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The influence of yeasts and copper sulphate addition on the aroma of Riesling wines

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ABSTRACT

The influence of two yeast strains and the addition of copper sulphate on the aroma of Riesling wines was monitored. Two commercially available yeast strains, Zymaflore VL3 and X-Pure, were applied. General composition, volatile thiols, norisoprenoids, free terpenes, aroma fermentation, sulphur compounds and copper content were determined, and the sensory attributes were assessed by 22 tasters in a balanced incomplete block design (BIBD). The VL3 yeast produced a higher concentration of thiols than the X-Pure yeast. Wines treated with copper sulphate showed a higher concentration of 3MH than the wines produced without copper sulphate addition for both yeast strains. Yeast strain and copper sulphate addition both influenced the amount of β-damascenone in Riesling wines significantly, but they showed no significant influence on the level of terpenes. Five out of eight rated sensory attributes showed significant differences. Yeast strain and copper sulphate addition were decisive factors for the sensory profile of experimental wines.

KEYWORDS

Riesling, volatile thiols, norisoprenoids, free terpenes, yeast strains, copper sulphate

INTRODUCTION

Riesling is a very important grapevine variety with very large areas of it planted in Germany, Australia, North America (California) and France (Alsace). In Austria, the area planted with Riesling is quite small (1,900 hectares; Austrian Wine Marketing Board, 2020), but the quality of Austrian Rieslings is renown worldwide. The aroma of Riesling is very complex and ranges from apricots and peaches to reductive flintstone. Riesling is known as a variety that closely reflects the soil of its origin. The authenticity and typicity of Riesling wines are strongly influenced by the terroir (Bauer *et al.*, 2011). Riesling is a semi-aromatic variety and contains a few important classes of aromatic substances: Norisoprenoids, terpenes and volatile thiols. Norisoprenoid 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) is responsible for the petrol and kerosene aromas in Riesling wines. The precursors of TDN are glycosidic derivatives (Williams *et al.*, 1982). Enzymatic or acid hydrolysis can provoke the cleavage of norisoprenoids from their bound form. Sunlight, shade and degree of ripeness, along with region, storage time and temperature all influence the level of norisoprenoid precursors in Riesling wines (Marais *et al.*, 1992a; Marais *et al.*, 1992b). Monoterpenes are present in higher quantities than norisoprenoids in young Riesling wines less than 2 years old (Black *et al.*, 2015). Linalool, alpha-terpeniol, nerol and geraniol are important terpenes in Riesling wines; they possess floral and fruity aromas, their precursors are also glycosidic derivatives and their levels in wine are influenced by many factors. The volatile thiols of Sauvignon blanc and a few other varieties are very well known and they are also present in Riesling wines. The most studied volatile thiols are 4-mercapto-4-methylpentan-2-one (4-MMP), 3-mercaptohexan-l-ol (3-MH) and 3-mercaptohexyl acetate (3-MHA). They possess different types of odours: broom, box-tree and grapefruit respectively. The precursors of 4-MMP and 3-MH are bound with cysteine and glutathione and they can be released as a result of beta-lyase activity (Tominaga *et al.*, 1998). During alcoholic fermentation, yeasts are able to convert 3MH to 3MHA. The esterification reaction between 3MH and acetic acid is catalysed by the enzyme ester forming alcohol acetyltransferase (Swiegers *et al.*, 2007). The precursors of volatile thiols and the volatile thiols levels are influenced by the following viticultural and oenological factors: ripening, water deficit, nitrogen level, *Botrytis cinerea*, machine harvest, SO_2 addition, oxygen exposure, skin contact, higher maceration temperature, pressing, fermentation, yeast strain, fermentation temperature, oxidation, copper addition and bottle aging (Coetzee and Du Toit, 2011). Fermentation aroma has an impact on Riesling wines in general, but has higher impact on young Riesling wines.

The winemaker can choose between inoculated and uninoculated (indigenous microflora) alcoholic fermentation. Different Riesling wines can be produced by using different yeast strains (Pavelescu *et al.*, 2016). The influence of the yeast strain on the aroma of Riesling wines is considerable, but the changes in aroma do not mask the varietal grape character (Egli *et al.*, 1998). In Austria many consumers prefer young wines; therefore many wines are bottled after 6 or 8 months of aging. When a Riesling wine is produced with a yeast strain that can release a high level of volatile thiols, the aroma- of the wine is often associated with a so-called reduced off-flavour (rotten egg) in the first year of aging. Many low molecular sulphur compounds are implicated in the reduced off-flavour, the most important ones being Hydrogen sulphide, Methanethiol, Ethanethiol, Dimethyl sulphide and Diethyl sulphide. Copper sulphate is usually used to treat wines against the reduced off-flavour. Copper sulphate has no selective removal properties as it can react with positive (volatile thiols) and negative (low molecular sulphur compounds) aroma compounds (Swiegers and Pretorius, 2007). The addition of copper sulphate can be a temporary solution for reduced off-flavour, but in the long-term it can increase hydrogen sulphide concentration after bottling. Ugliano *et al.* (2011) reported increased levels of hydrogen sulphide in bottled Sauvignon blanc stored 6 months after copper addition. Similar findings were reported for Chardonnay wines over a 12-month period: concentrations of hydrogen sulphide and dimethylsulfide were higher after copper addition (Wilkes *et al.*, 2013).

Previously, it was assumed that copper fining removes or reduces sulfur-containing compounds that cause off-flavours because of the very low solubility of copper sulfides and copper mercaptides in aqueous solutions and the possibility of physical separation (in particular filtration before filling). It is known that racking does not completely separate copper sulfides and copper mercaptides.

Kreitman *et al.* (2017) have shown that volatile sulphur compounds responsible for off-odours cannot be readily removed via filtration from wine as an insoluble complex after copper fining.

The aim of this study was to determine aroma levels in Riesling wines and to investigate the influence of copper addition on the aroma profile of the experimental wines. The secondary aims were to determine if there is any connection between sensory attribute “reduction” and the volatile thiols in the experimental wines and to compare the different yeast strains in the Riesling wines.

MATERIALS AND METHODS

1. Must origin and treatments

The Riesling grapes (Winery Mayer am Pfarrplatz, Alsegg single vineyard, Vienna, Austria) were harvested on 23 October 2016. A whole-berry press was carried out and the juice was prepared for fermentation. The following were added to the must: 1 g/hl Rapidase CB (DSM Food Specialties, Delft, The Netherlands), 10 g/hl Granucol GE (Erbslöh, Geisenheim, Germany), 100 ml/hl must Gelatine (Erbslöh, Geisenheim, Germany). After 12 hours, the must was racked and the sediment was filtrated with a lees filter (Mori, 60 x 60 model, 2016).

2. Yeast strains

The yeast strains used were Zymaflore VL3 and X-Pure (Laffort, Bordeaux, France) Dosage 25 g/hl. The yeasts were rehydrated according to the manufacturer's instructions and added to the must. The yeasts were rehydrated in water (10 times the yeast weight) at 37 °C. After 20 minutes, the acclimatisation phase was started by gradually adding must.

3. Fermentation procedure and fermentation kinetics

Fermentation took place in 30 l glass carboys at temperatures of 18.5 to 20 °C. Twenty-four hours after fermentation had begun, 20 g/hl Vitaferm Ultra F3 (Erbslöh, Geisenheim, Germany), a mixture of important nutrients, amino acids, peptides, special fermentation co-factors, mannoproteins and other adsorptive polymers, was added. Two more additions of 20 g/hl of the multi-nutrient complex were made on the 48th and 72nd hours of the fermentation process, with the final nutrient addition on day 6 of the fermentation process. The total amount of nutrients used was 70 g/hl. When the sugar level was below 5 g/l, fermentation was considered completed. The fermentation kinetics were monitored by means of portable density meter DMA 35 (Anton Paar, Graz, Austria). All the wines were replicated three times.

The wines were stored in a 10 l carboy for 7 months at 16 to 17 °C. Then the wines were racked and prepared for bottling without filtration. 0,375 l bottles were used and closed with screw caps.

4. Copper sulphate

Kupfat (Erbslöh, Geisenheim, Germany) was added at a dosage of 0,2 g/hl 6 months after fermentation had ended according to the manufacturer's instructions. It was dissolved in a small quantity of water before being thoroughly stirred into the wine in the carboys.

5. General composition and aroma substances

The general composition of the wine, volatile thiols, norisoprenoids, free terpenes, aroma fermentation, sulphur compounds and copper content were determined two and a half months after bottling by Excell Laboratories (Bordeaux, France; https://www.sarco.fr/en/).

6. Sensory analysis

A sensory analysis was performed 8 months after the end of fermentation by a panel of 22 expert wine tasters comprising 7 women and 15 men. The panelists gave a score for the intensity of each descriptor on a scale of 0 for low or no intensity to 10 for high intensity. The mean values for each descriptor were used to obtain a profile of the experimental wines. The panelists were presented with three samples per round, for a total of three rounds. The sensory analysis was performed in sensory booths in the tasting room at the Klosterneuburg Viticulture School. Wines were maintained at 10 °C and 30 ml of each sample was served. Seven aroma attributes were assessed by the tasters (duplicate fermentation replicates): “fruity”, “freshness”, “apricots”, “herbs (herbal)”, “reductive” (hydrogen sulphide aroma), “acidity” and “bitterness”. Two tastings sessions were carried out one week and two weeks after bottling.

7. Experimental design

The design consisted of two fixed factors, VL3 and X-Pure yeast, each with or without added copper sulphate ($CuSO_4$), as well as a blocking factor for the tasters. The blocking factor was regarded as a random factor and was included in the model to correct for any bias of each individual taster.

There were three replicates of each of the fixed factor combinations. There are several ways of analysing such a design. One option is to use a randomized complete block design (RCBD) which results in a linear mixed effects model with a random blocking factor and a fixed treatment factor with 12 levels.

To keep efficiency high and the total number of experiments reasonably low, a balanced incomplete block design (BIBD) was used with 22 tasters (blocks) and a block size of six. It should be noted that in the complete case (i.e., if each taster is to score every wine), this would result in 264 experiments (i.e., 22 × 2 × 2 × 3). However, this is not always feasible due to time restrictions or limitations in the tasters' concentration.

Additionally, the research hypotheses (effect of yeast strains and $CuSO_4$ addition, and possible interactions) can still be tested by linear constraints (Mangiafico, 2015). The wines were served to each taster in a random order, although this increases the effort of wine serving and data management during and after the tasting.

8. Statistical analyses

Statistical analyses were performed using the software R (R Core Team, 2020). A univariate linear mixed effects model was fit to the data using the R package nlme (Pinheiro *et al.*, 2020). Effects were tested by linear constraints using the R package multcomp (Hothorn *et al.*, 2008). The results were additionally analysed by a two-way ANOVA with interactions. p-values lower than 0.05 were regarded as significant.

Further, principal component analysis (PCA) was applied to the mean attribute ratings obtained from the panel.

RESULTS AND DISCUSSION

1. Fermentation kinetics and general composition of the experimental wines

Many rainy days during the summer influenced the 2016 vintage in Vienna. During the ripening period the weather was cold and therefore the Riesling grapes contained lower amounts of sugar than usual. Chaptalisation was carried out; the sugar level was 185 g/l and was adjusted to 210 g/l by adding sucrose.

The following values were obtained for the must: density = 1.087 g/dm3, pH = 3.2, TA = 7.5 /l, free SO_2 = 9 mg/l and total SO_2 = 30 mg/l before the fermentation started. The fermentation was completed after 14 days. Both the VL3 and X-Pure yeasts were able to ferment the must to dryness (Figure 1). The same fermentation rates were observed for both yeasts, with almost no differences in the fermentation curves of the samples. Between days 4 and 6 the temperature during fermentation dropped by 3 °C, resulting in less sugar being consumed by the yeasts.

2. General wine composition

The pH values were low, which is normal for the Riesling grape variety and the 2016 vintage. The alcohol level was very similar in all the wines in the trial. All samples were totally dry (1 g/l sugar; Table 1). The acidity and volatile acidity values

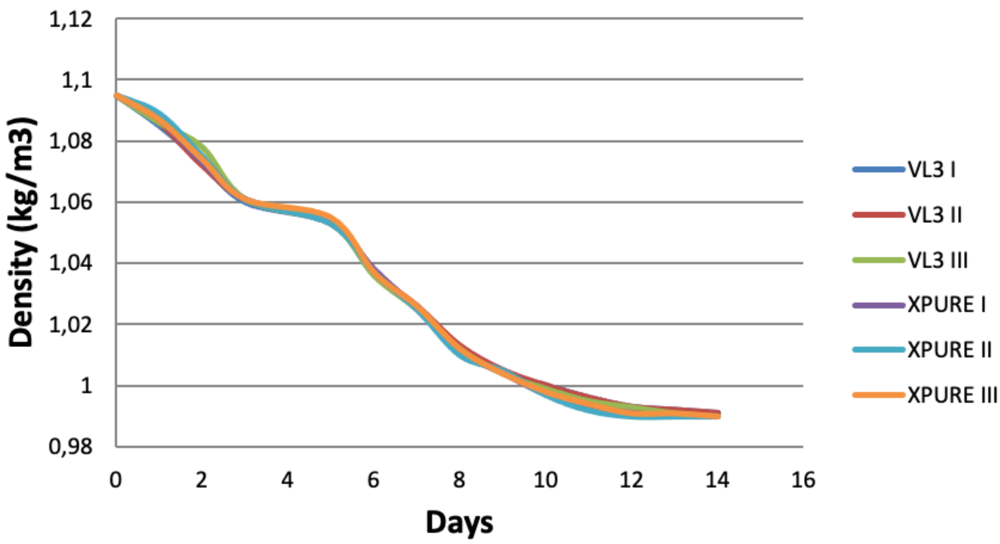

FIGURE 1. Daily fermentation monitoring in all carboys.

TABLE 1. General wine composition of Riesling wines produced with two different yeasts with or without $CuSO_4$ addition.

Parameter	VL3 with $CuSO_4$	VL3 without $CuSO_4$	X-Pure with $CuSO_4$	X-Pure without $CuSO_4$
pH	3.06 (0.01)	3.03 (0.02)	3.06 (0.01)	3.04 (0.01)
Alcohol (% v/v)*	13.64 (0.03)	13.68 (0.01)	13.71 (0.01)	13.7 (0.01)
Sugar (g/l)	1 (0.03)	1.07 (0.03)	1 (0.03)	1 (0.06)
Acidity (g/l)	7.22 (0.08)	7.4 (0.19)	7.21 (0.03)	7.23 (0.06)
Volatile acidity (g/l)	0.38 (0)	0.39 (0.01)	0.41 (0.01)	0.4 (0.02)
Free SO_2 (mg/l)	31.67 (2.19)	31.67 (0.88)	31.33 (2.19)	32.67 (1.67)
Total SO_2 (mg/l)	103 (3.38)	105 (0.58)	95,67 (5.04)	99.67 (0.67)
Copper (mg/l)	0,32	nd	0,33	nd

Values in brackets represent standard deviations (n = 3) and the statistical significance is indicated as *($P < 0.05$).

were similar for all the samples. No copper was detected in the wines without $CuSO_4$ addition (Table 1).

3. Volatile thiols

The concentrations of volatile thiol 3MH were higher than the perception thresholds in all the Riesling wines produced in the trial. The volatile thiol 3MH is a very powerful odorant. The concentration of 3MH was between 300 and 450 ng/l and the perception threshold was 60 ng/l. The content of 3MHA (the product of reaction between 3MH and acetic acid; 8 ng/l) was only higher than the perception thresholds in the wines produced with the yeast VL3 without $CuSO_4$ addition for which the perception thresholds was 4 ng/l (Figure 2). The concentration of 4MMP was lower than the perception thresholds in all the Riesling wines produced. These results are in accordance with those obtained by Tominaga *et al.* (2000), who reported high levels of 3MH in Riesling wines from Alsace. A difference between the two yeasts was also observed: wines obtained from the VL3 yeast contained higher concentrations of thiols than those from the X-Pure yeast. The factor "yeast strain" influenced the amount of 3MH in Riesling wines significantly. An additional observation is that the samples with copper sulphate added six months after fermentation showed a higher concentration of 3MH than the wines produced without copper sulphate addition with both yeast strains. Copper sulphate influenced the concentration of 3MH and 3MHA in Riesling wines significantly. The wines produced using the X-Pure yeast and with added copper sulphate showed higher amounts of 3MHA and 4MMP, but this increase was not significantly influenced by the copper sulphate. As expected, 3MHA and 4MMP decreased in the wines produced using VL3 yeast and with copper sulphate addition. The significant increase in 3MH in the unfiltrated Riesling samples with the copper sulphate addition is very difficult to explain; it is in contradiction to Ugliano *et al.* (2011), who reported a rapid decrease in 3MH after copper addition in filtrated Sauvignon blanc wines from Adelaide Hills, Australia. Further research is recommended to investigate the impact of filtration on the 3MH after copper sulfate treatment.

4. Norisoprenoids

Damascenon and β-ionone were analysed in Riesling wines (Figure 3). The concentrations of β-damascenone in the wines were higher than the perception thresholds. The levels of β-damascenone were between 2000 and 3400 ng/l and the perception thresholds is 50 ng/l (Guth, 1997). Kwasniewski *et al.* (2010) reported a high concentration of β-damascenone of between 5000 and 9000 ng/l in Riesling wines from Finger Lakes region of New York State, USA. β-damascenone possesses a flowery, sweet odour (Acree *et al.*, 1981). Higher concentrations of β-damascenone were found in the wines made using VL3 yeast than in those using X-Pure yeast, as well as in the samples with added copper sulphate compared to those without copper sulphate. Yeast strain and copper thus influenced the amount of β-damascenone in Riesling wines significantly. These results are in accordance with those published by Loscos *et al.* (2007), who also found significant differences in β-damascenone

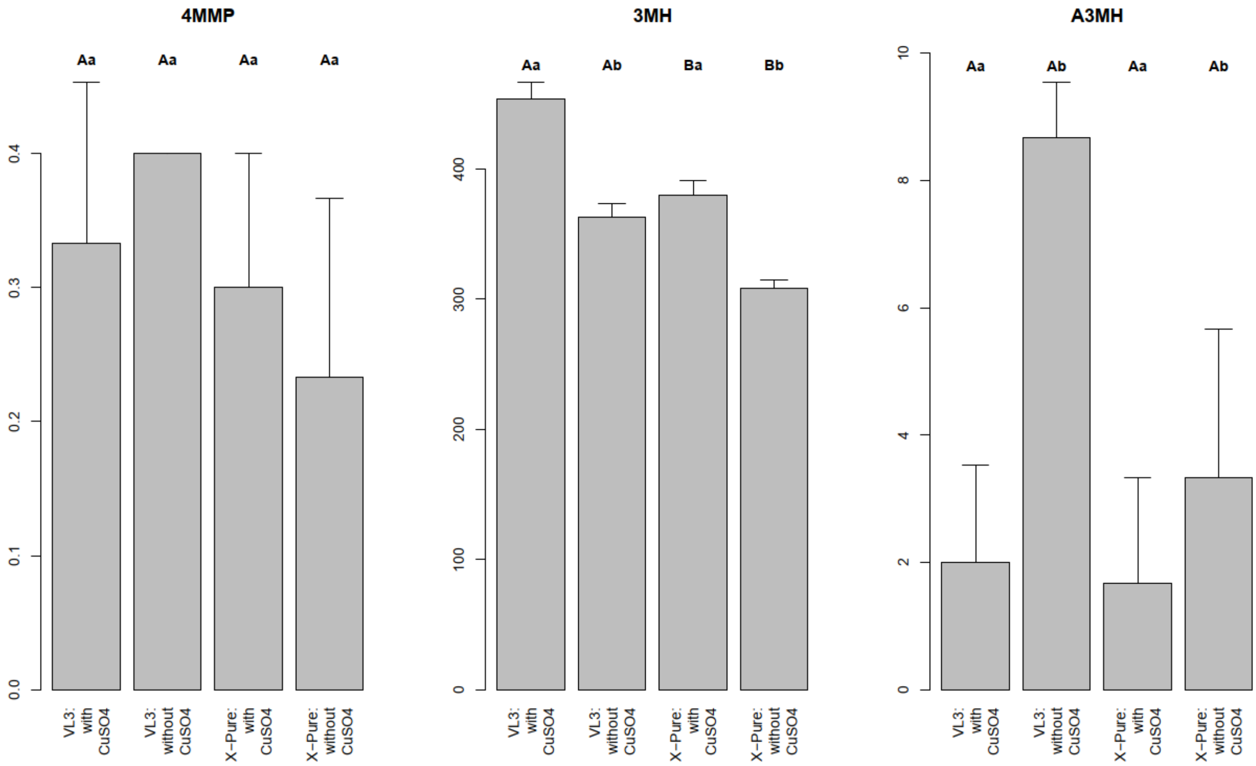

FIGURE 2. The content of 4-MMP (4-mercapto-4-methylpentan-2-one), 3-MH (3-mercaptohexan-1-ol) and 3-MHA (3-mercaptohexyl acetate) (ng/l) in Riesling wines produced using two different yeasts with or without $CuSO_4$ addition.

Capital letters refer to the yeast, and lower-case letters to the addition of $CuSO_4$. The same letters indicate that the null hypothesis of the corresponding factor cannot be rejected.

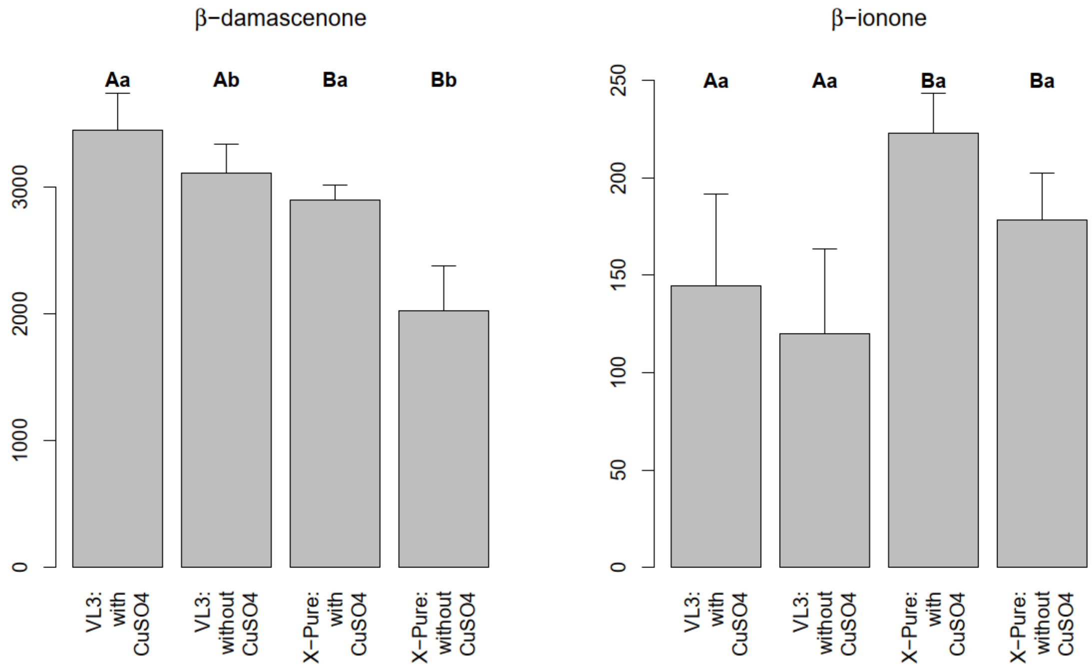

FIGURE 3. The content of β-damascenone and β-ionone (ng/l) in Riesling wines produced using two different yeasts with or without $CuSO_4$ addition (averages of three repetitions).

Capital letters refer to the yeast, and lower-case letters to $CuSO_4$ addition. The same letters indicate that the null hypothesis of the corresponding factor cannot be rejected.

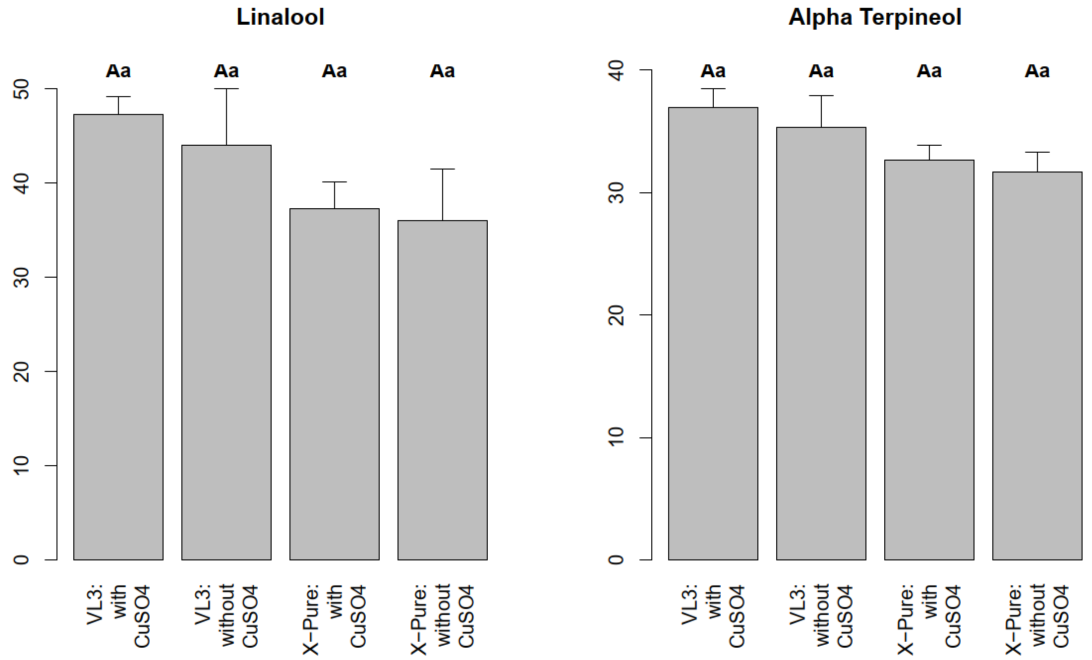

FIGURE 4. The content of linalool and alpha terpineol (µg/L) in Riesling wines produced with two different yeasts with or without $CuSO_4$ addition.

Capital letters refer to the yeast and lower-case letters to $CuSO_4$ addition. The same letters indicate that the null hypothesis of the corresponding factor cannot be rejected.

concentrations in wines produced from their experiment. Conversely, β-ionone concentrations were lower than the perception threshold; the former were between 120 and 220 ng/l and the latter was 800 ng/l (Ribéreau-Gayon *et al.*, 2006). Yeast strain and copper therefore had no significant influence on β-ionone levels.

5. Terpenes

The following terpenes were analysed: linalool, alpha-terpineol, citronellol, nerol and geraniol. Only linalool and alpha terpineol were found in higher levels than the detection limit (Figure 4). Both detected terpenes showed lower levels than the perception thresholds. The factors yeast strain and copper presented no significant influence on the level of terpenes. The detected concentrations of linalool were similar to the findings of Sacks *et al.* (2012).

6. Sulphur compounds

The following sulphur compounds were not detected in Riesling wines: hydrogen sulphide, carbon disulphide, ethanethiol, dimethyl disulphide and diethyl disulphide (Table 2). The level of methanethiol was from 1.5 to 3.9 µg/L higher than the perception thresholds in white wines (3.1 µg/L; Solomon *et al.*, 2010). As expected, the Riesling wine samples with added copper sulphate had a lower concentration of methanethiol. Higher levels of methanethiol were found in the wine made using VL3 yeast than in those using X-Pure yeast. Yeast strain, copper and the interaction between them influenced the amount of methanethiol in Riesling wines significantly. The concentration of dimethyl sulphide was between 2.5 and 9.3 µg/L, which is lower than the perception thresholds in white wines (29 µg/L; Goniak and Noble, 1987). Slightly higher concentrations of dimethyl sulphide were found in the wine made using X-Pure yeast than in those using VL3 yeast. Dimethyl sulphide can enhance the fruity character of wines at low concentrations (Spedding and Raut, 1982). Copper had a significant influence on the amount of dimethyl sulphide in the wine, which were between 3 and 5 µg/L and higher than the perception threshold (0,9 µg/L; Goniak and Noble, 1987). The wine produced using the VL3 yeast contained higher levels of diethyl sulphide than that made using the X-Pure yeast. Meanwhile, the Riesling wine samples with added copper sulphate had lower concentrations of diethyl sulphide. Yeast strain, copper and the interaction between them influenced the amount of diethyl sulphide in Riesling wines significantly. Our results contradict those of Wilkes *et al.* (2013), who observed no

TABLE 2. Sulphur compounds in Riesling wines produced with two different yeasts and with or without $CuSO_4$ addition.

Parameter (µg/L)	VL3 with $CuSO_4$	VL3 without $CuSO_4$	X-Pure with $CuSO_4$	X-Pure without $CuSO_4$
Hydrogen sulfide	nd	nd	nd	nd
Carbon disulfide	nd	nd	nd	nd
Ethanethiol	nd	nd	nd	nd
Dimethyl disulfide	nd	nd	nd	nd
Diethyl disulfide	nd	nd	nd	nd
Methionol	nd	nd	nd	nd
Methanethiol *	1.57 (0.07)	3.93 (0.09)	1.53 (0.12)	2.43 (0.15)
Dimethyl sulfide *	3.8 (0.25)	7.9 (0.15)	2.5 (0.06)	9.37 (0.2)
Diethyl sulfide *	3.07 (0.09)	5.07 (0.18)	3.1 (0.12)	3.97 (0.0)

Values in brackets represent standard deviations (n = 3) and the statistical significance is indicated as *(P < 0.05).

TABLE 3. Concentrations of esters in Riesling wines produced with two different yeasts with or without $CuSO_4$ addition.

Parameter	Aroma	Perception thresholds (mg/l)	VL3 with $CuSO_4$	VL3 without $CuSO_4$	X-Pure with $CuSO_4$	X-Pure without $CuSO_4$
2-phenylethanol	rose	14,00	34.00 (2.31)	34.33 (3.38)	38.33 (1.33)	36.67 (2.6)
Phenylethyl acetate	rose	0,25	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)
Isoamyl acetate	banana	0,03	1.27 (0.09)	1.13 (0.17)	1.47 (0.09)	0.93 (0.12)
Hexyl acetate	pear	0,70	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)
Ethyl butanoate		0,01	0.37 (0.03)	0.3 (0)	0.3 (0)	0.3 (0)
Ethyl hexanoate	green apple	0,01	2.33 (0.26)	2.27 (0.27)	2.1 (0.49)	2.33 (0.32)
Ethyl octanoate	floral	0,20	8.3 (0.42)	8.73 (0.52)	9.37 (0.44)	8.73 (1.15)
Ethyl decanoate	floral	0,20	3.07 (0.18)	3.73 (0.41)	3.3 (0.21)	3.5 (0.1)
Ethyl propionate	pineapple	0,40	0.2 (0)	0.2 (0)	0.2 (0)	0.2 (0)
Ethyl 2-methylpropanoate	pineapple	0,40	0.3 (0)	0.27 (0.03)	0.27 (0.03)	0.27 (0.03)
Ethyl 2-methylbutanoate *	green apple	0,45	0.1 (0)	0.07 (0.03)	0.03 (0.03)	0 (0)
2-phenylethanol	rose	14,00	34.00 (2.31)	34.33 (3.38)	38.33 (1.33)	36.67 (2.6)
Phenylethyl acetate	rose	0,25	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)
Isoamyl acetate	banana	0,03	1.27 (0.09)	1.13 (0.17)	1.47 (0.09)	0.93 (0.12)

Values in brackets represent standard deviations (n = 3) and the statistical significance is indicated as *(P < 0.05).

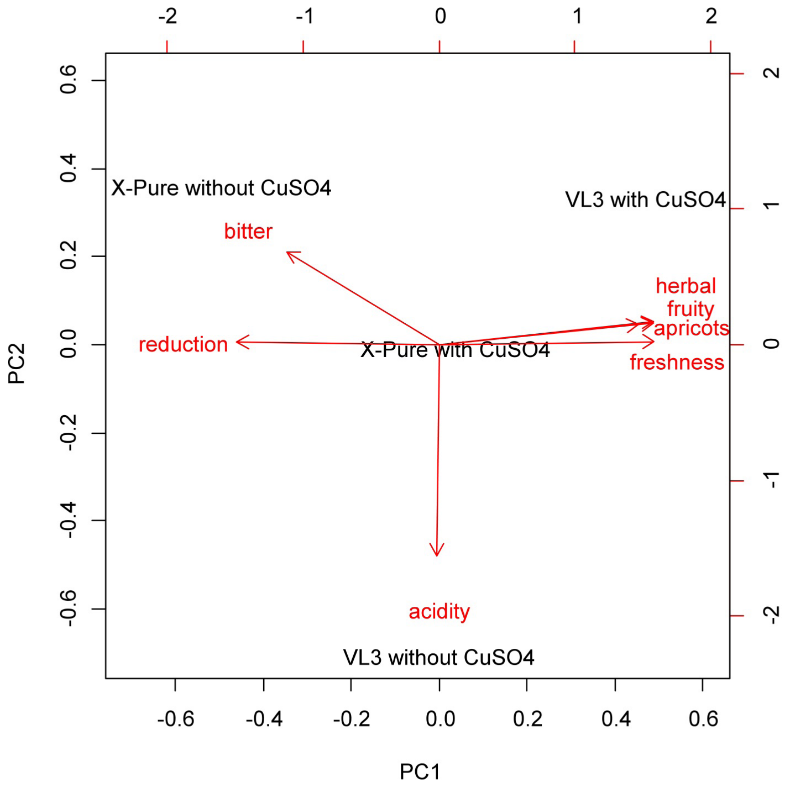

FIGURE 5. Principal component analysis of Riesling wines produced with two different yeast strains with or without $CuSO_4$ addition.

significant effect of copper on the concentration of dimethyl sulfide. However, this may be due to the influence of certain mechanisms; for example, the promotion of oxidation by copper (Viviers *et al.*, 2013) and the generation of dimethyl sulfide by cysteine in wines without added copper (Baldus *et al.*, 2017). Further research is needed for elucidation.

7. Esters

The ester content of the Riesling wines produced with the two yeasts was similar. The following esters showed higher concentrations than the perception thresholds: 2-phenylethanol, Isoamyl acetate, Ethyl butanoate, Ethyl hexanoate, Ethyl octanoate and Ethyl decanoate. Isoamyl acetate content was significantly influenced by copper. The wine samples with added copper sulphate showed higher levels of isoamyl acetate. The significant differences found in ethyl 2-methylbutanoate content were related to yeast strain. No major differences were noted between volatile esters composition in the Riesling wines produced.

8. Sensory analysis

The principal component analysis and the analyses of variance of attributed scores were carried out with the software R (version 3.6.3), revealing major differences between the Riesling wines. Five out of 8 rated attributes showed significant differences between the two yeast strains, as well as between the treatments with and without copper sulphate addition: “fruity”, “freshness”, “apricots”, “reduction” and “total impression”. Furthermore, no significant interaction effects were found. The results of the principal component analysis on the assessed aroma attributes and the different yeast strains with the applied treatments are shown in Figure 5. The wines made from VL3 yeast and with added copper sulphate received the highest scores for intensity of the attributes “fruity”, “freshness”, “apricots” and “herbs”. The wines produced with the X-Pure yeast with added copper sulphate and those produced with the VL3 yeast without copper sulphate addition showed similar intensities for the attributes “fruity”, “freshness”, “apricots”; however, the former wines were perceived as being slightly higher in intensity for all three attributes. Meanwhile, the wines produced with the X-Pure yeast without added copper sulphate were found to be the lowest in intensity for these three attributes. The attributes “herbs”, “acidity” and “bitterness” were found to be similar in intensity in all the Riesling wines produced in this trial, with no significant differences. Large differences between

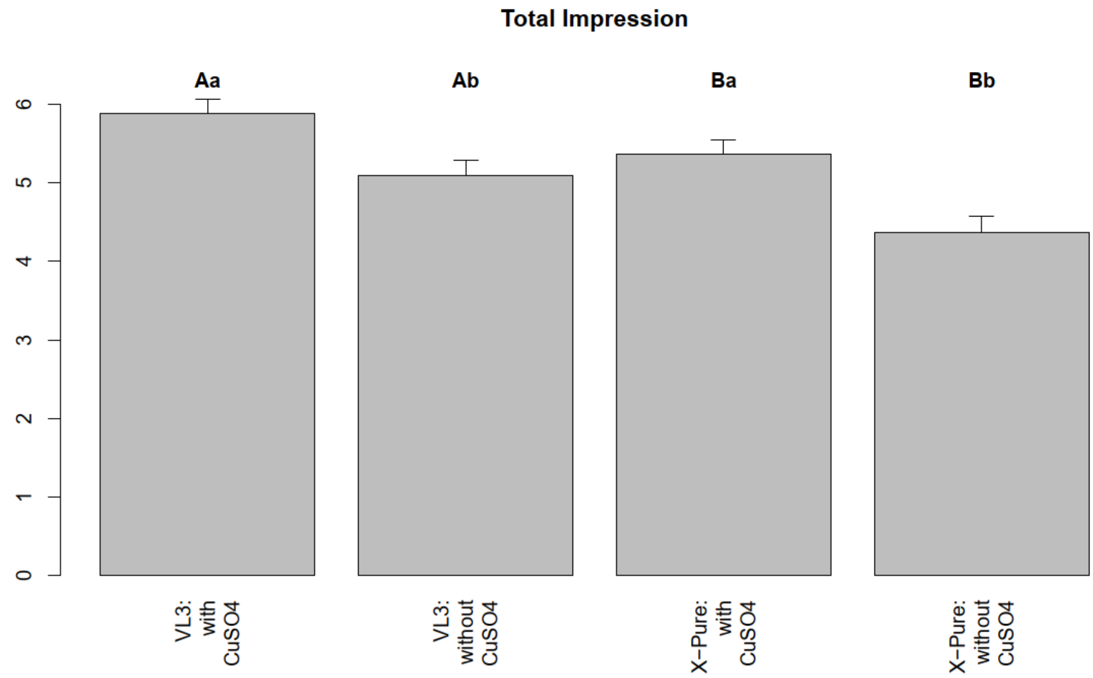

FIGURE 6. Overall sensory impression Riesling wines produced with two different yeast strains with or without $CuSO_4$ addition.

Capital letters refer to yeast, and lower-case letters to the $CuSO_4$ addition. The same letters indicate that the null hypothesis of the corresponding factor cannot be rejected.

the wines were found in the attribute "reduction", and the tasting panel perceived the wines with added copper sulphate to be low in intensity for this attribute.

The wines produced with the VL3 yeast without added copper sulphate were found to have medium intensity for the attribute "reduction and those produced with the yeast X-Pure without added copper sulphate the highest intensity. The attributes "fruity", "freshness", "apricots" and "herbs" were closely correlated, but rarely associated with reductive notes. The highest scores for overall impression were given to the wines produced with the yeast VL3 with added copper sulphate (Figure 6), while the lowest were given to those produced with the X-Pure yeast without added copper sulphate.

The wines produced with either of the yeasts and added copper sulphate were found to have the highest 3MH content (fruity, grapefruit odour) and the highest intensity for the attributes "fruity", "freshness" and "apricots". This observation contradicts the fact that 3MH content should decrease with copper sulphate addition. The copper content of the wines with added copper sulphate was 0.33 mg/l and no copper was detected in the wines without added copper. The wines were not filtrated when the copper was added; they were in contact with fine lees. If any copper was in the wines from vineyard spraying, it was probably bound by the lees. The significant increase in 3MH in unfiltrated Riesling wines after copper sulphate was added is still to be explained. There are at least two hypotheses: the existence of enzymatic activities catalysed by copper and copper catalysed desulphurization of cysteine (Luh and Ni, 1990); neither has yet been confirmed. A high number of volatile thiols in Riesling wines can be helpful when the wines start to develop petrol and kerosene aromas. The fruitiness and freshness of thiols can counterbalance the petrol aroma of mature Riesling wines. A high intensity in such attributes is deemed unacceptable by consumers.

CONCLUSIONS

To our knowledge, the increase in 3MH in Riesling wines after the addition of copper sulphate during the maturation on fine lees has been reported for the first time in this study. The results indicate that this type of maturation with copper sulphate addition can lead to a different style of Riesling. Volatile thiols are of major importance in the aroma of Riesling wines. The wines produced with the VL3 yeast had higher levels of thiols than those produced using the X-Pure yeast.

Copper sulphate influenced the concentration of 3MH and 3MHA in Riesling wines significantly; the significant increase in 3MH in the wines with copper sulphate addition requires further research. In particular, the impact of the time of copper sulphate treatment during the vinification process and of filtration before filling should be studied.

Only three volatile sulphur compounds (methanethiol, dimethyl sulphide and diethyl sulphide) were detected in the Riesling wines, but a reductive aroma was perceived by the panel. This suggests that other aroma substances play a role in the reduction attribute.

No correlation was observed between the reduction sensory attribute and the volatile thiols content. The yeast strain plays a major role in the aroma of Riesling wines. The VL3 yeast produced higher amounts of 3MH and β-damascenone than the X-Pure yeast. The levels of free terpenes were lower than the sensory thresholds, but it is possible that there was synergy between the aroma compounds. The sensory profile of Riesling wines was influenced by yeast and added copper sulphate; 5 out of 8 rated sensory attributes revealed significant differences.

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