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Effect of the Addition of Non-*Saccharomyces* at First Alcoholic Fermentation on the Enological Characteristics of Cava Wines

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Abstract: Background: Cava is considered to be a high-quality wine internationally. Hence, it has undergone consistent improvement and/or the preservation of its aromatic qualities, bouquet, color, and foamability, throughout its elaboration and aging. Methods: This study investigates the use of different *Saccharomyces* and non-*Saccharomyces* yeasts strains (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) in Chardonnay and Xarel.lo cava wines. The usual enological parameters, the volatile composition, the protein contents, and foamability were determined, and sensory analyses were also performed for all of the vinifications (both before tirage and after 18 months of aging on the lees). Results: the protein and foamability results show that there is a direct relationship between both parameters, with better foam persistence achieved in some non-*Saccharomyces* fermentation. *M. pulcherrima* base wines showed a high protein content, improving foamability and foaming persistence. In addition, the results of the aromatic composition and the sensory analysis showed that the use of *T. delbrueckii* at first fermentation produced interesting cavas from an aromatic perspective. These cavas showed the highest values of ethyl isovalerate (120–126 µg/L), providing aromatic fruity notes, especially fresh green apple. Conclusions: the use of non-*Saccharomyces* yeasts in the base wine fermentation can be an alternative to produce cavas with differentiated aromatic characteristics and interesting foaming ability.

Keywords: non-*Saccharomyces*; yeast; cava; foamability; protein; aroma; volatile

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1. Introduction

In the last century, *Saccharomyces* was the only genus of yeasts used in wine cellars and, although grapes have a great diversity of species and yeasts strains, the dominance of *Saccharomyces cerevisiae* during alcoholic fermentation was expected and desired. Traditionally, the use of *Saccharomyces* yeasts together with non-*Saccharomyces* species during alcoholic fermentation influences the final composition of the wine. Thus, the contribution to the final quality of the wine of some yeast strains that acted during fermentation can be negative, because in some cases increases in ethyl acetate, volatile acidity, and acetaldehyde are observed, among others [1,2]. Conversely, they may present a positive contribution, increasing some desirable metabolites, such as acetate esters [3], which generally provide greater organoleptic complexity. Different genera and species of non-*Saccharomyces* yeasts are now emerging to improve the winemaking process and the quality and differentiation of wines. Thus, many research topics are based on the use of non-*Saccharomyces* species to improve wine quality and aromatic profile or to modulate the composition of the wine [4,5].

Among the non-*Saccharomyces* yeasts, the most studied genera are *Candida*, *Metschnikowia*, *Pichia*, *Torulaspora*, *Starmerella*, and *Lachancea*, because they help improve the organoleptic characteristics of wines, such as color, aroma, and sensory characteristics.

It is described that, when non-*Saccharomyces* yeasts are added in the early stages of grape must fermentation, the aroma wine quality improves because of the resulting metabolic products, such as terpenoids, esters, higher alcohols, glycerol, acetaldehyde, and organic acids [4,5]. Comitini et al. [6] reviewed the use of different non-*Saccharomyces* yeasts in winemaking and, in addition to this aromatic improvement, they noted other benefits, such as a reduction in the undesirable microflora, alcohol degree, and the amounts of sulfur dioxide, hydrogen sulfide, acetaldehyde and copper [6]. Therefore, because non-*Saccharomyces* yeasts produce wines with many distinctive characteristics, there are progressively more commercially available for different yeast species, such as *T. delbrueckii*, *M. pulcherrima*, and *P. kluyveri*.

The literature currently shows that many studies that have used non-*Saccharomyces* yeasts during alcoholic fermentation to produce different types of wines. However, few studies have examined the effect of these yeasts on the quality of sparkling wines, such as cava. The most characteristic note of these appreciated wines is their bubbles, generated in a second fermentation, which provides many other distinctive organoleptic properties. This second fermentation is not easy because, once the first fermentation is complete, the yeasts face a hostile environment due to high alcohol content (around 11%), high pressure (5–6 bars), lack of nutrients, low pH, and the presence of SO_2. Furthermore, the yeast must be able to ferment at low temperatures and must have both a good flocculation and autolytic ability. As the *Saccharomyces* genus meets all of these requirements, it has been the most commonly used yeast for this purpose. However, recently, other species have been also studied, such as *Schizosaccharomyces pombe* [1], which shows a better adaptation to these specific circumstances, allowing the transformation of malic acid into ethanol, significantly reducing the levels of biogenic amines, and presenting the ability to ferment the sparkling base wine to dryness without producing aromatic defects [7–9].

T. delbrueckii is another non-*Saccharomyces* yeast that is able to carry out the second fermentation with interesting results because it enhances the sensory profile of the sparkling wine obtained compared to that provided by *S. cerevisiae.* This is due to a higher production of esters with subsequent high scores for some positive aromatic descriptors [10].

Finally, it should be noted that some non-*Saccharomyces* yeasts have been also used to obtain base wine for sparkling wine production by the sequential inoculation of non-*Saccharomyces* and *S. cerevisiae*. Gonzalez-Royo et al. [11] studied the influence of *T. delbrueckii* and *M. pulcherrima* when used for the first fermentation and the results showed different interesting characteristics. Thus, when *T. delbrueckii* was used, the volatility acidity of the base wine decreased, the glycerol amount increased, and positive effects on foaming properties were found. These results agree with those found by Medina-Trujillo et al., who also detected improvements in foam and effervescence [12]. Regarding *M. pulcherrima*, the results showed that this yeast produces high amounts of β-glucosidase, reduces the volatile acidity, and implies an increase in medium chain fatty acids, esters, terpenols, and glycerol. Therefore, when it is inoculated in the first fermentation, it can improve the aroma profile of the base wine obtained, in addition to the foaming characteristics [12].

Thus, because non-*Saccharomyces* yeast can modify the amounts of amino acids, ammonia, glycerol, volatile aromatic compounds, and proteins, which results in a possible improvement in the wine's flavor and foaming capacity, it is unsurprising that there is growing interest in the use of these yeasts to produce sparkling wine.

The aim of this work was to compare the use of both *Saccharomyces* and non-*Saccharomyces* yeasts in the fermentation of base wine, in terms of the aromatic quality, protein content, and foaming capacity of cava.

2. Materials and Methods

2.1. Winemaking Process

Chardonnay (CH) and Xarel.lo (XA) grape varieties were used for the production of the studied cavas. For each grape variety, 100 kg of grapes harvested in 2018 was destemmed, crushed and distributed into 50 L stainless steel fermenters. The basic chemical composition

of the musts was as follows: for CH, brix grade 16.9; nitrogen assimilable by yeast 297 mg/L; ammonium 122 mg/L; pH 3.41; total acidity 8.23 g/L expressed as tartaric acid; and malic acid 5.7 mg/L, and for XA, brix grade 16.3; nitrogen assimilable by yeast 193 mg/L; pH 3.15; total acidity 6.45 g/L expressed as tartaric acid; and malic acid 1.9 mg/L. During grape processing, 40 mg/L of SO_2 (Sulphur 18, Agrovin S.A., Ciudad Real, Spain) was added to prevent oxidation and for microbiological control. The vinifications were carried out in duplicate for each variety on a pilot scale in the experimental VITEC winery. For each vinification, 26 L of must obtained from 40 kg of varietal grapes was used (65% yield). The cold settling was carried out with active pectolytic enzymes (Endozym Éclair, AEB Iberica S.A.U., Barcelona, Spain) at 11 °C overnight and the alcoholic fermentation was carried out at a controlled and constant temperature (17 °C) in cold water baths. To carry out the alcoholic fermentation to obtain the base wine, five different commercial yeasts were used: three different strains from *S. cerevisiae*, Y1 (IOC 18-2007, Institut Oenologique de Champagne, Epernay Cedex, France) indigenous yeast selected from the Champagne vineyards, Y2 (Viacell Rhône 4600, Lallemand SAS, Blagnac Cedex, France) yeast selected by the Inter-Rhône Technical Service in the northern Cotes du Rhône region, and Y3 (Sensy Yseotm Lalvin, Lallemand SAS, Blagnac Cedex, France) indigenous yeasts selected by natural crossing, and two non-*Saccharomyces* yeasts, Y4 (Flavia MP346, Lallemand SAS, Blagnac Cedex, France) pure culture of *Metschnikowia pulcherrima* and selected from nature by the University of Santiago de Chile (USACH), and Y5 (Level Biodiva, Lallemand SAS, Blagnac Cedex, France) pure culture of *Torulaspora delbrueckii*. The addition of each of the yeasts was carried out as recommended by the manufacturer. The course of the fermentations was monitored by the consumption of sugar, and it was considered complete when the residual sugar concentrations were below 0.5 g/L. In all cases, fermentation began 24–48 h after inoculation and lasted about 10 days. After fermentation, base wines were sulphited to reach 30 mg/L of free SO_2. After 24 h, wines were racked and clarified with the addition of 40 g/hL bentonite (Bentogran, AEB Iberica S.A.U., Barcelona, Spain). After that, and to carry out the second fermentation, base wines were bottled in glass bottles of 750 mL and, finally, cavas were obtained. For this, *S. bayanus* (Uvaferm PMA, Lallemand SAS, Blagnac Cedex, France) was added in the tirage solution. Tirage also contained 40 g/L adjuvant (Adjuvant 92, Station Oenotechnic of Champagne, France) and 20 g/L sugar (glucose). Finally, after disgorgement, cavas were aged on their lees for 18 months. Analyses were made in base wines (BW) just before tirage, and in cavas after 18 months of aging on lees (18M).

2.2. Enological Analysis

The enological parameters of samples were quantified by applying the methods recommended by the International Organization of Vine and Wine (OIV) [13]. These methods were the color intensity (CI) and the chromatic characteristics (measured by Helios-α spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) (OIV-MA-AS2-07B); total acidity (OIV-MA-AS313-01) and pH (OIV-MA-AS313-15) (measured potentiometry by using an automatic titrator, TitroMAtic Hach by Crison®, Barcelona, Spain); the content of glucose-fructose (OIV-MA-AS311-02) (measured by enzymatic reaction with a Y15 BioSystems device and Control Wine®, BioSystems S.A., Barcelona, Spain); the content of malic acid (OIV-MA-AS313-10) (also measured by enzymatic reaction).

The values of the alcoholic strength and the volatile acidity were obtained using a Fourier transform infrared spectroscopy (FTIR) system (WineScan™ by FOSS, Hilleroed, Denmark), internally calibrated according to OIV [13].

2.3. Protein Analysis

2.3.1. Total Protein Concentration Determined by UV Spectrophotometric Method

Protein quantification was performed using a UV-visible spectrophotometer (BioDrop µLite, from Thermo Fisher Scientific, Waltham, MA, USA) for micro sample volumes. For measurement and quantification, all of the protein powder extracted from 16 mL of wine

sample according to the preparation described by Silvestri et al. [14] was used. Thus, 40 µL of 0.16 M TRIS-HCL (pH 6.8) was added to the protein powder in a 1.5 mL tube and then placed in an ultrasound bath for 60 min at 50 °C. The measurement of the absorbance of the proteins was carried out at 280 nm using 3 µL of sample. Previously, 3 µL of 0.16 M TRIS-HCL (pH 6.8) was used as a blank.

2.3.2. Wine Protein Composition Evaluated by SDS-PAGE

Samples of base wines were analyzed by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure S1). A previous sample extraction treatment was carried out, which was used both for its quantification by UV and for gel electrophoresis, as described by Silvestri et al. [14]. The proteins of the extracted wine samples were separated using SDS-PAGE (15% resolving gel and 5% stacking gel) in a vertical electrophoresis unit with an applied voltage of 100 V. The marker used was Precision Plus Protein™ Dual color Standards (Bio-Rad, Hercules, CA, USA). The gels were stained with Coomassie Brilliant Blue R-250 (0.05%, *w*/*v*) in methanol/acetic acid/water (25:10:65, *v*/*v*/*v*) and decolorized in the same solution without the colorant.

2.4. Foamability

The foamability of the different cavas was measured using the Mosalux procedure [15]. Briefly, before making the first measurement, it was necessary to regulate CO_2 flow at 7 L/h and the pressure at 1 atm for 15 min. Then, the gas injection was stopped. To measure the foamability of the cavas, 100 mL of sample was used, which was previously filtered through a membrane with a porosity of 0.65 µm and warmed to 18 °C for 12 h. The sample was then placed in the Mosalux cylinder and CO_2 was injected through the glass frit. The maximum height reached by the foam (maximum) was first measured and then, when the foam sample showed stable persistence, the height was measured in a steady state (minimum). Both measurements were performed in duplicate for each sample.

2.5. GC-MS Analysis

The volatile compounds of base wines (BW) and cavas (18 M) were extracted using SPME (DVB/CAR/PDMS) and analyzed using a GC 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an inert mass spectrometer 5975C MSD (Electronic Shock Source Triple Axis Detector) according to the method developed by Torrens et al. [16]. The chromatographic conditions were those previously optimized and described [17]. The results of the volatile compounds were semi-quantitative data and expressed as µg/L in relation to the response provided by the internal standard (2-octanol). All analyses were performed in duplicate.

2.6. Sensory Analysis

The quantitative descriptive analysis (QDA) was performed by a trained tasting panel (by ISO 8586: 2012) in a sensory standardization room (ISO 8589: 2007). A total of ten cavas were tasted after 18 months of aging in the bottle on lees (18M). The analysis was carried out over two sessions, one session to taste Chardonnay cavas and the other session to taste Xarel.lo cavas. Prior to the sensory analysis, seven descriptors were selected to guide to the panelists when performing the evaluation. These were: fresh, candied, and dried fruit, floral, cake shop, honey, and evolution. The perceived aroma intensity was rated by each panelist from 0 to 5 and the data obtained were processed with the FIZZ software (V.2.47B Biosystems, Barcelona, Spain). A specific tasting sheet was designed according to the aforementioned olfactory descriptors.

2.7. Statistical Analysis

A simple analysis of variance (ANOVA) was carried out using the StatGraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA) program. Tukey's procedure was used and the differences at p-values < 0.05 were considered significant.

3. Results and Discussion

3.1. Enological Analysis

The values of glucose and fructose, malic acid, total acidity, and pH were determined for both Chardonnay and Xarel.lo of all base wines (BW) were analyzed before tirage (Table S1). In addition, the alcoholic strength, volatile acidity, and color characteristics were analyzed of all base wines and also of all cavas after 18 months of aging on lees (18M) (Table 1).

Table 1. Basic analysis of Chardonnay (CH) and Xarel.lo (XA) base wines (BW) and cavas after 18 months of aging on lees (18M), with the addition of five different *S. cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces* (Y4 and Y5) yeasts. Different letters indicate significant differences between samples with different yeasts for each grape variety, and in each of the analysis times. Units: alcoholic strength (%vol) and volatile acidity (g/L). Samples (n = 2).

CHARDONNAY						
	Alcoholic Strength	**Volatile Acidity**	**Abs. 420 nm**	**Abs. 520 nm**	**Abs. 620 nm**	**Color Intensity**
BW						
Y1	10.1 a ± 0.0	0.40 c ± 0.00	0.099 a ± 0.005	0.036 ab ± 0.002	0.011 ab ± 0.003	0.15 a ± 0.01
Y2	9.9 a ± 0.3	0.38 bc ± 0.00	0.179 a ± 0.098	0.047 b ± 0.002	0.013 b ± 0.001	0.24 a ± 0.10
Y3	10.1 a ± 0.0	0.52 d ± 0.01	0.090 a ± 0.012	0.023 a ± 0.006	0.006 a ± 0.002	0.12 a ± 0.02
Y4	10.5 a ± 0.6	0.33 a ± 0.01	0.118 a ± 0.011	0.035 ab ± 0.006	0.012 ab ± 0.000	0.17 a ± 0.02
Y5	9.7 a ± 0.0	0.37 b ± 0.01	0.122 a ± 0.023	0.038 ab ± 0.008	0.013 b ± 0.001	0.17 a ± 0.03
18M						
Y1	11.3 c ± 0.0	0.54 b ± 0.02	0.146 e ± 0.001	0.065 c ± 0.007	0.029 c ± 0.001	0.24 c ± 0.01
Y2	11.2 b ± 0.0	0.48 ab ± 0.04	0.123 d ± 0.001	0.036 b ± 0.001	0.019 abc ± 0.001	0.18 b ± 0.00
Y3	11.5 e ± 0.0	0.72 c ± 0.01	0.110 b ± 0.000	0.023 a ± 0.001	0.007 a ± 0.001	0.14 a ± 0.01
Y4	11.1 a ± 0.0	0.44 a ± 0.01	0.106 a ± 0.001	0.037 b ± 0.001	0.022 bc ± 0.001	0.16 b ± 0.01
Y5	11.3 c ± 0.0	0.41 a ± 0.01	0.119 c ± 0.001	0.030 ab ± 0.001	0.015 ab ± 0.007	0.16 b ± 0.01
XAREL.LO						
	Alcoholic Strength	**Volatile Acidity**	**Abs. 420 nm**	**Abs. 520 nm**	**Abs. 620 nm**	**Color Intensity**
BW						
Y1	9.9 a ± 0.0	0.26 a ± 0.02	0.075 a ± 0.013	0.016 ab ± 0.001	0.004 a ± 0.001	0.09 a ± 0.01
Y2	9.9 a ± 0.0	0.26 a ± 0.00	0.064 a ± 0.006	0.015 a ± 0.002	0.005 a ± 0.002	0.08 a ± 0.01
Y3	11.7 d ± 0.0	0.31 b ± 0.00	0.065 a ± 0.001	0.023 b ± 0.001	0.006 a ± 0.001	0.09 a ± 0.00
Y4	10.0 b ± 0.0	0.25 a ± 0.00	0.067 a ± 0.005	0.017 ab ±0.004	0.005 a ± 0.001	0.09 a ± 0.01
Y5	10.1 c ± 0.0	0.29 ab ± 0.01	0.081 a ± 0.001	0.019 ab ± 0.001	0.004 a ± 0.001	0.10 a ± 0.00
18M						
Y1	11.1 b ± 0.0	0.30 b ± 0.01	0.075 bc ± 0.000	0.017 b ± 0.001	0.008 c ± 0.000	0.10 c ± 0.01
Y2	11.4 e ± 0.0	0.29 b ± 0.01	0.074 b ± 0.001	0.018 b ± 0.001	0.003 a ± 0.001	0.09 b ± 0.00
Y3	11.3 d ± 0.0	0.35 c ± 0.01	0.065 a ± 0.001	0.010 a ± 0.000	0.002 a ± 0.000	0.08 a ± 0.1
Y4	11.0 a ± 0.0	0.24 a ± 0.00	0.078 c ± 0.001	0.016 b ± 0.001	0.005 b ± 0.000	0.10 c ± 0.1
Y5	11.2 c ± 0.0	0.30 b ± 0.01	0.087 d ± 0.001	0.027 c ± 0.000	0.015 d ± 0.001	0.13 d ± 0.01

All of the base wines (BW) had glucose and fructose values below 0.5 mg/L, which indicated that they were completely dry base wines. In the case of CH wines, they showed a total acidity between 7.4 and 8.4 g/L. The amount of malic acid varied between 5.3 and 5.5 mg/L, and pH values were around 3.5 units. The base wines made from XA showed lower values than those of CH. The total acidity concentration ranged from 5.3 to 6.3 g/L, malic acid values from 1.2 to 1.6 mg/L, and pH from 3.1 to 3.2.

Regarding the effects of the different yeasts on the wines, the results showed that the CH base wines did not present significant differences in terms of their alcoholic strength and color intensity values, regardless of the yeast used. However, the volatile acidity parameter showed significant differences for all of the yeasts used, with Y3 yeast providing the highest concentration, and non-*Saccharomyces* yeasts (Y4 and Y5) provided the lowest concentrations. This behavior is consistent with previous studies that also found that some non-*Saccharomyces*, such as *S. pombe*, *T. delbrueckii*, and *L. thermotolerans*, produced lower

concentrations of volatile acidity than some *S. cerevisiae* (approximately 0.1 g/L) [4,11]. The XA base wines also presented a similar trend, with wines fermented with Y3 presenting the highest volatile acidity, and the lowest concentration was found in wines fermented with the addition of *M. pulcherrima* (Y4). Wine fermented with the addition of *T. delbrueckii* (Y5) also showed low values on the volatile acidity content.

In addition, these same parameters were analyzed in the cavas after 18 months of aging on the lees (18M). All of them presented significant differences. In the CH variety, the cavas elaborated with Y3 presented the highest values of alcoholic degree and volatile acidity, while the cavas with *M. Pulcherrima* presented the lowest values, as already happened in BW. Additionally, the cavas elaborated with addition to *T. delbrueckii* presented very low concentrations of volatile acidity. In the XA variety, as in CH, the cavas with the addition of Y3 presented the highest values of alcoholic strength and volatile acidity. However, the cavas with Y4 present the lowest values of both parameters. Finally, regarding color intensity, few differences were observed. The highest value of color intensity was obtained in CH cava with Y1.

Previous studies [18,19] already pointed out that the use of yeasts other than *Saccharomyces* can reduce the alcoholic content of wine, which is in agreement with our data. Furthermore, it is well known that volatile acidity is one of the important parameters that influence the quality of wine or cava and also depends to a great extent on the type of yeast that undergoes alcoholic fermentation. As has been observed in recent studies [20] there are different non-*Saccharomyces* yeast strains related to producing a low content of volatile acidity, as occurs in our study, with the yeast *M. Pulcherrima* (Y4), which shows that they may have or generate desirable oenological properties in wines and cavas.

3.2. Protein Analysis

3.2.1. Total Protein Concentration Determined by UV-Visible Spectrophotometry

The results obtained sowed that the quantity of protein was more influenced by variety than by the yeast strain added. Specifically, whereas the total concentrations ranged from 78.2 to 44.5 mg/L in the CH samples, these ranged from 36.3 to 21.3 mg/L in the XA base wines. This relationship between the variety and the amount of protein was previously verified by Cilindre et al. who found a higher content of protein in Chardonnay than in Pinot Meunier base wines [21].

In addition, Dambrouck et al. also observed that a large portion of the wine protein came directly from grapes, which in turn was influenced by the variety; however, it should be taken into account that other proteins were produced by yeasts during the fermentation process [22]. This last consideration explains why, in the present study, non-*Saccharomyces* yeasts provided similar or even higher protein values than some *Saccharomyces* yeasts for the two grape varieties. The protein content obtained with *M. pulcherrima* (Y4) in XA and CH was as high as the highest value obtained by one of *the S. cerevisiae* (Figure 1).

3.2.2. Wine Protein Composition Evaluated by SDS-PAGE

Proteins released by yeast have been reported as exerting a positive effect on the foaming capacity of sparkling wines [11]. Soluble protein fractions of CH and XA base wines were evaluated to study their relationship with foam characteristics. For CH variety, the MW values ranged between 15.9 and 97.7 KDa, and between 18.2 and 92.2 KDa for the XA variety (Table 2). In general, SDS-PAGE did not show notable differences in the distribution and intensity of protein bands between proteins extracted with *Saccharomyces* (Y1, Y2, Y3) and non-*Saccharomyces* yeasts (Y4, Y5), as was observed in previous studies [23].

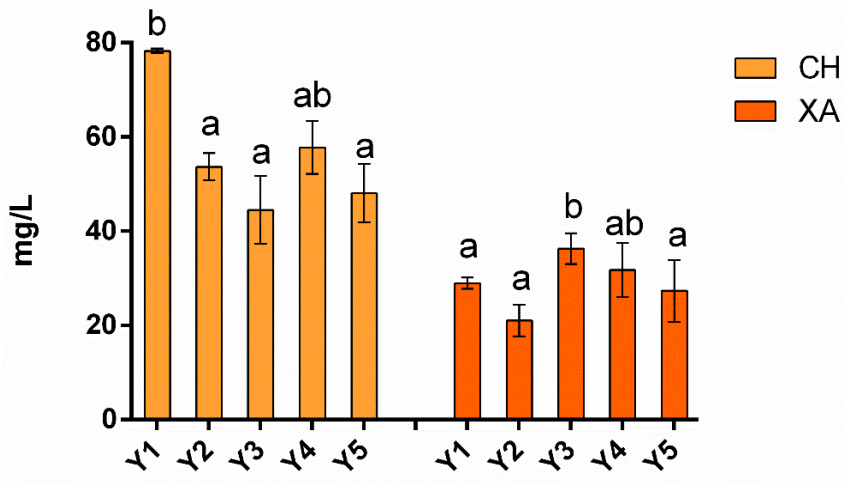

Figure 1. Total protein content base wines of Chardonnay (CH) and Xarel.lo (XA) elaborated with the addition of different *S. cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces* yeasts, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5). Different letters indicate significant differences between samples with different yeasts for each variety, Chardonnay (CH) and Xarel.lo (XA). Samples (n = 2).

Table 2. Analysis of the protein profile by SDS-PAGE and quantification in terms of the percentage of protein bands fractions (%) by detecting their molecular weights (MW), of the base wine of Chardonnay (CH) and Xarel.lo (XA) elaborated with different yeast strains: *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-Saccharomyces, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5).

CH Y1 (Bands)	V12	V13	V14	V15	V16	V17		
MW (Kda)	96.1	59.4	28.6	26.1	22.8	19.4		
%	12.8	2.7	9.1	7.4	63	5.1		
CH Y2 (Bands)	V30	V19	V20	V21	V22	V23		
MW (Kda)	97.7	64.3	27.2	24.9	22.1	18.6		
%	10.1	2.7	11.6	25.2	48.4	2		
CH Y3 (Bands)	V19	V20	V21	V22	V23	V24	V25	V26
MW (Kda)	66.7	57.4	47.2	40.4	32.7	26.4	21	18
%	14.6	12.8	5.7	5.7	7.2	7.2	32.2	2.2
CH Y4 (Bands)	V11	V12	V13	V14	V15	V16	V17	V18
MW (Kda)	74.8	53.9	36.3	28.2	23.4	20.2	18.7	15.9
%	28.8	4.5	2.4	12.2	42.9	3.7	4.3	1.2
CH Y5 (Bands)	V1	V2	V3	V4	V5	V6	V7	V8
MW (Kda)	68.5	48.7	35.9	26.8	23.1	20.3	18	16.2
%	20.2	2.9	5.7	25.1	30	3.7	3.2	9.3
XA Y1 (Bands)	V29	V30	V31	V32				
MW (Kda)	68.4	26.9	21.4	18.2				
%	27.6	33.9	26.5	12.1				
XA Y2 (Bands)	V22	V23	V24	V25				
MW (Kda)	73.2	29.5	27.6	25.5				
%	43.9	26.8	19.5	9.8				
XA Y3 (Bands)	V11	V12	V13	V14	V15			
MW (Kda)	92.1	32.9	28.9	25.3	22.9			
%	10.5	14.4	3.2	70.4	1.5			
XA Y4 (Bands)	V1	V2	V3	V4	V5			
MW (Kda)	91.6	31.7	29.5	27.6	23.9			
%	24.9	3.3	38.2	15.4	18.3			
XA Y5 (Bands)	V25	V26	V27	V28	V29			
MW (Kda)	92.2	31.5	28.7	25	20.8			
%	28.1	50.2	11.3	6.1	4.3			
10% <						< 80%		

As shown in Table 2, Chardonnay samples showed the most intense bands around molecular weights between 21 and 24 KDa. When observing the yeast effect, CH base wines fermented with the addition of Y1 presented up to 63% of their total content with an MW of 22.8 KDa. A similar trend was observed in wines with the addition of Y2, which showed 48.4% protein of 22.1 KDa and 25% of 24.9 KDa of MW. The wine with the addition of Y3 showed differences with respect to the previous wines, because although it presented 30% protein with MW of 21 KDa, this sample also presented around 30% of the proteins with higher molecular weights of 66.7 and 57.4 KDa. Wines fermented with the addition of non-*Saccharomyces* yeasts (Y4 and Y5) presented the highest content in proteins with high MW (28.8% of 74.8 KDa in the case of Y4 and 20.2% of 68.5 KDa in the case of Y5). Regarding base wines produced by Xarel.lo, we can observe that the Y1 and Y2 wines presented the highest MW percentages at 68.4 and 26.9 KDa values for Y1, and 73.2 and 29.5 KDa values for Y2. Y3 wine showed up to 70% of its proteins with MW of 25.3 KDa. However, and as happened in the CH variety, the wines with the Y4 and Y5 yeasts presented between 30% and 50% protein with MW around 30 KDa, and between 20% and 30% protein with the highest MW, 91.6 and 92.2 KDa, respectively. Therefore, it can be highlighted that the Chardonnay variety presented a greater number of bands (protein fractions) compared to the Xarel.lo variety, which could be influenced by the different quantity of proteins extracted from the grape. González-Royo et al. [11] observed that wines elaborated with sequential inoculation with *T. delbrueckii* and *M. pulcherrima* had higher values of lower molecular weight (LMW, molecular weight < 60 kDa) compared to the control wines (wines without addition of non-*Saccharomyces* yeasts), which related to the improved foam parameters observed in wines produced with non-*Saccharomyces* yeasts. In our study, this trend was observed in XA cavas produced from base wines of Y4 (*M. pulcherrima*), which presented higher values of LMW (molecular weight <60 kDa). This fact could explain the height of foam stability (HS) value observed in Y4 (Figure 2).

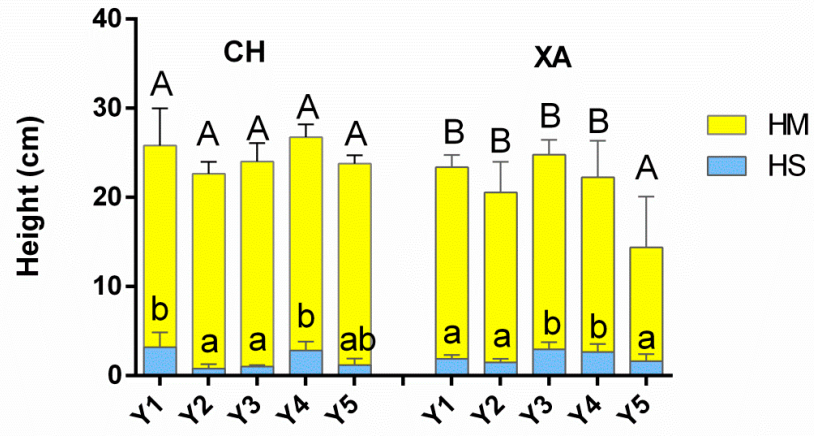

Figure 2. Foamability measurements of the different Chardonnay (CH) and Xarel.lo (XA) base wines made with different yeasts strains, *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces*, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5), using the Mosalux procedure. HM indicates the maximum height reached by the foam and HS the height in a steady state. Different letters indicate significant differences between samples with different yeasts. Uppercase letters for HM and lowercase letters for HS. Samples (n = 2).

3.3. Foamability

Figure 2 shows the values of the parameters HM (maximum foam height) and HS (height of foam stability) for each of the studied cavas (CH and XA). In general, as can be seen, CH cava presented slightly higher HM values than XA, as was observed in the previous studies by Andrés-Lacueva et al. [24] and Vanrell et al. [25], in which Chardonnay cavas showed higher HM values than Macabeo, Xarel.lo, and Parellada cava wines. However, the HS values were similar in all varieties.

By comparison, when the possible influence of the different yeasts on foamability was studied, it was observed that the highest HM and HS measurements were obtained when using Y1 and Y4 for the Chardonnay variety and Y3 and Y4 for the Xarel.lo variety. As can be seen, the samples that provided the highest HM values coincided with those with the highest values of protein content, so it can be concluded that there is a direct relationship between both parameters. These results agree with previous studies which reported that proteins and mannoproteins released by yeasts exert a positive effect on the foam [26], and that an increase in the amount of protein could lead to an increase in foamability [26] and lower velocities of foam dissipation [27]. Therefore, in this study, the results indicate that the wines examined with the addition of *M. pulcherrima* at first fermentation presented good foamability. Furthermore, this may be related to the high protein content, which can stabilize the bubble film due to its surface properties [26].

3.4. Volatile Composition

Among the different volatile compounds, a total of 18 were selected because they represent the four most abundant families (esters, acetates, alcohols, and fatty acids) of wines after carrying out the alcoholic fermentation. As shown in Table 3, these were analyzed in base wines (BW) and cavas, after 18 months of aging bottled on lees (18M).

Table 3 shows the total volatile concentration of the base wines and cavas. In general, and as expected, there was an aromatic decrease between the analysis times, before tirage in base wines (BW) and after 18 months of aging on lees (18M) as previously observed in other studies [28]. When observing BW values, a higher content of volatiles was found with the XA variety. However, when comparing the different yeasts, both varieties of wine showed the same trend. Thus, the volatile content was similar regardless of the yeast used, except when Y3 was used, which presented significant differences with respect to the other yeasts and provided the lowest volatile concentration.

Regarding the values after 18 months of aging on the lees, the aromatic content values were similar between varieties; thus, considering its higher volatile contents in base wine, XA suffered a greater decrease. When observing each of the varieties independently, greater significant differences were observed between the five yeasts used with respect to BW. In addition, the yeast *T. delbrueckii* (Y5) stood out in both varieties, with which high values of aroma concentrations were obtained, similar and/or higher than those obtained by some *Saccharomyces* yeasts.

To better study the results obtained, the different chemical families were separately studied. Thus, in relation to esters, in general these showed the same trend as the one described above for total aromas (Table 3). This behavior is due to the fact that the esters are the most abundant chemicals found in wine aroma and, therefore, are those that contribute the most to the total aroma. Furthermore, this family is of great importance because it provides the two important descriptors, fruit and flowers [29,30]. The base wines with non-*Saccharomyces* yeasts (Y4 and Y5) presented the highest concentrations of esters in CH, and values equal to or higher than the other *Saccharomyces* yeasts in XA. After 18 months of aging on the lees, higher amounts of esters were found when using Y2 and Y5 and, when dealing with XA cavas, also Y1. With reference to the different esters found, the highest concentrations of ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were observed in Y2 and Y5 in CH at 18 M (Table S2). Y5 also showed the highest value of ethyl isovalerate in XA cavas. These compounds are directly related to the fruity notes of pineapple, apple, pear, anise, and flowers (Table S2). Furthermore, in general, all of these compounds presented concentrations well above their sensory limit (Table S2), with the exception of ethyl butyrate, which only exceeded it in some cases. Therefore, non-*Saccharomyces* yeasts, such as *T. delbrueckii* (Y5), presented and conserved the same or higher concentrations of esters than some *Saccharomyces* yeasts.

Table 3. Families of volatile compounds (μg/L) from Chardonnay and Xarel.lo base wines (BW) and cavas, after 18 months of aging on the lees (18M), with the addition of five different yeasts in the alcoholic fermentation (Y1, Y2, Y3, Y4, Y5). Different letters indicate significant differences between samples with different yeasts for each grape variety, and in each of the analysis times. Samples (n = 2).

	CHARDONNAY					XAREL.LO				
Sample BW	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
ESTERS	26,557 b ± 387	29,560 b ± 311	17,351 a ± 209	29,375 b ± 357	29,256 b ± 825	30,854 ± 1266	32,710 b ± 1173	21,101 a ± 101	29,373 ab ± 2103	30,109 b ± 3834
Ethyl butyrate	164 b ± 2	208 b ± 8	88 a ± 11	210 b ± 17	201 b ± 16	599 ab ± 94	669 b ± 127	329 a ± 2	391 ab ± 26	411 ab ± 81
Ethyl isovalerate	267 b ± 4	233 b ± 0	111 a ± 19	265 b ± 35	241 b ± 8	279 b ± 13	278 ab ± 15	161 a ± 1	179 ab ± 19	270 ab ± 60
Ethyl hexanoate	5143 b ± 251	5272 b ± 134	2182 a ± 217	5326 b ± 704	5265 b ± 160	7149 b ± 244	7988 b ± 100	3755 a ± 14	6730 ab ± 336	7171 b ± 1283
Ethyl octanoate	14,015 b ± 172	15,199 b ± 56	8683 a ± 1498	15,457 b ± 41	15,060 b ± 192	14,242 b ± 464	15,093 b ± 738	9426 a ± 15	13,970 b ± 815	13,765 b ± 1901
Ethyl decanoate	6825 ab ± 286	8481 b ± 222	6027 a ± 399	7903 ab ± 1034	8321 b ± 463	7939 a ± 412	8065 a ± 634	7003 a ± 67	7602 a ± 78	7895 a ± 477
Ethyl dodecanoate	123 a ± 16	152 ab ± 5	243 b ± 47	198 ab ± 36	153 ab ± 4	621 a ± 42	595 a ± 79	413 a ± 3	468 a ± 119	556 a ± 35
Diethyl succinate	16 a ± 1	14 a ± 2	18 a ± 1	14 a ± 2	14 a ± 1	24 ab ± 3	21 a ± 4	13 a ± 0	34 b ± 4	36 b ± 3834
ACETATES	13,319 b ± 229	13,029 b ± 274	9219 a ± 319	14,189 b ± 1565	12,988 b ± 426	17,177 a ± 1237	16,816 a ± 1096	14,721 a ± 685	13,728 a ± 1091	16,104 a ± 783
Ethyl acetate	2809 a ± 45	3060 a ± 107	2902 a ± 67	2928 a ± 151	3016 a ± 150	4819 b ± 273	4492 b ± 49	4801 b ± 67	3195.3 a ± 250	4234 b ± 254
Isoamyl acetate	10,270 b ± 185	9741 b ± 158	6190 a ± 249	11,043 b ± 1412	9745 b ± 264	12,137 b ± 934	12,063 b ± 101	9724 a ± 621	10,342.3 ab ± 826	11,603 b ± 517
Hexyl acetate	81 bc ± 0	88 c ± 2	43 a ± 0	71 b ± 1	75 b ± 5	62 b ± 9	65 b ± 3	41 a ± 0	53.4 ab ± 4	58 ab ± 3
2-phenylethyl acetate	159 c ± 1	140 b ± 7	83 a ± 2	146 bc ± 1	151 bc ± 7	159 a ± 20	195 a ± 31	154 a ± 3	137 a ± 11	208 a ± 10
ALCOHOLS	3703 ab ± 59	3217 a ± 150	3823 ab ± 9	3944 b ± 132	3682 ab ± 339	7611 a ± 4	7671 a ± 147	8166 a ± 189	8282 a ± 711	7965 a ± 341
Isoamyl alcohol	3147 ab ± 54	2705 a ± 139	3291 ab ± 9	3408 b ± 120	3125 ab ± 306	6609 a ± 55	6592 a ± 137	7014 b ± 176	7146 b ± 623	6914 ab ± 280
Isobutanol	117 b ± 1	105 a ± 2	103 a ± 2	95 a ± 1	101 a ± 5	78 ab ± 1	81 ab ± 8	64 a ± 1	100 c ± 1	85 bc ± 5
Benzyl alcohol	2 a ± 0	2 cd ± 0	2 b ± 0	2 c ± 0	3 d ± 0	5 a ± 0	6 ab ± 0	5 a ± 0	6 ab ± 0	7 b ± 0
2-phenylethyl alcohol	437 a ± 6	405 a ± 16	426 a ± 1	439 a ± 11	452 a ± 29	919 a ± 50	991 a ± 19	1082 b ± 14	1030 b ± 86	959 a ± 56
FATTY ACIDS	924 b ± 19	1016 b ± 18	543 a ± 3	963 b ± 12	1032 b ± 55	1362 b ± 31	1626 b ± 131	819 a ± 4	1353 b ± 110	1640 b ± 102
Hexanoic acid	169 b ± 10	168 b ± 2	90 a ± 0	151 b ± 1	172 b ± 5	223 b ± 2	260 bc ± 8	117 a ± 1	223 b ± 21	282 c ± 22
Octanoic acid	511 b ± 3	550 b ± 12	273 a ± 2	516 b ± 15	561 b ± 29	786 b ± 17	930 b ± 69	461 a ± 4	790 b ± 62	944 b ± 56
Decanoic acid	243 b ± 6	299 c ± 3	180 a ± 1	296 c ± 4	299 c ± 19	353 ab ± 16	435 b ± 54	242 a ± 0	339 ab ± 37	414 b ± 24
TOTAL AROMAS	44,503 b ± 538	46,824 b ± 138	30,937 a ± 2410	48,471 b ± 1327	46,957 b ± 5	57,004 ab ± 2538	58,823 b ± 2547	44,808 a ± 601	52,737 ab ± 4015	55,819 ab ± 5061
	CHARDONNAY					**XAREL.LO**				
Sample 18M	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
ESTERS	11,917 a ± 140	15,320 c ± 13	10,801 a ± 107	11,867 a ± 438	13,842 b ± 487	13,274 d ± 118	13,837 d ± 192	9072 a ± 297	10,390 b ± 134	11,931 c ± 12
Ethyl butyrate	305 b ± 3	437 d ± 6	258 a ± 14	283 ab ± 3	370 c ± 2.	417 d ± 0	423 d ± 4	237 a ± 0	328 b ± 1	387 c ± 7
Ethyl isovalerate	64 a ± 1	113 bc ± 2	71 a ± 0	110 b ± 4	126 c ± 7	67 b ± 0	89 c ± 2	51 a ± 1	64 b ± 1	120 d ± 3
Ethyl hexanoate	3452 a ± 89	4261 c ± 39	3240 a ± 71	3271 a ± 42	3792 b ± 47	4110 cd ± 49	4353 d ± 65	2505 a ± 103	3625 b ± 66	3875 bc ± 45

Table 3. *Cont.*

	CHARDONNAY					XAREL.LO				
Sample 18M	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Ethyl octanoate	6105 ab ± 19	7805 c ± 35	5351 a ± 34	6044 a ± 347	7013 bc ± 390	6761 d ± 11	7549 e ± 238	4562 a ± 120	5370 b ± 29	6121 c ± 48
Ethyl decanoate	1938 a ± 27	2649 d ± 13	1824 a ± 13	2112 b ± 41	2475 c ± 40	1854 c ± 154	1353 b ± 20	1660 bc ± 70	943 a ± 35	1369 b ± 4
Ethyl dodecanoate	19 b ± 0	18 a ± 0	20 c ± 0	25 d ± 0	53 e ± 0	38 b ± 1	37 b ± 0	20 a ± 0	37 b ± 2	37 b ± 0
Diethyl succinate	33 c ± 1	36 d ± 0	37 d ± 1	21 b ± 0	12 a ± 0	25 a ± 0	33 b ± 1	36 b ± 2	24 a ± 1	23 a ± 0
ACETATES	9613 b ± 2	12,472 d ± 28	13,010 e ± 268	9086 a ± 90	10,955 c ± 701	9724 b ± 85	10,018 bc ± 172	8491 a ± 96	8132 a ± 38	10,387 c ± 31
Ethyl acetate	5715 b ± 68	7168 b ± 23	8496 c ± 170	4554 a ± 15	5853 b± 24	5703 b ± 6	5490 b ± 75	5432 b ± 35	4500 a ± 16	5402 b ± 37
Isoamyl acetate	3776 a ± 67	5136 c ± 42	4399 b ± 92	4430 b ± 73	4961 c ± 37	3931 c ± 78	4405 d ± 95	2978 a ± 61	3535 b ± 21	4828 e ± 5
Hexyl acetate	104 bc ± 2	143 d ± 2	95 b ± 5	67 a ± 1	117 c ± 9	51 b ± 1	67 c ± 1	28 a ± 0	51 b ± 0	97 d ± 1
2-phenylethyl acetate	17 a ± 1	24 ab ± 6	20 a ± 0	34 b ± 0	23 ab ± 0	39 a ± 0	56 d ± 1	52 c ± 0	46 b ± 0	59 e ± 0
ALCOHOLS	5437 b ± 88	6353 c ± 7	7507 d ± 180	4964 a ± 24	5621 b ± 39	6153 b ± 16	6063 b ± 105	6175 b ± 78	6066 b ± 14	5657 a ± 25
Isoamyl alcohol	4879 b ± 77	5780 c ± 9	4806 b ± 15	4465 a ± 21	5110 b ± 18	5402 c ± 16	5241 bc ± 94	4960 a ± 45	5089 ab ± 7	4960 a ± 24
Isobutanol	114 ab ± 5	146 cd ± 2	168 d ± 13	97 a ± 0	134 bc ± 1	135 bc ± 0	132 b ± 1	99 a ± 5	147 cd ± 4	149 d ± 2
Benzyl alcohol	2 a ± 0	4 c ± 0	6 e ± 0	5 d ± 0	3 b ± 0	4 b ± 0	4 c ± 0	4 a ± 0	5 d ± 0	4 a ± 0
2-phenylethyl alcohol	441 b ± 6	424 ab ± 0	527 c ± 15	396 ab ± 3	374 a ± 3	611 b ± 0	686 c ± 10	1111 e ± 30	826 d ± 19	544 a ± 1
FATTY ACIDS	845 b ± 3	988 d ± 2	625 a ± 14	877 c ± 4	853 bc ± 0	875 b ± 5	1068 d ± 4	709 a ± 10	945 c ± 14	971 c ± 9
Hexanoic acid	214 c ± 1	243 d ± 1	150 a ± 0	201 b ± 3	210 c ± 0	223 b ± 3	259 c ± 2	144 a ± 3	231 b ± 7	236 b ± 4
Octanoic acid	497 b ± 3	589 d ± 0	352 a ± 7	525 c ± 1	509 b ± 0	511 b ± 0	628 e ± 11	404 a ± 2	554 c ± 2	584 d ± 0
Decanoic acid	133 a ± 1	156 b ± 1	122 a ± 6	151 b ± 1	133 a ± 0	141 a ± 2	181 c ± 5	160 b ± 5	160 b ± 5	151 ab ± 5
TOTAL AROMAS	27,811 a ± 47	35,134 c ± 9	31,943 b ± 569	26,794 a ± 556	31,271 b ± 596	30,026 d ± 225	30,987 d ± 89	24,446 a ± 481	25,534 b ± 200	28,946 c ± 15

Regarding acetates, their highest concentration in CH base wine was shown with yeast Y4 but, in contrast, in XA this yeast provided the lowest concentration. In general, the main compounds that showed significant differences were isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate (Table 3), which provide aromas of banana, pear, and green tea, respectively (Table S2) [31,32]. Regarding the 18M analysis time, the highest concentrations of acetates were observed in the Chardonnay variety with the yeasts Y2, Y3, and Y5. It should be noted that, for cavas with Y2 and Y5 yeasts, the main compounds responsible for these high concentrations were isoamyl acetate and hexyl acetate. In the case of cava with yeast Y3, the increase was due to the ethyl acetate. This compound should be carefully considered because it provides positive aromas such as sweet fruits when it appears at low concentrations, but its presence at high concentrations implies undesirable aromas, such as glue or solvent. In this case, the cava with the Y3 yeast presented a high concentration of this compound, with a concentration of 8496 μg/L (Table 3), and was the only sample to exceed the sensory limit (LS of ethyl acetate 7500 μg/L) [33]. This indicates that there are some species of *S. cerevisiae* which provide certain aromatic deviations in cavas, unlike other non-*Saccharomyces* yeasts which do not produce them. The Xarel.lo cavas presented the highest concentrations of acetates with the three yeasts Y1, Y2, and Y5. These high values were mainly due to the isoamyl acetate compound, with values well above its sensory limit (670 μg/L) [32]. This could give the cavas a marked tropical aromatic profile because banana is their main aromatic descriptor. In addition, in cavas with Y2 and Y5, higher concentrations of isoamyl acetate and 2-phenylethyl acetate were observed. All of these results show that the yeast *T. delbrueckii* (Y5) is also capable of providing a more marked tropical character in cavas, unlike others in which only *Saccharomyces* yeasts were used.

Regarding aromatic alcohols, a different trend between the two grape varieties was observed. Whereas Xarel.lo presented a slight decrease in alcohol concentrations during aging, the Chardonnay variety showed a slight increase in the concentration of alcohol. However, despite these different trends, after 18 months of aging on lees, it was observed that both varieties present similar values of total alcohols, with a concentration range between 4964 and 6353 μg/L. In reference to the analyses of alcohols in Chardonnay BW, all of the base wines presented similar values, obtaining the highest value with yeast Y4 and the lowest concentration with Y2. For the Xarel.lo variety, the high concentrations of alcohols produced by Y3 and Y4 yeasts stand out. These high values were mainly due to two of the studied compounds: isoamyl alcohol and 2-phenylethyl alcohol. These compounds provide floral aromas of roses, pollen, and perfume [1,34]. Regarding the 18M time, in the Chardonnay variety, the cava obtained with Y2 had the highest concentration followed by Y5, Y3, and Y1. This is mainly due to the obtained concentrations of the isoamyl alcohol compound (Table 3). In XA, all of the cavas generally presented similar values, with the exception of the cava with yeast Y5, which presented a slightly lower value. Therefore, with respect to the chemical family of alcohols, it could be noted that the yeast *M. pulcherrima* (Y4) implied the highest alcohol content of BW but, after aging on lees, these values were generally similar to those obtained by the *Saccharomyces* yeasts, as observed in the XA variety.

Finally, in the family of fatty acids, the cava obtained with the Y3 yeast presented the lowest concentrations of fatty acids (in all studied cases), with significant differences compared to the other yeasts. In Chardonnay, four base wines presented similar values (Y1, Y2, Y4, Y5). However, for the Xarel.lo variety, the highest concentrations of fatty acids obtained when using Y2 and Y5 stood out. It should be noted that *T. delbrueckii* (Y5) presented the highest fatty acid values. These high values were mainly due to the concentrations obtained for hexanoic acid and octanoic acid (Table 3). These compounds give aromas of soap, cheese, and yogurt (Table S2). By comparison, after 18 months of aging on the lees, the cava with the Y2 yeast presented the highest concentrations of fatty acids in both varieties. However, it could be noted that non-*Saccharomyces* yeasts (Y4 and Y5) presented similar or higher values than those produced by other *Saccharomyces* yeasts (Y1 and Y3).

3.5. Sensory Analysis

Figure 3 shows the results of sensory analysis of the cavas after 18 months of aging in the bottle on lees. The Chardonnay variety showed the highest values of the fresh fruit descriptor when the Y2 and Y5 yeasts were used. This behavior may be directly related to the high concentrations of esters and acetates that were observed in the analytical analysis. Furthermore, it was observed how cava obtained with Y2 and Y4 presented the highest scores for bakery and floral descriptors, in addition to Y5 for the latter. These aromatic descriptors are related to the high concentrations of fatty acids and alcohols, which are related to lactic and rose aromas, respectively. In comparison, it was shown that the cava obtained with the yeast Y3 was the most evolved sensorial and also showed the highest scores of candied fruit and evolution descriptors, which could be due to its high content of ethyl acetate. In the Xarel.lo variety, it was observed that the cavas with the highest fresh fruit descriptors were those made with Y4 and Y5 yeasts, followed by those obtained with Y2. This can be directly related to the high concentrations of esters and acetates found in gas chromatography analysis. As happened with the Chardonnay variety, Xarel.lo cava obtained with Y3 was the most sensorially evolved. Therefore, it can be emphasized that non-*Saccharomyces* yeasts provided more fruity and fresh aromatic profiles, especially in the case of *T. delbrueckii*, and much less evolved than most *Saccharomyces* yeasts.

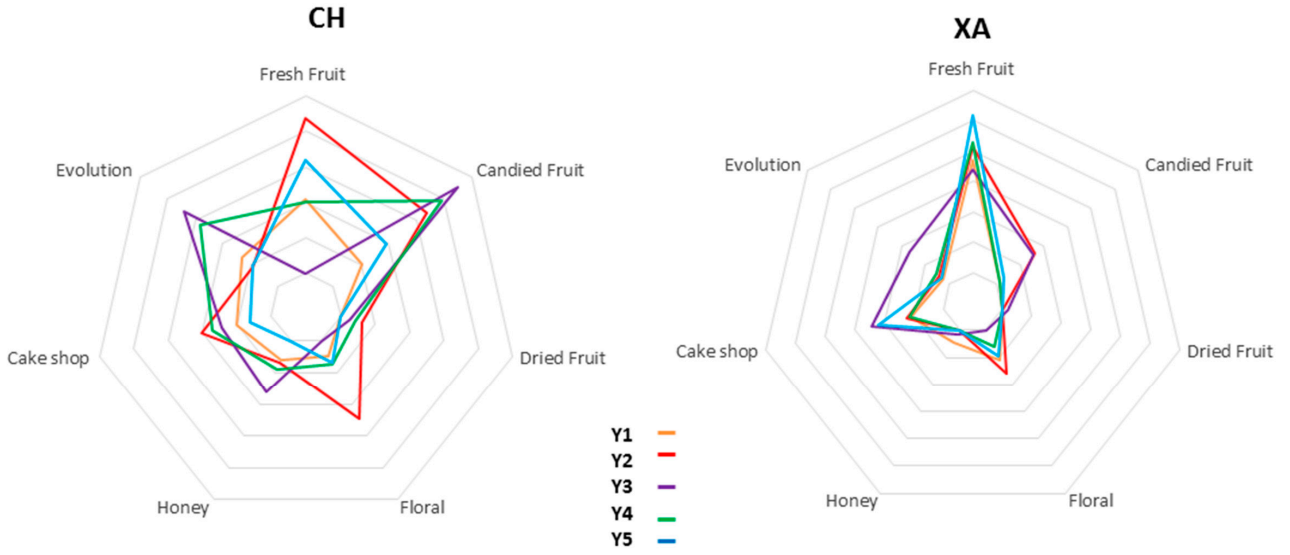

Figure 3. Aromatic profiles of the cavas obtained after 18 months of aging on lees, of the Chardonnay (CH) and Xarel.lo (XA) varieties made with five different yeasts strains (Y1, Y2, Y3, Y4, Y5). Results obtained by the mean and standard deviation of the scores given by the tasters.

4. Conclusions

The use of different *Saccharomyces* and non-*Saccharomyces* yeasts strains for the production of cavas allows products with certain distinctive chemical–physical and sensory attributes to be obtained. In addition, the different aromatic profiles of the wines not only appear after the moment of vinification with the addition of different yeasts, but certain aromatic, taste, and visual distinctions with respect to foamability also appear, after a long time of aging on the lees. In this study, it was possible to corroborate that the use of non-*Saccharomyces* yeasts allowed cavas with a similar or even better organoleptic quality to be obtained than those made with *Saccharomyces*. This was the case of the base wine made with *M. pulcherrima* yeast (Y4), which provided cavas with better persistence in foaminess. Moreover, the cavas made with *T. delbrueckii* preserved a higher concentration of aromas after 18 months of aging on the lees, with the subsequently more complex sensory properties, than those obtained with respect to *S. cerevisiae*. These results open the door to new

studies that allow for longer aging periods to be evaluated, testing the behavior of other varieties, and verifying the effect of the combination of yeasts other than *Saccharomyces*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fermentation7020064/s1, Table S1. SDS-PAGE of the proteins of Chardonnay (CH) and Xarel.lo (XA) base wines elaborated with different yeasts strains, *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces*, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5).

Author Contributions: A.M.M.: participated on the analysis of samples, manuscript redaction and editing. M.P.: performed and designed the experiment. I.A.: participated in the manuscript. N.E.: participated in the analysis of samples. S.d.L.: performed and designed the experiment, and also contributed to obtaining the funding resources. M.M.: participated in the redaction and revision of the manuscript, R.F.-G.: participated in the sample analysis, redaction, revision and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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

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