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Impact of Ageing on Ultrasound-Treated Lees on Volatile Composition and Sensory Properties of Red Sparkling Base Wine

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Abstract: Ageing on lees can be a good technique to enhance the quality of red sparkling base wines. Ultrasound treatment of the lees, prior to addition to the wine, can improve the releasing of their components into the wine. This study carries out a four month ageing on lees of a red sparkling base wine by the addition of lees sonicated at different amplitude levels: 30%, 60% and 90% for 10 min. The ageing on ultrasound-treated lees improved the quality of the red base wine, with a greater impact the higher the amplitude of the applied ultrasound. Sonicated lees at an amplitude of 90% enlarged the concentration of neutral polysaccharides in the wine and reduced its astringency, which was evaluated chemically. Furthermore, this treatment enhanced the concentration of some volatile compounds in the wine, mainly acetates, esters and terpenes with floral and fruity aromatic notes. This trend was also found for some fused alcohols, contributing to the aromatic complexity of wines, as well as for 2-phenylethanol, an alcohol with a rose-like aroma, and also for C6-alcohols with a green-herbaceous aroma. The results indicate that ultrasonication is a promising tool to increase the benefits of ageing on lees on the quality of red sparkling base wines.

Keywords: aroma; mannoproteins; lysis; organoleptic properties; sonication; volatile compounds

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1. Introduction

One of the strategies to produce adequate base wines for red sparkling wines is based on early harvesting to obtain the optimum levels of alcohol content, acidity and pH for the second fermentation. These wines show little colour intensity and are unbalanced, with overly bitter, green and astringent notes, and with an inadequate structure in the mouth. To solve this, an ageing on lees of the base wine can be carried out.

Among the different oenological practices to produce sparkling wine, the traditional champenoise method, consisting of a second fermentation and the ageing on lees of a base wine in a bottle for a variable period, is characterized by the development of a more complex aromatic profile compared with other sparkling wines. Generally, the evolution of the aromatic components from the base wine to the final mature sparkling wine is associated with a long-lasting period of ageing, as it occurs with the best millésimes from French Champagne and Spanish Cava. Research has so far focused mainly on the ageing period of these wines; nevertheless, a recent study emphasizes the importance of the base wine in the characteristics of the final sparkling wine, independently of the time of contact with the lees [1].

Ageing on lees is an oenological technique, widely used in both still and sparkling wines, that consists of keeping the wine in contact with its fermentation lees for a variable period of time in order to allow the autolytic process to take place [1,2]. Autolysis consists

of a self-degradation of dead yeasts by their own enzymes, releasing compounds from the cytoplasm and cell wall to the wine (mainly glycoproteins, polysaccharides, nucleic acids and lipids) [3]. In traditional sparkling wines, the ageing on lees period must be of at least 9–12 months [4], with a significant impact on the production costs. For this reason, several researchers have focused their activity on finding technological strategies to reduce this ageing time by accelerating the autolytic process [5].

Ultrasound waves of high frequencies (above 20 kHz) have been used to disrupt cells [6], enhancing the release of macromolecules from yeast lees (glycoproteins and polysaccharides, mainly) [7,8]. Del Fresno et al. [9] were the only researchers, to our knowledge, to evaluate the effect of sonicated lees in the ageing on lees process of a red wine, finding that several oenological parameters were positively affected. However, as mentioned above, the physico-chemical and sensory characteristics of a red base wine are different from a conventional red wine, mainly due to the early harvesting used in the production of the former. In this context, the hypothesis of this study was that the use of ultrasound-treated lees during the ageing of a red base wine would improve the wine quality. To confirm this, a specific study on the effect of sonicated lees on the ageing on lees of a red base wine has been carried out.

In previous studies, we compared the ageing on lees of a model wine with lees treated by ultrasound and high hydrostatic pressure. After 42 days of ageing, our study found better results with sonicated lees than with pressurised lees, as well as significant differences between the wines aged on sonicated and unsonicated lees [10]. In this study, our research was carried out with a red base wine of Tempranillo variety added with sonicated lees during an ageing period of four months. Oenological parameters, volatile organic components and sensory properties of the wines were evaluated.

2. Materials and Methods

2.1. Chemical Reagents

All chemicals were analytical quality grade and purchased from Panreac, S.A. (Madrid, Spain), except the internal standard (2-octanol and methyl nonanoate) used for volatile compound analysis, which were provided by Merck (Darmstadt, Germany).

2.2. Yeast Strain

The *Saccharomyces cerevisiae* strain used in this study was a wine yeast commercialized as a dried active yeast (Lalvin EC1118, Lallemand S.A., Montréal, QC, Canada).

2.3. Preparation of Lees of Yeasts by Thermal Treatment

Lees of yeasts were prepared in a model wine composed of water and ethanol 10% (*v/v*), malic acid (3 g/L), acetic acid (0.1 g/L), tartaric acid (4.0 g/L), potassium sulphate (0.1 g/L) and magnesium sulphate (0.025 g/L). The pH was adjusted to 3.0 with 1 M NaOH [11]. A concentration of 0.8 g/L of yeast in the model wine was heated at 30 °C with agitation (100 rpm) in an orbital shaker (Orbital Shaker SO1, Stuart Scientific, Stone, UK). After 64 h, the samples were centrifuged at 4000 rpm for 15 min (Sorvall ST 8R Centrifuge, Osterode am Harz, Germany), the supernatant was removed, and the lees were obtained.

2.4. Preparation of Lees of Yeasts by Ultrasound (US)

Sonication was performed by a UP400S ultrasonic processor (400 W and 24 kHz) (Hielscher Ultrasonics, Teltow, Germany) fitted with a titanium S24d22D sonotrode (22 mm diameter and depth of 30 mm) applying an 80% on-off pulse. A volume of 330 mL of the model wine with 0.8 g/L yeasts was treated by US at 25 °C [7]. Three different amplitude levels were used: 30%, 60% and 90%, with a processing time of 10 min.

2.5. Ageing on Lees of Red Base Wine

A red base wine of the Tempranillo variety from the 2021 vintage was used. The oenological composition of the wine was as follows: 11.4% (*v/v*) of alcoholic degree, 0.16 g/L

of volatile acidity, 7.36 g/L of total acidity, 25.7 mg/L of total sulphur and 0.13 mg/L of free sulphur. The base wine was adjusted before ageing on lees at 30 mg/L free sulphur. The assays of ageing on lees were carried out, in duplicate, in 20 L stainless steel tanks at a concentration of dry lees of 0.8 g/L and during 4 months at room temperature. One time per week, the different tanks were manually stirred for 1 min. Five experiments were carried out: C (control wine without lees); L (wine with heat-treated lees); 30, 60 and 90 (wine with ultrasound-treated lees at 30%, 60%, and 90% of amplitude for 10 min, respectively).

2.6. Composition of Wines

The oenological parameters of wines, such as total and volatile acidities, pH, alcohol degree, free and total SO_2 and total polyphenolic index were analysed according to OIV methods [12]. Hydroxycinnamic acids and flavonols were measured using UV absorbance at 320 and 365 nm, respectively [13]. Glories' method was used to determine the chromatic characteristics. This method is based on the determination of the absorbance of wines at 420, 520 and 620 nm. Colour intensity was obtained by calculating the sum of the three absorbances, tonality was determined as the ratio between the absorbance at 420 and 520 nm, and % yellow, % red and % blue colours were the ratio between the absorbance at 420, 520 or 620 nm, respectively, and the sum of the three absorbances [14]. Total proteins were analysed by the colorimetric method described by Bradford [15]. The formol titration method was employed for the quantification of free amino nitrogen. Total polysaccharides were evaluated according to phenol-sulfuric acid method [16]. Total tannin and anthocyanin contents were quantified following the methodologies described by Hidalgo [17] Astringency measurement was carried out using a method based on the precipitation of wine tannins with ovalbumin [18]. All analysis were carried out in triplicate.

2.7. Quantification of Volatile Organic Compounds (VOCs) of Wine by Headspace-Solid-Phase Microextraction-Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS)

A CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) connected with a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a 5977 mass selective detector (Agilent Technologies) was used to determine wine VOCs. HS-SPME was carried out for the extraction of wine volatile compounds following the method proposed by Massera et al. [19] with minor modifications. Five mL of wine was transferred into a vial of 20 mL. An amount of 50 µL of methyl nonanoate (0.059 mg/L) as an internal standard and 3 g of sodium chloride (NaCl) were added to each vial. The vials were sealed with a magnetic screw cap provided with a PTFE/silicone septum and incubated at 40 °C for 15 min with agitation (250 rpm). Then, a 50/30 µm DVB/CAR/PDMS fibre (Supelco, Inc., Bellefonte, PA, USA), preconditioned at 270 °C for 15 min before daily use, was exposed to the head space of each vial at 40 °C for 30 min with agitation (250 rpm). After equilibration, the fibre was injected into the injector of the chromatograph, and 15 min were allowed for the desorption of volatiles. The injector temperature was 250 °C, working in splitless mode (1 min). The chromatographic separation of the volatiles was performed on a HP-Innowax column (60 m, 0.250 mm, 0.5 µm) (J & W Scientific, Folsom, CA, USA). The oven temperature program was as follows: 40 °C held for 5 min, then ramped up to 230 °C at 2.5 °C/min and finally held for 20 min. Helium gas at a flow of 1.2 mL/min (pressure of 22.413 psi) was used as carrier gas. The MS detector was operated in full scan mode over a range of m/z of 30–500. Compounds were identified by cross-referencing their mass spectra with pure standards and with spectral data from the NIST08 y Wiley7 libraries. Identification was carried out with only both spectral data libraries when no standards were available [20]. Quantification was carried out using the internal standard quantification method as equivalents [21,22] of 2-octanol.

2.8. Descriptive Sensory Analysis

A trained panel composed of ten judges (5 men and 5 women) participated in this study. All panellists were selected and trained according to the ISO standard [23] as a reference and their performance was assessed in wine sensory descriptive analysis studies.

The samples were served as 25 mL aliquots in standardized wineglasses [24], which were coded with 3-digit numbers, and used a randomized complete block design. The serving temperature of the samples was 16 ± 1 °C. Water was provided to rinse the mouth between evaluations. All sensory evaluations were carried out at the Sensory Science Laboratory of the School of Agricultural Engineering at the University of Valladolid, Palencia (Spain), in individual booths designed in accordance with ISO 8589 [25].

The questionnaire was composed of 12 sensory descriptors grouped in two visual descriptors (tonality and layer intensity), four olfactory descriptors (odour intensity, fruit, herbaceous, and lactic) and six descriptors in the mouth (alcoholic, bitter, astringency, mouthfeel, flavour intensity, and persistence). The different descriptors were quantified using 10-cm unstructured intensity scales, where 0 corresponded to very low intensity and 10 to high intensity for the respective attribute. The wines were evaluated in duplicate by each assessor.

2.9. Statistical Analysis

The results were expressed as a mean ± standard error. Variance analysis (ANOVA) and statistically significant differences between the averages using the Tukey test, calculated at a confidence level of 95%, were employed. ANOVA and Principal component analysis (PCA) of the data were carried out using Statgraphics Centurion (v.19, Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results and Discussion

3.1. Analysis of the Oenological Composition of the Base Wines

Table 1 shows the oenological composition of the red base wines after 4 months of ageing on lees. The analysis of variance of the data showed statistically significant differences among the wines for neutral polysaccharides and astringency. For the other parameters, certain trends could be seen according to the treatment applied to the lees (Figure 1).

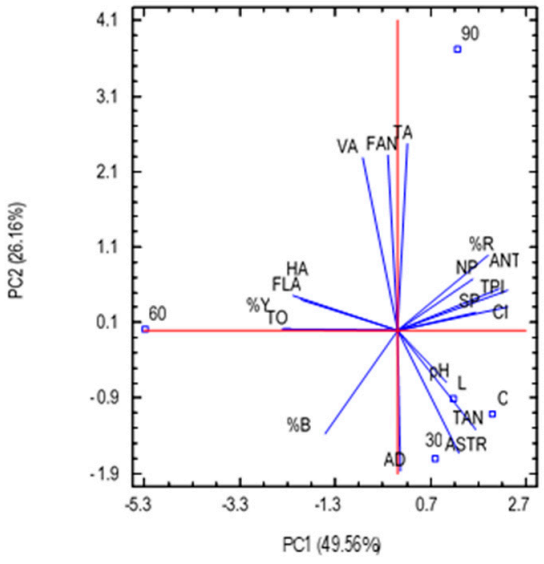

Figure 1. Principal component analysis of wines and oenological parameters. Symbols. C: Control; L: Ultrasound-untreated lees; 30, 60, 90: ultrasound-treated at an amplitude level of 30%, 60%, and 90%, respectively. Code of oenological parameters (see Table 1).

Table 1. Oenological parameters of the wines aged on ultrasound-treated and untreated lees (different letters indicate significant differences between samples ($p < 0.05$)).

Code	Parameters	Control (C)	Lees (L)	Ultrasound-Treated Lees		
				30%	60%	90%
TA	Total acidity (g/L)	5.3 ± 0.0	5.3 ± 0.1	5.3 ± 0.2	5.3 ± 0.1	5.5 ± 0.1
pH	pH	3.39 ± 0.04	3.50 ± 0.08	3.37 ± 0.26	3.35 ± 0.37	3.37 ± 0.03
AD	Alcohol degree (%, *v*/*v*)	11.4 ± 0.3	11.4 ± 0.0	11.5 ± 0.0	11.4 ± 0.0	11.4 ± 0.2
VA	Volatile acidity (g/L)	0.44 ± 0.06	0.42 ± 0.04	0.43 ± 0.05	0.47 ± 0.05	0.50 ± 0.03
ANT	Total anthocyanins (mg/L)	190 ± 44	195 ± 52	176 ± 69	138 ± 19	197 ± 61
NP	Neutral polysaccharides (g/L)	2.04 ± 0.33 ^{a}	2.11 ± 0.51 ^{a}	2.18 ± 0.39 ^{a}	2.03 ± 0.06 ^{a}	2.46 ± 0.23 ^{b}
SP	Soluble proteins (g/L)	0.40 ± 0.04	0.39 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.38 ± 0.02
FAN	Free amino nitrogen (mg/L)	92 ± 5	96 ± 4	93 ± 4	96 ± 10	100 ± 7
TAN	Tannins (g/L)	2.00 ± 0.26	2.23 ± 0.56	2.29 ± 0.58	1.70 ± 0.15	1.89 ± 0.27
%Y	% yellow	30.06 ± 0.33	30.05 ± 0.35	30.04 ± 0.21	30.29 ± 0.08	30.05 ± 0.34
%R	% red	54.31 ± 0.65	54.42 ± 0.60	54.60 ± 0.84	53.92 ± 0.20	54.77 ± 1.22
%B	% blue	15.63 ± 0.32	15.53 ± 0.26	15.36 ± 0.63	15.79 ± 0.12	15.18 ± 0.88
TO	Tonality	0.55 ± 1.43	0.55 ± 1.39	0.55 ± 2.58	0.56 ± 0.17	0.55 ± 2.78
CI	Colour intensity	12.30 ± 0.01	12.64 ± 0.01	12.33 ± 0.01	10.96 ± 0.00	12.60 ± 0.02
TPI	Total polyphenol index ($A_{280\,nm}$)	7 ± 0	7 ± 1	7 ± 1	6 ± 0	7 ± 1
HA	Hydroxycinnamic acids ($A_{320\,nm}$)	20.55 ± 1.41	21.5 ± 1.9	20.8 ± 1.1	22.1 ± 2.6	21.1 ± 2.2
FLA	Flavonols ($A_{365\,nm}$)	6.88 ± 0.53	7.13 ± 0.74	6.93 ± 0.53	7.40 ± 1.20	7.05 ± 1.06
ASTR	Astringency (g/L)	0.44 ± 0.05 ^{b}	0.41 ± 0.06 ^{b}	0.37 ± 0.04 ^{ab}	0.34 ± 0.05 ^{a}	0.33 ± 0.06 ^{a}

PCA analysis of the data was performed to evaluate the impact of ageing on ultrasound-treated lees on the oenological composition of the base wines. PC1 explained 49.56% of the variance, while PC2 explained 26.16%. The PCA plot (Figure 1) shows a considerable dispersion of the wine samples, with the wine aged on sonicated lees at an amplitude of 90% (sample 90) located at positive values for both PC1 and PC2, the wine aged on lees treated at 60% (sample 60) at negative value of PC1 and positive value of PC2, and a group of three wines (wine aged on lees treated at 30% (sample 30), wine aged on ultrasound-untreated lees (sample L) and control wine without lees (sample C)) at positive values of PC1 and negative of PC2.

Sample 90 was characterized by high values on parameters related to wine colour, such as % red, colour intensity, anthocyanins and total polyphenol index (Figure 1), although no statistically significant differences were found among the wines. These results are in line with those published by del Fresno et al. [9], which reported that ageing on sonicated lees increased the concentration of anthocyanins compared to untreated lees; this was probably due to a decrease in the adsorption of these compounds on the ultrasound-treated lees, although these authors also observed a decrease in the colour intensity of wine without changes in its tonality and total polyphenol index. High concentrations of polymers released during yeast autolysis, such as polysaccharides and proteins, the latter with no statistically significant difference among the wines, were also found in this wine (sample 90).

In contrast, some other parameters (% yellow, tonality, as well as the phenolic families hydroxycinnamic acids and flavonoles) were associated with sample 60. Finally, the wine aged on ultrasound-treated lees at the amplitude of 30% (sample 30), the wine aged on ultrasound-untreated lees (L) and the control wine (C) showed high values for parameters related to wine astringency (tannins and astringency), although no statistically significant difference was found for the tannins among the wines.

These results show that the application of ultrasound at high amplitudes improves to some extent the chromatic and polyphenolic features of the wine, and more notably it enhances the concentration of neutral polysaccharides, reducing its astringency (chemically evaluated). It seems that the application of ultrasound at 30% of amplitude (sample 30) is not sufficient to enhance the physico-chemical characteristics of wine.

3.2. Analysis of VOCs in Base Wines

The volatile components of the five lots of base wine were analysed by SPME-GC-MS. As can be seen in Table 2, 59 volatile components were detected and they were grouped in acetates, methyl and branched esters, ethyl esters, fused alcohols, C6-alcohols, acids, terpenes, phenols, aldehydes and ketones. According to their number, the ethyl ester group was the most numerous (17 compounds), followed by 13 alcohols, 7 acids, 5 acetates and 5 phenols.

Table 2. Volatile organic compounds (VOCs) (mg/L) of wines aged on ultrasound-treated and untreated lees (different letters indicate significant differences between samples ($p < 0.05$).

Code	Parameters	Control (C)	Lees (L)	Ultrasound-Treated Lees		
				30%	60%	90%
A1	Ethyl acetate	1.626 ± 0.131 ^{a}	1.489 ± 0.173 ^{a}	2.007 ± 0.048 ^{b}	1.979 ± 0.015 ^{b}	2.079 ± 0.014 ^{b}
A2	3-methylbutyl acetate	2.176 ± 0.160 ^{a}	1.893 ± 0.733 ^{a}	2.744 ± 0.329 ^{a}	2.650 ± 0.031 ^{a}	2.977 ± 0.267 ^{b}
A3	Hexyl acetate	0.259 ± 0.010	0.217 ± 0.070	0.312 ± 0.070	0.321 ± 0.038	0.378 ± 0.044
A4	Ethyl 2-phenylacetate	0.022 ± 0.009 ^{a}	0.021 ± 0.007 ^{a}	0.029 ± 0.009 ^{a}	0.042 ± 0.01 ^{b}	0.031 ± 0.021 ^{b}
A5	2-phenylethyl acetate	0.572 ± 0.079 ^{a}	0.585 ± 0.034 ^{a}	0.777 ± 0.121 ^{b}	0.816 ± 0.039 ^{b}	0.850 ± 0.023 ^{b}
E1	Pentyl 2-hydroxypropanoate	0.053 ± 0.004 ^{a}	0.058 ± 0.005 ^{ab}	0.070 ± 0.010 ^{bc}	0.081 ± 0.001 ^{c}	0.082 ± 0.001 ^{c}
E2	3-methylbutyl octanoate	0.080 ± 0.031	0.078 ± 0.010	0.061 ± 0.000	0.064 ± 0.006	0.061 ± 0.014
E3	Methyl decanoate	0.012 ± 0.001	0.015 ± 0.001	0.011 ± 0.001	0.013 ± 0.005	0.011 ± 0.001
E4	3-methylbutyl decanoate	0.033 ± 0.001	0.032 ± 0.010	0.027 ± 0.007	0.034 ± 0.005	0.028 ± 0.004
Et1	Ethyl butanoate	0.122 ± 0.017 ^{a}	0.100 ± 0.012 ^{ab}	0.165 ± 0.007 ^{bc}	0.142 ± 0.032 ^{bc}	0.169 ± 0.000 ^{c}
Et2	Diethyl butanedioate	0.614 ± 0.048 ^{a}	0.714 ± 0.038 ^{a}	0.849 ± 0.066 ^{b}	0.960 ± 0.028 ^{bc}	0.987 ± 0.029 ^{c}
Et3	Ethyl 2-hydroxypropanoate	0.542 ± 0.010 ^{a}	0.606 ± 0.019 ^{a}	0.767 ± 0.067 ^{b}	0.746 ± 0.031 ^{b}	0.769 ± 0.011 ^{b}
Et4	Ethyl 4-hydroxybutanoate	0.011 ± 0.003 ^{a}	0.011 ± 0.002 ^{a}	0.018 ± 0.000 ^{ab}	0.017 ± 0.001 ^{ab}	0.020 ± 0.006 ^{b}
Et5	Ethyl hexanoate	2.998 ± 0.012 ^{a}	2.662 ± 0.500 ^{ab}	3.566 ± 0.502 ^{bc}	3.869 ± 0.174 ^{bc}	4.115 ± 0.280 ^{c}
Et6	Ethyl heptanoate	0.045 ± 0.005	0.036 ± 0.003	0.050 ± 0.008	0.060 ± 0.016	0.065 ± 0.005
Et7	Ethyl octanoate	10.928 ± 0.707	9.924 ± 0.095	10.409 ± 1.425	11.800 ± 3.032	11.265 ± 0.589
Et8	Ethyl nonanoate	0.348 ± 0.017	0.325 ± 0.052	0.365 ± 0.007	0.326 ± 0.146	0.352 ± 0.008
Et9	Ethyl decanoate	3.779 ± 0.177	3.592 ± 1.194	2.995 ± 0.307	3.565 ± 1.468	2.965 ± 0.199
Et10	Ethyl dodecanoate	0.358 ± 0.056	0.309 ± 0.022	0.243 ± 0.044	0.337 ± 0.083	0.242 ± 0.051
Et11	Ethyl tetradecanoate	0.391 ± 0.140	0.388 ± 0.033	0.301 ± 0.060	0.293 ± 0.016	0.195 ± 0.050
Et12	Ethyl pentadecanoate	0.046 ± 0.017	0.054 ± 0.016	0.028 ± 0.008	0.027 ± 0.001	0.018 ± 0.003
Et13	Ethyl hexadecanoate	2.069 ± 0.697	2.192 ± 0.310	1.796 ± 0.513	1.623 ± 0.042	1.283 ± 0.024
Et14	Ethyl (E)-hexadec-9-enoate	0.038 ± 0.011	0.051 ± 0.047	0.029 ± 0.017	0.095 ± 0.052	0.274 ± 0.307
Et15	Ethyl octadecanoate	0.149 ± 0.045	0.173 ± 0.028	0.203 ± 0.046	0.182 ± 0.004	0.173 ± 0.006
Et16	Ethyl benzoate	0.025 ± 0.000 ^{a}	0.028 ± 0.001 ^{a}	0.027 ± 0.000 ^{b}	0.030 ± 0.001 ^{c}	0.030 ± 0.000 ^{c}
Et17	Ethyl furan-2-carboxylate	0.019 ± 0.001 ^{a}	0.021 ± 0.002 ^{ab}	0.023 ± 0.000 ^{b}	0.024 ± 0.003 ^{b}	0.024 ± 0.001 ^{b}
C6-1	Hexan-1-ol	0.629 ± 0.018 ^{a}	0.706 ± 0.044 ^{a}	0.906 ± 0.056 ^{b}	0.975 ± 0.028 ^{bc}	1.021 ± 0.007 ^{c}
C6-2	(E)-hex-3-en-1-ol	0.011 ± 0.000 ^{a}	0.013 ± 0.001 ^{ab}	0.015 ± 0.001 ^{b}	0.019 ± 0.001 ^{c}	0.019 ± 0.000 ^{c}
C6-3	(Z)-hex-3-en-1-ol	0.103 ± 0.023 ^{a}	0.110 ± 0.005 ^{ab}	0.135 ± 0.007 ^{bc}	0.160 ± 0.002 ^{cd}	0.165 ± 0.002 ^{d}
Alc1	Propan-1-ol	0.065 ± 0.013	0.057 ± 0.003	0.095 ± 0.024	0.079 ± 0.029	0.086 ± 0.015
Alc2	2-methylpropan-1-ol	0.615 ± 0.015 ^{ab}	0.552 ± 0.143 ^{a}	0.714 ± 0.033 ^{ab}	0.747 ± 0.040 ^{b}	0.789 ± 0.033 ^{b}
Alc3	3-methylsulfanylpropan-1-ol	0.031 ± 0.012	0.029 ± 0.009	0.032 ± 0.006	0.034 ± 0.002	0.031 ± 0.003
Alc4	Butan-1-ol	0.024 ± 0.000	0.021 ± 0.004	0.044 ± 0.024	0.035 ± 0.004	0.034 ± 0.010
Alc5	3-methylbutan-1-ol	8.324 ± 0.084 ^{a}	8.792 ± 0.113 ^{a}	11.379 ± 0.057 ^{c}	11.504 ± 0.233 ^{c}	11.959 ± 0.067 ^{d}
Alc6	3-methylpentan-1-ol	0.014 ± 0.000 ^{a}	0.016 ± 0.001 ^{a}	0.019 ± 0.003 ^{ab}	0.023 ± 0.001 ^{b}	0.022 ± 0.004 ^{b}
Alc7	Heptan-1-ol	0.074 ± 0.014	0.062 ± 0.006	0.070 ± 0.001	0.084 ± 0.004	0.073 ± 0.003
Alc8	Octan-1-ol	0.092 ± 0.010 ^{a}	0.103 ± 0.019 ^{a}	0.122 ± 0.023 ^{ab}	0.151 ± 0.009 ^{b}	0.149 ± 0.003 ^{b}
Alc9	Nonan-1-ol	0.142 ± 0.010	0.126 ± 0.039	0.131 ± 0.005	0.133 ± 0.006	0.129 ± 0.008
Alc10	Decan-1-ol	0.023 ± 0.005	0.028 ± 0.007	0.033 ± 0.006	0.027 ± 0.003	0.029 ± 0.002
Alc11	Dodecan-1-ol	1.056 ± 0.004 ^{c}	1.116 ± 0.062 ^{c}	1.409 ± 0.159 ^{b}	0.039 ± 0.021 ^{a}	0.028 ± 0.002 ^{a}
Alc12	Phenylmethanol	0.073 ± 0.011	0.074 ± 0.007	0.087 ± 0.002	0.113 ± 0.004	0.089 ± 0.041
Alc13	2-phenylethanol	4.637 ± 0.157 ^{a}	4.915 ± 0.119 ^{a}	5.817 ± 0.145 ^{b}	6.442 ± 0.068 ^{c}	6.284 ± 0.461 ^{c}
Ac1	2-methylpropanoic acid	0.045 ± 0.002 ^{a}	0.050 ± 0.003 ^{ab}	0.070 ± 0.010 ^{bc}	0.086 ± 0.011 ^{c}	0.085 ± 0.010 ^{c}
Ac2	Butanoic acid	0.026 ± 0.002 ^{a}	0.027 ± 0.002 ^{a}	0.038 ± 0.007 ^{ab}	0.043 ± 0.005 ^{b}	0.043 ± 0.007 ^{b}
Ac3	3-methylbutanoic acid	0.092 ± 0.003 ^{a}	0.100 ± 0.003 ^{a}	0.140 ± 0.003 ^{b}	0.157 ± 0.026 ^{b}	0.169 ± 0.003 ^{b}
Ac4	Octanoic acid	2.710 ± 0.229 ^{a}	2.977 ± 0.445 ^{ab}	3.723 ± 0.515 ^{bc}	4.097 ± 0.340 ^{c}	4.154 ± 0.286 ^{c}
Ac5	Nonanoic acid	0.072 ± 0.007	0.050 ± 0.010	0.090 ± 0.016	0.082 ± 0.033	0.084 ± 0.027
Ac6	Hexanoic acid	0.770 ± 0.014	0.851 ± 0.051	1.137 ± 0.125	1.321 ± 0.004	1.360 ± 0.082
Ac7	Decanoic acid	0.316 ± 0.091 ^{a}	0.380 ± 0.155 ^{a}	0.370 ± 0.079 ^{b}	0.411 ± 0.071 ^{c}	0.356 ± 0.065 ^{c}
Ter1	3,7-dimethylocta-1,6-dien-3-ol	0.045 ± 0.024 ^{a}	0.046 ± 0.050 ^{a}	0.096 ± 0.031 ^{b}	0.152 ± 0.012 ^{c}	0.127 ± 0.050 ^{c}
Ter2	3,7-dimethyloct-6-en-1-ol	0.018 ± 0.000 ^{a}	0.016 ± 0.004 ^{a}	0.024 ± 0.002 ^{b}	0.025 ± 0.007 ^{b}	0.021 ± 0.006 ^{b}
Phe1	Phenol	0.019 ± 0.000	0.020 ± 0.000	0.023 ± 0.004	0.021 ± 0.002	0.021 ± 0.006

Table 2. *Cont.*

Code	Parameters	Control (C)	Lees (L)	Ultrasound-Treated Lees		
				30%	60%	90%
Phe2	4-ethylphenol	0.184 ± 0.018 [a]	0.217 ± 0.125 [a]	0.243 ± 0.020 [a]	0.309 ± 0.014 [ab]	0.245 ± 0.067 [a]
Phe3	4-ethyl-2-methoxyphenol	0.069 ± 0.011	0.098 ± 0.027	0.089 ± 0.012	0.120 ± 0.004	0.100 ± 0.037
Phe4	2,4-ditert-butylphenol	0.085 ± 0.021	0.077 ± 0.013	0.134 ± 0.063	0.117 ± 0.043	0.121 ± 0.045
Phe5	1,1′-biphenyl	0.010 ± 0.002 [a]	0.011 ± 0.003 [a]	0.020 ± 0.003 [b]	0.018 ± 0.005 [b]	0.018 ± 0.004 [b]
Ald1	Acetaldehyde	0.122 ± 0.024	0.100 ± 0.032	0.186 ± 0.082	0.149 ± 0.006	0.152 ± 0.049
Ald2	Nonanal	0.036 ± 0.006	0.025 ± 0.003	0.021 ± 0.003	0.024 ± 0.006	0.021 ± 0.008
Ke1	Octan-2-one	0.014 ± 0.002 [a]	0.013 ± 0.003 [a]	0.021 ± 0.002 [b]	0.027 ± 0.003 [c]	0.022 ± 0.007 [b]

The volatile composition of the wines aged on ultrasound-untreated lees (L) and the control wine (C) displayed high values for 10 VOCs, accounting for 17% of total VOCs, although without statistically significant differences with the group of wines aged on sonicated lees. These compounds are the following: three methyl esters (3-methylbutyl octanoate, methyl decanoate and 3-methylbutyl decanoate) and five long-chain ethyl esters (ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl pentadecanoate and ethyl hexadecanoate), volatile compounds with a soapy, oily or wax odour [26], nonan-1-ol (green odour) and nonanal (soap-like odour) [27]. No statistically significant differences in volatile composition could be observed between both wines (samples C and L). This behaviour, found also in wine physico-chemical composition, may be explained by the fact that the ageing treatment was not intense enough, either because the ageing conditions (temperature, contact time of the lees with wine, lees stirring, etc.) were mild or because the concentration of lees was low. However, it seems that the most intense ultrasound treatment increased yeast autolysis, as an enhanced concentration of polysaccharides was observed in the wine aged on sonicated lees at 90% (Table 1).

Regarding the amplitude used during treatment of lees by ultrasound, wines aged with lees treated at 60% and 90% of amplitude (samples 60 and 90, respectively) showed high values for most of the VOCs located in the first quadrant of the PCA score plot (Figure 2).

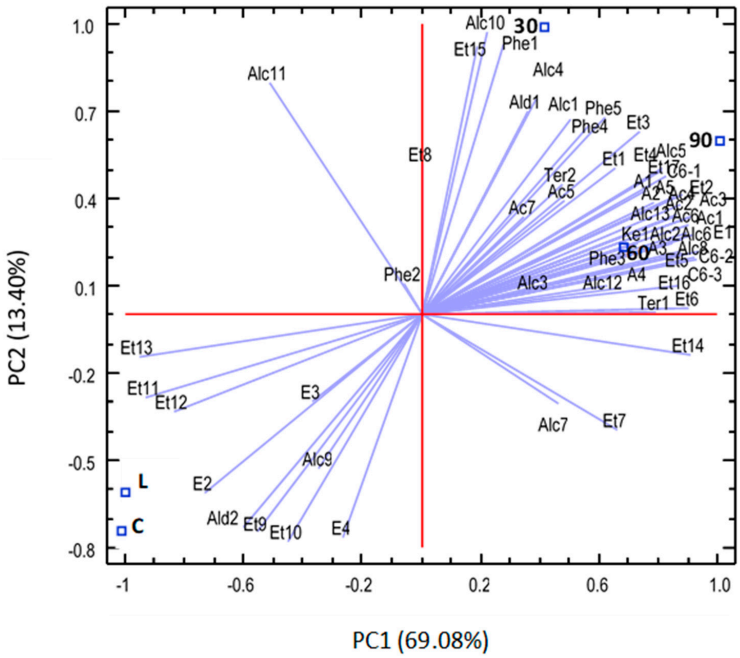

Figure 2. Principal component analysis of wines and volatile compounds. Symbols. C: Control; L: Ultrasound-untreated lees; 30, 60, 90: ultrasound-treated at an amplitude level of 30%, 60%, and 90%, respectively. Code of volatile compounds (see Table 2).

Statistically significant higher concentrations of four acetates (ethyl, 3-methylbutyl, ethyl 2-phenyl and 2-phenyethyl acetate), four short-medium chain esters (diethyl butanedioate, ethyl 2-hydroxypropanoate, ethyl 4-hydroxybutanoate and ethyl hexanoate), ethyl benzoate, ethyl furan-2-carboxylate, and the two terpenes (3,7-dimethylocta-1,6-dien-3-ol(linalool) and 3,7-dimethyloct-6-en-1-ol(citronellol)) were found in the wine aged on lees treated at the amplitude of 90% (sample 90) than in the control wine (sample C) and the wine aged on unsonicated lees (sample L) (Table 2). These compounds could contribute to the fruity and floral notes of the wine aroma [26].

The wines aged on sonicated lees at the amplitudes of 60% and 90% (samples 60 and 90, respectively) showed a high concentration of C6-alcohols, compounds responsible for the herbaceous and vegetal notes of wines (Figure 2 and Table 2) [26].

Regarding fused alcohols, the treatment of the lees with ultrasound at the highest amplitude (90%) increased the concentration of some alcohols, such as 2-methylpropan-1-ol, 3-methylbutan-1-ol, 3-methylpentan-1-ol and octan-1-ol in wine (sample 90). These compounds at concentrations below 300 mg/L contribute to the aromatic complexity of wines [26]. Similar results were found for 2-phenylethanol, an alcohol with a rose-like aroma [26], showing a remarkable increase in this compound in the wines aged on ultrasound-treated lees, especially in samples 60 and 90, compared with the wine aged on ultrasound-untreated lees (sample L) and the control wine (sample C).

Ultrasound treatment of the lees also had an impact on the concentration of some acids, noting a higher concentration of 2-methylpropanoic, butanoic, 3-methylbutanoic, octanoic and hexanoic acids in the wines aged on ultrasound-treated lees at an amplitude of 60% and 90% (samples 60 and 90, respectively) than in the wine with ultrasound-untreated lees (sample L). A similar trend was found for phenylbenzene (1,1′-biphenyl) (floral note) and octan-2-one ketone (floral and fruity note).

A detailed review of the literature on this subject has highlighted the limited knowledge that exists on the impact of ageing on ultrasound-treated lees on the volatile composition of the wine. Similar to our results, del Fresno et al. [9] found that the concentrations of total esters, 2-phenyl ethanol and acetaldehyde were higher in wines aged on sonicated lees compared to wines aged on ultrasound-untreated lees after 30 days of ageing.

It is recognised that glycoproteins and polysaccharides from yeast autolysis can interact with the volatile compounds of wine, modifying their volatility and perception [3,28] and modulating the aromatic wine profile [3]. However, this interaction depends on the structure and the composition of these biomolecules, as well as the type and the concentration of the volatile compounds [2,29]. As discussed above, the concentration of each volatile compound (Table 2) was not lower in the wine aged on ultrasound-untreated lees (sample L) than in the control wine without lees (sample C) despite the reported capacity of the lees to interact with aroma compounds [29]. Therefore, it could not be inferred whether ultrasound treatment increased the release of volatile compounds from the lees.

It has been reported that yeast lees can consume significant amounts of oxygen after alcoholic fermentation [30]. Therefore, the increased content of the volatile compounds in the wine aged on lees treated at the amplitude of 90% (sample 90) could be due to a great reducing power of these lees that could prevent the oxidation of the volatile compounds. Ultrasound may have caused a chemical and structural change in the lees improving their ability to consume oxygen. Del Fresno et al. [9] suggest that the ultrasound treatment of lees increases the antioxidant capacity of the wine aged on lees due to an enhanced release of proteins and glucans with antioxidant activity from the sonicated lees.

Moreover, some enzymes may be released from yeast cells during autolysis, such as esterases involved in acetate and ester synthesis [26,31], increasing the concentration of these compounds in the wines aged on ultrasound-treated lees. Concerning fused alcohols, they can be synthesised from their corresponding amino acids [26]. It is plausible that ultrasound enhanced the reported release of amino acids during yeast autolysis [32], raising the concentration of fused alcohols in the wines aged on ultrasound-treated lees.

From the Figure 2, wines aged with lees treated at the lowest amplitude (sample 30) showed high values for fewer VOCs (decan-1-ol (fruit note), phenol (phenolic note), ethyl octadecanoate (waxy note), butan-1-ol (fusel note), acetaldehyde (fruit note at low concentration and pungent at high), propan-1-ol (fruity note), and 2,4-ditert-butylphenol (phenolic note) [26,27,33], although without statistically significant differences between wines (VOCs were ranked in order of highest to lowest PC2 value).

3.3. Analysis of Sensory Characteristics of Base Wines

As for the sensory evaluation of the wines, it must be point out that no significant differences were found among the wines. However, some interesting findings were obtained, corroborating the results obtained in the chemical analysis.

PCA was also carried out with the sensory data (Table 3). The first principal component (PC1) and the second principal component (PC2) expressed 38.09% and 28.22% of the total variance, respectively. Wine aged on sonicated lees at 90% of amplitude (sample 90) was placed at positive values of both PC1 and PC2 (Figure 3). This sample presented high scores on layer intensity related to high values for colour intensity, anthocyanins and total polyphenolic index (Figure 1). This wine was also characterized by high scores on odour and flavour intensity, alcoholic and bitter.

Table 3. Sensory evaluation of the wines aged on ultrasound-treated and untreated lees.

Code	Parameters	Control (C)	Lees (L)	Ultrasound-Treated Lees		
				30%	60%	90%
	Visual phase					
Tona	Tonality	6.8 ± 2.1	6.3 ± 2.3	6.4 ± 2.5	6.1 ± 2.7	6.2 ± 2.3
Lay	Layer intensity	6.5 ± 1.8	6.9 ± 1.5	6.9 ± 1.4	6.4 ± 1.8	5.8 ± 1.8
	Olfactory phase					
Odo	Odour intensity	4.5 ± 2.0	4.8 ± 1.6	5.0 ± 1.3	5.1 ± 1.6	4.1 ± 1.6
Fru	Fruit	3.6 ± 1.5	3.4 ± 1.3	4.0 ± 1.3	3.9 ± 1.6	3.9 ± 1.3
Her	Herbaceous	3.8 ± 1.7	3.3 ± 1.4	3.5 ± 1.6	4.1 ± 1.8	3.6 ± 1.7
Lac	Lactic	3.8 ± 1.8	3.4 ± 1.5	3.2 ± 1.4	3.8 ± 1.7	3.2 ± 1.2
	Gustatory phase					
Alco	Alcoholic	3.7 ± 1.3	3.7 ± 1.1	4.1 ± 1.6	3.9 ± 1.6	3.3 ± 1.4
Bit	Bitter	3.0 ± 1.5	3.5 ± 2.0	3.1 ± 1.4	3.0 ± 1.2	2.7 ± 1.2
Ast	Astringency	2.9 ± 1.4	3.3 ± 1.5	2.7 ± 1.8	2.4 ± 1.3	3.0 ± 1.4
Mou	Mouthfeel	3.6 ± 1.1	3.8 ± 0.9	3.6 ± 1.1	3.2 ± 1.1	3.2 ± 0.7
Flavo	Flavour intesity	4.0 ± 1.1	3.9 ± 1.2	3.9 ± 1.4	3.4 ± 1.1	3.5 ± 1.0
Per	Persistence	3.5 ± 1.1	3.9 ± 1.1	4.1 ± 1.6	3.6 ± 1.2	3.7 ± 1.5

High scores on odour and flavour intensity found in this wine are consistent with their increased concentrations of volatile compounds (Figure 2). These results are similar to those reported by del Fresno et al. [9], which showed a higher score on aromatic intensity in wines aged on sonicated lees after 60 days of ageing.

Sample 60 was located at a positive value of PC1 and negative of PC2, and was characterised by high scores on three olfactory parameters: fruity, herbaceous and lactic. In fact, this wine showed high concentrations of volatile compounds responsible for fruity odour, such as acetates and ethyl esters [26] and C6-alcohols with herbaceous notes [34] (Figure 2). Moreover, the tonality of this wine was high, and it was confirmed by spectrophotometric analysis (Figure 1).

In contrast, the base wine aged on lees treated at 30% of amplitude was characterised by low values of all sensory parameters. Finally, samples C and L, located at negative values of PC1 and positive of PC2, were characterised by high levels of astringency, persistence and mouthfeel. Astringency was verified by chemical analysis, and it could be the cause of the increased persistence and mouthfeel observed in these wines. Del Fresno et al. [9] also observed that an ageing on sonicated lees reduced the perceived astringency of wine, while increasing the body of the wine.

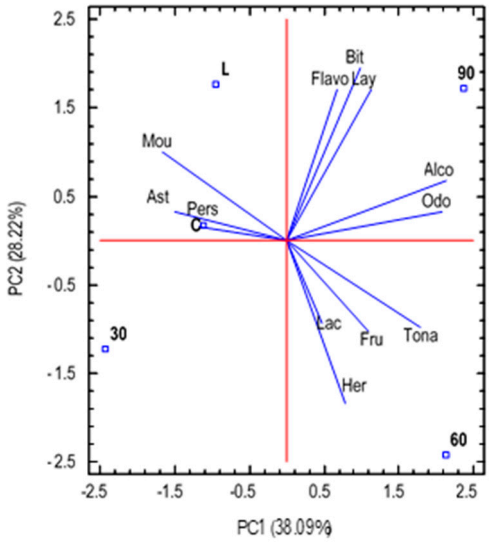

Figure 3. Principal component analysis of wines and sensory data. C: Control; L: Ultrasound-untreated lees; 30, 60, 90: Ultrasound-treated at an amplitude of 30%, 60%, and 90%, respectively. Code of sensory parameters (see Table 3).

4. Conclusions

Our results show that ageing on ultrasound-treated lees improved the red base wine quality with a greater favourable impact when the ultrasound treatment was more intense. The ageing on sonicated lees at the highest amplitude enhanced the concentration of neutral polysaccharides and some volatile compounds in the wine, mainly acetates, esters and terpenes with floral and fruity aromatic notes, and reduced the chemically-assessed astringency. Regarding the sensory analysis, although no significant differences were observed among the different wines elaborated, it should be remarked that the wines aged on sonicated lees at the highest amplitude showed high scores on layer intensity, odour and flavour intensity, and low on astringency. Taken together, the application of ultrasound-treated lees from *Saccharomyces cerevisiae* during the ageing of wines could be a promising alternative to enhance the quality of red sparkling base wines. Further research is needed to confirm these results, either using other red grape varieties or non-*Saccharomyces* yeast lees, as well as increasing the concentration of lees in the wines.

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