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STUDY OF THE POSSIBILITY OF DISCRIMINATION BY AN ELECTRONIC NOSE OF FETEASCA REGALA WINES PRODUCED WITH NEUTRAL AND AROMATIC YEASTS

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Abstract

Feteasca regala is a Romanian grape variety with a specific, non-mistakable aroma. This wine aroma is appreciated by many consumers accustomed with it, but there are also some consumers who tend to avoid it. In order to make this wine more acceptable for a greater number of consumers the aromatic profile may be manipulated by fermenting the grape juice with yeasts able to produce certain flavour compounds, which can cover the original specific varietal aroma. In this study, a highly aromatic yeast (Flavour 2000), a low aromatic yeast (Premium blanc) and a neutral yeast (Montrachet) were used to produce Feteasca regala wines with various aromatic profiles. In some samples, tannin was also added at fermentation time, as a supplementary protection against oxidation, so that the freshness of the wines would be increased. Two sets of samples were produced at one week interval. Aside of the maturation degree of the grapes all other experimental parameters remained unchanged. When analysed with a specialized gas chromatograph working on the principle of an electronic nose, the samples produced with the neutral yeast were clearly discriminated from the other samples produced with aroma inducing yeasts, irrespective of the addition of tannin at fermentation. The other yeasts and the presence of tannins lead to a lower degree of discrimination between samples, although Discriminant Factor Analysis (DFA) was able to differentiate the samples produced with tannin from those fermented in the absence of tannin. The electronic nose is a very sensitive instrument, therefore we can assume that, at least as far as the volatile profile of the wines is concerned, whenever the electronic nose cannot make a distinction, the human nose will not be able to do it either. However, human sensory analysis may find differences between samples by using the other senses rather than the olfactive sense. This research shows, however, that the aromatic profile of Feteasca regala can be modified to a certain degree, compared to the varietal profile, by using appropriate yeasts for fermentation and that tannin also helps to protect the volatile compounds from being oxidized, thus also benefiting the final aromatic profile.

Keywords: *aroma profile, electronic nose, Feteasca regala, wine*

INTRODUCTION

Feteasca regala is a Romanian grape variety, resulted supposedly from local varieties Feteasca alba and Grasa crossing [7, 11], very wide spread in the country, being present in all 8 viticultural zones of Romania [11, 18, 19] and covering over 13000 ha, ranking first among the cultivated varieties as far as the surface is concerned [9]. It is very popular in Romania for its semi-aromatic white wines with a specific varietal aroma [8, 7, 12], with floral and spicy notes. However, exactly this type of aroma makes it so different, compared to many of the most white wines sold abroad, and for this reason these wines are sometimes avoided by people outside our country. In this research the fermentation in the presence of some ester-producing yeast strains was used as a solution intended to induce some changes in the typical aroma of Feteasca regala wines. Actually, the usage of selected yeasts is often criticized on the international market for making typical wines of various regions more uniform [10]. However, uniformity or at least falling into a standardized pattern is not always a bad thing, many consumers preferring to buy products with "normal", recognizable aroma [17]. Fermentation aroma introduced in wines by esterogenic yeasts or yeasts having more β-glycosidasic enzymes, sometimes hide the varietal aroma of some

wines and make the wines more acceptable for the mentioned category of consumers.In this study, wines of Feteasca regala were produced by using 3 different yeasts and their volatile fingerprints were compared by using a specialized gas-chromatograph working on the principle of an electronic nose. The analysis with the electronic nose is more rapid than the sensory analysis done by a panel of winetasters and is more reliable too. For this type of preliminary study the electronic nose is the perfect tool, because it only assesses differences between samples and identifies the ones having a different aromatic profile, without expressing judgements regarding which one is superior or preferable.

MATERIAL AND METHOD

Raw materials

The wine samples were obtained in 2010 in the experimental vineyard of USAMV Bucharest. Two batches were produced at intervals of one week from each other, the grapes being harvested at September 27th and October 3rd. The grapes harvested in September accumulated sugars up to 22.1 °Brix and a total acidity of 8.39 g/l expressed as tartaric acid, being at the full maturity. The grapes harvested in October were in the early phase of over-ripening, a significant drop of acidity being recorded at that time (total acidity 5.20 g/l tartaric acid), while the sugar remained almost constant. Feteasca regala is a grape variety that does not benefit from overmaturation, because it does not accumulate more sugar as in the case of other varieties, while the aroma and the acidity decrease rapidly, the resulting wines losing a lot of freshness as a result.

Oenological substances and reagents

In modern technologies, for the fresh young wines which are not meant to be kept for aging, ascorbic acid is also added, aside of potassium metabisulphite, for antioxidant protection. A combined product was provided by Enologica Vason company, under the commercial name of Flavour-save [16], containing 65% of potassium metabisulphite (E224) and 35% of L-ascorbic acid (E300). The dosage of 15 g/hl was used in this study for all the wine samples, and the product was added before crushing, on the grapes. Flavour-save maintains the varietal aroma unoxidized, contributing to regulation of the rH of wines [14], allowing for a longer period of reducing action, provided that the free sulphur dioxide content is periodically checked and adjusted [5], otherwise the ascorbic acid itself will lead to oxidation in the wine.

Bentonite gel was prepared from sodium bentonite (Bentogran, AEB Group [13]) by adding 950 g/l bentonite in 6 litres of water and mixing for 4 hours in a plastic bucket until a gel of creamy consistency results. A dosage of 100 g/l bentonite was used to clarify the must (0.6 kg of this gel was added in 100 l must).

The yeasts used for fermentation were Montrachet (a neutral yeast), Premium blanc (a low aromatic yeast with β-glycosidasic enzymes) and Flavour 2000 (a highly aromatic ester producing yeast) all from Enologica Vason. All dry yeasts were rehydrated as follows: 20 g yeasts were added in 200 ml warm water, resulting a yeast suspension of 10%.

Montrachet [20] is a neutral *Saccharomyces cerevisiae* yeast strain selected in California for musts with low nitrogen content and high content of sulphur dioxide, giving clean aroma, due to low acetaldehyde and volatile acidity production. It respects the varietal aroma complexity of the grape, being used as control wine typical for Feteasca regala variety.

Premium blanc [21] is a *Saccharomyces cerevisiae* strain, but with killer phenotype and fast growth, ensuring its rapid prevalence against the wild yeasts. It has β-gycosidasic enzymes, contributing to an increase in of the normal aroma of the grape variety, by splitting the gycosidasic bonds of glucose combined volatile substances, especially of the terpenic ones. It is recommended for wines obtained from semi-aromatic grape varieties, such as Sauvignon, Rhein Riesling, Traminer, suggesting it is also suitable for Feteasca regala.

Flavour 2000 [15] is also a *Saccharomyces cerevisiae* yeast, considered “aromatic” due to its metabolic activity which leads to the

production of esters and some other secondary fermentation compounds. It also introduces in wines a rose-like aroma due to the production of β-phenyl-ethanol, a substance very appreciated by some consumers. Normally it enhances the aroma of neutral varieties and here it was intended to change the aroma type of the semi-aromatic variety Feteasca regala.

The yeast suspensions were activated by adding as nutrient 50 g/l suspension of V-Activ Premium [23] containing 40% yeast hulls, 30% granulated cellulose, 30% diammonium sulphate and diammonium phosphate and 0.06% thiamine chloride. In the must, along with the yeast suspension, 20 g/hl fermentation activator V-Activ [24] was added, containing diammonium sulphate 55%, acid ammonium sulphate 33%, filtration add 11.8% and thiamine chloride 0.2%.

For additional antioxidant protection, in some wine samples tannin was also added at fermentation time. The selected tannin was Ti Premium [22], a granulated cathequinic tannin extracted from green tea, used in a dosage of 2 g/hl. Although extracted from green tea leaves, Ti Premium is a cathequinic tannin with a chemical structure similar to that of the tannin extracted from grapes. It protects against oxidation not only due to its chemical antioxidant power, but also because it is highly reactive towards proteins, inactivating the oxidizing enzymes such as laccase ((EC 1.10.3.2, p-diphenol oxidase). This tannin improves wine aroma also by reacting and thus removing mercaptans and other sulphur containing compounds.

Winemaking sequence

The grapes were harvested in plastic boxes of 10 kg each and crushed immediately after having been brought into the experimental wine cellar of the university. The dosage of 15 g/hl of Flavour-save was used in this study for all the wine samples, and the product was added before crushing, on the grapes. The entire quantity of grapes was destemmed and crushed with a laboratory manual destemmer-crusher. The resulted must was collected in a plastic barrel of 130 l and treated with 100 g/hl bentonite and left to clarify at 15°C for 24 hours.

The clarified must was distributed the next day in equal quantities of 40 litres in a battery of 5 small stainless steel tanks of 50 l volume. The tanks are connected to a cooling system, fermentation being conducted at controlled temperature.

Each stainless steel tank was inoculated with 2.5 ml yeast suspension (10%). In each tank 5 g of V Activ was also added, to provide enough nutrients for the freshly inoculated yeast. Each stainless steel tank was inoculated with a different selected yeast strain, and in some tanks also 2 g/hl of Ti Premium tannin was added. The variants produced in the available stainless steel tanks are summarized in Table 1.

Table 1. Experimental wine variants of Fetească regala

Variant coding	Harvest date, year 2010	Yeast type and dosage	Tannin type and dosage
MoY-v1*	September 27th	Montrachet, 20 g/hl	-
MoY-TIF-v2*	October 3rd		TI Premium, 2 g/hl
PBY-v1	September 27th	Premium blanc, 20 g/hl	-
PBY-v2	October 3rd		
PBY-TIF-v1	September 27th	Premium blanc, 20 g/hl	TI Premium, 2 g/hl
PBY-TIF-v2	October 3rd		
FY-v1	September 27th	Flavour 2000, 20 g/hl	
FY-v2	October 3rd		
FY-TIF-v1	September 27th	Flavour 2000, 20 g/hl	TI Premium, 2 g/hl
FY-TIF-v2	October 3rd		

* Due to the fact that for each harvest time only 5 tanks were available for fermentation, the samples fermented with the neutral yeast, Montrachet, were produced without tannin for the first harvest time and in the presence of tannin at the second harvest time. Montrachet wine samples are considered control wines, this neutral yeast respecting the varietal aroma and giving typical wines.

Electronic nose

The analysis of the wine samples was performed with a dual column flash gas chromatograph produced by Alpha Mos company and called Heracles analyzer. Working on the principle of an electronic nose, this apparatus provides information regarding the volatile profile of a sample and allows the data processing in a similar way as

the brain does with the perceived volatile substances in a complex mixture. In this way, the result is a general, overall olfactory fingerprint of the volatile matrices and does not attempt the identification of each volatile component in the blend. For this apparatus, the "sensors" are the chromatographic peaks themselves, recorded and stored for each sample and later processed with a specialized software, Alpha Soft v. 11.0, allowing for a rapid and reliable discrimination between products.

The samples are introduced in 10 ml vials with metal cap. Each wine sample is analyzed in triplicate. The apparatus also includes a HS 100 auto-sampler from which 64 different samples can be automatically processed from two trays. In each vial 4 ml of wine is added and sealed. As in the sensory analysis, the volatiles analyzed from the wines are only those released from the wine in the atmosphere above the liquid (the headspace). The injected volatiles from the vial headspace are concentrated by passing through a Tenax trap, then the separation of the volatile compounds is performed on both columns (an non-polar column, DB-5 and a medium polar column, DB-1701) and detected simultaneously with two FID detectors. As combustion gas pure hydrogen produced with a gas station is used.

The analytical method applied is developed in our laboratory [6, 2, 1, 3,] and uses the following parameters: incubation temperature 60°C, incubation time 600 s, injected volume 2500 μl, injector temperature 200°C, detector temperature 220°C, measurement time 20 s, trap temperature: initial 40°C and 250°C at desorption, preheating trap time 20 s, baking time 60 s, pre-purging time 5 s. The programme of the gas chromatograph starts at a temperature of 40°C maintained for 2 s and increased by 5°C /s up to 200°C where it is also maintained for 5 s, then cooled down. The data acquisition time per sample is 40 s.

RESULTS AND DISCUSSIONS

The Heracles electronic nose based on dual-column gas-chromatography was used to differentiate Feteasca regala variants produced with various yeasts and tannin based on their volatile profiles. Knowing that each chromatographic peak corresponds to a volatile substance detected, these peaks can be considered virtual sensors specialized to detect a certain substance. The large number of peaks obtained on both chromatographic columns with complementary properties ensures a good sensitivity. However, not all peaks are taken into consideration for the analysis, but only those which are most important for the discrimination of one sample from the others. The common small peaks are generally disregarded.

By analyzing the database obtained for our samples, we applied two methods of multivariate statistics, PCA – principal component analysis and DFA – discriminating factor analysis. Both methods allow for graphic representations where the discrimination between samples or groups of samples is easily understandable. A parameter named "discrimination index" is correlated with the degree of discrimination success.

The first comparison was done for the samples harvested at the moment *v1*, September 27th, moment representing full maturation for the Feteasca regala grapes in 2010. In order to do this, both PCA and DFA analyses were performed and the results are presented in Fig. 1-4.

PCA analysis aims to reduce the number of variables by the formation of some new variables as linear combinations of the initial ones, resulted by direct analyses. In this way, the great variability of the initial experimental data can be easier explained and interpreted.

As seen in Fig. 1, in our case the software identified a principal component 1, which explains 68.33% of the variability induced by the initial data (the volatiles recorded as chromatographic peaks).

This principal component is a linear combination of the sensors (peaks) with discrimination power between 0.92 and 1.0, taken into account for this analysis from the multitude of the peaks recorded. The second principal component, placed perpendicular on the first one, explains 29.95% of the variability of the initial data. This fact shows that both the yeasts and tannin influenced the aromatic profile of the wines.

A good discrimination is observed for the wines produced with the neutral yeast, Montrachet (*MoY*), clearly separated from the wines produced with aromatic yeasts both in the presence and the absence of tannin. The wines fermented with the yeast Premium blanc (*PBY*), which, due to the presence of *β*-glucosidases, releases more aromatic compounds from those bound to glucose, but providing an aromatic profile based also on the varietal aroma, are also clearly separated from the wines produced with esterogenic yeast Flavour 2000 (*FY* and *FY-TIF*). The presence of tannin makes the samples more uniform in aroma, therefore, the wine variants *PBY-TIF* are not anymore discriminated from those produced with Flavour 2000, *FY-TIF*. The discrimination index is however small, 0.01, meaning that the differences between groups are quite small and very likely unnoticeable by a human subject.

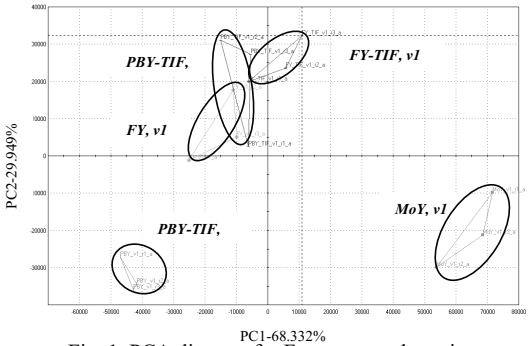

Fig. 1. PCA diagram for Feteasca regala variants harvested at full maturation (harvest moment *v1*). The discrimination power of the selected sensors for analysis was up to 0.92.

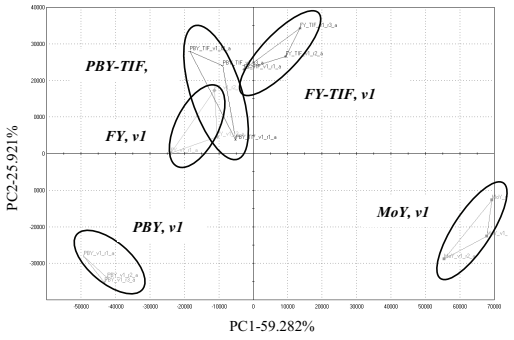

Fig.2. PCA diagram for Feteasca regala variants harvested at full maturation (harvest moment *v1*). The discrimination power of the selected sensors for analysis was up to 0.50.

In order to test the importance of the peak number selection, in Fig. 2 we present the same analysis as in Fig. 1, except for the fact that the peaks selectes as sensors were more numerous, being selected all those with a discrimination power superior to 0.50. Generally it is to be expected that a larger number of sensors will increase the sensitivity and provide a better discrimination. In this case too, an increas in the discrimination index is observed, from 0.01 to 0.3, but this is not sufficient to justify the increase in the number of chromatographic peaks taken into account.

In Fig. 3 the PCA analysis performed for the same samples as above is shown, but based on the sensors automatically selected by the Alpha Soft software, that is only those which have high discrimination power (peaks which are present in some of the samples and absent in others, due to the variations in the winemaking procedures). As expected, the discrimination index for this analysis increased to 88. However, the qualitative result is no different than the one obtained in the previous analyses, meaning that the selection of sensors has no big influence on the results, provided we select sensors with enough discriminant power. Again *MoY* variant is singled out as a separate group, while the samples fermented with non-neutral yeasts are grouped closer together.

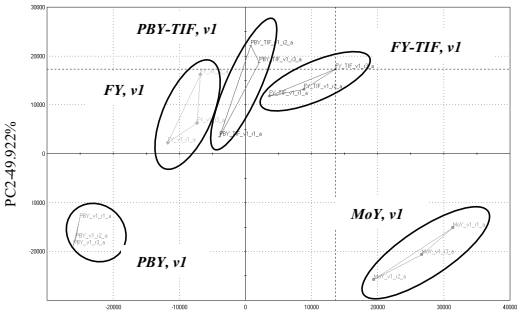

Fig.3. PCA diagram for Feteasca regala variants harvested at full maturation (harvest moment *v1*). The selected sensors for analysis were decided automatically by the Alpha Soft software based on their discrimination power.

In the case of the other analytical method, DFA (discriminant function analysis), other linear combinations of the initial variables, named discriminant functions, are computed, ensuring a separation (discrimination) as good as possible of the analyzed groups of samples. The DFA diagram depicted in Fig. 4 and obtained based on the same sensors as in the case of PCA diagrams in Fig. 1 - 3 shows a better discrimination of the groups of samples. In this case the five groups of samples are clearly separated, but still with a low discrimination power. Of all the samples, those fermented in the presence of the neutral yeast Montrachet are discriminated clearly by the electronic nose, giving hope for a possible change in aroma profile of Feteasca regala wines by fermentation with "aromatic" yeasts.

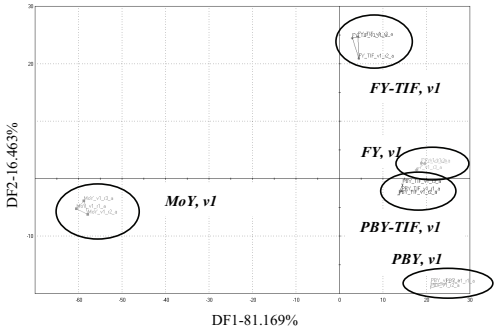

Fig. 4. DFA diagram for Feteasca regala variants harvested at full maturation (harvest moment *v1*)

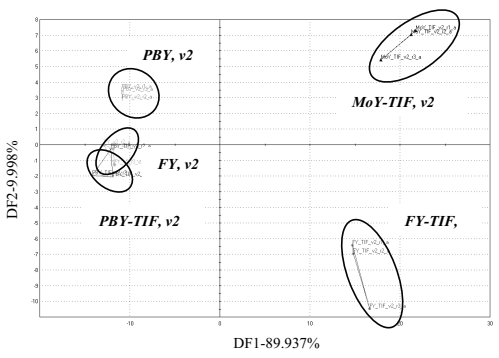

Fig. 5. DFA diagram for Feteasca regala variants harvested at over-maturation (harvest moment *v2*).

For the other harvesting moment, over-maturation moment (*v2*, October 3rd), a similar discrimination of the Montrachet fermented wines is observed (Fig. 5). In this diagram, because the samples produced with Montrachet yeast contained also tannin (*MoY-TIF, v2*), the profile is also influenced by tannin, therefore it is understandable that the distribution of the samples in the diagram is different than in the case of full maturation moment (*v1*, September 27th), where the Montrachet samples were produced in the absence of tannin (*MoY, v1*).

In order to assess further the influence of the tannin, we disregarded the harvest moment, grouping samples with the same technological treatment in the same group irrespective of the harvest time, resulting the following groups: *PBY (v1+v2), PBY-TIF (v1+v2), FY (v1+v2), FY-TIF (v1+v2), MoY (v1), Mo-TIF (v2).* The DFA analysis based on these groups showed (Fig. 6) that the tannin has its influence on the aroma of the wines, samples produced in the presence of the tannin (left part of the diagram) being grouped separately from those produced in the absence of tannin (right part of the diagram). Because of the low discrimination power showed on the graph scales we can safely assume that these differences would not be perceived by human observers. The tannin has its protection value as an antioxidant, therefore, after a period of evolution, significant differences in wine aroma could develop, so that the differences may become apparent also for consumers.

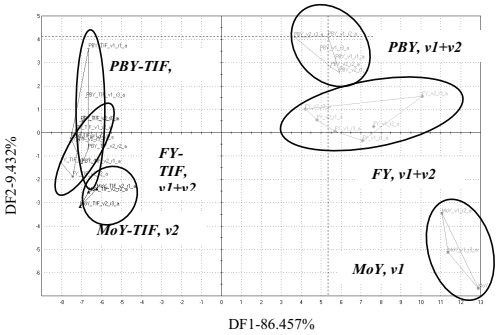

Fig. 6. DFA diagram for Feteasca regala variants with similar technological treatments, irrespective of the harvesting moment.

From the analyses performed it became apparent that the Montrachet yeast fermented wines have an easily detectable different aromatic profile than the wines produced with

the “aromatic” yeasts. This conclusion was also tested by calculating the distances, in odor units, of these wines as compared to the wines produced with Flavour 2000 and Premium blanc yeast and the analysis confirmed it (Fig. 7). The test was performed on wines produced in the absence of tannins, in order to assess only the influence of the yeast on the volatile profile.

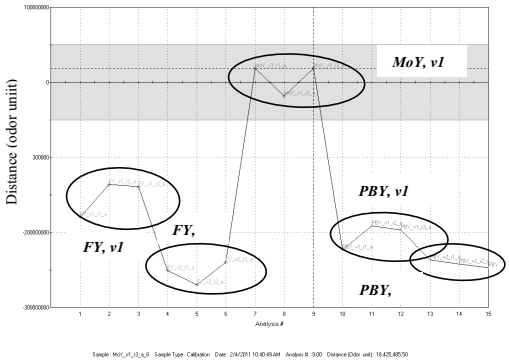

Fig. 7. Distance in odor units of the Feteasca regala wines produced with neutral yeast Montrachet (*MoY*) versus the wines produced with “aromatic” yeasts Flavour 2000 (*FY*) and Premium blanc (*PBY*)

CONCLUSIONS

Small differences in the aromatic profiles of wines are induced by the aromatic yeast, but these olfactory differences alone may not be noticeable by consumers, the varietal aroma of Feteasca regala being still the most important in the aromatic profile of the wines. The electronic nose is able to detect differences between groups of samples, even with small discrimination indexes (0.01 – 88.00), but human panelists may not be able to observe these differences. Some analyses showed that of all the samples the electronic nose discriminated clearly those fermented in the presence of the neutral yeast Montrachet from the other produced wines, indicating a possible significant modification in the volatile profile of Feteasca regala wines by fermentation with “aromatic” yeasts.

The treatment with tannin has a clear influence on the aroma of the wines, and the electronic nose discriminates these small differences, even though at this power of discrimination it can be safely assumed that these differences would not be perceived by human observers on young wines. However, the treatment with tannin should not be disregarded as a potential modulator of wine aroma, since with its antioxidant protection power tannin may lead to significant differences in wine aroma after a period of evolution, as compared to wines unprotected with tannin. In time, these small differences detected by the electronic nose may become apparent for consumers, too.

It is obvious that some yeasts can modify the varietal aroma of the wines. It was expected that the aroma profile induced by the ester-producing Flavour 2000 yeast through fermentation would be totally different than that of Premium blanc yeast, which only enhances the varietal aroma of a wine by releasing more aroma from bound compounds. Due to these aroma producing mechanisms, the Premium blanc fermented wines should have been closer in profile to the varietal aroma wines produced with the neutral yeast, Montrachet. However, the electronic nose showed that Montrachet is indeed different from all others, but the wines obtained with “aromatic yeasts”, in spite of their different aroma producing mechanisms, were grouped together.

Therefore, we can conclude that varietal aroma of Feteasca regala wines can be influenced by the presence of an aromatic yeast during fermentation, but not significantly erased so that the variety become unidentifiable. If this type of effect is desired, in order to make an impact on human observers, some other yeasts or treatments should also be tested to significantly change the varietal aroma of these wines.

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