fermentation

MDPI

Article

Application of Autochthonous Yeast *Saccharomyces cerevisiae* XG3 in Treixadura Wines from D.O. Ribeiro (NW Spain): Effect on Wine Aroma

Pilar Blanco [1,*], María Vázquez-Alén [2], Teresa Garde-Cerdán [3] and Mar Vilanova [4]

[1] Estación de Viticultura e Enoloxía de Galicia (EVEGA-AGACAL), Ponte San Clodio s/n, 32428 Leiro-Ourense, Spain
[2] Fundación Juana de Vega, Rúa Salvador Allende, 92, San Pedro de Nós, A Coruña, 15176 Oleiros, Spain; mariavazquezalen@hotmail.com
[3] Instituto de Ciencias de la Vid y del Vino (CSIC, Universidad de La Rioja, Gobierno de La Rioja). Ctra. de Burgos, Km. 6, 26007 Logroño, Spain; teresa.garde@icvv.es
[4] Misión Biológica de Galicia (MGB-CSIC), El Palacio, Salcedo, 36143 Pontevedra, Spain; mvilanova@mbg.csic.es
* Correspondence: pilar.blanco.camba@xunta.gal; Tel.: +34-988788091

Abstract: Yeast plays an essential role in winemaking. *Saccharomyces cerevisiae* strains involved in fermentation determine the chemical and sensory characteristics of wines. *S. cerevisiae* XG3, isolated in Galicia (NW Spain), has desirable oenological potential, which has been proved at a pilot scale to produce quality wines. This study applies XG3 as active dry yeast at an industrial scale for Treixadura wine elaboration, and compares it with commercial yeast and spontaneous fermentation within three wineries included in Denomination of Origin Ribeiro over two vintages. Fermentations are monitored using conventional methods, and microbiological implantation controls are carried out by mtDNA-RFLPs analysis. Wine basic chemical parameters are determined using OIV official methodology, and volatile aroma compounds are determined by GC-MS. Finally, wine sensory analysis is also performed. *S. cerevisiae* XG3 shows an acceptable implantation ability—as compared to commercial control strains. The wines from XG3 have a higher total acidity and lower alcohol content. Their volatile composition differs from control wines, since XG3 produces significantly higher concentrations of acetates, volatile acids, esters and volatile phenols, depending on the vintage and winery. However, lower differences are perceived at the sensory level, where fruity and floral descriptors are perceived by the panellists in XG3 wines. Therefore, XG3 constitutes an alternative to differentiate Treixadura wines.

Keywords: *Saccharomyces cerevisiae*; XG3; fermentation kinetics; yeast implantation; Treixadura; wine; aroma composition; sensory profile

Citation: Blanco, P.; Vázquez-Alén, M.; Garde-Cerdán, T.; Vilanova, M. Application of Autochthonous Yeast *Saccharomyces cerevisiae* XG3 in Treixadura Wines from D.O. Ribeiro (NW Spain): Effect on Wine Aroma. *Fermentation* **2021**, 7, 31. https://doi.org/10.3390/fermentation7010031

Academic Editor: Ronnie Willaert

Received: 29 January 2021
Accepted: 20 February 2021
Published: 25 February 2021

1. Introduction

Wine quality/characteristics are determined by numerous factors, including grape variety and technological practices applied in the winery. The handling of microorganisms involved in fermentation is a key factor in producing wine of acceptable quality for the current demanding market. Wine yeasts are responsible for alcoholic fermentation, a complex process by which they not only transform sugar into ethanol and carbon dioxide, but also into a range of minor secondary metabolites, including higher alcohols, fatty acids, esters, carbonyl compounds, volatile phenols, sulphur-containing compounds and thiols [1]. The range and quantity of compounds produced, which is yeast species and strain-dependent, shape the chemical and sensory profile of wine [2–4]. The succession of yeast populations during spontaneous fermentation is well documented. Thus, several yeast species of *Hanseniaspora*, *Candida*, *Pichia* and *Metschnikowia*, among other genera,

initiate fermentation, and they are present at early stages; however, as fermentation progresses, these non-*Saccharomyces* species are sequentially replaced by *Saccharomyces* which is a good fermenter and more alcohol tolerant [5,6]. For a long time, the competitiveness of *Saccharomyces cerevisiae* during winemaking was attributed to its high fermentative power and ability to withstand the harsh environmental conditions throughout fermentation. However, recent studies have evidenced other defensive strategies of *S. cerevisiae*, such as cell-to-cell contact and secretion of antimicrobial peptides to surpass other microbial species [7].

Bearing in mind the important role of yeast species/strains during fermentation, most wineries employ commercial starters to ensure fermentation control and wine quality. In this sense, the market offers a wide range of selected yeast strains, as active dry yeasts (ADY), with specific properties adapted to different wine styles. However, these strains are not always able to control fermentations; moreover, their use could lead to the standardisation of wines. The use of autochthonous yeasts is an interesting alternative to make unique wines, highlighting the so valued regional character of certain wines, because they are believed to be better adapted to the must's properties and to the environmental conditions [8–10].

In this context, the Estación de Viticultura e Enoloxía de Galicia (Evega-Agacal) has available a collection of oenological yeasts that have been obtained from musts and spontaneous fermentations around Galicia. The yeasts were identified at species and strain level and the oenological potential of several *S. cerevisiae* strains was evaluated at laboratory and pilot scale. The results showed that the autochthonous strain *S. cerevisiae* XG3 stood out for its fermentative vigour and its positive influence on the chemical and sensory characteristics of wines elaborated using the main white cultivars grown in Galicia [10,11].

Treixadura is one of the main traditional white cultivars grown in Galicia (NW Spain), particularly in Denomination of Origin Ribeiro (DO Ribeiro) [12]. This cultivar has been characterised by fruity and floral descriptors in basis to high concentration of ethyl esters and volatile acids [13,14].

Therefore, this work aims to evaluate the potential of *S. cerevisiae* XG3, an autochthonous yeast strain isolated at Estación de Viticultura e Enoloxía de Galicia (Evega-Agacal) for the production of Treixadura wine at an industrial scale. *S. cerevisiae* XG3 was tested and produced as active dry yeast by Lallemand. Then, it was applied during two consecutive years to ferment must from Treixadura in three industrial wineries from DO Ribeiro and compared to spontaneous fermentations or commercial yeast strains commonly used in this area. Fermentation ability, yeast implantation and chemical and sensory characteristics of the resulting wines were evaluated. To the best of our knowledge, this is the first study undertaken at an industrial scale that uses an indigenous yeast strain, as active dry yeasts, in Galicia.

2. Materials and Methods

2.1. Grapevine Cultivars and Yeast Strains

Fermentations were carried out during two consecutive years using must from *Vitis vinifera* L. 'Treixadura' from Galicia (NW Spain). *Saccharomyces cerevisiae* XG3 and other *S. cerevisiae* strains (named LSA1 to LSA5), available in the market, were used as inocula. Spontaneous fermentation (Esp) was also carried out. The genetic profile of these strains was established by mtDNA-RFLPs, according to Querol et al. [15]. *S. cerevisiae* XG3 is a yeast strain that has been isolated and selected in Evega. Preliminary studies at a pilot scale had confirmed its desirable fermentation ability and its positive impact on wine quality [10,11,16]. This strain was produced as active dry yeast at Lallemand facilities (Toulouse, France). Preliminary studies carried out in Evega confirmed that XG3 retained its oenological properties under this format (data not shown).

Genetic profiles (mtDNA-RFLPs) of *S. cerevisiae* strains used in the study (XG3 and LSA) are shown in Figure 1.

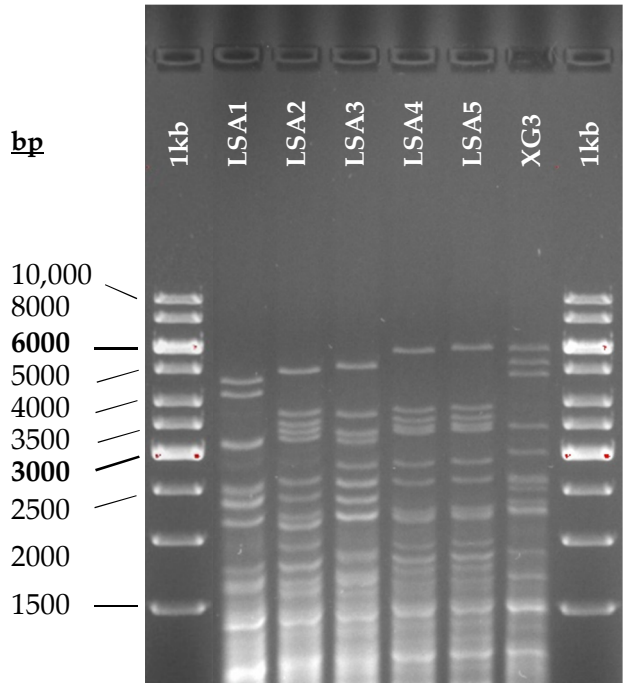

Figure 1. Genetic profile (mtDNA-RFLPs) of *Saccharomyces cerevisiae* strains used as inocula in this study. kb—1 kb DNA ladder molecular weight marker (Thermo Fisher Scientific, Madrid, Spain).

2.2. Fermentations Trials

Fermentations were carried out in three different wineries within DO Ribeiro: Priorato de Razamonde (PR), O'Ventosela (VT) and Gandalera (GD) during 2019 and 2020 vintages. The volume and yeast strain used in each winery are summarised in Table 1.

Table 1. Fermentation trials: Volume and yeast strains used to inoculate Treixadura musts in each winery and vintage.

Winery (Code)	2019		2020	
	XG3	LSA	XG3	LSA
Priorato de Razamonde (PR)	1000 L	5000 L (LSA1)	1000 L	10,000 L (LSA2)
O'Ventosela (VT)	5000 L	10,000 L (Esp)	10,000 L	40,000 L (LSA3)
Gandarela (GD)	4000 L	5000 L (LSA4)	2800 L	5000 L (LSA5)

Grapes from 'Treixadura' were manually harvested and transported to the winery facilities. At the winery, the grapes were de-stemmed, crushed and pressed in a pneumatic press to extract the juice. During grape processing, 50 mg/L of SO_2 were added to prevent oxidation and for microbiological control. In addition, pectinolytic enzymes were added to enhance clarification of musts and to facilitate settling. After 48 h of cold settling, the clean must was homogeneously distributed into two stainless steel tanks.

In each winery, two tanks were inoculated: One of them with *S. cerevisiae* XG3 and the other with a commercial yeast strain, as indicated in Table 1. Yeast inocula were added as recommended by the manufacturer. In particular, a dose of 50 g/hL of XG3 was rehydrated in a 10× volume of clean, chlorine-free water at 35–40 °C and gently stirred until a homogenous suspension was achieved. After 20 min, it was gradually mixed with a little juice to adjust the yeast suspension to within 5–10 °C of the must temperature. Finally, the suspension was inoculated into the tanks at a temperature no lower than 14 °C and fermentations were carried out at 16–18 °C. Density and temperature were measured to follow the evolution of alcoholic fermentation. In addition, samples for microbiological control were taken from the clarified must and at the initial (Fi), tumultuous (Ft) and final

(Ff) stages of fermentation. When the fermentations ended, the wines were racked to a new tank and sulphited (25 mg/L of free SO_2). A few samples were bottled and stored until further chemical and sensory analysis.

2.3. Microbiological Control

Must samples and those taken at different fermentation stages were used for yeast count and to monitor the implantation ability of the strains used in each winery. The samples were serially diluted in 2% *w*/*v* buffered peptone water and spread on WL Nutrient Agar medium (Scharlau Microbiology, Barcelona, Spain) [17]. Plates were incubated at 28 °C until visible colonies appeared, and those containing between 20 and 200 colonies were used to quantify the total viable cells. Then, a representative number of colonies (20–25 for each sample) were selected randomly and isolated on YPD for further characterisation. *Saccharomyces* and non-*Saccharomyces* yeasts were distinguished by growth on Lysine medium (Oxoid, Thermo Fisher Scientific, Madrid, Spain), since the former cannot grow on this medium.

S. cerevisiae isolates were characterised at the strain level by analysis of mitochondrial DNA restriction profiles (mtDNA-RFLPs). Total yeast DNA was obtained as described by Querol et al. [15], and digested with the restriction endonuclease Fast digest *Hinf*I (Thermo Fisher Scientific, Madrid, Spain). The restriction fragments were separated by gel electrophoresis on a 0.8% (*w*/*v*) agarose gel in 1X TBE containing Red Safe™ nucleic acid staining solution. DNA pattern bands were visualised under UV light and documented using a Molecular Imager® Gel Doc™ XR+ imaging system (BIO-RAD, Madrid, Spain).

2.4. Musts and Wines Chemical Analysis

Must parameters, including °Brix, sugar content, total acidity, pH, malic acid and tartaric acid, were determined using the official methodology [18]. In addition, α-amino nitrogen and ammonia, were quantified by enzymatic reaction followed by absorbance determination at 340 nm using a multiparameter analyser LISA 200 (TDI, Barcelona, Spain). Treixadura musts characteristics are summarised in Table 2.

Table 2. Characteristics of Treixadura musts from the different wineries used in this study.

Parameter	2019			2020		
	PR	**VT**	**GD**	**PR**	**VT**	**GD**
°Brix	21.5	21.2	23.2	21.4	22.6	19.6
Sugars (g/L)	209.0	205.5	228.5	207.8	221.6	187.4
Total acidity (g tartaric acid/L)	5.7	5.0	4.7	5.6	4.8	5.9
pH	3.51	3.69	–	3.56	–	3.57
Malic acid (g/L)	3.1	3.2	2.5	3.3	2.8	3.6
Tartaric acid (g/L)	3.3	–	4.2	3.4	3.5	4.2
α-Amino nitrogen (mg/L)	152.2	150.8	134.8	152.7	133.4	147.1
Ammonia (mg/L)	92.3	83.70	123.1	88.2	67.2	120.5
Total sulphur dioxide (mg/L)	70	45	99	76	50	92
Yeast population (log CFU/mL) *	4.93	4.66	4.93	3.14	4.97	4.84

* Total yeast count in must samples taken after must clarification, before the addition of yeast inoculum.

Basic parameters of wines (alcohol content, reducing sugars, pH, titratable and volatile acidities, tartaric, malic and lactic acids) were determined by Fourier transform infrared spectrometry (FTIR) using a Wine Scan FT120 analyser (FOSS Electric, Barcelona, Spain) calibrated according to OIV [18]. Free and total sulphur dioxides were also quantified using the OIV methods [18].

2.5. Identification and Quantification of Wine Volatile Compounds

In a 10 mL culture tube (Pyrex, ref. 1636/26MP), 8 mL of sample, clarified by centrifugation if necessary, 2.46 µg of internal standard (4-nonanol, Merck ref. 818773), and a magnetic stir bar were added. Extraction was done by stirring samples with 400 µL of dichloromethane (Merck, ref. 106054), at room temperature for 15 min, using a magnetic stirrer as described by Coelho et al. [19]. The tubes were placed vertically, and agitation was regulated to maintain dispersion of solvent micro-drops without reaching the sample surface. After cooling at 0 °C for 10 min, the magnetic stir bar was removed, and the organic phase was detached by centrifugation (5118× *g*, 5 min, 4 °C). Using a glass Pasteur pipette, the extract was recovered into a vial, dried with anhydrous sodium sulphate (Merck, ref. 1.06649), and transferred to a new vial for storage at −20 °C until analysis. Volatile compounds were extracted from each of the wines in triplicate

Gas chromatographic analysis of volatile compounds was performed using an Agilent GC 6890 N chromatograph, CA, USA, coupled to mass spectrometer Agilent 5975C. A 1 µL injection was made into a capillary column, coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 µm film thickness, Chrompack). The temperature of the injector was programmed from 20 °C to 250 °C, at 180 °C /min. The oven temperature was held at 40 °C for 5 min, then it was programmed to rise from 40 °C to 250 °C, at 3 °C/min, then it was held for 20 min at 250 °C, and finally, it was programmed to go from 250 °C to 255 °C at 1 °C/min. The carrier gas was helium N60 (Air Liquide) at 103 kPa, which corresponds to a linear speed of 180 cm/s at 150 °C. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 to 360 m/z, and an acquisition rate of 610 ms.

The compounds were identified using WSearch Free Software, by comparing mass spectra and retention indices with those of pure standard compounds. Pure standard compounds were purchased from Sigma-Aldrich (Darmstadt, Germany) with purity higher than 98%. Semi-quantitative data were obtained by calculating the relative peak area in relation to the internal standard (4-nonanol).

2.6. Sensory Evaluation

Treixadura wines were submitted to aroma sensory analysis by eleven trained panellists from Galicia (Spain). All the judges (5 males and 6 females aged between 33 and 60 years old) were experienced wine tasters, and all of them have previously taken part in similar studies. The sensory analysis was performed in the professional-standard room of Evega in agreement with the ISO Norm 8589 [20]. The evaluation was carried out using the QDA method [21]. A descriptive score card, including five aroma descriptors (intensity, nose quality, floral, fruity and herbaceous), was used. A constant sample volume of 30 mL of each wine was evaluated in wine-taster glasses [22] at 12 °C. During the analysis, the judges smelled and tasted the samples, and the perceived descriptors were indicated. Then, they scored the intensity of each attribute using a 10-point scale, where 10 indicated a very high intensity. The relative frequency (F), relative intensity (I) and geometric mean (GM) of the different descriptors were calculated for each wine. GM was calculated as the square root of the product between I and F, where I corresponds to the sum of the intensities given by the panel for a descriptor, divided by the maximum possible intensity for this descriptor; and F is the number of times that the descriptor was mentioned divided by the maximum number of times that it could be mentioned.

2.7. Statistical Analysis

All data were analysed using the software XLStat-Pro (Addinsoft, Paris, France, 2011). An analysis of variance was used to assess the effects of yeast (XG3 vs LSA/Esp) on wine volatile composition by winery and vintage. Differences among the intensity of aroma descriptors by winery were calculated separately for each season according to the least significant difference from Tukey´s test with a confidence interval of 95% ($p < 0.05$). Principal Component Analysis (PCA) was applied to the wines' composition to study the possible global grouping of wines based on volatile composition.

3. Results and Discussion

3.1. Fermentations

3.1.1. Fermentation Kinetics

Fermentation performance is a key trait for oenological yeast starters. In this study, the results evidenced that the fermentative activity of *S. cerevisiae* XG3 was similar to the commercial strains used, although the required time to complete fermentation was vintage and winery dependent. For instance, fermentations in Priorato de Razamonde in 2019 took about 16 days to end (Figure S1a); however, in 2020, both fermentations had finished after 12 days (Figure S1b). XG3 showed a slightly lower fermentative speed in 2019 and also at the beginning of 2020, probably due to the lower temperature until day 6. The later observation indicated that the differences among fermentations could be due to technical management at the wineries rather than to the fermentative power of the yeasts under evaluation. Nevertheless, in previous trials carried at microvinification level and pilot scale in Evega using fresh biomass, XG3 had shown a lower fermentative speed than LSA for Albariño fermentations, but not with Godello [11]. Accordingly, preliminary studies, including XG3, as ADY and as fresh cells, confirmed their similar fermentative efficiency; however, they showed a lower fermentation speed when compared to a commercial ADY (data not shown).

3.1.2. Yeast Population Dynamic during Fermentation

Yeast population in Treixadura musts was similar in all samples, ranging from 4.66 to 4.97 log CFU/mL (Table 2). These data agree with the values usually reported for musts in the literature [5], and particularly for Treixadura [23,24]. Must from PR in 2020 was an exception; it only contained 3.14 log CFU/mL, probably due to strong clarification. After yeast inoculation, the number of viable cells increased until the fermentation started and then followed different patterns depending on the winery (Figure 2). Thus, in Priorato de Razamonde (PR), the number of yeasts reached very high values at the beginning of fermentation (7.80 log CFU/mL) and remained at high density until the end of the process (Figure 2a). However, in O'Ventosela (VT), the number of yeasts when fermentations started (Fi) was lower (from 6.59 to 7.08 log CFU/mL) than in PR, but it increased until the tumultuous stage (Ft) and remained at high density until the end, especially in 2020 (8.40 log CFU/mL) (Figure 2b). Finally, in Gandarela (GD), yeast counts achieved high values at the initial stages of fermentation, as in PR; then, they slightly increased until Ft, but the number of viable yeasts decreased at the end of fermentations (Figure 2c). In general, the yeast population reached higher levels in 2020 than in 2019 for all strains and wineries, indicating that the addition of a yeast protector during the rehydration process benefited the yeast viability during fermentation.

The evolution of yeast population at a quantitative level during fermentation follows the typical pattern of microorganism's growth in a batch culture, which includes a lag phase, exponential phase, stationary phase and death phase [5]. Particularly in winemaking, differences in fermentation kinetics can be due to lag phase, growth rate and the number of cells reached, and duration of the decline phase. The growth curves observed in Gandarela followed the pattern described above and in other studies [8,24], whereas in PR and Ventosela a decline phase was not observed. The diminution of viable yeast at the end of fermentation has been attributed to the harsh environmental conditions and/or the presence of toxic metabolites [7]. The fact of maintaining high population levels at the end of fermentation indicated a good resilience of the strains to the winery and wine characteristics.

3.1.3. Yeast Implantation

The diversity and frequency of *S. cerevisiae* strains during fermentation were determined by means of mtDNA-RFLPs analysis. A total of 68 different genetic profiles were identified and named from P1 to P68. The results (for must and fermentations) in each winery and campaign are represented in Figure 3. In Priorato de Razamonde all inocula

(XG3, LSA 1 and LSA 2) shown an excellent implantation ability: 97% and 94% for XG3 and LSA1, respectively in 2019, and 100% for both XG3 and LSA2, in 2020 (Figure 3a). Non-*Saccharomyces* yeasts were identified at low frequency (<10%) in PR-LSA1 during fermentation, and in the must in 2020. Besides, the must contained eight different *S. cerevisiae* strains in 2019, but only four strains in 2020. The lower diversity found in 2020 could be related to the lower number of cells in this case (Figure 2a).

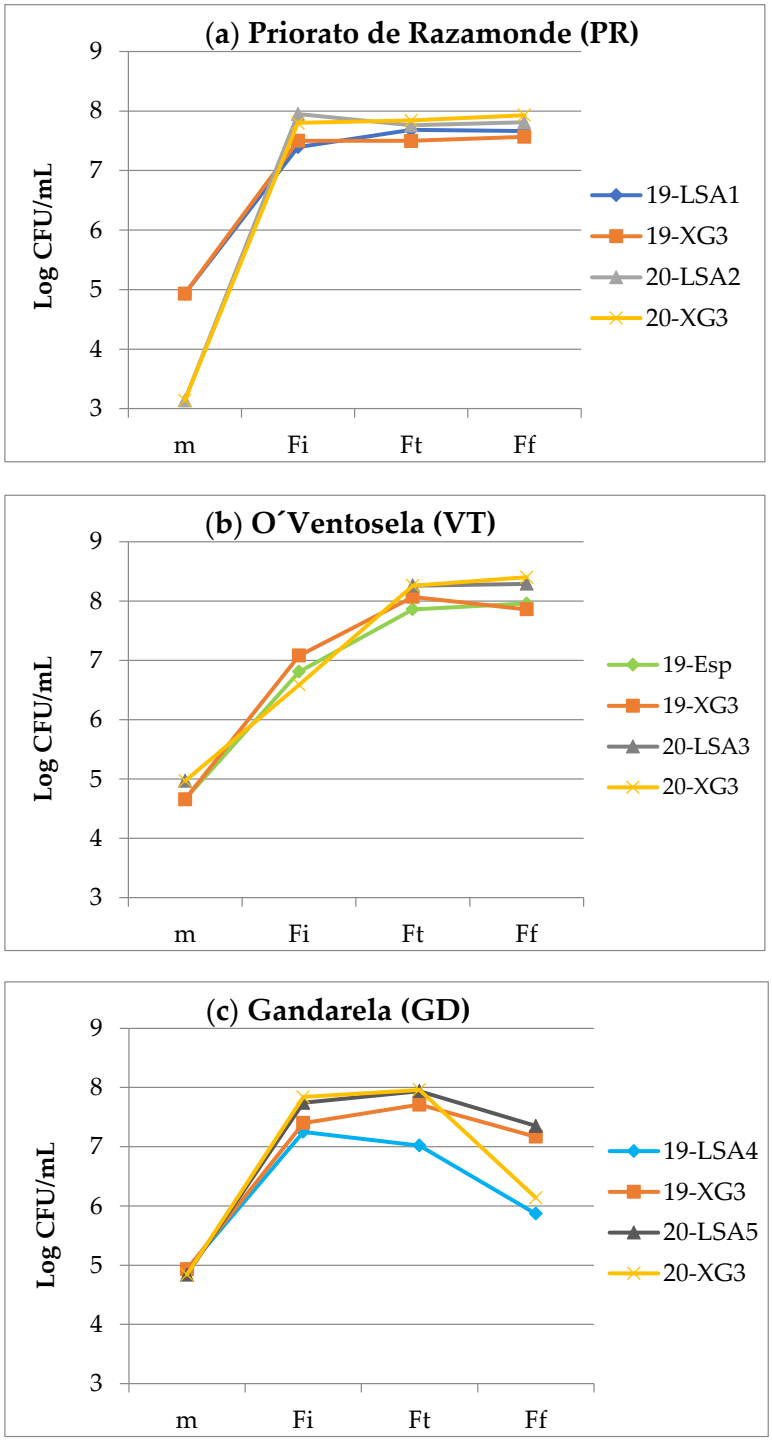

Figure 2. Evolution of yeast population in fermentations from (**a**) Priorato de Razamonde, (**b**) O'Ventosela, and (**c**) Gandarela in 2019 and 2020 campaign. M, must; Fi, Ft, and Ff are the initial, tumultuous and final stages of fermentation, respectively.

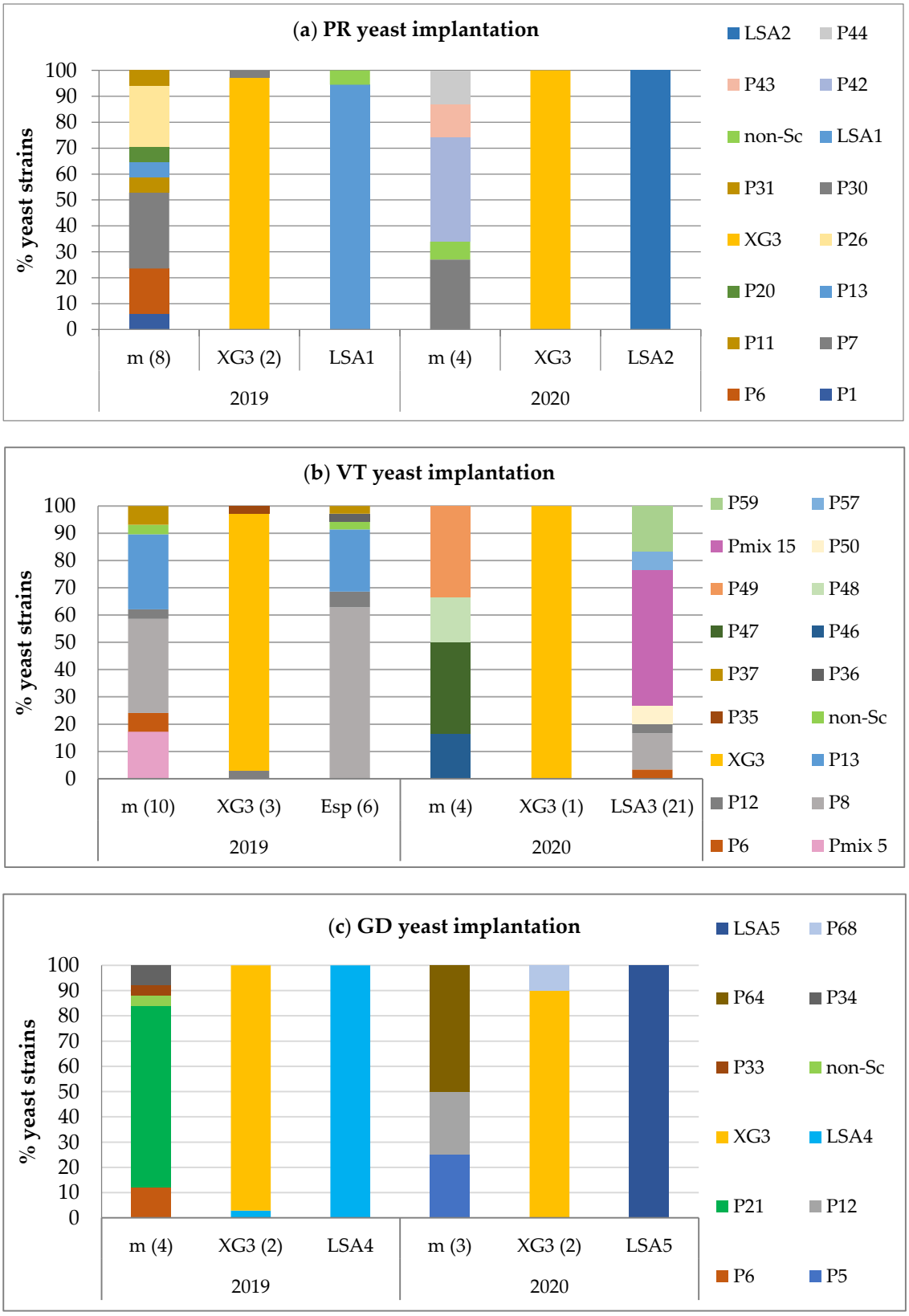

Figure 3. Inoculum implantation: Frequency of yeast strains in must (m) and fermentations with different yeast in 2019 and 2020. (**a**) Priorato de Razamonde (PR), (**b**) O'Ventosela (VT) and (**c**) Gandalera (GD). P1 to P68, XG3 and LSA1 to LSA 5 are different strains of *S. cerevisiae*. Non-Sc is non-*Saccharomyces* species. Pmix 5 and Pmix 15—the sum of frequencies of 5 and 15 different strains, respectively.

In O'Ventosela *S. cerevisiae* XG3 was also the main yeast strain in those fermentations where it was added as a starter culture. Its dominance reached 94% in 2019 and 100% in 2020. Two other strains were also identified in the 2019 vintage, but with frequencies lower than 5% (Figure 3b). In this winery, a spontaneous fermentation was used as a control in 2019. The microbiological analysis of this vinification revealed the presence of at least six different strains of *S. cerevisiae*, with two of them (P8 and P13) at higher frequency (63% and 23%, respectively). Both strains were also the predominant yeasts in the must from this winery. Next vintage (2020), the control tank was inoculated with the commercial strain named LSA 3: However, it was unable to outcompete the resident yeast population in must. Thus, a total of 21 strains were found during this fermentation, being P59 (17%) and P8 (13%) the major strains; the remaining strains appeared at frequencies lower than 10% (P50 and P57) or lower than 5%.

Yeast diversity found in Gandarela fermentations was lower than in O'Ventosela. The must contained 4 and 3 different *S. cerevisiae* strains in 2019 and 2020, respectively (Figure 3c). In addition, several non-*Saccharomyces* species were identified in the must sample in 2019. Under these conditions, both commercial inocula, LSA4 and LSA5, were the dominant strains in their respective fermentations, showing a 100% implantation rate. Regarding the autochthonous strain, *S. cerevisiae* XG3 reached frequencies of 97% and 90% in 2019 and 2020, respectively.

The results of yeast diversity in musts proved the differences in the resident *S. cerevisiae* population among wineries (in both number of strains and frequency) as previously reported in several wineries from Galicia [25]. This fact, together with the chemical characteristics of must and the oenological practices in each winery could have influenced the dominance of yeast inocula [5,16]. The implantation success of XG3 in all wineries and vintages confirmed its suitability to ferment Treixadura musts, despite the differences found in the basic characteristics of musts. The commercial yeast added in Priorato de Razamonde and Gandarela also showed excellent results both in implantation ability and quality of resulting wines.

However, the data obtained in O'Ventosela were completely different. First, the control tank-fermented spontaneously in 2019. Under these conditions, the genetic analysis evidenced the presence of several *S. cerevisiae* strains as widely reported in this type of fermentations [10,25]. Surprisingly, similar results were observed in 2020 despite having inoculated the must with a commercial starter (LSA3); even more, the diversity observed was greater than in 2019. Several reasons could explain this failure, including mingy dose, poor yeast batch or a wrong choice in the inoculum selection. In this sense, the availability of a local yeast strain as XG3 well adapted to local must characteristics represent a useful tool for winemakers. Moreover, this study confirmed that XG3 was able to overgrowth the natural microbial population in musts and became the dominant strain during fermentations at an industrial scale.

Indeed, the addition of commercial yeast as starters in industrial fermentation is a common practice among wineries. However, their use does not guarantee that they will control fermentation. The latter depends on several factors, including the dose added, rehydration conditions, inoculation temperature, and competition with indigenous yeasts [5,26]. Despite the widespread use of commercial active dry yeasts, there is little information about their implantation success. The available results indicated that the addition of an ADY did not ensure its dominance throughout the fermentation. Considering as effective an implantation rate of 80% and unsuccessful below 50% [27], the evaluation of implantation percentages in wines from DO "Vinos de Madrid" indicated that inoculations were effective only in 33% of the fermentations studied [26]. Similarly, Barrajon et al. [28] found successful implantations in 44% of the white vats analysed in wineries from Castilla-La Mancha, whereas in 28% of the vats yeasting was ineffective; the added strains were scarce or even non-existent (70%) during the process, as we found in VT fermentations. Lange et al. [29] also reported a low dominance of inoculum in some Canadian wineries; and

they highlighted a year-to-year variation in inoculum implantation, as well as differences among winery as we found in DO Ribeiro.

3.2. Chemical Characteristics of Wines

Table 3 summarised the basic chemical characteristic of Treixadura wines obtained in 2019 and 2020 with different yeast strains in each winery. The results showed trends for some parameters among wines depending on the winery—as was expected from the data obtained for the original musts (Table 2). Thus, the wines from PR showed the highest total acidity in 2019, as observed in must. Regarding the influence of yeast, the autochthonous strain XG3 tended to produce wines with higher acidity as reflected the values for total acidity and pH in all wineries. Similarly, this strain tended to reduce the alcohol content and increased the amount of glycerol in PR and VT, but not in Gandarela. No remarkable differences or trends were appreciated for lactic, malic and tartaric acids. Finally, the presence of sugars in wines from PR explained the slow down observed at the end of fermentation (Figure S1a) in 2019. The influence of XG3 on the basic characteristics of wines was confirmed in 2020. All wines obtained with the native strain presented lower alcohol content than controls with commercial strains. Besides, in two wineries, the total acidity of wines was higher with XG3. These results support the additional interest of *S. cerevisiae* XG3 as a tool to mitigate the consequences of climate change in wines, such as high alcohol content and low acidity [30]. Volatile acidity was lower than 0.30 g acetic acid/L in all cases. The presence of lactic acid in some wines indicate that the yeast strains used do not interfere with the malolactic fermentation performance, if desired. The low content of SO_2 could explain this activity.

Table 3. Characteristics of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 and 2020 vintages.

	Priorato Razamonde				**O'Ventosela**				**Gandarela**			
Parameter	**PR-LSA**		**PR-XG3**		**VT-LSA** §		**VT-XG3**		**GD-LSA**		**GD-XG3**	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
TA * (g/L)	6.1	5.5	6.8	6.3	4.5	4.6	4.7	5.5	5.6	4.9	5.9	4.4
VA ** (g/L)	0.27	0.30	0.26	0.28	0.25	0.19	0.26	0.18	0.20	0.26	0.16	0.20
Lactic acid (g/L)	0.1	0.1	0.1	0.1	0.1	1.0	0.1	0.2	0.2	0.2	0.1	0.3
Malic acid (g/L)	2.6	2.7	2.4	2.5	3.0	1.7	2.7	3.0	2.3	2.8	2.6	2.9
Tartaric acid (g/L)	2.5	2.2	3.3	3.1	1.8	2.8	1.9	2.1	2.6	1.6	2.4	1.4
Glucose + fructose (g/L)	3.2	0.2	3.9	0.7	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.2
Glycerol (g/L)	4.3	5.0	5.0	5.1	4.0	4.8	5.0	5.7	6.3	6.5	6.2	5.5
Alcohol content (% *v*/*v*)) (%vol.)	13.1	13.2	12.8	13.0	13.2	12.8	13.0	12.6	12.2	14.0	12.9	12.9
pH	3.32	3.50	3.24	3.31	3.71	3.54	3.67	3.65	3.67	3.82	3.59	3.84
Free SO_2 (mg/L)	43	44	42	36	10	10	10	10	12	10	10	12
Total SO_2 (mg/L)	92	125	93	96	69	118	52	83	92	48	69	68

* TA-total acidity expressed as g tartaric acid/L; ** VA-volatile acidity expressed as g acetic acid/L; § spontaneous fermentation in 2019.

The results of the preliminary microvinification studies comparing XG3, as ADY and as fresh cells, and a commercial LSA had already highlighted the effect of this strain on the ethanol content and the acidity of the wine (data not shown), as subsequently observed at an industrial scale. However, no significant differences were observed for these parameters when XG3 was compared to other local yeast strains in Treixadura assays [10]. Nor were differences found for alcohol content and total acidity in Godello and Albariño wines obtained at a pilot scale with different yeast strains, including XG3 [11]. It is important to note that the different tank volume between fermentations (Table 1) could have had an effect on wines chemical composition.

3.3. Wine Aroma Composition

Figure 4 shows the total volatile concentration (as the sum of alcohols, C6 compounds, acetates, ethyl esters, volatile acids, volatile phenols, lactones, aldehydes and C_{13}-norisprenoids) of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts over two consecutive vintages (2019–2020).

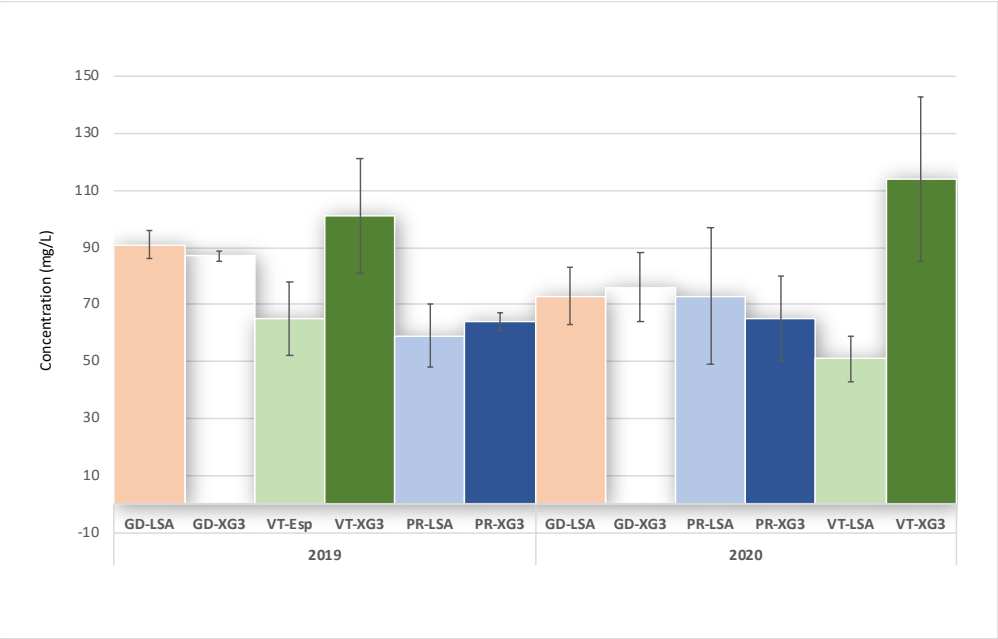

Figure 4. Total volatile concentration (mg/L) of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeast in 2019 and 2020 vintages.

Wines from VT-XG3 shows the higher concentration of total volatiles. In contrast, a trend to decrease the concentration of volatiles was shown for PR-LSA and VT-LSA in 2019 and 2020, respectively. In the O'Ventosela winery, wines fermented with XG3 tended to increase the concentration of total volatiles in both vintages vs LSA and Esp.

Table 4 summarises the volatiles concentration of Treixadura wines by chemical groups of compounds (alcohols, C6 compounds, acetates, ethyl esters, volatile acids, volatile phenols, lactones, aldehydes and C_{13}-norisprenoids) by winery and vintage. Higher alcohols were the principal group of volatiles for all Treixadura wines, followed by volatile fatty acids and acetates.

The effect of yeasts on chemical groups was studied by winery and season; a higher significant effect was observed for Gandarela wines vs O'Ventosela and Priorato de Razamonde. Treixadura wines from Gandarela winery fermented with XG3 showed a significant increase in alcohols and aldehydes in the 2019 vintage, acetates, ethyl esters and phenol volatiles in the 2020 vintage and C6-compounds in both vintages. Alcohols and phenol volatiles in the 2020 vintage and acetates in both vintages showed a significant influence by yeast in O'Ventosela winery, increasing their concentration when the wines were fermented with autochthonous XG3. However, only acetates and lactones from wines of Priorato de Razamonde in the 2019 vintage were affected by yeast, where lactones showed the highest level in XG3-wines.

Table 4. Volatiles concentration (μg/L) by chemical groups identified in Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 and 2020 vintages.

		Priorato de Razamonde			O'Ventosela			Gandarela		
Chemical Group	**Year**	**PR-LSA**	**PR-XG3**	**Sig.**	**VT-LSA**	**VT-XG3**	**Sig.**	**GD-LSA**	**GD-XG3**	**Sig.**
Higher Alcohols	2019	23,947	34,727	ns	31,145	61,864	ns	45,665	59,500	*
	2020	41,824	34,218	ns	35,865	86,599	*	46,669	43,471	ns
C6 compounds	2019	537	704	ns	1451	1151	ns	825	1049	**
	2020	734	561	ns	1210	1163	ns	410	706	*
Acetates	2019	13,708	8468	**	6647	19,068	**	17,913	10,106	***
	2020	10,860	10,150	ns	1693	10,595	**	8405	10,704	*
Ethyl esters	2019	2676	2778	ns	5186	3443	ns	3853	2286	***
	2020	4236	4292	ns	3337	4150	ns	3065	4305	**
Volatile acids	2019	17,732	17,365	ns	20,684	15,346	ns	22,355	13,773	***
	2020	15,168	15,230	ns	9113	11,329	ns	14,682	15,918	ns
Volatile Phenols	2019	392	345	ns	254	402	ns	379	395	ns
	2020	163	673	**	194	643	*	133	334	**
Lactones	2019	47	73	*	157	126	ns	131	76	**
	2020	42	51	ns	38	72	ns	25	49	ns
Aldehydes	2019	24	32	ns	nd	nd	-	76	139	**
	2020	nd	nd	-	nd	nd	-	nd	nd	-
C_{13}-norisoprenoids	2019	nd	nd	-	nd	nd	-	nd	nd	-
	2020	nd	nd	-	99	127	ns	92	93	ns

nd: not detected. Significance: *, **, and *** indicate significance at $p \leq 0.05$, 0.01, and 0.001, respectively; ns: not significant.

Differences in Treixadura musts characteristics and vinification process may be the reason for the differences between wines fermented with the same yeast from different wineries. Parameters as temperature and molecular oxygen availability during fermentation, the nitrogen availability for yeast growth, as well as the nature and quantity of the solids derived from the grapes, are recognised to have an influence on final wine composition [31,32]. In particular, yeast assimilable nitrogen (YAN) concentration in grape must is a vital parameter not only for the completion of the fermentation, but also for the production of volatile metabolites [33,34]. In this sense, the characteristics of Treixadura musts from the different wineries used in this study (Table 2) showed a higher maturation in Gandarela winery in the 2019 vintage, however the higher ripening in 2020 was observed for O'Ventosela. Moreover, the level of YAN (expressed as the sum of the α-amino acid nitrogen plus ammoniacal nitrogen) was also different among musts from different wineries (Table 2), where the higher level of YAN was observed in musts from Gandarela winery for both vintages.

Higher alcohols are quantitatively the largest group of aroma compounds in alcoholic beverages, and are secondary products of alcoholic fermentation [35]. Alcohols concentrations below 300 mg/L often contribute to the desirable complexity of wine; however, when their concentration exceeds 400 mg/L they are regarded as a negative influence on the quality of the wine [36]. In our study, all Treixadura wines showed concentration values of higher alcohols below 300 mg/L.

Two chemical groups of volatiles were significantly affected by the yeast independently to the winery: Acatetes, represented mainly by isoamylacetate (Tables S1 and S2) and phenol volatiles, represented by two compounds, 4-vinylguaiacol and 4-vinylphenol (Tables S1 and S2).

Acetates showed a significative increase in O'Ventosela wines in the 2019 vintage when the fermentation process was carried out with XG3. In contrast, XG3 produced a decrease in acetates in GD and PR wineries that year. A previous study also shows the increase in isoamyl acetate in Treixadura wines fermented with XG3 [10]. Acetate esters make the greatest contribution to the desirable fermentation bouquet of wine [36]. The characteristic fruity odours of fermentation bouquet are primarily due to esters and acetates mixture where isoamyl acetate gives a banana-like aroma [36]. Isoamyl acetate is synthesised from isoamyl alcohol and acetyl coenzyme A by alcohol acetyltransferase in

Saccharomyces cerevisiae and is hydrolysed by esterases at the same time [37]. Therefore, the balance of these two enzyme activities is important for isoamyl acetate accumulation.

The concentration of ethyl esters increased in the 2020 vintage when the wines were fermented with autochthonous yeast XG3 in all wineries, but this increase was only significant for Gandarela. Similarly, a tendency to increase the volatile acids in Treixadura wines fermented by XG3 was observed in the 2020 vintage in all wineries. In agreement with this result, previous studies showed the increase in ethyl esters and fatty acids concentration in Albariño and Godello wines when fermentations were performed with the autochthonous yeast XG3 [11].

The volatile phenols group was also affected by the yeast in the 2020 vintage, increasing their concentration in all wineries when the fermentation was carried out with XG3. Vinylphenols (4-vinylphenol and 4-vinylguaiacol) are natural constituents of wine and can play a role in wine aroma [38]. White wines contain important quantities of these volatile substances which, at a certain concentration (limit threshold = 725 μg/L of the sum of both), may be responsible for a 'phenolic' or 'pharmaceutic' characteristic depreciating the wine aroma. *S. cerevisiae* possesses enzymic activity, which is capable of transforming the phenolic acids of the must (coumaric and ferulic acids), by non-oxidative decarboxylation, into the corresponding vinylphenols. Moreover, it has been shown that glycosidic combinations of 4-vinylguaiacol, 4-vinylphenol and eugenol may exist in certain grape varieties [39]. Regarding 4-vinylguaiacol, this compound can play an important role in the varietal expression of certain white cultivars [40]. In our study none of the yeasts produced high levels of vinylphenols.

Finally, the C6-compounds group showed a significant increase in wines from XG3 in Gandarela winery; however, it was not affected in VT and PR. C6-compounds derive from grape polyunsaturated fatty acids, linoleic and-linolenic, through enzymatic reactions and can be esterified to produce esters. C6-compounds are formed during pre-fermentative steps [41].

Principal component analysis (PCA) was applied on the data of compounds identified and quantified in Treixadura wines grouped by chemical families (Figure 5). The application of PCA allowed stablishing groups of wines and vintages in basis to chemical groups analysed.

The PCA applied to volatiles explained 52.51% of the total variance. The principal component 1 (F1) explained 30.23% of the variance, and the second principal component (F2) explained 22.28%. The PCAs results showed the relevance of the vintage effect on the volatile composition of Treixadura wines. Thus, two groups of wines are shown in Figure 5. The first group corresponding to the 2019 season was positioned on the positive side of F1 and characterised for high concentrations of ethyl esters, volatile phenols, lactones, acetates, aldehydes and volatile acids. The second group, corresponding to all wines from the 2020 season, was positioned on the negative side of F1, and it was characterised by alcohols and C_{13}-norisoprenoids.

It can be noted that, for both vintages, the wines tended to group by the winery, mainly in the 2019 vintage. Wines from the 2019 vintage showed a tendency to have a higher concentration of most groups of volatile compounds.

In addition, the PCA showed good separation between wines fermented with XG3 and LSA in O'Ventosela and Gandarela in 2020. However, a clear differentiation of wines by yeast was not observed in the 2019 vintage.

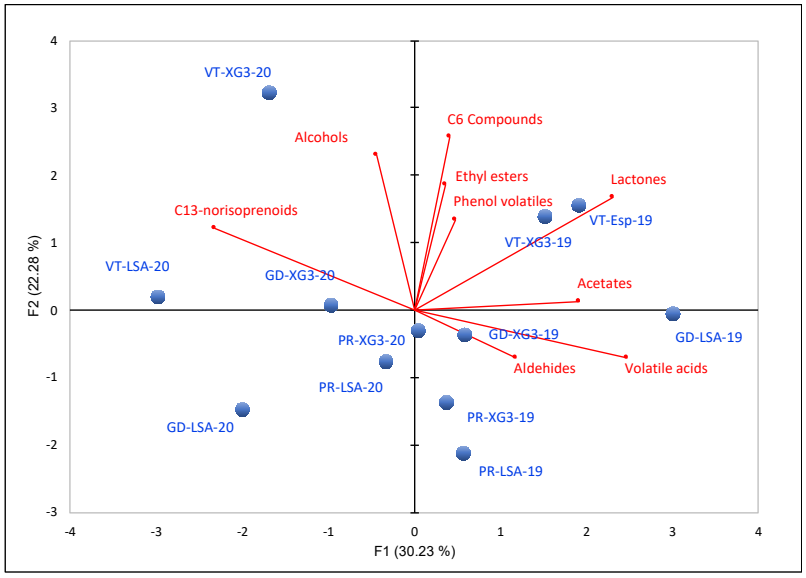

Figure 5. Principal Component Analysis from Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 and 2020 vintages.

3.4. Sensory Evaluation of Treixadura Wines

Results from aroma sensory analysis of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 and 2020 vintages are shown in Table 5 and Figure 6. Table 5 summarises the intensities of aroma descriptors. The values represented by the geometric means (GM%) for aroma descriptors (aroma intensity, aroma quality, fruity, floral and herbaceous) are shown in Figure 6.

Table 5. Aroma descriptors intensity of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 and 2020 vintages.

Year	Wines	Intensity	Quality	Fruit	Floral	Herbaceous
2019	GD-LSA	7.0 ab	7.0 a	6.1 a	5.3	4.8
	GD-XG3	4.9 c	3.8 b	3.5 ab	3.7	3.2
	PR-LSA	8.0 a	6.7 a	6.1 a	4.1	5.4
	PR-XG3	5.7 bc	6.1 ab	5.0 ab	4.1	3.4
	VT-LSA	5.0 bc	4.7 ab	3.3 b	4.5	3.4
	VT-XG3	4.8 c	4.2 b	3.7 ab	4.7	3.6
2020	GD-LSA	6.6 a	6.8 a	6.5 a	4.9 a	3.3
	GD-XG3	5.7 ab	5.6 ab	5.2 ab	4.1 ab	3.2
	PR-LSA	6.1 ab	5.0 ab	4.8 ab	3.7 ab	2.9
	PR-XG3	6.1 ab	5.2 ab	4.8 ab	4.0 ab	2.8
	VT-LSA	4.9 b	4.1 b	3.7 b	2.2 b	3.1
	VT-XG3	5.7 ab	4.5 b	4.5 ab	3.1 ab	2.6

Different letters indicate significant differences among wines from wineries by Tukey´s test with a confidence interval of 95% ($p < 0.05$); ns, not significant.

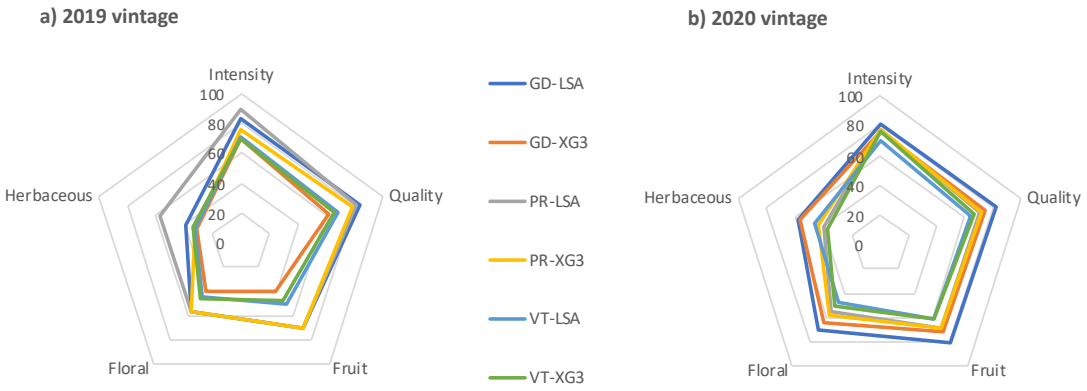

Figure 6. Aroma sensory analysis of Treixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in 2019 (**a**) and 2020 (**b**) vintages.

Significant differences ($p < 0.05$) were found among wines by vintage (Table 5). In 2019 aroma intensity, quality and fruit descriptors showed differences among wines, reaching the highest values in wines from LSA in Priorato de Razamonde (PR) and Gandarela (GD). The same descriptors, in addition to floral, showed significant differences in the 2020 vintage. However, in 2020, differences in intensity were only found between wines from LSA in GD and VT. With respect to aroma quality, the highest values were shown in GD-LSA, PR-LSA and PR-XG3 in 2019 and GD-LSA and GD-XG3 in 2020. However, only GD-LSA and PR-LSA reached significant higher aroma quality than GD-XG3 and VT-XG3 in 2019, and GD-LSA vs VT-LSA and VT-XG3 in the 2020 vintage. Finally, significant higher intensity of fruit descriptor was exhibited among wines from LSA (GD-LSA and PR-SA vs VT-LSA) in 2019. In the 2020 vintage, fruit and floral intensity had the same behaviour showing differences between wines from LSA in GD vs VT.

Because many descriptors can be rarely mentioned but which are very important in terms of the perceived intensity, and descriptors with a low perceived intensity but which are mentioned often, GM (%) was calculated (Figure 6a,b).

In the 2019 vintage (Figure 6a) the highest GM (%) values of aroma intensity and quality were observed for wines from LSA in all wineries; however, in the 2020 vintage (Figure 6b) this behaviour was only showed for Gandarela winery. Thus, wines from Priorato de Razamonde and O'Ventosela increased the intensity and quality descriptors when they were fermented with the autochthonous yeast XG3.

With respect to fruity and floral aroma descriptors, the highest values of GM (%) were found in wines from Gandarela in the 2020 vintage, where the GM of GD-LSA wines was higher than GD-XG3. However, in Priorato de Razamonde XG3 wines reached higher scores for fruity and floral descriptors than commercial LSAs in both vintages. Similarly, VT-XG3 wines also showed higher GM (%) of floral descriptor than VT-LSA wines in both vintages.

The perception of fruity aroma by the wine tasters disagrees with the data of ethyl ester and acetates production by the yeast in some wines. This situation could be due to the interaction (association or suppression) among wine volatile compounds. In fact, recent sensory research observations indicate that ethanol can suppress the fruit aroma attributes in wine [42]. In products with complex flavours, combinations of volatiles may yield different flavours than those expected from individual compounds [43]. In contrast, Albariño and Godello wines made with XG3 were characterised by flowery and fruity sensory descriptors, according to their high concentration of esters and fatty acids [11]. Finally, herbaceous descriptor was perceived with lower intensity and frequency by the wine tasters when Treixadura wines were fermented with the autochthonous yeast XG3, according to results obtained by Blanco et al. [10].

4. Conclusions

The results confirmed the oenological potential of *S. cerevisiae* XG3. This autochthonous yeast strain successfully fermented Treixadura musts at an industrial scale. In addition, XG3 was able to overgrowth the microbial population in musts being the dominant strains during fermentations, indicating a good adaptation to must conditions and the environmental conditions in wineries from DO Ribeiro. Regarding the influence on wine characteristics, its ability to reduce the alcohol content of wine and to increase the acidity of wines could help to obtain more balanced wines from overripe grapes. The evidence of significant differences in wine volatiles from XG3 vs LSA, highlighted the potential of XG3 to enhance the unique characteristics and to obtain singular wines. Moreover, our findings indicate that the yeast factor has a lower impact on wine characteristics than the vintage and winery location. Therefore, the yeast does not modify the wine characteristics provided by the variety and the season.

This study supports the suitability of *S. cerevisiae* XG3 to be used as a starter in industrial wineries for Treixadura fermentation. To advance toward its future availability for the wine industry, further research is required to evaluate whether XG3 could be also an appropriate strain to ferment musts from other cultivars traditionally grown in Galicia to obtain quality wines.

Supplementary Materials: The following are available online at https://www.mdpi.com/2311-5637/7/1/31/s1, Figure S1: Evolution of fermentations in Priorato de Razamonde in (a) 2019 and (b) 2020 campaign, Table S1: Volatile composition of Teixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in the 2019 vintage. Table S2: Volatile composition of Teixadura wines from Priorato de Razamonde (PR), O'Ventosela (VT) and Gandarela (GD) wineries obtained with different yeasts in the 2020 vintage.

Author Contributions: P.B.: Conceptualization, investigation, writing—original draft preparation, funding acquisition, project administration and supervision; M.V.-A.: formal analysis, data curation, investigation; T.G.-C.: funding acquisition, supervision; M.V.: investigation, formal analysis, supervision, writing—original draft preparation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by operational group number FEADER2018/042B from EIP (European Innovation Partnership) financed by FEADER (European Agricultural Funds for Rural Development), Galicia Government (Xunta de Galicia-Spain) and the Ministry of Agriculture and Fisheries, Food and Environment (MAPAMA) from Spanish Government.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: We are grateful to O'Ventosela, Priorato de Razamonde and Gandarela wineries for their collaboration in this project allowing us to use the strain *S. cerevisiae* XG3 at their facilities, technical support during fermentations and samples collection in their wineries. We also thank Manuel Marcos for the gas chromatography–mass spectrometry service from Scientific and Technological Research Assistance Centre (CACTI, Vigo University, Spain).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Swiegers, J.H.; Bartowsky, E.J.; Henschke, P.A.; Pretorius, I.S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173. [CrossRef]
2. Lambrechts, M.G.; Pretorius, I.S. Yeast and its importance to wine aroma—A review. *S. Afr. Enol. Vitic.* **2000**, *21*, 97–129. [CrossRef]
3. Vilanova, M.; Masneuf-Pomarède, I.; Dubourdieu, D. Influence of *Saccharomyces cerevisiae* strains on general composition and sensory properties of white wines made from *Vitis vinifera* cv. Albariño. *Food Technol. Biotechnol.* **2005**, *43*, 79–83.
4. Dubourdieu, D.; Tominaga, T.; Masneuf, I.; Peyrot des Gachons, C.; Murat, M.L. The Role of Yeasts in Grape Flavor Development during Fermentation: The Example of Sauvignon blanc. *Am. J. Enol. Vitic.* **2006**, *57*, 81–88.
5. Fleet, G.H.; Heard, G.M. Yeasts—Growth during fermentation. In *Wine Microbiology and Biotechnology*; Fleet, G.H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp. 27–54.
6. Fleet, G.H. Wine yeasts for the future. *FEMS Yeast Res.* **2008**, *8*, 979–995. [CrossRef]

7. Albergaria, H.; Arneborg, N. Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes: Role of physiological fitness and microbial interactions. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 2035–2046. [CrossRef]
8. Callejon, R.M.; Clavijo, A.; Ortigueira, P.; Troncoso, A.M.; Paneque, P.; Morales, M.L. Volatile and sensory profile of organic red wines produced by different selected autochthonous and commercial *Saccharomyces cerevisiae* strains. *Anal. Chim. Acta* **2010**, *660*, 68–75. [CrossRef]
9. Tristezza, M.; Vetrano, C.; Bleve, G.; Grieco, F.; Tufariello, M.; Quarta, A.; Mita, G.; Spano, G.; Grieco, F. Autochthonous fermentation starters for the industrial production of Negroamaro wines. *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 81–92. [CrossRef]
10. Blanco, P.; Mirás-Avalos, J.M.; Suárez, V.; Orriols, I. Inoculation of Treixadura musts with autochthonous *Saccharomyces cerevisiae* strains: Fermentative performance and influence on the wine characteristics. *Food Sci. Technol. Int.* **2013**, *19*, 177–186. [CrossRef] [PubMed]
11. Blanco, P.; Mirás-Avalos, J.M.; Pereira, E.; Orriols, I. Fermentative aroma compounds and sensory profiles of Godello and Albariño wines as influenced by *Saccharomyces cerevisiae* yeast strains. *J. Sci. Food Agric.* **2013**, *93*, 2849–2857. [CrossRef]
12. Bouzas-Cid, Y.; Falqué, E.; Orriols, I.; Mirás-Avalos, J.M. Effects of irrigation over three years on the amino acid composition of Treixadura (*Vitis vinifera* L.) musts and wines, and on the aromatic composition and sensory profiles of its wines. *Food Chem.* **2018**, *240*, 707–716. [CrossRef]
13. Falqué, E.; Fernández, E.; Dubourdieu, D. Volatile components of Loureira, Dona Branca, and Treixadura wines. *J. Agric. Food Chem.* **2002**, *50*, 538–543. [CrossRef]
14. Vilanova, M.; Oliveira, J.M. *El Potencial Aromático de Las Variedades de Vid Cultivadas en Galicia*; Xunta de Galicia: Santiago de Compostela, Spain, 2017; pp. 66–67.
15. Querol, A.; Barrio, E.; Huerta, T.; Ramón, D. Molecular Monitoring of Wine Fermentations Conducted by Active Dry Yeast Strains. *Appl. Environ. Microbiol.* **1992**, *58*, 2948–2953. [CrossRef]
16. Blanco, P.; Mirás-Avalos, J.M.; Orriols, I. Effect of must characteristics on the diversity of *Saccharomyces* strains and their prevalence in spontaneous fermentations. *J. Appl. Microbiol.* **2012**, *112*, 936–944. [CrossRef]
17. Pallmann, C.L.; Brown, J.A.; Olineka, T.L.; Cocolin, L.; Mills, D.A.; Bisson, L.F. Use of WL Medium to Profile Native Flora. *Am. J. Enol. Vitic.* **2001**, *52*, 198–203.
18. OIV: International Organisation of Vine and Wine. Compendium of International Methods of Wine and Must Analysis. Vol 1 y 2. Paris, France. 2018. Available online: http://www.oiv.int (accessed on 7 May 2020).
19. Coelho, E.; Lemos, M.; Genisheva, Z.; Domingues, L.; Vilanova, M.; Oliveira, J.M. Simple and quick LLME/GC-MS methodology to quantify minor volatile compounds in alcoholic beverages. *Molecules* **2020**, *25*, 621. [CrossRef] [PubMed]
20. ISO Norm 8589. *Sensory Analysis: General Guidance for the Design of Test Rooms*; International Organization for Standardization: Geneva, Switzerland, 1988.
21. Lawless, H.T.; Heymann, H. Sensory evaluation of food. In *Principle and Practices*; Kluwer Academic/Plenum Publishers: Boston, MA, USA, 1998.
22. ISO Norm 3591. Sensory analysis. In *Apparatus Wine Tasting Glass*; International Organization for Standardization: Geneva, Switzerland, 1977.
23. Castrillo Cachón, D. Estudio de la Diversidad de Levaduras en uva de Cultivo ecológico y Convencional en Galicia: Patrones Biogeográficos e Influencia en las Características Químicas y Sensoriales del vino. Ph.D. Thesis, Universidad de León, León, Spain, 2018.
24. Blanco, P.; Rabuñal, E.; Neira, N.; Castrillo, D. Dynamic of *Lachancea thermotolerans* Population of in Monoculture and Mixed Fermentations: Impact on Wine Characteristics. *Beverages* **2020**, *6*, 36. [CrossRef]
25. Castrillo, D.; Neira, N.; Blanco, P. *Saccharomyces cerevisiae* Strain Diversity Associated with Spontaneous Fermentations in Organic Wineries from Galicia (NW Spain). *Fermentation* **2020**, *6*, 89. [CrossRef]
26. Gil-Diaz, M.; Valero, E.; Cabellos, J.M.; García, M.; Arroyo, T. The impact of active dry yeasts in commercial wineries from the Denomination of Origen "Vinos de Madrid", Spain. *3 Biotech* **2019**, *9*, 382. [CrossRef]
27. Delteil, D. Aspectos prácticos del levadurado en condiciones mediterráneas.Técnica de inoculación y relación entre la población seleccionada y la población indígena. *Rev. F. d'Oenol.* **2001**, *189*, 14–19.
28. Barrajón, N.; Arévalo-Villena, M.; Rodríguez-Aragón, L.J.; Briones, A. Ecological study of wine yeast in inoculated vats from La Mancha region. *Food Control* **2009**, *20*, 778–783. [CrossRef]
29. Lange, J.N.; Faasse, E.; Tantikachornkiat, M.; Gustafsson, F.S.; Halvorsen, L.C.; Kluftinger, A.; Ledderhof, D.; Durall, D.M. Implantation and persistence of yeast inoculum in Pinot Noir fermentations at three Canadian wineries. *Int. J. Food Microbiol.* **2014**, *180*, 56–61. [CrossRef] [PubMed]
30. Varela, C.; Dry, P.R.; Kutyna, D.R.; Francis, I.L.; Henschke, P.A.; Curtin, C.D.; Chambers, P.J. Strategies for reducing alcohol concentration in wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 670–679. [CrossRef]
31. Fleet, G.H. Yeast interactions and wine flavour. *Int. J. Food Microbiol.* **2003**, *86*, 11–22. [CrossRef]
32. Swiegers, J.H.; Pretorius, I.S. Yeast modulation of wine flavor. *Adv. Appl. Microbiol.* **2005**, *57*, 131–175. [CrossRef] [PubMed]
33. Vilanova, M.; Ugliano, M.; Varela, C.; Siebert, T.; Pretorius, I.; Henschke, P.A. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeast. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 145–157. [CrossRef]

34. Barbosa, C.; Mendes-Faia, A.; Mendes-Ferreira, A. The nitrogen source impacts major volatile compounds release by *Saccharomyces cerevisiae* during alcoholic fermentation. *Int. J. Food Microbiol.* **2012**, *160*, 87–93. [CrossRef]
35. Amerine, M.A.; Ough, C.S. Alcohols. In *Methods for Wine and Must Analysis*; John Wiley and Sons, Inc.: New York, NY, USA, 1980; pp. 74–127.
36. Rapp, A.; Mandery, H. Wine aroma. *Experentia* **1986**, *42*, 873–884. [CrossRef]
37. Fukuda, K.; Yamamoto, N.; Kiyokawa, Y.; Yanagiuchi, T.; Wakai, Y.; Kitamoto, K.; Inoue, Y.; Kimura, A. Balance of activities of alcohol acetyltransferase and esterase in *Saccharomyces cerevisiae* is important for production of isoamyl acetate. *Appl. Environ. Microbiol.* **1998**, *64*, 4076–4078. [CrossRef]
38. Chatonnet, P.; Dubourdieu, D.; Boidron, J.N.; Lavigne, V. Synthesis of Volatile Phenols by *Saccharomyces cerevisiae* in Wines. *J. Sci. Food Agric.* **1993**, *62*, 191–202. [CrossRef]
39. Winterhalter, P.; Sefton, M.A.; Williams, P.J. Two dimensional GC-DCCC analysis of the monoterpenes, norisoprenoids and shikimate derived metabolites from Riesling wine. *J. Agric. Food Chem.* **1990**, *38*, 1041–1048. [CrossRef]
40. Versini, G. Sull'aroma del vino 'traminer aromatico' o 'Gewiirztraminer'. *Vignevini* **1985**, *12*, 57–65.
41. Oliveira, J.M.; Faria, M.; Sá, F.; Barros, F.; Araújo, I.M. C6-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Acta* **2006**, *563*, 300–309. [CrossRef]
42. Robinson, A.I.; Ebeler, S.E.; Heymann, H.; Boss, P.K.; Solomon, P.S.; Trengove, R.D. Interactions between Wine Volatile Compounds and Grape and Wine Matrix Components Influence Aroma Compound Headspace Partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313–10322. [CrossRef] [PubMed]
43. Chambers, E.; Koppel, K. Associations of Volatile Compounds with Sensory Aroma and Flavor: The Complex Nature of Flavor. *Molecules* **2013**, *18*, 4887–4905. [CrossRef]