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Effect of Yeast Derivatives and β-Glucanases on Ageing over Lees Process of Tempranillo Red Sparkling Wine

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Abstract: This study focuses on improving the second fermentation and the in-bottle ageing over lees process for 9 and 21 months of a red sparkling wine. The aim of the study was to enhance wine quality and try to make it more pleasant for consumers. For this purpose, four different yeast derivatives (yeast walls, yeast walls with tannins, inactivated yeasts and mannoproteins) and β-glucanases were added to a red base sparkling wine and were aged over lees during two different periods: 9 and 21 months. Oenological parameters, total polysaccharides, total proteins, free amino nitrogen, phenolic composition, foaming properties, and volatile compounds were analysed in conjunction with a sensory evaluation. Results show the different incidences of the studied adjuvants on the final parameters, highlighting the importance of the interactions of the studied adjuvants over time. In fact, time itself turned out to be one of the main factors affecting the final characteristics of the wine, so influencing consumers' opinions and modifying the wine's acceptability and quality.

Keywords: red sparkling wine; yeast derivatives; β-glucanases; ageing over lees; second fermentation

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1. Introduction

Sparkling wines represent a crucial wine category with significant economic value in various wine regions globally. In recent years, there has been a continuous increase and diversification in the trend for sparkling wines [1]. Among the different production methods for natural sparkling wines, the most common involve second fermentation of a base wine (BW) in a bottle (traditional or champenoise method), which is recognized for producing the highest quality sparkling wines.

The elaboration of sparkling wines using the traditional method consists of a series of well-differentiated phases aimed at obtaining carbon dioxide (CO_2) of endogenous origin. This elaboration requires producing a BW with suitable characteristics, such as relatively low alcohol content (9.5–10.5% *v*/*v*), medium-high total acidity (>5.0 g/L in tartaric acid) and low pH (<3.40) [2]. Once the BW is obtained, it undergoes a second alcoholic fermentation process within a bottle, which is triggered by the addition of a *tirage liqueur* (TL). After this second fermentation the wine is kept in contact with the dead yeasts for a variable period of time, called ageing over lees, in order to transfer the compounds resulting from the enzymatic degradation of these dead yeasts in the autolysis process to the wine.

Producing quality red sparkling wines requires a series of strategies to obtain a must suitable for this type of wine. Technological and phenolic maturity must be taken into account. Achieving a balance between these two maturities is not an easy task, so several strategies have been proposed to obtain a suitable BW, either from a must with optimal characteristics or by applying different techniques to the BW [3–5].

With regard to must production, the approaches to be followed in the case of early harvests are mainly aimed at eliminating or masking the polyphenolic compounds that cause herbaceous, bitter and astringent sensations [6–12].

Another strategy may be to determine the harvest date once the optimal polyphenolic maturity stage has been reached, which would result in musts with excessively high potential alcoholic strength and could hinder the second fermentation due to the high demand on the yeasts to withstand high alcoholic strength. In this case, the reduction of the alcohol content has been studied by means of various biotechnological and physical strategies [11,13–16].

The use of *Saccharomyces* yeasts, as well as yeasts of other genera, may allow a partial reduction of the final alcoholic strength [17]. There are *Saccharomyces* yeasts with lower ethanol production, such as a strain that has the capacity to produce up to 0.7% (*v*/*v*) less ethanol, together with a lower concentration of volatile acidity, in Malvasia wines [13]. The use of some non-*Saccharomyces* yeasts strains also makes it possible to obtain wines with a lower alcohol content. Studies by Röcker et al. [14] showed that a decrease of up to 3.8% (*v*/*v*) can be achieved by inoculation of *Metschnikowia pulcherrima* alone.

Different oenological techniques were analyzed on Tempranillo red base sparkling wines by Perez-Magariño in 2019 [3]. These techniques included prefermentative cold maceration with dry ice and *delestage* with early-harvested grapes, as well as sugar reduction in must and partial dealcoholization of wine with mature grapes. According to the study, the use of these oenological techniques resulted in greater reduction in variation in the wine's volatile composition than factors such as grape maturity and ageing time.

Several researchers have focused on the management of the second fermentation and subsequent ageing over lees. On the one hand, it has been suggested that one of the key technological and qualitative characteristics of yeast strains for sparkling wine production is their autolytic capacity [18]. Yeast metabolism is responsible for the quality of the sparkling wines, producing volatile compounds and releasing mannans, mannoproteins, and high molecular weight nitrogen compounds. Evaluating the impact of *Saccharomyces cerevisiae* strains on traditional sparkling wines production, Di Gianvito et al. [19] demonstrated substantial diversification with respect to the amount and type of aromatic compounds they produced. According to this result, principal component analysis of the data obtained by Martínez-García [20], when producing sparkling wines using the same must and different yeast strains, indicated significant sample segregation with respect to ageing time and yeast strain. Interestingly, Tufariello [21] found a significant, specific impact of indigenous yeast strains in the production of regional sparkling wines on both their aroma and metabolome when compared to those made using commercial strains. Monitoring yeast autolysis in sparkling wines from nine consecutive vintages produced by the traditional method, Pons-Mercadé [22] confirmed that yeast enriches sparkling wines with key macromolecules (polysaccharides and proteins) but in low proportions compared to the typical concentrations of these compounds in sparkling wine. Regarding non-*Saccharomyces* yeasts, the use of adapted strains with varying autolytic potentials has been shown to be a valuable approach to enhancing the production of sparkling wine through traditional means and promoting its distinctiveness [23].

On the other hand, the utilization of lees, yeast derivatives, and β-glucanases in the ageing over lees procedure have drawn the attention of numerous researchers. La Gatta et al. [24] proposed an innovative technology to improve the sensory profiles and reduce the ageing time of Bombino traditional sparkling wine. The technology entailed adding different volumes of lees recovered from the first fermentation into the TL for the second fermentation. The addition of up to 60 mL/L to the BW had a positive effect on some sensory characteristics. The finesse and complexity of wines notably improved, exhibiting heightened intensities of attributes such as structure, body, aftertaste, and persistence. Sensory evaluations also noted an elevated perception of delicate aromas such as unripe fruitiness and distinct notes of yeast, while ripe fruitiness, overripe fruitiness, and olfactory intensity showed a consistent decline. Our previous research revealed that

the application of ultrasound treatment of lees prior to adding them to the BW improved the extraction of their components [25]. Sonicated lees produced a decrease of astringency and an increase of neutral polysaccharides in wine. In addition, higher concentrations of volatile compounds, such as acetates, esters, and terpenes, result in increased levels of floral and fruity aromas. Concerning yeast derivatives, the addition of 5 g/hL of yeast protein extract and inactivated yeast from a *Torulaspora delbrueckii* has been investigated, proving its ability to aid in maintaining the esters responsible for the fruity flavors in wines that underwent 9 and 18 months of lees ageing. The incorporation of the yeast autolysate resulted in greater polysaccharide enrichment and antioxidant activity [26].

In a prior study conducted by our research group, the sensory qualities and acceptability of white Verdejo sparkling wines, which were supplemented with β-glucanases or yeast derivatives (autolyzed yeasts and yeast cell walls) during the tirage phase, were enhanced after a 22-month ageing process [27,28]. The supplemented sparkling wines exhibited greater total and neutral polysaccharide levels than the control. Furthermore, the use of yeast derivatives led to an increase in fruity and floral traits, while the addition of β-glucanases heightened the aroma with a yeasty character. The impact of these adjuvants was more pronounced in long-aged sparkling wines compared to short-aged ones.

For this study, we aim to explore the potential for enhancing the quality of red sparkling wines. For this purpose, different yeast derivatives and β-glucanases were added to a Tempranillo red wine after the second fermentation. The wine was then aged over lees for 9 and 21 months, resulting in varying outcomes.

2. Materials and Methods

2.1. Adjuvants

The adjuvants used were kindly provided by the company Agrovin S.A. (Ciudad Real, Spain). These were β-glucanases (BG) (Enozym Glucan, 0.05 g/L), yeast walls with tannins (YWT) (ManoArome, 0.375 g/L), mannoproteins (MN) (Mannoplus, 0.1 g/L), inactivated yeast (IY) (SuperBouquet, 0.3 g/L) and yeast walls (YW) (SuperBouquet MN, 0.3 g/L). Doses of various adjuvants were used in accordance with the recommendations of the manufacturers, using the highest concentrations suitable for producing sparkling wine.

2.2. Vinification Process

To obtain the BW, 200 kg of red Tempranillo grapes from the Valles de Benavente DO were processed. It was made using the standard red vinification method [29] by sulphiting the paste at a rate of 35 mg/L of total SO_2. To increase the extraction of phenolic compounds, the pectolytic enzymes, Lallzyme HC® and Lallzyme EX® (Lallemand, France), were added at a rate of 1.5 and 2.5 g/100 kg, respectively. After alcoholic fermentation with *S. cerevisiae* var. *bayanus* yeast (Viniferm BY®, Agrovin, Spain), the paste was pressed and malolactic fermentation occurred after inoculation with *Oenococcus oeni* (Lalvin VP41, Lallemand). To obtain the sparkling wine, the BW was chaptalized using a TL composed of 25 g/L of sucrose, 40 g/hL of *S. cerevisiae* var. *bayanus* yeast (Viniferm BY®,) and 20 g/hL of bentonite (Bengel®, Agrovin). A total of 5 batches (YW; YWT; BG; IY; MN) were prepared, to which adjuvants were added with the TL, except for MN which were added during disgorging with the expedition liqueur according to the manufacturer's instructions. At the same time, control wine (C) was also elaborated without adding adjuvants (Figure 1). During the second fermentation the bottles were horizontally positioned at 17 °C. Out of the 168 bottles collected, half were aged for 9 months, and the remaining ones for 21 months, after which the wine underwent disgorging, the lost wine was replenished with wine from each batch, and the bottles were recapped again.

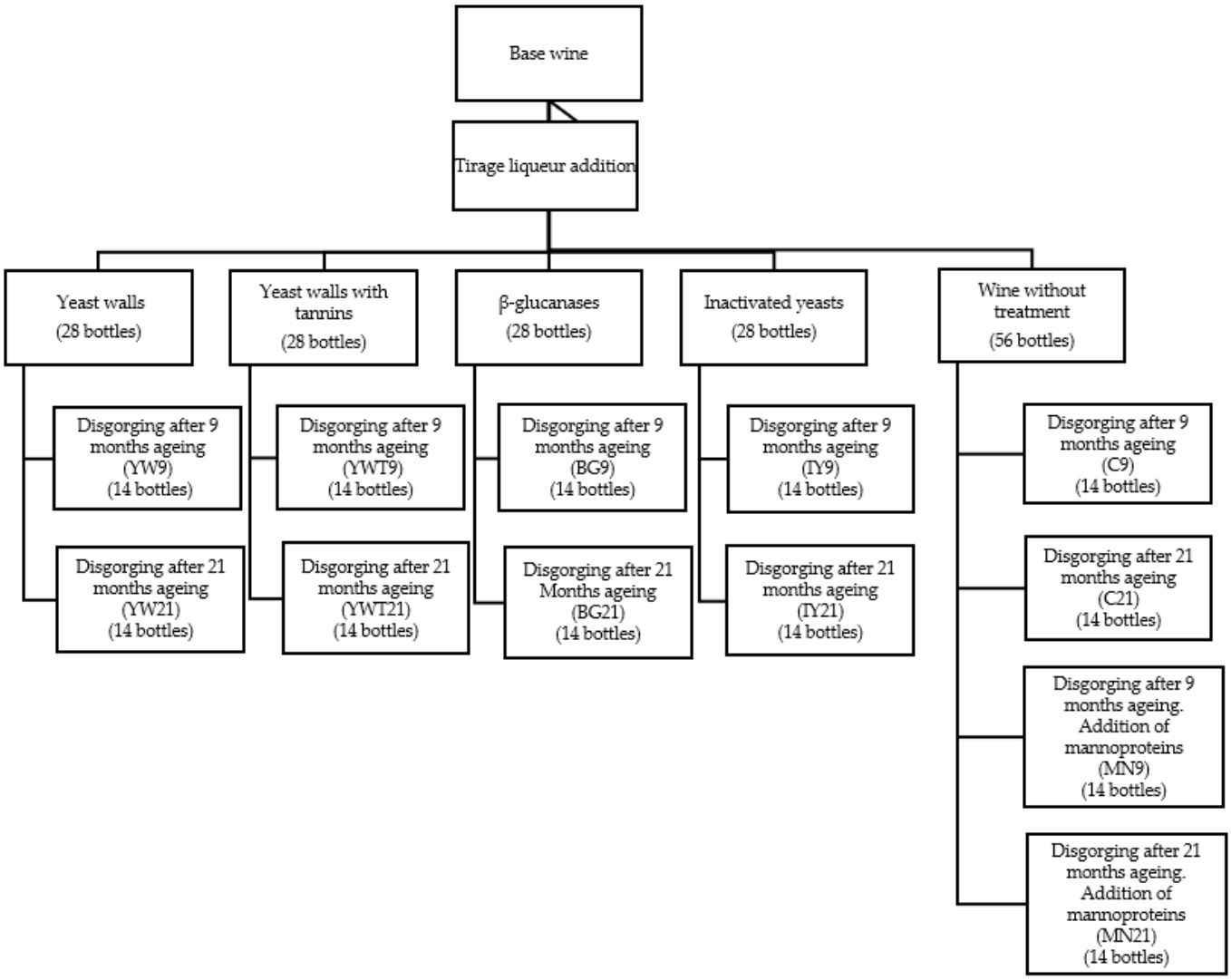

Figure 1. Scheme of the experiments carried out using the different adjuvants to produce the sparkling wines.

2.3. Chemical Reagents

The analytical quality reagents used were provided by Panreac S.A. (Madrid, Spain), except for the internal standards used for the analysis of organic volatile compounds (octan-2-ol and methyl nonanoate) which came from Merck (Darmstadt, Germany).

2.4. Physicochemical Analyses of Sparkling Wines

The analyses used to characterize sparkling wines such as total and volatile acidity, total polyphenol index (TPI), pH, reducing sugars and alcoholic strength, were carried out according to OIV techniques [30]. Analyses of hydroxycinnamic acids and flavonols were conducted by measurements of UV absorbance at 320 and 365 nm, respectively [31]. Total polysaccharides were evaluated according to the phenol-sulfuric acid method [32]. The amounts of total proteins were determined using the Bradford method modified by Murphey [33]. The formol titration method was employed for the quantification of free amino nitrogen [34]. The methods described by Hidalgo [35] were used for the analysis of tannins and anthocyanin. All analyses were performed in two bottles of each treatment, in triplicate, two months after disgorging.

2.5. Foaming Properties

The foaming properties were assessed through the utilization of the Mosalux method, described by Maujean et al. [36], in triplicate. For the measurement, the wines were previously degassed and 100 mL of sample was placed in a glass tube and then subjected to a constant flow of CO_2 (7 L/h). The resulting parameters obtained were foamability (F) (mm), which represents the maximum height reached by the foam; foam persistence (FP) (mm), being the stable height of the foam; and foam stability (FS) (s), which is the time it takes for the foam to disappear once the CO_2 flow is finished and is related to the foam persistence.

2.6. Analysis of Volatile Organic Compounds (VOCs)

Headspace Solid-Phase Microextraction (HS-SPME) was used for VOC extraction using a CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland). Thus, 5 mL of wine, a standard of 50 µL of methyl nonanoate (0.059 mg/L) and 3 g of sodium chloride were added to a 20 mL vial. Subsequently, the vial was stirred at 250 rpm for 15 min at a temperature of 40 °C. Then, a DVB/CAR/PDMS fiber (50/30 µm, Supelco, Inc., Bellefonte, PA, USA) was introduced into the headspace of the vial for 30 min at 40 °C with agitation at 250 rpm. The fiber was then desorbed into the injector of the gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA) for 15 min at 250 °C. The injection into the column (HP-Innowax, 60 m, 0.250 mm, 0.5 µm, J & W Scientific, Folsom, CA, USA) was performed in splitless mode for 1 min. The oven program was set as follows: 40 °C (5 min), 2.5 °C/min to 230 °C and 230 °C (20 min). Helium was used as the carrier gas at 1.2 mL/min [37]. VOC identification was performed using a mass selective detector (5977, Agilent Technologies) scanning the m/z range from 30 to 500. Mass spectra of pure standards and NIST08 v. 2.4 and Wiley7 libraries were used for the identification of VOCs [38]. Data were expressed as equivalents [39,40] using the internal standard quantification method. Analyses were performed in triplicate at the Laboratory of Instrumental Techniques at the University of Valladolid (Valladolid, Spain).

2.7. Sensory Analysis

A panel of 108 consumers from 19 to 65 years old, of which 60% were women and 40% were men, carried out the sensory evaluation of the wines. They performed an acceptability test in which they had to evaluate different parameters such as color, smell, taste, persistence, pungency and overall acceptability on a 9-point hedonic scale (from "1—dislike extremely" to "9—like extremely") [41]. In all sessions, the samples were served as 25 mL aliquots in high-quality sparkling flute-type wine glasses and were presented monadically using a randomized complete block design with three-digit codes. The serving temperature of the samples was 10 ± 1 °C. Consumers were provided with water to rinse their mouths between samples. The consumer sensory assessments took place in individual booths in a tasting room according to the ISO 8589 Standard [42], at the Sensory Science Laboratory in the School of Agricultural Engineering, University of Valladolid, Palencia (Spain).

2.8. Statistical Analysis

The data obtained were expressed as the mean ± standard error. Analysis of variance (ANOVA) for two factors (ageing time and treatment) and their interaction was carried out and differences were determined using Tukey's test with 95% confidence. Principal component analysis (PCA) was also performed on sensory analysis data to characterize the samples. Analyses were performed using Statgraphics Centurion (v.19, Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results and Discussion

3.1. Oenological Parameters

No statistically significant differences were found between the treatments for either the alcohol strength or the residual sugar content at 9 and 21 months (Table 1).

Regarding volatile acidity, no statistically significant differences were found among the applied treatments. However, and in agreement with our own results in a previous study in a short ageing period [43], ageing time and the interaction term (ageing time × treatment) showed statistically significant differences in volatile acidity (Table 1), with the control wine showing the highest increase from 9 to 21 months of bottle age and the BG treatment showing the best behavior, with a small decrease in the so mentioned parameter. All values were between 0.29 g/L and 0.41 g/L, indicating the good preservation of the bottled wines.

Slight differences were found in total acidity and pH over time, with an increase in both parameters, in accordance with the results of Sartor et al. [44], who found that the pH tended to rise a little during ageing over lees of rosé wines treated with mannoproteins. The pH level increase could be attributed to acid crystallization resulting from the formation of insoluble complexes like calcium and potassium tartrate. Gnoinski et al. [45] reported similar pH and total acidity levels at 6 and 12 months of bottle ageing, and significantly lower pH and total acidity after 18 months of ageing of a white Chardonnay and Pinot noir sparkling wine. However, in a previous study [27], our group detected no statistically significant differences between white sparkling wines produced with yeast derivatives and β-glucanases after 22 months of ageing, while Ubeda et al. [17] observed an increase in pH throughout the ageing period (0, 3, 6, 9 and 12 months) similar to our own findings in a previous study in a short ageing period of 9 months [28]. Alternatively, the elevation in total acidity could be explained by the release of compounds such as peptides and proteins during the autolysis process. These substances are protonated in the wine's pH conditions [46], so they do not affect the pH measurement, but they do affect the total acidity due to their deprotonation caused by the endpoint pH for wine titrations, in which the carboxylic acids groups will contribute to total acidity [47,48].

In any case, differences in pH and titratable acidity would not result in perceived sensory differences in wine (pH—3.76 to 3.84; total acidity—4.02 to 4.52 g/L).

3.2. Total Polysaccharides, Total Proteins and Free Amino Nitrogen

In the case of total polysaccharides, time was the only factor that had a statistically significant effect on their content, showing a marked increase (Table 2). These results could be attributed to enhanced yeast autolysis, bringing about a rise in the number of polysaccharides from yeast cell walls [27]. Lambert-Royo et al. [26] conducted a study with five yeast derivatives during an 18-month ageing period and found a statistical interaction between yeast derivatives and dosage, concluding that the release and removal of polysaccharides during the elaboration and ageing of sparkling wines seems to depend on complex phenomena that have not yet been elucidated.

Total protein concentration did not show statistically significant differences between the control and the treated wines (Table 2). In agreement with the results of Gnoinski et al. [45], the only factor that shows statistically significant differences is ageing time, showing a significant increase (Table 2). However, Gnoinski et al.'s findings said that this increase stopped at 12 months of ageing, whereas our results reveal a continuous increase throughout the ageing process up to the 21 months under study, similar to what happened in our previous study with short ageing [28]. These results contrast with those obtained by Lambert-Royo et al. [26] using several yeast derivatives, which revealed that the addition of the different adjuvants generally resulted in a decrease in protein content.

Table 1. Oenological parameters determined in the sparkling wines at 9 and 21 months of ageing over lees.

Parameters	AT	YW	YWT	BG	MN	IY	C	AT	T	AT × T
Alcoholic strength	9	12.25 ± 0.86	12.65 ± 1.32	12.65 ± 1.44	12.60 ± 1.27	12.40 ± 1.38	12.65 ± 1.55	ns	ns	ns
	21	11.50 ± 0.00	11.20 ± 0.00	11.50 ± 0.00	11.50 ± 0.00	11.50 ± 0.00	11.50 ± 0.00			
Reducing sugars	9	1.42 ± 0.43	1.72 ± 0.48	1.32 ± 0.75	1.55 ± 0.31	1.75 ± 0.45	1.12 ± 0.62	ns	ns	ns
	21	1.55 ± 0.33	1.22 ± 0.68	1.97 ± 0.61	1.67 ± 0.93	1.32 ± 0.53	1.45 ± 0.52			
Volatile acidity	9	0.31 ± 0.03	0.29 ± 0.02	0.30 ± 0.06	0.27 ± 0.03	0.30 ± 0.01	0.27 ± 0.04	*	ns	*
	21	0.41 ± 0.07	0.32 ± 0.05	0.29 ± 0.02	0.29 ± 0.03	0.39 ± 0.09	0.40 ± 0.03			
pH	9	3.76 ± 0.06 ^{a}	3.82 ± 0.00 ^{bc}	3.76 ± 0.04 ^{ab}	3.77 ± 0.04 ^{ab}	3.84 ± 0.02 ^{bc}	3.82 ± 0.02 ^{bc}	*	ns	ns
	21	3.87 ± 0.22	3.90 ± 0.09	3.94 ± 0.13	3.93 ± 0.11	3.92 ± 0.13	3.92 ± 0.10			
Total acidity	9	4.00 ± 0.14 ^{a}	4.02 ± 0.15 ^{ab}	4.30 ± 0.18 ^{de}	4.27 ± 0.15 ^{cde}	4.52 ± 0.26 ^{e}	4.25 ± 0.12 ^{abc}	*	ns	ns
	21	4.69 ± 0.18	4.52 ± 0.14	4.97 ± 0.60	4.82 ± 0.33	4.73 ± 0.47	4.43 ± 0.27			

Alcoholic strength (% *v*/*v*); reducing sugars (g/L); volatile acidity (g acetic acid/L); total acidity (g tartaric acid/L); AT—ageing time; T—treatment; AT × T—ageing time × treatment. Different letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). Asterisk (*) indicates significance at $p < 0.05$ and ns indicates no significance determined by two-way ANOVA.

Table 2. Total polysaccharides, total proteins and free amino nitrogen determined in the sparkling wines at 9 and 21 months of ageing over lees.

Parameters	AT	YW	YWT	BG	MN	IY	C	AT	T	AT × T
Total polysaccharides	9	3.70 ± 0.22	2.77 ± 1.11	3.62 ± 0.38	3.68 ± 1.59	4.28 ± 0.75	3.66 ± 1.47	*	ns	ns
	21	4.71 ± 1.36	4.76 ± 1.94	5.73 ± 0.86	5.16 ± 1.20	7.50 ± 0.42	5.97 ± 1.96			
Total proteins	9	145.32 ± 8.76	141.11 ± 4.14	152.33 ± 5.36	147.42 ± 9.93	143.68 ± 5.78	137.14 ± 35.94	*	ns	ns
	21	152.61 ± 5.65	152.17 ± 4.71	152.55 ± 6.27	150.86 ± 11.99	157.23 ± 8.61	154.89 ± 7.20			
Free amino nitrogen	9	56.00 ± 1.20 ^{a}	70.00 ± 0.06 ^{b}	84.00 ± 0.31 ^{c}	70.00 ± 0.61 ^{b}	70.00 ± 0.93 ^{b}	63.00 ± 9.89 ^{ab}	*	*	*
	21	65.33 ± 6.60	58.33 ± 8.73	56.00 ± 0.00	60.66 ± 6.98	58.33 ± 9.85	64.86 ± 4.62			

Total polysaccharides (g/L); total proteins (mg/L); free amino nitrogen (mg/L); AT: ageing time; T—treatment; AT × T—ageing time × treatment. Different letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). Asterisk (*) indicates significance at $p < 0.05$ and ns indicates no significance determined by two-way ANOVA.

Pons-Mercadé et al.'s findings [22] must be also taken into consideration when analyzing the content of the compounds released by the lees of nine consecutive vintages. The proportion of polysaccharides and proteins obtained from the lees was low in the youthful sparkling wines, specifically, 2–3% during the initial year of ageing and 7% at the end of 3 years of ageing. This implies that the contribution of polysaccharides and proteins from lees autolysis in sparkling wines disgorged before the end of the first year of bottle ageing should be low.

It must be taken into account that autolysis is a catabolic process that involves breaking down macromolecules within cells. In parallel, the irreversible degradation of cell wall components, such as glucans and mannoproteins, and increases the porosity of the cell wall, which facilitates the release of the degrading components into the wine. This may explain why the treatment with BG showed the highest levels of total proteins at 9 months, while remaining unchanged at 21 months, whereas the other treatments continued to show increased release [49].

For free amino nitrogen, statistically significant differences were found with respect to ageing time, treatment and their interaction (Table 2). Statistically significant differences were observed between treatments for free amino nitrogen after 9 months. BG exhibited the highest levels, followed by YWT, MN, IY, and the control, with YW producing the lowest levels. This is likely due to the brief period of ageing over lees during the second fermentation, in which yeasts utilize some of the free amino acids, while autolysis releases some into the medium. During this brief period, β-glucanase enzymes, by breaking the cell wall, facilitate the release of compounds from autolysis, such as amino acids and other free amino nitrogen compounds. The utilized yeast derivatives lack hydrolytic activity; therefore, the increase in free amino nitrogen content, in comparison to the control, during this brief ageing period, will be contingent on the particular makeup of the commercial preparation employed. During extended ageing, the free amino nitrogen content tends to decrease, potentially due to the breakage reactions of amino acids (deamination) or the formation of other compounds [49]. Our results are in agreement with the findings of Gnoinski et al. [45] for Chardonnay and Pinot noir sparkling wines, when yeasts subjected to different lysis treatments were added to the TL during the second fermentation, revealing that these enzymes represent tools that could potentially be used to induce the release of yeast compounds into the wine. In contrast, we found that yeast walls produced the lowest levels of free amino nitrogen (Table 2).

3.3. Phenolic Composition

Total polyphenol index showed a statistically significant decrease over time, in parallel with flavonol content, total anthocyanins and tannin content. Hydroxycinnamic acids showed no significant differences. These changes in wine ageing may stem from the hydrolysis of esterified compounds, resulting in the creation of free hydroxycinnamic acids or participation in polymerization reactions with anthocyanins. This process can ultimately lead to the formation of pyranoanthocyanins [50,51]. Copigmentation, depolymerization or polymerization reactions of these compounds with other phenolics that occur during wine ageing can also be involved in the obtained results [47].

No statistically significant differences were found, either with regard to the adjuvants used or interactions of adjuvants with ageing time, except total anthocyanins, which showed differences in this interaction (Table 3). These results are consistent with our previous results [28] and may be explained by the fact that the protective effect against phenol oxidation provided by the yeast derivatives and the products released by the β-glucanases diminishes over time. Lambert-Royo et al. [26] found no significant differences in color intensity or browning between the different yeast derivatives and dosages during sparkling wine ageing. On the contrary, Gnoinski et al. [45] found an increase in total polyphenol content during the ageing process, independently of the treatment, and Sartor et al. [52] reported statistically significant differences in color parameters, antioxidant activity, and individual concentrations of phenolic compounds in mannoprotein-treated

rosé sparkling wines during ageing over lees, although with variable effects throughout ageing, and mainly observed at the end of the ageing over lees process. Rinaldi et al. [53] concluded that using commercial mannoproteins may be a way to improve the mouthfeel and color of high tannin wines. In our study, it is likely that the contact time between the mannoproteins and the wine was too short to affect the final wine characteristics.

3.4. Influence on Foaming Properties

The foamability shows a statistically significant difference between adjuvants and increases with the ageing process (Table 4). In agreement with Ubeda et al. [17], who found no significant differences in the parameters of foamability, crown persistence, and bubble velocity and size between samples analysed after 3, 6, 9, and 12 months of ageing over lees, the duration of ageing seems to be a critical factor in terms of foamability. As can be seen in Table 4, at 9 months treatments BG, MN and YW have the highest values, while YWT has the lowest. At 21 months, however, treatment BG still has the highest values and MN reaches the second value (Table 4), while all other samples show a much smaller increase in foamability compared to 9 months. This could indicate that to improve the foaming conditions of red sparkling wine, it is advisable to add β-glucanases or mannoproteins.

Following the Mosalux procedure, Lambert-Royo et al. [26] found that the effect of the studied yeast derivatives on the foaming properties differed considerably depending on the ageing time, with yeast autolysates giving the highest values of foamability. Their findings, like ours, demonstrate the strong relationship between mannoproteins and foaming properties.

Regarding the foam persistence, Table 4 shows the statistically significant differences over time between the adjuvants and adjuvant x time interaction. The foam persistence improves with time. At 9 months, the YWT treatment has the lowest values and the others are all similar, while at 21 months they become the best together with the IY, followed by the MN and finally all the other adjuvants and the control. This may be due to the fact that the tannins in the yeast walls have reacted with the polyphenols in the wine, which has an effect on the foam persistence.

It is known that proteins with a weight of 420 KDa have a foaming-enhancing effect due to the physical properties of hydrophobicity [54]. Conversely, amino acids at wine pH have a positive charge and act as surfactants containing hydrophobic and hydrophilic groups. Amino acids are retained at the air-liquid interface of the bubbles, thereby reducing the surface tension of the wine and improving its foaming capacity [55]. Moreover, an improvement in foam quality has been attributed to the rise in polysaccharide concentration in the wine [56]. These molecules, owing to their hydrophobicity, can interact with the gas bubble walls and enhance the foam's properties [57]. We need to consider the potential interaction between the phenolic compounds in our red wine and those found in commercial preparations that may enhance foaming properties.

3.5. Evolution of VOCs

Almost all the alcohols studied show only statistically significant differences with time of ageing, with a slight decreasing tendency, except for octan-1-ol, which also showed significant differences with adjuvants (Table 5). The control sample (C) had the highest value at 9 months and the treatment BG had the lowest value at 9 months, while at 21 months there were no differences between samples. Ubeda et al. [17] did not find any noticeable changes in volatile composition during an ageing process of 18 months. Gallardo-Chacón et al. [58] postulated that yeast lees have a low capacity for alcohol retention on their surface. However, there is no consensus in the literature as to whether higher alcohols have a positive or negative contribution, although they are considered to be the aromatic compounds with the strongest effect on overall wine aroma. Though suspected of having a negative impact, this has been reported to depend on the specific aromatic context [59].

Table 3. Phenolic composition determined in the sparkling wines at 9 and 21 months of ageing over lees.

Parameters	AT	YW	YWT	BG	MN	IY	C	AT	T	AT × T
Total polyphenol index	9	30.58 ± 3.58	28.55 ± 2.51	29.23 ± 4.05	27.88 ± 2.84	27.28 ± 1.74	28.83 ± 1.67	*	ns	ns
	21	26.5 ± 2.38	28.5 ± 4.65	26.25 ± 2.50	24.50 ± 3.42	24.00 ± 3.46	25.00 ± 1.15			
Hydroxycinnamic acids	9	11.68 ± 1.04	10.8 ± 0.98	11.45 ± 1.32	10.75 ± 0.50	10.65 ± 0.79	10.85 ± 0.60	ns	ns	ns
	21	11.25 ± 3.20	11.5 ± 3.70	11.00 ± 2.16	10.00 ± 0.82	10.00 ± 0.82	10.50 ± 1.73			
Flavonols	9	4.65 ± 0.35	4.20 ± 0.29	4.53 ± 0.51	4.33 ± 0.33	4.13 ± 0.39	4.18 ± 0.36	*	ns	ns
	21	3.95 ± 0.92	4.08 ± 0.94	3.97 ± 0.77	3.75 ± 0.57	3.86 ± 0.38	3.66 ± 0.62			
Total anthocyanins	9	141.97 ± 9.99	128.63 ± 7.36	115.72 ± 28.15	136.94 ± 8.76	121.19 ± 12.43	142.84 ± 9.54	*	ns	*
	21	75.47 ± 3.14 ^{a}	68.91 ± 1.94 ^{b}	76.78 ± 2.3 ^{a}	69.34 ± 2.61 ^{b}	65.84 ± 1.49 ^{bc}	60.16 ± 3.74 ^{c}			
Tannins	9	1.06 ± 0.17	1.13 ± 0.31	0.99 ± 0.29	0.98 ± 0.28	0.89 ± 0.24	1.01 ± 0.23	*	ns	ns
	21	0.82 ± 0.10 ^{a}	0.83 ± 0.06 ^{a}	0.71 ± 0.21 ^{b}	0.81 ± 0.10 ^{ab}	0.71 ± 0.09 ^{ab}	0.81 ± 0.10 ^{ab}			

Total polyphenol index (A280 nm); hydroxycinnamic acids (A320 nm); flavonols (A365 nm); total anthocyanins (mg/L); tannins (g/L); AT—ageing time; T—treatment; AT × T—ageing time × treatment. Different letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). Asterisk (*) indicates significance at $p < 0.05$ and ns indicates no significance determined by two-way ANOVA.

Table 4. Foaming properties, as determined by Mosalux, in the sparkling wines at 9 and 21 months of ageing over lees.

Parameters	AT	BG	YWT	MN	IY	YW	C	AT	T	AT × T
Foamability	9	3.50 ± 0.00 ^{d}	2.10 ± 0.14 ^{a}	3.30 ± 0.42 ^{cd}	3.15 ± 0.21 ^{bc}	3.40 ± 0.28 ^{cd}	2.90 ± 0.14 ^{b}	*	*	*
	21	35.50 ± 0.71 ^{d}	27.50 ± 2.12 ^{ab}	31.50 ± 0.70 ^{c}	28.50 ± 0.70 ^{b}	27.00 ± 1.41 ^{a}	26.50 ± 0.70 ^{a}			
Foam persistence	9	1.85 ± 0.07 ^{b}	1.50 ± 0.00 ^{a}	1.95 ± 0.35 ^{bc}	1.90 ± 0.14 ^{bc}	2.00 ± 0.00 ^{bc}	2.05 ± 0.07 ^{c}	*	*	*
	21	17.5 ± 0.70 ^{a}	20.00 ± 0.00 ^{c}	19.50 ± 0.70 ^{bc}	20.00 ± 0.00 ^{c}	18.50 ± 2.12 ^{ab}	17.50 ± 0.70 ^{a}			
Foam stability time	9	95.50 ± 10.60 ^{cd}	80.00 ± 7.07 ^{ab}	97.50 ± 12.02 ^{d}	93.00 ± 24.04 ^{bcd}	81.00 ± 7.07 ^{abc}	76.50 ± 6.36 ^{a}	*	*	ns
	21	35.50 ± 0.70 ^{e}	27.00 ± 0.00 ^{c}	33.50 ± 0.70 ^{d}	22.50 ± 0.70 ^{a}	24.50 ± 2.12 ^{b}	23.50 ± 0.70 ^{ab}			

Foamability (mm); foam persistence (mm); foam stability time (s); AT—ageing time; T—treatment; AT × T—ageing time × treatment. Different letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). Asterisk (*) indicates significance at $p < 0.05$ and ns indicates no significance determined by two-way ANOVA.

Table 5. VOCs (mg/L) in the sparkling wines after 9 and 21 months of ageing over lees.

Compounds	AT	BG	YWT	MN	IY	YW	C	AT	T	AT × T
Propan-1-ol	9	0.29 ± 0.03	0.30 ± 0.03	0.25 ± 0.02	0.30 ± 0.01	0.24 ± 0.05	0.27 ± 0.01	*	ns	ns
	21	0.11 ± 0.00	0.11 ± 0.00	0.14 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.02			
2-Methylpropan-1-ol	9	3.41 ± 0.23	3.74 ± 0.21	3.39 ± 0.24	3.58 ± 0.14	3.64 ± 0.13	3.29 ± 0.20	*	ns	ns
	21	1.16 ± 0.0	1.31 ± 0.08	1.71 ± 0.32	1.26 ± 0.19	1.20 ± 0.13	1.21 ± 0.19			
3-Methylbutan-1-ol	9	46.15 ± 2.89	50.45 ± 1.29	46.61 ± 1.80	47.73 ± 2.65	49.80 ± 1.08	47.88 ± 0.21	*	ns	ns
	21	26.40 ± 1.36	29.35 ± 2.44	34.09 ± 5.41	28.28 ± 3.41	28.13 ± 2.81	27.06 ± 4.06			
Hexan-1-ol	9	1.69 ± 0.04	2.08 ± 0.12	1.56 ± 0.57	1.84 ± 0.19	2.05 ± 0.08	2.02 ± 0.08	*	ns	ns
	21	0.92 ± 0.04	1.03 ± 0.04	1.15 ± 0.18	0.97 ± 0.09	1.03 ± 0.08	0.93 ± 0.14			
Heptan-1-ol	9	0.28 ± 0.03	0.35 ± 0.04	0.32 ± 0.00	0.30 ± 0.05	0.33 ± 0.02	0.34 ± 0.02	*	ns	ns
	21	0.18 ± 0.00	0.21 ± 0.00	0.24 ± 0.03	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.02			
Octan-1-ol	9	0.15 ± 0.01 ^{a}	0.22 ± 0.01 ^{ab}	0.24 ± 0.00 ^{ab}	0.19 ± 0.02 ^{ab}	0.23 ± 0.00 ^{ab}	0.28 ± 0.04 ^{b}	*	*	*
	21	0.09 ± 0.00	0.11 ± 0.00	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.00			
2-Phenylethanol	9	35.21 ± 0.64	37.53 ± 2.30	34.63 ± 0.41	34.89 ± 1.64	37.45 ± 0.53	37.10 ± 4.02	*	ns	ns
	21	19.06 ± 1.91	21.35 ± 0.08	23.88 ± 1.03	20.86 ± 5.06	17.77 ± 1.45	18.56 ± 0.04			
3-Methylsulfanylpropan-1-ol	9	0.50 ± 0.04	0.57 ± 0.01	0.51 ± 0.00	0.51 ± 0.01	0.62 ± 0.01	0.59 ± 0.09	*	ns	ns
	21	0.16 ± 0.00	0.17 ± 0.01	0.18 ± 0.00	0.18 ± 0.10	0.19 ± 0.03	0.13 ± 0.02			
(Z)-Hex-3-en-1-ol	9	0.20 ± 0.02	0.21 ± 0.00	0.18 ± 0.00	0.21 ± 0.03	0.22 ± 0.00	0.22 ± 0.01	*	ns	ns
	21	0.09 ± 0.00	0.09 ± 0.01	0.0 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.00			
Hexanoic acid	9	1.47 ± 0.07	1.65 ± 0.15	1.41 ± 0.04	1.40 ± 0.17	1.62 ± 0.06	1.51 ± 0.28	*	ns	ns
	21	0.59 ± 0.06	0.67 ± 0.01	0.80 ± 0.07	0.66 ± 0.19	0.48 ± 0.06	0.52 ± 0.05			
Octanoic acid	9	3.69 ± 0.39	4.34 ± 0.58	3.76 ± 0.24	3.43 ± 0.38	4.34 ± 0.00	4.88 ± 1.88	*	ns	ns
	21	1.57 ± 0.10	1.78 ± 0.20	2.09 ± 0.22	2.09 ± 0.38	1.43 ± 0.10	1.57 ± 0.12			
Ethyl acetate	9	3.26 ± 0.19	3.43 ± 0.08	3.03 ± 0.24	3.10 ± 0.04	3.26 ± 0.14	3.06 ± 0.12	*	ns	ns
	21	1.69 ± 0.01	2.01 ± 0.27	2.07 ± 0.40	1.85 ± 0.19	2.03 ± 0.09	1.84 ± 0.27			
3-Methylbutyl acetate	9	4.13 ± 0.25	7.61 ± 0.82	6.47 ± 1.90	5.04 ± 1.21	7.41 ± 0.50	5.27 ± 1.11	*	ns	ns
	21	4.86 ± 0.28	4.28 ± 0.22	5.93 ± 1.20	4.98 ± 0.18	5.45 ± 0.67	5.64 ± 0.25			

Table 5. *Cont.*

Compounds	AT	BG	YWT	MN	IY	YW	C	AT	T	AT × T
2-Phenylethyl acetate	9	1.80 ± 0.09	4.30 ± 0.41	3.56 ± 1.34	2.81 ± 1.19	4.21 ± 0.01	3.48 ± 0.10	*	ns	*
	21	1.88 ± 0.25	1.55 ± 0.35	2.21 ± 0.38	1.76 ± 0.18	1.55 ± 0.37	2.00 ± 0.51			
Ethyl 2-hydroxypropanoate	9	3.00 ± 0.17	3.09 ± 0.15	2.66 ± 0.13	3.04 ± 0.26	3.16 ± 0.02	2.88 ± 0.13	*	ns	ns
	21	0.80 ± 0.04	0.93 ± 0.09	0.96 ± 0.06	0.89 ± 0.08	0.88 ± 0.04	0.81 ± 0.12			
Ethyl butanoate	9	0.28 ± 0.01	0.36 ± 0.03	0.31 ± 0.01	0.30 ± 0.01	0.31 ± 0.06	0.29 ± 0.08	*	ns	ns
	21	0.17 ± 0.01	0.20 ± 0.01	0.22 ± 0.05	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.03			
Diethyl butanedioate	9	20.80 ± 0.59	22.13 ± 0.60	20.45 ± 0.47	20.62 ± 1.20	22.38 ± 0.18	21.83 ± 1.39	*	ns	*
	21	6.67 ± 0.21	8.95 ± 0.51	8.35 ± 0.76	7.55 ± 1.64	6.02 ± 0.86	6.64 ± 0.25			
Ethyl hexanoate	9	6.86 ± 0.57	8.75 ± 0.42	8.51 ± 0.29	7.55 ± 1.24	8.92 ± 0.68	8.12 ± 1.13	*	*	ns
	21	2.75 ± 0.08	3.23 ± 0.37	4.10 ± 0.71	3.09 ± 0.00	3.63 ± 0.51	3.09 ± 0.30			
Ethyl octanoate	9	27.64 ± 2.75	31.12 ± 2.06	33.55 ± 0.37	28.66 ± 0.06	33.62 ± 2.54	34.33 ± 3.48	*	*	ns
	21	5.31 ± 0.85 ^{a}	7.50 ± 0.19 ^{ab}	10.09 ± 0.92 ^{b}	7.36 ± 1.34 ^{ab}	5.85 ± 1.41 ^{a}	6.80 ± 0.25 ^{ab}			
Ethyl decanoate	9	5.20 ± 0.13	5.91 ± 0.57	6.47 ± 1.05	4.82 ± 1.59	5.61 ± 0.25	8.99 ± 0.85	*	*	*
	21	1.64 ± 0.20 ^{ab}	2.07 ± 0.11 ^{ab}	2.37 ± 0.36 ^{ab}	2.54 ± 0.17 ^{b}	1.30 ± 0.48 ^{a}	2.19 ± 0.14 ^{ab}			
Ethyl dec-9-enoate	9	1.56 ± 0.05 ^{b}	2.44 ± 0.11 ^{d}	2.06 ± 0.13 ^{cd}	1.17 ± 0.04 ^{a}	2.11 ± 0.13 ^{cd}	1.90 ± 0.05 ^{bc}	*	*	*
	21	0.56 ± 0.09	0.61 ± 0.01	0.81 ± 0.00	0.86 ± 0.22	0.43 ± 0.14	0.64 ± 0.02			
Ethyl dodecanoate	9	1.17 ± 0.31 ^{a}	0.89 ± 0.24 ^{a}	1.96 ± 0.04 ^{a}	0.71 ± 0.31 ^{a}	0.72 ± 0.01 ^{a}	3.73 ± 0.93 ^{b}	*	*	*
	21	0.53 ± 0.00	0.71 ± 0.03	0.38 ± 0.01	0.58 ± 0.30	0.31 ± 0.09	0.39 ± 0.09			
Ethyl tetradecanoate	9	0.75 ± 0.00 ^{a}	1.60 ± 0.39 ^{a}	2.37 ± 0.38 ^{ab}	0.74 ± 0.05 ^{a}	0.97 ± 0.19 ^{a}	4.09 ± 1.33 ^{b}	*	*	*
	21	0.42 ± 0.10	0.35 ± 0.01	0.32 ± 0.01	0.31 ± 0.07	0.35 ± 0.12	0.35 ± 0.03			
Ethyl hexadecanoate	9	3.19 ± 0.00	4.06 ± 0.09	3.76 ± 0.03	3.06 ± 0.01	4.41 ± 0.21	4.59 ± 0.40	*	*	*
	21	0.57 ± 0.03 ^{a}	0.83 ± 0.06 ^{abc}	0.96 ± 0.02 ^{bc}	1.03 ± 0.09 ^{c}	0.65 ± 0.10 ^{ab}	0.92 ± 0.13 ^{bc}			
Benzaldehyde	9	0.91 ± 0.30	0.61 ± 0.05	1.02 ± 0.24	0.59 ± 0.07	0.58 ± 0.00	0.68 ± 0.04	*	ns	ns
	21	0.57 ± 0.54	0.22 ± 0.00	0.21 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.21 ± 0.04			

AT—ageing time; T—treatment; AT × T—ageing time × treatment. Different letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). Asterisk (*) indicates significance at $p < 0.05$ and ns indicates no significance determined by two-way ANOVA.

Two of the alcohols usually considered to be positive for the aroma of sparkling wines are 2-phenylethanol and hexan-1-ol. The values present for 2-phenylethanol in all samples (34.6–37.5 mg/L after 9 months and 17.8-23.9 mg/L after 21 months of ageing) reach levels significantly higher than those found by Torchio et al. [60] in aromatic red, sweet Brachetto sparkling wines (7.8–8.7 mg/L), consistent with the metabolic origin of this compound from yeast degradation during ageing over lees. As regards hexan-1-ol, the values achieved (1.7–2.1 mg/L at 9 months and 0.9–1.1 mg/L at 21 months of ageing) were also higher than those reported by the same authors (0.4–0.5 mg/L).

As for the esters, the tendency to decrease in concentration during ageing was maintained, although for certain compounds an ageing time × treatment interaction was observed (Table 5). Thus, in the case of ethyl decanoate, at 9 months the highest concentration corresponds to the control sample, with the adjuvants behaving similarly to each other, while at 21 months the treatment IY maintains the highest level of ethyl decanoate compared to the YW with the lowest. Nevertheless, there were no statistically significant differences. For ethyl hexadecanoate, in addition to the statistically significant decrease due to time, there is also an effect due to the adjuvant factor and the time × adjuvant interaction (Table 5). Our findings are in line with those revealed by other authors, which reflect a decrease in the content of esters during the ageing of white sparkling wines attributable to the effect of adsorption by the lees and chemical hydrolysis processes due to their thermodynamic instability [17]. Lambert-Royo et al. [26] point out that the decrease in esters has been described by several authors as a clear marker of the ageing process.

The release of volatile compounds and the adsorption effect of yeast macromolecules are two of the most important modifications induced by the addition of yeast derivatives to wine, according to Comuzzo et al. [61], who studied the effect of various lysis treatments on the volatile compounds yielded by different yeast derivatives to a wine-like solution. The same authors emphasize the influence of the different yeast derivative extraction techniques and conditions on the characteristics imparted to the wine. Additionally, in our study it is important to take into consideration the interactions between volatile compounds and phenolic compounds contributed by skin maceration, since we are working with red sparkling wines. In this sense, Rigou et al. [59] stated that the degree of interaction between aroma compounds and yeast derived products (YDP) is modulated by their fraction purification degree and chemical composition. The macromolecules most involved in the hydrophobic interactions identified so far are yeast wall mannoproteins. To a lesser extent, yeast wall lipids are also responsible for the retention of lipophilic compounds. The retention capacity of these parietal macromolecules can vary mainly with the industrial treatment conditions used, which can cause significant changes in the parietal structure of yeast.

Sawyer et al. [62], when comparing Chardonnay and Pinot Noir BWs aged on or off lees for 6, 12, and 24 months after bottling, found that the duration of ageing significantly influenced compositional changes in both fermentative and oxidative flavor compounds. Ageing BWs off or on the lees produced similar aroma profiles, regardless of their parietal structure. These authors concluded that the contribution of autolysis products was not as strong as expected over 24 months. Ageing has a more pronounced effect on the volatile compounds than the yeast strain used to develop the second fermentation [20].

This effect seems to be less marked in red wines, probably due to the competitive effect of polyphenols on the retention of lipophilic compounds. However, the data available on red wines are very scarce, perhaps due to the shortcomings of the available analytical methods used to study how aroma evolves in such a complex matrix.

As a function of chemical group, treatment and ageing, the profile of volatile compounds showed different development, although most of the compounds tended to decrease over the course of the ageing process. This is consistent with the findings of Martín-Garcia et al. [63], who compared volatile compounds during ageing over lees and commercial storage of sparkling wine from AO Cava (Spain). There is a general tendency to decrease in concentration during ageing, although for certain compounds an ageing

time × treatment interaction was observed (Table 5). Thus, there is a greater dispersion observed between the values reached by the different adjuvants at 9 months. However, after 21 months, the effect of prolonged ageing results in a homogenization of the values.

3.6. Sensory Analysis

PCA was performed with all the sparkling wine samples and the sensory attributes analyzed (color, smell, taste, persistence, pungency, and overall acceptability). Principal component 1 (PC1) includes intertemporal differences (ageing time) while principal component 2 (PC2) includes intratemporal differences (technological adjuvants). PC1 and PC2 account for 58.4% and 21.8% of the variance, respectively, in the distribution of the samples analyzed as a function of their ageing time (months in contact with lees) and treatment. As shown in Figure 2, the PCA demonstrates two distinct groups categorized by the sample age: 9-month-old samples characterized by greater acceptability of color, and 21-month-old samples displaying higher scores for the parameters of smell, persistence, taste, pungency, and overall acceptability. Evidently, the length of the ageing process has a more significant effect on the sensory characteristics of the final wine than the oenological adjuvant utilized.

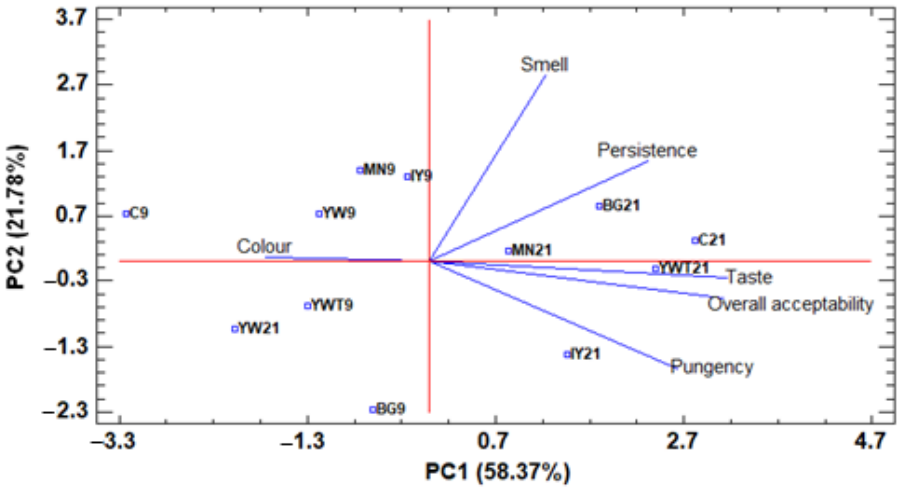

Figure 2. Principal component analysis (PCA) biplot of sensory attributes after 9 and 21 months of ageing over lees.

In accordance with Ubeda et al.'s findings [17], and consistent with the aforementioned statistically significant decrease in the total polyphenol index over time, ageing had a strong influence on the color of the sparkling wines, mainly due to the adsorption of pigment molecules on the cell walls of the yeast, which was perceived by the tasting panel. On the other hand, the longer ageing over lees period helped the consumers to appreciate the typical characteristics of a sparkling wine in terms of smell, taste, pungency, and persistence.

4. Conclusions

The present study aimed at evaluating the effect of four different yeast derivatives and β-glucanases on the ageing over lees process of a red sparkling wine. In summary, the effects of ageing had a greater impact than the use of the various adjuvants or their interaction with time. The length of time that wine spends ageing over lees has a significant impact on its oenological properties, leading to higher concentrations of polysaccharides, proteins, and free amino nitrogen due to the autolytic process. The wine treated with β-glucanase enzymes exhibited the greatest increase in total free amino acid content, confirming that these enzymes are useful in releasing yeast compounds into wine. Total polyphenol index, as well as total flavonols and tannins, experienced a notable decrease, which was confirmed by the observation of a decrease in color acceptability by the consumers.

The length of the ageing period appears to be a crucial factor concerning foamability. Our findings suggest that for enhancement of the foamability of red sparkling wine, incorporation of β-glucanases or mannoproteins is recommended. Yeast walls and inactivated yeasts display the best persistence at 21 months.

The volatile compound profile demonstrated diverse development based on chemical group, treatment, and ageing. Most of the alcohols and esters showed a decrease in concentration over time during ageing, although certain compounds exhibit a time x adjuvant interaction. An extended ageing over lees period allowed consumers to fully appreciate the characteristic taste, smell, pungency, and persistence of a sparkling wine, so increasing its overall acceptability.

Further research into various technical processes for producing a base red wine would offer more definitive insight into the effects of yeast derivatives and β-glucanases on sparkling wine quality.

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