fermentation

MDPI

Article

Diversity of *Saccharomyces cerevisiae* Yeast Strains in Granxa D'Outeiro Winery (DOP Ribeiro, NW Spain): Oenological Potential

Pilar Blanco [1,*], Estefanía García-Luque [2], Rebeca González [2], Elvira Soto [1], José Manuel M. Juste [2] and Rafael Cao [2]

[1] Estación de Viticultura e Enoloxía de Galicia (EVEGA-AGACAL), Ponte San Clodio s/n, 32428 Leiro-Ourense, Spain
[2] A Granxa D'Outeiro, Ctra. De Francelos a Filgueira s/n, 32418 Ribadavia, Spain; martinezjuste@gmail.com (J.M.M.J.)
* Correspondence: pilar.blanco.camba@xunta.gal; Tel.: +34-988788092

Abstract: Yeasts play an essential role in the aroma and sensory profiles of wines. Spontaneous fermentations were carried out at the newly built winery of Granxa D'Outeiro. Yeasts were isolated from must at different stages of fermentation. Colonies belonging to *Saccharomyces cerevisiae* were characterised at the strain level by mtDNA-RFLPs. General chemical parameters and aroma profiles of the wines were determined using official OIV methodology and GC-MS analysis, respectively. The diversity of *S. cerevisiae* per fermentation ranged from 5 to 13 different strains depending on the grapevine variety. Out of 24 strains, strain B was the dominant yeast in most fermentations at different proportions, but strains D, E, and H also reached up to 25% of the total population in some fermentations. The yeast diversity was higher in the Lado fermentation than in those containing Treixadura. The chemical compositions of the wines revealed differences among them, with Loureira and Albariño wines showing the highest content of volatile compounds. The evaluation of their technological properties revealed the oenological potential of some strains of *S. cerevisiae*. The strains showing the best scores were selected to be used in future vintages to enhance the typicality of wines in the Granxa D'Outeiro winery.

Keywords: indigenous yeasts; spontaneous fermentation; *Saccharomyces cerevisiae* strains; oenological traits; yeast selection; wine chemical composition

check for updates

Citation: Blanco, P.; García-Luque, E.; González, R.; Soto, E.; Juste, J.M.M.; Cao, R. Diversity of *Saccharomyces cerevisiae* Yeast Strains in Granxa D'Outeiro Winery (DOP Ribeiro, NW Spain): Oenological Potential. *Fermentation* **2024**, *10*, 475. https://doi.org/10.3390/fermentation10090475

Academic Editor: Chiara Nadai

Received: 26 July 2024
Revised: 3 September 2024
Accepted: 5 September 2024
Published: 13 September 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The success of winemaking involves not only the use of quality grapes but also the management of microorganisms and other technological practises in the winery. The fermentation of grape must to produce wine is a complex biochemical process where yeasts and bacteria play an essential role [1]. In particular, yeasts are responsible for alcoholic fermentation, a process by which sugar is converted into ethanol and carbon dioxide, and a range of minor, secondary metabolites that define the aroma and sensory characteristics of the wine [2].

Traditionally, fermentation was a spontaneous process carried out by the yeasts found on grape berries and in the winery environment. The succession of yeast populations during spontaneous fermentation is a well-established phenomenon. Non-*Saccharomyces* species are initially present but, as fermentation progresses, they are gradually replaced by *Saccharomyces*, which has a high fermentative power and tolerance to ethanol to overcome the microbial species naturally present in musts [3–5]. Several studies have shown that spontaneous fermentations can be differentiated from wines produced by inoculation with selected starter cultures [6–8]; however, this practise increases the risk of having a slow or stuck fermentation and the formation of off-flavours [9]. Given the crucial role of yeast species/strains during the fermentation process, the use of commercial starters as active

dry yeasts (ADYs) has become a common practise worldwide in winemaking. ADYs have been selected for their desirable oenological properties to ensure a rapid fermentation and the production of high-quality wines [10]. Nevertheless, despite the benefits previously mentioned, some studies indicate that commercial ADYs are not always able to overgrow natural yeasts during fermentation [11,12], and their use has been linked to the homogenisation of wines [3,8,10]. In this sense, indigenous yeasts offer an interesting alternative because they are believed to be better adapted to must properties and to environmental conditions, and they also contribute to maintaining the unique regional characters of wines [13–16]. In addition, these practises help to preserve the native yeast populations and to explore their contributions as enhancers of wine typicality from a given region.

The selection of indigenous yeasts involves the isolation of a representative number of yeasts from grapes or from spontaneous fermentation processes, the identification of the isolates at the strain level, and the evaluation of their oenological potential. The selection criteria of yeasts for winemaking include properties related to their fermentative ability, low production of volatile acidity, resistance to stress conditions, and secretion of desirable metabolites [17–21]. Following these premises, the *Saccharomyces cerevisiae* population has been characterised, and some strains have been selected in traditional wine-producing regions in Spain and tested at the winery scale to confirm their benefits [22–24]. Similarly, numerous studies report the diversity of *S. cerevisiae* strains and, in some cases, their potential for winemaking in different wine-producing areas around the world [25–37]. Furthermore, aware of the importance of local yeasts, winemakers demand their own starters and have implemented yeast strain selection programs in their wineries to obtain exclusive wines [38–40].

The wine industry in Galicia constitutes an important sector both from an economic and a social point of view since it contributes to the enhancement of rural areas and to environmental preservation [41]. A study conducted at the experimental winery of Estación de Viticultura e Enoloxía de Galicia (Evega) showed the occurrence of *S. cerevisiae* populations during spontaneous fermentations of must from different traditional varieties in this region [42]. The oenological potential of these strains was tested at the laboratory scale, and some of them were evaluated at the pilot scale [43–45]. Finally, one of them, *S. cerevisiae* XG3, was produced as an ADY and applied at the industrial scale [46]. Moreover, a survey on yeast populations in organic wineries from Galicia using spontaneous fermentations revealed a high diversity of *S. cerevisiae* strains, which could be related to the complexity and differentiation of organic wines [47,48].

Granxa D'Outeiro is a recovered (since 2016) ancient vineyard located in the heart of the Ribeiro Protected Denomination of Origin (DOP Ribeiro, Galicia, NW, Spain). Seven traditional white grapevine varieties grow under sustainable management at this farm: Treixadura, Albariño, Torrontés, Lado, Loureira, Godello, and Caíño blanco. Recently, a winery was built at the farm, and it was used for the first time in 2023. The purpose of Granxa D'Outeiro is to take advantage of their varietal resources in the vineyard by means of sustainable management and the use of indigenous yeasts to obtain an exclusive wine representative of the farm. To achieve this goal, this study aimed to evaluate the diversity of *S. cerevisiae* yeasts in the new winery during spontaneous fermentations through (i) the isolation and identification of indigenous *S. cerevisiae* at the strain level, (ii) the characterisation of the oenological potential of *S. cerevisiae* strains, and (iii) the selection of suitable strains for future utilisation as starters in the winery. The diversity of indigenous *S. cerevisiae* strains in the Granxa D'Outeiro winery and their oenological potential constitute a valuable resource for producing wines with distinctive characteristics.

2. Materials and Methods

2.1. Grape Origin and Processing

Vitis vinifera L. Treixadura, Torrontés, Godello, Albariño, Loureira, Lado, and Caíño blanco are traditional white grapevine varieties of preferential use in DOP Ribeiro, Galicia (NW Spain) [49]. All these cultivars are grown in the Granxa D'Outeiro vineyard

(42.2745221, −8.1626866) under sustainable management. In this study, grapes from these varieties in an optimal state of ripeness were harvested manually in September 2023, and transported to the Granxa D'Outeiro winery located within the same farm. The winery is newly built and it was used for the first time in this campaign.

Each variety was processed separately. Grapes were destemmed, crushed and sulphited (24 mg SO_2/Kg grape) in the must pump. Then, the paste was cooled in the heat exchanger (icespedes, Pontevedra, Spain) at 10 °C, transferred to a pneumatic press (Bucher Vaslin, Pontevedra, Spain) and sulphited again (6 mg SO_2/Kg grape). The must was transferred to a stainless-steel tank for a static settling for 48 h at 13.4 °C. Lysis VC (2 g/100 L) and Phylia Cys (20 g/100 L) enzymes were added to favour settling. After settling, the clean must was racked to a stainless-steel tank equipped with a cooling jacket (González Carballal, Ourense, Spain) for fermentation. Nine fermentations were carried out in total, some of them as single-varietal musts whereas some others contained a mixture of musts from different cultivars as indicated in Table 1.

Table 1. Fermentation codes, grapevine varieties, and characteristics of the musts.

Code	Grapevine Variety	Must Characteristics		
		Total Acidity (g Tartaric Acid/L)	PAC * (% vol)	YAN ** (mg/mL)
G + T	Godello + Treixadura	5.8	12.5	
L + T	Loureira + Treixadura	5.2	12.9	188
CB + T	Caíño blanco + Treixadura	4.6	12.9	241
ALB	Albariño	5.9	13.4	193
TRX	Treixadura	4.5	12.4	250
LD	Lado	5.5	13.5	288
M + T	Moscatel + Torrontés	4.6	11.5	185
LOU	Loureiro	6.6	12.8	
CB	Caíño blanco	6.9	13.2	177

* PAC–predicted alcohol content; ** YAN–yeast-assimilable nitrogen.

2.2. Fermentations

Musts were allowed to ferment spontaneously at 17 °C with no addition of commercial yeast starters in order to favour the proliferation of the winery's own yeast strains. Fermentation kinetics were monitored by measuring density and temperature every 12 h. Samples for microbiological control of the fermentations were taken in 100 mL sterile containers from grape juice at the beginning, middle, and final stages of fermentation. To ensure the nutritional needs of the yeasts and optimise their fermentative activity, the musts were supplemented at the beginning of fermentation at a density of 1070 g/L with diammonium phosphate and Actimax Natura (20 g/hL). In addition, at a density of 1050 g/L, bentonite (60 g/hL) was added to favour clarification. When fermentations were at the final stages, the wines were racketed to a new tank and Actimax Vit (20 g/hL) was added to prevent stuck and/or sluggish fermentations. Finally, when the fermentations had finished, the wines were racked off the lees and sulphited (60 mg/L of SO_2).

2.3. Yeast Isolation and Characterisation

Samples from musts at different stages of fermentation were serially diluted in 2% *w*/*v* buffered peptone water and spread on WL Nutrient Agar media (Scharlau Microbiology, Barcelona, Spain) [50]. The plates were incubated at 28 °C until visible colonies appeared, with those containing between 20 and 200 colonies used for viable yeast count. Then, a representative number of colonies (20–25 per sample) were selected randomly and isolated on YEPD [42] for further characterisation. All isolates were replicated on Lysine media (Oxoid, Thermo Fisher Scientific, Madrid, Spain) in order to distinguish between *Saccharomyces* and non-*Saccharomyces* yeasts, since the former cannot grow on this medium.

The characterisation of *S. cerevisiae* isolates at the strain level was carried out by analysis of the mitochondrial DNA restriction profiles (mtDNA-RFLPs). The total yeast DNA

was obtained following the protocol described by Querol et al. [51] with some modifications [42]. Then, DNA was digested with the restriction endonuclease Fast digest *Hinf* I (Thermo Fisher Scientific, Madrid, Spain), and the restriction fragments were separated by electrophoresis on a 0.8% *w*/*v* agarose gel in 1× TBE containing Red Safe™ (iNtRON Biotechnology, Inc.; supplied by Celta Ingenieros, A Coruña, Spain) nucleic acid staining solution. DNA pattern bands were visualised under UV light and documented using a Molecular Imager® Gel DocTM XR+ imaging system (BIO-RAD, Madrid, Spain).

2.4. Wine Chemical Analysis

Basic parameters of wines (alcohol content; reducing sugars; pH; titratable and volatile acidity; tartaric, malic, and lactic acids) were determined by Fourier transformed infrared spectrometry using a WineScan FT120 analyser (FOSS Electric, Barcelona, Spain) previously calibrated according to the official methods for wine analysis (OIV 2023) [52]. In addition, the free and total sulphur dioxide (SO_2) in the wines were also quantified using the OIV methods. The official methodology (OIV 2023) was also applied to determine the predicted alcohol content, pH, yeast-assimilable nitrogen (YAN), and total SO_2 in must [52].

Volatile compounds of wines were quantified by gas chromatography–flame ionisation detection (GC–MS) according to the protocol described by Lopez et al. in 2002 [53]. Fermentative volatiles in wines from microvinifications were determined following the methodology of Torrens et al. [54].

2.5. Technological Properties of S. cerevisiae Strains

The oenological potential of *S. cerevisiae* strains was evaluated by microvinification assays, carried out in duplicate using Treixadura grape juice which had been frozen during harvest time. The characteristics of the thawed must were a predicted alcohol content of 11.8% *v*/*v*, pH 3.59, and 37 mg/L of total SO_2. The must was distributed in 1 L bottles containing 900 mL of must each. Yeast inocula, prepared by growing each strain in 5 mL of YPD at 28 °C overnight, were added to the must to reach a final concentration of 1×10^6 cel/mL and allowed to ferment at 18 °C in a cold room. Fermentation kinetics were followed by daily measurements of °Brix using a digital refractometer (Schmidt + Haensch, Berlin, Germany). When the fermentations finished (Brix repeated for 3 days), the wines were centrifuged, sulphited (25 mg/L of free SO_2), and stored until further chemical analysis. Several criteria included in the resolution OIV-OENO 370–2012 [55] were considered. The fermentative vigour, or the speed at which a particular yeast starts the fermentation, was expressed as grams of sugar consumed per day during the first three days of fermentation. The ethanol yield of *S. cerevisiae* strains was calculated as grams of sugar necessary to produce one degree of alcohol.

The killer activity of *S. cerevisiae* strains was tested using plates of YEPD medium buffered at pH 4.2 with citrate–phosphate buffer and supplemented with 0.02% *w*/*v* methylene blue (Panreac Química S.L.U., Barcelona, Spain) [45]. Plates were seeded with a lawn of the sensitive strain *S. cerevisiae* CECT 1890. Then, a patch (approximately 1 cm in diameter) of the strains was inoculated, and the plates were incubated at 24 °C for 2–4 days. Killer strains were surrounded by a clear growth inhibition halo of the sensitive strain. *S. cerevisiae* EX73P was used as a positive killer control [56].

The ability of *S. cerevisiae* strains to produce H_2S (hydrogen sulphide) was evaluated on Nickerson Agar (BiGGY agar) (Scharlau Microbiology, Barcelona, Spain). H_2S was estimated according to the appearance of colonies after 3 days of incubation at 28 °C. A five-level scale was used for colour evaluation: 1—white-beige, 2—light brown, 3—brown, 4—dark brown, and 5—black.

Finally, to select those strains endowed with desirable oenological traits for future trials in the winery, the values of different parameters were scored by ranges (Table S1). Thus, the total and volatile acidity, sugar, alcohol, and glycerol of the resulting wines, as well as the time of fermentation and the fermentation vigour, were scored from 1 to 5. In

addition, the killer ability and the H_2S production were considered in the evaluation, but they were not included in the final score.

2.6. Data Analysis

To assess the diversity of *S. cerevisiae* strains during each spontaneous fermentation, the patterns of strains obtained and their relative abundance were used to calculate the classical Shannon (H') and evenness (*e*) ecology indexes [57]. The Shannon index includes the number of different strains and their proportion of the total population for each fermentation. Evenness (*e*) considers how similar the amount of each strain is in a given fermentation; thus, *e* is 1 when there are similar proportions of all yeast strains, but the value decreases when some strains appear at higher percentages. Diversity indexes were calculated using PAST Version 4.17 (2023). The differences in the chemical compositions of the wines, considering the yeast strain as a factor, were determined by one-way ANOVA. The Tukey HSD test was used to separate means. These analyses were carried out using SPSS18.0 for Windows. Principal component analysis (PCA) was used to separate the wines according to their volatile composition by considering the main fermentative compounds and/or group of compounds using previous standardisation of the data. PCA was performed using PAST Version 4.17 (2023).

3. Results

3.1. Yeast Population in Spontaneous Fermentations: Diversity and Occurrence of Each Strain

The genetic analysis of a total of 473 isolates allowed the identification of 24 different profiles, that is, 24 different strains of *S. cerevisiae*, named A, B, . . ., W. The number of isolates and strains found from each fermentation, the Shannon and evenness indexes, and the percentage of the most abundant profile are summarised in Table 2.

Table 2. Diversity of *S. cerevisiae* strains during spontaneous fermentations in the Granxa D'Outeiro winery.

Fermentation *	Number of *S. cerevisiae* Isolates	Number of Strains **	H	*e*	Number of Strains (Profiles) *** within Percentage Range		
					>25%	5–25%	<5%
G + T	54	9	0.95	0.29	1 (B)	1 (E)	7
L + T	52	8	1.19	0.41	1 (B)	2 (E,H)	5
CB + T	51	9	1.47	0.48	1 (B)	3 (D,E,H)	5
TRX	54	5	1.09	0.60	1 (B)	3 (C,D,H)	1
ALB	54	6	1.33	0.63	1 (B)	2 (D,E)	3
LD	49	13	2.07	0.61	1 (B)	3 (D,E,L)	9
M + T	53	11	1.90	0.61	1 (D)	4 (B,E,H,P)	6
LOU	53	6	1.01	0.46	1 (B)	2 (D,E)	3
CB	53	8	1.12	0.38	1 (B)	2 (C,H)	5
Total	473	24	1.71	0.23	1 (B)	3 (D,E,H)	20

* Codes indicated in Table 1. ** Each strain has a different mtDNA-RFLP profile. H—Shannon index; *e*—evenness. *** B; C, D,E,H,L,P are different strains of *S. cerevisiae*.

The diversity of *S. cerevisiae* ranged between 13 strains (H = 2.07) in Lado (LD) fermentation and 5 strains (H = 1.09) in Treixadura (TRX) (Table 2). Strain B was the dominant yeast in all fermentation processes except in M + T vinification, which was mainly controlled by strain D (Table S2). The proportion of strain B ranged from 29% in LD fermentation to 80% in G + T fermentation. Strains D, E, and H reached percentages between 5 and 25% in some fermentations; therefore, they also contribute to the final characteristics of wines. Strain L appeared at an abundance of <10% in LD fermentation, and the proportion of strain C was higher than 7% in TRX and CB assays. The presence of the remaining strains was anecdotal (<5%); however, they may own oenological properties of interest. In addition, Figure 1 shows the cumulative percentages of *S. cerevisiae* strains in each fermentation. Strain B

was the dominant yeast (frequency > 50%) in most fermentations, but in LD and M + T it appeared in codominance with other strains.

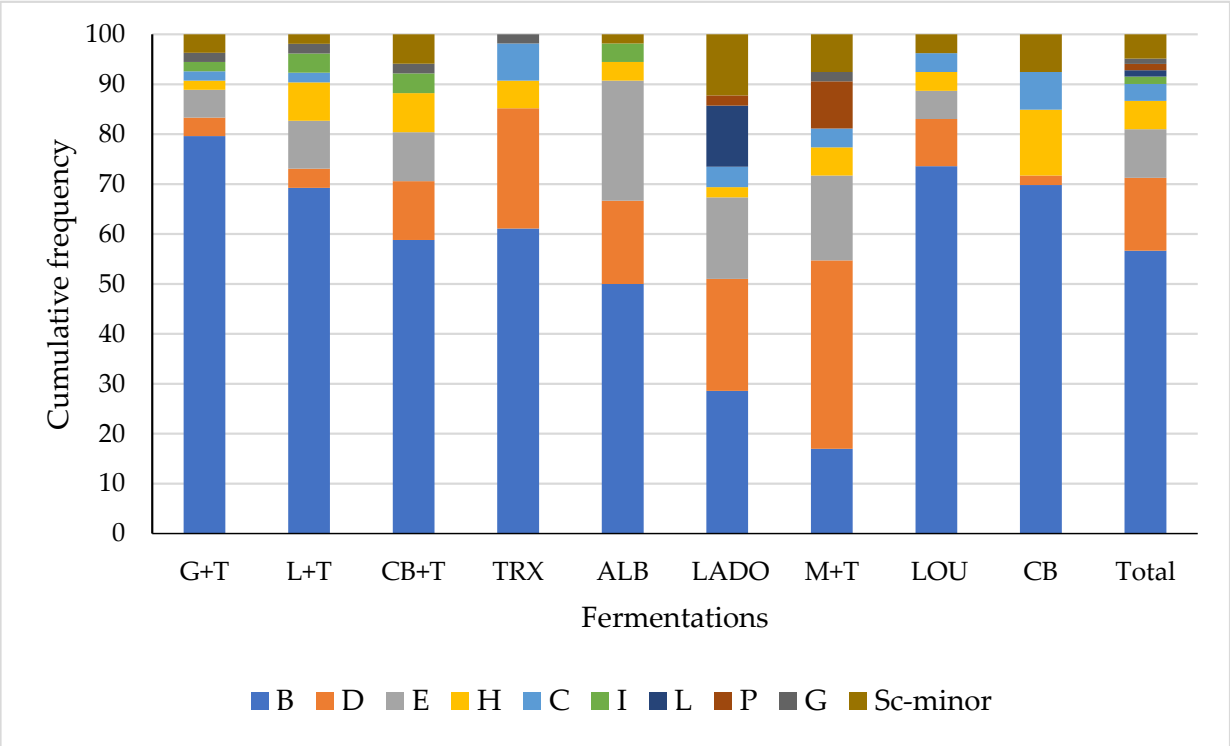

Figure 1. Cumulative percentages of *Saccharomyces cerevisiae* strains isolated from spontaneous fermentations in the Granxa D'Outeiro winery. Sc-minor: the sum of the strains found at proportions < 5% in each wine.

3.2. Chemical Composition of Wines from Spontaneous Fermentations

The basic chemical characteristics of the wines obtained by spontaneous fermentation are summarised in Table 3. The total acidity ranged from 4.6 g/L in wines that included a mix of varieties to 7.0 g/L in wine elaborated with Caíño blanco.

Table 3. Chemical characteristics of wines obtained by spontaneous fermentation in the Granxa D'Outeiro winery.

Parameter	LGT *	L + T	CB + T	ALB	LD	M + T	LOU	CB
Total acidity (g tartaric acid/L)	4.6	5.1	5.2	5.9	4.9	4.6	5.7	7.0
Volatile acidity (g acetic acid/L)	0.38	0.4	0.45	0.45	0.36	0.26	0.28	0.47
Lactic acid (g/L)	0.2	0.3	0.3	0.2	0.2	0.3	<0.1	<0.1
Malic acid (g/L)	2.2	1.9	2	2.3	2.5	2	1.6	2.5
Tartaric acid (g/L)	1.6	2.0	1.8	2.3	1.3	1.5	2.6	2.6
pH	3.60	3.55	3.52	3.53	3.58	3.47	3.20	3.22
Glucose + fructose (g/L)	0.9	4.8	7.1	6.7	0.8	0.4	4	14.5
Glycerol (g/L)	4.3	4.4	4.5	4.7	4.4	4.7	4.4	4.9
Alcohol content (% *v*/*v*)	13.5	13.4	13.0	13.9	14.2	12.4	12.8	12.6
Free SO_2 (mg/L)	24	22	27	23	<10	<10	18	11
Total SO_2 (mg/L)	115	122	128	126	42	56	63	68

* LGT—mix of G + T + LD.

Accordingly, volatile acidity also varied among wines, as well as the content of malic and tartaric acid and pH. None of the wines underwent malolactic fermentation, as indicated by the low lactic acid values. Most wines achieved an alcohol content according

to their must composition, but some of them still contained fermentable sugars and their alcohol degree was lower than expected.

The volatile composition of the wines is summarised in Table S3. The wines were separated using PCA by considering the main volatile compounds and/or groups of volatile compounds (Figure 2). All wines containing Treixadura were grouped together in the positive part of PC1. Each monovarietal wine was plotted in a different quadrant. Albariño was located in the first quadrant characterised by a high content of acetates, esters, and acids. Lado was in the second quadrant due to its isobutanol and gamma-butyrolactone content. Wine elaborated with Caíño blanco did not stand out for any volatile compound and appeared in the third quadrant. Finally, Loureira wine, the most aromatic one (Table S3), was located in the fourth quadrant characterised by the highest content of 2-phenyl ethanol, linalool, isoamyl alcohol, and ethyl lactate.

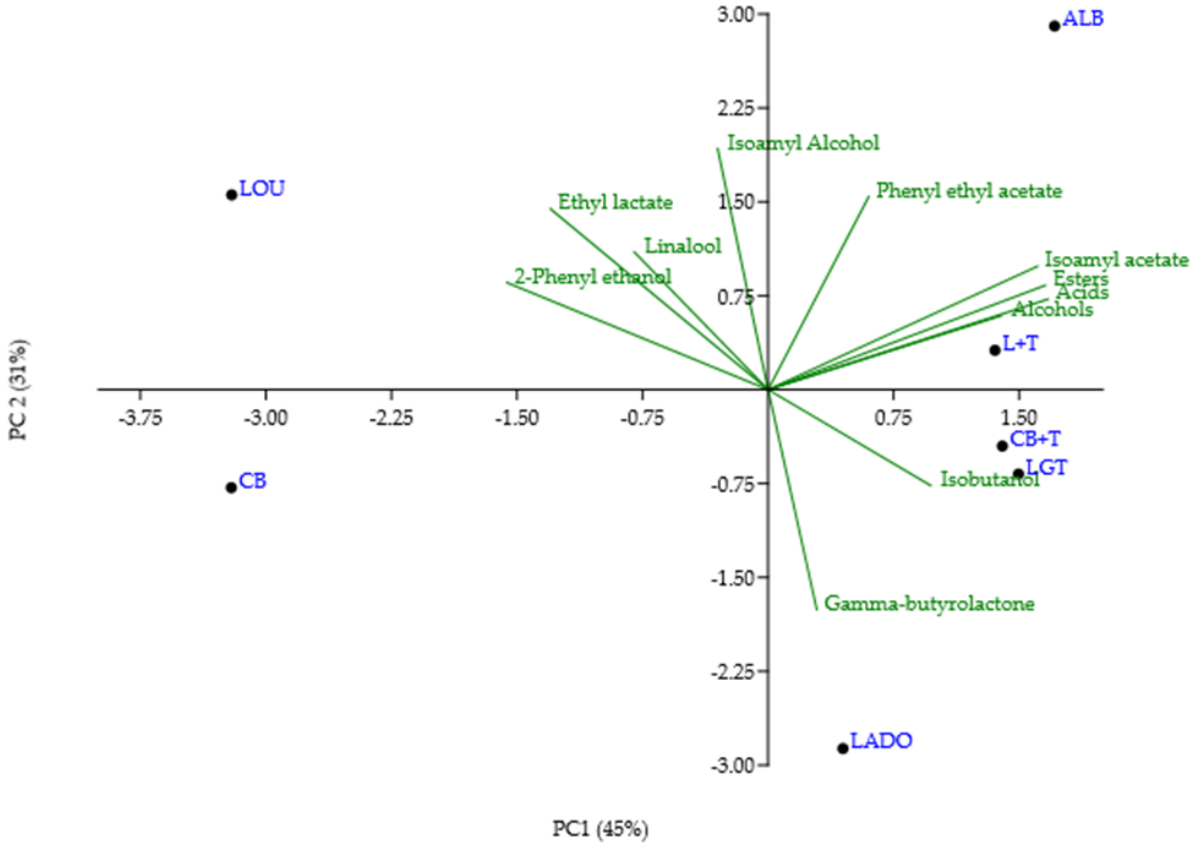

Figure 2. Principal component analysis (PCA) of wines from the Granxa D'Outeiro winery based on their main volatile compounds.

3.3. Oenological Potential of S. cerevisiae Strains from Granxa D'Outeiro

The fermentative ability of *S. cerevisiae* strains isolated in Granxa D'Outeiro differs between strains (Table S4). Fermentations lasted from 22 to 29 days, although some strains failed to complete the process, as indicated by the presence of sugars in the resulting wine. Accordingly, the fermentation rate also varied, ranging from 8.6 to 29 g sugar/day, with some strains consuming >35% of sugars during the first 3 days of fermentation (Table S4). These latter strains required 16 g of sugar to produce one alcoholic degree. Significant differences were also found for the total and volatile acidity of wines, alcohol content, and glycerol among the strains tested. All these technological traits were scored according to the ranges established in Table S1. Table 4 shows the values achieved by the different *S. cerevisiae* strains. In addition, the results of killer ability and the production of H_2S were included in this evaluation. Most of the strains presented killer activity or were neutral, whereas strain J was the only one found to be sensitive to this toxin (Table S4).

Regarding H_2S production, the strains isolated in this study showed a moderate production of this compound. Considering all these criteria, the strains L, K, M, and P achieved the highest scores (Table 4). None of these strains were found as the dominant yeast in Granxa D'Outeiro fermentations; they were all isolated in Lado fermentation at a low abundance except for strain L, which reached 12% (Table S2). Strain P achieved a proportion of 10% in M + T fermentation.

Table 4. Heatmap of the scores obtained by *Saccharomyces cerevisiae* strains for different selection criteria.

S. cerevisiae Strain	TA	VA	G + F	AC	Gly	Ft	Fv	Score
A	3	3	3	3	2	2	2	18
B	3	2	1	2	2	2	2	14
C	2	3	3	1	1	2	1	13
D	1	5	5	2	1	4	3	21
E	2	4	4	1	2	5	3	21
F	4	2	4	1	4	5	4	24
G	3	1	4	1	3	5	3	20
H	2	3	5	1	2	5	3	21
I	2	5	4	4	4	2	3	24
J	4	1	3	4	4	1	4	21
K	4	5	5	5	3	2	5	29
L	5	5	5	5	3	3	5	31
M	4	4	5	3	3	5	4	28
N	3	3	4	2	3	4	4	23
Ñ	4	2	5	4	5	2	4	26
O	3	1	3	5	4	2	3	21
P	3	5	5	2	5	4	4	28
Q	3	5	3	2	3	2	1	19
R	2	3	5	1	3	4	2	20
S	3	2	2	2	1	1	1	12
T	3	2	2	4	2	1	2	16
U	3	2	3	4	3	1	2	18
V	3	1	3	3	4	4	3	21
W	3	2	4	3	3	2	4	21

Selection criteria were TA—total acidity; VA—volatile acidity; AC—alcohol content; Gly—glycerol; Ft—time of fermentation; and Fv—fermentative vigour. Score points for each criterion were awarded following the range included in Table S4.

In addition, the fermentative aroma compounds of wines produced with each *S. cerevisiae* strain were quantified (Table S5). The results for total volatiles and the main families of compounds are represented in Figure 3. Wines from strains B, C, Ñ, and D presented the highest content of fermentative volatiles, mainly due to the high concentration of ethyl esters, related to the desirable sensorial properties of wines. Of those strains, B and D were found as predominant yeasts in Granxa D'Outeiro fermentations (Table S2, Figure 1). However, these strains did not show a high score in the aforementioned technological evaluation. Similarly, the *S. cerevisiae* strains that achieved the best scores (Table 4) did not produce wines with a high content of aroma compounds.

Figure 3. Concentrations (mg/L) of the main families of fermentative volatile compounds in wines obtained with different *S. cerevisiae* strains isolated from the Granxa D'Outeiro winery.

4. Discussion

The significant role of yeasts during alcoholic fermentation and their impact on the chemical and sensory qualities of wine have been widely recognised [1,2]. In this sense, the contribution of native yeasts during spontaneous fermentation to the complexity of wine, and how the use of ADYs leads to the standardisation of wines, are also well known [3,6–8,10]. Other advantages of natural yeasts over ADYs include their adaptation to the properties of the must and the environmental conditions of a given winery, as well as their contribution to the regional character of the wines [13–15]. Furthermore, evidence of the role microorganisms plays in the concept of terroir and their impact on the typicity of wine (microbial terroir) [15,58,59] has awakened the interest of wineries in having their own starters to differentiate their wines. Recently, the existence of different microbial terroirs has been reported within DOP Ribeiro [60].

Granxa D'Outeiro is aware of the above tendency; therefore, they are developing a program of isolation and selection of yeast strains that takes advantage of the varietal resources, sustainable management in the vineyard, and the use of indigenous yeasts in order to obtain an exclusive wine representative of the farm. This study includes the results of *S. cerevisiae* yeast diversity, wine characterisation, and evaluation of the *S. cerevisiae* strains' oenological potential. It is important to note that the winery is newly built and was used for the first time in a 2023 vintage; therefore, commercial yeasts have never been used in this winery's facilities.

Considering these conditions, the mtDNA-RFLP analyses of 473 colonies isolated from 9 spontaneous fermentations revealed 24 different strains of *S. cerevisiae*, but only 4 of them reached abundances higher than 10%. The number of strains identified per fermentation varied depending on the grapevine variety. This has already been proven for traditional cultivars in Galicia in the experimental winery of Evega [42], although ADY addition and spontaneous fermentations were used. Several studies have reported the impact of ADY inoculation in the indigenous yeast population in industrial wineries [31–61]. In this sense, the survival of commercial yeasts and their prevalence during spontaneous fermentations was evidenced in Evega [62]. For instance, the number of *S. cerevisiae* strains observed in spontaneous fermentations of Lado in Evega was lower than in Granxa D'Outeiro [63].

However, some authors found that winery- and vineyard-associated *S. cerevisiae* strains were detected despite the use of ADYs [31]. The opposite was also observed: autochthonous, resident yeasts well adapted to the winery environment were able to conduct fermentations to their final stages, or they were implanted in the fermentations year after year, imposing themselves over those entering with the grape [64–66]. In addition, the presence and abundance of yeast strains during fermentation is influenced by the must characteristics [67]. In this study, the same strain (B) was dominant in most of the fermentations except in the M + T and Lado trials. These two fermentations were characterised by a higher diversity of *S. cerevisiae* strains than the remaining fermentations, both in the number of different strains and in their abundance (as indicated by the high H and *e* indexes) (Table 2).

Regarding the diversity of *S. cerevisiae* strains, a survey in organic wineries from Galicia that carried out spontaneous fermentations showed higher diversity indexes for some wineries than the ones reported in this work [47]. Studies from other regions have shown variability on yeast diversity in spontaneous processes depending on the type of management, winemaking practises in the winery, and vintage or grapevine varieties, among others factors [39,64–66]. Our results also highlight the dominance of a few strains as suggested by the H indexes. In this sense, it has been proposed that several factors, including grapevine variety (must characteristics), resistance to ethanol and sulphur dioxide, temperature adaptation, killer activity, and cell-to-cell interactions are involved in diversity and the dominance of a given *S. cerevisiae* strain during spontaneous fermentation [5,67–69]. Thus, it is assumed that dominant strains are more competitive due to their ability to adapt to the changing environmental conditions during fermentation.

The persistence of certain yeasts in a winery over years, the so-called "winery effect", has been related to the distinctive characteristics of wines; therefore, the knowledge of local yeast diversity and the selection of strains with oenological potential is a topic of interest for winemakers. Traditionally, the criteria for yeast selection included evaluation of their fermentative ability, low production of volatile acidity, residual sugars, resistance to sulphur dioxide and other stressful conditions, killer phenotype, and secretion of desirable metabolites, among others [17,55]. However, there have arisen new selection criteria due to the consequences of climate change on wines and also the diversification of consumer preferences [18]. In this study, we have rated fermentative competence, wine total and volatile acidity, sugar consumption, and glycerol and ethanol production. The results indicate that none of the *S. cerevisiae* strains that achieved the highest scores (L, K, M, and P) were dominant yeasts in Granxa D'Outeiro fermentations. Surprisingly, the score obtained by *S. cerevisiae* strain B (the dominant one in most fermentations) was quite low, as well as the score of the other strains found at abundances > 5% (C, D, E, and H) (Table 4). Since this winery was used in 2023 for the first time, more campaigns will be necessary to establish an accurate "winery effect" in Granxa D'Outeiro facilities, as the effect has been reported in other wineries where no ADYs have been used [66,70]. However, in a study in La Rioja involving samples from 11 wineries located in different sub-zones over a period of 3 or 4 consecutive years, no evidence of representative yeasts from the winery or the area were found [71].

The chemical characteristics of wines from fermentations at the winery scale confirm the results obtained at the lab scale with the dominant *S. cerevisiae* strain B–that is, reducing sugars > 2%, low alcohol and glycerol content, and moderate content of total and volatile acidity (Table S4 and Table 4), which were penalised in the range scale (Table S1). Thus, all wines from fermentations with B as the dominant strain contained the above-mentioned characteristics (Table 3), except LD and M + T wines. These two were fermented by different *S. cerevisiae* strains in codominance (Table S2). In general, the results obtained for the basic chemical parameters of wines from Granxa D'Outeiro match the average values found in a survey of white wines from DOP Ribeiro (unpublished data). However, the content of glycerol was lower than the average observed in Ribeiro wines (5.7 g/L), while the total acidity and alcoholic degree varied depending on the grapevine variety. For instance, Caíño blanco, Loureira, and Albariño wines showed higher acidity than those of Treixadura,

as expected. A careful blend of these monovarietal wines will be necessary to obtain a balanced wine that fulfils the requirements of DOP Ribeiro while keeping the distinctive character of Granxa D'Outeiro products.

Regarding the volatile composition of the wines, the results clearly indicate the influence of the grapevine varieties used. Treixadura, Torrontés, Godello, Albariño, Loureira, Lado, and Caíño blanco are all traditional white grapevine varieties of preferential use in DOP Ribeiro, Galicia (NW Spain), with Treixadura being the main one [49]. Loureira and Albariño are also grown in this region, and their wines are the most aromatic in Galicia [72], as confirmed in this study. The oenological potential of Lado, whose growth is restricted to a limited area within the DOP Ribeiro, is less known due to its minority character [63,73]. The highest diversity of yeasts was found with this last variety.

Yeasts play a key role during alcoholic fermentation. Not only do they convert the grape sugars into ethanol, but they also produce minor, secondary metabolites that define the aroma of wines. Therefore, the production of fermentative aromas is an important criterion to keep in mind for the selection of yeasts. In particular, the content of higher alcohols, esters, and volatile acids in wine is influenced by the yeast responsible for fermentation, mainly *S. cerevisiae*, but the formation of these volatile compounds is strain-dependent [1,2]. From these compounds, esters have a great impact on wine aroma because they provide fruity and/or floral notes to wine [74]. The results of fermentative aroma production in this study show the highest concentrations in wines fermented with strains B, C, D, and Ñ, mainly due to the ethyl esters content (Figure 3, Table S5). In contrast, the wines obtained with the best scored strains did not stand out for their content of volatile compounds (Figure 3). In this sense, it is interesting to mention that if esters are present in excess amounts, they could mask varietal aromas [1]; therefore, these strains owning positive technological traits could be useful for fermentation while preserving the varietal potential of each cultivar.

Taking all these findings together, we can conclude that the diversity of *S. cerevisiae* strains in the newly built winery of Granxa D'Outeiro constitutes an interesting tool for wine differentiation. The strains showing desirable technological properties at the laboratory scale will be multiplied and used as starters in future campaigns in order to evaluate their potential at the industrial scale. This, together with the varietal resources of the farm, could help to obtain singular wines. A blend of these monovarietal wines will allow the Granxa D'Outeiro winery to obtain an exclusive wine that expresses the biodiversity from its terroir.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation10090475/s1. Table S1: Range of punctuation established for different parameters used to score *S. cerevisiae* strains; Table S2: Heatmap of frequencies found for each *S. cerevisiae* strain in spontaneous fermentations from Granxa D'Outeiro winery; Table S3: Volatile composition (μg/L) of wines obtained by spontaneous fermentation in Granxa D'Outeiro winery; Table S4: Chemical characteristics of wines elaborated with different *Saccharomyces cerevisiae* strains from Granxa D'Outeiro, and other technological properties; Table S5: Fermentative aromas (μg/L) of wines obtained with different *S. cerevisiae* strains.

Author Contributions: Conceptualisation, P.B., J.M.M.J. and R.C.; methodology, E.G.-L., R.G., P.B. and E.S.; software, E.G.-L., P.B. and E.S.; validation, P.B. and E.S.; formal analysis, E.G.-L., P.B. and E.S.; investigation, E.G.-L., R.G. and P.B.; resources, P.B. and E.S.; data curation, E.G.-L., P.B. and E.S.; writing–original draft preparation, P.B.; writing–review and editing, P.B.; visualisation, P.B. and E.G.-L.; supervision, P.B., J.M.M.J. and R.C.; project administration, P.B. and R.C.; funding acquisition, P.B. and R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FEADER (75%), Xunta de Galicia (22.5%), and the Ministry of Agriculture, Fisheries, and Food (MAPA) (2.5%), grant number FEADER 2022/009A.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank T. Blanco and D. Noriega for their peer review and English corrections.

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

References

1. Belda:, I.; Ruiz, J.; Esteban-Fernández, A.; Navascúes, E.; Marquina, D.; Santos, A. Moreno-Arribas, M.V. Microbial contribution to Wine aroma and its intended use for Wine quality improvement. *Molecules* **2017**, *22*, 189. [CrossRef] [PubMed]
2. 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]
3. 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.
4. Fleet, G.H. Wine yeasts for the future. *FEMS Yeast Res.* **2008**, *8*, 979–995. [CrossRef]
5. 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] [PubMed]
6. Varela, C.; Siebert, T.; Cozzolino, D.; Rose, L.; Mclean, H.; Henschke, P.A. Discovering a chemical basis for differentiating wines made by fermentation with "wild" indigenous and inoculated yeasts: Role of yeast volatile compounds. *Aust. J. Grape Wine Res.* **2009**, *15*, 238–248. [CrossRef]
7. Jolly, N.P.; Varela, C.; Pretorius, I.S. Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* **2014**, *14*, 215–237. [CrossRef]
8. Welsh, B.L.; Eisenhofer, R.; Bastian, S.E.P.; Kidd, S.P. Monitoring the viable grapevine microbiome to enhance the quality of wild wines. *Microbiol. Aust.* **2023**, *44*, 13–17. [CrossRef]
9. Bisson, L.F. Stuck and sluggish fermentations. *Am. J. Enol. Vitic.* **1999**, *50*, 107–119. [CrossRef]
10. Mas, A.; Beltran, G.; Torija, M.J. Microbiological control of alcoholic fermentation. *Ecocycles* **2020**, *6*, 57–72. [CrossRef]
11. 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]
12. Gil-Díaz, M.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*, 382–394. [CrossRef]
13. 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]
14. 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] [PubMed]
15. Bokulich, N.A.; Collins, T.S.; Masarweh, C.; Allen, G.; Heymann, H.; Ebeler, S.E.; Mills, D.A. Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *mBio* **2016**, *7*, e00631-16. [CrossRef] [PubMed]
16. Gao, J.; Wang, M.; Huang, W.; You, Y.; Zhan, J. Indigenous *Saccharomyces cerevisiae* Could Better Adapt to the Physicochemical Conditions and Natural Microbial Ecology of Prince Grape Must Compared with Commercial *Saccharomyces cerevisiae* FX10. *Molecules* **2022**, *27*, 6892. [CrossRef] [PubMed]
17. Regodón, J.A.; Peréz, F.; Valdés, M.E.; De Miguel, C.; Ramírez, M. A simple and effective procedure for selection of wine yeast strains. *Food Microbiol.* **1997**, *14*, 247–254. [CrossRef]
18. Suárez-Lepe, J.A.; Morata, A. New trends in yeast selection for winemaking. *Trends Food Sci. Technol.* **2012**, *23*, 39–50. [CrossRef]
19. Sidari, R.; Ženišová, K.; Tobolková, B.; Belajová, E.; Cabicarová, T.; Bučková, M.; Puškárová, A.; Planý, M.; Kuchta, T.; Pangallo, D. Wine Yeasts Selection: Laboratory Characterization and Protocol Review. *Microorganisms* **2021**, *9*, 2223. [CrossRef]
20. Romano, P.; Siesto, G.; Capece, A.; Pietrafesa, R.; Lanciotti, R.; Patrignani, F.; Granchi, L.; Galli, V.; Bevilacqua, A.; Campaniello, D.; et al. Validation of a Standard Protocol to Assess the Fermentative and Chemical Properties of *Saccharomyces cerevisiae* Wine Strains. *Front. Microbiol.* **2022**, *13*, 830277. [CrossRef]
21. Tzamourani, A.P.; Taliadouros, V.; Paraskevopoulos, I.; Dimopoulou, M. Developing a novel selection method for alcoholic fermentation starters by exploring wine yeast microbiota from Greece. *Front. Microbiol.* **2023**, *14*, 1301325. [CrossRef]
22. Morata, A.; Arroyo, T.; Bañuelos, M.A.; Blanco, P.; Briones, A.; Cantoral, J.M.; Castrillo, D.; Cordero-Bueso, G.; del Fresno, J.M.; Escott, C.; et al. Wine yeast selection in the Iberian Peninsula: *Saccharomyces* and non-*Saccharomyces* as drivers of innovation in Spanish and Portuguese wine industries. *Crit. Rev. Food Sci. Nutr.* **2022**, *63*, 10899–10927. [CrossRef] [PubMed]
23. Torija, M.J.; Rozes, N.; Poblet, M.; Guillamon, J.M.; Mas, A. Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years. *Antonie Van Leeuwenhoek* **2001**, *79*, 345–352. [CrossRef] [PubMed]
24. Beltran, G.; Torija, M.J.; Novo, M.; Ferrer, N.; Poblet, M.; Guillamón, J.M.; Rozès, N.; Mas, A. Analysis of yeast populations during alcoholic fermentation: A six year follow-up study. *Syst. Appl. Microbiol.* **2002**, *25*, 287–293. [CrossRef] [PubMed]

25. Börlin, M.; Venet, P.; Claisse, O.; Salin, F.; Legras, J.-L.; Masneuf-Pomarède, I. Cellar associated *Saccharomyces cerevisiae* population structure revealed high diversity and perennial persistence in Sauternes wine estates. *Appl. Environ. Microbiol.* **2016**, *82*, 2909–2918. [CrossRef] [PubMed]
26. Capece, A.; Pietrafesa, R.; Siesto, G.; Romaniello, R.; Condelli, N.; Romano, P. Selected Indigenous *Saccharomyces cerevisiae* Strains as Profitable Strategy to Preserve Typical Traits of Primitivo Wine. *Fermentation* **2019**, *5*, 87. [CrossRef]
27. Vaudano, E.; Quinterno, G.; Costantini, A.; Pulcini, L.; Pessione, E.; Garcia-Moruno, E. Yeast distribution in Grignolino grapes growing in a new vineyard in Piedmont and the technological characterization of indigenous *Saccharomyces* spp. strains. *Int. J. Food Microbiol.* **2019**, *289*, 154–161. [CrossRef]
28. Nikolaou, E.; Soufleros, E.H.; Bouloumpasi, E.; Tzanetakis, N. Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. *Food Microbiol.* **2006**, *23*, 205–211. [CrossRef]
29. Karampatea, A.; Tsakiris, A.; Kourkoutas, Y.; Skavdis, G. Molecular and Phenotypic Diversity of Indigenous Oenological Strains of *Saccharomyces cerevisiae* Isolated in Greece. *Eng. Proc.* **2021**, *9*, 7. [CrossRef]
30. Cheng, E.; Martiniuk, J.T.; Hamilton, J.; McCarthy, G.; Castellarin, S.D.; Measday, V. Characterization of Sub-Regional Variation in *Saccharomyces* Populations and Grape Phenolic Composition in Pinot Noir Vineyards of a Canadian Wine Region. *Front. Genet.* **2020**, *11*, 908. [CrossRef] [PubMed]
31. Martiniuk, J.T.; Pacheco, B.; Russell, G.; Tong, S.; Backstrom, I.; Measday, V. Impact of commercial strain use on *Saccharomyces cerevisiae* population structure and dynamics in pinot noir vineyards and spontaneous fermentations of a Canadian winery. *PLoS ONE* **2016**, *11*, e0160259. [CrossRef]
32. Goddard, M.R.; Anfang, N.; Tang, R.; Gardner, R.C.; Jun, C. A distinct population of *Saccharomyces cerevisiae* in New Zealand: Evidence for local dispersal by insects and human-aided global dispersal in oak barrels. *Environ. Microbiol.* **2010**, *12*, 63–73. [CrossRef] [PubMed]
33. Gayevskiy, V.; Goddard, M.R. Geographic delineations of yeast communities and populations associated with vines and wines in New Zealand. *ISME J.* **2012**, *6*, 1281–1290. [CrossRef] [PubMed]
34. González, M.L.; Chimeno, S.V.; Sturm, M.E.; Becerra, L.M.; Lerena, M.C.; Rojo, M.C.; Combina, M.; Mercado, L.A. Populations of *Saccharomyces cerevisiae* in Vineyards: Biodiversity and Persistence Associated with Terroir. *Fermentation* **2023**, *9*, 292. [CrossRef]
35. Schuller, D.; Alves, H.; Dequin, S.; Casal, M. Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde Region of Portugal. *FEMS Microbiol. Ecol.* **2005**, *51*, 167–177. [CrossRef]
36. Drumonde-Neves, J.; Franco-Duarte, R.; Vieira, E.; Mendes, I.; Lima, T.; Schuller, D.; Pais, C. Differentiation of *Saccharomyces cerevisiae* populations from vineyards of the Azores Archipelago: Geography vs. Ecology. *Food Microbiol.* **2018**, *74*, 151–162. [CrossRef]
37. Hu, L.; Ji, X.; Li, J.; Jia, Y.; Wang, X.; Zhang, X. Selection of *Saccharomyces cerevisiae* Isolates from Helan Mountain in China for Wine Production. *Fermentation* **2023**, *9*, 376. [CrossRef]
38. Ut, C.; Berbegal, C.; Lizama, V.; Polo, L.; García, M.J.; Andrés, L.; Pardo, I.; Álvarez, I. Isolation and characterisation of autochthonous *Saccharomyces cerevisiae* from 'Pago' Merlot wines of Utiel-Requena (Spain) Origin. *Aust. J. Grape Wine Res.* **2021**, *28*, 330–346. [CrossRef]
39. Agarbati, A.; Canonico, L.; Comitini, F.; Ciani, M. Ecological Distribution and Oenological Characterization of Native *Saccharomyces cerevisiae* in an Organic Winery. *Fermentation* **2022**, *8*, 224. [CrossRef]
40. Berbegal, C.; Ferrer, S.; Polo, L.; Pardo, I.; García-Esparza, M.J.; Andrés, L.; Álvarez, I.; Lizama, V. Diversity of Indigenous *Saccharomyces cerevisiae* Yeast Strains in Cabernet Sauvignon Fermentations from Utiel-Requena Region (Spain) as a Resource to Improve Wine Distinctiveness. *Fermentation* **2023**, *9*, 654. [CrossRef]
41. OIVE Interprofesional del Vino de España. La Relevancia Económica Del Sector Vitivinícola En GALICIA 2023. Available online: https://interprofesionaldelvino.es/galicia-la-comunidad-autonoma-con-mayor-numero-de-viticultores-de-espana/ (accessed on 4 July 2024).
42. Blanco, P.; Ramilo, A.; Cerdeira, M.; Orriols, I. Genetic diversity of wine *Saccharomyces cerevisiae* strains in an experimental winery from Galicia (NW Spain). *Antonie Van Leeuwenhoek* **2006**, *89*, 351–357. [CrossRef]
43. 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]
44. 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] [PubMed]
45. Blanco, P.; Mirás-Avalos, J.M.; Pereira, E.; Fornos, D.; Orriols, I. Modulation of chemical and sensory characteristics of red wine from Mencía by using indigenous *Saccharomyces cerevisiae* yeast strains. *OENO One* **2014**, *48*, 63–74. [CrossRef]
46. 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. [CrossRef]
47. 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]
48. Castrillo, D.; Blanco, P. Peculiarities of the Organic Wine in Galicia (NW Spain): Sensory Evaluation and Future Considerations. *Beverages* **2023**, *9*, 89. [CrossRef]
49. Designation of Origin Ribeiro. Available online: https://www.ribeiro.wine/types-of-grapes (accessed on 19 July 2024).

50. 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 Fermentations. *Am. J. Enol. Vitic.* **2001**, *52*, 198–203. [CrossRef]
51. Querol, A.; Barrio, E.; Huerta, T.; Ramon, D. Molecular monitoring of wine fermentations conducted by active dry yeast strains. *Appl. Environ. Microbiol.* **1992**, *58*, 2948–2953. [CrossRef] [PubMed]
52. OIV. Office International De La Vigne Et Du Vin Compendium of International Methods of Wine and Must Analysis. Volume 1 and 2. Paris, France. 2023. Available online: http://www.oiv.int (accessed on 31 July 2023).
53. López, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **2002**, *966*, 167–177. [CrossRef]
54. Torrens, J.; Riu-Aumatell, M.; López-Tamames, E.; Buxaderas, S. Volatile compounds of red and white wines by head-space-solid phase microextraction using different fibers. *J. Chromatogr. Sci.* **2004**, *42*, 310–316. [CrossRef]
55. OIV. Guidelines for the Characterization of Wine Yeasts of the Genus Saccharomyces Isolated from Vitivinicultural Environments. Available online: https://www.oiv.int/public/medias/1429/oiv-oeno-370-2012-en.pdf (accessed on 8 July 2024).
56. Maqueda, M.; Zamora, E.; Álvarez, M.L.; Ramírez, M. Characterization, ecological distribution, and population dynamics of *Saccharomyces* sensu stricto killer yeasts in the spontaneous grape must fermentations of Southwestern Spain. *Appl. Environ. Microbiol.* **2012**, *78*, 735–743. [CrossRef] [PubMed]
57. Castrillo, D.; Rabuñal, E.; Neira, N.; Blanco, P. Yeast diversity on grapes from Galicia, NW Spain: Biogeographical patterns and the influence of the farming system. *OENO One* **2019**, *53*, 573–587. [CrossRef]
58. Knight, S.; Klaere, S.; Fedrizzi, B.; Goddard, M.R. Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Sci. Rep.* **2015**, *5*, 14233. [CrossRef]
59. Belda, I.; Zarraonaindia, I.; Perisin, M.; Palacios, A.; Acedo, A. From Vineyard Soil to Wine Fermentation: Microbiome Approximations to Explain the "Terroir" Concept. *Front. Microbiol.* **2017**, *8*, 821. [CrossRef] [PubMed]
60. Blanco, P.; Rodríguez, I.; Fernández-Fernández, V.; Ramil, M.; Castrillo, D.; Acín-Albiac, M.; Adamo, I.; Fernández-Trujillo, C.; García-Jiménez, B.; Acedo, A.; et al. Physicochemical Properties and Microbiome of Vineyard Soils from DOP Ribeiro (NW Spain) Are Influenced by Agricultural Management. *Microorganisms* **2024**, *12*, 595. [CrossRef]
61. Santamaría, P.; Garijo, P.; López, R.; Tenorio, C.; Gutiérrez, A.R. Analysis of yeast population during spontaneous alcoholic fermentation: Effect of the age of the cellar and the practice of inoculation. *Int. J. Food Microbiol.* **2005**, *103*, 49–56. [CrossRef]
62. Blanco, P.; Orriols, I.; Losada, A. Survival of commercial yeasts in the winery environment and their prevalence during spontaneous fermentations. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 235–239. [CrossRef]
63. Blanco, P.; Vázquez-Alén, M.; Losada, A. Influence of yeast population on characteristics of the wine obtained in spontaneous and inoculated fermentations of must from Vitis vinifera Lado. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 183–188. [CrossRef]
64. Tello, J.; Cordero-Bueso, G.; Aporta, I.; Cabellos, J.M.; Arroyo, T. Genetic diversity in commercial wineries: Effects of the farming system and vinification management on wine yeasts. *J. Appl. Microbiol.* **2011**, *112*, 302–315. [CrossRef]
65. de Celis, M.; Ruiz, J.; Martín-Santamaría, M.; Alonso, A.; Marquina, D.; Navascués, E.; Gómez-Flechoso, M.; Belda, I.; Santos, A. Diversity of *Saccharomyces cerevisiae* yeasts associated to spontaneous and inoculated fermenting grapes from Spanish vineyards. *Lett. Appl. Microbiol.* **2019**, *68*, 580–588. [CrossRef]
66. Granchi, L.; Ganucci, D.; Buscioni, G.; Mangani, S.; Guerrini, S. The Biodiversity of *Saccharomyces cerevisiae* in Spontaneous Wine Fermentation: The Occurrence and Persistence of Winery-Strains. *Fermentation* **2019**, *5*, 86. [CrossRef]
67. 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] [PubMed]
68. Pérez-Torrado, R.; Rantsiou, K.; Perrone, B.; Navarro-Tapia, E.; Querol, A.; Cocolin, L. *Saccharomyces cerevisiae* strains: Insight into the dominance phenomenon. *Sci. Rep.* **2017**, *7*, 43603. [CrossRef] [PubMed]
69. Ganucci, D.; Guerrini, S.; Mangani, S.; Vincenzini, M.; Granchi, L. Quantifying the Effects of Ethanol and Temperature on the Fitness Advantage of Predominant *Saccharomyces cerevisiae* Strains Occurring in Spontaneous Wine Fermentations. *Front. Microbiol.* **2018**, *9*, 1563. [CrossRef]
70. Agarbati, A.; Comitini, F.; Ciani, M.; Canonico, L. Occurrence and Persistence of *Saccharomyces cerevisiae* Population in Spontaneous Fermentation and the Relation with "Winery Effect". *Microorganisms* **2024**, *12*, 1494. [CrossRef]
71. Santamaría, P.; López, R.; Garijo, M.P.; Escribano, R.; González-Arenzana, L.; López-Alfaro, I.; Gutiérrez, A.R. Biodiversity of *Saccharomyces cerevisiae* yeasts in spontaneous alcoholic fermentations: Typical cellar or zone strains? In *Advances in Grape and Wine Biotechnology*; Morata, A., Ed.; IntechOpen: London, UK, 2019; Volume 1. [CrossRef]
72. Vilanova, M.; Oliveira, J.M. *El Potencial Aromático de las Variedades de Vid Cultivadas en Galicia*; Xunta de Galicia, Consellería do Medio Rural: Santiago de Compostela, Spain, 2017; pp. 66–67.
73. Díaz-Fernández, Á.; Díaz-Losada, E.; González, J.M.D.; Cortés-Diéguez, S. Part II—Aroma profile of Twenty White Grapevine Varieties: A Chemotaxonomic Marker Approach. *Agronomy* **2023**, *13*, 1168. [CrossRef]
74. Lambrechts, M.G.; Pretorius, I.S. Yeast and its Importance to Wine Aroma—A Review. *South Afr. J. Enol. Vitic.* **2000**, *21*, 97–129. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.