Oxygen and SO_2 consumption rates in white and rosé wines. Relationship with and effects on wine chemical composition

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Abstract

This paper addresses the study of O_2 and SO_2 consumption rates of white and rosé wines, their relationship to the initial chemical composition and their effects on the chemical changes experienced by wine during oxidation. Eight wines were subjected to five consecutive air-saturation cycles. O_2 was monitored periodically; SO_2, color and antioxidant indexes were determined after each cycle, and the initial and final composition of the wines were thoroughly determined. Wines consumed oxygen at progressively decreasing rates. In the last cycles, after a strong decrease, consistent increases of oxygen levels were seen. Oxygen consumption rates were satisfactorily modelled, being proportional to wine Copper, quercetin and kaempherol contents, and negatively proportional to cinnamic acids. SO_2 consumption rates were highly diverse between wines and were positively related to free SO_2, Mn and pH, among others. In the last saturations, SO_2 consumption took place regardless O_2 consumption, implying that SO_2 should reduce chemical species oxidized in previous saturations. Some volatile phenols seem to be the endpoint of radical-mediated oxidation of polyphenols taking place preferably in the first saturation.

Key words: *acetaldehyde, copper, oxidation mechanisms, flavonols, flavanols*

Introduction

Oxygen management is crucial in winemaking, since it can cause significant improvements or irreversible defects. Oxidation and reduction reactions occur in several moments during the wine-making process causing important changes in color, aroma and taste.[1] In white and rosé wines, it is not usual to oxidize on purpose, except for some specific styles of wines, so that if these wines are accidentally exposed to air, their quality will be damaged.[2] Because of a number of reasons, such as the smaller levels of polyphenols, and the oxygen-sensitive nature of the varietal aroma of many white and rosé wines,[3] the wine resistance to oxidation and the use of sulfur dioxide (SO_2) and other antioxidants remain an important issue.

Oxidation mechanisms in wine have been recently reviewed [4-12] and it is now accepted that SO_2 does not directly reacts to O_2. When oxygen is dissolved in wine, a cascade of oxidative reactions catalyzed by metals such as copper and iron, oxidizes phenolic compounds.[2, 5, 6, 13] During this process, highly reactive species such as quinones and hydrogen peroxide are formed, being SO_2 a key component reacting to both intermediates. The first step of the oxidation mechanism is proposed to be the activation of dissolved oxygen by catalytic action of metal ions, principally Fe (II), but in which Cu (II) exerts a demonstrated enhancing effect. As a result, the hydroperoxyl radical ($HO_2^{\bullet}$) is thought to be formed. Following, this radical is supposed to react with the catechol moiety of phenols, leading first to the formation of semiquinones and finally of quinones, leaving hydrogen peroxide (H_2O_2) as the main by-product. If SO_2 is present, it reacts with H_2O_2, reducing it to water (H_2O) and oxidizing itself to sulfate (SO_4^{2-}). Besides, SO_2 can react to quinones, either to reduce them back to catechols or by a nucleophilic reaction to produce catechol sulfonate.[3] If SO_2 is not available, H_2O_2

triggers Fenton reaction, where Fe (II) transform H_2O_2 in the hydroxyl radical ($HO^{\bullet}$), one of the most reactive oxygen radicals, which is able to abstract hydrogen atoms from organic compounds to become H_2O. This radical $HO^{\bullet}$ is the main responsible for the oxidation of ethanol to acetaldehyde which if accumulates, will impart to wine a characteristic oxidative odor. The consequences of these reactions are important modifications in wine composition affecting to phenolic and aromatic composition.[4-12]

The key role played by SO_2 explains why this compound is the most important exogenous wine antioxidant. However, some allergic symptoms in humans have been associated to SO_2, which has triggered a general tendency to reduce the amounts of this antioxidant and eventually to replace it by a different one with the same efficiency and less toxicity. [14-16] This has not happened at present [17] and it can be hypothesized that reducing SO_2 levels while keeping or improving wine resistance to oxidation is a long term goal will require a deep understanding about the different processes directly or indirectly linked to the consumption of O_2 by wine.

In this regard, the main goals of the present work are to identify the chemical components with major effect on the rates at which white and rosé wines consume O_2 and SO_2, to describe the chemical changes associated to the consumption of oxygen and to assess how these changes are related to the protection levels of SO_2.

Materials and Methods

Wines and Samples

Five white wines and three rosé wines were purchased at a local wine store. Wines were from different Spanish Denominations of Origin, two of them were from Rueda, and one sample each from Navarra, Rias Baixas, Rioja, Cariñena, Calatayud and

Somontano. The detailed list of samples, including sample information is shown in Table 1.

Wine oxidation was performed in a five-cycle forced oxidation experiment. Wines were extensively analyzed at the beginning and after the five cycles. In addition, after each cycle a more limited set of analytical parameters was monitored (see details in "analytical characterization"). The bottles containing the wines for the experiment were opened inside a glove chamber from Jacomex (Dagneux, France) in which atmospheric oxygen was hold under 0.002% (<3 ppm). The contents of 2 bottles were mixed in a beaker and samples for analysis representing initial time were then taken in closed vials. The remaining wine was taken out of the chamber and 500 mL volumes of each wine were saturated with air until O_2 levels rose above 6 mg/L. Saturation was performed by shaking the wine in a 1 L flask 3 times for 10 s, opening the cap after each shake. Then, the 500 mL were distributed in eight 60 mL-screw capped clear glass vials supplied by WIT-France (Bordeaux, France), three of them containing PreSens PSt3 oxygen sensors from Nomacorc S.A. (Thimister-Clermont, Belgium). No headspace was left in the vials. Previous studies confirmed that the amount of O_2 passing through those closures was negligible for the purposes of the experiment (<0.05 mg/L per week). Wines were stored in the dark in an incubator at 25 °C and dissolved oxygen level was monitored with a Nomasense oxygen analyzer from Nomacorc S.A. (Thimister-Clermont, Belgium) every day. When oxygen reached 10% of the initial concentration (or a week later in cases in which no more significant decrease was observed in the oxygen concentration), the vials from a given wine sample were introduced inside the oxygen-free chamber, opened and mixed. Samples for intermediate analyses were taken from the mixture and the remaining volume of wine

was taken out of the chamber for a new saturation, being distributed this time in a smaller number of tubes. This process was repeated five times. In all the saturation steps, at least two vials containing a PreSens oxygen sensor were used in order to control the reproducibility of the process. This was assessed from the 286 pairs (or trios) of replicate measurements collected during the process.

Reagents, standards and materials

Solvents and Chemical Standards

Solvents for gas chromatography dichloromethane, methanol, hexane and diethyl ether (gas chromatography quality) were purchased from Merck (Darmstadt, Germany). Ethanol was from Panreac (Barcelona, Spain). Acetone, methanol, formic acid, ethanol, acetonitrile and sulphuric acid solvents for high-performance liquid chromatography were of HPLC grade from Scharlab (Barcelona, Spain). Water with resistance of 18.2 MΩ·cm at 25 °C was purified in a Milli-Q system from Millipore (Bedford, Germany).

Chemicals used for the analytical characterization were of analytical reagent grade and were supplied by Merck, Panreac, Sigma-Aldrich (Madrid, Spain), Lancaster (Eastgate, UK), Scharlau (Barcelona, Spain), Oxford Chemicals (Hartlepool, UK), Fluka (Madrid, Spain), ChemService (West Chester, PA, USA), Extrasythèse (Genay, France) and SAFC (Steinheim, Germany). Purity of chemical standards is over 95% in all cases and most of them are over 99%. TSK Toyopearl gel HW-50F was purchased from Tosohaas (Montgomery-ville, PA, USA). [18-24]

Analytical Characterization

Analyses of the 8 original wines and after each one of the five saturations included absorbances at 420, 520 and 620 nm, pH, free and total sulfur dioxide, free

acetaldehyde, total polyphenol index (TPI), Trolox equivalent antioxidant capacity (TEAC) and Folin-Ciocalteu index. Complementary analyses were performed at the beginning and at the end of the experiment (after oxygen is depleted in the fifth saturation): metals (Fe, Cu, Mn, Zn, Al), polyphenols (hydroxycinnamic acids, benzoic acids, stilbenes, flavanols and flavonols) and aroma compounds.

Color determination and Total Polyphenol Index (TPI)

Chromatic parameters were determined following the recommendation of the OIV for white and rosé wines.[25] Absorbances at 420 nm, 520 nm and 620 nm were determined without any further dilution with a 1 cm path length. Total Polyphenol Index (TPI) was estimated as absorbance at 280 nm. For the TPI determination, rosé wines were diluted 1:50 and white wines 1:20 and 1 cm path length cuvettes were used. All absorbance measurements were taken in triplicate in a UV-VIS spectrophotometer UV-17000 Pharma Spec from Shimadzu (Kyoto, Japan).

Determination free and total sulfur dioxide and free acetaldehyde

Total sulfur dioxide was determined by the aspiration oxidation method (Rankine method) following the recommendation of the OIV.[26]

Nominally free sulfur dioxide and free acetaldehyde were determined by headspace - gas chromatography - mass spectrometry (HS-GC-MS) as described recently,[27] since this method provides higher accuracies and limits of detection than the aspiration-oxidation method for free SO_2. HS-GC-MS analyses were performed using a GCMS-QP2010 from Shimadzu with a DB-WAX (30 m x 0.25 mm i.d. x 0.25 µm film thickness) column from J&W Scientific (Agilent Technologies, Santa Clara, CA, USA) as described in the reference.[27]

Trolox equivalent antioxidant capacity (TEAC)

TEAC assay is based on decolorization of the radical cation $ABTS^{\bullet+}$ when it is reduced to ABTS by an antioxidant. The assay was performed following the procedure described by Rivero-Perez *et al*. [18] White and rosé wines were diluted 1:10 in 0.075 M phosphate buffer (PBS) at pH 7.4. In a test tube 200 µL of each diluted sample was mixed with 9800 µL of $ABTS^{\bullet+}$ previously prepared to give an absorbance value of 0.70 ± 0.02 at 734 nm. Absorbance measurements were taken at 734 nm in duplicate with 1 cm path length cuvettes in a UV-VIS spectrophotometer UV-17000 Pharma Spec from Shimadzu.

Folin-Ciocalteu assay

Folin–Ciocalteau assay was performed as described by Singleton *et al.* [19] White and rosé wines were diluted 1:5 with Milli-Q water. An aliquot of 750 µL of the sample was mixed with 500 µL of Folin- Ciocalteau reagent (Sigma-Aldrich) and 2 mL of a Na_2CO_3 solution at 20% in water. The mixture is brought to 10 mL with Milli-Q water. The reaction takes place in darkness at room temperature for 2 hours and absorbance is then measured at 760 nm in 1 cm cuvettes using a UV-VIS spectrophotometer UV-17000 Pharma Spec from Shimadzu. The assay was performed in duplicate and results of phenolic content were expressed in mg of gallic acid equivalents per liter of wine.

Quantitative analysis of metals

Metal analyses included the determination of iron, copper, manganese, zinc and aluminum. Microwave-assisted digestion was used as sample treatment and they were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) as is described by Gonzalvez *et al.* [20]

Analysis of polyphenols

Polyphenolic matter was analyzed following the method described by Gonzalez-Hernandez et al. in 2014. [21] Two mL of wine were filtered by 0.45 µm and fractionated by Gel Permeation Chromatography (GPC) with a Vantage L column (120 mm x 12 mm) from Millipore (Bedforf, Ma, USA) packed with TSK Toyopearl gel HW-50F (Tosohaas, Montgomery Ville, PA, USA) to obtain 2 fractions. In fraction 1, low molecular weight phenolics were quantified by UPLC–MS, including flavonols, flavanols, hydroxycinnamic acids, phenolic acids, aconitic acid and resveratrol. Analyses by UPLC-MS were performed on a liquid chromatograph Shimadzu Nexera 30AD coupled to a mass spectrometer QTRAP AB Sciex 3200 (AB SCIEX, MA, USA), with a triple quadrupole and an electrospray as ionization source (ESI Turbo VTMSource). The column was a BEH-C18 Acquity UPLC (1.7 µm, 2.1 mm x 100 mm) from Waters (Milford, MA, USA). The second fraction was not analysed as it contains polymeric matter and is not important in white and rosé wines.

Aroma compounds analysis

For major aroma compound determination, a liquid-liquid microextraction with dichloromethane published was carried out.[22] Analyses were performed in a gas-chromatograph with flame ionization detection model CP-2800 GC from Varian (Walnut Creek, CA, USA). For minor and trace aroma compounds analysis, a solid-phase extraction was carried out based on the procedure described by Lopez et al. [23] An aliquot of 15 mL of wine were extracted in a 65 mg LiChrolut® EN cartridge (Merck, Darmstadt, Germany), cleaned up with 1.5 mL of a 30% methanol in water at pH 3 and further eluted with 0.6 mL of dichloromethane-5% methanol (v/v). Extracts were directly analyzed by gas chromatography with ion trap mass spectrometry detection in a GC-MS model 450-GC and Saturn 2200 GC/MS from Varian.

184 Statistical analysis and data treatment

185 Simple correlations and Partial Least-Squares (PLS) regressions were carried out using
186 Excel 2013 (Microsoft, WA, USA) and The Unscrambler 9.7(CAMO Software AS, Oslo,
187 Norway) respectively. PLS modeling was carried out using cross-validation criteria. In
188 this strategy, the model is built leaving out one of the samples, and the predicted
189 result for the sample out is computed as residual. The process is repeated with every
190 sample of the calibration set, and so on until every sample has been left out once; then
191 all prediction residuals are combined to compute the validation residual variance and
192 Root Mean Square Error of Prediction (RMSEP).

Results and discussion

Oxygen consumption in air saturation cycles

Wine oxidation was carried out following a procedure based on consecutive air-saturation cycles, with daily oxygen monitoring with oxygen sensors placed in screw-capped clear vials. A typical plot representing oxygen consumption versus time for a particular wine is illustrated in Figure 1. The reproducibility of the process was assessed by means of the duplicate measurements taken from the independent tubes in which volumes of the same wine were distributed during oxidation, as detailed in reference. [24] In the present case, the average standard deviation for the 286 series of duplicate measurements was σ = 0.29 mg O_2/L, which can be considered satisfactory and in fact, the plots obtained with different sensors were nearly superimposable.

As can be seen in figure 1, oxygen is continuously consumed at decresing rates in the three first saturations, while in the last two ones O_2 is consumed very fast in the first hours, but after the first measurement all the readings indicated that levels of O_2 were increasing. These increases were consistently observed in the 8 wines considered in this work (see S1 in Supporting Information). To the best of our knowledge this weird phenomena has never been reported. However, it could be consistent with the oxidation mechanism recently proposed by Danilewicz[28] based on previous reports on $[Fe^{II}(EDTA)]$ oxidation mechanims.[29, 30] Attending to such proposal, schematized in Figure 2a, the activation of oxygen with Fe(II) with the help of Cu(II) produces the $[Fe^{III}\text{-}O_2^{\bullet}]^{2+}$ radical complex (reaction 1). This complex can be reduced by Fe(II) into a $diiron^{III}$-dioxygen complex (reaction 2) which would finally rend H_2O_2 and Fe(III) (reaction 3). In the presence of oxidizable catechols this Fe(III) would be reduced back

to Fe(II), restoring the catalytic cycle. However, if the reduction fails due to lack of catechols in white and rosé wines, Fe(III) would accumulate and would oxidize back the $[Fe^{III}\text{-}O_2^{\bullet}]^{2+}$ superoxo complex releasing O_2 (reaction 4 in Figure 2a), which would explain the plot in Figure 1. A second alternative explanation for the observed increases in oxygen levels is based in the Fenton reaction shown in figure 2b,[29] in which ethanol is oxidized to acetaldehyde through a radical mechanism. In this case, the reaction takes place when there is no SO_2 available to scavenge H_2O_2 –and levels of free SO_2 in the last saturations are very low-. Attending to the scheme, the hydroxyethyl radical would react to O_2 to yield as reaction subproducts hydrogen peroxide and oxygen.

In any case, it is obvious that oxygen consumption rates in these wines cannot be interpreted by simple first or pseudo first order kinetic models.

Oxygen consumption rates

When the accumulated oxygen consumed is represented vs. time, a typical pattern such as the one shown in Figure 3, emerges. It can be seen that the amount of oxygen consumed in each saturation cycle becomes progressively smaller, in agreement with old reports.[33] These functions were fitted to a second-grade polynomial, which was further used to determine the oxygen consumed at 5, 20 and 30 days. The corresponding average oxygen consumption rates (OCRs) are given in Table 2. It can be observed, that although average OCRs decrease with time for all samples, decreases are more pronounced for the samples showing fastest initial OCRs. In consequence, the ranges in which those rates span shrinks from 0.258-0.833 for the 5 days OCR to

238 0.235-0.563 for the 30 days OCR. Rates were in general much smaller than those
239 observed for red wines.[24]

240 Correlation analysis revealed that just a limited set of chemicals was related to the
241 different OCRs. The 5-days OCRs were significantly and positively correlated ($P<0.05$)
242 to gallocatechin and copper. 20-days OCRs were positively correlated just with copper.
243 30-days OCRs were positively correlated to coutaric, *trans*-cinnamic, *cis*-ferulic acids
244 and to copper (data not shown). Following, PLS models with a quite satisfactory
245 prediction ability could be built for the three OCRs, as summarized in table 3 (models 1
246 to 3). All models are quite similar in structure, explain between 89.7% and 95.9% of the
247 original variance by cross-validation, and suggest that copper and flavonols and, to a
248 lesser extent, hydroxycinnamic acids are the key compounds determining OCRs in
249 whites and rosés. While, to the best of our knowledge, there are no previous reports
250 suggesting the role of flavanols on OCRs, copper is confirmed as the main and more
251 universal responsible for the ability of a given wine to consume oxygen.[7, 32]

252 It is worth mentioning, that models do not identify any relevant influence of SO_2 or Fe
253 contents on the wine OCRs, in spite of the known role played by these compounds in
254 wine oxidation.[7, 33] This apparent incongruence may imply that these compounds are
255 present in wine at levels at which they are not kinetically limiting. Alternatively, it may
256 be thought that what determines OCRs are the activities of Fe^{2+} and Fe^{3+}, or their
257 ratios as suggested by Danilewicz in 2016,[34] and the level of "truly" free SO_2. In
258 contrast, the parameters measured in the present work are total Fe and "nominally"
259 free SO_2, [27] which may not reflect the levels of the kinetically relevant parameters.

260 *Consumption of sulfur dioxide*

261 The evolution of the total SO_2 content of the wines during oxidation is summarized in
262 Figure 4. As can be seen, in most samples SO_2 decreases following a linear trend during
263 several saturation cycles, ending in some cases with a steeper SO_2 consumption in the
264 last saturations. The slopes of the linear segments represent the mg/L of SO_2
265 consumed per mg/L of O_2 consumed during the first saturations and range between
266 1.2 and 5.4. Transformed into molar ratios, the ratios consumed SO_2: consumed O_2 for
267 these wines in those linear periods ranged between 0.3 and 2.7 as detailed in Table 4.
268 This last unexpected value (case of W3), significantly above the maximum theoretical
269 value of 2, could be most likely due to the fact that in that wine there was so much
270 free SO_2 (see Table 1) that some was lost by evaporation during the air saturation of
271 the wines. The lowest molar ratio corresponds to Rs2 which contained already lowest
272 levels of free SO_2. Leaving aside this particular case, molar ratios are quite diverse,
273 ranging from 1 to 2, and were not significantly correlated to any single wine
274 compositional parameter, indicating that the ability of a wine to consume its own SO_2
275 during oxidation depends on several factors.

276 These were assessed by PLS modeling. A model with a quite satisfactory explaining
277 ability (94% by cross validation) could be built and is summarized in Table 3 (model 4).
278 The largest coefficient of the model is given to free SO_2, meaning that a first obvious
279 requisite for a wine to consume SO_2 is having it in free form. This was not observed in
280 red wines, for which total SO_2 seems to be most influential in SO_2 consumption. This
281 may be attributed to the smaller activity of free SO_2 in red wines as a consequence of
282 complexes with anthocyanins and also to their higher ability to remove free
283 acetaldehyde by condensation reactions which facilitates the dissociation of bound
284 SO_2. The model also suggests that the ability of the wine to consume SO_2 during

285 oxidation is positively related to its pH, TPI, Folin-Ciocalteu index and the wine content
286 in Mn.

287 Remarkably, in three of the wines (Rs1, W1 and particularly W5) the slopes of the
288 functions in Figure 4 become strikingly steeper, meaning that in those last saturations
289 more SO_2 is being consumed per unit of O_2. The extreme case is that of W5 for which
290 the consumption of O_2 in the last two saturations is very low (0.45 mg/L) and yet there
291 is a strong consumption of SO_2 (14 mg/L). If instead of consumed O_2, the plot in Figure
292 4 is re-plotted representing time in abscissas, the functions become strictly lineal (see
293 SI). This reveals that SO_2 had been consumed at a constant temporal rate in all wines,
294 while O_2 was consumed at progressively smaller rates in the experiment. This
295 unexpected result suggests that in some wines, notably in W5, some of the chemical
296 species oxidized in the first three saturations were reduced back by SO_2 two weeks
297 later.

298 A satisfactory and quite simple PLS model explaining SO_2 consumption rates was also
299 built (model 5 in Table 3). The model explains 92% of the variance by cross-validation
300 and the main variables are free SO_2 and the Folin-Ciocalteau (FC)/TEAC ratio, both with
301 positive sign. This ratio can be roughly attributed to the ratio general
302 antioxidant/scavenger contents of the wine. Therefore, the model suggests that while
303 most wine polyphenolics are oxidized with concomitant consumption of SO_2,
304 compounds with scavenging activities may compete with SO_2 for some radicals. The
305 existence of SO2 competitors has been previously suggested.[24]

306 *Chemical changes caused by oxidation*

As in a previous paper,[35] the changes in the chemical composition caused by oxidation have been studied by two simple statistical techniques. First, paired t statistical comparisons were applied to determine which changes were, in average, significant. Second, the correlation between the magnitude of the change with the O_2 consumed, segregated into several categories, was studied by correlation analysis. The categories in which consumed O_2 was segregated were "O_2 in SO_2", "O_2 not SO_2", "O_2 pre SO_2" and "O_2 at free SO_2 below 10 mg/L". The two first ones are complementary and represent the consumed O_2 which can (O_2 in SO_2) or cannot (O_2 not SO_2) be attributed to the total SO_2 consumed by the wine, assuming a 2:1 molar ratio (SO_2:O_2). The third one, O_2 pre SO_2, is similar to O_2 not SO_2 but referred just to the first saturation cycle. The last one, O_2 at free SO_2 below 10 mg/L, represents the amount of O_2 consumed by the wine at low free SO_2 levels, situation in which the presence of free radicals is expected.

The major changes introduced by oxidation in the phenolic composition of the wines were relevant increases in the levels of phenolic acids and decreases in those of flavanols, and flavonols, in accordance with previous reports.[36] Levels of benzoic acids increased in average 5.2 mg/L (39%) and those of hydroxycinnamic acids 4.0 mg/L (10%), although increases could also be related to the hydrolysis from their tartaric esters. Average levels of flavanols decreased not significantly by a 4%, while those of flavonols by a 2%. Increases in cis and trans-ferulic acids were negatively and significantly correlated to "O_2 in SO_2", indicating that their formation takes preferably place when SO_2 consumption is low. Contrarily, the decrease of kaempferol-3-rutinoside was significantly and negatively correlated to O_2 at SO_2 <10.

Regarding aroma, levels of most non polar aroma compounds decreased during the process (data not shown), which should be attributed to losses by evaporation during the experiment, as previously discussed.[24] Other changes were correlated to some of the parameters related to oxygen and SO_2 consumption and are summarized in table 5. The most remarkable change is the increment of free acetaldehyde (increases of other oxidation-related aldehydes were discussed in a previous reference).[37] Such increment is strongly correlated to the O_2 not SO_2, and negatively correlated to the SO_2:O_2 molar ratio. This result was expected and is in complete agreement with the Fenton-based radical-mediated oxidation. It should be noted, however, that in red wines the opposite correlation was found, which was attributed to the many polyphenols able to react with acetaldehyde present in reds.[38]

Not many other changes were related to this "O_2 not SO_2" parameter; levels of δ-octalactone, 4-ethyl phenol and 4-vinylguaiacol bear also positive correlations, which may suggest that these compounds may be the endpoint of radical-mediated oxidation of different precursors, such as fatty acids and polyphenols. It is also remarkable, that in contrast with red wines, the changes related to the category "O_2 preSO_2" were much limited and affected particularly to volatile phenols, such as guaiacol, 4-ethylphenol and 4-vinylguaiacol. The relatively smaller effect of this category may support the smaller real availability of free SO_2 in red wines as a consequence of the complexes with anthocyanins,[39] while the fact that most correlations are positive support that these compounds are the endpoint of the radical-mediated oxidation of some polyphenols which, surprisingly, seems to take place in the first saturation. All these observations confirm the need for analytical methods able to measure the real availability of SO_2 [40] and for a deeper study of the first phase of oxidation.[41]

354 Finally, the total amounts of O_2 consumed during the oxidation, and the O_2 not SO_2
355 parameter, have been related to the major changes suffered by the wine during the
356 oxidation using PLS modeling. Results are summarized in Table 3 (models 6 and 7). The
357 model for total O_2 consumed stated, as expected, that O_2 is invested mostly in
358 oxidizing SO_2 and in producing acetaldehyde and acetic acid, both the major oxidation
359 products of ethanol. The model for the amount of O_2 consumed without concomitant
360 SO_2 consumption, specifies that in addition to oxidize ethanol, O_2 goes into the
361 degradation of flavonols and the production of hydroxycinnamic acids.

362 In conclusion, whites and rosés consume oxygen at smaller rates than reds, and OCRs
363 decrease continuously with consecutive saturation cycles. In the last cycles, O_2 levels
364 decrease sharply in the first hours, but later consistently increase, which suggests an
365 oxidation mechanism in which O_2 can be regenerated by reversion of slow reactions.
366 OCRs were satisfactorily modelled, being proportional to wine copper, quercetin and
367 kaempherol contents, and negatively proportional to cinnamic acids. The molar ratio
368 consumed O_2:SO_2 is quite variable and depends on a number of factors, being the most
369 important the free SO_2 content, followed by pH, Folin-Ciocalteu index, Mn, and TPI.
370 Wines consume SO_2 at a constant temporal rate; as some wines were nearly unable to
371 consume O_2 in the last saturations, this may imply that chemical species oxidized in the
372 first three saturations are reduced back by SO_2 two weeks later. Changes in aroma
373 compounds suggested that some volatile phenols are the endpoint of radical-mediated
374 oxidation of some polyphenols taking place preferably in the first air-saturation which
375 confirm the need for studying the first phase of oxidation with analytical tools able to
376 measure the real availability of SO_2.

Funding Sources

This work has been funded by the Spanish Ministry of Economy and Competitiveness (Project AGL2014-59840). V.C. has received a grant from the Spanish FPU program. Funding from Diputación General de Aragón (T53) and Fondo Social Europeo is acknowledged.

Associated content. Plots showing the evolution of the levels of oxygen and SO_2 in the eight different wines during the experiment are given as Supporting Information.

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Figure captions

Figure 1. Average oxygen concentration measured in the set of tubes containing the same wine (sample W4 in the plot) throughout the experiment. Standard deviation is shown as error bars.

Figure 2. Scheme for a) the reduction of oxygen by Fe(II) to produce hydrogen peroxide and possible reversion by Fe(III); b) Fenton reaction for oxidation of ethanol and involvement of oxygen to regenerate hydrogen peroxide and oxygen. Adapted from reference.[29]

Figure 3. Plot relating cumulated oxygen consumed versus time in a white wine (sample W4). Standard deviation is shown as error bars.

Figure 4. Plot relating the evolution of total SO_2 levels, measured at the end of each saturation, to the amount of O_2 consumed by each wine. Standard deviation is shown as error bars.

Tables

Table 1. Wine samples used in the study: type, origin and relevant information regarding their oxidative behavior

Wine Code	Color	Denomination of origin	Grape Variety	Vintage	Ethanol (v/v)	pH	TPI	TEAC (eq. Trolox mM)	Folin (eq. Gallic acid mg/L)	Total SO_2 (mg/L)	Free SO_2 (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)
Rs 1	Rosé	Somontano	CS	2012	13.5	3.23	14.00	7.06	597.9	90.4	23.1	0.178	1.981	1.222
Rs 2	Rosé	Navarra	Ga	2012	13	3.19	11.45	6.94	437.8	61.6	5.1	0.185	12.52	0.766
Rs 3	Rosé	Rioja	Ga	2012	13.5	3.27	12.75	5.79	488.4	102.4	17.7	0.227	9.376	0.761
W 1	White	Calatayud	Ma	2012	14	3.31	10.59	5.11	410.6	105.6	26.9	0.345	3.038	0.571
W 2	White	Cariñena	Ch	2012	13.5	3.48	11.66	5.03	378.3	107.2	18.7	0.140	2.252	1.161
W 3	White	Rías Baixas	Al	2012	12.5	3.27	11.48	5.98	509.3	153.6	47.3	0.208	1.773	1.466
W 4	White	Rueda	Ve	2012	13	3.29	10.15	4.19	353.6	99.2	24.3	0.180	2.628	1.508
W 5	White	Rueda	Ve	2012	12	3.28	7.57	6.34	387.8	163.2	31.8	0.188	1.908	1.376

CS: Cabernet-Sauvignon; Ga: Garnacha; Ma: Macabeo; Ch: Chardonnay; Al: Albariño; Ve: Verdejo.

Table 2. Oxygen Consumption Rates (OCRs) in White and Rosé Wines.

	R^2 (2^{nd} degree Polynomial Regression)	5-days OCR (mg O_2/L/ day)	20-days OCR (mg O_2/L/ day)	30-days OCR (mg O_2/L/ day)
Rs 1	0.984	0.631	0.409	0.306
Rs 2	0.989	0.514	0.387	0.321
Rs 3	0.995	0.562	0.459	0.393
W 1	0.998	0.833	0.678	0.563
W 2	0.996	0.258	0.249	0.235
W 3	0.998	0.364	0.339	0.310
W 4	0.999	0.662	0.552	0.472
W 5	0.992	0.503	0.377	0.283
Average	0.995	0.541	0.435	0.368
Maximum	0.999	0.833	0.678	0.563
Minimum	0.989	0.258	0.249	0.235
Max/Min		3.226	2.718	2.396

Table 3. PLS models explaining different kinetic parameters related to the O2 and SO2 consumption of wines as a function of the chemical composition of the wines or of the major changes introduced by oxidation. Values between brackets are the model quality parameters obtained by cross validation

Nº	Parameter	R^2	RMSE	PLS Regression Model
1	5 days OCR	0.984 (0.897)	0.021 (0.061)	OCR = 0.541 + 0.15 Quercetin-3-glucuronide + 0.172 Cu – 0.064 t-Cinnamic acid
2	20 days OCR	0.993 (0.953)	0.010 (0.031)	OCR = 0.426 + 0.025 Quercetin-3-glucuronide + 0.05 Kaempferol-3-galactoside + 0.108 Cu – 0.002 Coutaric acid
3	30 days OCR	0.977 (0.959)	0.015 (0.023)	OCR = 0.357 + 0.024 Quercetin-3-glucoside + 0.039 Kaempferol-3-galactoside + 0.089 Cu
4	Molar ratio (SO_2:O_2)	0.9874 (0.9365)[a]	0.0755 (0.1937)[a]	Molar ratio (SO_2:O_2) = 1.529 + 0.385 Free SO2 + 0.231 TPI + 0.295 pH + 0.257 Folin-Ciocalteu Index + 0.255 Mn
5	SO_2 consumption rate ($mgSO_2$/L/day)	0.9499 (0.9248)[a]	0.0767 (0.1073)[a]	SO_2 consumption rate = 1.072 + 0.244 Free SO_2 + 0.189 (Folin-Ciocalteau/TEAC)
6	Total O_2 consumed (mg/L)	0.975 (0.868)	0.508 (1.338)	O_2 consumed = 11.4 + 3.85 ΔAcetaldehyde + 1.89 ΔAcetic – 2.3 Δtotal SO_2 – 0.314 ΔTotal hydroxycinnamic acids – 0.16 ΔTotal flavonols
7	O_2 not SO_2 (mg/L)	0.958 (0.849)	0.728 (1.58)	O_2 not SO_2= 2.74 + 4.22 ΔAcetaldehyde + 1.32 ΔAcetic + 0.777 ΔTotal hydroxycinnamic acids – 0.779 ΔTotal flavonols

Table 4. Consumption of total SO_2 during wine oxidation: Consumed SO_2/Consumed O_2 molar ratios estimated in the linear regions of plots equivalents to those shown in Figure 4 but in molar concentration (Total SO_2 vs. Consumed O2) and SO_2 consumption rates.

	Total SO_2 vs. Consumed O_2				Total SO_2 vs. Time		
	Saturations within the linear range	R	Slope (SD)	Molar ratio (SO2:O2)	R (saturations 0-5)	Slope (SD)	SO_2 consumption rate ($mgSO_2$/L/day)
Rs 1	0-5	-0.954	-2.11 (0.33)	2.11	-0.979	-1.13 (0.12)	1.13
Rs 2	0-3	-0.979	-0.32 (0.09)	0.32	-0.943	-0.33 (0.06)	0.33
Rs 3	0-5	-0.985	-1.49 (0.13)	1.49	-0.988	-1.09 (0.08)	1.09
W1	0-3	-0.998	-1.02 (0.04)	1.02	-0.994	-1.27 (0.07)	1.27
W2	0-5	-0.979	-1.89 (0.20)	1.89	-0.983	-0.87 (0.08)	0.87
W3	0-5	-0.998	-2.69 (0.09)	2.69	-0.993	-1.61 (0.10)	1.61
W4	0-5	-0.983	-1.37 (0.13)	1.37	-0.996	-1.23 (0.06)	1.23
W5	0-3	-0.990	-1.34 (0.13)	1.34	-0.998	-1.06 (0.03)	1.06

Table 5. Changes in the levels of aroma compounds observed during the oxidation experiment and observed correlations with the different O_2 consumption parameters

Compounds	Average Increment (µg/L)	Relevant Correlation
Acetaldehyde [a]	14.0 (1274%) ***	**0.908 ** (O_2 not SO_2)**; 0.916 ** (O_2 preSO_2); -0.856 ** (SO_2:O_2 Molar ratio)
Isobutanol [a]	-1.01 (-6%) **	0.746 * (total O_2)
Ethyl isobutyrate	ns	-0.781 * (O_2 preSO_2)
δ-octalactone	ns	**0.778 * (O_2 not SO_2);**
Ethyl cinnamate	ns	-0.831 * (total O_2)
Guaiacol	5.46 (40%) *	**0.805 * (O_2 preSO_2)**
4-ethylphenol	ns	**0.748 * (O_2 not SO_2)** ; **0.930 ***(O_2 preSO_2);** -0.752 * (SO_2:O_2 Molar ratio)
4-vinylguaiacol	ns	**0.708 * (O_2 not SO_2)** ; **0.841 **(O_2 preSO_2)**
4-vinylphenol	9.89 (89%) **	
Syringaldehyde	0.14 (53%) *	
Vanillin	7.30 (48%) **	
Ethyl vanillate	0.70 (14%) *	

[a] : Concentration in mg/L. ns: not significant. *P < 0.05. **P < 0.01. ***P < 0.001. O_2 in SO_2in the O_2 consumed with equivalent consumption of SO_2 assuming a 2:1 molar ratio. SO_2:O_2 O_2 not SO_2 is the O_2 consumed without equivalent consumption of SO_2 assuming a 2:1 molar ratio SO_2:O_2. O_2 preSO_2 is the O_2 consumed without equivalent consumption of SO_2 in the first saturation.

Figure graphics

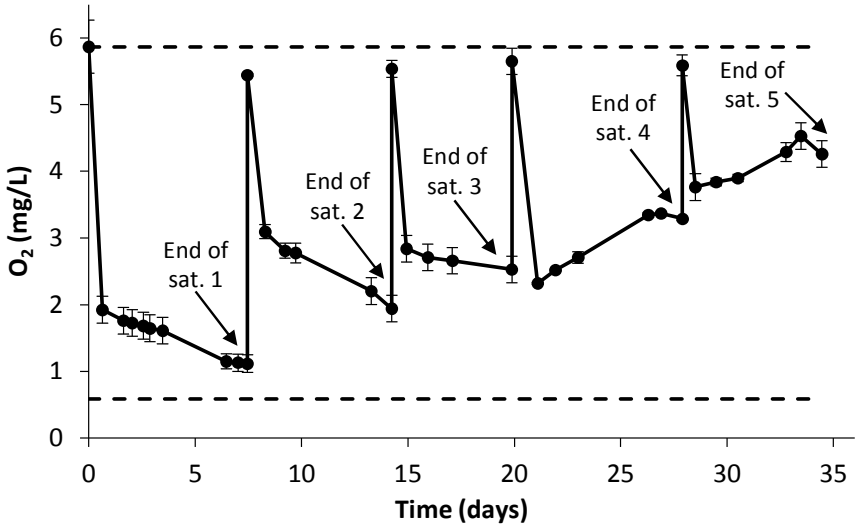


Figure 1

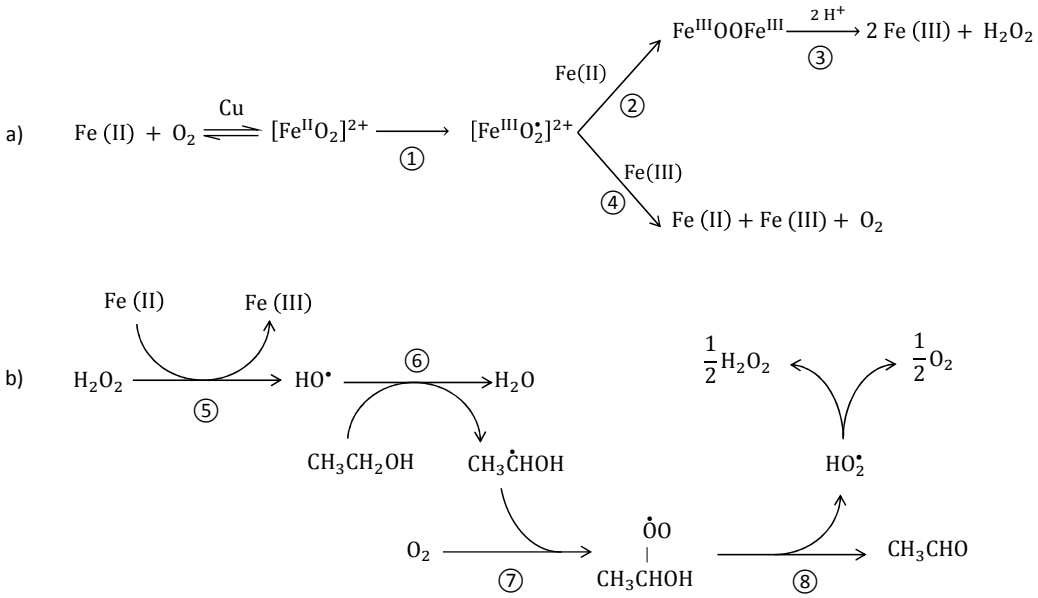

a)
Fe (II) + O2
Cu
[Fe^II O2]2+
①
[Fe^III O2•]2+
Fe(II)
②
Fe^III OO Fe^III
2 H+
③
2 Fe (III) + H2O2
Fe(III)
④
Fe (II) + Fe (III) + O2
b)
Fe (II)
Fe (III)
H2O2
⑤
HO•
⑥
H2O
CH3CH2OH
CH3ĊHOH
O2
⑦
ȮO
CH3CHOH
⑧
CH3CHO
1/2 H2O2
1/2 O2
HO2•

Figure 2

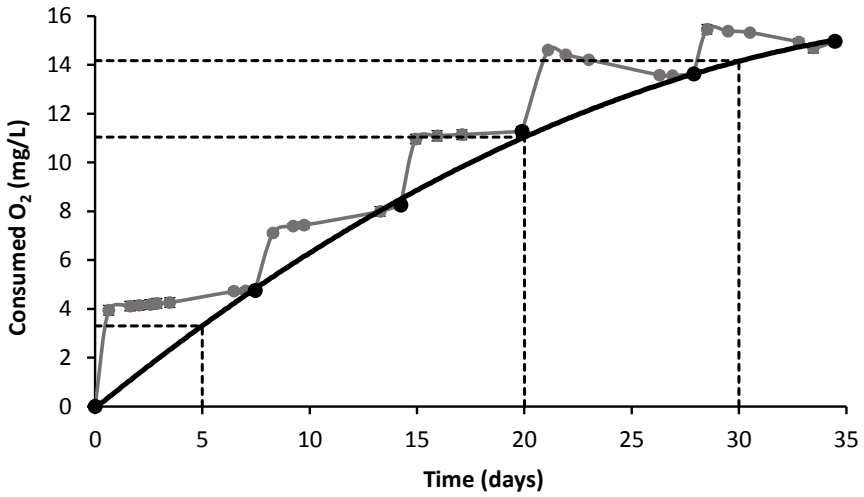


Figure 3

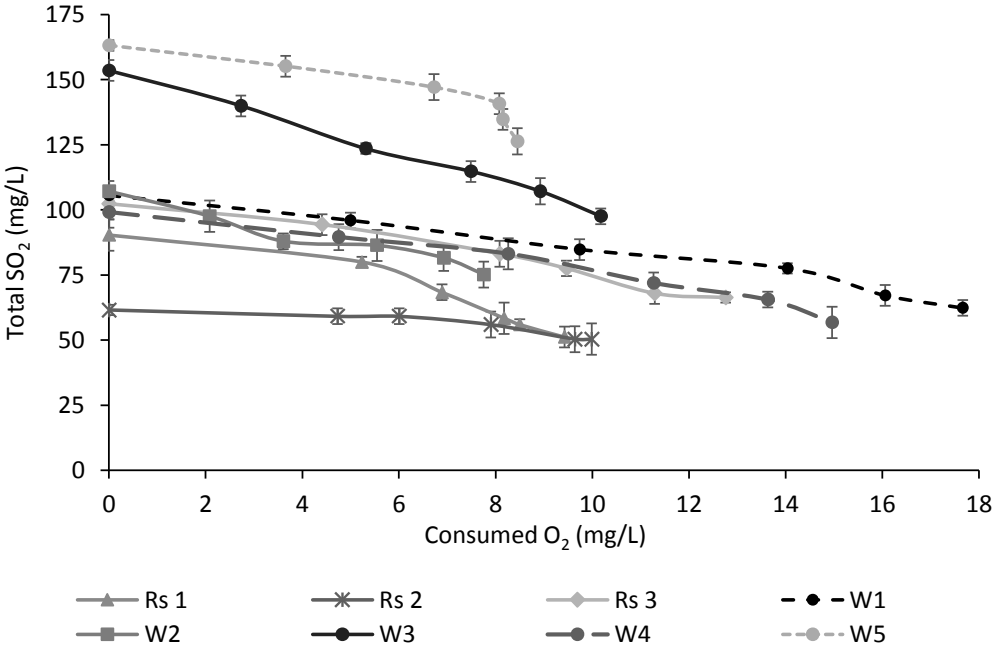
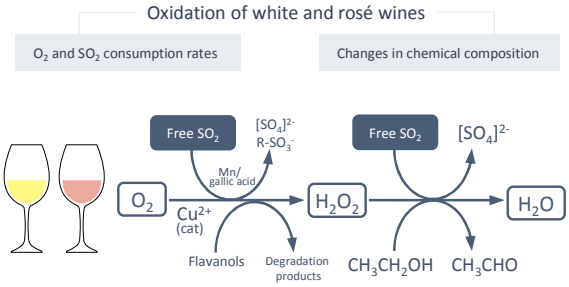

175
150
125
100
75
50
25
0
Total SO2 (mg/L)
0
2
4
6
8
10
12
14
16
18
Consumed O2 (mg/L)
Rs 1
Rs 2
Rs 3
W1
W2
W3
W4
W5

Figure 4

TOC graphic


Oxidation of white and rosé wines
O2 and SO2 consumption rates
Changes in chemical composition
Free SO2
[SO4]2-
R-SO3-
Mn/
gallic acid
O2
Cu2+
(cat)
H2O2
Free SO2
[SO4]2-
H2O
Flavanols
Degradation
products
CH3CH2OH
CH3CHO

Oxygen and SO2 consumption rates in white and rosé wines. Relationship with and effects on wine chemical composition

Journal:	*Journal of Agricultural and Food Chemistry*
Manuscript ID	jf-2017-027629.R2
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Carrascón, Vanesa; Universidad de Zaragoza, Department of Analytical Chemistry Bueno, Mónica; University of Zaragoza, Department of Analytical Chemistry Fernandez-Zurbano, Purificación; University of Rioja, Chemistry Ferreira, Vicente; University of Zaragoza, Analytical Chemistry