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Ultrasound and microwave techniques for assisting ageing on lees of red wines

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Abstract:	Ageing on lees is a slow process that carries microbiological and economic risks in the wineries. This study evaluates the possibility of enhancing the extraction of different compounds from the lees, using combined strategies, such as ultrasound (US) or microwaves (MW) and the addition of inactive dry yeasts (IDY), to reduce the lees ageing time. The complete chemical analysis of the wine was done, amino acids, polysaccharides, colour and volatile compounds, together with the sensory analysis. The combined treatments increased the release of total polysaccharides, mannoproteins and total monosaccharides in the wines, and some amino acids like proline. However, wines treated with US and MW, with and without lees, showed a decrease in tannins and colour intensity, and in some volatile compounds like fatty acid esters, acetates and terpenes. The wines treated with IDY and MW were the best valued for their floral and red berry flavours and less astringency.
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Ultrasound and microwave techniques for assisting ageing on lees of red wines

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ABSTRACT

Ageing on lees is a slow process that carries microbiological and economic risks in the wineries. This study evaluates the possibility of enhancing the extraction of different compounds from the lees, using combined strategies, such as ultrasound (US) or microwaves (MW) and the addition of inactive dry yeasts (IDY), to reduce the lees ageing time. The complete chemical analysis of the wine was done, amino acids, polysaccharides, colour and volatile compounds, together with the sensory analysis. The combined treatments increased the release of total polysaccharides, mannoproteins and total monosaccharides in the wines, and some amino acids like proline. However, wines treated with US and MW, with and without lees, showed a decrease in tannins and colour intensity, and in some volatile compounds like fatty acid esters, acetates and terpenes. The wines treated with IDY and MW were the best valued for their floral and red berry flavours and less astringency.

Keywords:

Lees ageing

Red wine

Ultrasounds

Microwaves

Volatile compounds

Non-volatile compounds

1. Introduction

Ageing on lees is a technique that has traditionally been used in the production of sparkling and red wines, in which the wine is kept in contact with the yeast for several months after fermentation, favouring the release of compounds from the autolysis of yeasts and improving the organoleptic characteristics of wines (Martínez-Rodríguez & Pueyo, 2009).

Yeast autolysis is a slow process, so ageing on lees implies immobilization of the wine in the cellar for a long time, increasing economic and microbiological hazards. The use of inactive dry yeasts (IDY) has become widespread in the wine industry to replace the yeast lees, avoiding the microbiological and organoleptic risks, and reducing the slow and complex process that entails the yeast autolysis (Pozo-Bayón et al., 2009; Pérez-Serradilla & Luque de Castro, 2008).

Inactive dry yeasts are obtained by thermal inactivation and drying of the yeasts, that have grown in a medium with a high concentration of sugar under aerobic conditions (Comuzzo et al., 2012). The most commercial inactive dry yeast (IDY) is made up of insoluble compounds, as inactive yeasts, yeast membranes and walls, and a soluble fraction formed by free cellular metabolites released after yeast lysis, as amino acids, peptides and proteins, polysaccharides, nucleotides, fatty acids, vitamins and minerals, which can be released into the wine during the lees ageing process (López-Solís et al., 2017). In IDY preparations, mannoproteins (MP), from the cell wall of yeasts, are the main components, showing a positive effect on wine sensory characteristics. In fact, MP improve the aromatic profile (Del Barrio-Galán et al., 2012), reduce astringency and bitterness, increase the body, structure, and roundness (Guadalupe et al., 2010; Poncet-Legrand et al., 2007) and influence the colour of red wines (Escot et al., 2001).

In order to accelerate the ageing process on the lees, in recent years emerging technologies have been investigated to replace traditional stirring or "batonnage", increasing the efficiency of the process. Among them, the use of high-power ultrasound (HPU) and microwave (MW) could be most promising (Lui et al., 2016). The high-power ultrasound technique is based on the application of mechanical sound waves with frequencies between 20 kHz and 100 MHz inducing acoustic cavitation in a liquid medium. The intense pressure and temperature gradients accelerate chemical and physical changes, causing cell rupture and allowing a greater matter transfer (Garcia-Martín. et al., 2013). While microwaves are non-ionizing electromagnetic waves that cause an increase in energy in the matrix produced by molecular friction, mainly by dipole rotation and ionic conduction, that can modify molecular structures and favour the migration of compounds (Clodoveo et al., 2016).

Both techniques have been used in the wine industry for different purposes such as microbiological stabilization (Clodoveo et al., 2016) and to reduce the maceration time increasing the extraction of grape compounds (polysaccharides, volatile compounds and polyphenols) (Pérez-Porras et al., 2021, Oliver et al., 2021; Muñoz et al., 2021; Muñoz et al., 2022). Additionally, the application of US and MW in wines during the ageing period increase the aromatic intensity of wood attributes and accelerate the ageing process (García-Martín et al., 2013).

Ultrasounds promote yeast autolysis by improving polysaccharide extraction in model solutions and wine (Cacciola et al., 2013; del Fresno et al., 2018), while no significant effect is observed in the case of microwave treatment (Liu et al., 2016). However, the same authors detected a reduction in aroma compounds due to the use of US, and a decrease in total polyphenols, which can affect the sensory characteristics of wines (Liu et al., 2016; del Fresno et al., 2018). It seems that the conditions used in the

93 treatment such as the type of yeast and the potency and duration of US treatment,
94 considerably affect the results obtained (García-Martín & Sun, 2013). No references have
95 been found on the effect that the use of microwaves in ageing on lees could have on the
96 volatile or phenolic compounds of the wine.

97 Therefore, the objective of this work is to obtain complete information on the
98 effect of US and MW treatments used as tools to accelerate the ageing of wine on lees,
99 using inactive dry yeasts (IDY), on the families of polysaccharides, the phenolic
100 composition and other wine components such as volatile compounds and amino acids on
101 which there is no prior information.

102 **2. Material and methods**

103 *2.1. Experiment design*

104 To carry out this experiment, a Mencía red wine produced at the "Instituto de la
105 Vid y el vino de Castilla-La Mancha" (IVICAM, Tomelloso, Ciudad Real, Spain) in the
106 2021 harvest was used.

107 The wine was distributed in 2 L flasks with a volume of 1.3 L per flask, forming
108 6 batches with different conditions, in triplicate. The first batch was kept without any
109 treatment as a control (sample C), in the second batch (sample IDY) inactive dry yeast
110 *Saccharomyces cerevisiae* (Lallemand) was added at 0.3 g/L per flask. The third batch
111 (sample US) was treated with ultrasounds (Ultrasons-HD, modelo 3000868, J.P. Selecta
112 S.A., Barcelona, Spain), at 400 W and a frequency of 40 Hz for 1 hour a day, 5 days a
113 week. The fourth batch (sample US-IDY) was subjected to the same ultrasound treatment
114 together with 0.3 g/L of inactive dry yeast per flask. The fifth batch (sample MW)
115 underwent microwave treatment (LG MJ3965ACS, Madrid, Spain) at a power of 700 W
116 and a frequency of 2,450 Mhz, for 1 min 4 times/day, 5 days a week. And in the last batch
117 (MW-IDY) the previous microwave treatment was applied, together with inactive dry

yeast (0.3 g/L). All flasks were kept for 3 months at a temperature of 20°C, after which the wines were decanted and arranged for the different analyses.

2.2. Conventional analysis

Conventional analysis (alcoholic degree, pH, total and volatile acidity, glucose and fructose, glycerol and organic acids (malic, lactic, citric, tartaric and succinic acids) and proline were determined by official analytical methods established in the International Organization of Vine and Wine (OIV, 2020).

2.2. Analysis of monosaccharides by GC–MS

Wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described (Guadalupe et al., 2012; Ayestarán et al., 2004). The monosaccharide composition was determined by GC–MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic methanolysis and derivatization as previously described (Guadalupe et al. 2012). GC was controlled by ChemStation software and equipped with a 7653B automatic injector consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Inc. Santa Clara, CA, USA) coupled to a 5975C VL quadrupole mass detector (MS). The content of each polysaccharide family was estimated from the concentration of individual glycosyl residues which are characteristic of structurally identified must and wine polysaccharides (Ayestarán et al., 2004).

2.3. Analysis of amino acids by HPLC

The determination of amino acids was carried out using the method described by Gómez-Alonso et al. (2007) with some modifications. Previously, samples were derivatized by mixing 1 mL of wine with 1.75 mL of 1 M borate buffer (pH=9), 30 µL of diethylethoxymethylenemalonate (DEEMM) and 750 µL of methanol in a screw cap test

142 tube for 30 min in an ultrasound bath. To allow complete degradation of excess DEEMM
143 and reagent by-products, the mixture heated at 70 ºC for 2 h.

144 A HPLC equipment was used to perform the analyses with a diode array detector
145 (Agilent, Model 1100; Agilent Technologies, Inc. Santa Clara, CA, USA). The
146 chromatographic separation was carried out on an ACE HPLC column (5 C18-HL),
147 particle size of 5 µm (250 mm × 2.1 mm), using a phase A: 25 mM acetate buffer, pH =
148 5.8 with 0.02% sodium azide; phase B: methanol and phase C: acetonitrile, and a flow
149 rate of 0.9 mL/ min. For detection, a photodiode array detector was used, monitored at
150 280 and 269 nm. Compounds were identified and quantified using the corresponding
151 standards (Sigma-Aldrich Chemie, Tres Cantos, Madrid, Spain).

152 *2.4. Chromatic parameters*

153 Spectrophotometric parameters: Colour intensity (CI) was calculated as the sum
154 of the absorbance at 620, 520 and 420 nm, following the method of Glories et al. (1984).
155 The hue was obtained by the ratio between the absorbance at 420 nm and at 520 nm. Total
156 phenol index (TPI) was calculated with absorbance analysis at 280 nm wavelength. Total
157 and polymeric anthocyanins were determined following the method of Ho et al. (2001)
158 determining the absorbance at 520 nm. Total methylcellulose precipitable tannins were
159 determined by the method of Smith (2005) being calculated by absorbance difference at
160 280 nm.

161 Determination of tannins by the phloroglucinolysis method: The samples were
162 analysed following the method of Busse-Valverde et al. (2010) using a Waters 2695
163 HPLC system (Waters, Milford, MA, USA) coupled to a Waters 2996 photodiode array
164 detector, and an Atlantis dC18 column (250 × 4.6 mm, 5 µm packing) with a guard
165 column of the same material (20 mm × 4.6 mm, 5 µm packing), kept at 30ºC. A
166 water/formic acid mixture (98:2, v/v) was used as solvent A, and acetonitrile/solvent A

(80:20 v/v) as solvent B, maintaining a flow rate of 0.8 mL/min. The injection volume was 10 µL. The analyses made it possible to determine the total tannin content, the mean apparent degree of polymerization (mDP), and the percentage of galloylation and the percentage of the epigallocatechin tannic subunit.

2.5. Volatile compounds analysis by GC-MS

Major volatile compounds (methanol, propanol, isobutanol and isoamyl alcohols) were analysed by direct injection, using a GC/MS Focus-ISQ chromatograph (Thermo Scientic, Milan, Italy). 4-methyl-2-Pentanol (41.5 mg/L) was added to wine (1:1 (v/v)) as internal standard. One microliter (1 µL) of wine was injected in split mode (1/25) onto a BP-21 (SGE) column (60 m × 0.32 mm × 0.25 µm). Helium (1.2 mL/min) was used as carrier gas. Injector temperature was set at 195 °C and the oven temperature program was 32 °C (2 min)- 5 °C/min to 120 °C- 75 °C/min to 190 °C (18 min).

Minor volatile compounds were extracted by Solid Phase Extraction (SPE) before de GC analysis using 500 mg styrene-divinylbenzene cartridges (Lichrolut EN Merck, KGaA, Darmstadt, Germany), previously conditioned with 10 mL of dichloromethane, followed by 5 mL of methanol, and 10 mL of 10% (v/v) aqueous ethanol. Then, 100 mL of wine were passed through the cartridge together with 40 µL of 4-nonanol (1 g/L) as internal standard. Hydrophilic compounds were removed using 50 mL of bidistilled Milli Q Plus water and minor volatile compounds were eluted with 10 mL of dichloromethane. The extracts were concentrated under a nitrogen stream and stored at −20 °C until analysis. One microliter (1 µL) of extract was injected in splitless mode (0.30 min) onto an Agilent 6890 GC System accoupled to an Agilent 5973 Mass Detector using a DB-WAX column (60 m × 0.25 mm × 0.25 µm) (Agilent Technologies, Inc. Santa Clara, CA, USA). Helium was used as carrier gas (1 mL/min). Column temperature: 70 °C (5 min)

191 rising at 1 ºC/min to 90 ºC (10 min) and then 2 °C/min to 210 °C (40 min). The injector
192 temperature was 250ºC.

193 In both cases the MS worked in the electron impact mode (70 eV), the ion source
194 temperature was 230 ºC and the scanning was made from 45 to 550 a.m.u. Identification
195 of the volatile compounds was executed by comparison with standards from Sigma-
196 Aldrich (Tres Cantos, Madrid, Spain). Compounds for which it was not possible to find
197 volatile references was tentatively identified using NBS75K and NIST14 libraries. The
198 response factor for each volatile compound was determined by injecting commercially
199 available standards into the analysis system at an intermediate concentration typically
200 found in wines. An equal amount of internal standard was added to both the standards
201 and the samples. In the case of compounds not commercially available the response factor
202 of compounds with similar chemical structures were used. Then, the different response
203 factors were used to calculate the concentration of each compound.

204 *2.6. Sensory descriptive analysis*

205 A panel made up of 8 expert tasters from the laboratory staff aged between 25 and
206 58 years old carried out the descriptive sensory analysis of the wines. The assessment
207 took place in a standard sensory analysis chamber (ISO 8589:2007) equipped with
208 separate booths and wine-tasting glasses (ISO 3591:1997). Previously the judges
209 individually generated the sensory terms that best described the samples, agreeing on the
210 following descriptors: red berry, herbaceous and floral flavours. Likewise, bitterness,
211 astringency, body and overall impression were evaluated. The panellists used a 10 cm
212 unstructured scale to rate the intensity of each attribute. The left extreme of the scale
213 indicated a null intensity of the descriptor and the right extreme the maximum value.

214 *2.7. Statistical analysis*

The statistical analysis was executed using the IBM SPSS statistics v.24.0 for Windows statistical package. Data set was analysed with the Student–Newman–Keul´s test to find significant differences between samples.

3. Results and discussion

3.1. Basic chemical composition and amino acids of wines

In general, the basic composition of the wine (Table 1) was little affected by the treatments carried out, but a slight increase in volatile acidity and acetic acid was observed in the samples treated with ultrasounds and lees (US, US-IDY and IDY) as has been described by other authors (García-Martín et al., 2016). Moreover, a small decrease in the content of succinic acid in the samples treated with microwaves (MW and MW-IDY) was observed, without any changes in the rest of the acids.

Table 2 shows the amino acids and ammonium concentration in the control and treated wines. The main amino acid in all wines was proline since it is the most abundant in the must and is also not usually metabolized by yeasts (Martínez-Rodríguez & Pueyo 2009). Alanine also stood out for its higher content, while the rest of the amino acids were found in small amounts.

Amino acids are generally released into the medium at the end of fermentation due to yeast autolysis. Guilloux-Benatier and Chassagne (2003), showed that the treatment of wine with inactive dry yeasts produced a greater release of amino acids due to the higher content of these compounds in the cells when they are grown in an aerobic medium. In our case, the amino acid most affected by the lees treatment was proline, which obtained a significant increase in all wines treated with lees (IDY, US-IDY and MW-IDY) compared to the control wine without treatment (C). The wine treated with US and lees (US-IDY) presented the highest amounts of proline, while the MW-IDY wine did not differ from the IDY control.

240 The treatment on lees (IDY) also caused a slight increase in other amino acids
241 (phenylalanine, ornithine, lysine, ammonium and glutamic acid + glutamine), compared
242 to the non-treated wine (C). In wines treated with ultrasounds and lees (US-IDY) this
243 increase was maintained and some amino acids such as β-alanine+arginine, methionine
244 or cysteine increased additionally, while in wines treated with microwaves and lees (MW-
245 IDY) there was lower changes and some amino acids decreased with respect to the IDY
246 wine.

247 The treatments with ultrasounds and microwaves without lees addition (US and
248 MW) also produced an increase in proline with respect to the control (C), especially in
249 the case of the ultrasound treatment. The same happened with other amino acids
250 (histidine, GABA, isoleucine), although not as noticeably, showing that these treatments
251 by themselves can affect the amino acid content of the wine, probably because they cause
252 their release from peptides or mannoproteins present in the wine.

253 *3.2. Wine polysaccharides*

254 When comparing control wines (without IDY addition), ultrasounds significantly
255 increased the total monosaccharide content (TMS) and the total polysaccharides families
256 (TPF) (Table 3). Concretely, it was observed a significant increase of the constituent
257 monosaccharides of pectic polysaccharides as galactose, arabinose, rhamnose and
258 glucuronic acid, which are the components of the pectic polysaccharides rich in arabinose
259 and galactose (PRAG), galacturonans, galactans, arabinogalactans, arabinogalactan
260 proteins and arabinans (Vidal et al., 2003). The content of 2-*O*-methyl-xylose, 2-*O*-
261 methyl-fucose, and Kdo also increased. These rare sugars are markers for the presence of
262 the pectic polysaccharides RG-II (Pérez et al., 2003; Vidal et al. 2003); the concentration
263 of rhamnose and fucose increased as they are components of RG-I or RG-II in the case
264 of rhamnose (Martínez-Lapuente et al., 2018), or RG-II in the case of fucose (Pellerin et

al., 1996). The content of galacturonic acid, principal constituent of homogalacturonans (HG) (Ayestarán et al., 2004), also increased. Mannose content in wines, which is attributed to mannoproteins (MP) from yeast cell walls (Guadalupe & Ayestarán, 2007; Martínez-Lapuente et al., 2018), was significantly higher in control wines treated with ultrasounds (US). These results showed that ultrasounds broke down the colloidal particles of the soluble pectic polysaccharides and the cell walls of the residual population of the yeast that were in the wine. However, this effect was not observed in control wines treated with microwaves and untreated, as they did not show significant differences in TMS, MP, PRAG, RG-II and TPF.

IDY treatment combined with ultrasounds and microwaves increased the content of TMS, TPF, MP, RG-II and PRAG compared to the control wines (US and MW) (Table 3). The combined treatment fragmented the soluble colloidal particles of galacturonans, galactans, arabinogalactans, arabinogalactan proteins and arabinans, which are the pectic polysaccharides of grapes. These results have not been described in the literature. In addition, the combined treatment favoured with greater intensity the solubility of MP from the cell walls of residual yeast in the wine and from the insoluble composition of IDY (inactive yeast and yeast walls). However, the only application of IDY (IDY) did not significantly increase the MP content in the wine compared to the control wine (C). But the IDY wines had similar glucose content, used to estimate the glucan content of the yeast cell walls (Pérez-Magariño et al., 2015). No significant differences in TMS. MP, PRAG, RG-II and TSP between IDY and C wines were observed. This result suggested that IDY does not interact with major wine pectic polysaccharides (PRAG and RG-II).

The combined US-IDY treatment was more effective in fragmentation of PRAG and RG-II colloidal particles than the MW-IDY treatment. Furthermore, the US-IDY

289 treatment was the most effective in the solubilization of MP. The use of IDY alone was
290 the least effective treatment in MP extraction.

291 *3.3. Chromatic characteristics of wines*

292 Chromatic parameters (spectrophotometric and chromatographic data) are shown
293 in Table 4. As it can be observed, the addition of lees to the wine (IDY) only produced
294 significant changes in tannin content, decreasing them. The application of ultrasounds or
295 microwaves (US and MW) produced a decrease in the colour intensity values of the wine,
296 especially when microwaves were used, associated with a decrease in anthocyanin
297 concentration possibly caused by the oxidation produced during the treatment (García-
298 Martín et al., 2016) and a decrease in tannins. The presence of lees in these wines (US-
299 IDY and MW-IDY) increased the effect of ultrasounds and microwaves, affecting wine
300 colour, due to slight losses in anthocyanin content and, above all, to a more significant
301 decrease in tannin content. This effect was also observed by Liu et al. (2016) and Del
302 Fresno et al. (2018) in whose studies ultrasounds were applied on red wine aged on lees,
303 considering the possibility that this decrease was due to oxidation phenomena of
304 anthocyanins due to an increase of the dissolved oxygen concentration. Along with it, the
305 agitation of the wine produced by the ultrasounds and the microwaves could have
306 generated a more intimate contact between the tannins and the yeast cell walls or other
307 components such as the plasma membrane (Mekou-Nguela et al., 2015), favouring in part
308 their adsorption and precipitation. Also, as ultrasounds and microwaves increased the
309 liberation of soluble polysaccharides (Table 3), they could also bind tannins and part of
310 these combinations could precipitate, especially those where high molecular weight
311 polysaccharides were involved, decreasing the tannin content in wine (Osete-Alcaraz et
312 al., 2020).

313 Regarding the concentration of tannins measured by phloroglucinolysis, there
314 were no significant differences between untreated wine (C) and lees-treated wine (IDY),
315 contrary to what was observed by spectrophotometry, indicating that the tannins mainly
316 affected were those that were oxidized and therefore, no depolymerizable tannins, rather
317 than those bound to anthocyanins, since no changes were observed in the values of
318 polymeric anthocyanins. Bautista-Ortín et al. (2014) also reported a higher adsorption of
319 oxidized tannins (with respect to non-oxidized tannins) by grape cell walls. With the
320 application of ultrasounds and microwaves, the tannin content showed a behaviour similar
321 to that observed by the measurements performed by spectrophotometry.

322 With respect to tannin composition, the application of lees to the wine (IDY) did
323 not produce changes in tannin composition. Contrary to our results, Mazauric and Salmon
324 (2005) observed a decrease in epigallocatechin due to aging on the lees, effect observed
325 in the present study when the ultrasounds were applied. Both, ultrasounds and
326 microwaves led to an increase in the percentage of galloylation, and in presence of lees
327 (US-IDY and MW-IDY) a more accentuated behaviour was observed in the variations of
328 these parameters.

329 *3.4. Volatile compounds of wines*

330 Volatile compounds formed during fermentation (acids, esters, lactones and
331 benzene compounds) as well as varietal compounds (terpenes, norisoprenoids and C_6
332 alcohols) were analysed in wines by GC-MS. Among the major alcohols (methanol,
333 propanol, isobutanol and isoamyl alcohols), isoamyl alcohols stand out for their higher
334 concentration, although they do not exceed levels that could negatively affect the aroma
335 of the wines. All the treatments used, alone or in combination (IDY, US, US-IDY, MW
336 and MW-IDY), caused a small decrease in propanol and isoamyl alcohols in the wines
337 (supplementary material). Liu et al., 2016 observed an opposite behaviour regarding

higher alcohols in wines treated with ultrasounds and lees depending on the yeast strain used.

Fig. 1 shows the total concentrations of the main groups of minor volatile compounds. While the minor alcohols did not present appreciable changes in the wines**,** the total esters increased in the wines with lees (IDY), but they remained constant in the other treatments with respect to the untreated wine (C). However, this behaviour is variable depending on the ester. Ethyl lactate did not show significant differences between the samples (supplementary material), however, fatty acid esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) decreased considerably in the wines treated with ultrasounds and microwaves, including those treated with lees (Fig. 1). The same effect was observed in the case of acetates, among which isoamyl acetate, with banana aroma, stands out for its high concentration (supplementary material). These compounds are of sensory relevance, influencing the fruity aromas of young wines mainly those from low-aromatic grape varieties (Ferreira, 2010).

Total fatty acids and lactones only increased in wines with lees (IDY), remaining constant in the rest of the wines. While the total benzene compounds, among which 2-phenylethanol (with a rose aroma) was the most abundant, increased slightly in all the treated wines (IDY, US-IDY and MW-IDY) (Fig. 1). Although some compounds, such as guaiacol, 4-vinylguaiacol and syringol, which can be related to spicy or medicinal aromas, had the opposite effect, decreasing with the treatments (supplementary material).

Regarding the varietal compounds, total C_6 alcohols showed an increase in all treated wines (Fig. 1), especially in the case of 1-hexanol, which was the main compound (supplementary material). These compounds have been linked to herbaceous aromas, although their concentrations in all wines were below their odour thresholds (Ferreira, 2010).

363 Terpenes and norisoprenoids are compounds of sensory relevance in wines due to
364 their floral and fruity aromas and low odour thresholds. Their tendency was towards a
365 decrease in the samples treated with ultrasounds (US and US-IDY), without any
366 significant changes in the rest of the treatments with respect to the control wine (C) (Fig.
367 1).

368 It has been described that the addition of less or IDY can affect the aroma of wines
369 in different ways. On the one hand, IDY can release volatile compounds into the medium
370 or soluble colloids that can affect their volatility (Comuzzo et al., 2012). On the other
371 hand, the cell walls of the yeasts, specifically the mannoproteins, have the capacity to
372 adsorb wine compounds, including odorant molecules, as well as their glycosylated
373 precursors (Pozo-Bayón et al., 2009). Additionally, the decrease in volatile compounds,
374 and especially esters, has been observed by several authors in wines treated with
375 ultrasounds and lees (Liu et al., 2016; Del Fresno et al., 2018). This effect may be due to
376 the increase in aeration produced during the ultrasound treatment, which can cause the
377 volatilization of some compounds or facilitate oxidative processes (García-Martín & Sun,
378 2013).

379 There are no references on the effect of microwaves on the volatile compounds of
380 wines, although based on our results the effect could be similar to that observed in the
381 ultrasound treatment.

382 *3.5. Sensory analysis*

383 Fig. 2 shows the results of the sensory analysis of the wines in the form of a spider
384 web. Wines treated with ultrasounds (US and US-IDY) had the lowest scores for floral
385 and red fruit flavour attributes, in agreement with other authors that observed lower
386 aromatic intensity and lower varietal character in wines treated with lees and ultrasounds
387 (Liu et al., 2016; Del Fresno et al, 2018). This may be related to the lower content of

388 volatile compounds in these wines, mainly esters and acetates. The microwave treatment
389 seems to have less effect on these attributes, while the lees treatment caused a small
390 increase in these attributes (IDY wines).

391 The herbaceous flavour decreased in all wines with lees, regardless of treatment.
392 This attribute can be considered negative if it is excessive and it has been related to C_6
393 alcohols. In our case an increase of C_6 alcohols was observed in the IDY samples, so it is
394 likely that other compounds associated with this attribute (sulfur compounds, pyrazines...)
395 could have been adsorbed by the lees (Pozo-Bayón et al., 2009).

396 In addition, the tasters detected a slight toasted aroma in the ultrasound treated
397 wines, which could influence the lower overall impression of these wines. This defect has
398 been observed in wines treated with ultrasounds due to oxidation phenomena (Del Fresno
399 et al. 2018). Del Fresno et al., 2019 carried out the ultrasound treatment of the lees prior
400 to their incorporation into the wine and obtained wines that were positively valued by the
401 tasters.

402 All the wines treated with lees (IDY, US-IDY and MW-IDY) had greater body,
403 and their astringency was considerably reduced, especially in the MW-IDY wine.
404 Bitterness was not detected in any of the wines treated with lees. This fact has been also
405 observed by other authors in wines aged on lees due to the increase in polysaccharides
406 and the decrease in tannins (Del Fresno et al., 2018, 2019). The best valued wines were
407 those treated with lees (IDY) and with lees and microwaves (MW-IDY), which best
408 preserved their floral and fruity flavours, reducing astringency and bitterness.

409 **4. Conclusions**

410 The ultrasound and microwave treatments applied to the lees aging significantly
411 improved the extraction of amino acids and polysaccharides from the yeast walls, being
412 the combination of ultrasound and IDY the most effective treatment, in both cases.

However, the US and MW treatments produced a decrease in color intensity, anthocyanins, and tannins, which was not observed in the wines treated only with IDY. This effect should be considered when these techniques are applied to wines with low polyphenol content.

Moreover, it is important to highlight that the treatments employed, particularly US, resulted in a decrease in some volatile compounds with sensory relevance in wines. Consequently, US-treated wines had lower scores in some olfactory attributes, such as red berry and floral, which negatively influenced their overall score. On the other hand, wines aged with microwaves and lees were the best valued, showing sensory characteristics very similar to the IDY control wine but with less astringency.

Further research will be required to evaluate the influence of the grape variety and different ultrasonic and microwave treatment conditions on wines aged on lees.

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Ultrasound and microwave techniques for assisting ageing on lees of ~~Mencía~~ red wines

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ABSTRACT

Ageing on lees is a slow process that carries microbiological and economic risks in the wineries. This study evaluates the possibility of enhancing the extraction of different compounds from the lees, using combined strategies, such as ultrasound (US) or microwaves (MW) and the addition of inactive dry yeasts (IDY), to reduce the lees ageing time. The complete chemical analysis of the wine was done, amino acids, polysaccharides, colour and volatile compounds, together with the sensory analysis. The combined treatments increased the release of total polysaccharides, mannoproteins and total monosaccharides in the wines, and some amino acids like proline. However, wines treated with US and MW, with and without lees, showed a decrease in tannins and colour intensity, and in some volatile compounds like fatty acid esters, acetates and terpenes. The wines treated with IDY and MW were the best valued for their floral and red berry flavours and less astringency.

Keywords:

Lees ageing

Red wine

Ultrasounds

Microwaves

Volatile compounds

Non-volatile compounds

1. Introduction

Ageing on lees is a technique that has traditionally been used in the production of sparkling and red wines, in which the wine is kept in contact with the yeast for several months after fermentation, favouring the release of compounds from the autolysis of yeasts and improving the organoleptic characteristics of wines (Martínez-Rodríguez & Pueyo, 2009).

Yeast autolysis is a slow process, so ageing on lees implies immobilization of the wine in the cellar for a long time, increasing economic and microbiological hazards. The use of inactive dry yeasts (IDY) has become widespread in the wine industry to replace the yeast lees, avoiding the microbiological and organoleptic risks, and reducing the slow and complex process that entails the yeast autolysis (Pozo-Bayón et al., 2009; Pérez-Serradilla & Luque de Castro, 2008).

Inactive dry yeasts are obtained by thermal inactivation and drying of the yeasts, that have grown in a medium with a high concentration of sugar under aerobic conditions (Comuzzo et al., 2012). The most commercial inactive dry yeast (IDY) is made up of insoluble compounds, as inactive yeasts, yeast membranes and walls, and a soluble fraction formed by free cellular metabolites released after yeast lysis, as amino acids, peptides and proteins, polysaccharides, nucleotides, fatty acids, vitamins and minerals, which can be released into the wine during the lees ageing process (López-Solís et al., 2017). In IDY preparations, mannoproteins (MP), from the cell wall of yeasts, are the main components, showing a positive effect on wine sensory characteristics. In fact, MP improve the aromatic profile (Del Barrio-Galán et al., 2012), reduce astringency and bitterness, increase the body, structure, and roundness (Guadalupe et al., 2010; Poncet-Legrand et al., 2007) and influence the colour of red wines (Escot et al., 2001).

In order to accelerate the ageing process on the lees, in recent years emerging technologies have been investigated to replace traditional stirring or "batonnage", increasing the efficiency of the process. Among them, the use of high-power ultrasound (HPU) ~~high-frequency ultrasound (HPU)~~ and microwave (MW) could be most promising (Lui et al., 2016). The high-power ultrasound technique is based on the application of mechanical sound waves with frequencies between 20 kHz and ~~10~~ 100 MHz inducing acoustic cavitation in a liquid medium. The intense pressure and temperature gradients accelerate chemical and physical changes, causing cell rupture and allowing a greater matter transfer (Garcia-Martín. et al., 2013). While microwaves are non-ionizing electromagnetic waves that cause an increase in energy in the matrix produced by molecular friction, mainly by dipole rotation and ionic conduction, that can modify molecular structures and favour the migration of compounds (Clodoveo et al., 2016).

Both techniques have been used in the wine industry for different purposes such as microbiological stabilization (Clodoveo et al., 2016) and to reduce the maceration time increasing the extraction of grape compounds (polysaccharides, volatile compounds and polyphenols) (Pérez-Porras et al., 2021, Oliver et al., 2021; Muñoz et al., 2021; Muñoz et al., 2022). Additionally, the application of US and MW in wines during the ageing period increase the aromatic intensity of wood attributes and accelerate the ageing process (García-Martín et al., 2013).

Ultrasounds promote yeast autolysis by improving polysaccharide extraction in model solutions and wine (Cacciola et al., 2013; del Fresno et al., 2018), while no significant effect is observed in the case of microwave treatment (Liu et al., 2016). However, the same authors detected a reduction in aroma compounds due to the use of US, and a decrease in total polyphenols, which can affect the sensory characteristics of wines (Liu et al., 2016; del Fresno et al., 2018). It seems that the conditions used in the

treatment such as the type of yeast and the potency and duration of US treatment, considerably affect the results obtained (García-Martín & Sun, 2013). No references have been found on the effect that the use of microwaves in ageing on lees could have on the volatile or phenolic compounds of the wine.

Therefore, the objective of this work is to obtain complete information on the effect of US and MW treatments used as tools to accelerate the ageing of wine on lees, using inactive dry yeasts (IDY), on the families of polysaccharides, the phenolic composition and other wine components such as volatile compounds and amino acids on which there is no prior information.

2. Material and methods

2.1. Experiment design

To carry out this experiment, a Mencía red wine produced at the "Instituto de la Vid y el vino de Castilla-La Mancha" (IVICAM, Tomelloso, Ciudad Real, Spain) in the 2021 harvest was used.

The wine was distributed in 2 L flasks with a volume of 1.3 L per flask, forming 6 batches with different conditions, in triplicate. The first batch was kept without any treatment as a control (sample C), in the second batch (sample IDY) inactive dry yeast *Saccharomyces cerevisiae* (Lallemand) was added at 0.3 g/L per flask. The third batch (sample US) was treated with ultrasounds (Ultrasons-HD, modelo 3000868, J.P. Selecta S.A., Barcelona, Spain), at 400 W and a frequency of 40 Hz for 1 hour a day, 5 days a week. The fourth batch (sample US-IDY) was subjected to the same ultrasound treatment together with 0.3 g/L of inactive dry yeast per flask. The fifth batch (sample MW) underwent microwave treatment (LG MJ3965ACS, Madrid, Spain) at a power of 700 W and a frequency of 2,450 Mhz, for 1 min 4 times/day, 5 days a week. And in the last batch (MW-IDY) the previous microwave treatment was applied, together with inactive dry

yeast (0.3 g/L). All flasks were kept for 3 months at a temperature of 20°C, after which the wines were decanted and arranged for the different analyses.

2.2. Conventional analysis

Conventional analysis (alcoholic degree, pH, total and volatile acidity, glucose and fructose, glycerol and organic acids (malic, lactic, citric, tartaric and succinic acids) and proline were determined by official analytical methods established in the International Organization of Vine and Wine (OIV, 2020).

2.2. Analysis of monosaccharides by GC–MS

Wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described (Guadalupe et al., 2012; Ayestarán et al., 2004). The monosaccharide composition was determined by GC–MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic methanolysis and derivatization as previously described (Guadalupe et al. 2012). GC was controlled by ChemStation software and equipped with a 7653B automatic injector consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Inc. Santa Clara, CA, USA) coupled to a 5975C VL quadrupole mass detector (MS). The content of each polysaccharide family was estimated from the concentration of individual glycosyl residues which are characteristic of structurally identified must and wine polysaccharides (Ayestarán et al., 2004).

2.3. Analysis of amino acids by HPLC

The determination of amino acids was carried out using the method described by Gómez-Alonso et al. (2007) with some modifications. Previously, ~~the~~ samples were derivatized by mixing 1 mL of wine with 1.75 mL of 1 M borate buffer (pH=9), 30 µL of diethylethoxymethylenemalonate (DEEMM) and 750 µL of methanol in a screw cap test

142 tube for 30 min in an ultrasound bath. To allow complete degradation of excess DEEMM
143 and reagent by-products, the mixture heated at 70 ºC for 2 h.

144 A HPLC equipment was used to perform the analyses with a diode array detector
145 (Agilent, Model 1101~~0~~0; Agilent Technologies, Inc. Santa Clara, CA, USA). The
146 chromatographic separation was carried out on an ACE HPLC column (5 C18-HL),
147 particle size of 5 µm (250 mm × 2.1 mm), using a phase A: 25 mM acetate buffer, pH =
148 5.8 with 0.02% sodium azide; phase B: methanol and phase C: acetonitrile, and a flow
149 rate of 0.9 mL/ min. For detection, a photodiode array detector was used, monitored at
150 280 and 269 nm. Compounds were identified and quantified using the corresponding
151 standards (Sigma-Aldrich Chemie, Tres Cantos, Madrid, Spain).

152 *2.4. Chromatic parameters*

153 Spectrophotometric parameters: Colour intensity (CI) was calculated as the sum
154 of the absorbance at 620, 520 and 420 nm, following the method of Glories et al. (1984).
155 The hue was obtained by the ratio between the absorbance at 420 nm and at 520 nm. Total
156 phenol index (TPI) was calculated with absorbance analysis at 280 nm wavelength. Total
157 and polymeric anthocyanins were determined following the method of Ho et al. (2001)
158 determining the absorbance at 520 nm. Total methylcellulose precipitable tannins were
159 determined by the method of Smith (2005) being calculated by absorbance difference at
160 280 nm.

161 Determination of tannins by the phloroglucinolysis method: The samples were
162 analysed following the method of Busse-Valverde et al. (2010) using a Waters 2695
163 HPLC system (Waters, Milford, MA, USA) coupled to a Waters 2996 photodiode array
164 detector, and an Atlantis dC18 column (250 × 4.6 mm, 5 µm packing) with a guard
165 column of the same material (20 mm × 4.6 mm, 5 µm packing), kept at 30ºC. A
166 water/formic acid mixture (98:2, v/v) was used as solvent A, and acetonitrile/solvent A

(80:20 v/v) as solvent B, maintaining a flow rate of 0.8 mL/min. The injection volume was 10 µL. The analyses made it possible to determine the total tannin content, the mean apparent degree of polymerization (mDP), and the percentage of galloylation and the percentage of the epigallocatechin tannic subunit.

2.5. Volatile compounds analysis by GC-MS

Major volatile compounds (methanol, propanol, isobutanol and isoamyl alcohols) were analysed by direct injection, using a GC/MS Focus-ISQ chromatograph (Thermo Scientic, Milan, Italy). 4 methyl-2-pentanol ~~2-pentanol-4-methyl~~ (41.5 mg/L) was added to wine (1:1 (v/v)) as internal standard. One microliter (1 µL) of wine was injected in split mode (1/25) onto a BP-21 (SGE) column (60 m × 0.32 mm × 0.25 µm). Helium (1.2 mL/min) was used as carrier gas. Injector temperature was set at 195 °C and the oven temperature program was 32 °C (2 min)- 5 °C/min to 120 °C- 75 °C/min to 190 °C (18 min).

Minor volatile compounds were extracted by Solid Phase Extraction (SPE) before de GC analysis using 500 mg styrene-divinylbenzene cartridges (Lichrolut EN Merck, KGaA, Darmstadt, Germany), previously conditioned with 10 mL of dichloromethane, followed by 5 mL of methanol, and 10 mL of 10% (v/v) aqueous ethanol. Then, 100 mL of wine were passed through the cartridge together with 40 µL of 4-nonanol (1 g/L) as internal standard. Hydrophilic compounds were removed using 50 mL of bidistilled Milli Q Plus water and minor volatile compounds were eluted with 10 mL of dichloromethane. The extracts were concentrated under a nitrogen stream and stored at −20 °C until analysis. One microliter (1 µL) of extract was injected in splitless mode (0.30 min) onto an Agilent 6890 GC System accoupled to an Agilent 5973 Mass Detector using a DB-WAX column (60 m × 0.25 mm × 0.25 µm) (Agilent Technologies, Inc. Santa Clara, CA, USA). Helium was used as carrier gas (1 mL/min). Column temperature: 70 °C (5 min)

192 rising at 1 ºC/min to 90 ºC (10 min) and then 2 °C/min to 210 °C (40 min). The injector
193 temperature was 250ºC.
194 In both cases the MS worked in the electron impact mode (70 eV), the ion source
195 temperature was 230 ºC and the scanning was made from 45 to 550 a.m.u. Identification
196 of the volatile compounds was executed by comparison with standards from Sigma-
197 Aldrich (Tres Cantos, Madrid, Spain). Compounds for which it was not possible to find
198 volatile references was tentatively identified using NBS75K and NIST14 libraries. The
199 response factor for each volatile compound was determined by injecting commercially
200 available standards into the analysis system at an intermediate concentration typically
201 found in wines. An equal amount of internal standard was added to both the standards
202 and the samples. In the case of compounds not commercially available the response factor
203 of compounds with similar chemical structures were used. Then, the different response
204 factors were used to calculate the concentration of each compound.

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205 *2.6. Sensory descriptive analysis*

206 A panel made up of 8 expert tasters from the laboratory staff aged between 25 and
207 58 years old carried out the descriptive sensory analysis of the wines. The assessment
208 took place in a standard sensory analysis chamber (ISO 8589:2007) equipped with
209 separate booths and wine-tasting glasses (ISO 3591:1997). Previously the judges
210 individually generated the sensory terms that best described the samples, agreeing on the
211 following descriptors: red berry, herbaceous and floral flavours. Likewise, bitterness,
212 astringency, body and overall impression were evaluated. The panellists used a 10 cm
213 unstructured scale to rate the intensity of each attribute. The left extreme of the scale
214 indicated a null intensity of the descriptor and the right extreme the maximum value.

215 *2.7. Statistical analysis*

The statistical analysis was executed using the IBM SPSS statistics v.24.0 for Windows statistical package. Data set was analysed with the Student–Newman–Keul´s test to find significant differences between samples.

3. Results and discussion

3.1. Basic chemical composition and amino acids of wines

In general, the basic composition of the wine (Table 1) was little affected by the treatments carried out, but a slight increase in volatile acidity and acetic acid was observed in the samples treated with ultrasounds and lees (US, US-IDY and IDY) as has been described by other authors (García-Martín et al., 2016). Moreover, a small decrease in the content of succinic acid in the samples treated with microwaves (MW and MW-IDY) was observed, without any changes in the rest of the acids.

Table 2 shows the amino acids and ammonium concentration in the control and treated wines. The main amino acid in all wines was proline since it is the most abundant in the must and is also not usually metabolized by yeasts (Martínez-Rodríguez & Pueyo 2009). Alanine also stood out for its higher content, while the rest of the amino acids were found in small amounts.

Amino acids are generally released into the medium at the end of fermentation due to yeast autolysis. Guilloux-Benatier and Chassagne (2003), showed that the treatment of wine with inactive dry yeasts produced a greater release of amino acids due to the higher content of these compounds in the cells when they are grown in an aerobic medium. In our case, the amino acid most affected by the lees treatment was proline, which obtained a significant increase in all wines treated with lees (IDY, US-IDY and MW-IDY) compared to the control wine without treatment (C). The wine treated with US and lees (US-IDY) presented the highest amounts of proline, while the MW-IDY wine did not differ from the IDY control.

The treatment on lees (IDY) also caused a slight increase in other amino acids (phenylalanine, ornithine, lysine, ammonium and glutamic acid + glutamine), compared to the non-treated wine (C). In wines treated with ultrasounds and lees (US-IDY) this increase was maintained and some amino acids such as β-ala~~na~~nine+arginine, methionine or cysteine increased additionally, while in wines treated with microwaves and lees (MW-IDY) there was lower changes and some amino acids decreased with respect to the IDY wine.

The treatments with ultrasounds and microwaves without lees addition (US and MW) also produced an increase in proline with respect to the control (C), especially in the case of the ultrasound treatment. The same happened with other amino acids (histidine, GABA, isoleucine), although not as noticeably, showing that these treatments by themselves can affect the amino acid content of the wine, probably because they cause their release from peptides or mannoproteins present in the wine.

3.2. Wine polysaccharides

When comparing control wines (without IDY addition), ultrasounds significantly increased the total monosaccharide content (TMS) and the total polysaccharides families (TPF) (Table 3). Concretely, it was observed a significant increase of the constituent monosaccharides of pectic polysaccharides as galactose, arabinose, rhamnose and glucuronic acid, which are the components of the pectic polysaccharides rich in arabinose and galactose (PRAG), galacturonans, galactans, arabinogalactans, arabinogalactan proteins and arabinans (Vidal et al., 2003). The content of 2-*O*-methyl-xylose, 2-*O*-methyl-fucose, and Kdo also increased. These rare sugars are markers for the presence of the pectic polysaccharides RG-II (Pérez et al., 2003; Vidal et al. 2003); the concentration of rhamnose and fucose increased as they are components of RG-I or RG-II in the case of rhamnose (Martínez-Lapuente et al., 2018), or RG-II in the case of fucose (Pellerin et

al., 1996). The content of galacturonic acid, principal constituent of homogalacturonans (HG) (Ayestarán et al., 2004), also increased. Mannose content in wines, which is attributed to mannoproteins (MP) from yeast cell walls (Guadalupe & Ayestarán, 2007; Martínez-Lapuente et al., 2018), was significantly higher in control wines treated with ultrasounds (US). These results showed that ultrasounds broke down the colloidal particles of the soluble pectic polysaccharides and the cell walls of the residual population of the yeast that were in the wine. However, this effect was not observed in control wines treated with microwaves and untreated, as they did not show significant differences in TMS, MP, PRAG, RG-II and TPF.

IDY treatment combined with ultrasounds and microwaves increased the content of TMS, TPF, MP, RG-II and PRAG compared to the control wines (US and MW) (Table 3). The combined treatment fragmented the soluble colloidal particles of galacturonans, galactans, arabinogalactans, arabinogalactan proteins and arabinans, which are the pectic polysaccharides of grapes. These results have not been described in the literature. In addition, the combined treatment favoured with greater intensity the solubility of MP from the cell walls of residual yeast in the wine and from the insoluble composition of IDY (inactive yeast and yeast walls). However, the only application of IDY (IDY) did not significantly increase the MP content in the wine compared to the control wine (C). But the IDY wines had similar glucose content, used to estimate the glucan content of the yeast cell walls (Pérez-Magariño et al., 2015). No significant differences in TMS. MP, PRAG, RG-II and TSP between IDY and C wines were observed. This result suggested that IDY does not interact with major wine pectic polysaccharides (PRAG and RG-II).

The combined US-IDY treatment was more effective in fragmentation of PRAG and RG-II colloidal particles than the MW-IDY treatment. Furthermore, the US-IDY

290 treatment was the most effective in the solubilization of MP. The use of IDY alone was
291 the least effective treatment in MP extraction.

292 *3.3. Chromatic characteristics of wines*

293 Chromatic parameters (spectrophotometric and chromatographic data) are shown
294 in Table 4. As it can be observed, the addition of lees to the wine (IDY) only produced
295 significant changes in tannin content, decreasing them. The application of ultrasounds or
296 microwaves (US and MW) produced a decrease in the colour intensity values of the wine,
297 especially when microwaves were used, associated with a decrease in anthocyanin
298 concentration possibly caused by the oxidation produced during the treatment (García-
299 Martín et al., 2016) and a decrease in tannins. The presence of lees in these wines (US-
300 IDY and MW-IDY) increased the effect of ultrasounds and microwaves, affecting wine
301 colour, due to slight losses in anthocyanin content and, above all, to a more significant
302 decrease in tannin content. This effect was also observed by Liu et al. (2016) and Del
303 Fresno et al. (2018) in whose studies ultrasounds were applied on red wine aged on lees,
304 considering the possibility that this decrease was due to oxidation phenomena of
305 anthocyanins due to an increase of the dissolved oxygen concentration. Along with it, the
306 agitation of the wine produced by the ultrasounds and the microwaves could have
307 generated a more intimate contact between the tannins and the yeast cell walls or other
308 components such as the plasma membrane (Mekou-Nguela et al., 2015), favouring in part
309 their adsorption and precipitation. Also, as ultrasounds and microwaves increased the
310 liberation of soluble polysaccharides (Table 3), they could also bind tannins and part of
311 these combinations could precipitate, especially those where high molecular weight
312 polysaccharides were involved, decreasing the tannin content in wine (Osete-Alcaraz et
313 al., 2020).

Regarding the concentration of tannins measured by phloroglucinolysis, there were no significant differences between untreated wine (C) and lees-treated wine (IDY), contrary to what was observed by spectrophotometry, indicating that the tannins mainly affected were those that were oxidized and therefore, no depolymerizable tannins, rather than those bound to anthocyanins, since no changes were observed in the values of polymeric anthocyanins. Bautista-Ortín et al. (2014) also reported a higher adsorption of oxidized tannins (with respect to non-oxidized tannins) by grape cell walls. With the application of ultrasounds and microwaves, the tannin content showed a behaviour similar to that observed by the measurements performed by spectrophotometry.

With respect to tannin composition, the application of lees to the wine (IDY) did not produce changes in tannin composition. Contrary to our results, Mazauric and Salmon (2005) observed a decrease in epigallocatechin due to aging on the lees, effect observed in the present study when the ultrasounds were applied. Both, ultrasounds and microwaves led to an increase in the percentage of galloylation, and in presence of lees (US-IDY and MW-IDY) a more accentuated behaviour was observed in the variations of these parameters.

3.4. Volatile compounds of wines

Volatile compounds formed during fermentation (acids, esters, lactones and benzene compounds) as well as varietal compounds (terpenes, norisoprenoids and ~~C6~~ C_6 alcohols) were analysed in wines by GC-MS. Among the major alcohols (methanol, propanol, isobutanol and isoamyl alcohols), isoamyl alcohols stand out for their higher concentration, although they do not exceed levels that could negatively affect the aroma of the wines. All the treatments used, alone or in combination (IDY, US, US-IDY, MW and MW-IDY), caused a small decrease in propanol and isoamyl alcohols in the wines (supplementary material). Liu et al., 2016 observed an opposite behaviour regarding

higher alcohols in wines treated with ultrasounds and lees depending on the yeast strain used.

Fig. 1 shows the total concentrations of the main groups of minor volatile compounds. While the minor alcohols did not present appreciable changes in the wines**,** the total esters increased in the wines with lees (IDY), but they remained constant in the other treatments with respect to the untreated wine (C). However, this behaviour is variable depending on the ester. Ethyl lactate did not show significant differences between the samples (supplementary material), however, fatty acid esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) decreased considerably in the wines treated with ultrasounds and microwaves, including those treated with lees (Fig. 1). The same effect was observed in the case of acetates, among which isoamyl acetate, with banana aroma, stands out for its high concentration (supplementary material). These compounds are of sensory relevance, influencing the fruity aromas of young wines mainly those from low-aromatic grape varieties (Ferreira, 2010).

Total fatty acids and lactones only increased in wines with lees (IDY), remaining constant in the rest of the wines. While the total benzene compounds, among which 2-phenylethanol (with a rose aroma) was the most abundant, increased slightly in all the treated wines (IDY, US-IDY and MW-IDY) (Fig. 1). Although some compounds, such as guaiacol, 4-vinylguaiacol and syringol, which can be related to spicy or medicinal aromas, had the opposite effect, decreasing with the treatments (supplementary material).

Regarding the varietal compounds, total ~~C6~~ C_6 alcohols showed an increase in all treated wines (Fig. 1), especially in the case of 1-hexanol, which was the main compound (supplementary material). These compounds have been linked to herbaceous aromas, although their concentrations in all wines were below their odour thresholds (Ferreira, 2010).

Terpenes and norisoprenoids are compounds of sensory relevance in wines due to their floral and fruity aromas and low odour thresholds. Their tendency was towards a decrease in the samples treated with ultrasounds (US and US-IDY), without any significant changes in the rest of the treatments with respect to the control wine (C) (Fig. 1).

It has been described that the addition of less or IDY can affect the aroma of wines in different ways. On the one hand, IDY can release volatile compounds into the medium or soluble colloids that can affect their volatility (Comuzzo et al., 2012). On the other hand, the cell walls of the yeasts, specifically the mannoproteins, have the capacity to adsorb wine compounds, including odorant molecules, as well as their glycosylated precursors (Pozo-Bayón et al., 2009). Additionally, the decrease in volatile compounds, and especially esters, has been observed by several authors in wines treated with ultrasounds and lees (Liu et al., 2016; Del Fresno et al., 2018). This effect may be due to the increase in aeration produced during the ultrasound treatment, which can cause the volatilization of some compounds or facilitate oxidative processes (García-Martín & Sun, 2013).

There are no references on the effect of microwaves on the volatile compounds of wines, although based on our results the effect could be similar to that observed in the ultrasound treatment.

3.5. Sensory analysis

Fig. 2 shows the results of the sensory analysis of the wines in the form of a spider web. Wines treated with ultrasounds (US and US-IDY) had the lowest scores for floral and red fruit flavour attributes, in agreement with other authors that observed lower aromatic intensity and lower varietal character in wines treated with lees and ultrasounds (Liu et al., 2016; Del Fresno et al, 2018). This may be related to the lower content of

volatile compounds in these wines, mainly esters and acetates. The microwave treatment seems to have less effect on these attributes, while the lees treatment caused a small increase in these attributes (IDY wines).

The herbaceous flavour decreased in all wines with lees, regardless of treatment. This attribute can be considered negative if it is excessive and it has been related to ~~C6~~ C_6 alcohols. In our case an increase of ~~C6~~ C_6 alcohols was observed in the IDY samples, so it is likely that other compounds associated with this attribute (sulfur compounds, pyrazines...) could have been adsorbed by the lees (Pozo-Bayón et al., 2009).

In addition, the tasters detected a slight toasted aroma in the ultrasound treated wines, which could influence the lower overall impression of these wines. This defect has been observed in wines treated with ultrasounds due to oxidation phenomena (Del Fresno et al. 2018). Del Fresno et al., 2019 carried out the ultrasound treatment of the lees prior to their incorporation into the wine and obtained wines that were positively valued by the tasters.

All the wines treated with lees (IDY, US-IDY and MW-IDY) had greater body, and their astringency was considerably reduced, especially in the MW-IDY wine. Bitterness was not detected in any of the wines treated with lees. This fact has been also observed by other authors in wines aged on lees due to the increase in polysaccharides and the decrease in tannins (Del Fresno et al., 2018, 2019). The best valued wines were those treated with lees (IDY) and with lees and microwaves (MW-IDY), which best preserved their floral and fruity flavours, reducing astringency and bitterness.

4. Conclusions

~~The treatments applied to the wines caused few changes in their basic composition, however, significant changes were found in other parameters studied. In the case of amino acids, the most affected was proline, which increased significantly in all~~

~~treated wines, especially in wines treated with ultrasounds and lees (US-IDY). Inactive dry yeast treatment combined with ultrasounds and microwaves also increased the content of monosaccharide content (TMS), total polysaccharides families (TPF), MP, and PRAG compared to the control wines (US and MW), while the use of IDY alone was the least effective treatment in MP extraction. On the other hand, the ultrasound and microwave treatments applied to the wines produced a decrease in colour intensity and anthocyanins, as well as in tannins, which was more pronounced with the addition of lees, especially in polymerized tannins. In addition, both treatments (US and MW) also produced a decrease in fatty acid esters and acetates, while varietal compounds such as terpenes, norisoprenoids and C6 alcohols were less affected. Wines treated only with lees (IDY) had the highest concentrations of total esters, fatty acids and lactones. From the sensory point of view, IDY addition reduced the sensation of astringency and bitterness in all cases, being wines aged with microwaves and IDY the best valued.~~

~~Although in most cases the ultrasound and microwave treatments accentuated the changes that usually occur in ageing on lees, it should be noted that these treatments could cause important changes in the chemical composition of the wines, which in the case of ultrasound treatment had a negative influence on its sensory evaluation.~~

The ultrasound and microwave treatments applied to the lees aging significantly improved the extraction of amino acids and polysaccharides from the yeast walls, being the combination of ultrasound and IDY the most effective treatment, in both cases. However, the US and MW treatments produced a decrease in color intensity, anthocyanins, and tannins, which was not observed in the wines treated only with IDY. This effect should be considered when these techniques are applied to wines with low polyphenol content.

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Moreover, it is important to highlight that the treatments employed, particularly US, resulted in a decrease in some volatile compounds with sensory relevance in wines. Consequently, US-treated wines had lower scores in some olfactory attributes, such as red berry and floral, which negatively influenced their overall score. On the other hand, wines aged with microwaves and lees were the best valued, showing sensory characteristics very similar to the IDY control wine but with less astringency.

Further research will be required to evaluate the influence of the grape variety and different ultrasonic and microwave treatment conditions on wines aged on lees.

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Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author CRediT Role Statement

R. Muñoz-García: formal analysis (equal); **L. Martínez-Lapuente:** formal analysis (equal); **Z. Guadalupe:** methodology (equal); funding acquisition (equal); **B. Ayestarán:** methodology (equal); writing—original draft preparation (equal); funding acquisition (equal); **L. Marchante:** formal analysis (equal); **M.C. Díaz-Maroto:** writing—original draft preparation (equal); writing—review and editing (equal); project administration (equal); funding acquisition (equal); **P. Pérez-Porras:** formal analysis (equal); **A.B. Bautista-Ortín:** methodology (equal); project administration (equal); funding acquisition (equal); **E. Gómez-Plaza:** conceptualization (equal); project administration (equal); funding acquisition (equal); **M. Soledad Pérez-Coello:** conceptualization (equal); writing—original draft preparation (equal); writing—review and editing (equal); project administration (equal); funding acquisition (equal); supervision (lead).

Table (Editable version)

Table 1. Basic chemical composition parameters of control and treated wines (mean ± SD).

Parameter	C	IDY	US	US-IDY	MW	MW-IDY
Alcoholic strength (% v/v)	14.56 ± 0.18	14.75 ± 0.21	14.37 ± 0.18	14.17 ± 0.29	14.76 ± 0.42	14.47 ± 0.17
Total acidity (g/L)	2.83 ± 0.07[a]	2.94 ± 0.05[a]	3.16 ± 0.03[a]	3.17 ± 0.06[a]	2.91 ± 0.01[a]	2.90 ± 0.01[a]
pH	4.10 ± 0.03	4.11 ± 0.01	4.05 ± 0.01	4.04 ± 0.01	4.09 ± 0.01	4.11 ± 0.05
Volatile acidity (g/L acetic)	0.21 ± 0.01[a]	0.31 ± 0.01[b]	0.29 ± 0.02[b]	0.26 ± 0.01[b]	0.21 ± 0.02[a]	0.20 ± 0.02[a]
Glucose + Fructose (g/L)	0.05 ± 0.01	0.06 ± 0.01	0.15 ± 0.03	0.17 ± 0.02	0.22 ± 0.14	0.11 ± 0.07
Acetic acid (g/L)	0.17 ± 0.05[a]	0.31 ± 0.03[b]	0.28 ± 0.02[b]	0.29 ± 0.02[b]	0.22 ± 0.01[a]	0.21 ± 0.02[a]
Malic acid (g/L)	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
Lactic acid (g/L)	1.02 ± 0.05[a]	1.06 ± 0.02[a]	1.16 ± 0.03[a]	1.24 ± 0.04[a]	1.10 ± 0.03[a]	1.07 ± 0.01[a]
Citric acid (g/L)	0.11 ± 0.01[a]	0.14 ± 0.01[a]	0.12 ± 0.01 [a]	0.13 ± 0.01 [a]	0.30 ± 0.20[b]	0.18 ± 0.01 [a]
Tartaric acid (g/L)	1.47 ± 0.12	1.61 ± 0.07	1.63 ± 0.07	1.57 ± 0.10	1.65 ± 0.27	1.57 ± 0.06
Succinic acid (g/L)	0.91 ± 0.02[b]	0.91 ± 0.03[b]	0.91 ± 0.02[b]	0.93 ± 0.01[b]	0.80 ± 0.17 [a.b]	0.71 ± 0.01[a]
Glycerol (g/L)	7.67 ± 0.21	7.65 ± 0.15	7.69 ± 0.13	7.72 ± 0.05	7.84 ± 0.25	7.49 ± 0.10

Different letters in the same row indicate significant differences between samples ($p \leq 0.05$). C: wines without any treatment; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

Table 2. Mean concentration (mg/L) and standard deviation of amino acids in control and treated wines.

Compound	C	IDY	US	US-IDY	MW	MW-IDY
Aspartic acid	6.61 ± 0.16	6.42 ± 0.29	6.10 ± 1.08	7.98 ± 0.56	6.26 ± 1.42	6.63 ± 0.99
Glutamic acid + Glutamine	7.27 ± 0.25^{a}	9.85 ± 0.54^{c}	7.72 ± 0.25^{a,b}	10.34 ± 0.31^{c}	8.21 ± 0.57^{b}	12.57 ± 0.48^{d}
Serine	1.48 ± 0.14^{a}	1.55 ± 0.10^{a}	2.84 ± 0.54^{c}	2.13 ± 0.05^{b}	1.44 ± 0.01^{a}	1.29 ± 0.06^{a}
Histidine	10.25 ± 0.13^{a}	11.43 ± 0.38^{b,c}	12.06 ± 0.48^{c}	12.02 ± 0.28^{c}	10.94 ± 0.55^{b}	11.17 ± 0.08^{b}
Glycine	7.47 ± 0.02	7.55 ± 0.15	7.42 ± 0.42	7.78 ± 0.06	7.37 ± 0.16	7.45 ± 0.04
Threonine	3.01 ± 0.13^{a}	3.95 ± 0.34^{b}	4.42 ± 0.26^{b}	4.32 ± 0.48^{b}	3.13 ± 0.34^{a}	2.91 ± 0.18^{a}
β-Alanine + Arginine	23.17 ± 0.54^{b}	8.48 ± 0.92^{a}	27.30 ± 1.26^{c,d}	27.87 ± 0.92^{d}	26.19 ± 0.21^{c,d}	25.60 ± 0.62^{c}
GABA	15.91 ± 0.39^{a}	19.12 ± 0.82^{b,c}	19.02 ± 1.09^{b,c}	19.77 ± 0.97^{c}	17.51 ± 0.53^{b}	18.16 ± 0.27^{b,c}
α-Alanine	184.61 ± 6.96^{b}	177.33 ± 7.41^{a,b}	173.47 ± 7.24^{a,b}	176.79 ± 4.26^{a,b}	168.38 ± 2.51^{a}	170.69 ± 3.96^{a,b}
Tyrosine	5.51 ± 0.88	6.17 ± 0.99	7.23 ± 0.64	6.70 ± 1.83	5.89 ± 0.85	5.31 ± 0.02
Ammonium	2.45 ± 0.43^{a,b}	5.11 ± 0.35^{c}	2.74 ± 0.24^{b}	2.68 ± 0.32^{b}	2.10 ± 0.25^{a,b}	1.95 ± 0.09^{a}
Valine	3.18 ± 0.47^{a}	4.09 ± 0.55^{a,b}	4.34 ± 0.17^{b}	4.23 ± 0.71^{a,b}	3.93 ± 0.02^{a,b}	3.26 ± 0.07^{a,b}
Methionine	2.39 ± 0.15^{b}	1.32 ± 0.18^{a}	2.11 ± 0.24^{b}	3.06 ± 0.31^{c}	3.35 ± 0.19^{c}	3..35 ± 0.13^{c}
Cysteine	2.57 ± 0.18^{a}	3.45 ± 0.43^{b}	4.30 ± 0.25^{c}	4.46 ± 0.02^{c}	3.60 ± 0.35^{b}	2.55 ± 0.16^{a}
Isoleucine	2.56 ± 0.28^{a,b}	3.60 ± 0.08^{b,c}	4.20 ± 1.22^{c,d}	4.92 ± 0.28^{d}	2.51 ± 0.57^{a,b}	1.61 ± 0.08^{a}
Tryptophan	0.57 ± 0.03	0.62 ± 0.02	0.70 ± 0.11	0.63 ± 0.11	0.58 ± 0.10	0.70 ± 0.15
Leucine	3.35 ± 0.07^{b,c}	3.87 ± 0.81^{b,c}	4.60 ± 0.20^{c}	4.49 ± 0.74^{c}	3.02 ± 0.86^{b}	1.94 ± 0.06^{a}
Phenylalanine	3.79 ± 0.03^{b}	5.42 ± 0.74^{c}	4.43 ± 0.12^{b}	4.23 ± 0.79^{b}	3.82 ± 0.13^{b}	2.08 ± 0.07^{a}
Ornithine	13.06 ± 2.50^{a}	37.88 ± 1.80^{b}	12.49 ± 0.36^{a}	12.42 ± 0.84^{a}	11.48 ± 1.14^{a}	10.44 ± 0.28^{a}
Lysine	1.24 ± 0.17^{a}	2.02 ± 0.06^{b}	2.93 ± 0.35^{c}	3.35 ± 0.23^{d}	1.13 ± 0.11^{a}	1.04 ± 0.01^{a}
Proline	409.68 ± 87.59^{a}	610.70 ± 37.43^{b}	561.15 ± 10.80^{b}	852.25 ± 60.52^{c}	576.91 ± 10.35^{b}	612.39 ± 39.81^{b}

Different letters in the same row indicate significant differences between samples ($p \leq 0.05$). C: wines without any treatment; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

Table 3. Monosaccharide composition (mg/L) and polysaccharides families (mg/L) in control and treated wines (mean ± SD).

Parameter	C	IDY	US	US-IDY	MW	MW-IDY
2-*O*MeFuc	4.42 ± 0.33^{ab}	3.75 ± 0.41^{a}	7.44 ± 0.34^{c}	8.38 ± 0.73^{d}	4.64 ± 0.02^{b}	7.31 ± 0.05^{c}
2-*O*MeXyl	2.37 ± 0.09^{ab}	1.98 ± 0.17^{a}	3.84 ± 0.03^{c}	4.23 ± 0.53^{c}	2.45 ± 0.02^{b}	3.84 ± 0.07^{c}
Api	1.45 ± 0.12^{a}	1.40 ± 0.20^{a}	1.38 ± 0.27^{a}	2.66 ± 0.27^{b}	1.41 ± 0.18^{a}	1.67 ± 0.66^{a}
Kdo	0.85 ± 0.04^{b}	0.51 ± 0.04^{a}	1.42 ± 0.08^{d}	1.47 ± 0.05d	1.17 ± 0.15^{c}	1.44 ± 0.07^{d}
Ara	67.18 ± 3.54^{a}	59.65 ± 3.61^{a}	109.89 ± 6.52^{c}	127.43 ± 12.70^{d}	68.32 ± 1.19^{a}	98.17 ± 3.44^{b}
Gal	314.45 ± 6.64^{a}	293.68 ± 15.03^{a}	428.36 ± 5.29^{b}	513.01 ± 46.63^{c}	306.20 ± 7.36^{a}	440.24 ± 3.65^{b}
GalA	66.74 ± 1.40^{b}	42.22 ± 1.92^{a}	105.44 ± 4.29^{e}	116.97 ± 1.12^{f}	78.59 ± 0.39^{c}	94.01 ± 6.21^{d}
GluA	15.01 ± 0.54^{b}	12.25 ± 0.09^{a}	20.03 ± 1.34^{c}	25.39 ± 2.09^{d}	15.72 ± 1.26^{b}	21.08 ± 1.96^{c}
Rha	28.37 ± 0.10^{a}	27.27 ± 1.96^{a}	48.91 ± 3.72^{b}	56.10 ± 8.08^{c}	30.66 ± 0.16^{a}	43.25 ± 1.77^{b}
Fuc	1.66 ± 0.00^{a}	1.60 ± 0.11^{a}	2.37 ± 0.14^{b}	2.69 ± 0.26^{c}	1.75 ± 0.01^{a}	2.29 ± 0.00^{b}
Xyl	8.51 ± 1.00^{ab}	6.55 ± 0.27^{a}	10.13 ± 1.59^{b}	9.68 ± 0.21^{b}	9.05 ± 1.11^{ab}	8.37 ± 2.76^{ab}
Glc	44.18 ± 0.62^{c}	45.91 ± 0.72^{c}	18.55 ± 3.02^{ab}	19.70 ± 1.43^{b}	46.14 ± 0.41^{c}	15.87 ± 1.45^{a}
Man	200.31 ± 4.05^{a}	195.91 ± 3.73^{a}	246.92 ± 3.00^{b}	303.39 ± 31.87^{d}	193.11 ± 8.78^{a}	273.85 ± 6.20^{c}
TMS	755.52 ± 5.88^{a}	692.70 ± 24.35^{a}	1004.71 ± 6.87^{b}	1191.14 ± 105.99^{c}	759.24 ± 16.40^{a}	1011.43 ± 17.55^{b}
MP	250.39 ± 5.06^{a}	244.89 ± 4.66^{a}	308.65 ± 3.75^{b}	379.24 ± 39.84^{d}	241.38 ± 11.09^{a}	342.32 ± 7.75^{c}
PRAG	462.78 ± 10.95^{a}	431.14 ± 21.65^{a}	641.98 ± 1.78^{b}	767.75 ± 70.50^{c}	451.93 ± 10.85^{a}	646.51 ± 7.88^{b}
RG-II	217.38 ± 13.68^{ab}	183.62 ± 18.89^{a}	361.51 ± 12.05^{c}	403.65 ± 40.58^{d}	227.19 ± 1.25^{b}	357.22 ± 4.09^{c}
HG	26.95 ± 1.57^{b}	8.45 ± 5.66^{a}	38.40 ± 1.21^{c}	41.54 ± 5.50^{c}	36.77 ± 0.23^{c}	28.15 ± 5.74^{b}
TSP	957.50 ± 39.53^{a}	868.11 ± 7.74^{a}	1350.55 ± 145.43^{b}	1592.18 ± 23.43^{c}	957.27 ± 25.46^{a}	1374.20 ± 281.56^{b}

2-*O*MeFuc: 2-*O*-CH_3-fucose, 2-*O*MeXyl: 2-*O*-CH_3-xylose, Api: apiose, Kdo: 2-keto-3-deoxyoctonate ammonium salt, Ara: arabinose, Gal: galactose, GalA: galacturonic acid, GluA: glucuronic acid, Rha: rhamnose, Fuc: fucose, Xyl: xylose, Glc: glucose, Man: mannose, TMS: total monosaccharides, MP: mannoproteins, PRAG: polysaccharides rich in arabinose and galactose, RG-II: rhamnogalacturonans type II, HG: homogalacturonans, TSP: total soluble polysaccharides. Different letters in the same row indicate significant differences between samples ($p \leq 0.05$). C: wines without any treatment; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

Table 4. Chromatic parameters of control and treated wines (mean ± SD).

Parameter	C	IDY	C-US	US-IDY	C-MW	MW-IDY
Colour intensity	12.70 ± 0.55^{c}	12.94 ± 0.04^{c}	11.63 ± 0.31^{b}	$11.21 \pm 0.04^{a,b}$	$10.94 \pm 0.13^{a,b}$	10.63 ± 0.34^{a}
Hue	0.58 ± 0.00^{a}	0.57 ± 0.00^{a}	0.63 ± 0.00^{d}	0.61 ± 0.01^{c}	0.60 ± 0.00^{b}	0.60 ± 0.00^{b}
Total polyphenol Index	37.71 ± 1.17^{c}	37.62 ± 0.30^{c}	$37.44 \pm 0.63^{b,c}$	$35.68 \pm 0.06^{a,b}$	34.94 ± 0.34^{a}	34.00 ± 0.90^{a}
Total anthocyanins	270.96 ± 17.87^{c}	278.64 ± 4.15^{c}	241.71 ± 9.45^{b}	$221.28 \pm 2.11^{a,b}$	212.05 ± 4.50^{a}	206.17 ± 6.80^{a}
Polymeric anthocyanins	91.39 ± 1.46^{c}	92.20 ± 0.28^{c}	79.63 ± 1.04^{a}	$80.81 \pm 0.80^{a,b}$	83.85 ± 0.48^{b}	$82.56 \pm 1.89^{a,b}$
Total methylcellulose precipitable tannins	1217.68 ± 26.48^{d}	1138.17 ± 7.70^{c}	1155.05 ± 25.98^{c}	1080.84 ± 11.79^{b}	1069.47 ± 12.16^{b}	992.27 ± 14.08^{a}
Total tannins by phloroglucinolysis method	405.21 ± 44.54^{c}	393.41 ± 33.53^{c}	$374.49 \pm 9.29^{b,c}$	$307.26 \pm 3.63^{a,b}$	$307.69 \pm 2.57^{a,b}$	275.67 ± 23.03^{a}
Mean degree of polymerization	4.54 ± 0.23^{a}	4.66 ± 0.07^{a}	4.64 ± 0.08^{a}	4.49 ± 0.02^{a}	4.44 ± 0.05^{a}	4.37 ± 0.03^{a}
Percentage of galloylation	3.69 ± 0.28^{a}	3.52 ± 0.03^{a}	$5.18 \pm 0.21^{b,c}$	5.34 ± 0.02^{c}	4.74 ± 0.06^{b}	$5.09 \pm 0.32^{b,c}$
Percentage of epigallocathequin	$14.49 \pm 0.17^{b,c}$	14.98 ± 0.07^{c}	12.71 ± 0.24^{a}	13.89 ± 0.45^{b}	14.80 ± 0.13^{c}	$14.52 \pm 0.14^{b,c}$

Different letters in the same row indicate significant differences between samples ($p \leq 0.05$). C: wines without any treatment; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

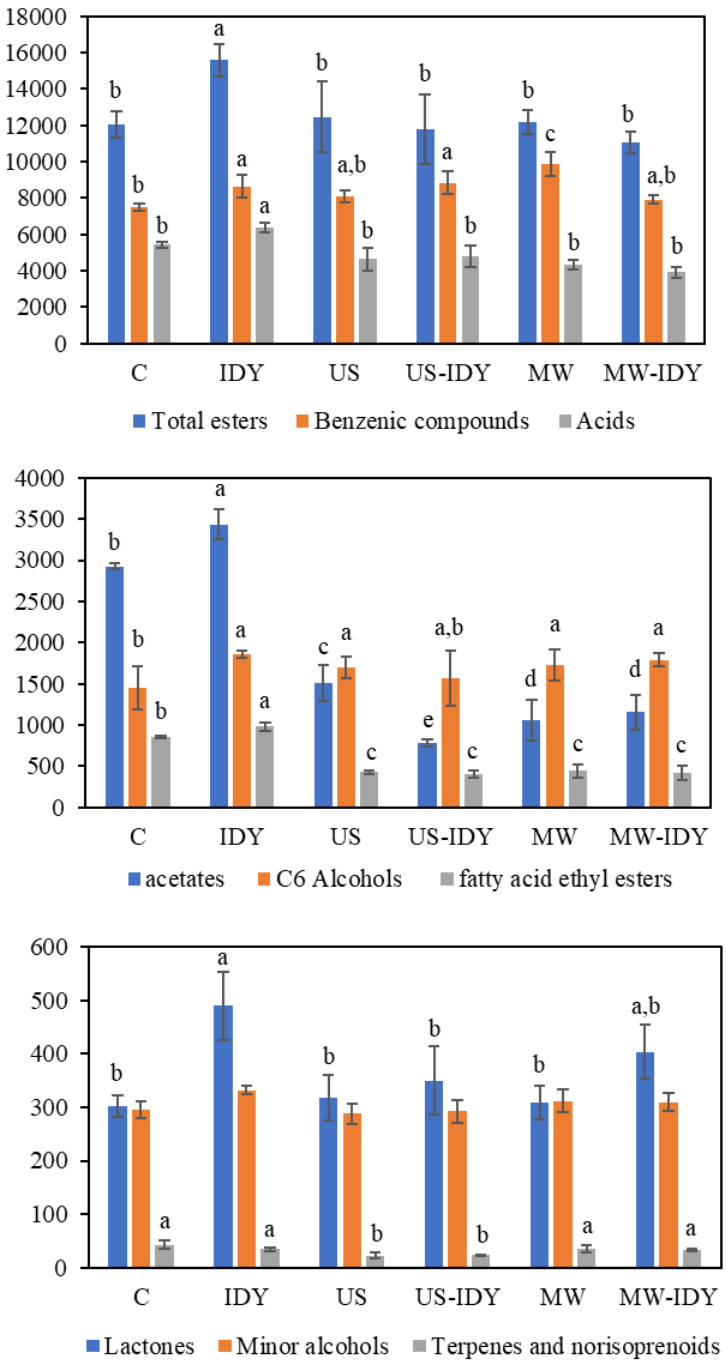

Fig. 1. Mean concentrations (µg/L) of main group of volatile compounds in control and treated wines. Different letters denote significant differences between treatments according to the Student-Newman-Keuls test ($p \leq 0.05$). C: wines without any treatment; IDY: wines treated with inactive dry yeast; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

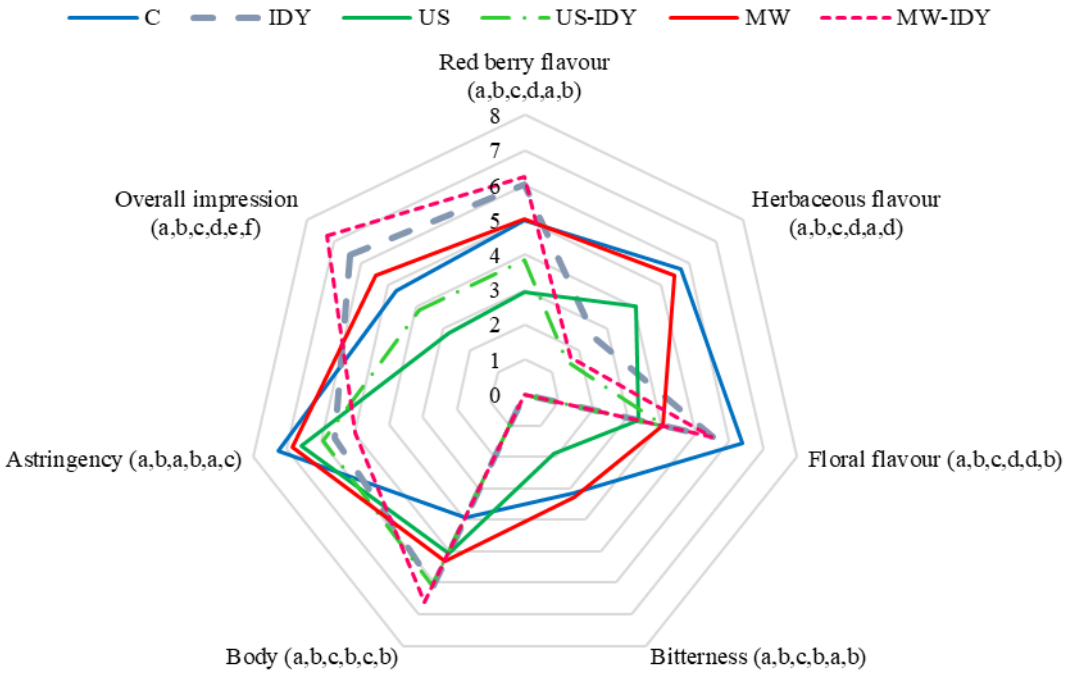

Fig. 2. Descriptive sensory analysis of control and treated wines. Different letters denote significant differences between samples according to the Student-Newman-Keuls test ($p \leq 0.05$) in the following order: C: wines without any treatment; IDY: wines treated with inactive dry yeast; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

Supplementary Table 1. Mean concentration (µg/L) and standard deviation of volatile compounds in control and treated wines. (*) Concentration in mg/L.

Volatile compound	RT	C	IDY	US	US-IDY	MW	MW-IDY
Esters							
Ethyl butanoate	1035	214.09 ± 6.05^{c}	262.99 ± 36.94^{d}	88.22 ± 13.19^{a,b}	57.79 ± 17.32^{a}	132.63 ± 25.40^{b}	110.24 ± 31.90^{a,b}
Isoamyl acetate	1122	2802.22 ± 55.40^{c}	3318.13 ± 177.33^{d}	1435.07 ± 200.52^{b}	696.41 ± 43.30^{a}	993.98 ± 258.21^{a}	1078.31 ± 214.40^{a}
Ethyl hexanoate	1233	268.10 ± 13.56^{b}	308.74 ± 13.58^{b}	149.56 ±15.42^{a}	147.87 ± 16.76^{a}	196.69 ± 55.24^{a}	203.33 ± 32.21^{a}
Hexyl acetate	1272	25.86 ± 2.10 ^{c}	32.85 ± 2.59^{d}	11.45 ±1.38^{b}	7.93 ± 1.78^{a}	5.21 ± 0.28^{a}	4.39 ± 0.74^{a}
Ethyl lactate	1347	7340.01 ± 670.51	10195.69 ± 104.56	9951.72 ±858.00	10017.16 ± 875.33	9737.14 ± 584.19	8684.57 ± 346.11
Ethyl octanoate	1435	273.67 ± 25.16^{c}	311.46 ± 5.95^{d}	163.64 ±21.51^{b}	166.73 ± 25.27^{b}	105.67 ± 12.43^{a}	89.92 ± 23.27^{a}
Ethyl 3-hydroxy-butyrate	1515	8.19 ± 0.70^{c}	7.48 ± 0.53^{a,b}	5.52 ± 0.31^{a}	5.53 ± 0.01^{a}	6.34 ± 0.94^{a,b}	6.61 ± 0.21^{a,b}
Ethyl 2-hydroxy-methylpentanoate	1547	15.43 ± 1.25^{a,b}	17.11 ± 1.68^{b}	12.64 ± 0.97^{a}	13.02 ± 0.69^{a}	14.86 ± 1.02^{a,b}	15.10 ± 0.33^{a,b}
Pentyl hydroxypropanoate	1610	37.33 ± 3.30^{b}	47.29 ± 2.99^{a}	34.07 ± 2.32^{b}	34.15 ± 2.68^{b}	37.66 ± 1.22^{b}	37.00 ± 0.75^{b}
Ethyl decanoate	1638	101.55 ± 23.59^{a}	97.92 ± 7.14^{a}	30.59 ± 0.38^{b}	33.50 ± 4.69^{b}	12.27 ±1.64^{b}	11.82 ± 2.00^{b}
Diethyl succinate	1671	581.52 ± 103.99^{b,c}	477.00 ± 17.36^{b}	348.51 ± 41.12^{a}	304.32 ± 83.05^{a}	660.29 ± 5.54^{c}	527.80 ± 6.25^{b}
Ethyl 3-hydroxy-hexanoate	1677	2.83 ± 0.66^{b,c}	2.06 ± 0.20^{a,b}	1.39 ± 0.20^{a}	2.25 ± 0.61^{a,b,c}	3.07 ± 0.76^{b,c}	3.52 ± 0.53^{c}
Ethyl 4-hydroxy-butyrate	1794	196.97 ± 53.35	158.00 ± 7.08	124.43 ± 28.52	169.08 ± 3.93	150.65 ± 41.38	161.14 ± 6.01
2-Phenyl ethyl acetate	1813	94.95 ± 16.51	84.98 ± 0.78	64.39 ± 18.42	80.47 ± 0.99	64.60 ± 16.89	71.55 ± 2.36
Major alcohols							
Methanol*	903	115.48 ± 1.49	120.25 ± 12.16	127.44 ± 1.61	117.06 ± 0.86	122.16 ± 4.30	127.71 ± 10.03
Propanol*	1036	47.51 ± 4.83^{b}	37.56 ± 5.89^{a}	34.41 ± 3.47^{a}	31.59 ± 0.92^{a}	36.33 ± 4.45^{a}	36.71 ± 4.38^{a}
Isobutanol*	1092	23.62 ± 1.16	28.32 ± 6.29	27.75 ± 5.55	22.72 ± 2.09	24.00 ± 4.01	26.40 ± 4.87
1-Butanol	1150	64.37 ± 8.36	67.75 ± 9.33	69.84 ± 12.75	71.92 ± 12.91	79.68 ± 5.02	75.14 ± 5.89
Isoamyl alcohols*	1200	236.40 ± 5.03^{c}	221.43 ± 13.37^{b}	210.72 ± 10.69^{a,b}	194.18 ± 3.73^{a}	205.78 ± 6.44^{a,b}	205.45 ± 2.25^{a,b}

Supplementary Table 1. Continued.

Minor alcohols							
4-Methyl-1-pentanol	1314	28.83 ± 0.40	30.75 ± 1.80	27.32 ± 1.80	27.52 ± 2.17	28.26 ± 4.40	30.82 ± 1.25
3-Methyl-1-pentanol	1325	68.67 ± 1.10	75.48 ± 7.26	67.20 ± 3.06	68.52 ± 5.75	70.70 ± 9.26	75.05 ± 5.61
1-Heptanol	1453	74.27 ± 10.81^{a}	91.37 ± 6.30^{b}	70.75 ± 3.04^{a}	73.43 ± 6.30^{a}	77.25 ± 4.17^{a}	79.58 ± 4.06^{a}
1-Octanol	1557	40.83 ± 1.50^{a}	50.63 ± 1.26^{b}	40.43 ± 3.81^{a}	38.78 ± 1.04^{a}	35.97 ± 0.50^{a}	36.07 ± 2.12^{a}
3-(Methylthio)-1-propanol	1719	18.50 ± 4.01^{a,b}	16.05 ± 0.83^{a,b}	12.20 ± 2.59^{a}	11.94 ± 1.81^{a}	20.06 ± 3.03^{b}	12.56 ± 2.61^{a}
Fatty acids							
2-Methylpropanoic acid	1570	249.60 ± 8.43^{a}	472.95 ± 54.28^{c}	356.44 ± 49.86^{b}	354.15 ± 48.86^{b}	333.52 ± 43.42^{b}	340.39 ± 15.09^{b}
3-Methylbutanoic acid	1662	65.67 ± 15.90^{b}	62.22 ± 1.30^{b}	32.66 ± 2.34^{a}	62.28 ± 0.74^{b}	61.86 ± 15.42^{b}	73.05 ± 2.09^{b}
Hexanoic acid	1846	2282.26 ± 61.98	2483.13 ± 72.24	2245.05 ± 250.25	2244.49 ± 241.34	2472.57 ± 191.39	2227.46 ± 56.87
Octanoic acid	2060	2303.92 ± 84.54^{c}	2760.41 ± 127.51^{d}	1921.64 ± 301.10^{b}	1855.97 ± 245.34^{b}	1426.11 ± 145.04^{a}	1225.24 ± 230.80^{a}
Decanoic acid	2276	529.53 ± 10.39^{c}	586.85 ± 60.22^{c}	85.74 ± 14.29^{a}	289.58 ± 66.66^{b}	40.82 ± 6.70^{a}	44.71 ± 7.32^{a}
Lactones							
γ-Butyrolactone	1632	267.47 ± 19.04^{a}	449.97 ± 88.14^{b}	279.25 ± 39.61^{a}	309.28 ± 59.88^{a}	269.84 ± 27.65^{a}	369.20 ± 52.28^{a,b}
γ-Nonalactone	2024	8.77 ± 0.02^{a,b}	9.83 ± 1.67^{a,b}	7.33 ± 1.00^{a}	7.54 ± 0.68^{a}	9.03 ± 0.47^{a,b}	8.89 ± 0.40^{a,b}
γ-Decalactone	2137	25.67 ± 1.84	30.18 ± 4.32	31.54 ± 3.47	33.10 ± 4.39	29.36 ± 3.52	25.31 ± 2.00
Benzenic compounds							
Benzaldehyde	1520	0.50 ± 0.05^{a}	0.55 ± 0.08^{a}	8.97 ± 1.67^{b}	9.85 ± 0.14^{b}	0.71 ± 0.17^{a}	0.75 ± 0.07^{a}
Guaiacol	1861	10.61 ± 1.49^{b}	4.58 ± 0.36^{a}	3.07 ± 0.80^{a}	3.37 ± 0.06^{a}	4.83 ± 0.51^{a}	3.26 ± 0.09^{a}
Benzyl alcohol	1870	94.51 ± 14.16	100.96 ± 4.13	88.78 ± 7.36	91.06 ± 10.43	99.50 ± 3.14	94.94 ± 2.18
2-Phenylethanol	1906	6501.65 ± 237.06^{a}	7713.79 ± 688.75^{b}	7429.59 ± 299.32^{,b}	7883.74 ± 639.22^{,b}	9365.43 ± 658.09^{b}	7533.51 ± 223.95^{b}
4-Vinylguaiacol	2188	81.21 ± 20.45^{b}	45.92 ± 7.41^{a}	27.08 ± 1.43^{a}	27.43 ± 2.27^{a}	40.91 ± 9.43^{a}	30.88 ± 1.54^{a}
Syringol	2273	172.83 ± 10.22^{d}	64.03 ± 8.03^{b,c}	55.59 ± 7.83^{a,b}	47.80 ± 8.16^{a,b}	66.85 ± 7.48^{c}	39.75 ± 1.27^{a}
Vanillin	2570	2.72 ± 0.65	2.06 ± 0.18	1.34 ± 0.19	2.84 ± 5.90	1.71 ± 0.44	1.18 ± 0.13
Ethyl vanillate	2654	97.40 ± 2.62^{a}	117.66 ± 9.09^{a}	110.14 ± 19.61^{a}	110.13 ± 18.08^{a}	136.25 ± 16.72^{a}	111.47 ± 4.75^{a}

Supplementary Table 1. Continued.

Terpenes and norisoprenoids							
Linalool	1547	2.03 ± 0.17^{a}	2.11 ± 0.18^{a}	1.72 ± 0.23^{a}	1.91 ± 0.26^{a}	2.47 ± 0.14^{a}	2.05 ± 0.07^{a}
α-Terpineol	1697	0.32 ± 0.05^{a}	0.24 ± 0.02^{a}	0.15 ± 0.03^{a}	0.23 ± 0.02^{a}	0.26 ± 0.08^{a}	0.25 ± 0.02^{a}
β-Citronellol	1765	7.91 ± 1.68^{b}	5.84 ± 0.17^{a,b}	4.43 ± 0.23^{a}	4.58 ± 0.27^{a}	7.28 ± 1.39^{b}	6.24 ± 0.16^{b}
β-Damascenone	1823	3.71 ± 1.09	3.25 ± 0.23	3.34 ± 0.55	3.19 ± 0.37	3.63 ± 0.24	3.27 ± 0.37
Geraniol	1847	12.35 ± 3.18^{b}	9.30 ± 0.30^{b}	5.84 ± 1.70^{a}	6.90 ± 0.11^{a}	6.84 ± 1.76^{a}	8.00 ± 0.17^{b}
Nerolidol	2034	15.59 ± 3.27^{b}	12.61 ± 1.00^{b}	6.36 ± 0.77^{a}	6.36 ± 0.73^{a}	14.42 ± 0.95^{b}	12.61 ± 0.55^{b}
3-OH-*β*-Damascone	2563	0.94 ± 0.15^{b}	1.02 ± 0.12^{b}	0.55 ± 0.14^{a}	0.77 ± 0.04^{b}	1.04 ± 0.15^{b}	0.82 ± 0.03^{b}
C6-Alcohols							
1-Hexanol	1355	1340.95 ± 363.76^{b}	1721.65 ± 33.03^{a}	1571.98 ± 120.61^{a}	1442.51 ± 423.02^{a,b}	1608.57 ± 174.49^{a}	1657.12 ± 71.26^{a}
cis-3-Hexen-1-ol	1373	33.97 ± 0.73	38.36 ± 2.07	35.21 ± 2.01	36.00 ± 3.63	34.60 ± 6.34	37.86 ± 0.64
trans-3-Hexen-1-ol	1380	76.68 ± 1.94	84.91 ± 5.29	74.10 ± 4.72	77.55 ± 6.75	79.05 ± 11.74	84.21 ± 3.96
cis-2-Hexen-1-ol	1405	0.70 ± 0.02^{a}	5.18 ± 0.96^{b}	7.31 ± 2.09^{b}	7.08 ± 2.05^{b}	1.35 ± 0.32^{a}	8.03 ± 0.21^{b}
trans-2-Hexen-1ol	1416	3.98 ± 0.65^{a}	5.48 ± 0.33^{b}	4.98 ± 0.39^{b}	5.40 ± 0.30^{b}	5.33 ± 0.50^{b}	5.69 ± 0.11^{b}

Different letters in the same row indicate significant differences between samples ($p \leq 0.05$). C: wines without any treatment; US: wines treated with ultrasounds; US-IDY: wines treated with ultrasounds and IDY; MW: wines treated with microwave; MW-IDY: wines treated with microwave and IDY.

RT: Retention indices (DB-Wax)