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**Volatile and fixed composition of sulphite-free white wines obtained  
after fermentation in the presence of chitosan**

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**RUNNING TITLE: Effects of chitosan on white grape must fermentation**

**Abstract**

Consumers are increasingly interested in healthier wines containing reduced levels or totally absent of sulphites. In the present investigation distinct fermentations of white musts either in the presence of chitosan or sulphur dioxide were carried out in order to compare the volatile and fixed composition of the wines produced, and evaluate the impact of chitosan as an alternative to sulphur dioxide.

Chitosan promoted a 24 h extended lag-phase and diminished the titratable acidity of wines by about 1 g L<sup>-1</sup> as a consequence of the absorption of tartaric and malic acids onto the polymer surface. The volatile composition of wines was analysed at the end of the alcoholic fermentation and then after 12 months of storage in glass bottle. Hexanoic, octanoic and decanoic acids were significantly higher in chitosan added wines, which further contained an increased amount of ethyl and acetate esters. Results demonstrated that, when added before the alcoholic fermentation, chitosan may affect both the acidic and volatile composition of wines, likely due to its polycationic behaviour and interaction with yeast wall constituents. This also suggests that attention to wine acidic balance should be paid before its use in other vinification steps such as must clarification or wine fining.

26    **Keywords:** chitosan; volatile compounds; sulphur dioxide; white wine; SPE-GC/MS

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28

## 29    **1. Introduction**

30    Sulphur dioxide is undoubtedly the most widely used preservative in oenology thanks to its  
31    antioxidant and antimicrobial properties, making it essential for the control of undesirable  
32    fermentations and oxidative spoilage in white and red wines.

33    In particular, for what concern oxidation, sulphite effectively counteracts both the phenolic and  
34    aromatic decay of wines (Bueno, Culleré, Cacho, & Ferreira, 2010; Waterhouse & Laurie, 2006),  
35    otherwise resulting in a decreased attractiveness of final products.

36    However, since seventies, the use of sulphite in foods is being questioned because of its  
37    allergenicity, which may cause asthma, dermatitis, urticaria, bronchoconstriction, or anaphylaxis in  
38    sensitive humans. (Vally, Misso, & Madan, 2009). Further, in the presence of specific contributory  
39    factors, sulphites have been linked to the onset of oncogenic processes. (EFSA, 2004; Lee et al.,  
40    2002).

41    Studies about efforts to replace sulphites in wines include physical, chemical or biological  
42    treatments. (Santos, Nunes, Saraiva, & Coimbra, 2012; Sonni, Cejudo Bastante, Chinnici, Natali, &  
43    Riponi, 2009). The prospective efficacy of some of those techniques has been claimed but further  
44    investigations are needed because a convincing alternative to sulphites is still waiting to be found.

45    Chitosan is the deacetylated product of chitin, a homopolymer of n-acetyl-glucosamine, extracted  
46    from shellfish wastes, insects or fungal sources. It has several applications in food and  
47    pharmaceutical industries, agriculture and water purification, due its features like metal chelation,  
48    lipid-lower activity, antimicrobial capacity, film-forming properties, multifaceted antioxidant and  
49    radical scavenging activities against hydroxyl and superoxide radicals (Dutta, Dutta, & Tripathi,  
50    2004; Yen, Yang, & Mau, 2008). Recently, the use of chitosan has been authorized in must and  
51    wine for microbial stabilization or metal and protein removal (EU Commission, 2011). In fermented  
52    beverages, chitosan can control the growth of *Brettanomyces spp.* yeasts and lactic bacteria, the  
53    both known to spoil wines. Intriguingly, some authors found that this polymer can also contrasts the  
54    browning onset and phenolic decay generated by both chemical and enzymatic oxidation in wines

55 and fruit juices (Abd & Niamah, 2012; Chinnici, Natali, & Riponi, 2014; Sapers, 1992; Spagna et  
56 al., 1996; Spagna, Barbagallo, & Pifferi, 2000), which makes chitosan a potential candidate for SO<sub>2</sub>  
57 replacement.

58 Chitosan can be used in several steps along the vinification process, from initial must clarification,  
59 to final wine stabilization just before bottling. Unprotected (e.g. sulphite-free ) white musts are  
60 prone to enzymatic oxidation or unwanted yeast and bacterial proliferation, which may drive to  
61 early browning development and sluggish fermentations (Bisson, 1999).

62 Interestingly, the addition of chitosan to free-run juices or during fermentation could acts as both an  
63 antioxidant and antimicrobial, in this way reproducing the two main functions that sulphites are  
64 called upon to play in the very first phases of winemaking. However, very little is known about the  
65 influence of the use of this polymer on musts, on fermentation kinetics and on the volatile  
66 composition of the obtained wines.

67 The aim of this work was, hence, to study the effects of the fermentative addition of chitosan on  
68 fixed and volatile compounds of sulphite-free white wines.

69 Chitosan was added just before yeast inoculation of white musts and resulting wines were evaluated  
70 after 12 months of storage in bottles and compared to wines treated with sulphur dioxide in the  
71 same step of the production process.

72

## 73 **2. Material and Methods**

### 74 *2.1 Chemicals*

75 Pure standards of volatile compounds, internal standard (2-octanol) and potassium metabisulphite  
76 were purchased from Sigma-Aldrich (Milano, Italy).

77 Dichloromethane and methanol (SupraSolv) were supplied by Merck (Darmstadt, Germany),  
78 absolute ethanol (ACS grade) was obtained from Scharlau Chemie (Sentmenat, Spain), and pure  
79 water was obtained from a Milli-Q purification system (Millipore, USA). LiChrolut EN resin for  
80 solid-phase extraction (SPE) prepacked in 200 mg cartridges (3 ml total volume) were purchased

from Merck (Darmstadt, Germany). Chitosan (low MW, 75-85% deacetylated, product #448869) was obtained from Sigma-Aldrich (Milano-Italy).

## 2.2 Microvinifications

Sulphite-free white musts were obtained at the experimental winery of the University of Bologna, from grapes cv. Trebbiano. Grapes were destemmed, crushed, pressed at 0.9 bars in a bladder press and cold-settled at 4°C for 24 h. The racked must was then filtered with Seitz-Supra EK1 filters from Seitz (Bad Kreuznach, Germany). The analytical parameters of the obtained must were as follow: sugars 205 g L<sup>-1</sup>; pH 3.05; titratable acidity 6.8 g L<sup>-1</sup>; total phenolics 107 mg L<sup>-1</sup>; O.D. 420 nm 0.146. Filtered must was placed in two litres laboratory glass fermentors, at room temperature, to start the fermentation. Trials were arranged in triplicate, before yeast inoculation, by adding potassium metabisulphite or chitosan to the musts at dosage of 60 mg L<sup>-1</sup> and 1 g L<sup>-1</sup> respectively. A further control fermentation (in triplicate) with no additions was also prepared. To avoid microbial contamination and oxygen entrance during fermentation, each fermentor was provided of a glass trap filled with 37% H<sub>2</sub>SO<sub>4</sub>. A *Saccharomyces cerevisiae* strain already characterized for its low SO<sub>2</sub> production (strain 1042 from University of Bologna – ESAVE collection) (Sonni et al., 2009) was inoculated after the rehydration of about  $1.5 \times 10^6$  CFU mL<sup>-1</sup> into 25 mL of sterilized must in 250-mL Erlenmeyer flasks plugged with cotton wool, incubated for 24 h. Fermentations were monitored by following the weight loss of samples. Once the weight loss stopped, chitosan and yeasts lees were left to settle down and the clarified wines were transferred by means of a peristaltic pump (VWR international, Milano, Italy) in 50 mL bottles, without headspace, and stored for 12 months at room temperature and in the darkness. Before the filling, air in the bottles was evacuated by a gentle nitrogen stream.

## 2.3 Oenological parameters

106 All the oenological parameters were determined according to OIV methods (International  
107 Organisation of Vine and Wine (OIV), 2015).

108 The pH was determined by using a pH-meter (Mettler Toledo, Spain). The alcoholic strength of  
109 wines was determined by using an oenochemical distilling unit (Gibertini, Italy). Total  
110 polyphenolics were spectrophotometrically determined (after wine filtration at 0.45  $\mu\text{m}$  with PTFE  
111 filters) at 280 nm using an Uvidec 610 spectrophotometer (Jasco, Japan) and results were expressed  
112 as  $\text{mg L}^{-1}$  of gallic acid (GAE). All the analyses were carried out in duplicate.

113

#### 114 *2.4 Organic acids, sugars and glycerol*

115 Quantification of organic acids, sugars and glycerol was conducted following the procedure  
116 described by Chinnici et al. (Chinnici, Spinabelli, Riponi, & Amati, 2005).

117 The HPLC used was a Jasco apparatus (Tokyo, Japan) equipped with a binary pump (PU 1580), a  
118 20  $\mu\text{L}$  loop, a Rheodyne valve (Cotati, CA, USA), a photodiode detector (PU MD 910; Tokyo,  
119 Japan), and a column oven (Hengood Mid Glamorgan, UK). The column was a Bio-Rad Aminex  
120 HPX 87H (300 mm $\times$ 7.8 mm), thermostatted at 35  $^{\circ}\text{C}$ . Isocratic elution was carried out with 0.005 N  
121 phosphoric acid at flow 0.4 mL/min. All the analyses were carried out in duplicate.

122

#### 123 *2.5 Wine volatile compounds*

124 Volatile compounds were extracted according to the method described and validated by Lopez et al.  
125 (López, Aznar, Cacho, & Ferreira, 2002). A 20 ml wine sample was added of 100  $\mu\text{L}$  of a 2-octanol  
126 solution at 500  $\text{mgL}^{-1}$  as internal standard and deposited on an Lichrolut EN cartridge previously  
127 activated. Analytes were eluted with 5 mL of dichloromethane, and concentrated to a final volume  
128 of 200  $\mu\text{L}$  under a stream of pure nitrogen ( $\text{N}_2$ ), prior to GC-MS analysis.

129 The Trace GC ultra apparatus coupled with a Trace DSQ mass selective detector (Thermo Fisher  
130 Scientific, Milan, Italy) was equipped with a fused silica capillary column Stabilwax DA (Restek,



131 Bellefonte, PA, USA; 30 m, 0.25mm i.d., and 0.25  $\mu$ m film thickness). The carrier gas was He at a  
132 constant flow of 1.0 mL/min.

133 The GC programmed temperature was: 45 °C (held for 3 min) to 100 °C (held for 1 min) at 3  
134 °C/min, then to 240 °C (held for 10 min) at 5 °C/min. Injection was performed at 250 °C in splitless  
135 mode and the injection volume was 1  $\mu$ L. Detection was carried out by electron ionization (EI)  
136 mass spectrometry in full scan mode, using ionization energy of 70 eV. Transfer line interface was  
137 set at 220 °C and ion source at 260 °C. Mass acquisition range was  $m/z$  30-400 and the scanning  
138 rate 1 scan  $s^{-1}$ .

139 Compounds were identified by a triple criterion: i) by comparing their mass spectra and retention  
140 time with those of authentic standards, ii) compounds lacking of standards were identified after  
141 matching their respective mass spectra with those present in the commercial libraries NIST 08 and  
142 Wiley 7, iii) matching the linear retention index (LRI) obtained under our conditions, with already  
143 published LRI on comparable polar columns (Table 1).

144 Quantification of compounds was carried out via the respective total ion current peak areas after  
145 normalization with the area of the internal standard. Calibration curves were obtained by duplicate  
146 injections of standard solutions, subjected to the above cited extraction procedure, containing a  
147 mixture of commercial standard compounds at concentrations between 0.01 to 200 mg  $L^{-1}$ , and  
148 internal standard at the same concentration as in the samples. The calibration equations for each  
149 compound were obtained by plotting the peak area response ratio (target compound/internal  
150 standard) versus the corresponding concentration.

151 For compounds lacking reference standards, the calibration curves of standards with similar  
152 chemical structure were used.

153 Analyses were done in duplicate and data were collected by means of Xcalibur software (Thermo  
154 Fisher Scientific, Milano, Italy)

155

156 *2.6 Statistical analysis*

157 Statistical analysis of the entire dataset was performed using the XLSTAT Software package  
158 (Version 2013.2, France). One-way analysis of variance (ANOVA) followed by a post hoc  
159 comparison (Tukey's HSD test) and Principal Component Analysis (PCA) were carried out.

160

### 161 **3. Results and discussion**

#### 162 *3.1 Fermentation and oenological parameters*

163 The evolution of fermentation was monitored checking the weight loss of fermentors. All the  
164 fermentations were completed in 10 days, even if the presence of chitosan resulted in initially  
165 slower fermentation rates (**Figure 1**). This was somehow expected since chitosan has already been  
166 reported to interfere variably on *Saccharomyces* ssp. growth kinetics (Allan & Hadwiger, 1979;  
167 Roller & Covill, 1999). In particular, Roller and Covill (Roller & Covill, 1999) found that the  
168 effects on *Saccharomyces* spp. cells growth of 0.4 g L<sup>-1</sup> soluble chitosan spanned from complete  
169 inactivation to a three days delayed lag phase, depending on the strain considered. These differences  
170 in fungi responses have been suggested to be linked to the polyunsaturated free fatty acids content  
171 of cells plasma membrane. In sensitive fungi, such as *Neurospora crassa* and *Saccharomyces*  
172 *cerevisiae*, the high content of polyunsaturated free fatty acids enhances membrane fluidity and  
173 permeabilization leading to augmented intracellular oxidative stress because of the chitosan  
174 entrance in the plasma (Lopez-Moya & Lopez-Llorca, 2016; Zakrzewska et al., 2007; Zakrzewska,  
175 Boorsma, Brul, Hellingwerf, & Klis, 2005). In our case, the fermentation of samples added with 1 g  
176 L<sup>-1</sup> of chitosan showed a 24 hours extended lag phase but, from day 8 and thereafter, their weight  
177 loss was similar to SO<sub>2</sub> or control samples (figure 1). This suggests that the strain used in this  
178 experiment was able to resume growth to levels comparable to those observed in untreated musts.  
179 At the end of fermentation, samples treated with chitosan had a decreased content of organic acids,  
180 with consequent higher pH values (augmented by 0.08 units) and lower titratable acidity (lessened  
181 by 1.1 g L<sup>-1</sup>) (**Table 2**). In particular the grape-derived tartaric and malic acids were reduced by  
182 about 0.30 g L<sup>-1</sup> and 0.50 g L<sup>-1</sup> respectively while, in the same wines, succinic acid amount was

0.25 g L<sup>-1</sup> lesser. The acid binding properties of chitosan had been already claimed and proposed for the treatment of coffee beverages, vegetable or fruit juices (Imeri & Knorr, 1988; Scheruhn, Wille, & Knorr, 1999). This feature is due to the electrostatic interaction between the positively charged amino groups of glucosamine and the anions coming from dissociated acids, whose pKa and hydroxyl content may also play a role (Mitani, Yamashita, Okumura, & Ishii, 1995).

Hence, this would be the reason for the diminution in native organic acids during the 10 days of fermentation. Succinic acid, however, does not come from grapes being produced by yeasts during alcoholic fermentation. Its low amount in KT wines could be the result of reduced fermentative excretion and/or the adsorption by chitosan. It still remains unclear whether one or both the phenomena occurred in our samples.

Alcohol content, volatile acidity and total phenolics index were not affected by the treatments while, as expected, the bleaching and antioxidant capacities of sulphite resulted in lighter yellow nuances of final wines if compared with control sample (see tab. 2, at O.D. 420 nm parameter). In this respect, Kt and SO<sub>2</sub> samples were not significantly different in color, suggesting that chitosan may have controlled the browning development, as already reported by other authors (Chinnici et al., 2014; Spagna et al., 2000).

### 3.2 Volatile compositions of wines

A list of volatile compounds found in wines before or after storage is reported in table 1. A total of 74 volatiles were elucidated while 12 further compounds lacking of standard and published LRI, were tentatively identified based on their mass spectrum (these compounds are flagged with “MS” in the last column of Table 1).

Table 3 reports the amounts of the most significant compounds found in wines at the beginning and at the end of bottle storage, grouped as chemical families, which will be separately discussed.

#### 3.2.1 Fatty acids

209 Our results indicate that treatments with chitosan enhanced the synthesis of three of the main  
210 medium chain fatty acids (MCFA), hexanoic, octanoic and decanoic acid (Table 3) that, according  
211 to sensory studies, can contribute to the aroma of white wines (Ferreira & Felipe, 2011). During  
212 winemaking, a mixture of fatty acids are produced, normally classified as short chain (C2-C4),  
213 medium chain (C6-C10), long chain (C12-C18) and branched-chain fatty acids. Metabolism of  
214 saturated fatty acids produces straight-chain fatty acids (C4-C12) as intermediate products.  
215 (Lambrechts & Pretorius, 2000). The final products, mainly C16 and C18 are incorporated into  
216 phospholipids, the backbone of cell membranes. The increased contents of MCFA in wines  
217 fermented with chitosan may be due to an augmented permeability of yeast membranes caused by  
218 the polysaccharide. As already commented, in fact, at wine pH most of the glucosamine units of  
219 chitosan are positively charged due to the protonation of amino groups which allows them to  
220 interact with the negatively charged components of cell surface (Zakrzewska et al., 2005).

221 This electrostatic interaction induces changes in the properties of membrane thus modifying, among  
222 other, the cell permeability (Hadwiger, Kendra, Fristensky, & Wagoner, 1986).

223 Evidences have been given that growing limiting factors, such as an increased membrane  
224 permeability, may cause an augmentation in the production of MCFA by the fatty acid synthase  
225 complex (Wakil, Stoops, & Joshi, 1983).

226 These C6 to C10 fatty acids at concentrations  $< 10 \text{ mg L}^{-1}$  impart mild and complex aroma to wine.  
227 However, at levels above  $20 \text{ mg L}^{-1}$ , their impact on wines becomes negative (Shinohara, 1985). At  
228 the end of fermentation, MCFA concentration in all the samples did not exceed that limit, as  
229 reported in table 3.

230 Fermentation conducted in the presence of chitosan showed a decrease in isobutyric and pentanoic  
231 acid amounts. The former acid is not produced by the fatty acid synthetic pathway, being derived  
232 from oxidation of the aldehydes formed during amino acid metabolism (Ugliano & Henschke,  
233 2009).

234 Unpaired acids though, are derived from propionyl-CoA likely formed via  $\alpha$ -ketobutyric acid, a  
235 metabolite in threonine degradation (Guitart, Orte, Ferreira, Peña, & Cacho, 1999). Their reduced  
236 contents in KT wines could be, hence, apparently related to a modification of the amino acid  
237 metabolism in yeasts.

238 Fatty acids in wines did not change substantially during the 12 months of bottle storage, confirming  
239 the relative stability of this class of compounds when stored at room temperature (Garde-Cerdán,  
240 Marsellés-Fontanet, Arias-Gil, Ancín-Azpilicueta, & Martín-Belloso, 2008).

241

### 242 3.2.2 Esters

243 Volatile esters produced during alcoholic fermentation are of great interest, because of their key  
244 role in the sensorial quality of wines, being responsible of fruitiness, candy and perfume-like aroma  
245 but also of negative notes like “glue-like” aroma (Lambrechts & Pretorius, 2000; Saerens et al.,  
246 2008).

247 Chitosan seemed to enhance the esters production, particularly isoamyl acetate, phenylethyl acetate  
248 and medium chain fatty acids (MCFA) ethyl esters, ethyl hexanoate, ethyl octanoate, ethyl  
249 decanoate and ethyl 3-hydroxybutanoate (Table 3). For ethyl esters, this done is in direct  
250 relationship with MCFA amounts in respective wines as the latter are the substrates and limiting  
251 factors for the syntheses of the former (Saerens et al., 2008).

252 Acetate esters are formed through the condensation of higher alcohols with acetyl-CoA catalysed in  
253 the cell by alcohol acetyltransferase (ATF) enzymes (Mason & Dufour, 2000). However, in KT  
254 samples, results did not show any relationship between higher alcohols and acetate esters  
255 production (table 3). The reason for the higher amounts of acetates in KT wines is, thus, not clear  
256 but it is worth mentioning that ATF enzymes are regulated by the levels of unsaturated fatty acids  
257 (UFA) in the medium and that low concentrations in UFA correspond to higher quantities of acetate  
258 esters (Saerens, Delvaux, Verstrepen, & Thevelein, 2010).

259 After alcoholic fermentation, a lesser amount of ethyl lactate, ethyl malate, mono and diethyl  
260 succinate was found in KT wines. These compounds comes from the esterification of the respective  
261 organic acids, whose lower amount in chitosan-treated wines (table 2) may well justify our results.  
262 The presence of sulphites led to enhanced production of ethyl-4-hydroxybutanoate, which could be  
263 directly related to higher amounts of  $\gamma$ -butyrolactone in  $\text{SO}_2$  added wines (Carrau et al., 2008)  
264 As expected, during storage, acetate esters drastically decreased while ethyl esters increased to  
265 various extents (table 3) in accordance with previous findings (Saerens et al., 2008).  
266 In particular, ethyl esters of organic acids significantly raised in concentration after 12 months of  
267 storage, and the presence of  $\text{SO}_2$  further contributed in promoting their generation as already stated  
268 by other authors (Garde-Cerdán et al., 2008)

269

### 270 3.2.3 Alcohols

271 Together with acids and esters, alcohols are a further important class of yeast-derived volatile  
272 compounds in wines, since they play a considerable role in wine aroma (Nykänen, 1986). At the  
273 end of fermentation, there were no significant differences in total alcohols content among samples  
274 even if differences for some volatiles were found.

275 Isobutyl alcohol and 3-methyl-1-butanol amounts were higher in  $\text{SO}_2$  added wines, confirming  
276 previous results that postulated that the presence of  $\text{SO}_2$  during fermentation favours a prompt  
277 consumption of amino acids (Herraiz, Martin-Alvarez, Reglero, Herraiz, & Cabezudo, 1989; Sonni  
278 et al., 2009).

279 Quite surprisingly, however, other alcohols deriving from amino acids, such as 2-phenylethanol and  
280 4-hydroxybenzenethanol, were not affected by the presence of  $\text{SO}_2$ , the reason for this behaviour  
281 remaining unclear.

282 Sulphites affected the amount of 3-ethoxy-1-propanol which, as already consistently reported  
283 (Herraiz et al., 1989; Sonni et al., 2009), is produced in lower quantities in the presence of  $\text{SO}_2$ .

284 For what concern chitosan, its pre-fermentative addition seemed not to have a considerable  
285 influence on alcohols contents, except for the lower levels of isobutyl alcohol and 3-methylthio-1-  
286 propanol, the both deriving from amino acid metabolism. This finding may be related to a reduced  
287 amino acid availability in musts due to the protein binding features of chitosan (Chatterjee,  
288 Chatterjee, Chatterjee, & Guha, 2004).

289 After 12 months of storage, total amount of alcohols in wines increased mostly due to 3-methyl-1-  
290 butanol and 2-phenetyl alcohol, without notable differences among samples. Most of the volatile  
291 compounds remained unchanged in quantity except 3-methylthio-1-propanol, benzyl alcohol and 4-  
292 hydroxy benzenethanol that decreased similarly to what has been already observed in previous  
293 works (Garde-Cerdán et al., 2008)

294

#### 295 3.2.4 Other compounds

296 In wine, acetylation occurring between acetaldehyde and glycerol gives raise to heterocyclic  
297 compounds such as 1,3-dioxane and 1,3-dioxolane isomers. These compounds, with herbaceous or  
298 green olfactory nuances, have been reported to increase in content during wine conservation and  
299 aging and have been proposed as markers of Madeira wine ages (Câmara, Marques, Alves, & Silva  
300 Ferreira, 2003). Results showed that the amounts of 1,3-dioxanes and 1,3-dioxolane increased  
301 drastically during the conservation in bottle but, in sulphite added wines this phenomenon was  
302 observed to a significantly lesser extent. This is due to the quenching of acetaldehyde by SO<sub>2</sub> that  
303 prevent the reaction with glycerol (Da Silva Ferreira, Barbe, & Bertrand, 2002).

304 Furans are another class of heterocyclic compounds in wine. They mainly originate from  
305 monosaccharides that, in acidic medium, degrade via enolization and subsequent dehydration or  
306 react with amino acids following the Maillard chemistry (Belitz, Grosch, & Schieberle, 2009).

307 Their presence usually increases with time and is related to sugars level in wine. Table 3 confirms  
308 the general augmentation of furanic compounds during storage, in particular for furfural, ethyl 5-

309 oxotetrahydrofuran-2-furancarboxylate and hydroxymethylfurfural that, complessly, tended to be  
310 higher in SO<sub>2</sub> samples.

311

### 312 *3.3 PCA Analysis of volatile compounds*

313 Figure 2 shows the results of the application of PCA (Principal Component Analysis) to the entire  
314 dataset of wines volatile compounds. In that figure, for the sake of clarity, only the variables with  
315 the highest contribution to the total variance have been plotted. The first component, which explains  
316 51.47% of variance, clearly discriminates the samples based on the storage time. On this  
317 component, samples at bottling are located in the left quadrants, where the highest variance is due  
318 to N-acetyltyramine, isoamyl acetate and 2-hexanol. On the right side, the wines stored for 12  
319 months are distinguishable for their content in ethyl esters of succinic, malic and lactic acids.  
320 Principal component 2 (31.29% of explained variance) produced a clear separation between KT and  
321 the others samples (Control and SO<sub>2</sub>) due to the contribution of hexanoic and octanoic acids and  
322 ethyl hexanoate, higher in KT wines, and  $\gamma$ -butyrolactone, isobutyric and pentanoic acids which  
323 characterized all the samples not containing chitosan.

324

## 325 **4. Conclusions**

326 The overall results demonstrated chitosan may affect the fermentation and composition of sulphite-  
327 free musts. When present all along the fermentation, chitosan may interacts with yeasts, delaying  
328 the lag phase, and with organic acids, producing a decrease in total acidity. This fact should be  
329 taken into consideration even in the case of its use for musts clarification or during the stabilization  
330 steps of wines.

331 Concerning the volatile compounds, KT wines had higher concentrations of medium chain fatty  
332 acids and related ethyl esters, probably due to the alteration of cell permeability and subsequent  
333 perturbation of the fatty acids synthase complex.



334 Except some compounds deriving from amino acids metabolism, alcohols were less affected by the  
335 addition of the polysaccharide. Furthermore, differences in volatile composition were maintained  
336 over a 12 months storage time. Further investigations are currently being carried out at a semi-  
337 industrial scale, which may permit, together with the phenolic characterization, the sensory  
338 evaluation of sulphite-free wines fermented in the presence of chitosan.

339

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342

343 **References**

- 344 Abd, A. J., & Niamah, A. K. (2012). Effect of chitosan on apple juice quality. *International Journal*  
345 *of Agricultural and Food Science*, 2, 153–157.
- 346 Allan, C. R., & Hadwiger, L. A. (1979). The fungicidal effect of chitosan on fungi of varying cell  
347 wall composition. *Experimental Mycology*, 3, 285–287.
- 348 Belitz, H. D., Grosch, W., & Schieberle, P. (2009). Carbohydrates. In *Food Chemistry* (pp. 248–  
349 339). Berlin, Heidelberg: Springer Berlin Heidelberg.
- 350 Bisson, L. F. (1999). Stuck and sluggish Fermentations. *American Journal of Enology and*  
351 *Viticulture*, 50, 107–119.
- 352 Bueno, M., Culleré, L., Cacho, J., & Ferreira, V. (2010). Chemical and sensory characterization of  
353 oxidative behavior in different wines. *Food Research International*, 43, 1423–1428.
- 354 Câmara, J. S., Marques, J. C., Alves, A., & Silva Ferreira, A. C. (2003). Heterocyclic acetals in  
355 Madeira wines. *Analytical and Bioanalytical Chemistry*, 375, 1221–1224.
- 356 Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A., & Dellacassa, E. (2008).  
357 Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts:  
358 Effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Research*, 8, 1196–  
359 1207.
- 360 Chatterjee, S., Chatterjee, S., Chatterjee, B. P., & Guha, A. K. (2004). Clarification of fruit juice  
361 with chitosan. *Process Biochemistry*, 39, 2229–2232.
- 362 Chinnici, F., Natali, N., & Riponi, C. (2014). Efficacy of Chitosan in inhibiting the oxidation of (+)-  
363 Catechin in white wine model solutions. *Journal of Agricultural and Food Chemistry*, 62,  
364 9868–9875.
- 365 Chinnici, F., Spinabelli, U., Riponi, C., & Amati, A. (2005). Optimization of the determination of  
366 organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. *Journal of*  
367 *Food Composition and Analysis*, 18(2–3), 121–130.
- 368 Da Silva Ferreira, A. C., Barbe, J. C., & Bertrand, A. (2002). Heterocyclic acetals from glycerol and

acetaldehyde in port wines: Evolution with aging. *Journal of Agricultural and Food Chemistry*, 50, 2560–2564.

Dutta, P. K., Dutta, J., & Tripathi, V. S. (2004). Chitin and chitosan : Chemistry , properties and applications. *Journal of Scientific & Industrial Research*, 63, 20–31.

EFSA. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. (2004). <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2004.32/epdf> accessed on 02 october 2017.

EU Commission. (2011). Regulation 53/2011 of 21 January 2011. *Official Journal of the European Union*. 2011., L19/1-L19/6.

Ferreira, V., & Felipe, J. (2011). Flavor of Wine. In Henryk Jelen (Ed.), *Food Flavors. Chemical, Sensory and Technological Properties* (pp. 269–300). Boca Raton, FL (USA): CRC Press 2011.

Garde-Cerdán, T., Marsellés-Fontanet, A. R., Arias-Gil, M., Ancín-Azpilicueta, C., & Martín-Belloso, O. (2008). Effect of storage conditions on the volatile composition of wines obtained from must stabilized by PEF during ageing without SO<sub>2</sub>. *Innovative Food Science and Emerging Technologies*, 9, 469–476.

Guitart, A., Orte, P. H., Ferreira, V., Peña, C., & Cacho, J. (1999). Some observations about the correlation between the amino acid Content of musts and wines of the Chardonnay variety and their fermentation aromas. *American Journal of Enology and Viticulture*, 50, 253-258.

Hadwiger, L. A., Kendra, D. F., Fristensky, B. W., & Wagoner, W. (1986). Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In *Chitin in Nature and Technology* (pp. 209–214). Boston, MA: Springer US.

Herbst-Johnstone, M., Nicolau, L., & Kilmartin, P. A. (2011). Stability of varietal thiols in commercial Sauvignon blanc wines. *American Journal of Enology and Viticulture*, 62, 495–502.

395 Herraiz, T., Martin-Alvarez, P. J., Reglero, G., Herraiz, M., & Cabezudo, M. D. (1989). Differences  
 396 between wines fermented with and without sulphur dioxide using various selected yeasts.  
 397 *Journal of the Science of Food and Agriculture*, 49, 249–258.

398 Imeri, A., & Knorr, D. (1988). Effects of chitosan on yield and compositional data of carrot and  
 399 apple juice. *Journal of Food Science*, 53, 1707–1709.

400 International Organisation of Vine and Wine (OIV). (2015). Compendium of international methods  
 401 of wine and must analysis. Paris (France). [http://www.oiv.int/en/technical-standards-and-](http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts/)  
 402 [documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-](http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts/)  
 403 [and-musts/](http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts/) Accessed on 3 october 2017.

404 Lambrechts, M. G., & Pretorius, I. S. (2000). Yeast and its importance to wine aroma - A Review.  
 405 *South African Journal of Enology and Viticulture*, 21, 97–129.

406 Lee, W. J., Teschke, K., Kauppinen, T., Andersen, A., Jäppinen, P., Szadkowska-Stanczyk, I., ...  
 407 Boffetta, P. (2002). Mortality from lung cancer in workers exposed to sulfur dioxide in the  
 408 pulp and paper industry. *Environmental Health Perspectives*, 110, 991–5.

409 López, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile  
 410 compounds in wine by solid-phase extraction and gas chromatography with mass  
 411 spectrometric detection. *Journal of Chromatography A*, 966, 167–177.

412 Lopez-Moya, F., & Lopez-Llorca, L. (2016). Omics for investigating chitosan as an antifungal and  
 413 gene modulator. *Journal of Fungi*, 2, 2-11.

414 Mason, A. B., & Dufour, J. P. (2000). Alcohol acetyltransferases and the significance of ester  
 415 synthesis in yeast. *Yeast (Chichester, England)*, 16, 1287–98.

416 Mitani, T., Yamashita, T., Okumura, C., & Ishii, H. (1995). Adsorption of benzoic acid and its  
 417 derivatives to swollen chitosan beads. *Bioscience, Biotechnology, and Biochemistry*, 59, 927–  
 418 928.

419 Nykänen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic  
 420 beverages. *American Journal of Enology and Viticulture*, 37, 84–96.

421 Roller, S., & Covill, N. (1999). The antifungal properties of chitosan in laboratory media and apple  
 422 juice. *International Journal of Food Microbiology*, 47, 67–77.

423 Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and  
 424 biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology*, 3,  
 425 165–177.

426 Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R.  
 427 (2008). Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during  
 428 fermentation. *Applied and Environmental Microbiology*, 74, 454–461.

429 Santos, M. M. C., Nunes, C., Saraiva, J. A., & Coimbra, M. A. (2012). Chemical and physical  
 430 methodologies for the replacement/reduction of sulfur dioxide use during winemaking: review  
 431 of their potentialities and limitations. *European Food Research and Technology*, 234, 1–12.

432 Sapers, G. M. (1992). Chitosan enhances control of enzymatic browning in apple and pear juice by  
 433 filtration. *Journal of Food Science*, 57, 1192–1193.

434 Scheruhn, E., Wille, P., & Knorr, D. (1999). Studies of acid binding properties of chitosan in coffee  
 435 beverages. *Nahrung - Food*, 43, 100–104.

436 Shinohara, T. (1985). Gas Chromatographic analysis of volatile fatty acids in wines. *Agricultural*  
 437 *and Biological Chemistry*, 49, 2211–2212.

438 Sonni, F., Cejudo Bastante, M. J., Chinnici, F., Natali, N., & Riponi, C. (2009). Replacement of  
 439 sulfur dioxide by lysozyme and oenological tannins during fermentation: influence on volatile  
 440 composition of white wines. *Journal of the Science of Food and Agriculture*, 89, 688–696.

441 Spagna, G., Barbagallo, R. N., & Pifferi, P. G. (2000). Fining treatments of white wines by means  
 442 of polymeric adjuvants for their stabilization against browning. *Journal of Agricultural and*  
 443 *Food Chemistry*, 48, 4619–4627.

444 Spagna, G., Pifferi, P. G., Rangoni, C., Mattivi, F., Nicolini, G., & Palmonari, R. (1996). The  
 445 stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food*  
 446 *Research International*, 29, 241–248.

447 Ugliano, M., & Henschke, P. A. (2009). *Wine Chemistry and Biochemistry*. (M. V. Moreno-Arribas  
 448 & M. C. Polo, Eds.), *Wine Chemistry and Biochemistry* (1st ed.). New York, NY: Springer  
 449 New York.

450 Vally, H., Misso, N. L. A., & Madan, V. (2009). Clinical effects of sulphite additives. *Clinical &*  
 451 *Experimental Allergy*, 39, 1643–1651.

452 Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983). Fatty acid synthesis and its regulation. *Annual*  
 453 *Review of Biochemistry*, 52, 537–579.

454 Waterhouse, A. L., & Laurie, V. F. (2006). Oxidation of wine phenolics: A critical evaluation and  
 455 hypotheses. *American Journal of Enology and Viticulture*, 57, 306–313.

456 Yen, M.-T., Yang, J.-H., & Mau, J.-L. (2008). Antioxidant properties of chitosan from crab shells.  
 457 *Carbohydrate Polymers*, 74, 840–844.

458 Zakrzewska, A., Boorsma, A., Brul, S., Hellingwerf, K. J., & Klis, F. M. (2005). Transcriptional  
 459 response of *Saccharomyces cerevisiae* to the plasma membrane-perturbing compound chitosan.  
 460 *Eukaryotic Cell*, 4, 703–15.

461 Zakrzewska, A., Boorsma, A., Delneri, D., Brul, S., Oliver, S. G., & Klis, F. M. (2007). Cellular  
 462 processes and pathways that protect *Saccharomyces cerevisiae* cells against the plasma  
 463 membrane-perturbing compound chitosan. *Eukaryotic Cell*, 6, 600–608.

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## 466 Figure Captions

467

468 Figure 1: Weight loss of fermentors during the fermentation

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470 Figure 2: Principal component analysis. Plot of the samples scores and the variables loadings in the  
471 plane defined by the first two principal components, at bottling (, gray labels) and after 12 months  
472 of storage (black labels). Samples labels:  $\triangle$  Control;  $\circ$  SO<sub>2</sub>;  $\square$  KT;

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Table 1

tR (min)	Compound	LRI	Identification <sup>a</sup>
5.04	ethyl 2-methylbutyrate	1078	Std, MS, LRI
5.39	ethyl isovalerate	1090	Std, MS, LRI
5.78	isobutyl alcohol	1104	Std, MS, LRI
6.74	isoamyl acetate	1127	Std, MS, LRI
7.19	n-butanol	1138	Std, MS, LRI
9.44	3-methyl-1-butanol	1194	Std, MS, LRI
10.28	ethyl n-caproate	1221	Std, MS, LRI
11.63	ethyl pyruvate	1265	Std, MS, LRI
12.00	methyl lactate	1281	MS, LRI
12.82	2-hexanol	1304	Std, MS, LRI
13.03	4-methyl-1-pentanol	1309	Std, MS, LRI
13.44	3-methyl-2-buten-1-ol	1319	Std, MS, LRI
13.51	3-methyl-1-pentanol	1321	Std, MS, LRI
14.19	ethyl lactate	1339	Std, MS, LRI
14.52	n-hexanol	1348	Std, MS, LRI
14.84	4-hydroxy-4-methyl-2-pentanone	1357	Std, MS, LRI
14.92	4-methyl-1,3-oxathiolane	1359	MS
15.35	3-ethoxy-1-propanol	1370	Std, MS, LRI
15.72	3-hexen-1-ol	1380	Std, MS, LRI
16.14	nonanal	1391	Std, MS, LRI
17.30	ethyl 2-hydroxy-isovalerate	1421	Std, MS, LRI
17.74	ethyl octanoate	1432	Std, MS, LRI
18.05	5-methyltetrahydro-2-furanyl-methanol	1440	MS, LRI
18.11	2-ethyl-2-methylbutanoic acid	1441	MS
19.03	Furfural	1464	Std, MS, LRI
20.19	cis-5-hydroxy-2-methyl-1,3-dioxane	1493	MS, LRI
20.36	2-mercaptoethanol	1498	Std, MS, LRI
21.05	ethyl 3-hydroxybutyrate	1514	Std, MS, LRI
21.36	2-methyl-3-thiolanone	1522	MS, LRI
21.47	2-(methylthio)ethanol	1524	Std, MS, LRI
22.89	1,3-Dioxolan-2-one	1558	MS
23.07	isobutyric acid	1563	Std, MS, LRI
23.80	propylene glycol	1580	Std, MS, LRI
23.93	ethyl 3-hydroxypropionate	1583	MS
24.35	trans-4-hydroxymethyl-2-methyl-1,3 dioxolane	1593	MS
24.94	γ-butyrolactone	1616	Std, MS, LRI
25.08	n-butyric acid	1623	Std, MS, LRI
25.23	ethyl decanoate	1631	Std, MS, LRI
25.35	N-ethyl acetamide	1637	MS, LRI
26.03	2-furanmethanol (furfuryl alcohol)	1672	Std, MS, LRI
26.25	pentanoic acid	1683	MS, LRI
26.44	diethyl succinate	1693	Std, MS, LRI
27.48	3-methylthio-1-propanol	1733	Std, MS, LRI
28.08	4-hydroxy-2-butanone	1754	MS
28.99	2-hydroxy-methyl ester benzoic acid = methyl salicylate	1787	MS, LRI
29.19	2,7-dimethyl-4,5 octandiol	1794	MS
29.24	ethylphenyl acetate	1796	Std, MS, LRI
29.79	ethyl 4-hydroxybutanoate	1822	Std, MS, LRI
30.01	2-phenylethyl-acetate	1833	Std, MS, LRI
30.11	trans-5-hydroxy-2-methyl-1,3-dioxane	1837	MS, LRI
30.16	4-methyl-2-pentanoic acid	1840	MS
30.76	hexanoic acid	1869	Std, MS, LRI
31.36	N-(3-methylbutyl)acetamide	1899	MS, LRI
31.45	benzyl alcohol	1902	Std, MS, LRI
31.98	ethyl 3-methylbutyl butanedioate	1921	MS, LRI
32.33	2-phenylethanol	1933	Std, MS, LRI
32.86	cinnamyl nitrile	1951	MS
33.35	benzyl oxytridecanoic acid	1967	MS, LRI
34.07	2H-piran-2,6 (3H)-dione	1992	MS
34.63	1H-Pyrrole-2-carboxaldehyde	2017	Std, MS, LRI
34.85	pantolactone	2029	Std, MS, LRI
34.97	diethyl malate	2035	Std, MS, LRI
35.32	octanoic acid	2053	Std, MS, LRI
37.30	N-acetylglycine ethyl ester	2170	MS
37.32	diethyle 2-hydroxypentanedioate	2172	MS
38.03	4-vinyl-2-methoxy-phenol	2213	Std, MS, LRI
38.82	ethyl 5-oxotetrahydrofuran-2-furancarboxylate	2250	MS, LRI
39.17	3-hydroxy-4-phenyl-2-butanone	2267	MS, LRI
39.31	decanoic acid	2274	Std, MS, LRI
39.39	ethyl 2-hydroxy-3-phenylpropanoate	2278	Std, MS, LRI
39.76	3,5-dihydroxy-2-methyl-4H-pyran-4-one	2295	MS, LRI
40.20	glycerin	2313	Std, MS, LRI
40.33	diethyl tartrate	2318	Std, MS, LRI
41.33	ethyl hydrogen succinate	2355	Std, MS, LRI
41.55	4-vinyl phenol	2364	Std, MS, LRI
42.53	2-furancarboxylic acid	2401	Std, MS, LRI
42.92	dodecanoic acid	2427	Std, MS, LRI
43.19	ethyl hydrogen fumarate	2445	MS, LRI
43.50	α-(phenylmethyl) benzeneethanol	2466	Std, MS
44.17	5-(hydroxymethyl)-2-furancarboxaldehyde	2514	Std, MS, LRI
44.25	benzenacetic acid	2521	Std, MS, LRI
46.20	tetradecanoic acid	2673	Std, MS, LRI
48.22	3,4-dimethoxyphenylalanine	2759	MS, LRI
49.39	n-hexadecanoic acid	2803	Std, MS, LRI
50.16	N-acetyltyramine	2840	Std, MS, LRI
50.73	1-H-indole-3-ethanol	2867	Std, MS, LRI
51.77	4-hydroxy-benzenethanol	2944	Std, MS, LRI

Table 1: List of identified compounds. <sup>a</sup> identification assignment: Std = comparing mass spectra, LRI and retention times with pure compounds, MS = by comparing mass spectra with NIST 08 and Wiley 7 spectral database, LRI = matching LRI on comparable polar columns  
(taken from the following publicly available databases: <https://pubchem.ncbi.nlm.nih.gov/>;  
<https://www.nist.gov/srd>; <http://www.flavornet.org/flavornet.html>)



Table 2

	Control	SO <sub>2</sub>	KT
Alcohol (% v/v)	12.07 a	11.99 a	11.97 a
Titrateable Acidity (g L <sup>-1</sup> )	6.52 a	6.23 ab	5.25 b
Volatile Acidity (g L <sup>-1</sup> )	0.39 a	0.36 b	0.42 a
pH	3.11 b	3.11 b	3.19 a
Total SO <sub>2</sub> (mg L <sup>-1</sup> )	1.92 a	48.7 b	2.56 a
Reducing sugars (g L <sup>-1</sup> )	< 2.0 a	< 2.0 a	< 2.0 a
Total phenolics (mg L <sup>-1</sup> )	42.3 a	42.3 a	40.7 a
O. D. 420 nm	0.092 a	0.082 b	0.085 ab
Citric acid (g L <sup>-1</sup> )	0.20 a	0.19 a	0.18 a
Tartaric acid (g L <sup>-1</sup> )	2.94 a	3.03 a	2.67 b
Malic acid (g L <sup>-1</sup> )	2.23 a	2.14 a	1.68 b
Lactic acid (g L <sup>-1</sup> )	0.18 a	0.23 a	0.18 a
Succinic acid (g L <sup>-1</sup> )	0.95 a	0.93 a	0.69 b
Acetic acid (g L <sup>-1</sup> )	0.36 a	0.39 a	0.41 a
Glycerol (g L <sup>-1</sup> )	9.37 a	9.74 a	9.30 a

Table 2: Enological parameters of wines at the end of alcoholic fermentation  
In the same row, different letters indicate significant differences according to Tukey's test (p<0.05). n=3.

Table 3

	Wines					
	End of fermentation			12 months of storage		
	Control	SO <sub>2</sub>	KT	Control	SO <sub>2</sub>	KT
Acids						
isobutyric acid	4.04 a	3.70 a	1.94 b	3.42 a	2.93 a	1.49 b
n-butyric acid	0.28 b	0.31 b	0.35 a	0.18 c	0.25 b	0.30 a
pentanoic acid	3.55 a	3.53 a	2.03 b	3.47 a	3.44 a	1.67 b
hexanoic acid	3.58 b	3.67 b	6.19 a	3.52 b	3.62 b	6.54 a
octanoic acid	3.84 b	3.85 b	7.08 a	3.27 b	3.32 b	6.80 a
decanoic acid	1.49 b	1.26 b	5.33 a	1.16 b	1.02 b	3.77 a
dodecanoic acid	0.20 a	0.21 a	0.18 a	0.05 b	0.05 b	0.10 a
benzenacetic acid	0.13 b	0.22 a	0.06 c	0.03 b	0.09 a	0.05 b
Total acids	17.11 b	16.75 b	23.15 a	15.09 b	14.72 b	20.72 a
Esters						
isoamyl acetate	1.16 b	1.04 b	1.64 a	0.34 a	0.36 a	0.33 a
ethyl hexanoate	0.25 b	0.29 b	0.65 a	0.40 b	0.36 b	0.75 a
ethyl pyruvate	0.06 a	0.06 a	0.06 a	0.13 b	0.19 a	0.10 b
methyl lactate	0.05 b	0.03 b	0.08 a	n.d.	n.d.	n.d.
ethyl lactate	1.08 b	1.30 a	0.86 c	3.92 a	3.39 b	3.44 b
ethyl octanoate	0.10 b	0.20 b	0.44 a	0.70 b	0.54 b	1.33 a
ethyl-3-hydroxybutyrate	0.12 b	0.07 b	0.17 a	0.12 b	0.16 a	0.16 a
ethyl decanoate	0.00 b	0.05 b	0.16 a	0.10 b	0.07 b	0.42 a
diethyl succinate	0.18 a	0.20 a	0.14 b	6.39 a,b	7.45 a	4.48 b
methyl salicylate	0.04 a	0.02 a	0.04 a	n.d.	n.d.	n.d.
ethyl 4-hydroxybutanoate	2.64 b	3.33 a	1.09 c	0.05 a,b	0.12 a	0.01 b
2-phenylethyl acetate	0.87 b	0.93 b	2.10 a	0.12 b	0.14 b	0.36 a
diethyl malate	0.40 a	0.41 a	0.28 b	6.89 b	11.43 a	7.15 b
diethyl tartrate	n.d.	n.d.	n.d.	0.67 b	1.17 a	0.40 b
ethyl hydrogen succinate	2.77 a	2.85 a	2.11 a	11.73 a	13.57 a	14.71 a
Total esters	9.71 a	10.79 a	9.83 a	31.59 a	38.98 a	33.64 a
Alcohols						
Isobutyl alcohol	20.27 b	28.23 a	13.46 c	27.54 a	20.46 b	14.83 c
2-hexanol	0.02 c	0.08 a	0.05 b	0.08 a	0.07 a	0.08 a
3-methyl-1-butanol	30.64 b	40.12 a	30.59 b	55.27 a	45.43 a	55.81 a
2-hexanol	0.05 a	0.05 a	0.05 a	0.03 a	0.03 a	0.03 a
4-methyl-1-pentanol	0.00 c	0.01 b	0.02 a	0.00 b	0.01 a	0.01 a
n-hexanol	0.09 a	0.10 a	0.07 b	0.08 a	0.09 a	0.06 b
3-ethoxy-1-propanol	0.19 a	0.11 b	0.17 a	0.18 a	0.09 c	0.15 b
3-hexen-1-ol	0.03 b	0.03 a	0.03 a,b	0.03 a	0.03 a	n.d.
3-methylthio-1-propanol	1.06 a	1.17 a	0.41 b	0.63 a	0.65 a	0.27 b
Benzyl alcohol	0.20 a,b	0.29 a	0.11 b	0.10 a	0.12 a	0.09 a
2-mercaptoethanol	n.d.	0.01 a	n.d.	n.d.	n.d.	n.d.
Phenethyl alcohol	38.97 a	38.36 a	37.40 a	63.50 a	72.55 a	76.03 a
4-hydroxy-benzenethanol	20.54 a	20.63 a	22.88 a	14.26 a	19.38 a	20.91 a
Total alcohols	112.08 a	127.20 a	108.25 a	161.69 a	158.89 a	168.26 a
Others						
cis-5-hydroxy-2-methyl-1,3-dioxane	0.03 b	0.05 a	0.04 a	1.75 b	0.87 c	3.19 a
trans-4-hydroxymethyl-2-methyl-1,3 dioxolane	0.02 b	0.10 a	0.04 b	0.76 b	0.44 c	1.26 a
trans-5-hydroxy-2-methyl-1,3-dioxane	0.04 a	0.05 a	0.04 a	1.64 b	1.02 c	2.59 a
γ-butyrolactone	0.28 b	0.37 a	0.12 c	0.19 b	0.26 a	0.09 c
Furfural	0.07 a	0.07 a	0.08 a	0.12 c	0.44 a	0.25 b
Furfuryl alcohol	0.10 a	0.13 a	0.12 a	0.06 a	0.03 b	0.07 a
4-hydroxy-2-butanone	0.88 a	0.76 b	0.55 c	-0.01 b	0.04 a	0.05 a
ethyl 5-oxotetrahydrofuran-2-furancarboxylate	0.79 a	0.86 a	0.31 b	1.01 b	1.61 a	0.93 b
2-furancarboxylic acid	0.08 b	0.17 a	0.08 b	0.19 a,b	0.23 a	0.13 b
5-(hydroxymethyl) 2-furancarboxaldehyde	n.d.	n.d.	n.d.	0.73 b	0.95 a,b	1.32 a
N-acetyltyramine	0.10 b	0.14 a	0.13 a	n.d.	n.d.	n.d.
Total others	2.40 a	2.69 a	1.51 b	6.43 b	5.90 b	9.87 a

Table 3. Concentration of the quantified volatile compounds (mg L<sup>-1</sup>) in wines at the end of the alcoholic fermentation and after 1 year of bottle storage.

In the same row, different letters indicate significant differences according to Tukey's test (p<0.05). n=3.

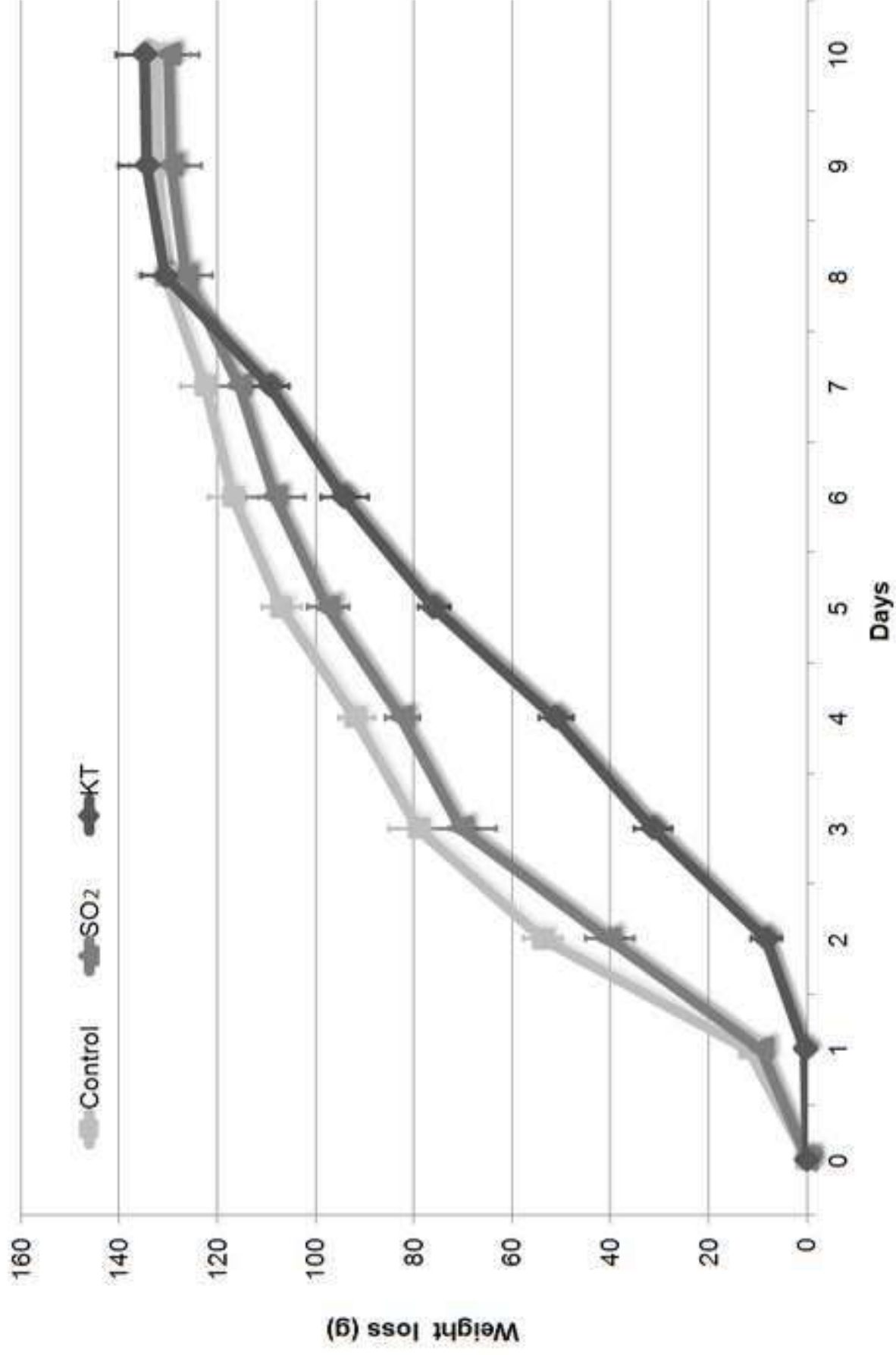


Figure 1

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Figure 2

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