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The diversity of effects of yeast derivatives during sparkling wine aging

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ABSTRACT

This study shows the monitoring of the physical, chemical and sensorial changes that occur in the sparkling wine along 18 months of aging due to different typology yeast-derived products; dry inactivated yeast from Saccharomyces (*Saccharomyces cerevisiae*) and non-Saccharomyces (*Torulaspora delbrueckii*) yeast strains, yeast autolysate, and yeast protein extract tested at two different doses. The addition of 5 g/hL yeast protein extract and inactivated yeast from *T. delbrueckii* helped to preserve esters in wines with 9 and 18 months of aging on lees. The addition of yeast autolysate achieved greater polysaccharide enrichment and gave rise to sparkling wines with the highest antioxidant activity. Effects on foaming properties were quite different depending on the aging time. Despite this, sparkling wines treated with 10 g/hL of yeast autolysate and Optimum White™ generally exhibited the highest foamability and foam stability. Further experiments with higher doses are needed to observe clear effects on sensory profile.

1. Introduction

The production of high-quality sparkling wines consists of a second fermentation process in steel tanks (*Charmat* method) or in bottles (*Champenoise*/traditional method). The second fermentation in the bottle is followed by an aging period on lees (mainly composed of yeast and tartaric acid; and inorganic matter, to a lesser extent), during which yeast autolysis occurs (Pérez-Serradilla & De Castro, 2008). This catabolic process is triggered by the conditions of low pH, lack of nutrients, carbon dioxide, high alcohol concentration, and low storage temperature and characterized by the hydrolysis action of internal proteases from dead yeast cells (Alexandre & Guilloux-Benatier, 2006). Autolysis occurs once the second fermentation is complete and the intracellular content is released two to six months after completion of this fermentation (Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2006); however, it is strongly dependent on aging conditions such as temperature, wine pH, ethanol content, and the nature of the yeast strain (Alexandre & Guilloux-Benatier, 2006). Positive sensory effects of the aging on yeast lees are easily perceived after 9 months of contact; therefore, this is the minimum period required in Europe for the labelling of sparkling wines produced by the *Champenoise* method such as Cava, Champagne, or Talento (Commission Regulation (EC) N°606/2009). From the beginning of autolysis, several interesting intracellular and cell wall constituents such as lipids, carbohydrates, nucleotides, amino acids, peptides, proteins, polysaccharides (mainly mannoproteins), and volatile compounds are released into the media, providing different characteristics to the wine (Alexandre & Guilloux-Benatier, 2006). Special interest has been paid to the study of the effects of these compounds in sparkling wines (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009; Martínez-García et al., 2020) because of their substantial contribution to the organoleptic properties of the final product (Comuzzo, Tat, Tonizzo, & Battistutta, 2006; Rodriguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2012). Proteins, peptides, and amino acids, (Kemp et al., 2019) as well as polysaccharides (Moreno-Arribas, Pueyo, Nieto, Martín-Álvarez, & Polo, 2000) have shown positive effects on the foaming properties. Changes in volatile

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compounds have also been studied during aging on lees. These compounds include the compounds excreted from the cell as a result of autolysis and the volatile compounds released from the aromatic precursors due to the action of the enzymes released from the dead yeast. In addition, the higher glutathione content in yeast has demonstrated antioxidant properties that prevent browning and reduce the amount of SO_2 used in the winemaking process (Bahut et al., 2020). Because of the importance of the presence of these compounds from yeast on the quality of sparkling wine, the winemaking industry has been employing yeast-derived products (YDs) to improve the organoleptic characteristics of this beverage (Núñez et al., 2006; Pérez-Magariño et al., 2015; Rodriguez-Nogales et al., 2012). These YDs are obtained from *Saccharomyces* and *non-Saccharomyces* and are commercialized as inactivated yeast, yeast autolysate, yeast protein extract, yeast cell wall, and yeast mannoprotein (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009). The study of the effects of these YDs on the properties of still wine are well known, but information on the effects of their addition before the second fermentation in sparkling wines is scarce. Rodriguez-Nogales et al. (2012) studied the changes in color, total phenols index, flavanols, proteins, and sensorial characteristics for 9 months after the addition of purified mannoproteins, autolysate, and cell walls. On the other hand, Pérez-Magariño et al. (2015) also determined the phenolic compounds, free amino acids, biogenic amines, mannoproteins, volatile compounds, foam properties, and sensory properties after 9 months of aging by testing different kinds of autolysates. More recently, Sartor et al. (2019) studied the changes in organic acids and the polyphenolic profile of sparkling wines treated with mannoproteins. Depending on the characteristics of the harvest and the base wine, besides the oenologist preferences, it may be difficult to choose the proper YD to achieve a specific effect in the sparkling wine, given that there is a lack of a comprehensive study providing a global vision including the analysis of chemical, physical and sensorial parameters along aging on lees of sparkling wine employing different typology of yeast-derived products. Starting from the hypothesis that each YD is going to fit better by enhancing certain wine characteristics and some of them will work better for short aging times and others for longer periods, the collection of this information would allow evaluating which type of YD to use depending on the characteristics that the oenologist want to upgrade in the resulting sparkling wine. Therefore, the aim of this work was to perform a comprehensive monitorization of chemical (volatile, polysaccharide, and protein composition), physical (colour and foaming properties), and sensorial properties changes after the addition of different YDs in the same trial/conditions that would allow comparing their individual effects in the wine. To the best of our knowledge, this is the only study including the treatment of base wine with dry inactivated yeast obtained from Saccharomyces (Saccharomyces cerevisiae) and non-Saccharomyces (*Torulaspora delbrueckii* NSC19) yeast strains, a yeast autolysate and a yeast protein extract, including the monitorization of several parameters throughout 18 months of bottle aging on lees employing two different doses of these products.

2. Material and methods

2.1. Sparkling wines production and e design

Sparkling wines were obtained from 300 L Chardonnay base wine made in the San Pedro de Tarapacá winery, located in the Casablanca Valley region of Chile from the 2017 vintage. This base wine had an alcoholic degree of 10.4 % (v/v) and a pH of 3.42. The wine was stabilized with 50 g/hL bentonite, the dose was determined by using the fast heat test (Esteruelas et al., 2009), racked off, and transferred to a clean stainless-steel tank. The *tirage* was carried out following the traditional method (*champenoise*). For this purpose, a preadapted yeast culture of Lalvin EC1118® *Saccharomyces cerevisiae bayanus* purchased from Lallemand (Chile) was used for the second fermentation in the bottle. Therefore, the *tirage* mixture in every bottle was composed of Chardonnay base wine, 24 g/L of sucrose, the preadapted yeast ($60x10^6$ ucf/mL, 5% v/v), and 3 g/hL activated sodium bentonite (SIHA® G, Eaton Industries, Dublin, Ireland) to facilitate riddling. Hence, this was the composition of the control bottles, and in the bottles for the treatments different Yeast -derived products (YDs) were added to this *tirage* mixture provided by Lallemand Inc. Among the five YDs employed, two of them are commercial specific dry inactivated yeast from *Saccharomyces cerevisiae*: Optimum White™ (**OW**) and Pure-Lees Longevity™ (**LO**) and the other three are experimental YDs: a yeast autolysate (**AL**), a yeast protein extract (**PE**) and an inactivated dry yeast from *Torulaspora delbrueckii* NSC19 (**TD**). These YDs are extensively described in Table S1. These products were added in the *tirage* properly diluted in the same wine after filling the bottles and before covering the bottle with the bidule and the crown cap. Two different concentrations of each YDs were tested: 5 and 10 g/hL. The second alcoholic fermentation finished after 4 weeks reaching 6.5 atm of pressure in the bottle. Sparkling wines had an average alcoholic degree of 12.1 % (v/v) and a pH of 3.50. The samples analysed were taken at 3, 6, 9, and 18 months of aging on lees, therefore, the bottles were disgorged after *remuage* at those aging times in one cycle with a Gyropalette® (Oenoconcept®, Epernay, Champagne, France) employing to refill the bottles the wine of the same type and closed again with a crown tap. All the analyses described in this study were carried out employing three biological replicates in the case of sparkling wines and an analytical triplicate in the case of the base wine.

2.2. General oenological parameters

Alcoholic degree and pH were analysed according to the official methods established by the International Organisation of Vine and Wine (OIV, 2021).

2.3. Solid phase Microextraction/Gas chromatography/Mass spectrometry (SPME/GC/MS)

The volatile compounds were extracted by Headspace Solid Phase Microextraction (HS-SPME) following Ubeda et al. (2019). Thus, 7.5 mL of sample was placed into a 20-mL glass vial with 1.5 g of sodium chloride and 10 μL of 4-methyl-2-pentanol (0.75 mg/L) (used as internal standard). First, a 2 cm 50/30 μm Carboxen/DVB/PDMS SPME fiber (Supelco, Bellefonte, p.a., USA) was cleaned and conditioned following the manufacturer's instructions. Then, after 20 min incubating at 45 °C and 500 rpm agitation speed, the adsorbent material of the fiber was exposed to the headspace of the vial for 40 min. The penetration into the vial was 30 mm and after this extraction, the fiber was desorbed in the injector using the spitless (3 min.) mode and a transfer line temperature of 280 °C. Afterward, gas chromatography analysis was performed in a 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA). For this analysis, a DB Wax capillary column (60 m × 0.25 mm, and 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) was used, and the carrier gas was helium at a flow rate of 1 mL/min. The program of the oven temperature was set as follows: 35 °C for 1 min, then increased to 130 °C at 12 °C/min and held for 1 min, then to 160 °C at 1 °C/min, and then to 220 °C at 10 °C/min (held for 10 min). The electron ionization mass spectra in the scan mode were recorded at 70 eV with the electron energy in the range of 35 to 300 amu.

All data were recorded using an MS ChemStation (Agilent Technologies, Palo Alto, CA, USA).

Authentic reference standards from Sigma-Aldrich (Germany) (ID: A; Table 1) were used for the compound identification as well as the matching with the 2.0 version of the standard NIST library and the linear retention indices from the literature (LRI). Linear retention indices (LRIs) were calculated by retention times of *n*-alkanes (C6-C30) under identical conditions for each analysis program.

Data were expressed in concentration (μg/L) obtained from calibration curves (r^2 = 0.9428–0.9949) with these reference standards

(relative area vs. concentration). The relative area was calculated by dividing the peak area of the major ion of each compound by the peak area of the major ion of the internal standard (4-methyl-2-pentanol). The compounds from every chemical group were calibrated in specific ranges of concentration based on the usual concentrations present in wine. Hence, esters were calibrated from 1 to 15000 μg/L (97.0–99.8% purity); alcohols from 1 to 50000 μg/L (98.0–99.9% purity) extended to 300 mg/L for isoamyl alcohol (99.0% purity); terpenes from 0.25 to 50 μg/L (95.0–98.5% purity); acids from 10 to 15000 μg/L (96.0–99.0% purity); furfural from 10 to 500 μg/L (99.0% purity) and norisoprenoids were expressed in relative areas.

2.4. Polysaccharides determination by High-Performance liquid chromatography (HPLC)

Polysaccharides were extracted from the wine matrix following the method of Ayestarán, Guadalupe, and León (2004). Briefly: after a degassing process, 10 mL of sparkling wine were concentrated until 2 mL by using a vacuum centrifuge. After that, samples were mixed with 10 mL of cold (4 °C) acidified (0.3 M HCl) ethanol and kept for 24 h at 4 °C to let the polysaccharides precipitate. Subsequently, samples were centrifuged (4.500×*g*, for 20 min), the liquid was carefully removed and the pellets containing the polysaccharides were washed three times with cold ethanol (0 °C). Then, the pellets were redissolved with 1 mL of ultrapure water and froze (−20 °C). Finally, the sample was lyophilized, and redissolved with 1 mL of aqueous ammonium formate 30 mM and filtered through a 0.45 μm pore size Millex-HV Hydrophilic PVDF membrane syringe filter (EMD Millipore, Billerica, MA). Finally, 100 μL of the sample was injected into the chromatographic system following the protocol described in Martí-Raga et al. (2016). Separation was carried out using two Shodex OHpak SB-803 HQ and SB-804 HQ columns connected in a series (300 mm × 8 mm I.D.; Showa Denko, Japan). The mobile phase was an aqueous solution of 30 mM ammonium formate with a constant flow rate of 0.6 mL min-1 for 60 min. The detection of polysaccharides was performed using a refractive index detector (RID). The ranges of molecular weight (Mn) for each fraction were estimated by using commercial standards of dextrans (From *Leuconostoc mesenteroides*, Sigma-Aldrich, Chile) with the following averaged number molecular weights: 3260, 8110, 18300, 35600, 55500, 100300, 164200, 236,300 and 332800 Da. Quantification was done by the external standard method using pectins from citric fruits (Sigma-Aldrich, Chile) and dextrans from *Leuconostoc mesenteroides* in a range from 0 to 2 g/L.

2.5. Proteins determination by fast protein liquid chromatography (FPLC)

For the determination of the proteins content, the method detailed in Pons-Mercadé et al. (2021) was followed. Briefly, sparkling wines were degassed to be dialyzed in tubes with a molecular weight cut-off of 3.5 kDa (Membrane Filtration Products Inc., San Antonio, TX, USA) separating the proteins from interferences. Fifteen mL of wine were first dialyzed against aqueous ammonium acetate (0.3 M) and then against ultrapure water. Afterward the samples were lyophilized and stored at −20 °C before HPLC-DAD analysis, and resuspended in 600 μL of 0.3 M ammonium acetate solution adjusted to pH 7.0 and followed by centrifugation at 12,000*g* at 4 °C for 2 min. The supernatant was filtered through 0.22 μm acetate cellulose filters (Merck Millipore, Darmstadt, Germany), and 100 μL of supernatant was then injected into the chromatographic system. The analyses were carried out in an HPLC Agilent 1200 Series system (Agilent Technologies, Barcelona, Spain) with a diode array detector (DAD) to monitor output at 230 and 320 nm. Separation was carried out at 20 °C using an S 165 Shodex gel permeation HPLC column (OHpak SB-803 HQ, 300 mm × 8 mm i.d.; Showa Denko, Tokyo, Japan). The calibration curve for quantification was made up using bovine serum albumin (Sigma-Aldrich, Madrid, Spain) ranging from 0 to 1 mg/mL as an external standard.

2.6. Mosalux system

The measurement of the foam properties was done following the Mosalux procedure (Maujean, Poinsaut, Dantan, Brissonet, & Cossiez, 1990). To carry out the measurement, the wines were degasified. Afterward, a test tube with a porous piece of glass at the bottom and a CO_2 entry, was filled with 100 mL of sample and a constant flow of CO_2 (10 L/h) was passed through the sample at a constant temperature of 16 °C. The parameters measured were HM which is the maximum height reached by the foam and represents the foamability, HS which is the stable height of the foam which represents the ability of the wine to produce stable foam/persistence of the foam collar, TM corresponding to the time needed to reach the maximum height, related with the sparkling wine effusiveness and finally TS that is the time needed for the foam to collapse after the gas flow has stopped, related with the persistence of the foam (Maujean et al., 1990). Measurements were done in three biological replicates of each treatment. The parameters HM and HS are expressed in mm and TS and TM in seconds.

2.7. Oxygen radical antioxidant capacity (ORAC)

The antioxidant activity of the sparkling wines aged for 18 months was measured by the oxygen radical antioxidant capacity (ORAC) following the protocol of Zulueta, Esteve, and Frígola (2009) with some modifications. ORAC-FL was performed in a black 96-well microplate (BD Falcon, BD Biosciences, UK), with a fluorimeter (FLX 800 Biotek, Winooski, Vermon). The excitation wavelength was set at 485 nm and the emission wavelength at 528 nm at 37 °C. First, a stock solution of fluorescein (Sigma-Aldrich, Germany) was prepared weighing 22 mg and dissolving in 50 mL of phosphate buffer solution (PBS) (75 mM, pH 7). This solution was stored in darkness and refrigerated. The working solution was prepared immediately before the analysis (0.167 mL of stock solution in 25 mL of PBS). The AAPH radical (Sigma-Aldrich (Germany) was prepared daily (221 mM) in PBS. The sample, sparkling wines, were centrifuged for 10 min at 12,500*g* (3500 rpm) and subsequently filtered with a grade 40 filter paper, 0.45 μm pore size (Whatman®). Then, a solution of 1:1000 was obtained from the filtered sample with PBS Buffer. Daily a reference standard trolox solution of 20 μM was prepared in PBS.

For each measurement, all the wells were filled with 25 μL of AAPH and 25 μL of the sample (buffer, Trolox, or assay sample) and finally, 150 μL of fluorescein was added to all the plates with the multichannel pipette. Fluorescence measures were taken at intervals of 5 min until the fluorescence intensity was<5% of the initial fluorescence. The results were expressed in μM of Trolox equivalents (μM TE) calculated by the formula:

$$\mu\text{MTE} = \frac{C_{Trolox}\cdot\left(AUC_{sample} - AUC_{Blank}\right)\cdot k}{\left(AUC_{sample} - AUC_{Blank}\right)}$$

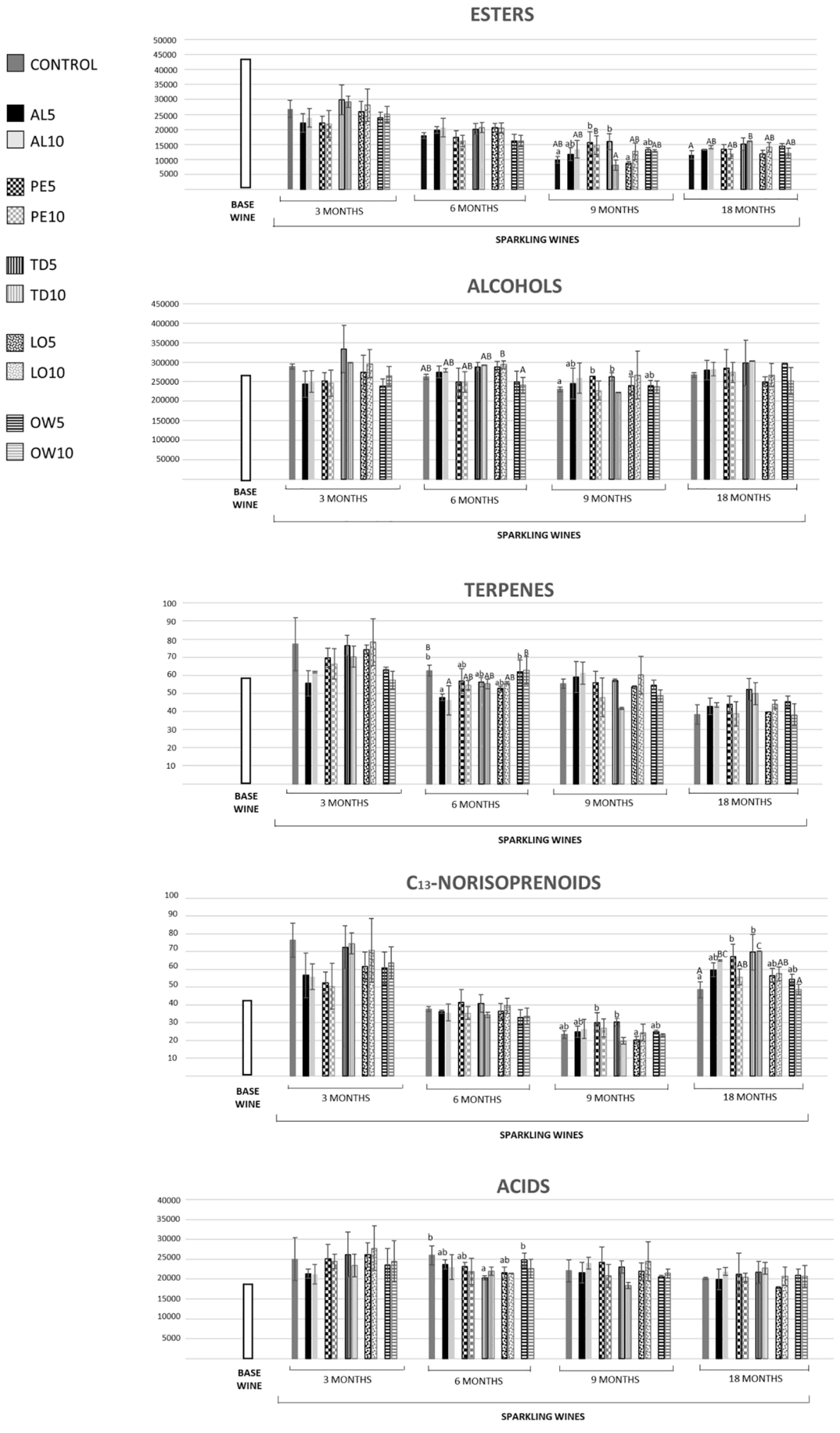
where C_{Trolox} is the concentration of trolox (20 μM), k is the sample dilution factor, and AUC is the area below the fluorescence decay curve of the sample, blank, and trolox, calculated as follows:

$$AUC = (0.5 + f_5/f_0 + f_{10}/f_0 + \ldots f_{n+5}/f)\cdot 5$$

where f_0 is the fluorescence at time 0 and f_n is the fluorescence at the end of the analysis.

2.8. Colour intensity and browning determination

Colour intensity (CI) and browning were measured as described in the literature (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). All the spectrophotometric measurements were performed with a UV–Vis spectrophotometer SHIMADZU UV-1700 PharmaSpec (Equilab Ltda., Santiago de Chile, Chile). The colour intensity was estimated as the sum of absorbance of the samples at 620 nm, 520 nm, and 420 nm,



(caption on next page)

Fig. 1. Evolution of the main groups of volatile compounds from 9 months to 18 months of aging on lees with the different yeast derivatives at 5 and 10 g/hL. Different lowercase letters indicate significant differences between YDs at 5 g/hL. Different capital letters indicate significant differences between YDs at 10 g/hL ($p < 0.05$) (Tukey test). Yeast autolysate (**AL**); yeast protein extract (**EX**); inactivated dry yeast from *Torulaspora delbrueckii* NSC19 (**TD**); Optimum White™ (**OW**); Pure-Lees Longevity™ (**LO**).

being the value of this last absorbance the browning determination. Measurements were performed using a 10-mm path length quartz cuvette and distilled water as a reference.

2.9. Sensorial analyses

Sparkling wines with 18 months of aging were selected to perform descriptive sensory analysis (Jackson, 2016). The sensorial panel was composed by 18 tasters (eight females and ten males). Panellists were chosen by our research group, being sparkling wine experts and producers who are very familiar with Chilean sparkling wines. Despite not being standardized, a *mise en bouche* was done to agree on the intensity of the attributes. Sparkling wines were evaluated at the Lallemand Chile facilities in Rancagua, Chile, organized in three sessions on the same day with a time-lapse of 30 min among them providing water to rinse the palate between tastings. For each evaluation, 50 mL of sparkling wine at 6–8 °C was served in each glass (model "Degustazione Champagne", Riedel®). First, four glasses marked with three-digit random numbers were presented to each taster. Panelists performed a descriptive analysis evaluating visual (foamability, bubble size, yellow component, foam stability), olfactory (aromatic intensity, fruity, floral, bread/toasted/yeast, complexity), and in mouth attributes (foam aggressiveness, acidity, bitterness, fruity flavor, persistence). Preliminary tasting sessions among members of the research group were performed to select by consensus the attributes for the evaluation. The selected attributes were on a tasting-card and panellists were asked to rank each descriptor on a 15-cm unstructured scale (being 0 = unnoticeable and 15 = very strong). Afterward, data obtained were treated as described in the Statistical analysis section.

2.10. Statistical analysis

Data obtained were compared using ANOVA and a post hoc Tukey test ($\alpha = 0.05$) ($p < 0.05$) employing the InfoStat software (version 2017p, FCA-Universidad Nacional de Córdoba, Argentina). Sensory analysis data were processed using PanelCheck V1.4.2.

3. Results and discussion

3.1. Effects on volatile compounds

The volatile compounds profile was analyzed in all the samples produced during this research, for all the aging times and doses of the YD treatments. From these samples, forty-six compounds were identified or tentatively identified in the Chardonnay base wines and sparkling wines. The different chemical groups exhibited different evolutions after aging as observed in Fig. 1. Esters tend to decrease after the second fermentation and during aging due to the release of enzymes such as esterases, which are involved in their breakdown (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2009; Di Gianvito et al., 2018), the adsorption of these compounds on lees (Gallardo-Chacon, Vichi, Lopez-Tamames, & Buxaderas, 2010), or because of their thermodynamic instability (Martínez-García, Mauricio, García-Martínez, Peinado, & Moreno, 2021). After 3 months of aging, the evolution of the sparkling wines in all the bottles remained heterogeneous and, therefore, statistically, there were no significant differences between the different treatments. After 6 months of aging, the ester concentration was very similar in all the treated wines. However, at 9 months of aging, the control wines, TD10 and LO5, exhibited the lowest ester concentrations. Finally, after 18 months of aging on lees, the effect of the YDs was revealed in all the treated wines, independent of the dose, presenting higher ester concentrations than the untreated sparkling wines. However, with respect to the doses applied, the effects of each treatment varied depending on the aging time. It is worth mentioning that the wines treated with 5 g/hL of TD exhibited a completely different behavior when compared to the 10 g/hL dose after 9 months. This difference was mainly due to the drastic decrease in isoamyl and hexyl acetates. Thus, for short aging of 9 months, PE5 and TD5 (5 g/hL) could help to preserve the esters such as ethyl butanoate, isoamyl acetate, and hexyl acetate (Table 1) and, therefore, the aromatic fruity character of the wine, considering that these esters have been related to the fruity character in other studies. Thus a decrease in esters, especially acetate esters, is designed as a clear aging marker (Torrens, Riu-Aumatell, Vichi, Lopez-Tamames, & Buxaderas, 2010; Ubeda et al., 2019). As Fig. 1 shows, there was a decrease in esters when PE was applied to the base wine; however, as previously mentioned, after 9 months this product appeared to have a protective effect. This may be due to an adsorption effect followed by a subsequent release of these compounds. As postulated by Pérez-Magariño et al. (2015), the effect of the adsorption phenomenon on the yeast lees and YDs is a reversible process. In the case of the longest aging period (18 months), TD best preserved the fruity character of the wines, not only because it released the compounds adsorbed in the previous stage, such as hexyl acetate, isoamyl acetates, or ethyl octanoate, but also because it contributed to a slight reduction of other compounds, as is the case of isoamyl octanoate and β-phenethyl acetate (Table 1). This reduction is probably due to the adsorption of esters by the cell walls, preventing their loss. Previous reports have described a YDs protective effect of the esters towards oxidation (Andújar-Ortiz, Chaya, Martín-Álvarez, Moreno-Arribas, & Pozo-Bayón, 2014). However, since the sparkling wines are covered with a crown cap during aging, we did not observe a higher concentration of these compounds in the sparkling wines treated with YDs rich in glutathione with respect to the rest, since only solubilized oxygen was present in the matrix during the *tirage*.

As usual, the volatile alcohols did not suffer noticeable changes during aging. After the second alcoholic fermentation, there tended to be an increase in terpenoids in both the control and treated samples probably due to the β-glycosidic bond break down due to β-glucosidase action of the yeast, which releases these compounds with respect to the base wine to start decreasing afterward along with the aging and because of acid hydrolysis at wine pH, including rearrangements of free terpenes present in the base wine increasing the amounts of some of them (Ganss, Kirsch, Winterhalter, Fischer, & Schmarr, 2011). It has been evidenced that compounds usually present as glycosidically bound aroma compounds, such as terpenes, can interact with the yeast walls to decrease the varietal character (Moio, Ugliano, Gambuti, Genovese, & Piombino, 2004). However, the addition of the autolysate (AL) compensated for this decrease after 9 and 18 months in all the treated samples, especially when the inactivated yeast from strain NSC19 of *Torulaspora delbrueckii* was added (Fig. 1), mainly due to the increase in linalool formate and *trans*-nerolidol (Table 1). Thus, this conservation and enhancing effect was observed in all the sparkling wines treated with YDs, in accordance with the results obtained in model wine (Rodríguez-Bencomo et al., 2014) and in sparkling wine (Pérez-Magariño et al., 2015).

C_{13}-norisoprenoids are also present in the berry as non-volatile carotenoid-derived precursors and the enzymatic action that occurs in the first and second fermentations liberates the volatile molecule to the media (Martínez-García et al., 2021; Torrens et al., 2010). This effect can easily be observed in Fig. 1. Following this increase during the second

Table 1
Volatile compounds determined in base wine and sparkling wines after 9 months (regular font numbers) and 18 months (bold font numbers) of aging on lees with 10 g/hL of YDs.

	LRI	ID	BASE WINE	SPARKLING WINES					
				CONTROL	Yeast autolysate	Yeast protein extract	*Torulaspora delbruekii*	Pure Lees Longevity™	Optimum White™
					AL	PE	TD	LO	OW
ESTERS									
Ethyl butanoate	1056	A	519 ± 35	154 ± 6b	37.8 ± 9.8a	304 ± 70c	53.3 ± 9.0a	17.4 ± 3.7a	59.5 ± 10.4a
				624 ± 65*	**779 ± 46***	**656 ± 111***	**765 ± 74***	**757 ± 94***	**571 ± 122***
Ethyl 2-methylbutanoate	1069	A	9.86 ± 1.50	2.12 ± 0.23a	2.33 ± 0.46a	5.50 ± 0.61bc	1.73 ± 0.18a	3.61 ± 0.51ab	5.87 ± 1.28c
				21.0 ± 4.2a*	**29.9 ± 0.3ab***	**24.8 ± 4.8ab***	**33.2 ± 1.7***	**28.3 ± 2.5ab***	**22.7 ± 5.2ab***
Isoamyl acetate	1139	A	5964 ± 189	957 ± 36ab	1106 ± 298ab	2270 ± 267b	512 ± 173a	1103 ± 249ab	1364 ± 222b
				2357 ± 273*	**2697 ± 70***	**2261 ± 187**	**2819 ± 95***	**2664 ± 175***	**2390 ± 368***
Methyl hexanoate	1189	B	1.87 ± 0.09	0.128 ± 0.007a	0.091 ± 0.024a	0.791 ± 0.261b	0.128 ± 0.033a	0.190 ± 0.046a	0.285 ± 0.048a
				1.44 ± 0.31*	**1.70 ± 0.01***	**1.51 ± 0.11***	**1.55 ± 0.02***	**1.78 ± 0.17***	**1.45 ± 0.17***
Ethyl hexanoate	1248	A	1420 ± 51	455 ± 37a	580 ± 185ab	940 ± 273b	268 ± 84a	452 ± 137a	561 ± 103ab
				1198 ± 240*	**1548 ± 52***	**1405 ± 213**	**1792 ± 9***	**1533 ± 203***	**1331 ± 231***
Hexyl acetate	1282	A	11377 ± 760	2512 ± 528ab	3417 ± 813ab	4124 ± 1034b	1824 ± 412a	2701 ± 483ab	3047 ± 375ab
				3256 ± 330	**3893 ± 273**	**3302 ± 464**	**4279 ± 5***	**4042 ± 459***	**3411 ± 435**
Ethyl heptanoate	1293	A	5.07 ± 0.51	1.67 ± 0.33	2.44 ± 0.56	2.98 ± 0.95	1.34 ± 0.48	2.12 ± 0.51	2.37 ± 0.26
				3.07 ± 0.45a*	**4.06 ± 0.17ab***	**3.48 ± 0.33ab**	**4.65 ± 0.01b***	**4.26 ± 0.49ab***	**3.54 ± 0.43ab***
Ethyl lactate	1331	A	0.535 ± 0.028	6.78 ± 0.80	8.08 ± 1.55	9.08 ± 2.64	8.44 ± 2.00	9.51 ± 2.17	8.39 ± 0.84
				9.31 ± 0.76*	**10.61 ± 0.19**	**9.08 ± 1.78**	**9.16 ± 1.10**	**9.12 ± 2.14**	**11.2 ± 2.2**
Methyl octanoate	1411	A	16.3 ± 2.6	3.44 ± 0.46	4.78 ± 1.42	5.44 ± 1.39	3.19 ± 0.21	4.12 ± 0.86	4.34 ± 0.78
				6.07 ± 0.88*	**6.16 ± 0.28**	**5.54 ± 0.25**	**6.43 ± 0.63***	**6.46 ± 2.64***	**4.88 ± 0.53**
Ethyl octanoate	1453	A	4865 ± 110	1118 ± 153	1656 ± 446	1699 ± 537	1214 ± 214	1408 ± 361	1517 ± 15
				1648 ± 214a*	**2172 ± 59ab**	**1848 ± 248a**	**2647 ± 55b***	**1966 ± 263a**	**1733 ± 254a**
Isoamyl hexanoate	1462	B	5.45 ± 0.48	nd	1.00 ± 0.17	1.10 ± 0.36	0.271 ± 0.019	0.731 ± 0.187	0.706 ± 0.143
				nda	**1.73 ± 0.10cd**	**0.829 ± 0.221ab**	**2.10 ± 0.14d***	**1.32 ± 0.37bcd**	**0.984 ± 0.327abc**
Vinyl octanoate	1525	B	178 ± 7	68.5 ± 12.8	61.0 ± 7.7	52.1 ± 10.1	54.0 ± 0.9	84.2 ± 18.2	55.5 ± 6.6
				69.8 ± 14.0a	**84.7 ± 0.8ab***	**80.1 ± 17.3a**	**117 ± 4b***	**91.1 ± 8.7ab**	**73.4 ± 8.2a***
Ethyl nonanoate	1549	B	36.9 ± 3.3	12.0 ± 2.3	16.3 ± 3.0	14.2 ± 3.2	13.5 ± 3.4	20.5 ± 4.8	19.5 ± 2.7
				7.69 ± 0.77ab*	**7.33 ± 1.03ab***	**5.73 ± 0.56a***	**10.2 ± 0.7b**	**7.07 ± 1.36ab***	**6.23 ± 1.58a***
Methyl decanoate	1586	A	7.78 ± 0.58	1.42 ± 0.06	1.81 ± 0.29	1.76 ± 0.17	1.47 ± 0.04	1.67 ± 0.27	1.65 ± 0.14
				1.30 ± 0.15	**1.40 ± 0.06**	**1.28 ± 0.05***	**1.36 ± 0.15**	**1.52 ± 0.08**	**1.24 ± 0.11***
Ethyl decanoate	1657	A	2037 ± 121	234 ± 51	408 ± 76	368 ± 83	327 ± 14	375 ± 110	369 ± 29
				149 ± 45a	**236 ± 10ab**	**168 ± 25a***	**294 ± 14b**	**206 ± 42ab**	**185 ± 29a***
Isoamyl octanoate	1673	A	10371 ± 963	1934 ± 480	3357 ± 805	2743 ± 614	2023 ± 688	3651 ± 988	3263 ± 29
				777 ± 215a*	**1314 ± 59ab***	**733 ± 177a***	**1544 ± 9b**	**1301 ± 303ab***	**1025 ± 271ab***
Ethyl *trans*-4-decenoate	1685	B	13.9 ± 1.0	38.1 ± 0.7abc	37.4 ± 5.3abc	25.3 ± 4.7a	28.0 ± 4.3ab	51.5 ± 12.1b	44.8 ± 1.9bc
				25.5 ± 1.2b*	**23.7 ± 1.1b***	**14.4 ± 1.1a***	**28.4 ± 0.2b**	**24.1 ± 3.1b***	**22.5 ± 2.9b***
Diethyl succinate	1700	A	19.3 ± 0.8	48.0 ± 4.4	47.7 ± 4.9	43.2 ± 5.1	40.5 ± 0.2	53.2 ± 10.2	45.7 ± 1.6
				74.9 ± 1.8*	**71.7 ± 3.6***	**76.9 ± 6.4***	**81.4 ± 2.6***	**74.7 ± 8.4***	**75.6 ± 11.5***
Ethyl phenylacetate	1832	A	1.50 ± 0.03	2.01 ± 0.12	2.18 ± 0.16	1.95 ± 0.23	1.75 ± 0.12	2.28 ± 0.38	1.99 ± 0.06
				2.11 ± 0.02a	**2.39 ± 0.04ab**	**2.39 ± 0.28ab**	**2.67 ± 0.10b***	**2.29 ± 0.11ab**	**2.25 ± 0.16ab**
β-phenethyl acetate	1870	A	5466 ± 186	2242 ± 268	2410 ± 310	2039 ± 344	1665 ± 57	2528 ± 438	2116 ± 89
				1100 ± 59ab*	**1140 ± 58ab***	**1117 ± 181ab***	**1428 ± 22b***	**1161 ± 101ab***	**1067 ± 102a***
Ethyl dodecanoate	1879	B	484 ± 32	34.1 ± 7.5a	65.7 ± 11.4b	58.9 ± 2.4ab	36.9 ± 5.5a	65.7 ± 13.7b	63.2 ± 5.5b
				58.4 ± 9.3a*	**68.9 ± 3.5ab**	**59.3 ± 5.3a**	**93.3 ± 8.8b***	**64.7 ± 10.7a**	**55.1 ± 8.2a**
Isoamyl decanoate	1884	A	791 ± 155	111 ± 28ab	195 ± 44b	162 ± 22ab	87.7 ± 10.1a	199 ± 47b	188 ± 13b
				72.3 ± 9.3	**78.1 ± 3.1***	**65.5 ± 3.8***	**85.4 ± 14.0**	**85.1 ± 24.6***	**65.1 ± 8.8***
ALCOHOLS									
Isobutanol	1105	A	29240 ± 1405	29151 ± 3546	33646 ± 4297	19279 ± 3811	25217 ± 6677	32821 ± 9171	27119 ± 8093
				33855 ± 2362ab	**37728 ± 1501b**	**29480 ± 7465ab**	**35644 ± 1408b**	**33791 ± 3011ab**	**22008 ± 4582a**
Butanol	1142	A	1851 ± 30	1902 ± 119a	2074 ± 176ab	2048 ± 263ab	2489 ± 7b	2351 ± 306ab	2007 ± 41ab
				2178 ± 58*	**2433 ± 31**	**2048 ± 230**	**2068 ± 219***	**2132 ± 106**	**2211 ± 51**
Isoamyl alcohol	1221	A	181414 ± 7314	144697 ± 13685	164420 ± 26571	153852 ± 15271	144511 ± 7919	169373 ± 37410	153755 ± 8741
				172180 ± 4905*	**181092 ± 11988**	**183432 ± 14802**	**200713 ± 2500***	**171296 ± 20115**	**170878 ± 24006**

(*continued on next page*)

Table 1 (*continued*)

	LRI	ID	BASE WINE	SPARKLING WINES					
				CONTROL	Yeast autolysate	Yeast protein extract	*Torulaspora delbruekii*	Pure Lees Longevity™	Optimum White™
					AL	PE	TD	LO	OW
Hexanol	1360	A	42096 ± 1817	40139 ± 2498	43138 ± 6428	37995 ± 4183	37602 ± 1417	45332 ± 10389	39170 ± 2064
				43523 ± 705*	**44556 ± 2654**	**42189 ± 4563**	**47369 ± 2214***	**44375 ± 4636**	**41345 ± 4791**
***trans*-3-Hexen-1-ol**	1368	B	13.6 ± 3.8	24.1 ± 5.8	28.8 ± 6.8	17.2 ± 6.1	19.2 ± 0.7	35.8 ± 8.6	21.2 ± 5.5
				20.9 ± 2.9	**22.1 ± 5.1**	**15.9 ± 2.9**	**21.0 ± 6.2**	**23.5 ± 2.7**	**18.7 ± 4.7**
3-Ethoxypropanol	1395	C	308 ± 27	370 ± 31	403 ± 36	440 ± 105	448 ± 142	442 ± 95	381 ± 21
				361 ± 24	**404 ± 5**	**384 ± 119**	**314 ± 70**	**374 ± 81**	**390 ± 3**
***cis*-3-Hexen-1-ol**	1405	A	373 ± 10	381 ± 20	405 ± 45	349 ± 22	376 ± 10	430 ± 82	349 ± 9
				381 ± 6	**389 ± 12**	**370 ± 20**	**394 ± 24**	**385 ± 26**	**375 ± 23**
2-Ethyl-1-hexanol	1513	A	3.27 ± 0.34	29.9 ± 7.4	21.9 ± 0.2	10.4 ± 1.3	27.8 ± 9.5	34.2 ± 4.3	22.2 ± 4.7
				1.23 ± 0.11*	**1.42 ± 0.37***	**0.40 ± 0.06***	**1.22 ± 0.33***	**1.78 ± 0.15***	**1.05 ± 0.30***
Decanol	1769	A	5.73 ± 0.20	5.02 ± 0.34b	5.42 ± 0.25a	4.30 ± 0.64ab	4.07 ± 0.12b	5.66 ± 1.002b	4.95 ± 0.38ab
				3.27 ± 0.49ab*	**4.01 ± 0.03b***	**3.48 ± 0.47a**	**4.71 ± 0.09ab***	**3.88 ± 0.35b***	**3.81 ± 0.33ab***
β-phenylethanol	1975	A	15602 ± 585	13618 ± 1821	15545 ± 1659	13506 ± 2289	11965 ± 224	16040 ± 3807	13960 ± 558
				14790 ± 815a	**16034 ± 901ab**	**15940 ± 836a**	**16785 ± 2578b**	**14634 ± 2377ab**	**15017 ± 1458ab**
TERPENES									
o-Cymene	1271	B	1.72 ± 0.21	1.47 ± 0.09	1.65 ± 0.23	1.61 ± 0.22	1.64 ± 0.14	1.69 ± 0.26	1.81 ± 0.09
				1.59 ± 0.31	**1.72 ± 0.28**	**1.58 ± 0.28**	**2.01 ± 0.51**	**1.87 ± 0.41**	**1.82 ± 0.30**
***trans*-2-pinanol**	1436	A	11.1 ± 0.3	3.75 ± 0.34	4.68 ± 0.30	5.30 ± 1.87	3.05 ± 0.97	2.50 ± 0.73	4.81 ± 0.45
				2.46 ± 0.61*	**3.21 ± 0.59***	**2.73 ± 0.30**	**3.45 ± 0.28**	**3.14 ± 0.64**	**4.27 ± 0.93**
Linalool formate	1518	B	23.8 ± 0.6	29.3 ± 2.1	31.2 ± 3.3	23.3 ± 5.4	21.6 ± 4.5	31.6 ± 6.7	25.1 ± 3.7
				23.6 ± 3.2	**26.6 ± 0.1**	**22.9 ± 4.8**	**29.6 ± 0.0**	**26.7 ± 1.6**	**19.7 ± 4.4**
Linalool	1557	A	7.66 ± 0.15	6.40 ± 0.46a	6.61 ± 0.71ab	4.67 ± 0.83ab	5.23 ± 0.19ab	7.05 ± 1.41b	4.76 ± 0.39ab
				2.88 ± 0.13*	**3.24 ± 0.14***	**2.91 ± 0.58***	**3.49 ± 0.33***	**3.22 ± 0.42***	**3.10 ± 0.23***
Citronellol	1789	A	5.06 ± 0.19	3.06 ± 0.31	3.17 ± 0.06	2.80 ± 0.67	2.25 ± 0.12	3.37 ± 0.53	2.74 ± 0.13
				1.41 ± 0.32a*	**1.91 ± 0.28ab***	**1.50 ± 0.36ab***	**2.11 ± 0.01b**	**1.78 ± 0.19ab***	**1.58 ± 0.09ab***
***trans*-nerolidol**	2048	A	8.25 ± 1.00	11.4 ± 0.6ab	14.0 ± 1.7b	10.1 ± 2.5ab	7.89 ± 0.37a	13.6 ± 2.8b	9.53 ± 1.32ab
				6.27 ± 1.14a*	**6.87 ± 0.44ab***	**7.09 ± 0.90ab**	**9.25 ± 0.61b**	**7.13 ± 0.45ab***	**7.54 ± 0.56ab**
ACIDS									
Hexanoic acid	1893	A	10606 ± 596	11578 ± 1606	12290 ± 704	10361 ± 735	10050 ± 240	12915 ± 2801	10807 ± 361
				11066 ± 610	**11440 ± 427**	**10597 ± 234**	**11183 ± 1282**	**10916 ± 1284**	**11073 ± 1251**
Octanoic acid	2072	A	13892 ± 551	9194 ± 1178	10315 ± 941	9095 ± 1902	7355 ± 532	10254 ± 1942	9320 ± 635
				8217 ± 256	**9363 ± 529**	**8774 ± 832**	**10388 ± 173***	**8780 ± 969**	**8660 ± 1335**
Decanoic acid	2255	A	3169 ± 79	1331 ± 194	1444 ± 98	1326 ± 286	945 ± 27	1339 ± 175	1294 ± 122
				864 ± 81*	**1073 ± 23***	**1004 ± 54***	**1173 ± 28***	**1019 ± 81***	**977 ± 85***
C_{13}-NORISOPRENOIDS									
Vitispirane A	1574	B	3.59 ± 0.17	6.21 ± 0.69	8.07 ± 1.82	7.47 ± 1.20	5.67 ± 0.95	6.42 ± 1.56	6.41 ± 0.32
				13.1 ± 1.7*	**16.8 ± 0.9***	**14.7 ± 1.3***	**17.4 ± 0.3***	**15.1 ± 1.8***	**12.7 ± 0.9***
Vitispirane B	1577	B	2.89 ± 0.29	4.76 ± 0.47	5.81 ± 1.21	5.48 ± 0.75	4.33 ± 0.34	4.83 ± 0.95	4.94 ± 0.23
				9.77 ± 1.02*	**11.4 ± 0.3***	**10.6 ± 0.8***	**12.4 ± 0.6***	**10.4 ± 0.8***	**9.47 ± 0.95***
TDN	1795	B	6.46 ± 0.21	3.54 ± 0.72a	4.87 ± 1.23ab	8.43 ± 2.30b	3.35 ± 0.21a	4.38 ± 0.91a	5.38 ± 0.96ab
				12.7 ± 1.9*	**21.7 ± 0.2***	**16.8 ± 0.7***	**24.0 ± 0.2***	**16.4 ± 1.7***	**13.1 ± 1.6***
β-Damascenone	1871	A	28.5 ± 1.3	8.74 ± 0.64b	7.62 ± 1.24ab	5.57 ± 1.20a	6.28 ± 0.34ab	8.69 ± 1.55b	6.17 ± 0.36ab
				12.9 ± 0.9*	**15.1 ± 0.4***	**13.4 ± 2.1***	**16.3 ± 0.9***	**15.4 ± 0.3***	**13.3 ± 0.3***
ALDEHYDES									
Furfural	1435	A	30.5 ± 2.9	86.5 ± 6.7a	95.5 ± 12.4a	87.7 ± 11.2a	119 ± 19b	102 ± 17ab	84.5 ± 5.1a
				320 ± 3*	**385 ± 14***	**375 ± 12***	**353 ± 16***	**326 ± 26***	**350 ± 22***

Results (average ± SD) are expressed as μg L^{-1}. C_{13}-Norisoprenoids are expressed in relative area. ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data; C, tentatively identified; mass spectrum agreed with mass spectral data base. n.d.: not detected.

Values with different superscript letter indicate statistically significant differences among the YDs treatments with the same months of aging ($p < 0.05$). *: statistically significant differences among 9 and 18 months of aging of the same YD treatment ($p < 0.05$)

Table 2
Determination of proteins (mg/L), antioxidant capacity (Trolox equivalents), color intensity and browning of sparkling wines after 18 months of aging on lees of the two dosages assayed: 5 and 10 g/hL. Yeast autolysate (AL); yeast protein extract (EX); inactivated dry yeast from *Torulaspora delbrueckii* NSC19 (TD); Optimum White™ (OW); Pure-Lees Longevity™ (LO).

DOSE	SAMPLE	PROTEINS (mg/L)	Antioxidant capacity (Trolox equivalents)	Colour Intensity	Browning
	CONTROL 18 M	46.6 ± 0.7bB	8160 ± 534aA	0.153 ± 0.003	0.121 ± 0.001
5 g/hL	**AL 18 M**	37.4 ± 3.7ab	12850 ± 1986b	0.158 ± 0.005	0.125 ± 0.003
	PE 18 M	30.7 ± 3.3a	8238 ± 400ab	0.156 ± 0.003	0.124 ± 0.002
	TD 18 M	31.1 ± 4.2a	8347 ± 1193ab	0.152 ± 0.005	0.120 ± 0.003
	LO 18 M	27.4 ± 6.1a	7619 ± 434ab	0.156 ± 0.005	0.123 ± 0.003
	OW 18 M	40.5 ± 1.4b	10415 ± 2467b	0.154 ± 0.001	0.120 ± 0.001
10 g/hL	**AL 18 M**	32.3 ± 4.4A	12146 ± 2934B	0.154 ± 0.005	0.122 ± 0.002
	PE 18 M	32.9 ± 6.2A	6770 ± 721A*	0.159 ± 0.002	0.125 ± 0.002
	TD 18 M	30.6 ± 3.5A	9170 ± 552A	0.149 ± 0.002	0.119 ± 0.001
	LO 18 M	32.4 ± 9.2A	8527 ± 1119A	0.155 ± 0.001	0.121 ± 0.001
	OW 18 M	47.4 ± 7.1B	9934 ± 1515AB	0.157 ± 0.008	0.121 ± 0.002

Values with different lower-case letter in the same column indicate statistically significant differences among treatments with the dosage of 5 g/hL and values with different capital letter in the same column indicate statistically significant differences among treatments with the dosage 10 g/hL ($p < 0.05$) (Tukey test. Asterisks indicates statistical difference among the two dosages of the same treatment.

fermentation and 3 months of aging, the tendency was to decrease after 6 and 9 months. For a short aging period of 9 months, the best treatment for preserving these compounds seemed to be yeast protein extract (PE) and the inactivated dry yeast from *Torulaspora delbrueckii* (TD) at the lower dose (5 g/hL). C_{13}-norisoprenoids are typical aging markers (Torrens et al., 2010; Ubeda et al., 2019), and accordingly, after 18 months of aging, the amount significantly increased. After 18 months, all the wines treated with the YDs used in this study presented greater C_{13}-norisoprenoids concentrations than the control wines, with the experimental treatments (AL, PE, and TD) obtaining better results than the commercial treatments (LO and OW) (Fig. 1, Table 1).

Collection and analysis of the isoprenoid data (terpenes and C_{13}-norisoprenoids) showed that, after 18 months in contact with lees and YDs, there was a greater concentration of isoprenoids in the TD sparkling wines (inactivated dry yeast made from the NSC19 strain of the non-*Saccharomyces* yeast, *Torulaspora delbrueckii*) when compared to the LO and OW sparkling wines (inactivated dry yeast made from *Saccharomyces* yeast).

The acids analyzed (hexanoic, octanoic, and decanoic) increased after the second fermentation but the tendency, from that point onwards, was to decrease. These straight-chain fatty acids have a fatty/rancid odor and are present in sparkling wines above their odor thresholds (Waterhouse, Sacks, & Jeffery, 2016). However, as described by Bábíková et al. (2012), they seem to have no negative sensory effect below 20–30 mg/L.

As we mentioned above, no fixed pattern could be derived for the difference between the two doses because the difference was dependent on the YDs applied and the aging time. On the one hand, the application of the higher dose of AL after 9 months of aging produced wines with a higher concentration of volatile compounds. However, for the rest of the YDs, the addition of a higher dose was counterproductive, generally resulting in wines with lower concentrations of volatile compounds. In these cases, the addition of smaller amounts may increase the volatility of some compounds, and higher doses may exert an adsorption effect (Comuzzo et al., 2006; Pozo-Bayón, Andujar-Ortiz, Alcaide-Hidalgo, Martin-Alvarez, & Moreno-Arribas, 2009). On the other hand, the time elapsed after 18 months in contact with the YDs blurred these trends, as they were also dependent on the effect of the chemical group. Pozo-Bayón, Andujar-Ortiz, Alcaide-Hidalgo et al. (2009) tested the effects of different inactive dry yeasts on volatile compounds and stated that the contact time with the yeast derivatives was definitory thus shorter contact times (2, 4, and 6 days) mainly promoted a "salting-out" effect and longer exposures (9 and 13 days) provoked a retention effect. Furthermore, an interesting common effect was observed in all the chemical groups after the addition of the autolysate at an early stage of autolysis (Fig. 1). As a result, after 3 months of treatment, the sparkling wines treated with yeast autolysate (AL) suffered a decrease in the concentration of volatile compounds. This could be associated with a rapid release of mannoproteins from the autolysates (Del Barrio-Galán, Ortega-Heras, Sánchez-Iglesias, & Pérez-Magariño, 2012) and direct adsorption of these molecules to them, possibly sequestered until 6–9 months.

3.2. Effects on antioxidant activity and color parameters

One of the demonstrated properties of yeast derivatives is the protection of wines against oxidation and, therefore, the protection of color, showing reduced absorbance values at 420 nm (Rodriguez-Nogales et al., 2012). In this study, we examined whether the yeast derivatives exhibited a protective effect on the wines during aging despite having a crown cap. To do this, we analyzed the antioxidant activity and color parameters in the final samples obtained after 18 months of aging. As observed in Table 2, the addition of yeast autolysate (AL) significantly increased the antioxidant activity of the sparkling wine with respect to the control regardless of the dose employed, followed by the sparkling wines treated with OW. The commercial yeast derivative OW was expected to have a high antioxidant activity because one of its claims is that it can prevent wine oxidation due to its high glutathione and polysaccharide concentration (Table 1, Supplementary material). The addition of glutathione has been reported to reduce oxidation in wines (Bahut et al., 2020). The greater antioxidant activity in sparkling wines with the addition of AL could be explained by the higher composition in high molecular weight cell wall polysaccharides since the presence of these molecules in sparkling wines has been positively correlated with the antioxidant activity (Rodriguez-Nogales et al., 2012). Despite the differences shown for the antioxidant activity, there were no significant differences in the color intensity or browning between the different YDs and doses. Nevertheless, more noticeable changes in color have been previously described for anthocyanin (rosé and red wines) than for non-anthocyanin compounds (white wines) in the case of the addition of glutathione-enriched YDs (Andújar-Ortiz et al., 2012).

3.3. Protein determination

As well as the analyses of colour parameters and antioxidant activity, the protein content analyses were performed only with the samples of 18 months of aging on lees. The addition of the YDs resulted, in a general diminution of the quantity of the protein in all cases except using Optimum White™ (OW) (Table 2). However, this decrease was statistically non-significant for AL dosed at 5 g/hL. The lower content of proteins for the sparkling wines treated with YD's could seem surprising, especially in the case of the yeast protein extract (PE), a yeast extract containing

Table 3
Determination of polysaccharides fractions F1 (Mn≈500 KDa), F2 (Mn≈100 KDa) and F3 (Mn≈10 KDa) and total polysaccharides of sparkling wines after 18 months of aging on lees of the two dosages assayed: 5 and 10 g/hL.

		F1 (mg/L)			F2 (mg/L)			F3 (mg/L)			TOTAL POLYSACCHARIDES (mg/L)		
SPARKLING WINES		**5 g/hL DOSAGE**	**10 g/hL DOSAGE**	**Global**	**5 g/hL DOSAGE**	**10 g/hL DOSAGE**	**Global**	**5 g/hL DOSAGE**	**10 g/hL DOSAGE**	**Global**	**5 g/hL DOSAGE**	**10 g/hL DOSAGE**	**Global**
CONTROL		–	–	**63.8 ± 4.6 a**	–	–	**46.7 ± 2.5 a**	–	–	**62.1 ± 7.6 bc**	–	–	**172 ± 14 a**
Yeast autolysate	**AL**	71.9 ± 6.3 a B	57.9 ± 3.9 a A	**64.9 ± 8.9 a**	57.1 ± 1.7b A	52.2 ± 3.6b A	**54.6 ± 3.7b**	76.3 ± 0.7c B	62.5 ± 5.2b A	**69.4 ± 8.3c**	205 ± 4b B	172 ± 12 a A	**189 ± 19b**
Yeast protein extract	**PE**	65.4 ± 1.4 a A	67.5 ± 0.2c A	**66.4 ± 1.5 a**	47.3 ± 1.7 a A	48.8 ± 0.3 ab A	**48.1 ± 1.4 a**	52.1 ± 1.4 ab A	58.3 ± 2.7ab B	**55.2 ± 3.9b**	164 ± 2 a A	174 ± 2 a B	**169 ± 5 a**
Torulaspora delbrueckii	**TD**	65.1 ± 4.3 a A	66.4 ± 1.5c A	**65.8 ± 3.0 a**	46.1 ± 1.1 a A	47.2 ± 1.1 a A	**46.7 ± 1.2 a**	60.6 ± 7.5b A	63.1 ± 2.2b A	**61.8 ± 5.1 bc**	171 ± 11 a A	176 ± 3 a A	**174 ± 8 a**
Pure Lees Longevity™	**OW**	62.8 ± 5.7 a A	59.3 ± 2.6 ab A	**61.0 ± 4.4 a**	48.2 ± 3.8 a A	44.7 ± 0.7 a A	**46.4 ± 3.1 a**	64.0 ± 4.9 bc A	55.7 ± 1.7ab A	**59.8 ± 5.6b**	175 ± 11 a A	159 ± 4 a A	**167 ± 11 a**
Optimum White™	**LO**	66.6 ± 1.4 a A	65.4 ± 2.5 bc A	**66.0 ± 1.9 a**	49.2 ± 0.0 a A	48.6 ± 0.6 ab A	**48.9 ± 0.5 a**	44.4 ± 6.7 a A	48.6 ± 5.4a A	**46.5 ± 5.9 a**	160 ± 8 a A	162 ± 7 a A	**161 ± 7 a**
GLOBAL		***66.4 ± 4.8B***	***63.3 ± 4.5 A***	**	***49.6 ± 4.4 A***	***48.3 ± 2.9 A***	*	***59.5 ± 12.0 A***	***57.6 ± 6.3 A***	**	***175 ± 17B***	***169 ± 9 A***	***

Values with different capital letter in the same row indicate statistically significant differences among dosages ($p < 0.05$) (Tukey test); values with different lower-case letter in the same column indicate statistically significant differences among YDs ($p < 0.05$) (Tukey test). Asterisks indicates p-value for the interaction between dosage and YD employed as a factors. $p < 0.05$ (*);$p < 0.01$ (**);$p < 0.001$ (***)

proteins. Proteins from base wine and from YDs could be metabolized by yeasts during the second fermentation, but they also could be removed besides bentonite and yeast lees during riddling and disgorging. Regarding the volatile compounds profiles of wines treated with PE, it does not seem that such treatment enhanced the production of volatiles during the second fermentation. Moreover, it has been reported that yeasts have small requirements of nitrogen compounds to complete the second fermentation when good nutrition was performed during the acclimatisation (Martí-Raga et al., 2016). Thus, the reported results point out that the addition of YDs during the tirage could exert a clarifying effect itself (González-Royo et al., 2017), or maybe they could increase the effectiveness of bentonite removing proteins from wine, which could be explained through macromolecular interactions between proteins and polysaccharides.

3.4. *Changes in soluble polysaccharide fractions*

The soluble polysaccharide content in sparkling wines was estimated using HRSEC-RID for the samples after 18 months of aging (Table 3). The polysaccharide profile of sparkling wines (Figure S1) accounted for three different fractions according to the molecular weight: F1, with an average molecular mass of 500 KDa, F2, with an average molecular mass of 100 KDa, and F3, with an average molecular mass of 10 KDa. The polysaccharide content of base wine depends on the balance between the release of cell wall polysaccharides (from both grapes and yeasts) during winemaking and their breakdown due to enzymatic degradation and precipitation phenomena. However, the base wine used for this trial was the same for all the samples. The polysaccharide content of sparkling wines depends –in addition to the base wine content– on the balance between the release by the yeast (during both second fermentation and yeast autolysis) and the added YDs, and the losses due to enzymatic degradation and bentonite retention during aging, riddling, and disgorging.

Considering the results, the doses assayed during this trial did not contrast enough to observe a dose effect on the polysaccharide content of the resulting sparkling wines. In fact, the lower dose (5 g/hL) resulted in wines that were greater in F1 and total polysaccharides, regardless of the YD employed, although such differences were quite small. Given that the treated wines did not show higher polysaccharides concentrations with respect to the untreated (control) wines, the polysaccharides from the YDs had to be partially removed from the wine, either by yeast metabolism/enzymatic degradation during the second fermentation or by precipitation with solid waste (bentonite and yeast lees) during disgorging.

Focusing on the overall effects of the YDs, AL sparkling wines showed the highest content of F2, F3, and total polysaccharides -regardless of the dose- indicating that these autolysates had the greatest potential for polysaccharide enrichment out of all the YDs tested. However, it should be noted that, during the disgorging process, we observed that the AL wines showed a noticeably greater potential to gush from the bottle, increasing wine losses and hindering the elaboration of sparkling wine with the traditional method. The effect of the YDs seems more evident in the oligosaccharide fraction (F3) regardless of the dose since the results indicate that the LO sparkling wines showed the lowest oligosaccharide concentration, while the AL, TD, and untreated wines had the highest concentrations. These results may be related to a different enzymatic activity throughout the second fermentation and bottle aging on lees. Wine oligosaccharides have been related to several sensory attributes (Apolinar-Valiente, Williams, & Doco, 2020) thus the differences reported for F3 between the wines treated with the different YDs could affect the organoleptic properties.

Finally, it should be noted that a statistical interaction exists between the YDs and the dose employed for all the polysaccharide fractions (F1, F2, and F3) as well as their total content, which indicates that the polysaccharide profile of sparkling wines is influenced differently by the same YD at different doses and vice versa. Thus, the release and removal

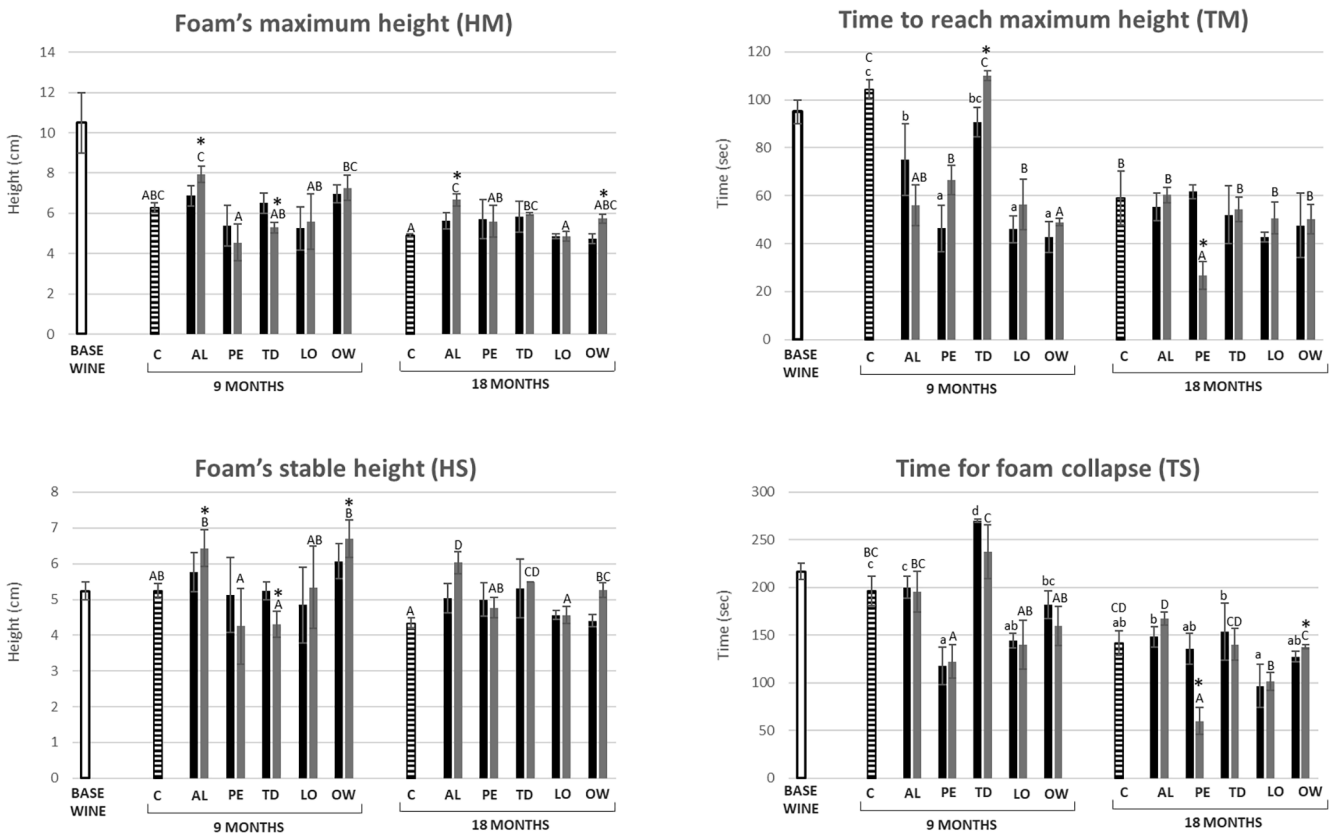


Fig. 2. Foaming properties determined by Mosalux method. HM: Maximum height; HS; Stable height; TM: Time spent to reach maximum height; TS: Foam stability time. Different lowercase letters indicate significant differences between YDs at 5 g/hL. Different capital letters indicate significant differences between YDs at 10 g/hL. Asterisks: significant differences between dosages of the same treatment ($p < 0.05$) (Tukey test). Yeast autolysate (**AL**); yeast protein extract (**EX**); inactivated dry yeast from *Torulaspora delbrueckii* NSC19 (**TD**); Optimum White™ (**OW**); Pure-Lees Longevity™ (**LO**).

of polysaccharides during sparkling wine production seem to be driven by complex phenomena that require additional research for further comprehension.

3.5. Influence on foaming properties

As observed in Fig. 2, the physical parameters characterizing the foam were measured in the 9 and 18-month samples after aging on lees for both tested doses. Foamability is usually identified by the maximum height reached by the foam (HM). As expected, the foamability of the wines decreased after the second fermentation due to the increase in ethanol content. The results obtained show that the addition of the yeast autolysate (AL) gave rise to sparkling wines with a higher HM than the control wines at 9 and 18 months, but these results were only statistically significant for the 10 g/hL dose of AL after 18 months. The addition of this YD followed a quantitative logic; the greater the dose, the higher the maximum height achieved. This fact coincides with the strong relation between mannoproteins, and the foam characteristics established by some authors (Núñez et al., 2006). Also, the absence of significant differences between the other treatments is also in line with other studies (La Gatta et al., 2016; Pérez-Magariño et al., 2015). Our results coincide with those obtained by Medina-Trujillo et al. (2017) who also employed the same TD and OW used in our study and observed similar results in HM after 9 months of aging on lees.

Measurements of the time needed to reach the maximum height (TM) did not achieve good repeatability, but the results showed that the effusiveness of the sparkling wines after 9 months increased with respect to the control and all the YDs except TD, and that these effects diminished after 18 months except for the TM of the foam of the sparkling wines treated with PE at 10 g/hL. It seems that the YDs potentiate the effusiveness of the foam after short aging periods; however, this effect is dispersed after longer periods. The contact with lees made from yeast used for the second fermentation had a greater effect on this parameter than the YDs.

Foam stability is equally important as foam height. The stable height of the foam (HS) represents the wine's ability to produce stable foam or persistence of the foam collar. Fig. 3, shows the similarities between the results for HS and HM. The addition of 10 g/hL of AL and OW produced sparkling wines with higher stable foam heights that were statistically significant with respect to the control wines after 18 months of aging. AL is a yeast autolysate produced from a *Saccharomyces cerevisiae* yeast selected for its high content of high molecular weight cell wall polysaccharides (Table 1, Supplementary material). AL may also be responsible for the high HM and HS, since polysaccharides have been characterized as molecules that improve foaming properties (Kemp et al., 2019).

Finally, after 9 months of aging, the highest foam stability, measured as the time required for the foam to collapse after the gas flow has stopped (TS), was observed in the wines treated with TD. In contrast, the foam quickly dissipated in the wines aged with PE and LO. After 18 months, the addition of 10 g/hL of PE and LO continued to negatively affect the foam characteristics.

3.6. Descriptive sensory analysis

Fig. 3 shows that the addition of the different YDs varied the sensory profile of the wines in most of the attributes. However, in Table S2 can be observed that the addition of YDs was significantly perceived in the sparkling wines only for some attributes, mainly in visual characteristics. As previously explained, the wine tasting was carried out by

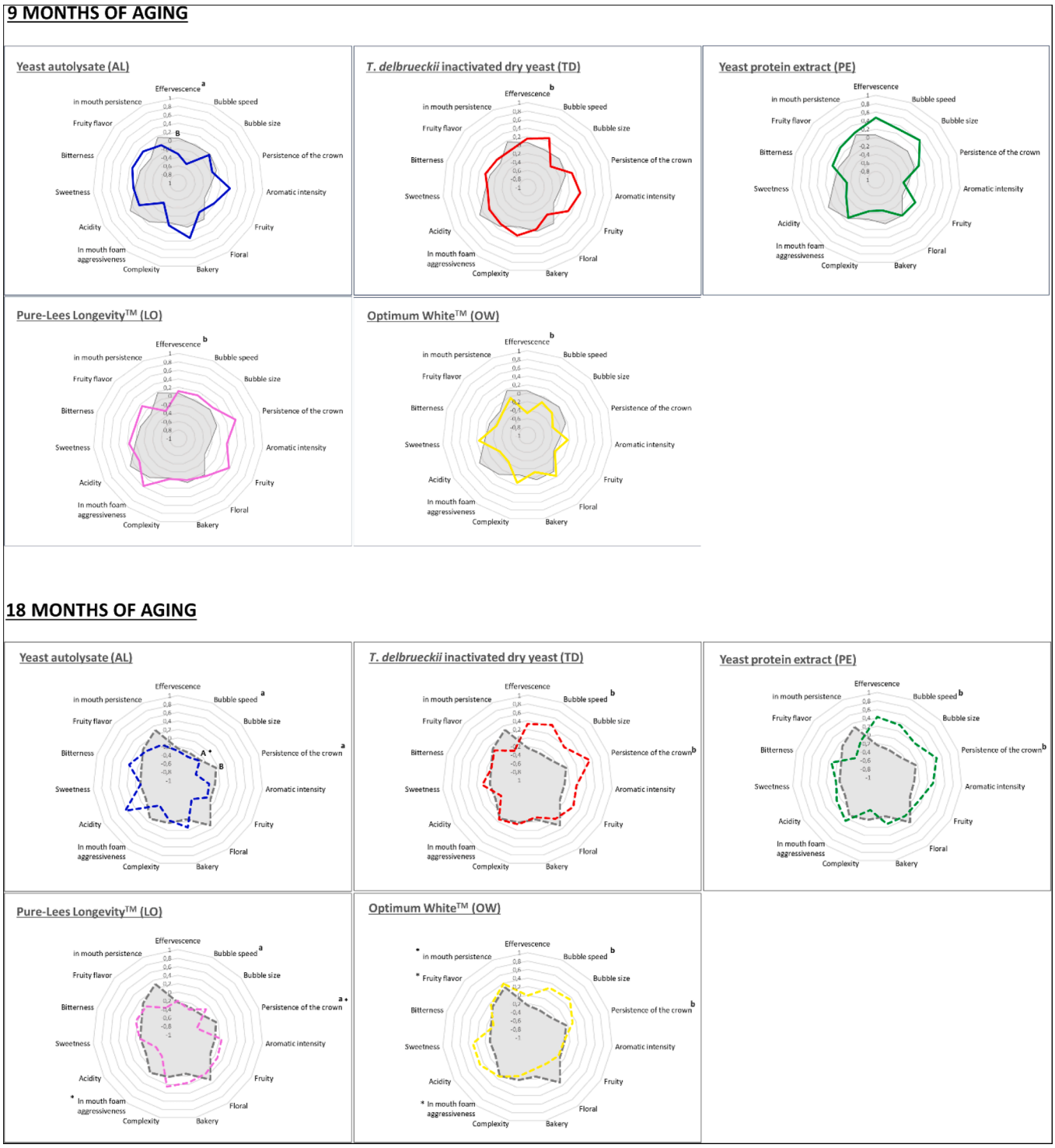


Fig. 3. Descriptive sensory analysis of the sparkling wines with the different YDs treatments (10 g/hL dosage) aged on lees during 9 and 18 months. All the graphics contain the representation of the control wine at the corresponding aging time (light grey shaded). Continuous lines: 9 months. Discontinued lines: 18 months. Different letters indicate differences between treatments applied to the sparkling wines within the same months of aging (capital letter belongs to control sample). Asterisks: significant differences between 9 and 18 months ($p < 0.05$) (Tukey test). Yeast autolysate (**AL**); yeast protein extract (**EX**); inactivated dry yeast from *Torulaspora delbrueckii* NSC19 (**TD**); Optimum White™ (**OW**); Pure-Lees Longevity™ (**LO**).

sparkling wine experts, oenologists, and producers. They were not trained by our research group; therefore, the variance among perception rates made it difficult to detect any appearance of significant differences. Despite this limitation, at first sight, the wines aged for 9 months treated with AL and OW showed less effervescence than the rest of the sparkling wines (Table S2). This result does not agree with the results obtained with the Mosalux system. This could indicate that the differences measured in these sparkling wines with the Mosalux system were not perceived by the tasters or that the time elapsed between the physical measurement of the foam properties and the sensory test could have affected the wine. In the sparkling wines treated with AL and the control aged for 18 months, the bubble speed was perceived as being slower

than that of the rest of the treatments (Table S2). Moreover, the persistence of the crown was lower in the wines treated with AL and LO. Again, these data do not coincide with the Mosalux measurements. This suggests that further research is needed in order to understand the correlation between the physical foam properties and how they are perceived by the consumer.

Among the YDs employed in this study, the commercial derivatives only showed significant differences in some of the attributes in relation to the aging time (Table S2). The addition of OW increased the in-mouth fruitiness, persistence, and foam in-mouth aggressiveness. On the other hand, foam aggressiveness decreased, and foam stability increased from 9 to 18 months with the addition of LO. There may be several reasons for the lack of more significant differences, as previously mentioned, such as the expert but non-standardized sensory panel, the time elapsed between disgorging and the sensory evaluation, in addition to the dose employed. The sparkling wines used for the sensory tests were treated with 10 g/hL. This dose may be sufficient to observe and measure some of the chemical and physical changes produced, but not enough to perceive them. Medina-Trujillo et al. (2017) employed the same YD from *Torulaspora delbrueckii* strain NSC19 and Optimum White™ used for this study but with a dose of 30 g/hL, and easily found differences with the control.

Despite the absence of statistical differences, some attributes followed common tendencies at 9 and 18 months of aging with the experimental YDs when compared to the control. For example, the addition of the yeast protein extract (PE) produced wines with greater bubble size and speed, more effervescence, and they were perceived as fruitier in nose.

The sparkling wines treated with AL were perceived as more intense in the bakery attribute and exhibited less aggressive foaming. Finally, the sparkling wines aged with TD had more persistence of the crown and bubble speed than the control.

4. Conclusions

In this study, we investigated the diversity of effects of different typology yeast-derived products in sparkling wine and showed that each one is suitable for different purposes. Moreover, the effects were not the same for the same YD at the different aging periods. In general, all the yeast derivative products employed during the *tirage* of Chardonnay sparkling wine produced chemical and physical differences with respect to the control wines. The perception of the changes was mainly sensorially appreciated in the sparkling wines with the commercial products of Optimum White™ and Pure-Lees Longevity™.

Our results point out that, to produce sparkling wines with a short aging time of 9 months, the addition of 5 g/hL of yeast protein extract and inactivated yeast from strain NSC19 of *T. delbrueckii* would help to preserve the volatile compounds responsible for its fruity characteristics. For longer aging periods of 18 months, strain NSC19 from *Torulaspora delbrueckii* would be the best option for this purpose. The effect produced by most of the YDs was a decrease in wine proteins; however, there were no significant differences in the doses employed. The autolysate had the highest potential for polysaccharide enrichment of all the YDs tested and it also resulted in sparkling wines with a notable antioxidant activity, potentially positioning it as a good product to prevent browning during long aging times. The impact of the YDs on the foaming properties was quite different depending on the aging time. Despite this, the sparkling wines treated with 10 g/hL of yeast autolysate and Optimum White™ generally exhibited the highest foamability and foam stability. However, the sensory analyses did not agree with the physical measurements, and differences between the treatments were barely perceived, likely because insufficient yeast derivative was added. The results from this study about the contributions of the YDs employed could be helpful deciding which one to use depending on the sparkling wine characteristics the oenologist wants to enhance. However, the study of higher doses would provide more conclusive information regarding the sensory repercussion of the use of these yeast derivatives on the quality of sparkling wines.

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CRediT authorship contribution statement

María Ignacia Lambert-Royo: Data curation, Investigation, Methodology. **Cristina Ubeda:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources. **Rubén Del Barrio-Galán:** Formal analysis, Investigation, Methodology. **Nathalie Sieczkowski:** Conceptualization, Visualization, Validation. **Joan Miquel Canals:** Investigation, Methodology, Validation. **Álvaro Peña-Neira:** Resources, Methodology, Validation. **Mariona Gil i Cortiella:** Conceptualization, Formal analysis, Investigation, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133174.

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