Effects of Blending and Co-inoculation on the Aromatic Profile of Wines

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Wines of Feteasca regala variety were obtained by inoculation and co-inoculation with several commercially available wine yeasts. The yeast growth rate was measured for each fermentation batch by isothermal calorimetry. Some blends of the mono-inoculated wines were also prepared after the completion of fermentation. The wines thus obtained were analyzed by a GC electronic nose, which provided a good differentiation of the wine groups in accordance with the yeast used for their fermentation. The volatile profile of the co-inoculated wines proved to be different from those mono-inoculated with the same yeasts, even though in some co-inoculations one of the yeasts was a killer strain. Some peaks of the volatile compounds on chromatograms clearly showed that the concentrations of several volatiles were similar in the killer yeast inoculated wine and its co-inoculation variant, but the overall profile of the wine was still differentiated through Discriminant Factor Analysis (DFA). The differentiation by DFA analysis applied to the volatile fingerprint of wines is discussed in correlation with the results of the sensory analysis performed by a trained tasting panel. Among the 10 yeasts used for fermentation, one was Saccharomyces bayanus and the rest were strains of Saccharomyces cerevisiae. The strain of S. bayanus produced wines with a significantly different aromatic profile compared to all the other wine yeasts, so that the mono-inoculated wines and the blends of S. bayanus wines made separate groups in the DFA analysis. The "bayanus character" is however lost in co-inoculations. Also, the ester-producing yeast strains were clearly differentiated by the electronic nose as well as by the tasting panel.

Keywords: blending, yeast co-inoculation, electronic nose, sensory analysis, wines

In the last decades at least, the fermentation of grape musts in order to obtain wine has been done with selected yeasts which are inoculated in doses of 20-30 g/hL dry yeast and thus become dominant and quickly eliminate all other yeasts present in the must [11]. However, changes are seen in this area. On one side there is renewed interest in the so called "natural fermentations", expected to perhaps better preserve in wines something of the local terroir [7]. The disadvantage in this case is that, along with the good outcomes (complex aroma, sometimes superior to wines fermented with selected yeasts) there are many accidents too – such as stuck fermentations or sensory deviations. On the other side there is the trend towards tailoring of yeast strains for wine fermentation [16].

Co-inoculation of musts with two different yeast strains [13, 15] can be a way to achieve a superior, complex aroma bouquet, while keeping the advantages of the dried yeasts and avoiding the accidents that might occur on natural fermentations. When choosing the yeasts for co-inoculation various factors need to be considered: fermentation rate and temperature, requirements of nitrogen and complex nutrients, killer character, production of certain alcohols and esters and ethanol tolerance. All these factors can influence the growth dynamics of the yeasts used for co-inoculation and, eventually , the general features of the resulting wine.

Knowing that the wine aroma is not only dependent on the grape variety, but also on the secondary metabolites released by the yeast strain [14], in this work experiments were carried out to study the effect of co-inoculation of several commercially available yeasts on the white wines of the Romanian variety Feteasca regala.

Experimental part

Materials and methods

The musts used for experiments were obtained from the grapes of the 2009 harvest at the University of Agronomical Sciences and Veterinary Medicine of Bucharest (Feteasca regala variety, must with 210 g/L sugars and 6.5 g/L acidity expressed as tartric acid). Before preparing the experimental variants the must was clarified with bentonite and cooled down to 10°C. Three repetitions were prepared for each experimental variant, in three consecutive days; all batches were of 4 L volume. The yeast dosage was 30 g/hL; the dried yeasts were rehydrated for 20 min in warm water before use.

Ten commercially available yeasts were used, alone or in combinations of two. A control sample of wine was also prepared, fermented with the indigenous yeasts naturally present in the must. The variants employed are presented in table 1.

Besides these variants, blends were prepared (in proportion 50%-50%) of the wines obtained by fermentation with a single yeast strain. For example, a "blend Y1+Y4" refers to the blend in equal proportions of the two wines obtained by fermentation with yeast Y1, and yeast Y4, respectively. This must be distinguished from the wine obtained by co-inoculation with the mix of yeasts Y1 and Y4, simply named Y1Y4.

Electronic nose analysis was applied for the comparison and classification of the produced wine samples. The chromatographic method developed in our laboratory is described in detail in a previous paper [2].

Sensory analysis was also performed on all wine variants, using a panel of 5 trained judges who evaluated

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No.	Variant	Yeast(s) used for fermentation	Yeast characteristics
1	Control (Y)	Indigenous yeasts	-
2	Yeast 1 (Y1)	*Saccharomyces cerevisiae* var. *bayanus* (Lalvin QA 23, Lallemand)	killer; ethanol tolerance 16%; temperatures 14-28°C; low nitrogen requirements; enhance citrus-fruit-type aromas (lime, grapefruit) in the aromatic white grapes (beta-glucosidase activity);
3	Yeast 2 (Y2)	*Saccharomyces cerevisiae* var. *cerevisiae* hybrid; (NT 116, Anchor)	killer; ethanol tolerance 16%; temperatures 12-16°C; low nitrogen requirements; enhances volatile thiol aromas (passion fruit, grapefruit and guava) and produces acetate esters;
4	Yeast 3 (Y3)	*Saccharomyces bayanus;* (AWRI 1176, Lallemand)	dominant in relation with other yeasts; ethanol tolerance 16%; temperatures 25-30°C; high nitrogen requirements; different volatile profile;
5	Yeast 4 (Y4)	*Saccharomyces cerevisiae* var. *cerevisiae;* (Uvaferm HPS, Lallemand)	neutral competitive factor; ethanol tolerance 16%; temperatures 18-30°C; medium nitrogen requirements; enhances varietal character (mannoprotein overproducer, tendency to candied fruit);
6	Yeast 5 (Y5)	*Saccharomyces cerevisiae* var. *cerevisiae;* (Lalvin ICV D254, Lallemand)	neutral competitive factor; ethanol tolerance 15-16%; temperatures 15-28°C; medium nitrogen requirements; enhances varietal character (intense fruit concentration);
7	Yeast 6 (Y6)	*Saccharomyces cerevisiae* var. *cerevisiae;* (Lalvin CLOS, Lallemand)	killer; ethanol tolerance 17%; temperatures 13-35°C; medium nitrogen requirements; enhances varietal character (aromatic complexity, structure and palatability);
8	Yeast 7 (Y7)	*Saccharomyces cerevisiae* var. *cerevisiae;* (Lalvin BM 45, Lallemand)	killer with slow start, ethanol tolerance 15%; temperatures 18-28°C; medium to high nitrogen requirements; enhances varietal character (fruit jams, rose and cherry liqueurs);
9	Yeast 8 (Y8)	*Saccharomyces cerevisiae;* (Motrachet, Vason)	ethanol tolerance 15%; temperatures 10-35°C; low nitrogen requirements; neutral strain;
10	Yeast 9 (Y9)	*Saccharomyces cerevisiae;* (Premium Blanc 12V, Vason)	killer; ethanol tolerance 13%; optimal temperatures 18-20°C or in range 10-35°C; medium to high nitrogen requirements; enhances varietal character (release of aroma precursors);
11	Yeast 10 (Y10)	*Saccharomyces cerevisiae;* (Flavour 2000, Vason)	ethanol tolerance 15%; temperatures 14-28°C; medium to high nitrogen requirements; ester-producing strain (β-phenylethanol)
12	Mix of yeasts 1-4 (Y1Y4)	Yeast 1 + Yeast 4 (1:1)	-
13	Mix of yeasts 2-4 (Y2Y4)	Yeast 2 + Yeast 4 (1:1)	-
14	Mix of yeasts 3-4 (Y3Y4)	Yeast 3 + Yeast 4 (1:1)	-
15	Mix of yeasts 2-3 (Y2Y3)	Yeast 2 + Yeast 3 (1:1)	-

Table 1
EXPERIMENTAL VARIANTS OF WINES OF FETEASCA REGALA OBTAINED USING VARIOUS YEASTS AND MIXTURES OF YEASTS

the wines mainly regarding their volatile profile characteristics, using a specially designed score sheet [3].

For all samples and variants the growth rate of the yeast was determined by isothermal calorimetry, using the apparatus and technique described elsewhere [5]. An electronic nose based on dual-column flash gas-chromatography, named Heracles, from Alpha MOS company, was used to analyze and differentiate all the variants based on their volatile profiles [1, 4].

Results and discussions

The electronic nose methodology allows for the discrimination between samples and grouping the samples with common traits (volatile profile), without a previous indentification of the volatile components, as it is in the case of other techniques such as gas chromatography-FID [12], gas chromatography–mass spectrometry [8, 10], NMR spectroscopy [6, 9, 17, 18].

By analyzing the data obtained from the chromatographic determinations with the electronic nose Heracles, the plot given in figure 1 is obtained, showing the differentiation of wines obtained by inoculation with a single yeast strain.

In figure 1 it can be seen that 3 groups of mono-inoculated samples are better discriminated compared to the others which are displayed in a rather compact area of the plot, having closer volatile profiles. These wines with a rather different volatile profile were sample Y (control, obtained with indigenous strains), Y1 (obtained with the yeast Lalvin QA 23) and Y3 (obtained with *S. bayanus* AWRI 1176). In fact, though, if these 3 wines are removed from analysis and the remaining compact group of samples are analyzed again – it can be seen (fig. 2) that the apparatus is capable of discriminating correctly all the remaining 8 variants obtained with indigenous yeasts and single yeast strains.

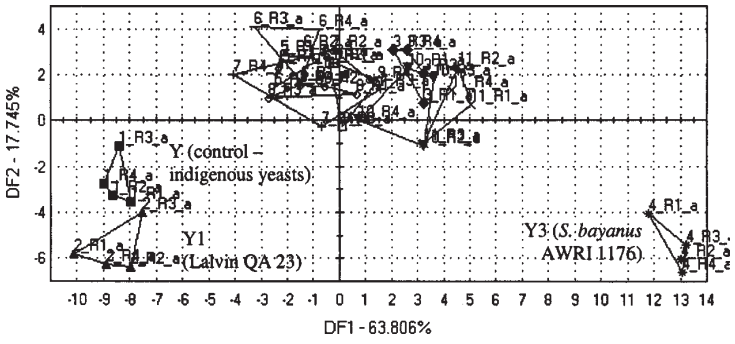

Fig. 1. Discriminant Factor Analysis (DFA diagram) obtained by multivariate analysis of the data provided by the Heracles electronic nose on the wine samples obtained with a single yeast strain (no blends, no co-inoculation)

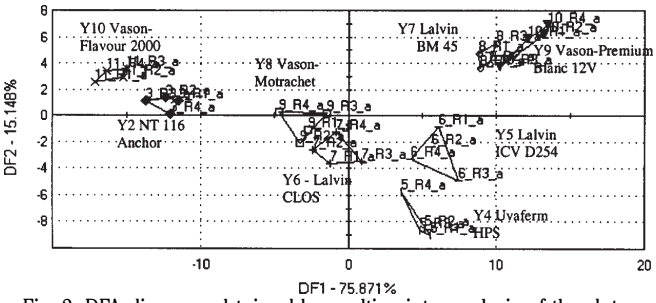

Fig. 2. DFA diagram obtained by multivariate analysis of the data provided by the Heracles electronic nose on the wine samples obtained with a single yeast strain (no blends, no co-inoculation) – after the elimination of variants Y, Y1 and Y3 which were more clearly discriminated in figure 1

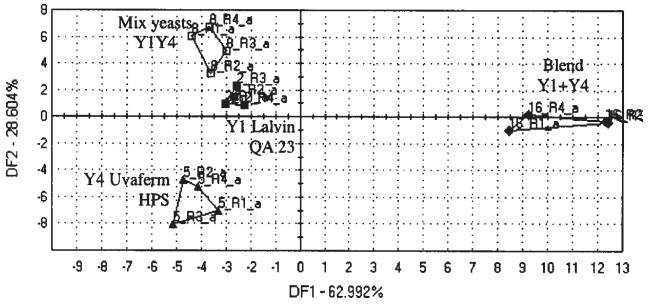

Fig. 3. DFA diagram obtained by the multivariate analysis of the experimental data corresponding to variants Y1, Y4, mix of Y1Y4 yeast strains and blend Y1+Y4 of the wines obtained with Y1 and Y4 yeast strains

Interesting aspects can also be derived by DFA analysis of certain groups of wine samples. For example, one such group is the one composed of the wines obtained by fermentation with Y1 and Y4 yeast strains respectively, a blend of these two wines (in equal proportions) Y1+Y4 and also the wine fermented with a 1:1 mix of Y1 and Y4 yeast strains, Y1Y4. When these samples are analyzed with the Heracles apparatus and its software, the DFA plot given in figure 3 is obtained.

From figure 3 it becomes apparent that the volatile profile of the wine made by co-inoculation (mix Y1Y4 of yeast strains) is closer to that of the wine made using only the yeast Y1 (Lalvin QA 23). This fact might be explained by the presence of the killer character in the yeast Y1: in the presence of another yeast that does not have the killer character, Y1 must have become dominant quite soon during the fermentation. This fact is also supported by the calculated growth rate from the calorimetric power-time curve, which shows a growth rate constant two times higher for Y1 ($\mu Y1 = 0.06424$) in comparison with Y4 ($\mu Y4 = 0.03195$). It is also interesting to notice that simply blending the wines obtained using Y1 and Y4 as single yeasts in fact leads to a distinct wine, with a different volatile profile, easily discriminated by the electronic nose.

The results suggested by figure 3 can be correlated with those obtained from the sensory analysis. Figure 4 presents the sensory profiles derived from the sensory analysis session, regarding the same wine samples referred to in figure 3 above.

Examining figure 4 it is apparent that, although there are some differences among all the samples evaluated by sensory analysis – the sensory profile of the wine obtained by co-inoculation (QA 23 x HPS in fig. 4) is closer in shape and size to that of the wine obtained by using the yeast Y1 (QA 23).

Moving to another group of samples, figure 5 presents the DFA diagram obtained when analyzing the wine variants Y2, Y4, a blend of these two wines (in equal proportions) and also the wine fermented with a 1:1 mix of Y2 and Y4 yeast strains.

This time, all four types of wines are clearly different – at least from the viewpoint of their volatile profile – and as a result the electronic nose is able to differentiate them easily. Although the yeast Y2 (NT 116 Anchor) was described as a dominant yeast in relation to other yeasts, from the viewpoint of the volatile profile we can see that it was not completely able to dominate Y4 (Uvaferm HPS) in the co-inculation experiment, fact also supported by a slower growth constant of this first yeast ($\mu Y2 = 0.01593$) in comparison with the latter ($\mu Y4 = 0.03195$).

A similar DFA diagram is given in figure 6 for the wine variants Y3 (*S. bayanus* AWRI 1176), Y4 (Uvaferm HPS), blend of these two wines, as well as co-inoculation with a mix of Y3 and Y4 yeasts. In this case too, the wine samples obtained by blending are easy to differentiate from those obtained by co-inoculation as well as from those fermented with a single yeast strain. It is interesting that *S. bayanus*

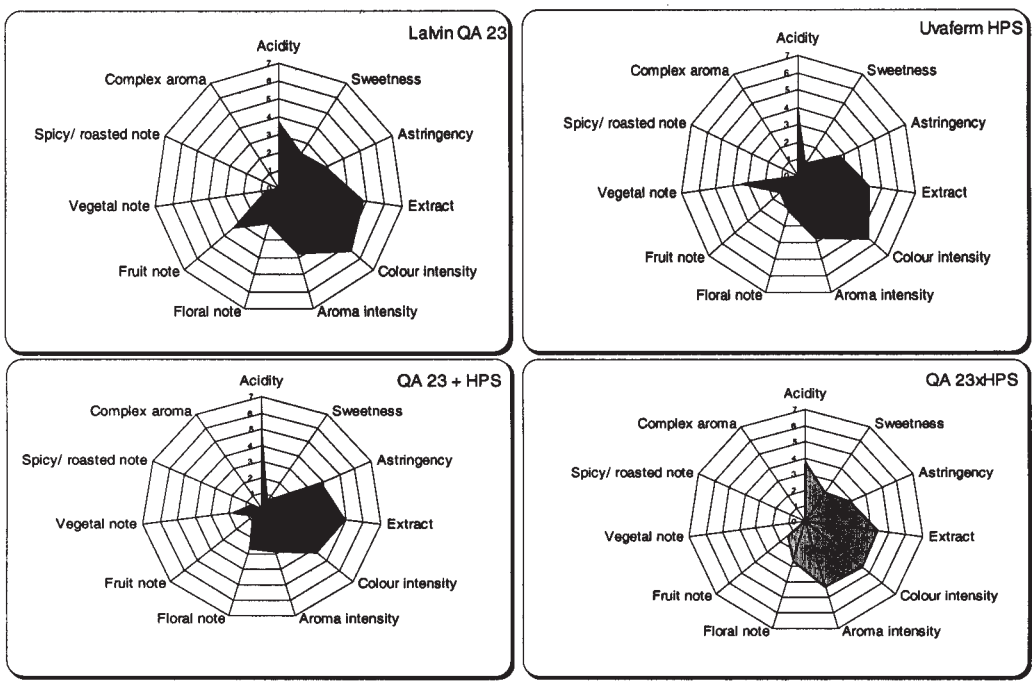

Fig. 4. Sensory profiles of the same wines depicted in figure 3 – obtained through sensory analysis.

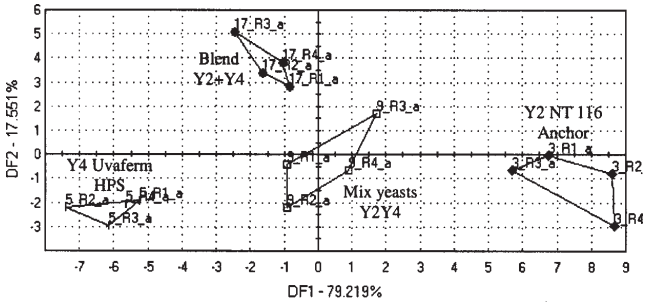

Fig. 5. DFA diagram obtained by the multivariate analysis of the experimental data corresponding to variants Y2, Y4, mix of Y2Y4 yeast strains and blend Y2+Y4 of the wines obtained with Y2 and Y4 yeast strains.

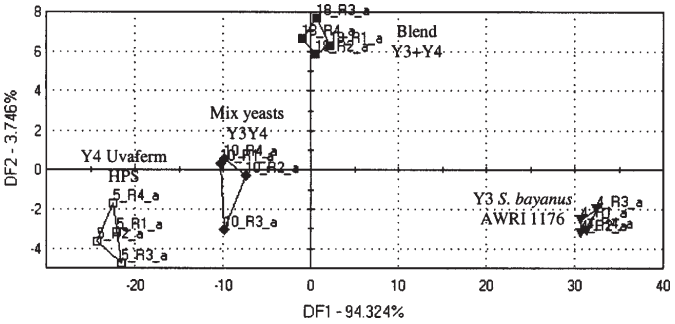

Fig. 6. DFA diagram obtained by the multivariate analysis of the experimental data corresponding to variants Y3, Y4, mix of Y3Y4 yeast strains and blend Y3+Y4 of the wines obtained with Y3 and Y4 yeast strains

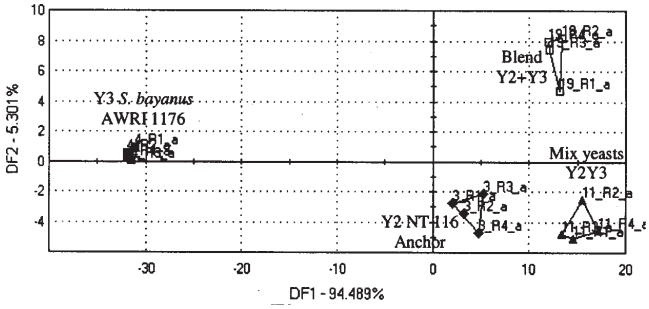

Fig. 7. DFA diagram obtained by the multivariate analysis of the experimental data corresponding to variants Y2, Y3, mix of Y2Y3 yeast strains and blend Y2+Y3 of the wines obtained with Y2 and Y3 yeast strains

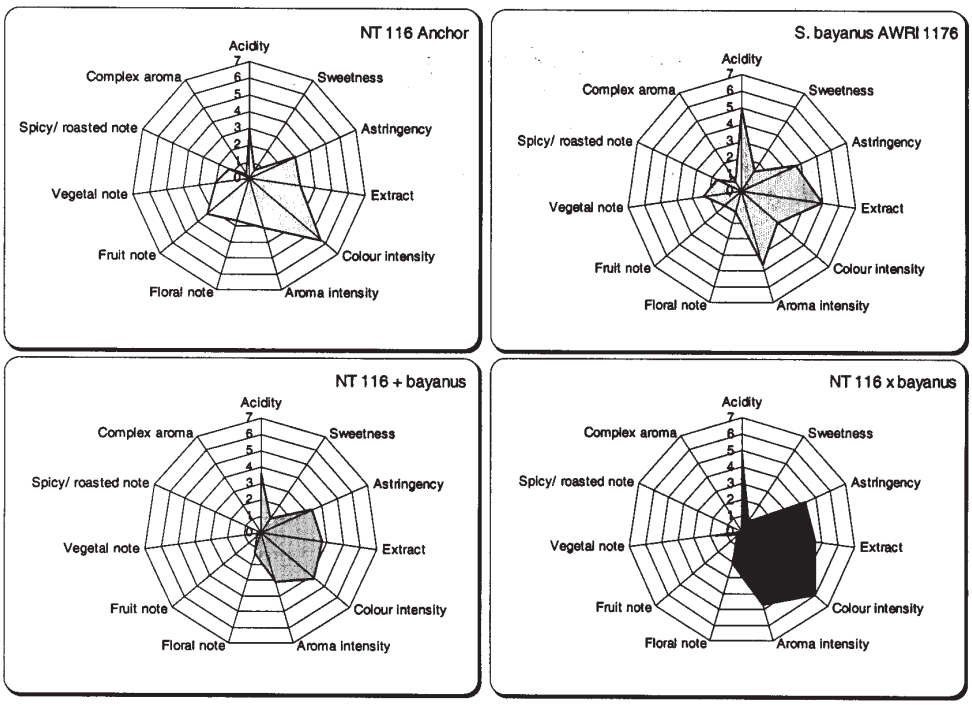

Fig. 8. Sensory profiles derived by sensory analysis for the same wine samples depicted in figure 7

appears to play an active role until the end of the fermentation determining a different volatile profile to the resulting wine. Moreover, compared to Y4 (μY4=0.03195), the yeast Y3 – *S. bayanus* – had in our must a higher growth rate constant of 0.05236.

Finally, a similar result is given in figure 7 regarding the wine samples obtained with yeast Y2 (NT 116 Anchor), Y3 (S. bayanus AWRI 1176), blend of these two wines (Y_2 + Y_3), as well as co-inoculation with a mix of Y2 and Y3 yeasts (Y_2 Y_3). All wine samples are clearly differentiated by the electronic nose, in spite of the fact that one sample is co-inoculated along with a killer yeast (NT 116 Anchor) that was expected to become dominant. With a growth rate three times higher (μY3=0.05236), *S. bayanus* managed to outgrow Y2 yeast (μY2=0.01593) in the sample co-inoculated with bowth strains.

Figure 8 presents the correlating aspects that could be derived from the results of the sensory analysis.

The sensory profiles given in figure 8 allow us to compare the wines obtained using single yeast strains (NT 116 and *S. bayanus*, respectively) to the wine obtained by co-inoculation (indicated by "NT 116 x bayanus") and to the blend obtained from the first two wines mentioned (indicated by "NT 116 + bayanus"). It can be said that *S. bayanus* appears to have a significant impact on the sensory profile of the co-inoculated wine, in spite of the fact that the other yeast has a killer character. However, the resulting wine is still different from each of those fermented with a single yeast – as well as from the blend obtained from these wines.

Conclusions

The analysis of the wine samples using the electronic nose Heracles indicates that co-inoculation with two yeast strains out of which one has killer character is likely to result in that strain reaching dominance very quickly – therefore the wine obtained is not very different, from the viewpoint of its aromatic profile, from the wine fermented with that single yeast strain alone. However, in all other cases, when one of the two yeasts used for inoculation did not eliminate the other, the wines obtained by co-inoculation were indeed different from those obtained with single yeast strains. The electronic nose also perceived as "different" the samples obtained by blending the wines fermented with single yeast strains.

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