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Modulation of Welschriesling wine volatiles through the selection of yeast and lactic acid bacteria

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ABSTRACT

Welschriesling is a variety common to Central and Eastern European wine-producing countries; however, its aromatic composition remains poorly understood. Our study aims to determine the volatile profile of Welschriesling by analysing varietal thiols, esters, higher alcohols and monoterpene alcohols in commercial wines. In addition, the selection of commercial yeast starters and lactic acid bacteria (LAB) was used to modulate Welschriesling wine volatile composition. Both the selection of yeast and LAB had a significant impact on the compositional parameters of the wines. Yeast starter Uvaferm 228 (UVF228) was the most effective in terms of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) production. For the ethyl esters of fatty acids (EEFAs), higher alcohol acetates (HAAs) and higher alcohols (HAs), no common inter-group behaviour could be observed based on yeast selection, however, as a group, EEFAs and HAs were highest in wines fermented with UVF228.

Malolactic fermentation (MLF) significantly influenced varietal thiols, irrespective of the LAB strain used. Concentrations of 3MH significantly increased with MLF, 4-mercapto-4-methyl-pentan-2-one (4MMP) concentrations decreased, while 3MHA concentrations remained unaltered. This study showed the specific influence of yeast and LAB strain on selected volatile compounds. MLF appeared to be a promising tool to boost the presence of 3MH in wines, however, the results need further confirmation across a range of fermentation conditions and strain selections.

KEYWORDS

fermentation, varietal thiols, esters, malolactic fermentation

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INTRODUCTION

Wine fermentation is a complex microbiological process in which yeast plays a fundamental role (Padilla *et al.,* 2016). Commercial yeast starters are used to tailor fermentation capacity, volatile production, nutrient requirement and to secure fermentation reliability with the expected outcome (Lambrechts and Pretorius, 2000; Swiegers *et al.,* 2005; Swiegers *et al.,* 2009; Kritzinger *et al.,* 2013). *Saccharomyces cerevisiae* is the main fermentation yeast in inoculated fermentations with a superior fermentation capacity due to its ability to survive in high alcohol fermentation media, low volatile acidity (VA) production and favourable aromatic outcomes (Ribéreau-Gayon *et al.,* 2007). Yeasts play an essential role in tailoring wine aroma by releasing volatiles from non-volatile precursors or *de novo* synthesis from amino acids, lipids and other nutrients found in must (Swiegers *et al.,* 2005).

Varietal thiols are released from cysteinylated precursors such as 3-S-cysteinylhexan-1-ol (cys-3MH) and S-3-cysteinyl(4-mercapto-4-methyl-pentan)-2-one (cys-4MMP) during fermentation through the activity of β-lyase, while glutathionylated precursors (des Gachons *et al.,* 2002) are released through γ-glutamyl transpeptidase and carboxypeptidase action (Pinu *et al.,* 2014). Lastly was identified pathway of thiols formation in grapes via C6 unsaturated compounds (Schneider *et al.,* 2006), however, the release of 3-mercaptohexan-1-ol (3MH) from S-3-(hexanal)-glutathione and its bisulfite was negligible in oenological conditions (Thibon *et al.,* 2016). Juice composition, particularly amino acids, significantly influence the production of varietal thiols as well as the 3-mercaptohexyl acetate (3MHA)/3MH acetylation rate (Pinu *et al.,* 2014), while yeast strains also differ in their ability to release varietal thiols from their respective precursors (Dubourdieu *et al.,* 2006; Swiegers *et al.,* 2007; Jenko *et al.,* 2013). Indeed, the ability of 82 different yeast strains to release varietal thiols from synthetic must differed 20-fold for 3MH and 35-fold for 4-mercapto-4-methyl-pentan-2-one (4MMP) (Cordente, 2017). Furthermore, co-inoculation of two different *S. cerevisiae* yeasts resulted in a modified wine volatile profile compared to single-strain inoculation (King *et al.,* 2008). In particular, co-inoculation with the VIN7/QA23 combination resulted in increased levels of 3MH and 3MHA in Sauvignon blanc wines (King *et al.,* 2008). Varietal thiols, in particular 3MH, 3MHA and 4MMP have been shown to be key odorants of Sauvignon blanc wines, with distinct aromas of grapefruit, tropical fruit, cat urine, passionfruit and box tree (Darriet *et al.,* 1995; Tominaga *et al.,* 1998). The presence of varietal thiols, especially 3MH and 3MHA, have also been reported in several other varieties, such as Pinot gris, Melon B., Petit and Gros Manseng, Colombard and Riesling, as well as in Merlot, Grenache and Cabernet-Sauvignon (Roland *et al.,* 2011; Tominaga *et al.,* 2000).

Not only alcoholic fermentation but also malolactic fermentation (MLF) influences the wine volatile profile. Similarly to alcoholic fermentation, the choice of lactic acid bacteria (LAB) strain, the time of inoculation, fermentation temperature, pre-MLF pH, wine matrix and other factors influence the final outcome (Costello *et al.,* 2012). It has been reported that LAB influence the fruity notes of red wines by manipulating concentrations of esters (Antalick *et al.,* 2012; Sumby *et al.,* 2013; Gammacurta *et al.,* 2018), or by masking the fruitiness of wines with increased levels of diacetyl, ethyl lactate and diethyl succinate (Lasik-Kurdyś *et al.,* 2018). *Oenococcus oeni* can cleave the bonds between glycosidic molecules and aromatic aglycones such as terpenes, C13 norisoprenoids, volatile phenols and C6 compounds, whereas this is also a common trait of other LAB strains. A recent study (Takase *et al.,* 2018) demonstrated the ability of *Lactobacillus plantarum* to convert cysteine-S-conjugates and cysteinyl-glycine-S conjugates to odorous 3MH. Enzymatic activity of LAB to release aglycones can positively affect wine aroma, while it can also have a negative effect, particularly on red wines, affecting the evolution of volatile phenols (Virdis *et al.,* 2021). While MLF is a common practice in red wine-making, the influence of MLF on the aromatic perception of white wine has been less well-researched.

Welschriesling (*Vitis vinifera* L.) is a variety grown in Central and Eastern Europe (Austria, Croatia, Hungary, Slovakia and Slovenia). Aromatic expression of the variety is considered to be strongly dependent on the planting material, geographical location, yield per vine, grape ripeness and winemaking practices (Flak *et al.,* 2003). Welschriesling's volatile profile has been described as reminiscent of green and ripe apple, tropical fruit aromas and meadow flowers (Flak *et al.,* 2006). In the same study, the authors also analysed a few esters, nerol and β-damascenone in Welschriesling wines.

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However, the results were presented only as peak area ratios, therefore they provided little information on measured metabolite abundance and their possible influence on wine perception.

This study aimed to highlight Welschriesling aromatic composition, by analysing several classes of wine volatiles in eight commercially available Welschriesling monovarietal wines from two vintages. In addition, it provides knowledge about yeast and LAB-derived modulations of Welschriesling wines by analysing the chemical profile of the wine after alcoholic fermentation with four *S. cerevisiae* yeast starter cultures and after MLF conducted with three LAB strains in combination with spontaneous microflora.

MATERIALS AND METHODS

1. Samples

1.1. Commercial Welschriesling wine samples

Welschriesling samples were purchased from stores or producers in October 2013. Eight monovarietal Welschriesling wines from four different producers in the Podravje Slovenian wine region were selected. A Welschriesling wine from the 2011 and 2012 vintages was purchased from each producer. Producers 1, 2, 3 and 4 were assigned a letter–A, B, C and D, respectively–followed by 11 for wine from the 2011 vintage and 12 for wines made in 2012. After purchasing, the wines were brought to the laboratory and stored at +12 °C in a dark room. All the wines were sealed with a screw cap. Wine chemical volatile analysis was performed in November 2013.

1.2. Alcoholic fermentation

Grape juice for the experiment was obtained from a commercial wine cellar in Podravje and pressed with a hyper-reductive press. During pressing, 30 mg/L of SO_2 was added in the form of a 5–6 % aqueous solution of sulphurous anhydride. The juice was transported to the experimental wine cellar in full stainless-steel containers. After arrival at the experimental cellar, the juice was transferred to a 150 L stainless steel tank, previously purged with N_2 gas, homogenized and closed with a floating lid with an inflatable tube. The juice was left for cold settling at +4 °C overnight. The following day 5 L glass fermenters were filled with 4.5 L of juice in the presence of N_2. Seven different fermentations, of which six were commercial starter cultures and one a spontaneous fermentation, were initiated in triplicate. Fermentations that fermented to a residual sugar of 5 g/L or below were considered for further analysis and are listed in Table 1. The selected yeast starter (40 g/hL) was added to each 5 L fermenter directly without previous yeast rehydration. The juice fermented with indigenous microflora was not inoculated. Fermentations were carried out in a temperature-controlled room at 16–18 °C. After an initial drop in the sugar of approximately 30 %, a yeast nutrient (Fermaid E Blanc®, Lallemand, Canada) was added to all the fermentations at a concentration of 40 g/hL. The progress of the fermentation was monitored using refractometric measurements of sugar in Oechsle degrees (°Oe). Towards the end of fermentation, residual sugars (g/L) were measured. When residual sugar was quantified to be below 5 g/L, the fermentations were sulphured to 50 mg/L SO_2 (administrated in the form of 5–6 % aqueous solution of sulphurous anhydride), transferred to +4 °C room to facilitate sedimentation, and after three days bottled in 0.5 L screw cap bottles. The wine volatile profile was analysed approximately 6 months after bottling.

1.3. Malolactic fermentation

In 2013, Welschriesling wine was obtained from the commercial wine cellar in Podravje ten days after the completion of alcoholic fermentation. The wine was fermented with Zymaflore® X5 (Laffort, France), inoculated at 30 g/hL and the fermentation temperature was held at between 15–18 °C. During fermentation, 15 g/hL of nitrogen nutrient was added at both the 1/3 and 1/2 stage of alcoholic fermentation, in the form of diammonium phosphate (DAP, Laffort, France). Alcoholic fermentation was completed in 14 days.

TABLE 1. List of starter cultures in the study fermenting to below 5 g/L of residual sugars.

Abbreviation	Commercial name	Yeast culture	Producer
AlchI	Alchemy I	*S. cerevisiae*—multiple strains	Anchor Yeast
AlchII	Alchemy II	*S. cerevisiae*—multiple strains	Anchor Yeast
UVF228	Uvaferm 228®	*S. cerevisiae*—multiple strains	Lallemand
LevulinS	Levuline Synergie	*S. cerevisiae*—multiple strains	Danstar Ferment AG

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The wine was brought to the experimental wine cellar before racking and SO_2 addition in a commercial wine cellar. The wine was transferred to 3 L glass bottles to evaluate the effect of MLF on the chemical composition of the wine. On the same occasion, a sample was taken to determine organic acids and varietal thiols in the initial wine. Three commercial *O. oeni* LAB preparations were selected to perform MLF: LACTOENOS® SB3 instant (LactSB3) (Laffort, France), Lalvin VP41™ (LalVP41) (Lallemand, Canada) and VINIFLORA® CH35 (VinCH35) (Christian Hansen, Horsholm, Denmark). The fourth treatment (Spont) was MLF, performed with indigenous LAB. The fermentations were inoculated with selected commercial LAB, i.e., LactSB3, LalVP41 and VinCH35, on 26.11.2013, at a concentration of 1 g/hL according to the manufacturer's instructions and without added LAB nutrients. For spontaneous MLF, no inoculation was performed. MLF was carried out in a temperature-controlled room at 22–23 °C in triplicate. The progress of MLF was monitored by measuring the organic acids in wines before inoculation with LAB (26.11.2013) and on four additional occasions (10.12.2013, 8.1.2014, 25.2.2014 and 5.5.2014). When malic acid dropped below 0.1 g/L, MLF was considered to be completed and the wines sulphured to 30 mg/L of free SO_2 administrated in the form of a 5–6 % aqueous solution of sulphurous anhydride. After sulphuring, the fermentations were transferred to a room at +4 °C for three days to facilitate sedimentation, and the wine was then bottled in 0.5 L screw cap bottles. At the last sampling on 5.5.2014, the remaining two treatments (Spont and LactSB3) were sulphured, despite malic acid concentration above 0.1 g/L in the Spont treatment. Thiols were quantified in the wines in May 2014.

2. Methods

2.1. Juice analysis: TSS, pH, titratable acidity and yeast assimilable nitrogen

Total soluble solids in the juice were measured using a digital refractometer (WM-7, Altago, Saitama, Japan). Juice pH was measured with a MeterLab PHM 210 (Radiometer Analytical, Lyon, France) and titratable acidity (TA) determined by sodium hydroxide titration and with bromothymol blue as an indicator of colorimetric change (European Commission regulation (EEC) No. 2676/90, 1990). Yeast assimilable nitrogen was measured using enzymatic kits (Megazyme, Bray, Ireland) with testing performed on an Agilent technologies 8453 spectrophotometer (Agilent Technologies, Palo Alto, CA, USA).

2.2. Analysis of basic wine parameters

Juice pH was measured with a MeterLab PHM 210 (Radiometer Analytical, Lyon, France) and TA determined by sodium hydroxide titration and with bromothymol blue as an indicator of colorimetric change (European Commission regulation (EEC) No. 2676/90, 1990). Ethanol content was measured using an alcohol meter (Alcolyzer Wine M, Anton Paar, Austria), wine density with density meter (Density Meter DMA 4500M, Anton Paar, Austria) while reducing sugars and VA were quantified using an enzymatic robot (BS-200, Mindray, China). Wine ash and wine total dry matter were measured following official OIV methods (Uradni list st. 43/2011 31.5.2001, 2001).

2.3. Analysis of organic acids

Tartaric, malic, lactic and citric acid were measured in wines before and during MLF using a liquid chromatograph (HPLC) 1100 Series (Agilent Technologies, Palo Alto, CA, USA) connected to a diode-array detector (DAD) (Agilent Technologies, Palo Alto, CA, USA). Wine samples were filtered through 0.45 µm filters (Merck Schnelldorf, Germany) and directly injected. The separation of organic acids was achieved on a Rezek RCM-Monosaccharide Ca+2 cation exchange column (Phenomenex Torrance, CA, USA) at 65 °C. Chromatographic conditions were identical to those already described (López & Gómez, 1996).

2.4. Analysis of varietal thiols

Thiols in wines were extracted using Dowex resin, according to previously published methods (Tominaga and Dubourdieu, 2006), with some slight modifications in sample preparation as described in (Jenko *et al.,* 2013). Briefly, 50 mL of wine sample, spiked with internal standards, i.e., 4-methoxy-2-methyl-2-mercaptobutane (Sigma Aldrich, Schnelldorf, Germany) for 4MMP quantification, [$^2H_2$]-3-mercaptohexyl acetate (Auckland University, New Zealand) for 3MHA quantification, and [$^2H_2$]-3-mercaptohexan-1-ol (Auckland University, New Zealand) for 3MH quantification. Wine pH was adjusted to 7 and the wine passed through a Dowex column and thereafter eluted with cysteine buffer as described in (Tominaga and Dubourdieu, 2006; Jenko *et al.,* 2013). Thiols were extracted from the cysteine buffer using liquid-liquid extraction with ethyl acetate and dichloromethane.

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The organic phase was collected, dried over anhydrous sodium sulphate and concentrated to a final volume of 50 µL (Jenko *et al.,* 2013). Samples were analysed using a gas chromatograph (GC) (Agilent Technologies 7890A, Palo Alto, CA, USA), coupled to a mass spectrometer (Agilent Technologies 5975C, Palo Alto, CA, USA). The chromatographic conditions were identical to those described in (Jenko *et al.,* 2013). One-point calibration was performed using calibration standards in an alcoholic solution with a final concentration of 65 ng/L of 4MMP, 650 ng/L of 3MHA and 1202 ng/L of 3MH, and injected after every ninth sample. The limit of quantification (LOQ) was 2, 4 and 60 ng/L for 4MMP, 3MHA and 3MH, respectively.

2.5. Analysis of higher alcohols, ethyl acetate, acetaldehyde and diethylacetal

Higher alcohols, ethyl acetate acetaldehyde and diethylacetal were analysed using GC coupled to a flame ionization detector (FID) (Hewlett Packard 6890, Germany) without previous extraction using CP-Wax, 57CB, 50m × 0.25 mm, film thickness 0.20 µm (Bavčar *et al.,* 2011). The wine sample (5 mL) was spiked with the internal standard 4-methyl-2-pentanol (Sigma Aldrich), vortexed and 1 µL directly injected into GC-FID. Validation of the method was carried out (Bavčar, 2011) and quantification was performed as previously reported (Bavčar, 2011; Bavčar *et al.,* 2011, Bavčar and Baša Česnik, 2011).

2.6. Analysis of esters, 1-hexenol, (Z)-3-hexenol, γ-butyrolactone and benzyl alcohol

Esters, C6 alcohols and other compounds were analysed as described previously (Bavčar *et al.,* 2011) after liquid-liquid extraction with dichloromethane and organic phase concentration to 1 mL, as described in (Bavčar *et al.,* 2011). Samples were analysed using GC (Hewlett Packard 6890, Waldbronn, Germany), coupled to an MS (Hewlett Packard 5973, Palo Alto, CA, USA) using CP-Wax 57CB 50 m × 0.25 mm, film thickness 0.20 µm Varian (Lake Forest, CA, USA) column coupled to a fused silica deactivated 2 m × 0.25 mm guard column (Agilent Technologies Palo Alto, CA, USA). Compound identification and quantification were performed as described in (Bavčar, 2011; Bavčar *et al.,* 2011; Bavčar and Baša Česnik, 2011).

2.7. Analysis of monoterpene alcohols

Monoterpene alcohols in wines were analysed using headspace-solid phase microextraction (HS-SPME), as described by (Bavčar *et al.,* 2011) using a GC (Agilent Technologies 7890A, Palo Alto, CA, USA) coupled to an MS (Agilent Technologies 5975C, Palo Alto, CA, USA) and equipped with a Gerstel MPS Autosampler (Gerstel, Mulhein an der Ruhr, Germany). Briefly, wine samples were diluted in a 1:4 ratio with MilliQ water. In an SPME vial, 5 ml of diluted wine was spiked with 4-nonanol, followed by 1.7 g NaCl. Compound separation was achieved on an INNOWax 30 m × 0.25 mm, film thickness 0.25 µm column (Agilent Technologies), coupled to a fused silica deactivated 2 m × 0.25 mm guard column (Agilent Technologies). The ions used for terpene quantification and method validation parameters are reported elsewhere (Bavčar, 2011; Bavčar *et al.,* 2011; Bavčar and Baša Česnik, 2011).

2.8. Statistical analysis

The Shapiro–Wilk normality test was used to check the normality of data, whereas the homogeneity of variances was tested using Leven's test. Significance was checked using parametric one-way analysis of variance (one-way ANOVA) for variables with normal distribution, and means were separated using Fisher's LSD test. For identified variables with non-homogeneous variances and non-normal distribution, the non-parametric Kruskall–Wallis test was used and the means were separated using the Bonferroni test. Different letters account for significant differences at $p \leq 0.05$. Asterisks denote the level of significance * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Data were analysed in R (version Ri386 4.0.5) using the R Commander package. Principal component analysis was conducted in R studio version 1.2.1335 (RStudio Team, 2019). Unit variation and mean centring was used to build PCA in the ggbiplot package.

3. Results

3.1. Commercial Welschriesling wines

Commercial Welschriesling wines were screened for 28 volatiles, deriving from fermentation (esters and higher alcohols), as well as for varietal thiols and four monoterpene alcohols. PCA was applied to all measured volatiles in commercial Welschriesling wines and explained 57.9 % of the variance in the dataset. A clear separation between wines from the two vintages was noticed

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TABLE 2. Concentration of measured volatiles in commercial Welschriesling wines.

	Odour detection threshold	Min	Max	Average
	Varietal thiols (ng/L)			
4MMP	0.8[1]	3.48	8.80	5.48
3MHA	0.0042[2]	198.5	349.7	277.5
3MH	60[1]	362.1	1056.5	820.1
	Ethyl esters of straight-chain fatty esters (µg/L)			
ethyl butyrate	20[5]	257.4	382.5	294.9
ethyl hexanoate	5[5]	640.9	910.3	814.2
ethyl octanoate	2[5]	965.9	1381.8	1106.5
ethyl decanoate	200[6]	377.7	676.9	508.4
ethyl dodecanoate	640[7]	0.71	18.7	8.1
	Higher alcohol acetates (µg/L)			
hexyl acetate	670[3]	17.1	125.3	49.5
isoamyl acetate	30[5]	359.6	2264.1	1077.8
2-phenylethyl-acetate	250[5]	32.2	171.9	86.5
	Monoterpene alcohols (µg/L)			
linalool	15[5]	5.93	28.93	14.64
α-terpineol	250[6]	41.24	101.49	68.39
citronellol	100[5]	3.66	13.77	8.41
geraniol	30[5]	-	129.5	65.02
	Higher alcohols (mg/L)			
1-hexanol	8000[5]	0.36	0.99	0.62
1-propanol	-	14.4	47.0	25.3
2-methyl propanol	40,000[5]	11.03	30.53	20.03
2-methyl butanol	30,000[5]	15.86	32.22	23.22
3-methyl butanol	30,000[5]	99.80	196.99	138.73
2-phenylethyl alcohol	10,000[5]	20.97	43.46	32.15
benzylalcohol	-	16.36	97.27	33.39
(*Z*)-3-hexen-1-ol	400[5]	0.028	0.071	0.049
	Other compounds			
benzaldehyde (mg/L)	91,000[4]	1.27	4.39	2.59
diethyl succinate (µg/L)	200,000[8]	790	1672	1322.9
γ-butyrolactone (µg/L)	-	1805	3306	2791
acetaldehyde (mg/L)	500[5]	2.9	22.1	14.8
ethyl acetate (µg/L)	7500[5]	35.2	54.5	45.4

Min indicates the lowest concentration measured in eight commercial Welschriesling wines, Max indicates the highest concentration measured in eight commercial Welschriesling wines and Average, the average concentration in the eight Welschriesling wines analysed.

[1] (Tominaga *et al.*, 2000), the odour thresholds reported were determined in 12 % ethanol, 5 g/L tartaric acid and pH 3.2.

[2] (Tominaga et al., 1996), the odour thresholds reported were determined in 12 % ethanol, 5 g/L tartaric acid and pH 3.2.

[3] (Pineau et al., 2009), the odour thresholds reported were determined in dearomatised red wine.

[4] (Yu et al., 2020), the odour thresholds reported were determined in 14 % w/w aqueous ethanol.

[5] (Guth, 1997), the odour thresholds reported were determined in 10 % w/w aqueous ethanol.

[6] (Ferreira et al., 2000), the odour thresholds reported were determined in 11 % v/v ethanol, 7 g/L glycerine, 5 g/L tartaric acid and pH 3.4

[7] (Salo, 1970), the odour thresholds reported were determined in 9.5 % w/w ethanol.

[8] (Etievant, 1991), the odour thresholds reported were determined in 12 % w/w aqueous ethanol.

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along principal component (PC)1, accounting for 36.4 % of the variation in the dataset. Not surprisingly, wines from the 2012 vintage were associated with the majority of quantified esters and 3MH, (Figure 1), whereas 3MHA and 4MMP were found in a positive dimension of PC1, together with Welschriesling wines from the 2011 vintage. Varietal thiols were measured in concentrations well above the reported detection threshold in all the wines analysed (Table 2). Concentrations of varietal thiols were in the range of 362–1056 ng/L, 198–349 ng/L and 3.48–8.80 ng/L for 3MH, 3MHA and 4MMP, respectively, see Table 2. Interestingly, concentrations of 3MH were correlated to PC2, and specifically, producer A showed the highest concentrations of 3MH in 2011 and 2012, whereas the concentrations of varietal thiols were consistently lowest in the wines from producer D. HAAs and EEFAs were another group of compounds contributing significantly to sample separation based on vintage (Figure 1).

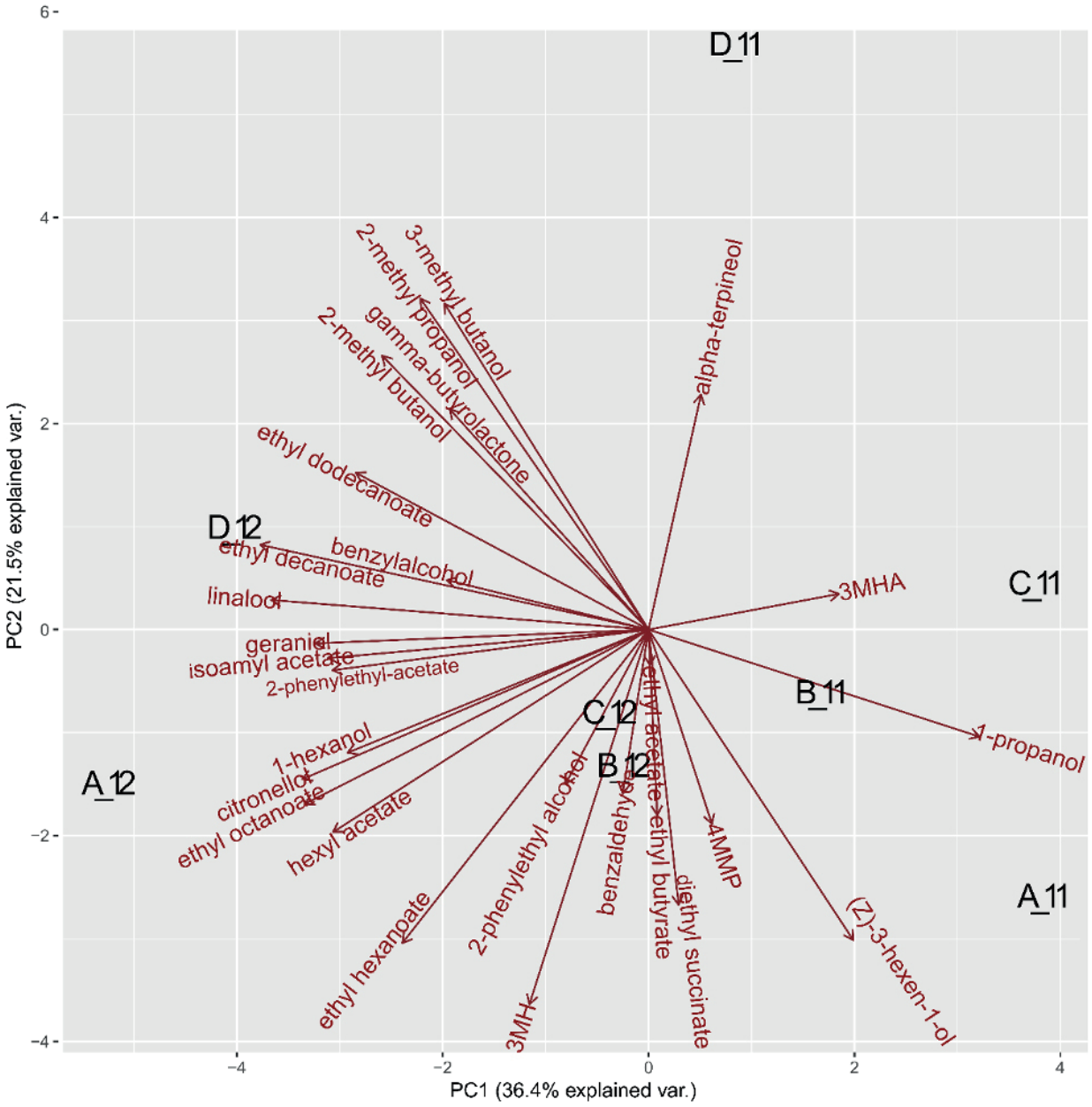


FIGURE 1. Principal component analysis (PCA) computed on all measured volatiles in commercial Welschriesling wines.

Letters A, B, C and D indicate producers 1, 2, 3 and 4, respectively, while 11 and 12 stand for the wine vintage, i.e., 2011 and 2012, respectively.

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3.2. Influence of commercial yeast on the chemical profile of the wine

Before yeast inoculation, the basic juice parameters were measured in all the fermentations. No significant differences were observed in the TSS (22.4 Brix), pH (3.26), TA (5.8 g/L as tartaric acid/ 3.9 g/L as sulphuric acid) and YAN (94.8 mg N/L) between treatments. AlchI and AlchII fermented to a residual sugar of around 2 g/L in wine, while LevulinS and UVF228 struggled to finish fermentation. The final reduced sugar in wines fermented with UVF228 and LevulinS was 4.9 and 4.5 g/L, respectively (Figure 2). The difference in the alcohol content of the wines was significant but relatively small. The lowest alcohol content of 13.85 ± 0.03 % vol was measured in wines fermented with UVF228, and the highest of 14.23 ± 0 % vol in AlchII inoculums (Figure 2). The commercial starter AlchII required 17.08 g of sugar for the production of 1 % vol of ethanol, which was the lowest, followed by the AlchII commercial starter, whereas the highest requirement of 17.33 g of sugar for the production of 1 % vol ethanol was calculated for UVF228. Therefore, if the yeast utilised all the available sugars, the highest probable alcohol level would be measured in wines fermented with AlchI (14.34 %vol) and the lowest in wines fermented with UVF225 (14.13 %vol). The production of acetic acid is an important qualitative trait in the yeast selection process. The differences in VA were significant between samples, with the lowest values of 0.28 ± 0 g/L quantified in UVF228 wines and the highest in the LevulinS treatment, where VA was 0.43 ± 0 g/L. To clarify the overall influence of yeast selection on the chemical composition of the wine, PCA was applied to the treatments and

TABLE 3. Evolution of organic acids concentrations in Welschriesling wines during MLF (g/L).

	Spont	LactSB3	LalVP41	VinCH35	p-value
Sampling 1 (0 days after LAB inoculation)					
Citric acid	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.00	0.25 ± 0.01	ns
Tartaric acid	2.43 ± 0.02ab	2.48 ± 0.03a	2.42 ± 0.02ab	2.42 ± 0.03b	*
Malic acid	1.90 ± 0.03b	2.04 ± 0.06a	1.93 ± 0.05b	1.93 ± 0.04b	*
Lactic acid	0.11 ± 0.00b	0.11 ± 0.00b	0.11 ± 0.00ab	0.11 ± 0.00a	*
Sampling 2 (14 days after LAB inoculation)					
Citric acid	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.00	0.29 ± 0.02	ns
Tartaric acid	2.51 ± 0.02	2.50 ± 0.00	2.48 ± 0.00	2.51 ± 0.04	ns
Malic acid	2.10 ± 0.03a	2.04 ± 0.06a	1.91 ± 0.04b	1.93 ± 0.07b	**
Lactic acid	0.11 ± 0.00c	0.16 ± 0.02b	0.21 ± 0.04a	0.21 ± 0.00a	**
Sampling 3 (43 days after LAB inoculation)					
Citric acid	0.32 ± 0.01a	0.19 ± 0.11ab	0.16 ± 0.02ab	0.08 ± 0.01b	*
Tartaric acid	2.55 ± 0.02	2.51 ± 0.02	2.53 ± 0.04	2.55 ± 0.02	ns
Malic acid	2.08 ± 0.06a	0.99 ± 1.03b	0.11 ± 0.01b	0.20 ± 0.09b	**
Lactic acid	0.14 ± 0.01b	0.98 ± 0.77ab	1.69 ± 0.04a	1.47 ± 0.07ab	**
Sampling 4 (91 days after LAB inoculation)					
Citric acid	0.31 ± 0.01a	0.14 ± 0.11b	-	0.06 ± 0.01c	*
Tartaric acid	2.57 ± 0.02ab	2.49 ± 0.03b	-	2.61 ± 0.03a	*
Malic acid	1.61 ± 0.55	0.58 ± 0.85	-	0.16 ± 0.02	ns
Lactic acid	0.47 ± 0.34	1.31 ± 0.69	-	1.68 ± 0.01	ns
Sampling 5 (160 days after LAB inoculation)					
Citric acid	0.25 ± 0.07a	0.06 ± 0.01b	-	-	**
Tartaric acid	2.56 ± 0.04	2.53 ± 0.03	-	-	ns
Malic acid	0.19 ± 0.09a	0.05 ± 0.00b	-	-	*
Lactic acid	1.22 ± 0.11b	1.59 ± 0.03a	-	-	*

ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$. All stated uncertainty is the standard deviation of three replicates per treatment. The asterisks indicate the level of significance: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ – indicates that samples were not measured, whereas ns indicates no significant differences.

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	LevulinS	AlchI	UVF228	AlchII	p value
Varietal thiols (ng/L)					
4MMP	3.67±0.16b	5.59±1.33a	4.03±0.39ab	3.80±0.29ab	*
3MHA	201.6±133.5	175.8±114.8	323.5±28.7	208.4±110.3	ns
3MH	723.7±77.8ab	713.2±12.1b	983.2±186.7a	738.0±15.9ab	*
Ethyl esters of straight chain fatty esters (µg/L)					
ethyl butyrate	333.6±1.0ab	298.7±2.6bc	351.1±2.5a	293.1±5.7c	*
ethyl decanoate	428.7±3.5c	452.4±7.3b	488.6±0.6a	474.4±19.5a	***
ethyl octanoate	793.9±1.5d	832.8±2.6c	1005±3a	854.1±25.3b	*
ethyl dodecanoate	17.3±0.18a	13.79±0.24c	10.08±0.26d	14.84±0.82b	***
ethyl hexanoate	1094±8a	995.8±6.8c	1061±3b	957.4±28.6d	***
SUM EEFAs	2675±13ab	2604±19b	2923±7a	2604±80b	*
Higher alcohol acetates (µg/L)					
hexyl acetate	55.8±0.1ab	61.2±0.1a	38.1±0.2c	54.7±1.2bc	*
isoamyl acetate	754.5±4.5d	1067±5a	827.0±3.6c	1027±27b	*
2-phenylethyl-acetate	60.0±0.8d	96.2±0.3ab	92.2±0.2c	98.8±3.07a	*
SUM HAAs	869.8±4d	1224±6a	957.3±14c	1180±31b	*
Monoterpene alcohols (µg/L)					
linalool	14.7±1.1a	12.2±1.1b	12.1±0.6b	11.3±1.1b	*
α-terpineol	27.7±0.9b	29.9±1.0a	28.3±0.8b	30.5±0.6a	*
citronellol	7.56±0.36b	8.67±0.9b	11.6±0.43ab	8.07±0.72ab	*
SUM	49.9±0.6	50.8±2.0	52.0±0.4	49.9±1.6	ns
Higher alcohols (mg/L)					
1-hexanol	0.63±0.2b	0.57±0.4c	0.66±0.2a	0.55±0.14d	***
benzyl alcohol	21.13±016b	20.35±0.13c	22.6±0.02a	18.8±0.57d	***
(Z)-3-hexen-1-ol	0.06±0.002a	0.06±0.01ab	0.06±0.001a	0.06±0.02b	*
1-propanol	26.7±0.1c	30.8±0.4a	20.9±0.4d	27.8±0.4b	***
2-methy propanol	19.8±0.1bc	19.5±0.3c	29.8±0.1a	20.7±0.1ab	*
1-butanol	0.75±0.1	0.77±0.1	0.73±0.1	0.76±0.3	ns
2-methyl butanol	21.8±0.3cd	26.2±0.1b	25.3±0.1c	26.4±0.1a	*
3-methyl butanol	128.6±1.6c	141.1±0.4b	149.5±0.4a	149.9±0.1a	***
2-phenylethyl alcohol	21.9±4.9	21.8±0.3	26.5±0.2	22.3±0.7	ns
SUM	240.8±7.1d	260.5±1.5c	275.5±0.8a	266.7±0.3b	***
Other compounds					
benzaldehyde (mg/L)	5.62±0.06b	4.66±0.9d	4.94±0.01c	6.07±0.15a	***
diethyl succinat (µg/L)	970±9c	1234±10ab	1820±2a	1190±45bc	*
γ- butyrolacton (µg/L)	4895±22b	4137±77c	6720±1a	3894±116d	*
acetaldehyde (mg/L)	21.2±0.4a	14.9±0.8b	16.9±1.7b	10.9±1.2c	***
ethyl acetate (µg/L)	71.5±1.1a	64.3±0.4b	54.5±0.3d	61.4±0.3c	***
ethanol (% vol)	13.92±0.09bc	14.10±0.07ab	13.85±0.03c	14.23±0a	*
reducing sugars (g/L)	4.48±0.01b	2.25±0c	4.93±0.01a	1.95±0.01d	*
total dry matter (g/L)	21.7±0.2b	19.7±0.1c	22.6±0.3a	18.7±0d	***
TA (g/L sulphric acid)	3.59±0b	3.57±0.04b	3.72±0a	3.46±0.06c	***
VA (g/L acetic acid)	0.43±0a	0.33±0b	0.28±0a	0.34±0.01b	***
pH	3.29±0.1a	3.30±0.0a	3.27±0b	3.3±0a	*
ash (g/L)	1.68±0.01a	1.67±0.01ab	1.65±0.02bc	1.54±0.04c	*

FIGURE 2. Effect of four commercial yeast starter cultures on Welschriesling wine volatiles.

Red represents max value; Yellow median value; Green min value.

ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$. All stated uncertainty is the standard deviation of three replicates per treatment. The asterisks indicate the level of significance: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, whereas ns indicates no significance.

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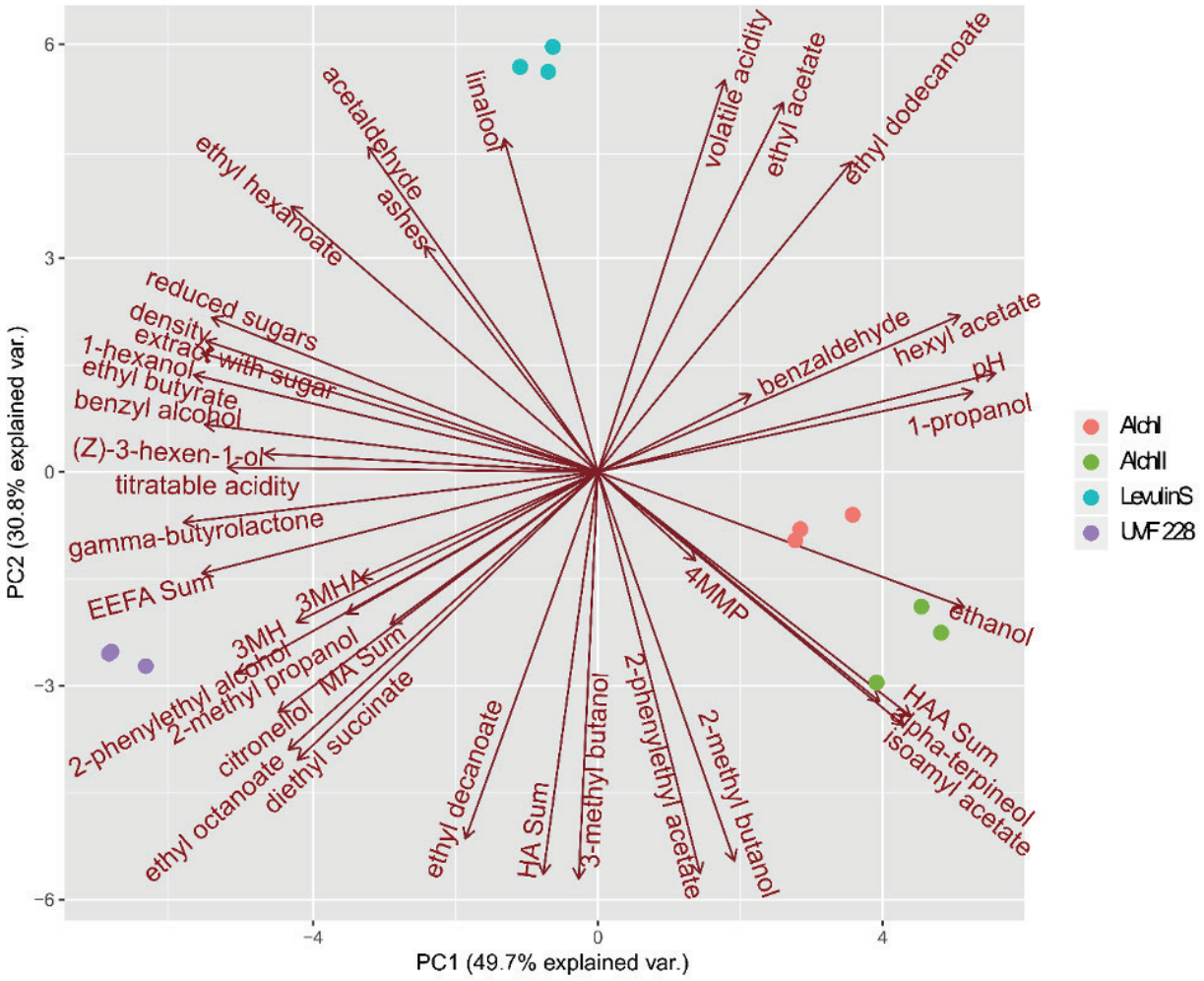


FIGURE 3. Principal component analysis (PCA) computed on all measured volatiles and non-volatiles in Welschriesling wines fermented with four different yeast starter cultures.
Dots of the same colour represent treatment replicates.

measured variables to observe possible trends in the data set (Figure 3). The selection of the yeast strain clearly modified the chemical composition of the wine. Wines fermented with the AlchI and AlchII commercial starters were closely grouped together, separated from wines fermented with UVF228 and LevulinS along PC1, which accounted for 49.7 % of the variability in the data set. 3MH, 3MHA, EEFAs and γ-butyrolactone were grouped together with wines from the UVF228 treatment. Wines fermented with UVF228 resulted in a 3MH concentration of 983 ± 186.7 ng/L, which was higher compared to other treatments (Figure 2). Similarly, concentrations of 3MHA were higher in the wines of the same treatment compared to the values measured in AlchI, AlchII and LevulinS wines, see Figure 2. The differences observed between treatments in terms of 4MMP concentrations were small. The highest concentrations of 5.59 ± 1.33 ng/L were measured in AlchI wines (Figure 2). Wines fermented with UVF228 also resulted in a higher concentration of total EEFAs, followed by wines fermented with LevulinS (Figure 2). Ethyl butyrate, ethyl octanoate and ethyl decanoate were the most abundant EEFAs in the UVF228 treatment, whereas ethyl hexanoate, the most abundant EEFAs in our study, was measured in significantly higher concentrations in the LevulinS treatment (Figure 2). In contrast to the trends observed for EEFAs, AlchI and AlchII strains resulted in higher HAAs compared to the UVF228 and LevuliS treatments. However, the same trend was not observed for HAs, which were significantly higher in the UVF228 treatment (Figure 2). Furthermore, γ-butyrolactone and diethyl succinate were most abundant in wines fermented with UVF228 (Figure 2). Concentrations of monoterpene alcohols were compound-specific. No trends could be observed for a specific yeast strain (Figure 2).

3.3. Influence of MLF on the chemical composition of wine

Welschriesling wine from the same commercial wine cellar was used for this experiment. The basic wine parameters before LAB inoculation were: ethanol content 13.4 % vol, residual sugar 1.1 g/L, TA 6.5 g/L as tartaric acid, 4.25 g/L as sulphuric acid, pH 3.24 and VA as acetic acid 0.30 g/L. The concentrations of organic acids before LAB inoculation can be seen in Table 3. The commercial LAB used in the study proved to be efficient in performing MLF in Welschriesling wines (Table 3). LalVP41 was the fastest to conclude MLF, followed by VinCH35 (Table 3). At the last sampling, malic acid was already below 0.1 g/L in the LactSB3 treatment, whereas in Spont, it was still 0.19 g/L (Table 3). The commercial LAB also utilized citric acid during MLF, while it remained unused in Spont treatment (Figure 3). The rate of citric acid utilization was highest in LactSB3 (Table 3).

The concentrations of 4MMP decreased in all MLF treatments, regardless of the LAB strain (Table 4). Interestingly, the lowest 4MMP concentrations were measured in wines inoculated with LalVP41, which was also the fastest strain to conclude MLF (Tables 3 and 4). On the other hand, indigenous LAB (Spont), with the slowest MLF, resulted in the highest 4MMP concentration, which was however lower than reported in wine before MLF (Table 4). Conversely, 3MH concentrations increased significantly in wines after MLF. Concentrations of 3MH doubled in wines inoculated with LactSB3, LalVP41and in spontaneous MLF, and were about 75 % higher in wines inoculated with VinCH35 compared to the wine before MLF (Table 4). Despite a significant increase in 3MH concentrations with MLF, 3MHA concentrations were not significantly affected (Table 4).

TABLE 4. Varietal thiols (ng/L) in Welschriesling wines after MLF

	Base wine	Spont	LactSB3	LalVP41	VinCH35	p-value
4MMP	12.4 ± 2.6a	9.9 ± 0.3b	9.6 ± 0.8b	6.3 ± 0.6c	8.3 ± 0.9bc	**
3MHA	53.7 ± 31.9	38.9 ± 5.3	56.0 ± 19.5	31.1 ± 3.9	128.9 ± 75.4	ns
3MH	544.1 ± 168.5c	1143.8 ± 106.8ab	1119.5 ± 266.2ab	1304.6 ± 76.5a	951.8 ± 132.5ab	**

ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$. All stated uncertainty is the standard deviation of three replicates per treatment. Asterisks indicate the level of significance: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, whereas ns indicates no significant differences.

DISCUSSION

1. Commercial Welschriesling wine volatile profile

Analysis of commercial wines revealed concentrations of varietal thiols 3MH, 3MHA and 4MMP well above their perception threshold; 60, 4.2 and 0.8 ng/L, respectively (Tominaga *et al.,* 2000) and in concentrations comparable to those reported in commercial Australian Chardonnay wines (Capone *et al.,* 2018). Capone *et al.* (2018) reported median 3MH concentration in more than 100 analysed commercial Australian Chardonnay wines to be 650 ng/L, whereas, in an older study, also conducted on Australian Chardonnay wines, the median value was 400 ng/L (Capone *et al.,* 2015). Interestingly, the median value for 3MHA concentration in the 2018 study was only just above 10 ng/L (Capone *et al.,* 2018), whereas in the Welschrielsing wines in our study the average concentration was 277 ng/L for 3MHA and 820 ng/L for 3MH. The presence of varietal thiols in Welschriesling wines was also reported by (Čuš and Zabukovec, 2017; Maslov Bandić *et al.,* 2018). The observed concentration range for varietal thiols was similar to that in our study (Čuš and Zabukovec, 2017). However, the 3MH and 3MHA concentrations measured in Chardonnay wines (Capone *et al.,* 2018) and our study were still below observed concentrations in commercial Sauvignon blanc wines from New Zealand, South Africa or France (Lund *et al.,* 2009). Chardonnay wines with higher varietal thiol content were more liked by consumers (Capone *et al.,* 2018). It could be assumed that varietal thiols may also play an important role in the varietal character of Welschrielsing wines, however additional sensorial studies are needed to confirm this assumption. A rapid decrease in fermentative aromas due to ester hydrolysis could also explain the separation of wine samples by vintage, as can be seen in Figure 1. Accentuated wine ageing has been reported for wines stored under supermarket-like conditions

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(higher ambient temperatures and illumination) (Arapitsas *et al.,* 2014; Grant-Preece *et al.,* 2017). It is therefore not surprising that Welschriesling wines from the 2012 vintage were associated with the majority of quantified HAAs and EEFAs in our study (Figure 1). Variability in growing conditions and winemaking practices are also likely to have contributed to variations in chemical composition between wines from the two vintages and the four producers. A clear distinction between Welschriesling wines from the two vintages based on the chemical composition of the wine was also noted by Flak *et al.,* 2006.

2. Influence of commercial yeast on the chemical profile of the wine

Yeast properties such as fermentation ability, generation of desirable aromatic profiles, sensitivity to osmotic pressure, ethanol tolerance, and oxygen requirements have a critical impact on fermentation outcomes. Lengthier fermentations were observed for the UVF228 and LevulinS strains, finishing fermentation at 4.93 and 4.48 g/L of residual sugar, respectively. The initial nitrogen content in the current study was at the lower end of the range (94.8 mg N/L) required for successful completion of alcoholic fermentation (Bell and Henschke, 2005), the juice was therefore supplemented with N in the form of a commercial yeast nutrient (for more details see the Materials and Methods section). Juice supplementation with nitrogen generally results in improved fermentation kinetics or fermentation time (Kemsawasd *et al.,* 2015). However *S. cerevisiae* strains differ significantly in terms of YAN requirements for the completion of fermentation, and the fermentation rate differs greatly in the same juice for different strains (Crépin *et al.,* 2012; Song *et al.,* 2020), explaining the selected yeast performance in our study. Lengthier fermentation is also invisibly correlated to YAN levels and maximum cell population (Bely *et al.,* 2003), resulting in increased VA levels. In our study, the LevulinS strain resulted in higher VA production and lengthier fermentation, however, the VA concentrations remained well below the legal limit of around 1.0 g/L in the EU (European Commission, EEC 606/2009) for white wines, and were below 0.5 g/L in all the treatments.

On examining the measured parameters, focusing primarily on wine volatile chemical constituents, it is immediately evident that yeast can influence the wine composition strain specifically (Swiegers *et al.,* 2009; Blazquez Rojas *et al.,* 2012; Jenko *et al.,* 2013). Similar trends were observed in wines fermented with AlchI and AlchII, indicating a similar cocktail of yeast strains in the inoculum, while UVF228 and LevulinS were significantly different. The highest 3MH concentration was measured in the UVF228 treatment. The ability of yeast to release 3MH and 4MMP from Cys-4MMP and Cys-3MH is limited by β-lyase activity (Swiegers *et al.,* 2007). Therefore, high variability among commercial *S. cerevisiae* strains in terms of the varietal thiols released is expected (Murat *et al.,* 2001; Swiegers *et al.,* 2009). On the other hand, 3MHA is derived from 3MH by acetylation in a reversible enzymatic process (Swiegers and Pretorius, 2007). Although the commercial yeasts VIN 7 (Anchor Oenology, South Africa) and VIN 13 (Anchor Oenology, South Africa) were reported as the yeasts with the highest 3MH and 4MMP production ability among those tested, QA23 (Lallemand, Canada) had the highest thiol conversion capacity (Swiegers *et al.,* 2009). In our study, the concentration of 3MHA did not differ significantly between treatments, however, the highest acetylation rate (3MHA/3MH) of 0.33 was observed for the UVF228 yeast starter culture, whereas it was 0.28 in wines fermented with the AlchI, AlchII and Levulin S yeast starter cultures. The conversion of 3MH to 3MHA was also significantly influenced by supplementing juice with various nitrogen sources, such as γ-aminobutyric acid, diammonium phosphate, glutamic acid and glutamine, as well as by the yeast strain, whereas the addition of succinic acid and GSH-3MH also influenced higher 3MHA concentrations in Sauvignon blanc wines (Pinu *et al.,* 2014). Several variables, such as nitrogen content, fermentation temperature, oxygen, lipid composition and differences in the expression of ATF1 and ATF2 responsible for ester synthesis, can explain the strain-specific ability to produce HAAs (Saerens *et al.,* 2008). In our study, significant differences between the different treatments were found for both individual and total HAAs. Wines fermented with AlchI followed by the AlchII treatment resulted in the highest total HAAs, whereas concentrations of higher alcohols were significantly higher in UVF228 fermented wines. This further strengthens previous observations that the limiting factor for the production of HAAs is the expression of the ATF1 and ATF2 and not the availability of higher alcohols and acetyl-CoA (Sumby *et al.,* 2010). All analysed EEFAs were also significantly influenced by the yeast starter culture selection. Our results confirm the variability of EEFAs concentration in wines fermented with different *S. cerevisiae* yeast starter cultures, which is in accordance with similar

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studies carried out with different *Saccharomyces* strains (Blazquez Rojas *et al.,* 2012) and sequential inoculations with non-*Saccharomyces* yeasts (Hranilovic *et al.,* 2018). The highest total concentrations of EEFAs were measured in the UVF228 treatment (Figure 2). However, in contrast with HAAs, expression of the ethyl ester biosynthesis genes EEB1 and EHT1 has been suggested not to be the limiting factor for ethyl ester production (Saerens *et al.,* 2010). A strain-specific influence on wine monoterpene alcohols was also observed, which could be attributed to the higher β-glycosidase activity or, as suggested by Carrau *et al.* (2005), to the yeast's ability to de-novo synthetise linalool, citronellol and α-terpineol.

3. Influence of MLF on wine thiols

Several studies have already been conducted on the influence of MLF on the varietal chemical and sensory composition of wine. Esters, higher alcohols, organic acids and fatty acid modulations as a consequence of MLF have received the most attention (Antalick *et al.,* 2012; Gammacurta *et al.,* 2018; Sumby *et al.,* 2010). However, only a few studies have tackled the effect of MLF on varietal thiols (Antalick *et al.,* 2012; Takase *et al.,* 2018), focusing only on 3MH concentrations. Under the MLF conditions in our study, 3MH concentrations increased significantly in selected commercial *O. oeni* strains and during spontaneous MLF. During AF only a small percentage of thiol precursors are released, so that after the end of AF wine represents an enormous pool of aromatic potential that could be explored (Coetzee and du Toit, 2012). An increase in 3MH concentration after MLF in red wine has already been observed for some commercial *O. oeni* strains (Antalick *et al.,* 2012). Another study reported the release of 3MH during MLF inoculated with *L. plantarum* from cys-S-3MH and S-3-(hexan-1-ol)-L-cysteinylglycine (3MH-S-cysgly) precursors (Takase *et al.,* 2018). The presence of *L. plantarum* in grape juice is well documented (Spano *et al.,* 2002), therefore this genus could possibly contribute to the increase in 3MH concentration in Welschriesling wines, especially in MLF with indigenous LAB. Additional research is needed to test the variability among *O. oeni* strains in terms of releasing 3MH from precursors and its dependence on initial wine composition. An increase of 3MH in wine can also have a non-microbiological background. In the study of (Makhotkina and Kilmartin, 2012), an increase in 3MH concentration was shown at the expense of a decrease in 3MHA during the early stages of wine ageing. However, in our study, 3MHA concentrations remained relatively unchanged during MLF and such chemical hydrolysis can be excluded. However, further research is needed to better understand the role of LAB in relation to varietal thiol concentrations in white wines.

CONCLUSION

This study has provided an insight into the volatile composition of Welschriesling wines and highlighted the potential importance of varietal thiols for the aromatic typicity of Welschriesling wines. This will need to be further explored in sensory studies using a larger set of wine samples. Furthermore, the selection of commercial yeast had a profound influence on the volatile composition of the wine, offering winemakers a tool for tailoring wine styles according to market demands. This study provides evidence of increased 3MH concentrations in wines after MLF, regardless of the LAB strain used. However, the results require further confirmation across the range of fermentation conditions (i.e., wine nitrogen status, LAB strain, level of available precursors) although MLF seems to be a promising tool to boost the presence of 3MH in wines.

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