oxide had the highest mean concentration (46.54 µg/L). Our work also shows that R-(−)-β-citronellol concentrations in the analysed 2012 vintage wines were the lowest concentration (0.47 µg/L). Dziadas and Jeleń (2010) reported α-terpineol to be one of the most dominant monoterpenes in 2006 and 2007 Riesling wines from Alsace, Germany and Hungary. Of the monoterpenes, this compound was not dominant in the wines of our study; this may be due to differences in vintage or region. The amount of cis/trans-linalool oxides was similar to those previously measured in other Riesling wines (Schüttler et al., 2015). It is also important to note that all of the wines were from the same vintage. The content of the measured monoterpenes is known to change as wine ages,

**FIGURE 3.** X-Y scatterplots of enantiomer pair concentrations (µg/L) in the 4 regions with fitted linear lines.

**DISCUSSION**

...
and therefore differences between our results and those of other work may be due to differences in wine age (Black et al., 2015).

The sensitivity of Riesling grape to environmental factors results in the production of wines with distinctly different flavours. The differences in monoterpene enantiomers depending on region are most likely due to a combination of environmental factors and choice of Riesling clone. Monoterpene biosynthesis in Riesling has been shown to be very sensitive to a range of environmental factors, including soil, light and grape cultivar (Burbott and Loomis, 1967; Friedel et al., 2016; Godshaw et al., 2019; Marciniak et al., 2017; Sweet, 2009). Grape genetics has also shown that different grape clones can result in different aroma composition of the same grape variety (Marais and Rapp, 2017; Versini et al., 1990). Therefore, the differences between monoterpene isomers in wines from different regions are most likely due to the specific environment of a given location and choice of grape clone. Winemaking choices may also impact the monoterpene content of wines, as the choice of yeast strain, the application of maceration and the addition of enzymes can all alter, and in many cases increase, the monoterpene content of wines (Baron et al., 2017; Kim and Park, 2017; Rossouw and Bauer, 2016; Schmidt, 2016). While we cannot pinpoint the exact environmental or winemaking factor that contributes to the differences in monoterpene enantiomer in this study, the fact that we saw such clear regional separations suggests that general environmental factors may be the cause of differences between regional wines.

The differences in monoterpene enantiomers in terms of wine styles are most likely due to harvest decisions or winemaking practices. Harvest dates for Riesling in Germany for the different wine styles are based on grape sugar content (Cole, 2010); grapes left on the vine longer contain more sugar. Higher temperature and solar exposure increase the monoterpene contents of grapes (Marais et al., 1999). Therefore, grapes used in sweeter wines will have higher monoterpene content than dryer wines. Additionally, some winemaking practices, such as choice of yeast strain, can alter the terpene content, which can differentiate the wines according to the desired wine style. Dessert Riesling fermentations have been found to contain a diverse array of both yeast and bacteria prior to alcoholic fermentation (Erasmus et al., 2004; Sipiczki et al., 2010), which can alter the monoterpene content.

**FIGURE 4.** Linear discriminant plot on monoterpene enantiomer fractions of Riesling wines in regions and styles.

Panel A, regions scores were represented by centroids surrounded by 95% confidence regions (the solid circle). Panel B, all enantiomer fractions vectors were shown based on varietal wines. Panel C, wine styles scores were represented by centroids surrounded by 95% confidence regions. Panel D, all enantiomer fractions vectors were shown based on wine style.
The chiral monoterpenes of the regional Rieslings measured in this study were found at concentrations both above and below their known olfactory perception thresholds, thus potentially being able to affect aroma perception and wine quality. For example, the Oregon wines contained higher concentrations of all oxide isomers, which have high aroma threshold values (Garneau et al., 2006) and therefore may not contribute as much to aroma perception as other terpenes, such as (+)-linalool, (-)-linalool and R-(+)-β-citronellol isomers, which have low aroma perception thresholds (Garneau et al., 2014; Gunata et al., 1988). The dominant monoterpenes in each region may contribute to specific aromas of these wines (Marais, 1983). Even those found at concentrations below their known thresholds may impact aroma, as combinations of different monoterpenes both at concentrations below and above their known perception thresholds in Pinot gris have been found to influence aroma perception (Tomasino et al., 2020).

The enantiomeric ratios of key aroma compounds have been successfully used to distinguish between natural and synthetic food products, to define geographic area and to identify plant variety (Ebele et al., 2001; Weber et al., 1995). Therefore, enantiomeric ratios could offer an interesting alternative to conventional flavour analysis methods for product authentication (Ebele et al., 2001; Marais and Rapp, 2017; Ruiz del Castillo et al., 2003). Enantiomeric fractions have successfully been used to determine the age of Bordeaux dessert wines with the R enantiomer of 2-nonen-4-olide dominating in aged wines and the S form more dominant in younger wines (Stamatopoulos et al., 2016). We attempted to determine whether region of origin is linked to EFs. Each measured monoterpane had similar enantiomeric fractions of linalool oxides. The low R2 values for linalool oxide EFs may be due to racemisation or rearrangement at wine pH (Park and Noble, 1993; Strauss et al., 1986). The sensitivity of some chiral monoterpenes to acidic conditions and to increases in temperature and storage time may cause the variation in linalool oxide concentrations (Whittaker, 1972). Linalool can easily be oxidised via an epoxide to form four linalool oxides (Ribéreau-Gayon et al., 1975). Linalool oxide has been reported to increase in wine stored at room temperature compared to that stored at 10 °C (Strauss et al., 1986). Similar reports in Riesling wines during aging have been observed in other research (Di Stefano, 1985; Strauss et al., 1986).

The storage time and conditions prior to purchase of the wines in this study are unknown. Despite the low R2 values for linalool oxide, the fact that the majority of EFs for the other monoterpenes had high R2 values suggests that, despite this potential variation, Riesling wines can be authenticated using chiral monoterpane EF. However, regional and stylistic differentiation had more variation when using overall enantiomer content, suggesting that enantiomeric content could be a better measurement for region and wine style than EF.

**CONCLUSIONS**

The results of this study suggest that it is possible to classify Riesling wines according to their geographical origin and style on the basis of the chiral monoterpane profile and enantiomer fractions. The ANOVA and linear discriminant analysis showed a clear impact of region and style on the chiral monoterpane profiles in Riesling wines. The majority of the studied wines contained similar EFs regardless of region or style, except for German wines. However, monoterpenes are affected by confounding factors, such as light and temperature, representing additional challenges for researchers, winemakers and viticulturists. Hence, it is strongly recommended to carry out further studies on the effects of region and style over several vintages and using a bigger sample size (taking into account the different oenological treatments, as well as the bottling technique and the type of bottle closure), in order to gain a better understanding of the factors involved in these regional and stylistic chiral monoterpane differences.

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