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Impact of different vinification techniques on the formation of reductive notes in *Vitis vinifera* cv. Vernatsch

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Abstract. The grape variety Vernatsch is prone to the formation of severe reductive notes during alcoholic fermentation (AF), spoiling the fruity aroma characteristic for this variety. We investigated the impact of eight different vinification treatments on the formation of volatile sulfur compounds (VSCs) and their impact on the sensorial quality of the wines in this susceptible grape variety. Without the addition of sulfur under the form of potassium metabisulfite ($K_2S_2O_5$) to the crushed grapes, wines were significant less reductive. The clarification treatment showed promising results for the diminution of reductive notes, but might not be a feasible strategy for commercial wineries. Changing fermentation temperature, adding air, bentonite or copper to fermenting wines increased the appearance of reductive notes. The addition sulfur prior AF increased reductive notes in Vernatsch wines and needs to be considered as a crucial factor for the formation of reductive notes.

1. Introduction

The formation of reductive notes during the winemaking process is a widespread problem in the wine industry. These reduced off-flavours are caused by volatile sulfur compounds (VSCs) [1]. Attributes such as rotten egg, onion, cabbage, or putrefaction are sensory descriptors for VSCs and are often considered to have a negative influence on the wine aroma [2,3]. Problems with reductive notes are reported in different winemaking areas, as well as for different grape varieties and winemaking techniques.

The most studied compound for the reduced sulfur off-flavour is hydrogen sulfide (H_2S). This low volatile sulfur compound is produced from wine yeast (*Saccharomyces cerevisiae*) during alcoholic fermentation as a by-product of amino acid synthesis [4]. Residues from sulfur containing pesticides are known to enhance the formation of reductive notes [5–9]. But also other factors, such as yeast strain [4,5], nutrient status and nutrient composition of the juice [4,10–12], sulfur precursor compound, fermentation kinetics [13] as well as the sulfite reductase enzyme [4].

However, recent works show there are other molecules than H_2S, which can enhance the appearance of reductive notes in wines [14]. Rauhut and Kürbel [15,16] report that wines with sulfur aroma defects had increased concentrations of methanethiol (MeSH), ethanethiol (EtSH), dimethyl disulfide (DMDS), methyl ethyl disulfide (MeSSEt), diethyl disulfide (DEDS), thioacetic acid-S-methyl ester (MeSAc) and thioacetic acid-S-ethyl ester (EtSAc) as well as other known and unknown S-compounds.

1.1. Issue Vernatsch

Among a wide range of cultivated varieties in South Tyrol, the autochthonous grape variety Vernatsch suffers the most from reductive notes [17]. Reductive notes have become an almost typical descriptor for this variety; almost all wines tend to get in reduction during or right after alcoholic fermentation. Reduced sulfur off-flavours are one of the main reasons for complaints in finished Vernatsch wines at the certification process for obtaining the designation of origin. Not only wine-technicians detect the default, also consumers claim the lack of fruitiness, or complain about the unpleasant aromas of cooked vegetables, onions and rotten eggs of concerned wines. The strategy winemakers have to prevent and to eliminate these undesired off flavours is to rack and aerate the Vernatsch wines several times after alcoholic fermentation. To carry out this operation several times with each wine is time consuming and the wines might not only loose negative low volatile sulfur compounds, but also other aroma compounds sensitive for oxidation processes. Not always aeration alone is effective enough to get rid of the reductive notes, especially if the problem remains untreated for a longer period of time. In these cases, copper containing fining agents are added to the wines. However, the not always efficient and non-selective action against a wide range of sulfur containing aromas, together with the accumulation of a heavy metal including the risk of a copper casse formation are the major drawbacks of this strategy. In some severe cases the above described interventions are not sufficient to produce faultless Vernatsch wines. If Vernatsch is more prone to the formation of reductive notes or simply shows the symptoms of reductive notes more easily compared to other varieties grown in the same area and under similar viticultural conditions has not been investigated yet.

The grape variety is known to be very susceptible to powdery mildew, a disease caused by the fungus *Uncinula necator*. As sulfur containing formulations have several advantages compared to alternatives, they are the most used pesticides to control powdery mildew (Savocchia et al. 2011). Although the high susceptibility, Vernatsch vineyards in South Tyrol are usually treated the same as other varieties with an additional refrain from late sulfur applications, as they are known as a possible risk for excessive H_2S formation in wines. Many vine growers even do a late copper spraying in Vernatsch vineyards to have higher copper concentrations in the pomace with the aim to prevent from sulfur off flavours. Further Vernatsch is known to have very low levels of yeast assimilable nitrogen (YAN), but shortage is usually balanced out with diammonium phosphate (DAP) and/or organic nitrogen and should not be a limiting factor for yeast development.

However, sensorial impressions of reductive notes in Vernatsch wines often seem to be different compared to reductive notes in other varieties.

A better understanding of the formation of these off-flavours in Vernatsch wines is necessary to improve the overall wine quality and to ensure the existence of this autochthonous grape variety in the future. Different winemaking techniques were applied to examine their impact on the formation of low volatile sulfur compounds in this susceptible cultivar.

2. Material and methods

In this paper results from one vineyard site and only the 2013 vintage are presented. Analyses of some of the most important VSCs, such as H2S, MeSH, EtSH, DMS and DMDS have been carried in Geisenheim University, but data is not shown in this paper. Eight different vinification treatments were imposed in triplicate as follows: (1) control treatment **Tc**; (2) without SO_2 addition before AF **TnoSO$_2$**; (3) pressing, sedimentation and addition of the clear juice to the pomace **Tsed**; (4) cool fermentation temperature **Tcool**; (5) warm fermentation temperature **Twarm**; (6) aeration during AF **Tair**; (7) preventive addition of $CuSO_4$ **TCu**; (8) addition of Bentonit before AF **Tbent**.

2.1. Winemaking

Vernatsch grapes from the province winery Laimburg were used for this experiment. Grapes are grown in an east-facing slope in the vineyard *"Ölleiten"* at an altitude of approximately 300 meters above sea level in the south of the village Caldaro. Trellis system is a single Pergola with 2 shoots per vine. The vineyard was planted 1995 with Grauvernatsch clone LB59 with 5556 plants per hectare. Yield was approximately 12 tons per hectare.

Grapes were processed according to the following procedure, except for the changes described for each treatment. Grapes were harvested manually at the beginning of October with 19.7 °Brix and were transported to the research station in 200 kg plastic bins where they were destemmed and crushed immediately. After homogenization, pomace was distributed equally on 34 L glass balloons each of them containing approximately 30 kg of pomace. 30 mg/kg SO_2 under the form of $K_2S_2O_5$ was added to each container, except for treatment (2) and inoculated with *Saccharomyces cerevisiae* Levuline BRG (Lallemand, Verona, Italy). Alcoholic fermentation (AF) took place at constant 25°C ± 1 except for treatment (4) and (5). 0.2 g/kg diammonium phosphate (DAP) was added on the second day of fermentation. When residual sugars dropped below 2 g/L, wines were racked under nitrogen (N_2) and pressed. For malolactic fermentation (MLF) wines were stored at 21°C and inoculated with *Oenococcus oeni* Viniflora Oenos (Hansen, Horsholm, Denmark). After MLF, wines were racked under N_2, sulfitized and stored at 14 °C in glass containers until first sensorial analysis took place six months after harvest. Free SO_2 was adjusted to 30 mg/L before wines were filtered and bottled in 500 mL glass bottles with screw cap and stored in dark conditions at 14 °C. For treatment (3) pomace was pressed after crushing using a 100 L pneumatic membrane press. After pressing, 30 mg/kg SO_2 was added to the recovered juice as well as to the pomace and stored at 2°C. After 12 hours of cold sedimentation the clear juice was racked, added to the pressed pomace and warmed up to 25°C before continuing with the above described winemaking protocol. For treatment (4) and (5) AF took place at 20°C ± 1 and 30°C ± 1 respectively. For treatment (6) the wines were aerated each day during AF with compressed air. Air was blown in for 1 minute with 0.2 bar pressure using a porous frit. Treatment (7) consisted of a preventive copper addition of 0.50 mg/L Cu in three steps during AF. The addition was made using Desulfin® (AEB, Brescia, Italy) which is a 0.1% concentrated $CuSO_4$ solution. 5 mL/hL

Table 1. Analytical Data (FTIR) of standard wine parameters of the wines obtained from the different treatments measured the day of sensorial analysis. Values expressed as average of the three replicates ± standard deviation.

Treatment	Ethanol [% vol]	pH	Titratable acidity [g/L]	Volatile acidity [g/L]
Tc	12.64 ± 0.05	3.90 ± 0.04	4.95 ± 0.14	0.57 ± 0.00
TnoSO$_2$	12.68 ± 0.03	3.90 ± 0.04	5.02 ± 0.12	0.61 ± 0.01
Tsed	12.66 ± 0.06	3.93 ± 0.06	4.79 ± 0.08	0.62 ± 0.01
Tcool	12.79 ± 0.04	3.90 ± 0.04	4.83 ± 0.16	0.55 ± 0.01
Twarm	12.64 ± 0.05	3.92 ± 0.03	4.93 ± 0.17	0.60 ± 0.02
Tair	12.63 ± 0.01	3.91 ± 0.04	5.03 ± 0.10	0.60 ± 0.02
Tcu	12.66 ± 0.06	3.91 ± 0.02	4.86 ± 0.10	0.57 ± 0.01
Tbent	12.62 ± 0.06	3.92 ± 0.04	4.79 ± 0.12	0.60 ± 0.02

were added at the beginning and the middle of AF, 10 mL/hL were added at the end of AF when wines were racked. Treatment (8) consisted of an addition of 1 g/kg Bentonit to the pomace before AF.

The applied winemaking techniques lead to minor differences in some wine parameters (compare Table 1). Ethanol content is slightly increased for treatment Tcool as cooler fermentation temperatures increase the alcohol yield. Titratable acidity is slightly lower in treatment Tsed and Tcool which can be explained by the more pronounced formation of potassium tartrate at lower temperatures and thus a loss of Titratable acidity. Volatile acidity was not affected from the different vinification methods. This puts in evidence that, wines were no potassium metabisulfite is added prior fermentation do not necessarily contain higher levels of volatile acidity. This is true for the above described experimental conditions, including the immediate processing of only healthy grapes, rapid inoculation with selected dry yeasts and the clean environment in the experimental cellar.

2.2. Wine and sensory analyses

Must and wine analyses were carried out using Fourier Transform Infrared Spectroscopy (FTIR) from FOSS (Denmark) do determine parameters such as alcohol content, residual sugar, malic acid, lactic acid, pH, total acidity, free and total SO_2.

Sensorial evaluation of the wines occurred six months after harvest from a well trained tasting panel. Approximately half of the panellists are Laimburg internal referees (all of them wine and/or viticultural technicians) and the other half are external wine technicians from private wineries or cooperatives. The number of panellists varied from minimal 11 to maximal 15, subdivided in two groups. Sensory evaluation was carried out using unstructured rating scales [18,19]. The used evaluation scheme contained six parameters (cleanness in the aroma, fruitiness, reductive notes, varietal typicality, full-bodiedness and overall quality) with five additional sub-parameters (masked/dusty, lees/yeast, cooked vegetables, onion and H_2S/rotten eggs) to better describe the reductive notes (results not shown). The range for each parameter was from 0 to 10 with a supposed optimum at 10. For each tasting session the same evaluation scheme was used. Each panellist was examined for each parameter on his sensitivity and stability of judgement using analysis of variance as described by Kobler [20].

2.3. Statistical analysis

Microsoft Excel (Microsoft Corporation – USA) was used for data preparation and the elaboration of overview tables. Statistical analysis of one factor ANOVA and Tukey-b test were performed using SPSS (IBM Inc., New York, USA).

3. Results

Different vinification methods lead to differences in the appearance of reductive notes in Vernatsch wines. The addition of sulfur to the pomace prior AF seems to enhance the formation of this off-flavour in Vernatsch.

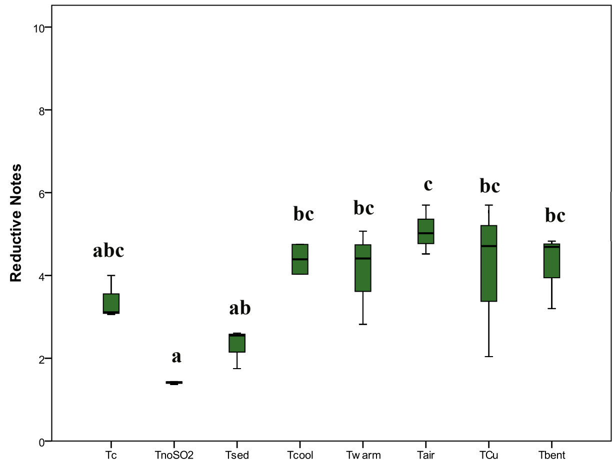

Figure 1. Boxplot of the intensity of reductive notes in the Vernatsch 2013 detected in the sensory analysis six months after harvest. Values shown for the three replicates of each treatment, averaged over the three tasting sessions. Different letters indicate statistically significant differences at $p < 0.01$.

As demonstrated in Fig. 1, the treatment $TnoSO_2$ without the addition of sulfur to the crushed grapes is significant less reductive compared to all other treatments in the trial. The formation of reduced sulfur off-flavours drops when no sulfur is added to the pomace. This effect can be seen best when comparing the treatment without sulfur addition to the control treatment. The extent of the difference in reductive notes can be attributed to the sulfur addition prior AF, as this is the only changing variable.

Beside the treatment $TnoSO_2$ also the clarification treatment Tsed, shows less reductive notes than the control treatment, although all wines, except treatment $TnoSO_2$, received sulfur prior fermentation. Ranking in between the Tc and $TnoSO_2$ treatments, the sedimentation process has a positive effect on the prevention from reductive notes in Vernatsch wines. Interesting is that Tsed is the only treatment where sulfur was added prior AF and reductive notes being less pronounced. Compounds which may have an impact on the formation of reductive notes, seems to have been removed or depleted by racking from the system. Other works have shown that must clarification reduced the appearance of reductive notes in white winemaking, but for red winemaking no evidence could be found in literature. If parts of the added sulfur have been removed during the clarification process and if this is the explanation for the observed effect has not been investigated in this work and remains the content of speculations. However, due to the labour intensive operation it might not be a feasible strategy for commercial wineries.

The remaining treatments (Tcool, Twarm, Tair, TCu and Tbent) show more reductive notes in the sensory analysis. Some of the interventions such as the addition of copper or air are known in praxis for their positive action against reductive notes; however, their impact on wine quality in this study was negative. Possible explanations for these observations are described in the discussion part of this paper.

Depending on the applied winemaking technique, not only reductive notes, but also three other sensorial

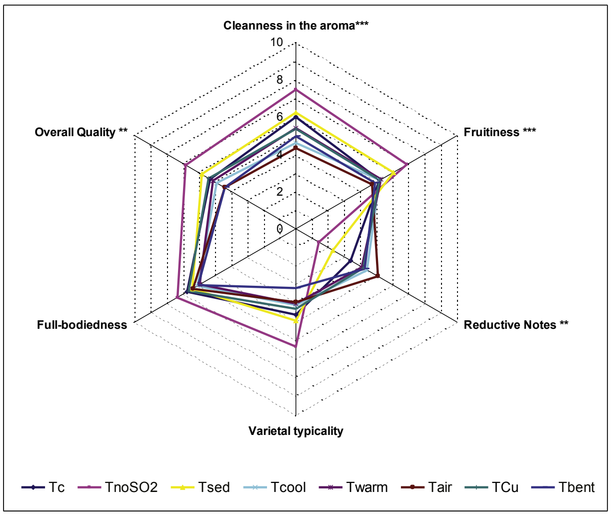

Figure 2. Main parameters of the sensory analysis of the Vernatsch 2013 six months after harvest. Values are the average of three sensory analysis sessions and the three replicates of each treatment. Statistical analysis: One-factor ANOVA. *Values are significantly different at $P < 0.05$; **Values are significantly different at $P < 0.01$; ***Values are significantly different at $P < 0.001$.

parameters (cleanness in the aroma, fruitiness and overall quality) are subject to significant changes (see Fig. 2).

The results for cleanness in the aroma are of the same order as discussed above for reductive notes, only reversed, as cleanness in the aroma is correlated negatively to reductive notes. The fact that treatment $TnoSO_2$ has the highest score for this parameter is a sign that even without the addition of SO_2 microbial spoilage prior AF was not a problem under the given circumstances. With other words, the positive effects of the non-occurrence of reductive notes overcame the possible faults of microbial spoilage due to the absence of sulfur prior AF. The same trend as for cleanness in the aroma is found for the parameter fruitiness. Differences among the all treatments where sulfur was added, except for Tsed are not as pronounced as for the two parameters described previously. The fourth parameter which showed significant differences among the treatments is overall quality. Also for this parameter the treatment $TnoSO_2$ performs best.

The only two parameters were differences among the different treatments are not significant are varietal typicality and full-bodiedness. Varietal typicality is a difficult concept to train a panel for, as it requires a lot of experience with the variety as well as knowledge about local and regional peculiarities, styles and vintage effects. For some of the panellists it might be true, that a low to moderate occurrence of reductive notes is part of the varietal typicality. This because Vernatsch wines with a reduced aroma is a widespread phenomenon in South Tyrol. There is no evidence shown, that the appearance of reductive notes impact on the body of a wine. The little differences for the body aren't significant at all for the different treatments. Nevertheless there is a clear trend that the $TnoSO_2$ has the highest scores for both parameters varietal typicality and body. Tsed showed slightly elevated scores for varietal typicality but no differences in the body compared to the other treatments.

Results demonstrate that the addition of sulfur before AF can lead to an increased formation of reductive notes and negatively impacts on the sensorial quality of Vernatsch wines.

4. Discussion

Different vinification methods lead to differences in the appearance of reductive notes in Vernatsch wines. Among the eight different vinification treatments, the addition of potassium metabisulfite to the pomace was the factor which had the biggest impact on the formation of reductive notes. Results demonstrate the addition of sulfur before AF leading to an increased formation of reduced sulfur off-flavours in the variety Vernatsch. The decision whether to add or not sulfur to the pomace was the factor with the biggest impact on the formation of reductive notes under the experimental conditions. There might be several reasons for the observed increment of reductive notes due to the utilization of potassium metabisulfite. A possible explanation might be the increased amount of available sulfur for yeast metabolism. Another explanation might be the shift in the redox potential as a consequence of the addition of a strong antioxidant to the pomace as it is the case with potassium metabisulfite. Gössinger and Steidl already described the impact of a sulfur addition to the must on the redox potential [21] and described that a low redox potential increased the formation of reductive off-flavours in Austrian white wines [22]. A similar effect on the formation of reductive notes due to sulfur residuals from crop protection has already been described from several authors.

To use copper or air, as often recommended, had a negative effect on the wine quality and enhanced the formation of reductive notes when added already during AF. Different hypothesis exist to explain these observations: The addition of air and thus oxygen in the beginning of fermentation conventionally leads to a stronger multiplication of yeasts resulting in a higher cell number. This increase in biomass also intensifies the competition for nutrients, this means mainly yeast assimilable nitrogen but also vitamins and micronutrients. Those are required for the synthesis of sulfur containing amino acids, such as methionin and cystein. This might be the reason why it comes to the accumulation of hydrogen sulfide and derivative compounds.

With the addition of copper ions some of the reduced sulfur compounds (H_2S) are removed from the system and yeasts are upregulating H_2S production to fulfil their own needs. This is an energy demanding process for yeasts and cannot be regulated down fast enough and thus could leads to an intensification of reductive notes. The effects of this process depend on the composition of the must/pomace and the yeast strain; in sufficiently supplied musts with nutrients it might barely or not even appear.

Timing of both interventions (air and copper) seems to be a crucial factor in the complex issue of reductive notes.

In red winemaking additional elements need to be considered; depending on the applied winemaking method, an increased input of oxygen can be expected to lead to reactions of H_2S and other thiols with phenolic compounds [23].

Sedimentation and racking showed promising results for the diminution of reductive notes. If this is because parts of the added and/or naturally present sulfur are removed from the system by racking remains the content of speculation. For white winemaking it is shown, that must clarification reduces the appearance of reductive notes, but for red winemaking no evidence could be found in literature. Anyway, due to the labour intensive operation it might not be a feasible strategy for commercial wineries.

The above discussed sensorial impressions have been underpinned with analysis of some of the most important volatile sulfur compounds. The results aren't shown in this paper as analyses have been extended to some more molecules. Data could not be elaborated within the deadline for this paper and will be published elsewhere. The evolution of the experimental wines during storage is tracked and the most promising treatments are repeated in following vintages on different vineyard sites.

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