Effect of short ageing on lees on the mannoprotein content, aromatic profile and sensorial character of white wines

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Abstract

In Albariño white wines, aging of wines on lees is a technique not used or only used empirically by some producers to obtain a distinctive character in the final wine. This study analyzes the influence of a short aging on lees on the chemical and sensorial parameters of this young white wine. Albariño grape must was inoculated with a locally selected yeast (*S. cerevisiae* 1) and the effect of a short aging on lees was studied during different times (10, 20, 30, 40 and 50 days). Mannoprotein content and the aromatic profile were determined and a sensorial analysis of the wines was conducted. Results showed that aging time was correlated with the concentration of some key aroma compounds and mannoproteins in Albariño wines. The best sensorial character was obtained in wines aged 20 days on lees. Further aging times decreased the sensorial quality of Albariño wine and modified its volatile profile and mannoprotein concentration.

Significance of study: The use of a short contact time during ageing on lees of young Albariño white wines could be a successful post-fermentative alternative to enhance their typical aromatic characteristics and to produce more distinctive wines.

Keywords: white wines, yeast, lees, aging on lees, aroma compounds, mannoproteins.

1. Introduction

Aging on lees is an oenological practice, which involves the contact of the wine obtained after alcoholic fermentation with resting dead yeast cells. Lees are formed by microorganisms (mainly yeast), and tartaric and inorganic matter (both in a minor proportion) (Perez-Serradilla and de Castro 2008). Traditionally, only some white wines mainly from Burgundy and sparkling wines produced by the traditional method are aged in contact with their lees (Loscos and others 2009) ,but nowadays, wine aging on lees is gaining importance in many wine production areas (Del Barrio-Galan and others, 2011; Pati and others 2012; Rodrigues and others 2012). The aim of this technique is to improve wine's sensorial character, as well as some technological aspects such as stability and foam ability. The yeast autolysis process, which takes place during wine aging produces breakdown of cells membranes, release of intracellular components, liberation of hydrolytic enzymes, and hydrolysis of intracellular biopolymers into low molecular weight products. Amongst compounds released by yeast during aging on lees, mannoproteins consists on small chains with one to four D-mannose residues that are linked to polypeptide chains on serine or threonine residues (Perez-Serradilla and de Castro 2008). Mannoproteins like other breakdown products released into wine can modify significantly its sensorial properties (Pozo-Bayon and others 2009).

In young white wines the aroma is one of the principal quality criteria, these wines are characterized by a high intensity of fresh and fruity notes which depends mainly on the content of terpenes present in the grape, in addition with

acetates and mono- and dicarboxylic acid ethylesters which appear during the fermentation process (Perez-Coello and others 2003). Additionally, yeast lees can influence the wine aroma contributing to its balance, thus affecting positively the wine quality. Nevertheless, the contact of wine with lees could also reduce their content in certain volatile compounds, which a consequent decrease in the quality of wine (Perez-Serradilla and de Castro 2008). This behavior seems to be correlated with several variables, such as the characteristics of lees and the time that wine stays in contact with lees. Loscos and others (2009) have found that lees from different yeast strains may have slightly different abilities to release volatile compounds derived from precursors. On the other hand, it has been observed that the contact of white wines with lees during 7 months has modified their sensorial properties, decreasing fruit and floral aromas (Bautista and others 2007). Using a short ageing time on lees (20 days), the behavior observed was dependent on the grape variety. While in Airen wines most of the compounds increased its concentration, in contrast in Macabeo wines decreased (Bueno and others 2006). The reported capacity of lees to interact with aroma compounds and potentially modify their sensory properties has also been associated to mannoprotein fraction, considering that some of them can retain aroma compounds (Chalier and others 2007; Juega and others 2012).

Albariño grape is a Galician typical variety recognized by its high quality. White wines from Albariño grapes are mainly produced as young wines with a high concentration of terpenes, and fruity and floral odors (Vilanova and others 2010; Carrascosa and others 2012). Until present day, there are not studies

about the impact of short aging times on lees in the aroma composition and final quality of these wines, although this is a practice used empirically by some producers with the purpose to obtain a distinctive character in the final wine. In the present work, we have studied the effect of different aging times on lees in Albariño white wines, assessing its impact on the mannoprotein content, aroma profile and sensorial character.

2. Materials and methods

2.1- Must, yeast and fermentation conditions. The grape must used in this study was from *Vitis vinifera* cv. Albariño grapes (vintage 2009) and was supplied by the winery Terras Gauda, Galicia, Spain. The composition of the must was the following: sugars 190 g/L, pH 3.38, total acidity 8.2 g/L and maturation index 22.3. The grape must was inoculated with *Saccharomyces cerevisiae* (*S. cerevisiae*) strain 1, a locally-selected yeast (Carrascosa and others 2012) and fermented in 30L stainless steel tanks. Fermentation experiments were carried out in triplicate. The temperature was set to 18ºC. Fermentation was followed by the sugar consumption, and the reducing sugar during fermentation was determined until 40 days. The obtained wines were aged on its lees during different periods: 10 days (W10), 20 days (W20), 30 days (W30), 40 days (W40) and 50 days (W50). A control wine (CW) was prepared without aging on lees. Once alcoholic fermentation was completed, the control wine was kept in the tank for 4 days to allow sedimentation of the gross lees. Following this, the wine was racked off and kept in the tank for 4-5

121 days to allow sedimentation of the fine lees. Predominance of the selected
122 yeast in the fermentation tanks was verified by studying the mitochondrial DNA
123 profile at the end of the fermentation (Querol and others 1992). 1500 mL
124 samples were taken from each tank and used in the experimental and sensory
125 analysis. They were prepared by centrifugation at 1800 x g, 15 min and kept at -
126 18º C until analysis. Conventional parameters in the wines (alcoholic grade,
127 total acidity, volatile acidity, pH, tartaric and malic acid) were determined by the
128 European Commission methods (EC 1990) at the end of the fermentation and
129 after 50 days of aging.
130
131 **2.2- Precipitation, hydrolysis, and quantification of mannoproteins.** The
132 procedure described by Segarra and others 1995, was used for the isolation of
133 the colloidal fraction containing mannoproteins. 40 ml of ethanol (96% v/v) and
134 400 µl HCL (1N) were added to 8 ml of wine. After 18 h of incubation at 22 ºC,
135 the tubes were centrifuged (1800 x g, 20 min), after which the supernatant was
136 discarded and the pellet was washed three times in ethanol (96%, v/v). For the
137 determination of the sugar composition of mannoproteins, the samples obtained
138 were hydrolysed at 100 ºC for 24 h in a closed vial containing 1 ml of 2 M
139 trifluoroacetic laudinaacid and 0.5 ml myo-inositol (0.1 % w/v, internal standard)
140 solution. After hydrolysis, the mixture was evaporated to dryness under
141 vacuum. The dried hydrolysed residue was silylated following the procedure
142 described by (Nunez and others 2006). Briefly, the sample was dissolved in 100
143 ml of anhydrous pyridine, and 100 ml of trimethylsilylimidazole, 100 ml of
144 trimethylclorosilane, 100 ml of n-hexane and 200 ml of deionized water were

145 sequentially added, shaking during each step. Trimethyilsilyl derivatives (1µl)
146 were analysed on a Hewlett-Packard 6890 Chromatograph (Palo Alto, CA,
147 USA), equipped with a flame ionization detector (FID) and split/splitless injector.
148 Samples were injected on a Carbowax 20M column (30 m X 0.25 mm) coated
149 with a stationary phase of 0.25 mm thickness. Temperatures were as follows:
150 injector and detector, 220 ºC; oven, held at 175 ºC for 15 min, then increasing
151 15 ºC/min to 200 ºC during 13 minutes and finally programmed at 30 ºC/min to
152 270 ºC during 20 minutes. The carrier gas was helium (10 psi, split 1/15).
153 Response factors were calculated with a series of pure standards at different
154 concentrations using myo-inositol as internal standard. The identification of the
155 mannose present in the samples was carried out by comparing the retention
156 time of the peaks with those of pure standard. Each sample was analyzed by
157 triplicate. Results were expressed as mg/L of polymeric mannose in the wine.
158 The concentration of protein moieties was determined following the Bradford
159 method (Bradford 1976), based in the reaction of the protein with the Coomasie
160 blue G-250. Absorbance was determined at 595 nm 15 min. after the addition of
161 the reactive. The results were expressed in mg of bovine seroalbumine (BSA)/L.
162
163 **2.3- Volatile Compounds**. The extraction of volatile compounds was
164 automatically performed by using a CombiPal system (CTC Analytics AG,
165 Zwingen, Switzerland) provided with a 50/30 µm
166 Divinylbencene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber of 2 cm
167 length (Supelco, Bellefonte, PA. USA). 5 ml of wine sample and 2 g NaCl were
168 placed in 15 ml sample vial sample vial with 10 µl of internal standard (methyl

nonanoate 15ppm). The vial was capped with a PTFE- silicon septum. The extraction was performed in the headspace of the vial for 20 minutes at 40 ºC. The desorption was performed in the injector of the GC chromatograph (Agilent 7890) in splitless mode for 12 minutes at 280 ºC. After each injection the fiber was cleaning for 30 minutes avoiding any memory effect. All the analyses were performed in triplicate. An Agilent MSD ChemStation Software was used to control the gas chromatograph (Agilent 7890). For separation, a fused silica CP-WAX 57CB column (50m X 0.25mm X 0.39mm film thickness) from Varian (Houten, The Netherlands) was used. Helium was the carrier gas (1 ml/min). The oven temperature was programmed as follows: 60 ºC as initial temperature, held for 5 minutes, followed by a ramp of temperature at 2 ºC/min to 120 ºC and 3 ºC/min to 215 ºC, and then held for 25 minutes. For the MS system (Agilent 5973N), the temperatures of the manifold and transfer line were 150 and 230 ºC respectively; electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 μA. The acquisitions were performed in selected-ion- monitoring (SIM) mode. The signal corresponding to a specific ion of quantification was calculated by the data system. Quantitative data were obtained by calculating the relative peak area (or TIC signal) in relation to that of the internal standard used for each compound. Calibration curves of each compound were performed using a model wine (4 g/L tartaric acid, 10 % v/v ethanol and pH=3) spiked with the commercial pure reference compounds at five different levels of concentration covering the concentration ranges expected in wines.

193 **2.4- Sensory analysis.** A panel of experts comprised of eight judges carried
194 out sensory evaluation of the wines. The tasting card used was the official Rías
195 Baixas index card (Carrascosa and others 2012) . Wine samples were
196 evaluated at 15º C. The scores used were penalizing scores so better quality
197 wines receive a lower score. Six variables (visual examination, aroma intensity,
198 aroma quality, taste intensity, taste quality and harmony) were proposed for
199 assessment, and a scale of 7 categories designed (excellent: 0–7, very good:
200 8–23, good: 24–44, correct: 45–52, ordinary: 53–78, defective: 79–90,
201 eliminated: >90). The mode of the scores given by the eight tasters was used to
202 arrive at the final score for each parameter corresponding to the sensorial
203 characteristics of wine.
204
205 **2.5- Statistical analysis.** Significant differences among the data obtained from
206 the volatile composition of the wines aged on lees during different periods were
207 estimated by applying analysis of variance (ANOVA). The Tukey least
208 significant differences (LSD) test was used to evaluate the significance of the
209 analysis. The program used was SPSS 16.0 for Windows, version 16.0.1 (Nov.
210 2007).
211
212 **3. Results and Discussion**
213
214 The inoculated strain prevailed during the elaboration process,
215 fermenting the grape must to dryness (1.2 g/L ± 0.0 residual sugars). Table 1
216 shows the values of different chemical wine parameters at the end of alcoholic

fermentation and after 50 days of aging on lees. According to results, no significant differences ($p<0.01$) were observed among them, highlighting that these parameters were not affected by the aging on lees. In all cases, the values obtained were according to normal ranges found for these wines (Zamúz and Vilanova 2006; Carrascosa and others 2012) and showed that the vinification was adequate.

Table 2 shows variations in protein and polymeric mannose concentrations during different ageing times on lees. Throughout the first 20 days of aging, no significant differences were found in protein concentration. At 30 days of aging a significant increase in protein concentration was detected in the wine, suggesting the beginning of the autolysis process. In previous studies, we have observed that proteins are reliable markers for autolysis process. In first process stages, there is a steady increase in wine protein concentration (Martinez-Rodriguez and Polo 2000). In the succeeding stages, previous released proteins were metabolized by freed proteases, producing small peptides and amino acids, which are not detected by Bradford protein analysis. These results agree with those obtained for 50 days of ageing, where a sharp decrease in proteins was detected, which indicates that the nature of proteins depends on the aging time on lees, being less polymerized while aging time increases (Martinez-Rodriguez and Polo 2000). In the case of the polymeric mannose fraction a similar behavior was detected. No significant changes were observed during the first 30 days of aging, decreasing the polymeric mannose concentration at 40 days of aging. It is known that during the first stages of autolysis, the β-glucanases act on the yeast cell wall releasing mannoproteins

241 covalently linked to the glucan in the cell wall (Pozo-Bayon and others 2009).
242 Subsequently, protein moieties of mannoproteins are hydrolysed by proteases
243 into low molecular peptides, while the β-glucanases degrades the glucans that
244 are still linked to mannoproteins, releasing peptidemannans into the wine
245 (Rodrigues and others 2012). These peptidemannans can be detected as a
246 new increase in polymeric mannose, as was observed for 50 days of aging.

247 In table 3 lists major volatile compounds identified in the wines aged on
248 lees at different times. Higher alcohols, ranging from to 2.93 mg/L to 181.59
249 mg/L, were the most abundant compounds. In all cases, the concentration of
250 this family of compounds was under 300 mg/L, which is the threshold at which
251 alcohols can negatively affect the wine (Flanzy 2003). 1-hexanol was not
252 modified during aging on lees, while 3-methyl-1-butanol and 2-phenylethanol
253 changed its concentration during the aging process. Some higher alcohols with
254 high molecular weight, such as 2-phenylethanol, can be absorbed on the yeast
255 cell wall and its concentration in the wine can be enhanced with the yeast cell
256 wall lysis (Masino and others 2008). In the present context, the first increase at
257 30 days of 2-phenylethanol and 3-methyl-1-butanol in wine agrees with some
258 analytical evidences of the yeast autolysis, suggesting that it can vary the
259 concentration of these compounds, which was observed at 40 and 50 days of
260 aging.

261 Esters and acetates were in terms of quantity the second group of
262 volatile compounds. These compounds are partially responsible for the fresh
263 and fruity aroma of young white wines (Antalick and others 2010). A total of 7 of
264 these compounds were identified in the wines tested: Isoamyl acetate, ethyl

hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, diethyl succinate, and 2-phenylethanol acetate. Most individual compounds presented a highest concentration after 20 days, suffering modifications afterwards. It has been described that the hydrolysis and esterification of esters can be strongly affected by esterase activity. Esterases, which are released after alcoholic fermentation, are also associated to autolysis process (Bueno and others 2006).

In the case of the terpenes and norisoprenoids identified (linalol, α-terpineol, terpin-4-ol, β-damascenone, α-ionone and β-ionone) the highest concentration for most of them was found at 20 days of aging on lees, except for nerol and α −ionone, which concentration remains uniform during aging on lees. A previous study, has demonstrated the capacity of the present locally yeast strain (*S. cerevisiae* strain1), selected to carry out the alcoholic fermentation and aging on less, to influence the volatile profile of white wines produced with Albariño grape must when they are used as single inoculum, increasing the final concentration of terpenes and norisoprenoids in the final wine (Carrascosa and others 2012). Apparently, some mannoproteins correspond with this behaviour, at least for some compounds such as geraniol and linalool, which can be absorbed by specific mannoproteins released by this locally yeast strain (Juega and others 2012). Aditionally, the β- glucosidases released during autolysis could contribute to increase the concentration of these compounds in early stages of autolysis. These enzymes are able to break the glycoside bound of terpenes and norisprenoids, releasing the free aromatic form that consequently contribute to the characteristic aroma in wine (Liberatore

289 and others 2010).

290 Butyrolactone was the only lactone identified in the wines with a
291 concentration ranged between 4.16 mg/L and 6.51 mg/L. At these levels,
292 lactones can contribute to the floral and fruity character of the wines (Perez-
293 Serradilla and de Castro 2008). The concentration of octanoic acid, which was
294 the main fatty acid quantified in wines, was highest in wines aging on lees for
295 20 days, coexisting with the first evidences of autolysis. It has been reported
296 that the presence of lees can increase the concentration of fatty acids in wine,
297 due to desorption phenomena occurred after fermentation and caused by yeast
298 autolysis (Bueno and others, 2006; Bautista and others, 2007).

299 The results obtained from the sensorial analysis of the wines are
300 represented in the figure 1. It can be observed that the aging time on lees can
301 influence the sensorial evaluation of the wines. A penalizing system was used
302 in order to score wines, being the wine with lowest scores the best evaluated by
303 tasters. In accordance with results, aging on less seems to increase the
304 acceptability of the wines, being the wines aged 20 days (W20) the best
305 considered. The visual aspect was similar in all the wines, pointing out that the
306 differences observed were mainly due to variations in aroma and taste. The
307 wines were sorted according to their preference in the following way:
308 W20>W30>W40>W50>W10>WC. This distribution points out that between 10
309 and 20 days of aging on lees, wines acquire their better properties, which
310 decrease afterwards. These results were consistent with those obtained from
311 chemical analysis, which indicates that chemical composition influences wine
312 sensorial behaviour. There was an optimum point for aging on lees associated

313 with the best sensorial quality of the wine. The best scored wines (W20) were
314 also wines with the highest concentration of terpenes, norisoprenoids, esters
315 and acetates, confirming that these compounds are significantly involved in the
316 quality of the sensorial attributes of this style of wines. After 20 days of aging on
317 lees, breakdown process related to yeast autolysis affected the chemical
318 composition and sensorial properties of wines. Likewise, overall results
319 comparing with those obtained for control wine (WC), points out that any time of
320 the aging was favourable for the sensorial character of the wine. The autolysis
321 process can contributes to modify positively the sensorial character of the wines
322 trough the aging time on lees, but also it can negatively affect the sensorial
323 properties of the white wines, mainly after several months of aging on lees
324 (Bautista and others 2007).The optimum aging time on lees will depends on
325 several variables related with the winery process, but the yeast strain has a
326 pivotal role and this point should be considered when this procedure is used
327 (Bautista and others 2007; Carrascosa and others 2012).
328
329 **4. Conclusions**
330 Locally selected yeast strain used in this study to carry out the alcoholic
331 fermentation and aging on less in Albariño white wines produces the wines with
332 the best sensorial character after 20 days of aging on lees. This time is also
333 related with the highest concentration of some key aroma compounds and
334 mannoproteins. Further aging times decrease the sensorial quality of the wine,
335 also modifying its analytical composition in both, aroma compounds and
336 mannoproteins. Although similar results were obtained in two different vintages

337 (data not shown), the identification of an analytical marker capable to define an
338 optimal aging time on lees could be interesting from the practical point of view,
339 avoiding the putative interference of the multiple variables involved in the
340 fermentation process.

341

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347

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Figure captions:

Figure 1. Mean scores of sensory profile of the wines aged on lees (W10, W20, W30, W40 and W50) and the control wine without aging (CW).

446

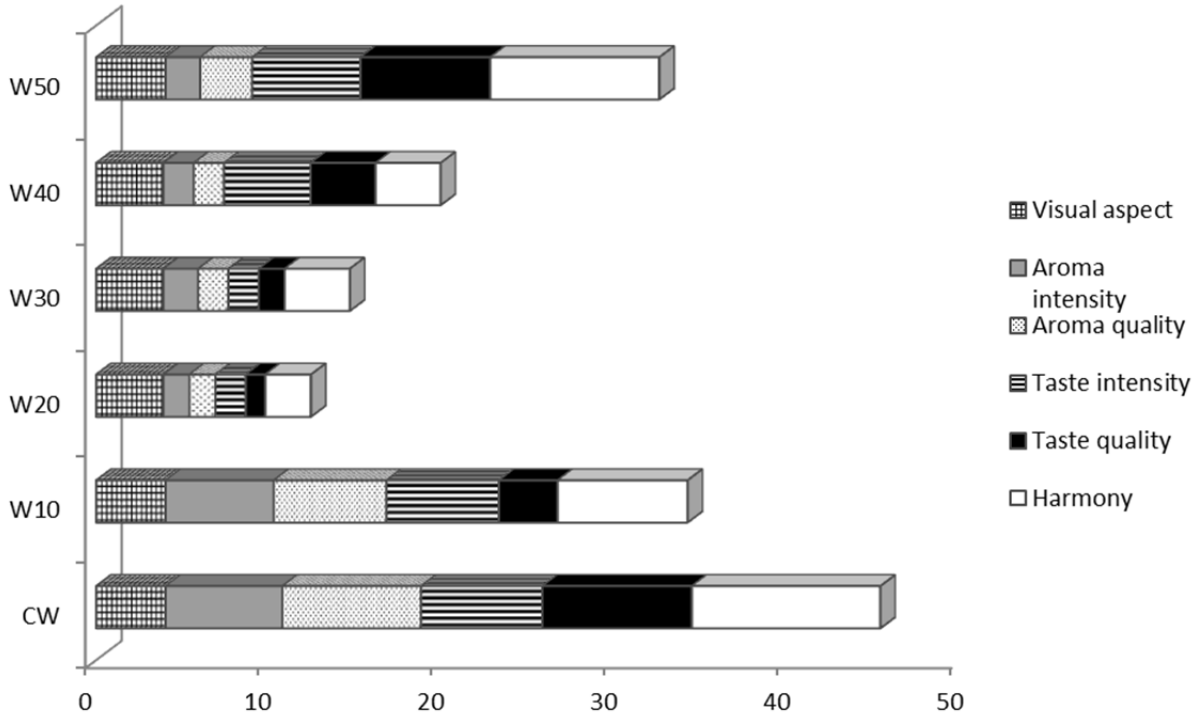

Figure 1. Mean scores of sensory profile of the wines aged on lees (W10, W20, W30, W40 and W50) and the control wine without aging (CW)

Table 1. Chemical parameters in Albariño wines, at the end of the fermentation (CW), and after 50 days of contact with wine lees (W50). They were not significant differences ($p < 0.05$) between samples.

Parameters	CW	W50
Ethanol (% v/v)	12.28 ± 0.06	12.37 ± 0.06
pH	3.44 ± 0.01	3.42 ± 0.02
Total acidity (g/L)	6.50 ± 0.00	6.52 ± 0.06
Volatile acidity (g/L)	0.22 ± 0.01	0.24 ± 0.01
Tartaric acid (g/L)	3.36 ± 0.12	3.38 ± 0.06
Malic acid (g/L)	3.38 ± 0.17	3.40 ± 0.15

Table 2. Concentration of proteins and polymeric mannose expressed in mg/L, in the wines aged on lees for different periods of time (0-50 days).

Aging time (days)	Proteins (mg/L)	Polymeric mannose (mg/L)
0	56.59b ± 2.39	173.41a ± 2.50
10	53.31b ± 0.74	163.31a ± 8.86
20	52.06b ± 1.58	175.73a ± 3.44
30	60.32a ± 5.13	169.76a ± 2.86
40	53.02b ± 1.39	122.69b ± 2.35
50	18.75c ± 1.92	162.75a ± 2.16

a, b, c,- Same letter in the same column indicates absence of significant differences ($p < 0.05$).

Table 3. Content of each aroma compounds identified in the wines in the control wine (CW) and in wines aged on lees at different times (10, 20, 30 40 and 50 days). Results are presented as mean ± SD

Concentration (mg/L)	CW	W10	W20	W30	W40	W50
Higher alcohols						
3-methyl-1-butanol	178.41a ± 5.92	152.50b ± 0.63	127.50c ± 2.47	181.59a ± 5.72	116.90c ± 10.85	161.05b ± 1.67
1- hexanol	3.33a ±0.11	3.06 a ±0.75	3.05a±0.06	3.93a±0.30	2.93a ±0.04	3.33a ±0.03
2-Phenylethanol	12.20a ±0.00	11.20a±0.02	10.90a±0.02a	14.60b±0.00	10.91a ±0.00	12.54a±0.00
Lactones						
Butyrolactone	5.70a±0.02	4.44bc±0.95	4.16b±0.01	6.51d±0.08	4.41bc ±0.01	5.19ac ±0.05
Esters and acetates						
Isoamyl acetate	2.03a ±0.15	1.80a±0.31	2.29a±0.12	2.05a±0.06	1.36b±0.06	2.05a±0.19
Ethyl hexanoate	0.80a±0.02	0.58b±0.09	0.86a±0.05	0.84a±0.03	0.56b±0.03	0.72a±0.00
Hexyl acetate	0.79a±0.00	0.70a±0.07	0.81a±0.03	0.74a±0.01	0.60b±0.02	0.84a±0.02
Ethyl octanoate	1.12a±0.01	0.57b±0.02	0.90c±0.01	1.03d±0.02	0.76e±0.00	0.64f±0.04
Ethyl decanoate	0.17a±0.00	0.08a±0.00	0.18a±0.00	0.16a±0.00	0.11a±0.00	0.18a±0.00
Diethyl succinate	3.65ab±0.10	3.84bd±0.00	4.86c±0.08	4.55c±0.07	4.16d±0.07	3.44a±0.09
2- phenylethanol acetate	0.22a±0.00	0.21a±0.02	0.24b±0.00	0.22a±0.00	0.18c±0.00	0.21a±0.00
Fatty acids						
Octanoic acid	2.27a±0.81	5.42b±0.36	6.76c±0.73	4.94b±0.38	4.67b±0.32	6.29c±0.26
Terpenes						
Linalool	0.041a±0.000	0.040a±0.000	0.051b±0.000	0.041a±0.001	0.038a±0.000	0.040a±0.001
Terpinen-4-ol	0.001a±0.000	0.030a±0.000	0.042b±0.000	0.030a±0.001	0.030a±0.000	0.031a±0.000
α-terpineol	0.020a±0.000	0.016a±0.000	0.021b±0.000	0.020a±0.000	0.015a±0.000	0.021b±0.000
Nerol	0.013a±0.000	0.009a±0.001	0.010a±0.000	0.009a±0.000	0.010a±0.000	0.009a±0.000
Eugenol	0.081a±0.000	0.083b±0.000	0.081a±0.000	0.082b±0.000	0.080a±0.000	0.083b±0.000
Norisoprenoids						
ß-Damascenone	0.002a±0.000	0.001a±0.000	0.003b±0.000	0.002a±0.000	0.002a ±0.000	0.002a±0.000
α-ionone	0.013a±0.000	0.013a±0.001	0.013a±0.000	0.013a±0.000	0.013a±0.001	0.013a±0.000
ß-ionone	0.040a±0.000	0.040a±0.001	0.042b±0.000	0.040a±0.001	0.040a±0.000	0.041b±0.000

a, b, c,- Same letter in the same row indicates absence of significant differences ($p < 0.05$).

Table 3. Content of each aroma compounds identified in the wines in the control wine (CW) and in wines aged on lees at different times (10, 20, 30 40 and 50 days). Results are presented as mean ± SD

Concentration (mg/L)	CW	W10	W20	W30	W40	W50
Higher alcohols						
3-methyl-1-butanol	178.41a ± 5.92	152.50b ± 0.63	127.50c ± 2.47	181.59a ± 5.72	116.90c ± 10.85	161.05b ± 1.67
1- hexanol	3.33a ±0.11	3.06 a ±0.75	3.05a±0.06	3.93a±0.30	2.93a ±0.04	3.33a ±0.03
2-Phenylethanol	12.20a ±0.00	11.20a±0.02	10.90a±0.02a	14.60b±0.00	10.91a ±0.00	12.54a±0.00
Lactones						
Butyrolactone	5.70a±0.02	4.44bc±0.95	4.16b±0.01	6.51d±0.08	4.41bc ±0.01	5.19ac ±0.05
Esters and acetates						
Isoamyl acetate	2.03a ±0.15	1.80a±0.31	2.29a±0.12	2.05a±0.06	1.36b±0.06	2.05a±0.19
Ethyl hexanoate	0.80a±0.02	0.58b±0.09	0.86a±0.05	0.84a±0.03	0.56b±0.03	0.72a±0.00
Hexyl acetate	0.79a±0.00	0.70a±0.07	0.81a±0.03	0.74a±0.01	0.60b±0.02	0.84a±0.02
Ethyl octanoate	1.12a±0.01	0.57b±0.02	0.90c±0.01	1.03d±0.02	0.76e±0.00	0.64f±0.04
Ethyl decanoate	0.17a±0.00	0.08a±0.00	0.18a±0.00	0.16a±0.00	0.11a±0.00	0.18a±0.00
Diethyl succinate	3.65ab±0.10	3.84bd±0.00	4.86c±0.08	4.55c±0.07	4.16d±0.07	3.44a±0.09
2- phenylethanol acetate	0.22a±0.00	0.21a±0.02	0.24b±0.00	0.22a±0.00	0.18c±0.00	0.21a±0.00
Fatty acids						
Octanoic acid	2.27a±0.81	5.42b±0.36	6.76c±0.73	4.94b±0.38	4.67b±0.32	6.29c±0.26
Terpenes						
Linalool	0.041a±0.000	0.040a±0.000	0.051b±0.000	0.041a±0.001	0.038a±0.000	0.040a±0.001
Terpinen-4-ol	0.001a±0.000	0.030a±0.000	0.042b±0.000	0.030a±0.001	0.030a±0.000	0.031a±0.000
α-terpineol	0.020a±0.000	0.016a±0.000	0.021b±0.000	0.020a±0.000	0.015a±0.000	0.021b±0.000
Nerol	0.013a±0.000	0.009a±0.001	0.010a±0.000	0.009a±0.000	0.010a±0.000	0.009a±0.000
Eugenol	0.081a±0.000	0.083b±0.000	0.081a±0.000	0.082b±0.000	0.080a±0.000	0.083b±0.000
Norisoprenoids						
ß-Damascenone	0.002a±0.000	0.001a±0.000	0.003b±0.000	0.002a±0.000	0.002a ±0.000	0.002a±0.000
α-ionone	0.013a±0.000	0.013a±0.001	0.013a±0.000	0.013a±0.000	0.013a±0.001	0.013a±0.000
ß-ionone	0.040a±0.000	0.040a±0.001	0.042b±0.000	0.040a±0.001	0.040a±0.000	0.041b±0.000

a, b, c,- Same letter in the same row indicates absence of significant differences ($p < 0.05$).