BIO Web of Conferences **9**, 02003 (2017) DOI: 10.1051/bioconf/20170902003
40th World Congress of Vine and Wine

Detection with flash gas chromatography electronic nose of the general influences of glutathione, ascorbic acid, tannin and carbon dioxide treatments on the volatile profiles of white wines of feteasca regala

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Abstract. The present work aimed to determine some of the influences of the treatments with glutathione (GSH), ascorbic acid (AA), catechinic tannin (T) and carbon dioxide (CO_2) during winemaking or bottling on the volatile profiles of white wines of Feteasca Regala. The study is based on the use of a flash gas chromatography electronic nose, which is able to discriminate various clusters of wine samples prepared with various combinations of antioxidants. The treatments that induce enough differences in the volatile profiles of the wine that the electronic nose is able to discriminate are likely to be discriminated also by the consumers. When the electronic nose does not discriminate the clusters of samples with certain treatments it is very likely that those treatments are not sufficient to induce a practically important difference in the aromatic profile of wines. The main detectable influences of the antioxidant treatments are presented and discussed. The treatment of musts with AA (50 mg/l) clearly influences the volatile profile of the wines, reducing their aromatic complexity. The treatment during bottling with catechinic tannin (20 mg/l) does not seem to have an important influence of the profile of the wines produced with the addition of AA and or GSH. The addition of carbon dioxide during bottling does not show much influence one year after bottling, but it shows that in the samples most prone to oxidation, a difference is present. The CO_2 treated wines belonging to the cluster of wines treated with 40 mg/l GSH and 50 mg/l AA have distinct profiles, different from those not protected and closer to the clusters of the less oxidised wines. The treatment of musts with GSH shows clear influences at the dose of 40 mg/l, but for the lower dose of 20 mg/l GSH in Feteasca regala wines it is difficult to identify differences compared to the wines included in the cluster of samples not treated with GSH. This finding is particularly relevant as the dose of 20 mg/l GSH is the maximum allowed at present to be added in must and wines.

1. Introduction

The protection of wine volatiles from oxidation is one of the most desirable effects achieved by the use of the antioxidants or other compounds in winemaking or during bottling. Among the oenological permitted practices are the addition of glutathione (GSH), ascorbic acid (AA) and catechinic tannin (T) in must or wines, as well as the bottling of the wines in the presence of carbon dioxide. For this reason, although not able to replace the sulphur dioxide in spite of many trials [7,10,11,13], these compounds have been widely used and assessed in winemaking in various combinations and doses, both for still [5,7–9,12,16,18–20] and sparkling wines [21, 22]. Taking into account the many possible combinations and reactions in wine, the effect of their application is difficult to assess by sensory analyses or by physico-chemical determinations. One rapid method for evaluating the effects of these compounds on the resulted wines is the comparison of the volatile profiles of wines by using a flash gas chromatography electronic nose. This technology allows for a precise and time-efficient evaluation of clusters of samples grouped based on similar treatments [1], such as combinations of antioxidants, or common product traits, such as origin [14,17] or other [2,4]. The technique does not directly identify the volatile compounds, but rather records non-target signals resulted from the chromatographic separation of the volatile fraction present in the sample headspace and uses them as variables (e-nose sensors) for multivariate statistical analysis.

Although the electronic nose does not tell which volatile profile is better from the viewpoint of the consumer, it can evaluate many samples in a short period of time and show if samples grouped in various clusters in accordance to the undergone treatments can be discriminated or not. If some clusters of samples cannot be discriminated by the electronic nose, most likely, from the viewpoint of the perceived volatile profile, they will not be discriminated by the consumers either. Thus, the presence of clusters which cannot be discriminated would mean that the treatments performed on the wines included in those clusters are not important enough to induce perceivable differences and may not be worth applying in the wine cellar.

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2. Materials and methods

The wines evaluated were obtained in normal wine cellar conditions from Feteasca regala wine grapes cultivated in the experimental field of the University of Agronomic Science and Veterinary Medicine of Bucharest, Department of Viticulture and Enology. The oenological materials used were: L-glutathione reduced (Carl Roth GmbH, purity min. 98% for biochemistry), L(+)-ascorbic acid (Carl Roth GmbH, purity min 99%, for biochemistry), catechinic tannin (Ti Premium, Enologica Vason), carbon dioxide (Buse Gaz, purity min. 99.99%) *Saccharomyces cerevisiae* yeast (Premium blanc 12 V yeast, Enologica Vason), fermentation activator (V Starter TF, Enologica Vason). The free-run must of Feteasca regala had the following parameters: 21.6% Brix; total titratable acidity of 82.7 meq/l; pH 3.33 and 135 mg/l YAN.

The experimental wine was obtained in stainless steel tanks of 50 l volume, with temperature control of fermentation. For the wine variants preparations reduced glutathione (GSH) was added in the musts in doses of 0, 20 and 40 mg/l. The sample variants with 20 and 40 mg/l GSH were prepared either without or with 50 mg/l AA added also before the onset of fermentation. In this way, after fermentation, 5 types of wines were obtained, coded in accordance to the oenological treatments of musts as G0, G20, G20A, G40 and G40A. After racking and cold stabilisation for 3 months in tanks, the variants were bottled in 0.75 l volume glass containers, at this stage dividing each variant in accordance with some supplementary treatments performed during bottling, as follows: addition of 0 or 20 mg/l catechinic tannin (T00 and T20) and addition or not of carbon dioxide in the headspace of the bottle.

Usual dosages of the sulphur dioxide were maintained in the musts and wines, being corrected when necessary, so that the average of the free sulphur dioxide in wines was 26 mg/l and the total sulphur dioxide 132 mg/l. Detailed wine preparation techniques and the complete results for the main wine physico-chemical parameters (measured at the moment of electronic nose assessment) are presented in another paper [3]. The coding for each wine and the treatments made are included in Table 1.

One year from the bottling time the wines were evaluated with an electronic nose working on the principle of gas chromatography (GC E-nose Heracles, Alpha MOS, France). The apparatus used is a flash GC endowed with a Tenax trap and two short chromatographic columns of 2 m length and 0.18 mm diameter – one non-polar (DB5 – 5% diphenyl, 95% dimethylpolysiloxane) and another of low/mid polarity (DB1701 – 14% cyanopropylphenyl, 86% dimethylpolysiloxane) working simultaneously. The separated compounds are detected with flame ionization detectors placed at the end of each chromatographic column. Hydrogen is used both as carrier and combustion gas.

The wines from each variant are introduced in triplicate (repetitions r1–r3) in vials of 10 ml, which are filled with a volume of 4 ml of wine sample and sealed with magnetic caps with silicone septa, specially designed for the autosampler HS 100 (Combi PAL Auto-Sampler System, CTC Analytics AG, Switzerland). The method of sample analysis involves a step of warming up the sample in the autosampler shaking oven for 10 minutes at 60 °C and 500 rpm, extraction with a HS syringe of a 2.5 ml gas sample from the head-space and injection into the GC. The chromatographic program used for the separation on both columns has the following parameters: trap temperature 40 °C, trap desorption temperature 250 °C, trap purge time 50 s, bake-out 50s, initial column temperature 40 °C with an increase rate of 5 °C/s up to 200 °C, FID temperature 220 °C, FID fuel pressure 35 psi. The aquisition time is 46 s, with 5 min between 2 sample analyses. Some other details related to the method and apparatus can be found in other papers [1,2,4]. The data processing was done using the apparatus software, AlphaSoft V12.42.

Table 1. Coding and antioxidant treatments of the wine samples.

Sample code	Glutathione (mg/l)	Ascorbic acid (mg/l)	Catechinic tannin (mg/l)	Carbon dioxide*
Stage of treatment	Winemaking		Bottling	
G00_A00_T00	0	0	0	no
G00_A00_T00_CO2	0	0	0	yes
G00_A00_T20	0	0	20	no
G00_A00_T20_CO2	0	0	20	yes
G20_A00_T00	20	0	0	no
G20_A00_T00_CO2	20	0	0	yes
G20_A00_T20	20	0	20	no
G20_A00_T20_CO2	20	0	20	yes
G20_A50_T00	20	50	0	no
G20_A50_T00_CO2	20	50	0	yes
G20_A50_T20	20	50	20	no
G20_A50_T20_CO2	20	50	20	yes
G40_A00_T00	40	0	0	no
G40_A00_T00_CO2	40	0	0	yes
G40_A00_T20	40	0	20	no
G40_A00_T20_CO2	40	0	20	yes
G40_A50_T00	40	50	0	no
G40_A50_T00_CO2	40	50	0	yes
G40_A50_T20	40	50	20	no
G40_A50_T20_CO2	40	50	20	yes

*(Bottle headspace filling).

Discriminant factor analysis (DFA) was selected as the most relevant statistical method to apply to separate the groups of samples.

To ensure that no information is lost, unless stated otherwise, for the statistical analysis all sensors (chromatographic peaks) identified by the apparatus were selected and used, irrespective of their discriminant power (Table 2).

As seen in Table 2, the sensors represent the chromatographic peaks determined by the AlphaSoft as being the most discriminant for the analysed samples and they are from both chromatographic columns (suffix 1A for the column DB5, 2A from the column DB1701).

3. Results and discussion

In order to compare wine samples containing various types of antioxidants, the variants were grouped in accordance with the main antioxidants used for their preparation. The variants treated with carbon dioxide were included in the clusters with similar samples not treated with CO_2, as the sensory analyses on the samples showed no perceivable influence [3]. Thus, the following clusters are formed (Table 3).

Table 2. The selection of the sensors (chromatographic peaks) for the DFA analysis.

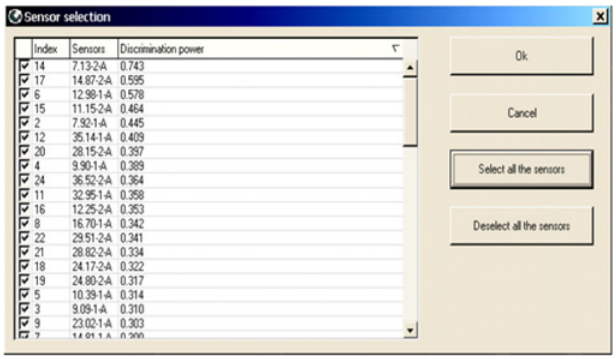

Table 3. Clusters of wines grouped in accordance with the main antioxidant treatments.

Cluster name	Sub-cluster name	Sample code	Color in figures
G0	**G0T0**	G00_A00_T00 (r1-r3)	Light
		G00_A00_T00_CO2 (r1-r3)	blue
	G0T20	G00_A00_T20 (r1-r3)	Dark
		G00_A00_T20_CO2 (r1-r3)	blue
G20	**G20**	G20_A00_T00 (r1-r3)	Light
		G20_A00_T00_CO2 (r1-r3)	green
	G20T20	G20_A00_T20 (r1-r3)	Dark
		G20_A00_T20_CO2 (r1-r3)	green
G20A	**G20AT0**	G20_A50_T00 (r1-r3)	Light
		G20_A50_T00_CO2 (r1-r3)	orange
	G20AT20	G20_A50_T20 (r1-r3)	Dark
		G20_A50_T20_CO2 (r1-r3)	orange
G40	**G40T0**	G40_A00_T00 (r1-r3)	Light
		G40_A00_T00_CO2 (r1-r3)	cyan
	G40T20	G40_A00_T20 (r1-r3)	Dark
		G40_A00_T20_CO2 (r1-r3)	cyan
G40A	**G40AT0**	G40_A50_T00 (r1-r3)	Light
		G40_A50_T00_CO2 (r1-r3)	magenta
	G40AT20	G40_A50_T20 (r1-r3)	Dark
		G40_A50_T20_CO2 (r1-r3)	magenta

The clusters take into account only the presence of GSH and AA, irrespective of the tannin treatment, while the sub-clusters take also into account the presence of tannin.

3.1. The influence of glutathione and ascorbic acid treatments

First we tried to discriminate the volatile profiles of the wines treated with various dosages of GSH in the presence or absence of AA. The clusters were formed based on the content of GSH and AA, irrespective of the presence of tannin, as described in Table 3 - first column (clusters G0, G20, G40, G20A, and G40A).

All the chromatographic peaks were selected for the DFA analysis, having discrimination power from 0.060 to 0.709.

As it can be seen in Fig. 1 the electronic nose discriminates the clusters of wines, and most of the variation is included in the discriminant function 1 (87.41%).

The treatment with AA clearly influences the volatile profile of the wines, thus the electronic nose separates in

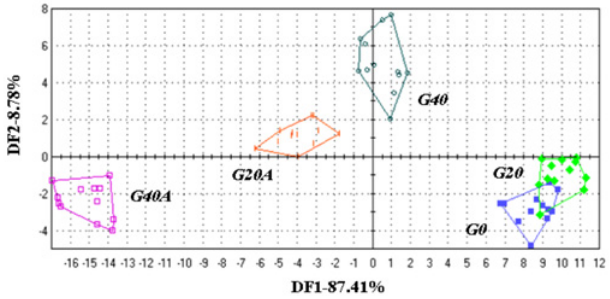

Figure 1. Discrimination of wines grouped in clusters in accordance to the glutathione and ascorbic acid treatments, irrespective of the presence of tannin. (GSH of 0, 20 and 40 mg/l (clusters G0, G20 and G40), GSH of 20 and 40 mg/l plus 50 mg/l AA (clusters G20A and G40A). Cluster colors: G0 - blue, G20 - green, G40 - cyan, G20A - orange, G40A - magenta.

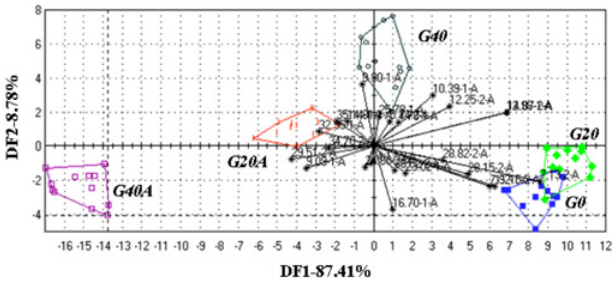

Figure 2. DFA diagram with loadings for the wine clusters discriminated in accordance to the glutathione and ascorbic acid treatments, irrespective of the presence of tannin. (GSH of 0, 20 and 40 mg/l (clusters G0, G20 and G40), GSH of 20 and 40 mg/l plus 50 mg/l AA (clusters G20A and G40A). Cluster colors: G0 - blue, G20 - green, G40 - cyan, G20A - orange, G40A - magenta.

the right part the wines containing only GSH from the wines containing both GSH and AA, which are included in the left part of the diagram (Fig. 1).

The presence of AA reduces the number of discriminative substances (chromatographic peaks) present in the headspace of the wine sample vials, suggesting that the volatile profiles of these wines are less complex. This can be qualitatively demonstrated by the diagram in Fig. 2, where the loadings of the chromatographic peaks are also represented along with the wine clusters. Fewer and shorter loadings determine the differentiation of the clusters G20A and G40A, those that contain samples with AA along with GSH.

Interestingly, the wines treated with only 20 mg/l GSH do not appear to have significantly different volatile profiles compared to the control wines. This can be seen in both Fig. 1 and Fig. 2, where the clusters G0 and G20 overlap. Also, if we take into account the discriminant function 3, same result is obtained (Fig. 3).

This observation is important, as the dose of 20 mg/l GSH is the maximum allowed at present for the treatments of musts and wines in accordance to the International Organisation of Vine and Wine recommendations [15].

The fact that the electronic nose does not discriminate the samples treated with 20 mg/l GSH from those not treated (control samples) means that from the viewpoint of the aromatic profile the consumer will most likely perceive no difference as well.

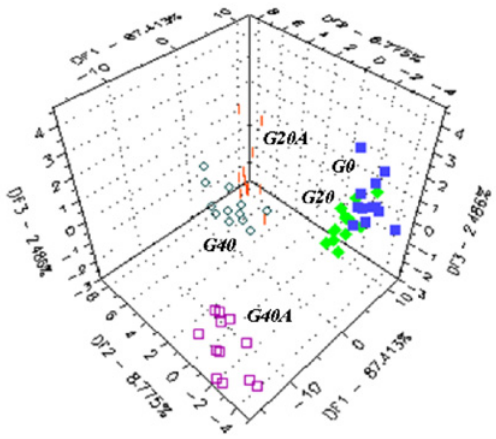

Figure 3. DFA 3D diagram for the wine clusters discriminated in accordance to the glutathione and ascorbic acid treatments, irrespective of the presence of tannin. (GSH of 0, 20 and 40 mg/l (clusters G0, G20 and G40), GSH of 20 and 40 mg/l plus 50 mg/l AA (clusters G20A and G40A.) Cluster colors: G0 - blue, G20 - green, G40 - cyan, G20A - orange, G40A - magenta.

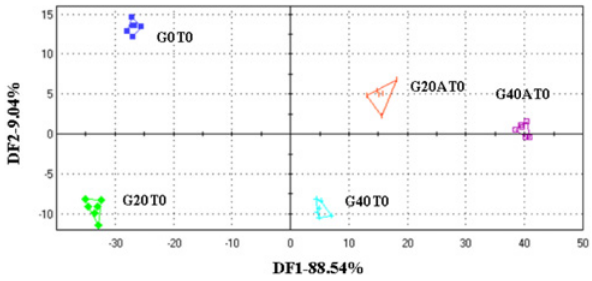

Figure 4. Discrimination of wines grouped in clusters in accordance to the glutathione and ascorbic acid in the absence of tannin. Sub-cluster colors: (G0T0 - light blue, G20T0 and G20T20 - light green, G40T0 - light cyan, G20AT0 - light orange, G40AT0 - light magenta.

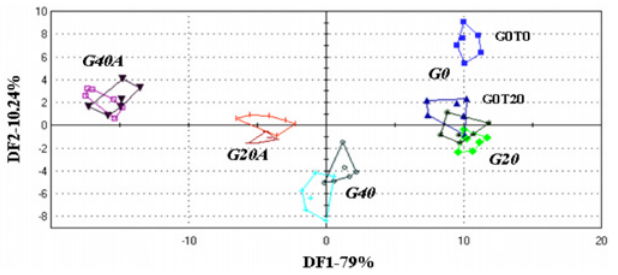

Figure 5. Discrimination of wines grouped in clusters in accordance to the glutathione, ascorbic acid and tannin treatments. GSH of 0, 20 and 40 mg/l (clusters G0, G20 and G40), GSH of 20 and 40 mg/l plus 50 mg/l AA (clusters G20A and G40A). Sub-cluster colors: G0T0 and G0T20 - light and dark blue, G20T0 and G20T20 - light and dark green, G40T0 and G40T20 - light and dark cyan, G20AT0 and G20AT20 - light and dark orange, G40AT0 and G40AT20 - light and dark magenta.

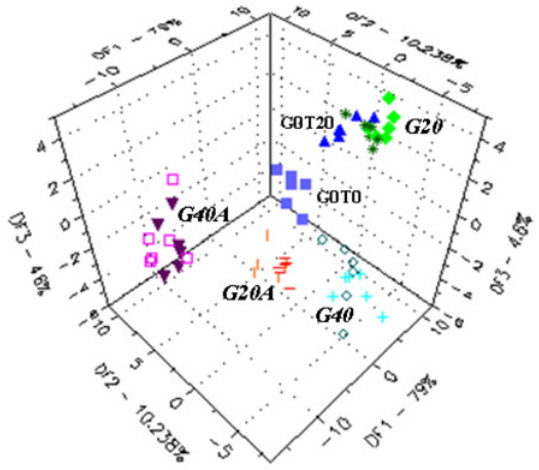

Figure 6. DFA 3D diagram for the wine clusters treated with glutathione, ascorbic acid and tannin treatments.

This effect may, however, be due to the influence of the tannin present in some of the samples included in the analysed groups.

If we eliminate the effect of tannin, by comparing clusters of wines groups only in accordance with the GSH and AA treatments and with no tannin added, the wines containing 20 mg/l GSH are also separated from the control wines. (Fig. 4), although the discrimination power of the most discriminant sensor was only 0.654 (the selected sensors ranged from 0.654 to 0.218).

Therefore, it is very clear that for the discrimination of the wine samples by the electronic nose and also by the consumers, the complexity of the wine matrix, and the combination of oenological treatments are important.

3.2. The influence of tannin treatment

To determine the influence of the treatment with tannin on the volatile profile of wines already vinified with GSH and AA the DFA analysis was performed by grouping the samples in clusters containing also tannin (0 or 20 mg/l), as described in Table 3 second column (sub-clusters G0T0 and G0T20, G20T0 and G20T20, G40T0 and G40T20, G20AT0 and G20AT20, G40AT0 and G40AT20).

All the sensors were selected for analysis, with discrimination power ranging from 0.078 to 0.754.

The results showed that the presence of 20 mg/l tannin was not important enough to have a perceivable impact on the on the wine samples, as compared to the GSH or AA treatments.

Figures 5 and 6 show that the clusters with and without tannin overlap in all groups with the exception of the control cluster (blue color) which is split in the G0T0 (light blue) and G0T20 dark blue) sub-clusters.

The fact that in the control cluster we observe a slight discrimination between samples treated with tannin and those not treated suggests that the tannin actually influences the volatile profile of the wines, but when the GSH or AA are also added, its effect is masked by the greater effect induced by these other antioxidants.

The main effect on the volatile profile of the wines is induced by the GSH and the combination of GSH and AA. This is more clearly demonstrated when only the sub-clusters containing tannin are compared (Fig. 7). The sensor selection ranged from 0.817 to 0.222 discrimination power.

In Fig. 7 to the clusters of samples treated with both GSH and AA (right) are separated from the clusters of samples treated only with GSH (left), while the dose

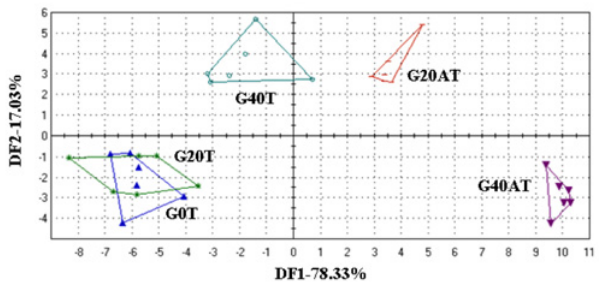

Figure 7. Discrimination of wines treated with tannin grouped in clusters in accordance to the glutathione and treatments. Sub-cluster colors: G0T20 - dark blue, G20T20 - dark green, G40T20 - dark cyan, G20AT20 - dark orange, and G40AT20 - dark magenta.

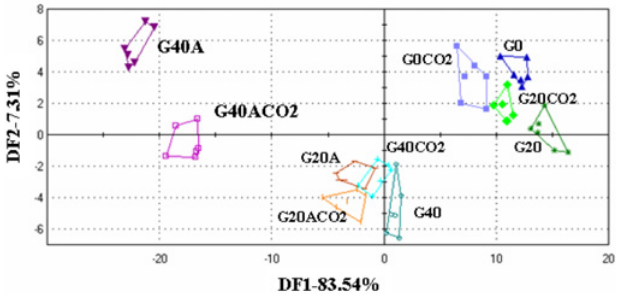

Figure 8. Discrimination of wines grouped in clusters in accordance to the glutathione, ascorbic acid and carbon dioxide treatments. GSH of 0, 20 and 40 mg/l (clusters G0, G20 and G40), GSH of 20 and 40 mg/l plus 50 mg/l AA (clusters G20A and G40A). Sub-cluster colors: G0CO2 and G0 – light and dark blue, G20CO2 and G20 - light and dark green, G40CO2 and G40 – light and dark cyan, G20ACO20 and G20A – light and dark orange, G40ACO2 and G40A– light and dark magenta.

of 20 mg/l GSH is not sufficiently important to ensure discrimination of samples from the control wines, not treated with GSH.

The results obtained under our experimental conditions seem to indicate that at least the treatment with 20 mg/l GSH in Feteasca regala does not lead to any benefit as far as the aromatic profile is concerned. Comparable results were obtained by other authors too [18], the 20 and 40 mg/l GSH treatments of certain white wines (also containing AA from the must treatment) leading to no significant difference in sensory parameters as compared to control wines.

3.3. The influence of carbon dioxide treatment

Although in a previous study the sensory analysis of the samples did not show any perceivable influence of the carbon dioxide on the aroma intensity and quality of these samples [3], the discrimination by electronic nose of the groups of samples with and without carbon dioxide added during bottling was also attempted (Fig. 8). The wines were grouped in the same clusters based on the treatments of GSH and AA, irrespective of the presence of tannin, but then, each cluster was split in sub-clusters with CO_2 (light colored) and without CO_2 (dark colored).

After 1 year from bottling time, the addition of carbon dioxide in the bottles did not influence significantly the aroma profile. All the sub-clusters containing wines with CO_2 overlapped with their corresponding sub-cluster without CO_2, with the exception of the samples with both 40 mg/l GSH and 50 mg/l AA, which, as shown in other studies too [6,23] are the most prone to oxidation. Thus, we can see that after 1 year in bottle, without CO_2 protection, the wines containing a larger dose of GSH and in the presence of AA (group G40A) show different profiles of the volatile substances as compared to all the other wines.

This result suggests that, in time, the effects of preservation with carbon dioxide will be more easily detectable and that the treatment is worth making.

4. Conclusions

The electronic nose can discriminate fast and reliably the samples of wines prepared with various oenological treatments.

Based on the statistical analysis of the discriminant factors (DFA) we were able to evaluate if the antioxidant treatments of white wines of Feteasca regala induced significant differences in the volatile profile 6 month after winemaking and 1 year after bottling.

Thus, in our study, the electronic nose helped to establish the following effects determined by the treatments with the antioxidants on Feteasca regala wines:Generally, the treatment with GSH in the must influences the volatile profile of the final wines only at doses larger than the 20 mg/l allowed at present by the OIV. The cluster of wines resulted from musts treated with 20 mg/l GSH was not discriminated by the electronic nose from the cluster without GSH added. A discrimination was however possible when the effect of tannin added was removed from the analysis, by excluding from the wine sample clusters the samples with added tannin.

The addition before fermentation of 50 mg/l AA in the musts treated with GSH, irrespective of the GSH dosage, reduced the complexity of the volatile profile of wines. The presence of AA in the samples determined the clear discrimination of the clusters containing wines with AA, from those without AA, irrespective of the other treatments.

The treatment with only 20 mg/l catechinic tannin during bottling was generally not important enough to induce perceivable effects on the volatile profile, in the context of addition of AA or/and GSH in the musts, which had a greater influence.

The treatment with carbon dioxide during bottling shows a promising effect in the long run. At 1 year form bottling, the wines mostly prone to oxidation (those included in the cluster of samples treated with 40 mg/l GSH and 50 mg/l AA) are split in sub-clusters of samples with and without CO_2 preservation. The sub-cluster with CO_2 is positioned closer to the samples less oxidised.

Of all the findings, the fact that the treatment of must with 20 mg/l GSH in Feteasca regala wines does not induce major differences in the volatile profiles of the resulted wines from the wines included in the cluster of samples not treated with GSH is particularly relevant as the dose of 20 mg/l GSH is at present the maximum allowed to be added in must and wines.

The authors declare no financial or commercial conflicts of interest.

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