Experiments with oenological methods to increase the spicy aroma in Austrian Grüner Veltliner wines

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Abstract. Grüner Veltliner is Austria's most important grape variety, accounting for 32.5% of the vineyard area (a total of around 14,500 ha). There is a noticeable trend with this variety away from fresh, fruity wines towards more spicy, single-vineyard wines. This is especially true for export-oriented producers. The aim of this scientific study is to test oenological methods for increasing the leading substance of the spicy aroma, the sesquiterpene rotundone and to sensory evaluate the wines. The methods skin contact, full and partial fermentation on the skins, addition of whole grapes and or leaves were tested in two independent experimental designs. The wines were analysed using a SPE-SPME-GC-TQMS method. The "retro-tratitional methods" of winemaking mentioned in the article can enhance the spiciness of the wines to a certain degree. Especially the addition of whole grapes as well as partial maceration gave the best results here. The increase in spiciness was observed both sensorially and analytically, as evidenced by higher rotundone contents. However, in summary, it must be stated that the results indicate that an exclusive analysis of rotundone, without the inclusion of phenols and other volatile substances, cannot contribute to a full understanding of the matter, which is based on the interaction of many components. Further analysis both sensory and analytical involving many volatile compounds and phenols are therefore absolutely necessary.

1 Introduction

Although Austria produce about only 1% of the global wine volume, it is consistently present worldwide as a well-known wine country. Our most important story revolves around the wines of the Grüner Veltliner variety, which is planted in about one third (exactly 32.5%) of all Austrian vineyards. According to [1], the aroma of the 'Grüner Veltliner' variety is characterised by fruity aromas (e.g. citrus, grapefruit, green or ripe apple, pear, quince, melon, dried fruit, baked apple), vegetal notes (e.g. pepper, wildflower, a hint of spice, acacia blossom, fresh grass, hay, straw, cooked green beans, asparagus, nut) as well as soil-associated impressions (e.g. mineral, earthy, loess-like). Hardly any other white wine variety is as often attributed with the spicy, peppery note as the 'Grüner Veltliner'. The term 'Pfefferl', which is frequently used in Austria, represents the trademark of the true typical Grüner Veltliner wine [2].

While Grüner Veltliner in Austria is often drunk fruity-fresh and above all young, there is a worldwide trend towards wines where winemakers try to bring out their origin and vineyard site with as little intervention as possible, but also with maceration or even fermentation on the skins. There is also a trend towards to try new experiments, such as the addition of whole grapes and even leaf addition to Grüner Veltliner wine during ist fermentation. The result is wines whose aroma profile is spicier and less fruit-driven. There are very few studies that look at the impact of what we call "retro traditional techniques" such as maceration, grape addition or even leaf addition to the fermentations of white wine compared to classical winemaking with or without skin contact. [3,4] studied the influence of fermentation on the skin of white wine while [5] studied the influence of stems and leaves on the content of rotundone and 1.8 cineole in Australian Syrah wine. The authors are not aware of a comparable study in Grüner Veltliner.

Rotundone, which is so important to the peppery aroma, is found at least 98% in the skin of the grape [6,7]. According to [7] most of the rotundone is extracted from the berries between the second and fifth day of fermentation. [6] found that only 10% of the rotundone contained in grapes is extracted during fermentation, while

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only 6% is recovered in bottled wine. A significant amount of rotundone is lost during wine treatment and filtration, probably due to its hydrophobic structure and binding to other materials. This hydrophobic character was also confirmed by [8]. Considering that maceration time affects the extraction of aroma compounds and other wine treatments influence the recovery in wine, it was of interest to investigate to what extent the rotundone concentration in Grüner Veltliner wines is affected. [9] therefore investigated the influence of maceration time, fermentation temperature, addition of sulphur dioxide, must clarification and yeast nutrition on the concentration of rotundone in Grüner Veltliner wines. While the prolongation of the maceration time led to a reduction of the contents, a higher fermentation temperature led to an increase of the concentration. No clear results could be generated for the other parameters tested. There were partly contradictory results in relation to the two trial years, also the wines were not sensory tested

The aim of the current study is to investigate in two experiments with regard to retro-traditional methods for wines of the Grüner Veltliner variety, on the one hand, the influence of partial and full skin fermentation and fermentation temperature in comparison to classical production (with and without skin contact time) and, on the other hand, the influence of grape and/or leaf addition on the content of rotundone. Therefore a proper method based on a SPE-SPME-GC-MS/MS instrument should be established and the wines will be analysed sensory on their spiciness.

2 Material and methods

2.1 Material

For the two experiments, grapes of the variety Grüner Veltliner were used as starting material from the Agneshof research station of the Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, Klosterneuburg, Austria from the Wagram wine-growing region (48°17' 44" N; 16°19'31" E).

2.2 Experimental design

Two separate experimental designs were used to test the influence of the different factors on the rotundone content. In a first experiment, grape addition (100 g/ 10 L must, with stem crushed) and/ or leaf addition (100 g/ 10 L) was compared with a control variant. In a second experiment, control variants with the factors skin contact for 12 h, partial mash fermentation (fermentation on the mash for 2-3 days with removal of free juice without pressing) and full mash fermentation (fermentation on the mash until the end of fermentation and pressing) were investigated, taking into account two fermentation temperatures (15 °C, 25 °C). Vinfication was carried out on a 30 liter scale under standardized conditions. After sterile filling, the wines were stored at 4 °C until analysis. All variantes were repeated in triplicate.

2.3 Rotundon analysis

The sample preparation for the analysis for rotundone is in accordance with [2]. Here, solid phase extraction (SPE) was performed by gravity on LiChrolut EN columns containing 500 mg solid phase (Merck, Vienna, Austria) and 6 ml total volume. The columns were previously cleaned and conditioned with 10 ml of n-pentane, 10 ml of methanol and finally with 20 ml of model wine (12% EtOH (ethanol absolute AnalaR NORMAPUR® ACS, VWR Chemicals, Radnor, USA) buffered to pH 3.2) (Fig. 2). 100 ml of wine sample was percolated through the column after mixing and homogenization with 100 µl of internal standard (5,7-dimethyltetralone; concentration 1 mg/l). The column was then washed with deionized water and the (-) rotundone was eluted with 10 ml of N-pentane/dichloromethane (4:1 ratio). The organic solvent was removed under vacuum using a rotovapor (Büchi R-200, Flawil, Switzerland). The remaining residue was dissolved in 1 ml of 50% ethanol. This absorbed residue was transferred to 14 ml of deionized H2O in a 20 ml "headspace vial". This solution was analyzed using SPME-GC-TQMS technology in MRM mode rather than SPME-GC-SIM-MS, in contrast to the protocol of Nauer et al. (2018). The masses 218→163 and 218→161 were used as transitions. Furthermore, a d6-redundon (50ng/l) with transitions 224→169 and 224→167 was used as internal standard. These adaptations lowered the LOD of the original method from 3 ng/l to 2 ng/l and LOQ from 10 ng/l to 7 ng/l and reduced the relative standard deviation of method precision (RSD) from 10.5% to 8.2% with model wine calibration. The recovery for different wine samples spiked at 100 ng/l ranged from 97 to 108% with the new method instead of 102 to 124% [2].

2.4 Sensory analyse

Sensory testing was focused on the question of whether the wines had increased in spiciness. For this purpose, the wines were tasted in comparison with the respective control sample. The test was carried out by 24 panelists (19 male, age 21-48 years). The panelists were students of the university course Viticulture, Enology and Wine Management of the University of Natural Resources and Applied Life Sciences, employees of the Federal College and Research Institute for Eonology and Pomology in Klosterneuburg or winemakers. All panelists were official and certified wine tasters and were also part of the sensory panel for DAC Weinviertel (Grüner Veltliner) wines and stated that they regularly drink Austrian Grüner Velltiner wines and know about the typicity of the wines. Sensory analysis was performed in an ISO 17025 accredited sensory laboratory. The tasting categories can be found in Table 1 and also the points for calculating the results.

Table 1. Tasting categories and corresponding points.

Categorie	Points
much more spiciness	+2 points
more spiciness	+1 point
the same level of spiciness	0 point
less spiciness	-1 points
much less spiciness	-2 points

2.5 Statistic

Statistical analysis was performed using SPSS 23.0 from IBM. Rotundon analysis results in experiment one were compared using simple ANOVA and post-hoc test Tukey B, and in experiment two the effects (temperature and treatment) were compared using a general linear model (univariate test). The significance level was always assumed to be alpha 0.05. Normal distribution and variance homogeneity of the variants were assumed.

3 Results and Discussion

3.1 Results of the rotundone analysis

While the addition of whole grapes increased the rotundone content by 57%, the addition of leaves reduced the content by 42%. With the addition of grapes and leaves, these effects offset each other again to a certain amount. To summeraze, the addition of grapes can increase the spiciness of the wines, while the addition of leaves, the spiciness decrease significantly (Fig. 1).

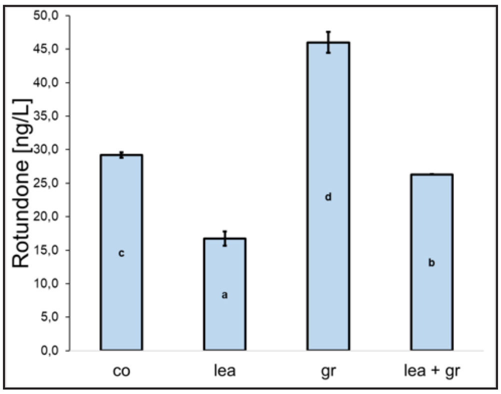

Figure 1. Rotundone concentration experiment 1.

The Rotundon, so important for the spiciness, is at least 98% in the skins of the grape [6,7]. This is the reason why the content in the classic wines and in the wines with maceration is significant lower in comparison with the skin fermentation variants (Fig. 2). Most of the rotundone is extracted from the berries between the second and fifth days of fermentation [7], which is why partial maceration without pressing (3 days of fermentation at 15 °C, 2 days of fermentation at 25 °C) did not perform worse compared to full maceration with pressing. In this period enzymes are very active and ethanol content increases.

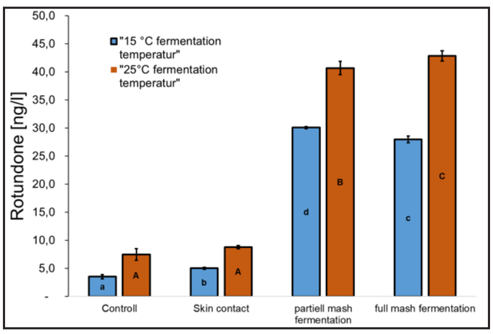

Figure 2. Rotundone concentration experiment 1.

That is, mash fermentation can greatly increase (more than 5-fold) the content of rotundone. Also, a higher fermentation temperature tends to result in higher contents; at higher temperatures, the hydrophobic character of rotundone is likely to decrease due to the higher kinetic energy. In contrast, maceration time had hardly any influence on the content of rotundone. To summarize, both factors treatment and temperature are significant

3.2 Results of the sensory study

Figure 3 shows the sensory analysis of the various factors in comparison with corresponding controls, where positive values indicate an increase in spiciness, while negative values lead to a decrease. If we try to compare these results with the Rotundon values, first of all it is noticeable that the results correlate positively to a certain degree, but with some exceptions. The fermentation with skins was positive only in the partial mash fermentation, while in the full mash fermentation the contents decreased again, this phenomenon is particularly drastic at higher fermentation temperature. Furthermore, it is noticeable that the skin contact is much better than could be expected from the Rotundon results. The addition of whole grapes has shown to be the most suitable, the addition of leaves, however, the worst. The two effects are likely to add up, which has already been found in the analysis. The wine matrix is very complex and other aromatic substances and phenols may have a crucial influence on the sensory perception of spiciness.

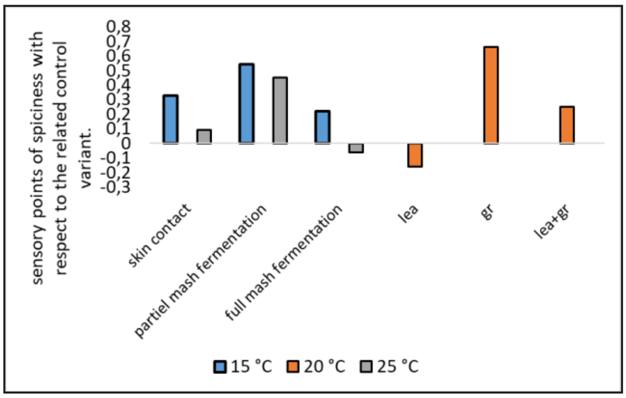

Figure 3. Results of the sensory study: average points of 24 panelists with Figure 3. The rating (much more spiciness: +2 points; more spiciness: +1 point; same spiciness level: 0 points; less spiciness: -1 points; much less spiciness -2 points).

4 Conclusion

The "retro-tratitional methods" of winemaking mentioned in the article can enhance the spiciness of the wines to a certain degree. Especially the addition of whole grapes as well as partial maceration gave the best results here. The increase in spiciness was observed both sensorially and analytically, as evidenced by higher rotundone contents. However, in summary, it must be stated that the results indicate that an exclusive analysis of rotundone, without the inclusion of phenols and other volatile substances, cannot contribute to a full understanding of the matter, which is based on the interaction of many components. Further analysis both sensory and analytical involving many volatile compounds and phenols are therefore absolutely necessary. Also, of course, the impact on the overall quality of the wines must be assessed.

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