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Evaluation by Flash GC Electronic Nose of the Effect of Combinations of Yeasts and Nutrients on the Aromatic Profiles of Feteasca Regala Wines after Two Years of Storage

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Abstract: Feteasca regala is a semi-aromatic Romanian white grape variety, which can benefit from technological interventions aiming to modulate its aromatic profile. In this study, two specific yeast nutrients, designed to increase the esteric and thiolic aromatic potential, respectively, were added at appropriate times, before or during fermentation. The musts were inoculated with two different strains of yeast, specially selected to favour the formation of an esteric or a thiolic volatile profile. The resulting wines were bottled and analysed two years later by Heracles flash GC electronic nose (from Alpha MOS), which provided a good discrimination of the samples based on the peaks of volatile molecules identified on the two chromatographic columns. The electronic nose showed that, in the aged wines, the influence of the yeast inoculated for fermentation was more evident than the impact of the yeast nutrients added. Using the AroChemBase software module from Alpha MOS, some volatile esters and other compounds were identified, and their importance for the discrimination of the wines and for the aroma profile is discussed. However, because the GC electronic nose can identify only some volatile compounds, but not all, sensory analysis was also applied to evaluate the wine samples, showing that the yeast, as well as the nutrients, have a clear influence on the perceived aromatic profiles. As intended, samples prepared with any of the technological interventions showed different volatile/aromatic profiles than the control wine prepared by natural fermentation and were clearly separated by the electronic nose, even after two years of storage. However, due to the limitations of the chromatographic columns used, the electronic nose could not provide an overall description of the aromatic profile of the produced wines, which is why the expertise of panelists was still needed to evaluate wines.

Keywords: Heracles GC electronic nose; Feteasca regala; esters; thiols; yeast nutrients; wine; aromatic profile

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1. Introduction

Feteasca regala is a Romanian variety of grape for white wines with a wide cultivation, covering 12,277.71 ha in 2021 and representing 12.13% of the total *Vitis vinifera* vineyards in Romania, which puts it in first place regarding surface area [1] (data not public). As occurs with such representative grape varieties, oenologists have attempted to modulate its aromatic profile to obtain various styles of wine and thus to address the preferences of different consumer segments. However, the modulation of the aromatic profile of Feteasca regala is less researched and reported.

One of the most frequently used methods to modulate the aromatic profile of a wine is the use of specific selected yeasts. Many authors have studied the impact of yeast fermentation on volatile profiles for several other varieties, such as Arinto [2], Tempranillo [3], Muscat Ottonel [4], Shiraz [5], and Pošip [6], or for a defined medium which simulates standard grape juice [7].

Most yeasts lead to wines with esteric profiles, as the esters are the main aroma compounds generated during wine fermentation [8]. Some other yeasts may enhance the thiolic profile if they have increased β-lyase activity [9], and there are also yeasts which can modulate the aroma profile in both directions. This is the case for a *Saccharomyces cerevisiae* strain which has an overexpression of the *ATF1* gene, giving it the possibility of enhancing both the thiol and ester aromas in wine [10], as well as a *Saccharomyces cerevisiae* strain which expresses protein Ure2, which functions as a nitrogen metabolism regulator and as a glutathione-dependent peroxidase [11].

Furthermore, yeasts themselves respond differently when their growth environment is different. In the absence of sufficient nutrients, yeasts tend to increase production of higher alcohols [12], leading to an aromatic profile less preferred by the consumers. Therefore, adding nutrients to the must in fermentation is also an intervention which may allow the modulation of the wine aroma. By adding nutrients based on nitrogen, yeast growth is regulated and more esters are produced, which in turn confers more fruity and floral characteristics to the wine aroma [13]. Although the response to the concentration of nitrogen is strain-dependent [14] and time-dependent [15], it has been observed that some amino acids, such as phenylalanine, may enhance fruity aromas, while leucine and isoleucine lowered ethanol production during fermentation [16].

When amino acids are insufficient, the yeast tends to split proteins and then produce sulfur compounds as a result of catabolism of amino acids with sulfur, especially methionine [17], leading to compounds with undesirable flavours (such as H_2S and low molecular volatile thiols— methanethiol, ethanethiol). It is documented that adding amino acid-based nutrients in the grape must leads to the production of more esters, increasing the floral aromatic profile of wine [18], even though there is no direct correlation between the amount of the esters formed and the added amino acids [19,20].

Furthermore, glutathione, a natural antioxidant compound which is present in grapes, but also in yeasts [21], can serve to further modulate the aromatic profile of the wine [6] if the winemaker increases the must concentration in glutathione or its precursors. As opposed to the small molecules of thiols, the volatile thiols with higher molecular weight positively contribute to the wine aroma profile [22]. Several mechanisms are involved in the production of thiolic aroma in wines, from interventions in the vineyard aiming to increase thiolic precursors in grapes, to interventions in the oenological practices designed to increase extraction by longer maceration or to use *Saccharomyces cerevisiae* yeast with higher β-lyase activity [22].

Thus, this research attempted to combine these two types of interventions, inoculation of selected yeasts and addition of specific nutrients, to induce some desirable changes in the volatile compounds produced during fermentation.

The aroma profile of wines changes over time due to various chemical reactions that take place during wine maturation in bottles. Young wines are more intense in aroma, but after a year in bottle, the fermentation aroma is already known to be significantly decreasing [6]. For various reasons, the wine is not always sold immediately after fermentation or while young, in the first year after production. Thus, it makes sense to determine if the differences in the volatile profile induced by technological interventions are still detectable. Therefore, this study focuses on the differentiation of the volatile profile of wines after two years of storage in bottles and the impact of storage on the sensory perception of the wine aroma. The volatile profile discrimination and the identification of some of the volatile compounds were performed by an electronic nose based on the principle of gas chromatography, while the sensory evaluation was performed by a panel of human tasters. Many studies of wine aroma are based on evaluations done by GS, GS-MS, two-dimensional GC, olfactometry [23–25] and similar methods.

The electronic nose technology is very good at sensing minute differences among the experimental variants, and many studies take advantage of its discrimination power [26–29], its rapidity [30] and also attempt to eliminate human evaluators [31]. However, for our study, the compounds that the electronic nose was able to separate and identify were

not sufficient to thoroughly describe the aroma profile of the wines; therefore, combining both approaches—electronic nose and sensory evaluation—led to a better evaluation and discrimination of the samples.

2. Materials and Methods

2.1. Raw Material and Winemaking

Feteasca regala grapes were harvested from the experimental vineyard of the University of Agronomic Sciences and Veterinary Medicine of Bucharest on 10 September 2019. The vinification process was performed in the winery of the university, observing the usual white wine technology. The grapes were destemmed and crushed with a destemmer with a stainless-steel perforated cylinder (Enoveneta, Piazzola sul Brenta, Padova, Italy) and transferred to a hydraulic bladder press with a stainless-steel cage (Fratelli Marchisio & C., Pieve di Teco, Imperia, Italy), from which the free run juice was se-parated and used for the preparation of wine variants.

The resulted must was treated with a dose of 50 mg/L SO_2 using a 10% solution of potassium metabisulfite, and also with 2 g/hL of the pectolytic enzyme Zimafruit and 10 g/hL PVVP, all from Enologica Vason, Corrubbio, Verona, Italy. The juice was cooled below 10 °C and left to settle in the cold for 24 h to allow for clarification up to a turbidity of 25 NTU.

The acidity of the limpid must, with an initial dry content of 26.5° Brix and 4.23 g/L titratable acidity expressed as tartaric acid, was adjusted by adding 1.5 g/L tartaric acid.

From the limpid must, four wine variants were prepared by using two different commercially available selected dry yeasts and also two combinations of specific yeast nutrients, designed to favour the formation during fermentation of either an esteric or a thiolic volatile profile. For the rehydration of dry yeasts, the nutrient Go Ferm Protect Evolution from Lallemand, Montreal, Canada, consisting of *Saccharomyces cerevisiae* inactivated yeast and autolysate, was added in a dose of 25 g/hL.

The alcoholic fermentation was conducted in tanks of 50 l volume at a temperature of 16 ± 1.5°C and lasted about 4 weeks. Afterwards, on 17 October, the newly obtained wines were racked and left for maturation on the fine lees for another 4 months. During the first two of the four months of maturation, the wines were stirred twice a month to increase contact with the lees, for a better extraction of compounds with effects on wine aroma.

Adjustments of wine acidity were performed twice (1 week after racking and 2 months after racking), with 1 g/L tartaric acid each time. A small dose of 50 mg/L sulfur dioxide was added in each tank 2 months after racking, and this was supplemented 2 months later with another 20 mg/L on the occasion of bottling to the level of 110 mg/L total sulphur dioxide. The wines thus prepared were left to age in bottles for 2 years at 16°C.

2.2. Wine Yeasts and Yeast Nutrients

The wine yeasts, nutrients and enzymes used for the experimental protocols are obtained from Lamothe-Abiet, France and are as follows:

- *Yeast Excellence* ***STR*****:** *POF*(-) *Saccharomyces cerevisiae* yeast strain obtained without using GMO technology through a technique called direct breeding. Lacking the POF (phenol off flavour) gene, this strain preserves better the varietal expression of the grapes. Hereafter, it is abbreviated as STR;
- *Yeast Excellence* ***TXL*****:** a *URE2*(-), *POF*(-) and *SSU1*-R(+) *Saccharomyces cerevisiae* yeast strain obtained without using GMO technology through a technique called targeted breeding, which involves crossing and backcrossing with other strain until a descendant is obtained which shares 93% of the original target strain, as well as the desired genes of the other strain. For our study, the trait for which this strain was selected is the deletion of the *URE2* gene, which removes the so-called "nitrogen catabolic repression" and allows the yeast to use the nitrogen from the amino acids bound to thiols as aroma precursors, in this way increasing the production of the varietal volatile thiols. Other traits of this yeast include increased resistance to sulfur dioxide

due to incorporation of the *SSU1-R* gene and minimal production of the undesirable compounds vinylphenol and vinyl guaiacol due to the deletion of the *POF* gene. Hereafter, it is abbreviated as TXL.

- *OptiEsters*: inactivated *Saccharomyces cerevisiae* yeasts naturally rich in amino a-cids and ergosterols, which stimulate the production of precursors of higher alcohol acetates, modulating the production of esters in the wines. Hereafter it is abbreviated as *Ester* in the codename of the variants.
- *OptiThiol*: inactivated *Saccharomyces cerevisiae* yeasts naturally rich in glutathione (GSH) precursors with reducing effects, such as cysteine, N-acetyl-cysteine, homocysteine, glycine-cysteine, glutamate-cysteine and glutathione. These GSH precursors ensure the protection of thiol aromas, increasing their quantity after alcohol fermentation. Hereafter, it is abbreviated as *Thiol* in the codename of the variants.
- *Oenozym Thiols*: pectolytic enzymes from *Aspergillus niger*, such as arabanases, cellulases and hemicellulases, rich in secondary activities but with no undesirable cinnamoyl-esterase activity. The enzyme enhances the release of thiol aromas from precursors linked to cysteine or glutathione and may increase their preservation time in wines. It was used for the *Thiol* variants as treatment during fermentation and post-fermentation as part of the thiolic winemaking protocol.

The experimental protocols with the wine yeasts, nutrients and enzymes used, as well as the fermentation conditions and post-fermentative treatments, are summarized in Table 1.

Table 1. The experimental wine variants with the sequence in which the materials were used.

Variant [1]	Pre-Fermentative Treatments	Yeast for Fermentation	Fermentation Temperature	Treatments during Fermentation	Post-Fermentative Treatments
Control		No added yeast (spontaneous fermentation)	20 °C		- Racking from the lees, bentonite 0.62 g/L, racking, addition of 80 mg/L SO_2 (three weeks from grape harvesting) - Racking from the bentonite, addition of 30 mg/L SO_2 (two months after grape harvesting) - Fine filtration (0.8 to 1.5 µm) with JPOR J1500 filter pads (JU.CLA.S, Settimo Verona, Italy) and bottling (three months after grape harvesting)
Ester-STR		*Excellence* ***STR***, 20 g/hL	15 °C	*OptiEsters*, 30 g/hL (one week from grape harvesting, when must had 18° Brix)	
Ester-TXL		*Excellence* ***TXL***, 20 g/hL			
Thiol-STR	*OptiThiol*, *30 g/hL*	*Excellence* ***STR***, 20 g/hL	18 °C	*Oenozym Thiols*, 4 mL/hL (6 days from grape harvesting) *Oenozym Thiols*, 2 mL/hL (36 days after the first addition)	
Thiol-TXL		*Excellence* ***TXL***, 20 g/hL			

[1] The yeasts are abbreviated STR and TXL and the nutrients *Ester* and *Thiol*, respectively.

The temperature for fermentation has a great impact on yeast metabolism; therefore, it was carefully selected and controlled to allow the development of the targeted aromatic profile. It is known that lower temperatures lead to better development of ethylic esters [32,33]; therefore, for the fermentations *Ester*-STR and *Ester*-TXL, a fermentation temperature of 15 °C was selected to favour the development of esteric aromas. For *Thiol*-STR and *Thiol*-TXL, the fermentation temperature selected was 18 °C to avoid the formation of hydrogen sulphide by the yeast in the presence of the thiolic nutrient based on sulphur compounds.

The control wine was fermented in the usual conditions for white wines, the slightly higher temperature of 20 °C being necessary for the development of the typical terpenic profile of this variety, which is otherwise inhibited by lower temperatures [33]. Additionally, the control wine was fermented with its natural yeasts to reveal the exact aromatic profile obtained in the region by the classical winemaking technology.

2.3. Analyses

2.3.1. Determination of the Main Wine Parameters

The main wine parameters were determined in accordance with the OIV methods [34]: alcoholic concentration was determined by distillation method OIV-MA-AS312-01A; volatile acidity by method OIV-MA-AS313-02; sugar concentration in wine by the chemical method OIV-MA-AS311-01A; total acidity and pH by potentiometric method OIV-MA-AS313-01; total dry extract by method OIV-MA-AS2-03A; glycerol, L-malic acid, L-lactic acid and citric acid by enzymatic methods OIV-MA-AS312-05, OIV-MA-AS313-26, OIV-MA-AS313-25, and OIV-MA-AS313-09, respectively; free and total sulfur dioxide by titrimetry methods OIV-MA-AS323-04A1 and OIV-MA-AS323-04A2; and density by method OIV-MA-AS2-01A. The total polyphenols were determined spectrophotometrically and expressed in mg/L gallic acid equivalents. The method is based on a modification by Singleton et al. [35] of the Folin–Ciocalteau method and is described in detail in Artem et al. [36].

2.3.2. Electronic Nose Analysis

The volatile profiles of the wine variants were determined by using a gas chromatograph with two columns working as an electronic nose. The equipment, called the Heracles e-nose analyser, was produced by Alpha MOS, Toulouse, France. The apparatus is endowed with a Tenax trap which allows for the pre-concentration of volatile organic compounds. The sample is extracted from the headspace and simultaneously introduced in two short capillary columns, which allow for a fast chromatographic analysis, the volatile compounds being detected by flame ionization detectors (FID) placed at the end of each column. The columns are of different polarities, as follows: a non-polar column DB5 is composed of 5% diphenyl and 95% dimethylpolysiloxane, and a low/mid polarity column DB1701 is composed of 14% cyanopropylphenyl and 86% dimethylpolysiloxane. High-purity hydrogen is used as the carrier gas, as well as for the combustion for the FIDs.

The volatile samples were taken from the headspace of 10 mL vials filled with 4 mL of wine and sealed with magnetic caps. For each variant, the analysis was run in triplicate.

Additional information on the Alpha MOS Heracles analyser and methods used can be found in previous papers [29,30,37–41]. The software used by the Heracles electronic nose for data recording and processing was AlphaSoft v12.42, and the library AroChemBase version 2010 (Alpha MOS, Toulouse, France) was used for the identification of chemical compounds based on retention Kovats indices.

The e-nose experimental parameters, examples of chromatograms obtained on both columns with this e-nose, the method for selecting the sensors (represented by chromatographic peaks), and the method for calibration and calculation of Kovats indices can be found in a previous paper [29].

2.3.3. Sensory Analysis

The sensory analyses were performed based on an evaluation sheet developed in a previous research project and registered as part of a patent [42,43].

The perceptions of acidity, sweetness, astringency, bitterness, extract, colour intensity and aroma intensity were evaluated by a panel of trained winetasters on continuous intensity scales with a maximum value of 10, anchored with appropriate descriptors for each parameter. The perception of specific aromas, such as vegetal, fruity, floral, and spicy, with associated descriptors where certain aromas were identified, was evaluated on discontinuous scales of 5 points, for which the values of 2, 4, 6, 8 and 10 were allocated during data analysis.

2.3.4. Statistical Analyses

For the e-nose data, the software AlphaSoft v12.42 (Alpha MOS, Toulouse, France) was used for performing discriminant factor analyses (DFA), as they allow the separation

of the groups of samples and the identification of possible patterns correlated with the yeasts used for fermentation and/or the added nutrients.

The sensory analysis data were statistically processed with the Origin 9.0 software package (OriginLab, Northampton, Massachusetts, USA), calculating the significance of the mean differences and performing principal component analysis (PCA). PCA allows the separation of the sample groups in accordance to the main components which, in turn, are computed based on the descriptors most associated with each applied treatment.

For both statistical analysis and peak areas of the compounds separated and identified by the e-nose, one-way ANOVA and Tukey post-hoc analyses were used for multiple comparison of the mean differences. The significantly different values at $p > 0.05$ are indicated in the text with different letters.

3. Results

3.1. Main Physico-Chemical Parameters

The wines produced with various technological interventions were stable after two years of storage in bottles, with the exception of some tartaric acid precipitation, which is a normal phenomenon. For each wine variant, only a bottle of the entire batch was analysed to determine the main wine quality parameters, the goal being to ascertain if the wines preserved well and had no major deviance. The main physico-chemical analyses showed that all the wine parameters (Table 2) were in the normal range as defined by legislation [44].

Table 2. The main wine parameters of the experimental variants obtained with two different yeasts (TXL and STR) and two different added nutrients (*Ester* and *Thiol*).

Parameter	Control	*Ester*-STR	*Ester*-TXL	*Thiol*-STR	*Thiol*-TXL
Alcohol, % vol	13.4	15.5	15.7	15.5	15.2
Total dry extract, g/L	20.6	18.8	18.6	19.0	19.4
Density at 20 °C	1.0044	0.9887	0.9889	0.9889	0.989
Reducing sugar, g/L	35.85	2.85	3.85	3.20	3.05
Total acidity, g/L tartaric acid	5.2	4.6	4.6	4.9	4.9
Citric acid, g/L	0	0	0	0	0
L-malic acid, g/L	0	1.75	2.05	1.91	2.11
L-lactic acid, g/L	0.82	0.05	0.21	0.03	0
Volatile acidity, g/L acetic acid	0.49	0.64	0.52	0.51	0.51
pH	3.52	3.62	3.61	3.60	3.60
Glycerol, g/L	0.64	0.63	0.64	0.63	0.63
Free SO_2, mg/L	9.1	18.2	16.9	13.0	23.4
Total SO_2, mg/L	72.8	66.3	72.8	65.0	63.7
Total polyphenols, mg GAE/L	69	81	80	84	96

The values provided in Table 2 show conformity with the legislation for quality wines. As can be seen, the independent parameters fluctuate in narrow ranges for all the sample variants, which would not be normally differentiated by human tasters in sensory tests done with these compounds independently. Their impact in wines relies on their combination, and this effect is evaluated in Section 3.4, Table 5, in which the main sensory parameters are identified and statistical differences determined and discussed.

3.2. Relevant Volatile Compounds Identified in the Wines by GC Electronic Nose and the Library AroChemBase

For the identification of volatile compounds which participate in the aromatic profile of wines, three bottles of each variant were used for the GC electronic nose analysis. The sample for chromatography was taken from the headspace of the vials; therefore, the aromatic profile obtained only includes compounds with high volatility which can be perceived olfactorily when the wine is normally sniffed.

The main compounds which were unequivocally identified by using AroChemBase on at least one of the chromatographic columns of the e-nose apparatus are presented in Table 3 along with the respective chromatographic peak area, which allows for comparison between wine variants.

Table 3. Compounds identified with AroChemBase in the experimental variants obtained with combinations of two different yeasts (TXL and STR) and two different added nutrients (*Ester* and *Thiol*).

Identified Compounds	GC Column	* Peak Area (± Standard Error of Mean), Volts-Minute				
		Control	*Esters*_STR	*Esters*_TXL	*Thiols*_STR	*Thiols*_TXL
			Aldehydes			
5-Methylfurfural	DB5	399 ± 53 ^{a}	384 ± 27 ^{a}	375 ± 31 ^{a}	427 ± 41 ^{a}	331 ± 34 ^{a}
Benzaldehyde	DB1701	405 ± 54 ^{a}	n.d. **	n.d. **	437 ± 67 ^{a}	487 ± 80 ^{a}
			Alcohols			
(E)-3-Hexen-1-ol	DB1701	497 ± 33 ^{b}	631 ± 36 ^{ab}	537 ± 30 ^{ab}	737 ± 86 ^{a}	513 ± 56 ^{b}
			Esters			
Ethyl acetate	DB5	150,794 ± 10,693 ^{a}	165,236 ± 7434 ^{a}	170,030 ± 8310 ^{a}	171,157 ± 9175 ^{a}	170,417 ± 6238 ^{a}
Ethyl acetate	DB1701	112,187 ± 7625 ^{a}	124,774 ± 5417 ^{a}	129,553 ± 5520 ^{a}	128,729 ± 7181 ^{a}	128,248 ± 4577 ^{a}
Ethyl propionate	DB5	905 ± 109 ^{b}	1536 ± 136 ^{a}	1384 ± 136 ^{a}	1328 ± 75 ^{ab}	1169 ± 80 ^{ab}
Ethyl butyrate	DB5	6510 ± 189 ^{b}	8602 ± 406 ^{a}	8605 ± 298 ^{a}	6823 ± 126 ^{b}	6964 ± 159 ^{b}
Ethyl butyrate	DB1701	221,720 ± 6184 ^{a}	218,342 ± 9663 ^{a}	229,340 ± 7444 ^{a}	255,072 ± 8220 ^{a}	240,825 ± 12,067 ^{a}
Ethyl isobutyrate	DB1701	1692 ± 204 ^{a}	2286 ± 246 ^{a}	2806 ± 436 ^{a}	2471 ± 365 ^{a}	3220 ± 518 ^{a}
Isoamyl acetate	DB5	40,545 ± 1843 ^{c}	88,565 ± 4297 ^{ab}	77,337 ± 5543 ^{ab}	92,844 ± 5028 ^{a}	72,820 ± 4332 ^{b}
Isoamyl acetate	DB1701	29,256 ± 1705 ^{c}	66,743 ± 3468 ^{ab}	59,123 ± 4462 ^{ab}	70,907 ± 4138 ^{a}	55,640 ± 3326 ^{b}
Ethyl decanoate	DB5	37,371 ± 3128 ^{a}	27,427 ± 1491 ^{b}	34,244 ± 1995 ^{ab}	26,679 ± 1327 ^{b}	28,621 ± 1259 ^{b}
Ethyl decanoate	DB1701	26,960 ± 2469 ^{a}	19,414 ± 1307 ^{b}	24,010 ± 1680 ^{ab}	18,826 ± 1152 ^{b}	21,264 ± 1096 ^{ab}
3-Hexenyl acetate	DB5	1525 ± 83 ^{b}	5381 ± 358 ^{a}	5352 ± 475 ^{a}	4781 ± 348 ^{a}	4390 ± 324 ^{a}
3-Hexenyl acetate	DB1701	1280 ± 74 ^{b}	4668 ± 284 ^{a}	4636 ± 379 ^{a}	4004 ± 354 ^{a}	3717 ± 331 ^{a}
			Thiols			
3-Mercaptohexyl acetate	DB5	1426 ± 395 ^{a}	1858 ± 354 ^{a}	1738 ± 323 ^{a}	1853 ± 347 ^{a}	1752 ± 312 ^{a}
			Phenols			
4-Vinylguaiacol	DB5	544 ± 72 ^{a}	694 ± 154 ^{a}	666 ± 77 ^{a}	671 ± 124 ^{a}	355 ± 118 ^{a}
4-Ethylphenol	DB1701	284 ± 54 ^{b}	374 ± 46 ^{ab}	448 ± 76 ^{ab}	515 ± 62 ^{ab}	564 ± 68 ^{a}
			Other compounds			
1H-Indole	DB1701	1379 ± 355 ^{a}	733 ± 279 ^{a}	902 ± 221 ^{a}	781 ± 272 ^{a}	983 ± 193 ^{a}

* One Way ANOVA, post-hoc Tukey HSD $p < 0.05$ (the higher significant concentrations are emphasized in bold font, while the lowest significant ones are bold and italics). The significantly different values at $p > 0.05$ are indicated in the text with different letters. ** Groups excluded from statistical analysis, n.d. meaning that the substance was not detected under the analytical conditions used.

These compounds, mixed in various proportions in the wine variants, participate in the creation of the wine olfactory profile. Each compound, evaluated separately, can resemble the aroma of certain fruits, flowers, vegetables, spices or of unpleasant flavours. Even though their olfactory produced sensation may change in combination, knowing the aroma produced independently is still of value, as it gives an idea of what to expect when evaluating the wines. The expected sensory impact of the identified volatile compounds is, thus, summarized in Table 4.

Table 4. The main compounds identified in the wine variants and their sensory impact.

* Retention Time	GC Column	Sample Kovats Indices	Database Kovats Indices	Compounds	** Sensory Descriptors
			Aldehydes		
16.68	DB5	965.13	967	5-Methylfurfural	sweet, almond, caramel, spicy
20.67	DB1701	1086.00	1086	Benzaldehyde	bitter almond, burnt sugar, cherry, malt, sweet, roasted pepper
			Alcohols		
15.56	DB1701	958.80-2	960	(E)-3-Hexen-1-ol	grass, green fruit, green leaf, herb, unripe banana
			Esters		
4.78 5.73	DB5 DB1701	612.21 675.97	609 673	Ethyl acetate	aromatic, ethereal, anise, brandy, contact glue, grape, pineapple
7.00	DB5	709.16	709	Ethyl propionate	sweet, ethereal, wine-like, fruity, pineapple
9.93 11.49	DB5 DB1701	797.19 855.82	798 860	Ethyl butyrate	apple, butter, cheese, pineapple, strawberry
9.84	DB1701	815.81	813	Ethyl isobutyrate	citrus, strawberry
13.02 14.89	DB5 DB1701	874.45 942.02	874 941	Isoamyl acetate	apple, banana, pear, glue
32.90 34.62	DB5 DB1701	1389.99 1458.25	1395 -	Ethyl decanoate	brandy, grape, pear, oily
18.29 20.31	DB5 DB1701	1004.90 1075.93	1004 1080	3-Hexenyl acetate	green, vegetable, banana
			Thiols		
27.82	DB5	1249.33	1248	3-Mercaptohexyl acetate	black currant, grapefruit, mango, passion fruit, green, fruity, tropical
			Phenols		
30,37	DB5	1321.12	1319	4-Vinylguaiacol	clove, curry, spice, apple, wine-like, peanut
32.33	DB1701	1394.42	1395	4-Ethylphenol	leather, phenol, spice, stable, medicinal
			Othercompounds		
37.81	DB1701	1545.17	1549	1H-Indole (2,3-benzopyrrole)	burnt, mothball, vegetable, cheese, butter, fatty, chocolate, musty, earthy, fishy, animal, grape, wine-like, floral, honey, jasmine, vanilla

* Average values resulted from three recorded chromatograms (repetitions of the same sample/variant). ** Sensory descriptions taken from AroChemBase and other public databases, such as the FEMA flavour library [45], for each identified compound.

3.3. Differentiation of the Wine Samples Based on Their Aroma Profiles after Two Years in Bottle

Based on the main discriminant volatile compounds identified in our wine samples, DFA statistical analyses were performed to determine if the oenological interventions correlated with the aroma profile of the wines after aging in bottles for two years.

In order to see if the aroma profile of wine variants is distinct enough to separate the variants in specific groups, discriminant factor analysis (DFA) was performed, taking into account the volatile compounds established to have discriminant power.

Figure 1 shows a DFA diagram which attempts to separate all wine variants into groups, while the subsequent two figures show DFA diagrams with wines containing different nutrients separated into groups, irrespective of the yeast used (Figure 2), and

wines containing different yeasts separated into groups, irrespective of the nutrients used (Figure 3), respectively.

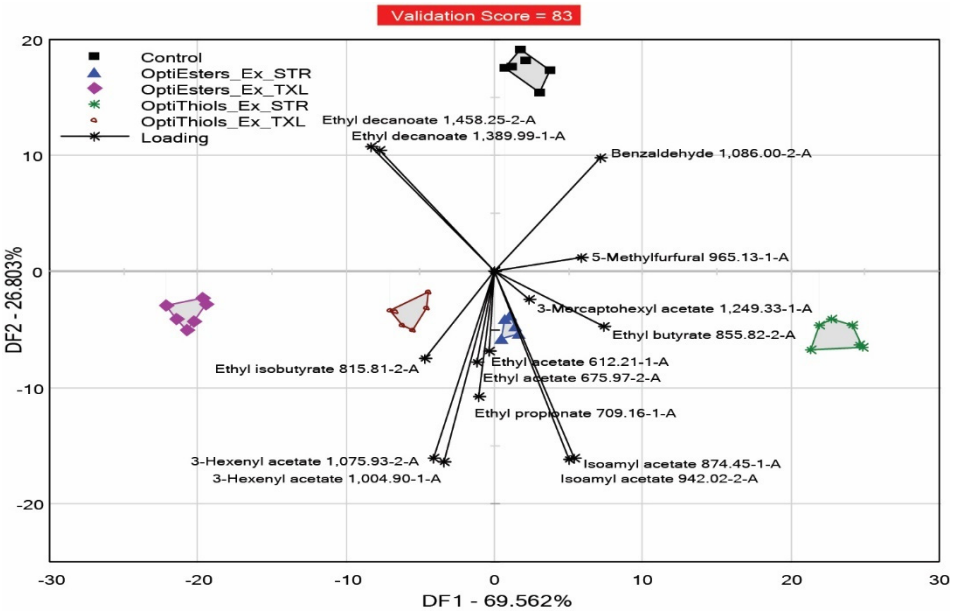

Figure 1. DFA diagram that shows the separation into groups of all wine variants obtained with combinations of two different yeasts (TXL and STR) and two different added nutrients (*Ester* and *Thiol*) in accordance to the volatile compounds determined to have discriminant power.

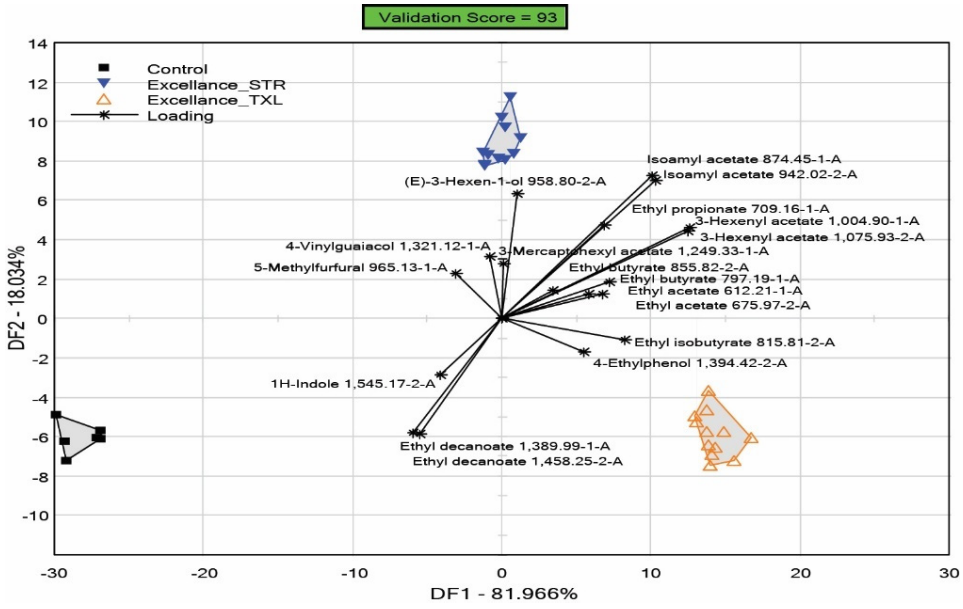

Figure 2. Discriminant factor analysis (DFA) showing the separation into groups of wines containing different yeasts (STR and TXL), irrespective of the nutrient used.

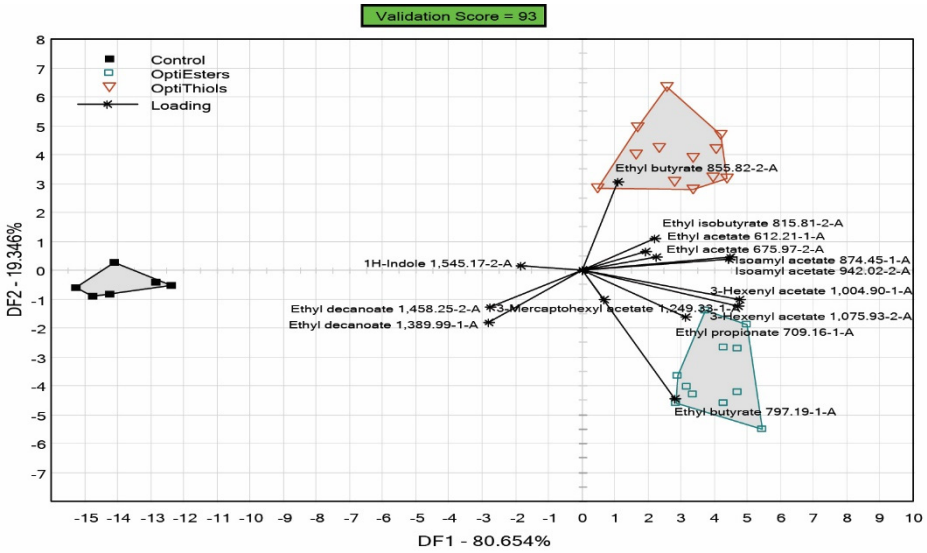

Figure 3. Discriminant factor analysis (DFA) showing the separation into groups of wines containing different nutrients (*Ester* and *Thiol*), irrespective of the yeast used.

3.4. Sensory Analysis of the Wine Samples after Two Years in Bottle

Considering that the chromatographic electronic nose determines aromatic profiles based only on the volatile compounds its columns are able to separate, and that, also, it is not able to take into account compounds with gustatory effect (not present in the headspace of the sample), sensory analysis was also performed in order to have a broader view on the wine samples' quality and their aromatic profile.

Table 5 presents the results for the main sensory parameters, which include the aromatic intensity, the colour intensity, as well as perceived intensities of gustatory parameters such as acidity, sweetness, astringency, bitterness, and extract.

Table 5. The main compounds identified in the wine variants and their sensory impact.

Sensory Parameter	Control	*Ester*-STR	*Ester*-TXL	*Thiol*-STR	*Thiol*-TXL
Acidity	4.17 ± 1.44 ^{a}	5.00 ± 1.50 ^{a}	4.53 ± 1.08 ^{a}	4.33 ± 1.15 ^{a}	4.53 ± 0.90 ^{a}
Sweetness	**3.83 ± 1.01 ^{a}**	0.73 ± 0.92 ^{b}	1.13 ± 0.85 ^{b}	1.10 ± 0.66 ^{b}	1.03 ± 0.68 ^{b}
Astringency	**5.93 ± 0.90 ^{a}**	4.73 ± 0.46 ^{b,c}	3.83 ± 1.04 ^{b,c}	3.17 ± 1.15 ^{c}	2.10 ± 1.15 ^{c}
Bitterness	2.37 ± 3.16 ^{a}	4.83 ± 0.29 ^{a}	3.63 ± 0.15 ^{a}	3.83 ± 1.04 ^{a}	3.17 ± 0.76 ^{a}
Extract	**6.43 ± 0.81 ^{a}**	4.70 ± 0.52 ^{b}	4.97 ± 0.06 ^{a,b}	4.67 ± 0.58 ^{b}	4.60 ± 0.69 ^{b}
Colour intensity	**6.40 ± 0.36 ^{a}**	5.50 ± 0.87 ^{a}	5.07 ± 0.31 ^{a}	4.33 ± 0.58 ^{a}	3.33 ± 1.44 ^{b}
Aroma intensity	5.43 ± 1.89 ^{a}	4.57 ± 1.22 ^{a}	5.23 ± 1.62 ^{a}	5.17 ± 2.31 ^{a}	4.20 ± 0.96 ^{a}

The significantly different values at $p > 0.05$ for One Way ANOVA, post-hoc Tukey HSD are indicated in the text with different letters. The most significant concentrations are emphasized in bold font.

Furthermore, a detailed aroma evaluation was quantified for each sensory descriptor obtained from the evaluation panel, and the relation between these descriptors and the aroma profile of each wine variant was best shown in a PCA analysis (Figure 4).

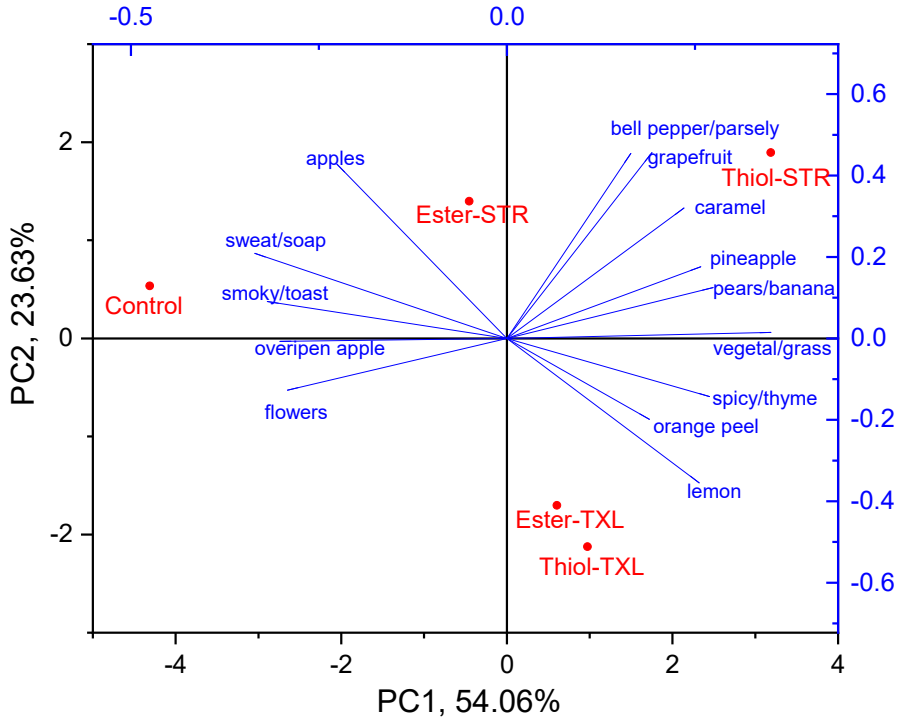

Figure 4. Principal component analysis (PCA) showing the main aromas perceived during sensory analysis, including the components which explain the variability of the wines obtained with combinations of two different yeasts (TXL and STR) and two different added nutrients (*Ester* and *Thiol*).

4. Discussion

4.1. Significance of the Main Physico-Chemical Parameters

As seen in Table 2, the grapes had high sugar content at harvest; therefore, the wines produced by fermentation with the selected yeasts reached concentrations of alcohol over 15% v/v, which is unusual for the Feteasca regala variety. However, this phenomenon is encountered more and more often in conjunction with the climatic changes observed in Bucharest [46].

Due to this high sugar content at harvest (26.5° Brix), the Control wine, produced with the yeasts naturally occurring on the grapes, was not fermented to dryness, as the fermentation stopped at a level of about 36 g/L reducing sugars, the result being a half-dry wine with a lower alcoholic strength of 13.4% v/v. The other variants, produced with selected yeast, finished the fermentations in good conditions and all achieved the potential alcoholic strength, the level of alcohol being between 15.2% and 15.7% v/v and the reducing sugars under 4 g/L, which means the wines are, in accordance with wine regulations, dry.

The other oenological parameters were within the normal ranges imposed for quality stable wines.

4.2. Importance of the Wine Aroma Profile of the Volatile Compounds Identified in the Wines by GC Electronic Nose and the Library AroChemBase

As can be observed in Table 3, among the identified volatile compounds still present in the aroma profile of wines after two years of storage in bottle are several esters, some aldehydes, alcohols, thiols and volatile phenols. The esters form the fermentation aroma of wines and are present also in aged wines [47,48]. Thiols, however, result mainly from the hydrolysis of the cysteinylated and coumaroylated thiolic compounds of grapes [49]. They are very fragile and easily oxidized; therefore, they are found in lower quantities in aged wines, especially when the wines are from varieties with low thiolic aroma precursors, such as Feteasca regala, in which they are not usually detected [29,50].

The volatile phenols are usually considered unpleasant in higher quantities, and this is the reason why selected yeasts which do not release this kind of compounds, as is the case with STR and TXL, are desirable for controlled wine fermentations. Our results showed that these yeasts kept 4-vinylguaiacol and 4-ethylphenol at lower levels (Table 3), as demonstrated by the peak areas of these substances being small (355–671 for 4-vinylguaiacol and 284–564 for 4-ethylphenol). The fact that for the *Thiols*-TXL wine sample, the value of 4-ethylphenol (564 ± 68) seems to be significantly different compared to the values of the other samples is not relevant, as all values are relatively low, which means that the aroma profile is clean, without the negative influences of excessive volatile phenolic compounds.

Similarly, the concentration of 3-hexenol, which brings aromas of grass, green fruit, green leaf, or herbs, is relatively low, being significantly higher only in the sample *Thiol*-STR (peak area 737 ± 86 mm^2). This is not high enough to induce a more specific green aroma in this sample than in the others. Additionally, a related aroma compound, 3-hexenyl acetate, which brings aromas of greens and vegetables, has, on both chromatographic columns, values that do not differ significantly among the samples, with the exception of the Control wine sample, where on both columns this compound was found to be lowest (1525 ± 83 on DB5 and 1280 ± 74 on DB1701). This means that the selected yeasts produce a higher concentration of this compound as compared to the yeasts naturally found on grapes. The selected yeasts produced higher concentrations of 3-hexenyl acetate (peak areas ranging from 3717 to 5381), adding some pleasant green/vegetable aromas to the overall profile of the wine samples produced with STR and TXL yeasts.

Among the esters, higher values are recorded for ethyl butyrate (peak area ranging from 218,342 to 255,072 on column DB1701) and ethyl acetate (peak area from 150,794 to 171,157 on column DB5 and from 112,187 to 129,553 on DB1701), but these compounds' concentrations are not significantly different among the samples. Only for ethyl butyrate on column DB5 (range 6510 to 8605) do we observe a tendency for the samples prepared with the nutrient specific for the generation of esteric aromas to have significantly higher concentrations (peak area 8602 ± 406 for *Ester*-STR and 8605 ± 298 for *Ester*-TXL). This ethyl butyrate specifically induces some fruity aromas into wines (apple, pineapple, strawberry) as well as some aromas reminiscent of butter and cheese, all of which are present in many white wines [51]. The fact that the nutrient OptiEster increases this compound in the final aroma profile is a positive aspect. Ethyl acetate is also a compound which is present in wines in significant amounts and tends to increase during wine aging; thus, it is no surprise to find it in all the samples. As long as this ester does not increase over a certain level, its effect is beneficial for the overall profile, inducing the aromas of grape, pineapple, anise, brandy. At higher concentrations, ethyl acetate becomes unpleasant, with its ethereal and glue notes. This compound correlates with the volatile acidity, being present in higher concentrations when the acetic acid is also high, which is not the case in our wines. In our samples, the volatile acidity is low (0.49 g/L acetic acid (Table 2), as compared to the maximum of 1.18 g/L allowed by legislation); thus, the concentration of acetic acid or ethyl acetate is of no concern, even after 2 years of storage.

The next ester quantitatively important in our Feteasca regala samples is isoamyl acetate. Present in many white wines with fruity aroma, this ester is very appreciated for its banana, pear and apple flavour. As is the case with other compounds, in excessive quantities this ester too can become unpleasant, inducing a glue-like aroma or creating the sensation that the wine has an atypical fruity aroma [52]. It can also cover the varietal aroma [53]. The yeasts and nutrients used in this study created a very balanced aroma of isoamyl acetate (area peaks between 29,256 and 70,907 on DB1701 and 40,545 to 92,844 on DB5), with the wine produced with the OptiThiol nutrient and STR yeast having the highest concentrations recorded on both chromatographic columns (70,907 ± 4138 on DB1701 and 92,844 ± 5028 on DB5). It is important to note that while the selected yeasts released good concentrations of isoamyl acetate, contributing to the quality aroma profile of the resulting wines, the yeasts which realized the spontaneous fermentation in the Control released

significantly lower quantities, only about 50% as compared to the concentrations of this compound in the wines produced with the selected yeasts.

Another quantitatively important ester, mostly synthesized by the yeasts during fermentation, is ethyl decanoate (peak area from 26,679 to 37,371 on DB5 and from 18,826 to 26,960 on DB1701), which releases into wines nice aromas of grape, pear and brandy. When its concentration becomes excessive, soapy and oily notes [54] can also be perceived. The concentrations which resulted in our samples are moderate, with significantly lower concentrations in the wines fermented with the yeast STR and the highest concentrations in the Control wines, produced without selected yeasts. This means that the selected yeast can contribute to a better-balanced aroma profile than the yeast naturally present on the grapes and in the must.

Ethyl isobutyrate was found on a single column, DB1701, with medium-low concentrations (peak areas from 1692 to 2806), with no significant difference among the samples. It brings a fruity aroma of citrus and strawberry which blends well in the overall fruity aroma of Feteasca regala wines.

The last ester identified is ethyl propionate, with its wine-like aroma and other sweet and fruity notes. The concentrations are rather low (peak area from 905 to 1536) and are only measured on the DB5 column, which means its influence is rather light and the induced aroma is delicate. Even so, the Control sample has significantly lower ethyl propionate concentrations, while the wines produced with the nutrient OptiEster have the highest levels, meaning that the addition of amino acids in the wines indeed enhances some of the esteric wine aroma.

The thiolic aroma, which is also sought in some wines by adding nutrients and yeasts which may increase the free thiols in the wine, is mostly dependent on the precursors present in the grape variety [55]. Additionally, these compounds are fragile; they are easily degraded during the storage of wine in bottles and gradually disappear [22]. Furthermore, the columns with which the Heracles e-nose is equipped are not very sensitive to the thiolic compounds, and if they are in low quantities, they are not separated and detected.

Among the thiolic compounds, only 3-mercaptohexyl acetate was detected in our case. It is a compound found in Sauvignon blanc [56] and other varieties famous for their thiolic aroma [57,58], but it is also present in low quantities in other grape varieties. It brings specific aromas of black currant, grapefruit and tropical fruits, such as mango, passion fruit, along with some green notes. It is a very elegant aroma which enhances the olfactory profile of wine. The values in our wines were moderate (peak area ranging from 1426 to 1858). At this stage of wine evolution, the concentration of this thiol showed no significant difference among the wine samples, irrespective of the treatment with nutrients such as OptiThiol, meant to stimulate an increase in the overall thiolic compounds. It is likely that the thiols produced due to the presence of the compounds with -SH group brought into the must by the nutrient OptiThiol were lost during aging in bottles. Although not significantly different, the value in the Control sample is the lowest (peak area of 1426 as compared to an average of 1800), indicating that this thiol is produced when nutrients and selected yeasts are properly used.

Another compound determined by chromatography in our Feteasca regala wines was indole, a compound of tryptophan degradation produced by yeasts during wine fermentation when the nitrogen concentration is low. At low concentrations, indole brings some complexity to wine aromas, with notes of jasmine, honey, vanilla, chocolate. At higher concentrations, it becomes unpleasant, with musty, earthy, fishy, animal aromas, and even a faecal odour [59]. In our wines fermented with nutrients and selected yeasts, the concentrations of indole were low (peak areas from 733 to 983), while in Control, where nutrients were not added, the concentration was a little higher (peak area 1379) but did not reach the level of significance.

4.3. Possibilities of Differentiation of the Wine Samples Based on Their Aroma Profiles after Two Years in Bottle

Discriminant factor analysis was performed to determine the possible influences of both the nutrients and yeasts used in combination for the controlled fermentations (Figures 1–3).

In Figure 1, all the variants are displayed in a DFA diagram in an attempt to discriminate the volatile profiles induced by all the combinations of nutrients and yeasts. All wine variants are discriminated, but the low validation score shows that the separation is not reliable enough, meaning that some yeast-nutrient combinations do not induce sufficiently different aroma profiles. The bi-plot shows some of the most influential substances for the discrimination of each type of samples. For example, for the Control wines, the most important factors appear to be higher ethyl decanoate and benzaldehyde coupled with lower 3-hexenyl acetate and isoamyl acetate; for the TXL groups it is the ethyl decanoate, ethyl isobutyrate and 3 hexenyl-acetate in higher concentrations coupled with lower contents of benzaldehyde and ethyl butyrate. For the STR groups, the most important factors are, vice versa, higher values of benzaldehyde and ethyl butyrate and lower concentrations of ethyl decanoate and ethyl isobutyrate.

To confirm that the electronic nose can clearly separate the volatile profiles of the wines produced with different yeasts, a DFA analysis (Figure 2) was performed only for three groups: Control (indigenous yeasts), the STR group (including wine variants *Ester*-STR and *Thiol*-STR) and the TXL group (including wine variants *Ester*-TXL and *Thiol*-TXL). This time, the validation score was sufficiently high to account for a good separation of the samples in the DFA diagram. It can be observed that the presence of relatively high contents of ethyl decanoate and indole, coupled with the relative scarcity of 3-hexenyl acetate and isoamyl acetate, are most discriminating for the Control wine group, while more complex combinations of volatile compounds are responsible for the discrimination of the STR and TXL groups.

In order to determine the influence of the yeast nutrients in the development and preservation of the aroma profile of the wine variants, Figure 3 shows the DFA analysis for which the following groups were used: Control (no added nutrients), the *Ester* group (including wine variants *Ester*-STR and *Ester*-TXL) and the *Thiol* group (including wine variants *Thiol*-STR and *Thiol*-TXL). Again, the Control wine group clearly differentiated itself by the increased contents of ethyl decanoate and indole and by lower concentrations of other odorant compounds, while the discrimination of nutrient groups was possible due to more complex and intricate influences of combinations of volatile compounds. In this plot, too, the validation index confirms that the discrimination is reliable. However, no compounds were identified as being specific for one or the other groups of variants, most being part of both DF1 and DF2 discriminant factors, which means that all the volatile compounds participate in the discrimination, but in different degrees for each wine.

Due to these results, it was concluded that the electronic nose is powerful enough and able to sense minute differences between the complex volatile compound combinations in each wine variant. However, the electronic nose only discriminates the samples, but cannot always manage to fully describe the aromatic profiles. For this reason, sensory analysis is still needed to describe the aroma perceived in each wine.

4.4. Possibilities of Differentiation of the Wine Samples Based on Sensory Analysis after Two Years in Bottle

The sensory analysis performed was able to show that the main influences of the fermentation yeasts and yeast nutrients were on the profiles of the volatile compounds and not so much on the other gustatory or visual parameters (Table 5).

As all samples are based on the same grape variety, the taste perception of acidity and bitterness of the experimental variants was similar (no significant difference). The taste of the Control sample was perceived as being different regarding the sweetness, astringency and extract, which had significantly higher values (on intensity scales from 0 to 10) compared to all other variants produced with technological interventions. Additionally,

the color intensity perception was higher for the Control sample. These higher intensity values for the Control samples do not show better quality in any way; on the contrary, a higher color intensity is correlated with a higher level of oxidation, while a higher astringency is a trait that is not desirable in white wines. The higher values of sweetness and extract are correlated with the residual sugar still present in the wine because the indigenous yeasts were not able to complete the fermentation (Table 2).

Olfactorily, the aroma intensity was not perceived as being discernably different from one variant to another, but the quality of the aroma was clearly different (Figure 4). The aroma descriptors provided by the panelists during the sensory analysis included aromas of flowers, orange peel, fruits (apple, lemon, grapefruit, pear, banana, pineapple), spices (thyme), vegetal aroma (bell pepper, parsley, grass), caramel, smoky, toast and even some undesirable flavors such as overripened apple, sweat or soap.

The sensory analysis also confirmed that the Control sample had lower quality, with an aroma profile associated with many unpleasant flavors (overripened apple, sweat, soap, smoky). The samples of the variant *Thiol*-STR are well-separated from the others in the PCA diagram (Figure 4) and are associated with the most pleasant aromas of tropical fruits, grapefruit and bell pepper, all being specific descriptors for thiolic aromas. It is true that the GC e-nose was not able to identify many thiolic compounds, most likely due to technical limitations, but the sensory analysis showed that this sample, *Thiol*-STR, produced in the presence of a thiol enhancing nutrient, actually demonstrated a thiolic profile. The other variant containing the *Thiol* nutrient *Thiol*-TXL was placed rather unexpectedly in a different part of the diagram, very close to the *Ester*-TXL sample, all of which displayed an elegant spicy and citrus fruit aromatic profile. The less-expressed thiolic character of the *Thiol*-TXL sample—unexpected because the use of the TXL yeast and the addition of the Thiol nutrient should have led to the contrary result—can be explained by a possible degradation or combination during storage of the thiolic compounds. There is a very fine redox balance in the white wines, especially when they contain glutathione (in our case from the OptiThiol nutrient), which enhances aroma in a reductive medium, but can act as an oxidant in higher concentrations [29]. Finally, the relative similarity and closeness in the sensory PCA space of samples *Ester*-STR and *Thiol*-STR on one hand and samples *Ester*-TXL and *Thiol*-TXL on the other hand point out that the wine aroma is more strongly modulated by the yeast, and to a lesser extent, by the added nutrients.

5. Conclusions

This study showed that both yeasts and their nutrients exert complex influences on the volatile compound profiles of the produced wines, and that these technological interventions can enhance certain types of aromas, helping to produce wines in accordance to specific consumer preferences. Even from the same variety of grapes harvested in the same vineyard, wines with quite different aromatic profiles can be produced.

The GC electronic nose can elucidate the main compounds making up the aroma profile and can also discriminate between wine samples produced with different interventions, even when their aromatic profile consists of combinations of the same volatile substances in different quantities.

Because the main influences of the technological interventions were at the olfactory level, it can be concluded that the use of the electronic nose for this type of evaluation is very appropriate.

Specifically, for the present research, we have found that after two years of storage in bottles, this evaluation methodology was able to differentiate and classify the wine variants produced with the intention of modulating the aroma profile by using specific nutrients and yeasts.

Compared with the variants with selected yeasts, the Control wine, produced by the classical technology with spontaneous fermentation, displayed:

- The highest concentrations of isoamyl acetate, a compound which in excess brings soapy and oily notes;

- The highest concentration of indole, which may contribute some earthy, fishy, or animal aromas;
- The lowest ethyl propionate concentrations, which could have enhanced wine-like aromas and other sweet and fruity notes;
- The lowest concentration of thiols (such as 3-mercaptohexyl acetate).

All these show that the selected yeast can contribute to a better-balanced aroma profile compared to the yeast naturally present on the grapes and in the must. This also shows that the fragile thiolic aroma is produced when nutrients and selected yeasts are properly used.

Both selected yeasts, STR and TXL, produced:

- Higher concentrations of 3-hexenyl acetate, adding some pleasant green/vegetable aromas to the overall profile of the wine samples;
- A very balanced aroma of isoamyl acetate, as, in the right concentrations, this compound is very appreciated for its banana, pear and apple flavour.

The nutrients had a lower overall impact, but we observed that the variants produced with the nutrient *OptiEster* contained:

- Increased ethyl butyrate concentrations, which specifically induce some fruity aromas into wines, as well as some aromas reminiscent of butter and cheese;
- The highest levels of ethyl propionate, enhancing the wine-like aroma and other sweet and fruity notes.

Even after two years of storage, all samples displayed in their aromatic profile:

- Low levels of undesirable volatile compounds, such as:
    - 4-vinylguaiacol and 4-ethylphenol, which are considered a major source for off-flavors in wines;
    - 3-hexenol, which may increase the perception of grass and green leaf aromas;
    - Ethyl acetate, which normally tends to increase during wine aging and induces ethereal and glue notes.

For the discrimination of the wine samples, we observed that:

- For the Control wines, the most important factors appear to be higher ethyl decanoate and benzaldehyde coupled with lower 3-hexenyl acetate and isoamyl acetate;
- For the wines produced with TXL yeast, the most important factors include the presence of ethyl decanoate, ethyl isobutyrate and 3 hexenyl-acetate in higher concentrations, coupled with lower concentrations of benzaldehyde and ethyl butyrate;
- For the wines produced with STR yeast, the most important factors include higher values of benzaldehyde and ethyl butyrate and lower concentrations of ethyl decanoate and ethyl isobutyrate;
- For the wines produced with specific nutrients, even though the electronic nose is able to separate them on the overall volatile profile, no compounds were identified as being specific for one or the other groups of variants.

Correlated to the results of sensory analysis, it could be concluded that the wine aroma is more strongly modulated by the yeast and to a lesser extent by the added nutrients.

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