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MODULATION BY YEASTS OF AROMA PROFILE OF 'FETEASCĂ REGALĂ' WINES PROTECTED WITH LOW CONCENTRATION OF SULPHUR DIOXIDE

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Abstract

Fermentation of grape juice with various yeasts inherently leads to wines with different aromatic profiles, which get superimposed onto the varietal aroma and may or may not be well suited to the grape variety used. Determining which commercial wine yeast to use for a certain grape variety is a challenge and a risk for the winemaker, especially when working with low doses of sulphur dioxide or when the yeast was not used before in the winery. A good option to select a suitable yeast is to perform micro-vinification to compare the sensory profile of the resulted wines. The sensory analysis can then be completed by a flash GC analysis to determine the differences in the odour intensity and in the volatile profile of the wines, compared with the wine variant fermented with a classic well-known yeast. In this study 5 yeasts were compared in order to select the most suitable ones to modulate the aroma of Romanian variety 'Fetească regală' so that the temperate climate fruits aroma, specific to this variety, is intensified. At the same time, it was important to also identify the yeasts able to minimize the oxidative aroma when the sulphur dioxide concentration used in winemaking was low. Among the yeasts used 2 were recommended for their ester producing aroma (Anchor Alchemy I and Renaissance Allegro AL-48), while the other 3 are mainly used for their ability to release thiols (Lalvin QA23, Anchor VIN7 and Renaissance TR-313). The sulphur dioxide added in all variants was only 70 mg/l in total. Sensory analysis performed 3 years after bottling showed that some, but not all, yeasts were able to produce intense fruity aroma which was well preserved. Our study shows that for 'Fetească regală', ester releasing yeasts are more suitable to produce stable aroma, less affected by oxidation during storage under preservation with low sulphur dioxide concentrations.

Key words: *Heracles GC electronic nose; 'Fetească regală'; commercial wine yeast, aromatic profile, low* SO_2.

INTRODUCTION

Aroma is an essential attribute involved in the recognition of quality of food products and beverages and its complexity is often directly linked with quality and consumer preference (Wang and Spence, 2018). Aroma is generated by the mixture of the volatile compounds present in the food matrix, which can be perceived by the nose and is evaluated by the brain. A volatile profile which induces a more pleasurable reaction will distinguish a specific product from other similar ones. Thus, in wine, for example, the process of winemaking is usually conducted to optimize the volatile compounds extracted from the grapes, as well as produced during fermentation and aging. Avoiding the oxidation of the volatile compounds is also important (Zironi et al., 2010), as the oxidation of aroma is perceived as a loss of quality. Beside the grape variety, which brings to wine specific aromatic compounds (primary aroma), yeast strains used for fermentation play a significant role in the final product aromatic profile (secondary aroma). As primary aroma is relatively constant for a grape variety grown in a certain region, intervention with an appropriate yeast for fermentation can induce more changes in the final volatile profile (Swiegers and Pretorius, 2005; Graham, 2008) and lead to a more or less favourable result, also depending on the winemaking conditions (Morgan *et al*., 2019). To evaluate the influence of interventions, yeasts which generate either more ester compounds or more thiol compounds were compared. Two yeast strains for ester production and three yeast strains for thiol production were selected to be used to modulate the aromatic profile of 'Fetească regală' white wine produced in the region of Bucharest, Romania. Considered among the semi-aromatic varieties, 'Fetească regală' benefits sometimes from maceration of the grape skins, as more aroma precursors are extracted, but at the same time, this skin contact also brings into the must more phenols, increasing bitterness and astringency as well. In order to limit phenolic extraction, separation of the skins immediately after grape crushing is the most used winemaking procedure, which, in turn, reduces the varietal aroma of wines. Therefore, to obtain a more intense aromatic profile, selecting suitable wine yeasts for fermentation is of utmost importance. Moreover, because the consumers also prefer to have wines with reduced SO_2 concentrations (Amato et al., 2017), to compensate for the less antioxidant protection it is desirable to select yeasts which generate compounds less oxidisable or confer aroma protection for longer times. The present study evaluates the aroma profile generated by 4 yeasts as compared to another one usually employed and considered the control, also evaluating the sensorial profile of these wines after 3 years from bottling with low SO_2 concentrations.

MATERIALS AND METHODS

The wine used for the experiment was obtained from grapes cultivated in the plantation of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and harvested in 2018 on September 11th. The wine variants were prepared in accordance to white wine technology. The harvested grapes were destemmed, crushed and the free run juice separated in a hydraulic press and treated with a dose of 50 mg/l SO_2 and then with a dose of 1 g/hl commercial pectolytic enzyme Zimafruit from Enologica Vason (web source 1). The collected must was left one day for settling in a stainless-steel tank. The limpid must, with a turbidity of 113 NTU, sugar of 23.2% Brix and titratable acidity of 3.94 g/l tartaric acid, was split in 5 smaller tanks, 40 l each and inoculated with selected commercial yeasts, as follows: Lalvin QA23 from Lallemand (web source 2), VIN7 (web source 3) and Alchemy I (web source 4) from Anchor Oenology, and TR-313 (web source 5) and Allegro (AL-48) (web source 6) from Renaissance. For these yeasts, the main characteristics are summarized in Table 1. The alcoholic fermentation was conducted in each tank at a temperature of 16 ± 1.5°C and lasted about 4 weeks. Afterwards, the newly obtained wines were racked on October 17th and left for maturation on the fine lees for another 4 months, in the first two months also being homogenised with the lees twice a month. Adjustments of acidity were made twice (one week after racking and 2 months after racking, respectively), with 1 g/l tartaric acid each time. A small dose of 50 mg/l sulphur dioxide was added in each tank 2 months after racking and this was supplemented two months later with another 20 mg/l on the occasion of bottling. The wines thus prepared were left for aging in bottle for 2 years.

Table 1. Main oenological characteristics of selected yeast strains

Comercial name	Lalvin QA23	Anchor VIN7	Renaissance TR-313	Anchor Alchemy I	Renaissance Allegro (AL-48)
Genus, species and variety	*S. cerevisiae var. bayanus*	*S. cerevisiae x S. kudriavzevii*	*S. cerevisiae*	*S. cerevisiae (mixture)*	*S. cerevisiae var. bayanus*
Aroma profile	Thiolic	Thiolic	Thiolic	Esteric	Esteric
Latency	Medium	Short	-	-	Short
Kinetics	Fast	Very fast	Fast	Fast	Moderate
Optimal temperature	14-18°C, without peaks over 28°C	13-16°C	14-18°C, without peaks over 25°C	13-16°C	15-18°C, without peaks over 28°C
Cold tolerance	<10°C	12°C	13°C	12°C	13°C
Alcohol tolerance	16.0% vol.	14.5% vol.	16.0% vol.	15.5% vol.	16.0% vol.
Conversion factor	16.5	16.2	16.3	16.2	16.3
Glycerol, g/100 ml ethanol	Medium (3.5-5)	Medium (3.5-5)	High (>5)	-	Medium (3.5-5)
Volatile acidity, acetic acid, g/100 ml ethanol	Low (<0.25)	Medium (0.25-0.4)	Low (<0.25)	Medium (0.25-0.4)	Low (<0.25)
Nitrogen requirements, mg N/g sugars	Low (0.75)	High (1.25)	Low – Medium (0.85)	Medium (0.90)	Medium (0.90)
Killer factor	Active (K2)	Sensitive	Active	Active / Neutral	Active
Flocculation	Low	Low	High	-	High
Foam production	Low	Moderate	Low	Low	Low
SO_2 production	Low (<20 ppm)	Low (<20 ppm)	Low (<20 ppm)	Low (<20 ppm)	None (0 ppm)
H_2S production	Low	-	None	-	None

The aroma profile of the wines was evaluated 2 years later by sensory analysis by a panel of winetasters, using a specific tasting sheet (Antoce and Namolosanu, 2007; Antoce and Cojocaru, 2017a) containing intensity scales for the main parameters (acidity, sweetness, astringency, bitterness, extract, colour intensity and aroma intensity) as well as discontinuous scales for various identified aromas.

The volatile compounds with main influence on the wine profile were also evaluated using a 2-column gas-chromatograph from Alpha MOS, France (Heracles e-nose), working on the principle of an electronic nose. A more detailed description of the apparatus and the method used is available elsewhere (Antoce and Namolosanu, 2011; Antoce and Cojocaru, 2017b, 2017c; Cojocaru and Antoce, 2019). The e-nose has its own software used for data acquisition and data analysis (AlphaSoft 12.42).

The main statistical analysis performed were the PCA (Principal Component Analysis), which allowed for non-hierarchic grouping of wines fermented with different yeasts strains and SQC (Statistical Quality Control analysis), which compares the total quantity of volatile compounds of a control wine with the ones of the other wines, based on the major volatile compounds determined to have discriminant powers above 0.5.

For the identification of the compounds separated by the chromatograph an integrated database, AroChemBase and Flavornet database (web source 7) were used. For each wine the chromatographic analysis was run 3 times.

RESULTS AND DISCUSSIONS

The Heracles e-nose, endowed with two short chromatographic columns of different polarities, allows for the separation of different volatile compounds on each column, but sometimes the same compound is separated and identified in both columns. The column DB5 is mainly suitable for separation of alkanes and other less-polar compounds, while the column DB1701 is better for the separation of alcohols, diols, esters, carboxylic acids, ethers, ketones, thiols or amines.

In Table 2 are included the volatile compounds separated and clearly identified with our flash chromatograph on the non-polar column DB5 and on the medium-polar column DB1701, respectively, to be important for the discrimination of the 'Fetească regală' wines fermented with the 5 selected yeasts. Kovats indices, necessary for the identification of the chromatographic peaks are also included, as reported in the literature (web source 7) and as determined previously by us on this Heracles apparatus (Cojocaru and Antoce, 2019). For each peak, the discrimination power calculated by the AlphaSoft show the impact of these compounds for the differentiation of wine samples fermented with the 5 different yeasts. Sensory descriptors of these volatile compounds were taken from ArochemBase and Flavornet (web source 7).

Table 2. Compounds identified on non-polar column DB5 and low/mid-polarity column DB1701 in 'Fetească regală' wine variants

Average RT	DB5 Kovats	DB1701 Kovats	Actual Heracles Kovats*	Compounds	Discrimination power (R^2)**	Sensory descriptors
8.96	769	-	769.08-*1*	*cis*-2-Penten-1-ol	0.718	green, plastic, rubber
9.84	795	-	795.32-*1*	Ethyl butyrate	0.891	banana, ethereal, pineapple, apple
11.54	-	859	858.98-*2*	Ethyl butyrate	0.921	banana, ethereal, pineapple, apple
12.93	874	-	873.77-*1*	Isoamyl acetate	0.947	banana, pear
14.80	-	940	940.60-*2*	Isoamyl acetate	0.952	banana, pear
16.63	967	-	964.51-*1*	5-Methylfurfural	0.890	sweet, almond, caramel, spicy
7.08	-	729	729.25-*2*	Isoamyl aldehyde	0.960	fruity
30.86	-	1354	1,354.30-*2*	2-Decenal	0.632	tallow, orange
33.38	-	1424	1,423.45-*2*	4-Oxodecanal	0.677	fatty

*DB5 = column 1 (suffix -1); DB1701 = column 2 (suffix -2); **Coefficient of determination R^2 (COD);

By taking into account these determined discriminant peaks it can be observed that the wine variants can clearly be separated by the PCA analysis (Fig. 1), with a high positive Discrimination Index calculated by AlphaSoft. Wines produced with esteric-aroma releasing yeasts (Alchemy I, Allegro AL-48) are placed in distinct parts of the biplot as compared to the wines produced with thiolic-aroma releasing yeasts (QA23, TR-313). VIN7 yeast however is placed between the thiolic and esteric-aroma producing yeasts, leading to wines with the lowest ethyl-butyrate concentration, but with moderate production of isoamyl-acetate (the highest observed among the 3 tested thiolic yeasts), confirming that this strain is also a good ester-producer, not only a thiol-releaser (Hart et al., 2017).

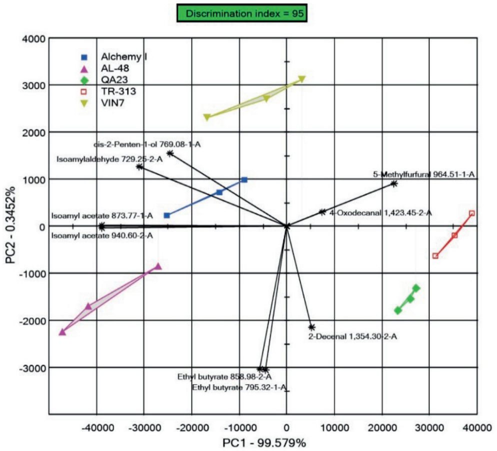

Figure 1. PCA biplot of wines fermented with 5 selected yeast strains and the main volatile compounds identified chromatographically

The other thiolic yeasts (QA23 and TR-313) lead to wines which were more easily affected by oxidation, the PCA biplot showing that especially TR-313 is clearly differentiated by 5-methylfurfural, a compound which is developed in aged (Dumitriu et al., 2019) or oxidized/prematurely aged wines (Escudero, et al., 2002; Tarko et al., 2020).

As seen from Table 1, in spite of the fact that thiol-releasing yeasts were used in some experimental variant, no thiols were chromatographically identified as being clearly present or discriminant for the variants. One explanation is that 'Fetească regală' variety has lower accumulation of thiols or precursors than the notorious Sauvignon Blanc variety or even other varieties such as Pinot Gris, Riesling, Chenin blanc, Colombard, Gewurztraminer, Semillon, Koshu and so on, which are known for the presence of thiols in the grapes (Roland et al., 2011). However, another explanation is also the lower protection from oxidation conferred by our attempt to lower the doses of sulphur dioxide in the final wine. Thus, the thiols, which are more sensitive to oxygen (Coetzee and Du Toit, 2015) or indirect oxidetion by means of catechins (Blanchard et al., 2004), had surely a higher rate of destructtion than the esters, which are more resistant to oxidation. Many thiols disappear in the first months of wine storage in bottles (Herbst-Johnstone et al., 2011).

This does not necessarily mean that the thiolic yeasts may not positively modulate the final aroma of 'Fetească regală' wines, given the right conditions.

Albeit the concentration of grape aroma precursors is highly important (Lee et al., 2008), the concentration of some thiols in wines are not strictly correlated with the must precursors (Pinu et al., 2012).

The magnitude of the volatile compounds' concentration was evaluated based on the peak areas, which are presented in Table 3.

Table 3. Peak area of the compounds identified in wines produced with different selected yeasts

Identified compounds	***Peak Area (± Standard error of mean)**					**Effect size (ω^2)**
	Wines fermented with thiolic yeasts			**Wines fermented with esteric yeasts**		
	QA23	**VIN7**	**TR-313**	**Alchemy I**	**AL-48**	
cis-2-Penten-1-ol (DB5)	596 ± 174^{b}	1291 ± 131^{a}	774 ± 128^{ab}	1345 ± 106^{a}	1017 ± 87^{ab}	0.589
Ethyl butyrate (DB5)	7508 ± 148^{a}	4827 ± 306^{c}	6547 ± 284^{ab}	6002 ± 180^{bc}	7857 ± 364^{a}	0.839
Ethyl butyrate (DB1701)	7067 ± 99^{a}	4584 ± 183^{c}	5988 ± 225^{b}	5650 ± 201^{b}	7384 ± 290^{a}	0.883
Isoamyl acetate (DB5)	50272 ± 962^{c}	75231 ± 4777^{b}	43297 ± 1791^{c}	82898 ± 3699^{b}	100179 ± 4653^{a}	0.920
Isoamyl acetate (DB1701)	41019 ± 663^{c}	60057 ± 3344^{b}	34236 ± 1328^{c}	66842 ± 3059^{b}	81266 ± 3822^{a}	0.928
5-Methylfurfural (DB5)	103 ± 5^{b}	182 ± 37^{b}	425 ± 44^{a}	163 ± 5^{b}	104 ± 0^{b}	0.829
Isoamyl aldehyde (DB1701)	3252 ± 85^{c}	5733 ± 184^{a}	2593 ± 167^{c}	4136 ± 196^{b}	5189 ± 188^{a}	0.940
2-Decenal (DB1701)	140 ± 19^{a}	n.d.**	122 ± 30^{a}	63 ± 32^{a}	132 ± 44^{a}	0
4-Oxodecanal (DB1701)	334 ± 17^{a}	297 ± 37^{a}	n.d.**	266 ± 58^{a}	n.d.**	0

*One Way ANOVA, post-hoc Tukey HSD p<0.05; **Groups excluded from statistical analysis.

To determine the statistical differences among the wines for each compound/ chromatographic peak, One-Way ANOVA ($p<0.05$) with Tukey HSD post-hoc was applied. For some of the discriminant compounds, ANOVA analysis too showed significant differences ($p<0.05$) among the wines (Table 3). For 2-decenal and 4-oxodecanal, which could not be identified in all samples, it seems that the influence of the yeast in their production is limited. Although the peak area values are small, it may be of importance to notice that 4-oxodecanal was not detected in the wines produced with any yeast from Renaissance, irrespective of their classification as a thiol or ester releasers. The other compounds, with a calculated effect size ($\omega^2>0.14$) can be safely be considered as being influenced by the fermentation yeasts, especially on the cases of the compounds with high ω^2 values, which explain a high percentage of variation among samples (94% for Isoamyl aldehyde, 92% for Isoamyl acetate, 88% for Ethyl butyrate). The fruitiness and banana-like aroma expected to be induced by the Isoamyl aldehyde and Isoamyl acetate is more present in both esteric-yeasts rather than in the other 3, the thiolic ones. Also, the banana-pineapple-like aroma of ethyl-butyrate is expected to be present in all wines, but with a lowest intensity in the wines fermented with VIN7.

The sensory analysis revealed that the major parameters of the final wines were not significantly affected by the yeast employed for the alcoholic fermentation (Table 4). The only noticeable exception was the total aroma intensity, which, after two years in bottles, was perceived as being lower for the wines fermented with the yeast conferring an esteric profile, especially in the case of Alchemy I.

Table 4. Main sensory characteristics of wines produced with different selected yeasts analysed after 2 years of aging in bottle

Sensory parameter*	Wines fermented with thiolic yeasts			Wines fermented with estric yeasts	
	QA23	VIN7	TR-313	Alchemy I	AL-48
Acidity	6.7 ± 0.6^a	6.6 ±0.9^a	5.2 ± 0.4^a	6.1 ± 0.6^a	6.7 ± 1.1^a
Sweetness	0.4 ± 0.6^a	1.8 ± 0.9^a	0.5 ± 0.6^a	1.3 ± 1.1^a	1.2 ± 1.0^a
Astringency	3.8 ± 1.5^a	5.3 ± 0.8^a	4.3 ± 1.2^a	5.5 ± 0.6^a	6.2 ± 1.1^a
Bitterness	1.2 ± 1.1^a	0.9 ± 0.6^a	2.5 ± 1.0^a	1.4 ± 1.1^a	2.4 ± 0.5^a
Extract	5.1 ± 0.4^a	4.7 ± 0 .7^a	4.0 ± 1.1^a	5.5 ± 0.8^a	6.3 ± 1.2^a
Colour intensity	5.0 ± 1.6^a	6.2 ± 0.8^a	5.2 ± 1.2^a	5.0 ± 1.4^a	5.3 ± 0.8 ^{a}
Aroma intensity	4.5 ± 0.8 ^{a,b}	5.9 ± 1.1^a	5.9 ± 0.9^a	3.7 ± 0.5^b	4.3 ± 1.3^{a,b}

* The values represent the sensory evaluation on intensity scales of maximum 10, expressed as means ± standard error of means.

In spite of a lower aroma intensity, the quality of aroma and the aromatic profile of wines was however more appreciated in the wines fermented with Alchemy I, being correlated with specific vegetal, lime and some fresh floral aroma, while the aromatic profile of wines fermented with the thiolic yeasts QA23 and TR-313 were more correlated with oxidized aromatic compounds described as sweet apple, caramel and toasted nuts (Figure 2). In sensory analysis too, the yeast VIN7 behaved differently than the typical thiolic or esteric yeasts, the wine aromatic profile generated by this being the most complex, with attributes related to flower, citric fruits, spices and temperate climate fruits (quince, apricots and pears), but with an overwhelming overripen apple aroma, showing that the low protection with sulphur dioxide was not beneficial for this particular wine.

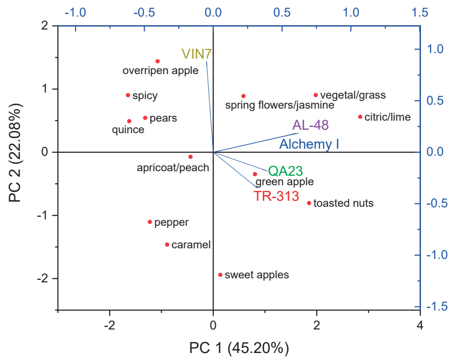

Figure 2. PCA biplot of wines fermented with 5 selected yeast strains and the main aroma descriptors identified by sensory analysis

Overall, the odor intensity conferred by the volatile compounds identified as being discriminatory for the wine fermented with the 5 selected yeasts proved that the esteric yeasts Alchemy I and Allegro AL-48 were clearly differentiated by a higher distance from the control QA23. Figure 3 shows diagram of odor distances of all wines fermented with selected yeasts as compared to the wines fermented with the QA23 control yeast obtained by statistical quality control analysis (SQC). The SQC analysis has taken into account only the peaks (representing sensors for the electronic nose) identified as having a high discriminative power (those presented in Table 2, with the exception of 2-Decenal and 4-Oxodecanal, which were not directly associated with yeast strains used). Figure 3 also shows that the thiolic TR-313 yeast was placed in a lower range than the control yeasts, proving again that the compounds it generates and releases are more sensitive to oxidative degradation than in the case of the estetic yeasts.

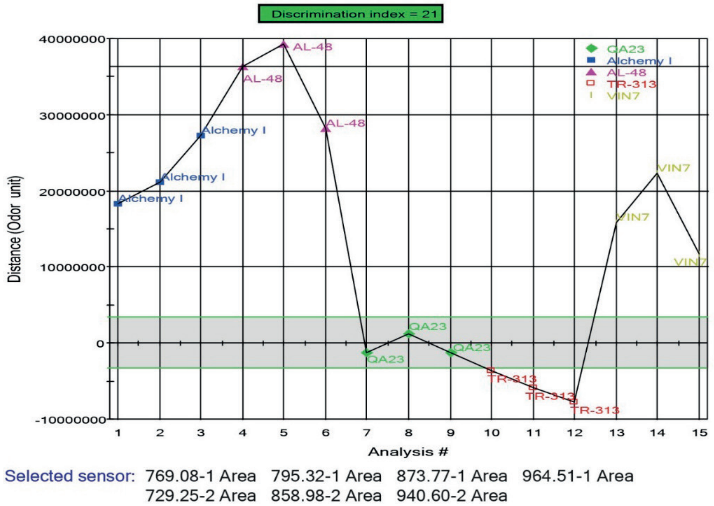

Figure 3. Diagram of odor distances of the wines fermented with several selected yeasts as compared to the wines fermented with the QA23 control yeast

Moreover, it should also be noted that a higher odor distance from the control is not necessarily associated with a higher odor quality. As it was the case of the wines fermented with VIN7, the odor distance determined by the electronic nose was mostly due to compounds showing premature aging/oxidation.

CONCLUSIONS

This experiment showed that the aromatic profile of the semi-aromatic variety 'Fetească regală' can indeed be influenced by winemaking, and the selection of the fermentation yeast leaves a specific mark on the final wine, also in direct dependence of the oxidative status of the wine. Selected yeasts classified as ester-releaser and thiols releasers, respectively, were compared with the classical QA23 thiolic yeast usually employed for this variety fermentation. While in other experiments, in which the wines were protected with the normal levels of sulphur dioxide permitted by legislation, the thiolic yeasts tended to confer a more complex and pleasant aromatic profile for 'Fetească regală', in the present experiment, lowering of the sulphur dioxide dose used at the bottling pointed to a more stable aromatic profile conferred by the esteric yeasts. Thus, to comply with the trend in lowering the sulphur dioxide concentrations in the bottled wines, the winemakers may need to resort to esteric yeasts, such as Alchemy I or Allegro AL-48.

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