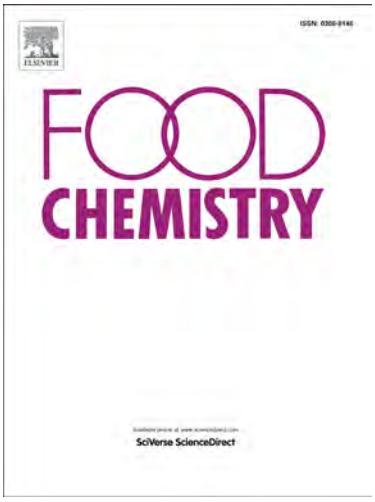
Accepted Manuscript



The kinetics of oxygen and SO_2 consumption by red wines. What do they tell about oxidation mechanisms and about changes in wine composition?

Vanesa Carrascón, Anna Vallverdú-Queralt, Emmanuelle Meudec, Nicolas Sommerer, Purificación Fernandez-Zurbano, Vicente Ferreira

PII: S0308-8146(17)31425-5
DOI: http://dx.doi.org/10.1016/j.foodchem.2017.08.090
Reference: FOCH 21639

To appear in: *Food Chemistry*

Received Date: 19 March 2017
Revised Date: 27 August 2017
Accepted Date: 28 August 2017

Please cite this article as: Carrascón, V., Vallverdú-Queralt, A., Meudec, E., Sommerer, N., Fernandez-Zurbano, P., Ferreira, V., The kinetics of oxygen and SO_2 consumption by red wines. What do they tell about oxidation mechanisms and about changes in wine composition?, *Food Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.foodchem.2017.08.090

The kinetics of oxygen and SO_2 consumption by red wines. What do they tell about oxidation mechanisms and about changes in wine composition?

Vanesa Carrascón[1], Anna Vallverdú-Queralt[2], Emmanuelle Meudec[2], Nicolas Sommerer[2], Purificación Fernandez-Zurbano[3], Vicente Ferreira[1,3]*

[1] Laboratory for Aroma Analysis and Enology. Instituto Agroalimentario de Aragón (IA2-Unizar-CITA). Department of Analytical Chemistry, Faculty of Sciences, Universidad de Zaragoza, 50009, Zaragoza, Spain

[2] Plate-Forme D'analyse des Polyphénols, UMR1083 Sciences Pour l'Œnologie, Institut National de la Recherche Agronomique, Montpellier 34060, France

[3] Instituto de Ciencias de la Vid y el Vino, (Universidad de La Rioja-CSIC-Gobierno de la Rioja), Finca La Grajera, Ctra. De Burgos Km. 6, 26007 Logroño, Spain.

* Corresponding author. Tel.: +34 976 762067; Fax: +34 976 761292. E-mail address: vferre@unizar.es (V. Ferreira)

ABSTRACT

This work seeks to understand the kinetics of O_2 and SO_2 consumption of air-saturated red wine as a function of its chemical composition, and to describe the chemical changes suffered during the process in relation to the kinetics. Oxygen Consumption Rates (OCRs) are faster with higher copper and epigallocatechin contents and with higher absorbance at 620 nm and slower with higher levels of gallic acid and catechin terminal units in tannins. Acetaldehyde Reactive Polyphenols (ARPs) may be key elements determining OCRs. It is confirmed that SO_2 is poorly consumed in the first saturation. Phenylalanine, methionine and maybe, cysteine, seem to be consumed instead. A low SO_2 consumption is favoured by low levels of SO_2, by a low availability of free SO_2 caused by a high anthocyanin/tannin ratio, and by a polyphenolic profile poor in epigallocatechin and rich in catechin-rich tannins. Wines consuming SO_2 efficiently consume more epigallocatechin, prodelphinidins and procyanidins.

KEYWORDS: red wine, oxygen consumption rate, sulfur dioxide, polyphenols, epigallocatechin, tannins, copper, amino acids

1. INTRODUCTION

Nowadays, the use of oxygen in winemaking is widespread around the world to obtain high quality wines. A mild oxidation is known to produce improvements in red wines: more color stability due to reactions of oxygen with anthocyanins (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Cano-Lopez, et al., 2008), softening of astringency and bitterness due to reactions of tannins (Cejudo-Bastante, Hermosin-Gutierrez, & Perez-Coello, 2011), aroma modulation and decrease of vegetative and green perceptions (Cejudo-Bastante et al., 2011; Ortega Heras, Rivero-Perez, Perez-Magarino, Gonzalez-Huerta, & Gonzalez-Sanjose, 2008). However, oxygen can also produce undesirable and unpredictable effects. In this context, research is required concerning the different effects that oxygen consumption has on wine composition, together with a better understanding about the mechanisms implied in wine oxidation. Undeniably, models able to predict how the wine is going to respond to contact with oxygen would be useful for helping winemakers to make wines more resistant towards oxidation.

Sulfur dioxide (SO_2) is the most important chemical used to prevent wine oxidation. It is used worldwide and it has not only antioxidant, but also antimicrobial and antioxidasic properties. Furthermore, SO_2 binds to certain compounds, such as aldehydes, preventing the detection of many oxidation-related off-odors even if they are already present (Bueno, Carrascón, & Ferreira, 2016). On the other hand, SO_2 produces allergic reactions in some individuals, so the maximum levels are legally restricted (European Comission Regulation 606/2009, 2009) and there is an increasing tendency to produce wines containing lower levels of sulfite. For all of this, SO_2 has been used for many years, and although many studies are now being conducted to replace it by new chemicals with similar properties but with less harmful effects (Guerrero & Cantos-Villar, 2015), no replacement has been yet found.

Recently, wine oxidation chemistry has been broadly studied (Danilewicz, 2007; Danilewicz, 2011; Danilewicz, Seccombe, & Whelan, 2008; Laurie, et al., 2012; Singleton, 1987; Ugliano, 2013), and the general mechanism has been already established. In the first place, molecular oxygen accepts electrons from iron and copper ions - Fe(II) and Cu(I) - which act as catalysts forming a superoxide ion $O_2^{\bullet -}$ (hydroperoxyl radical OH-O$^{\bullet}$ at wine pH). Phenolic compounds with a catechol group are oxidized by Fe(III) to a quinone, and the hydroperoxyl is reduced by Fe(II) to hydrogen peroxide. If sulfur dioxide is present, it reacts with hydrogen peroxide, giving sulfate and water, and with the quinone, reducing it back to the catechol form or forming a product with a sulfonate group. The precise outcome depends on pH and on the structure of the polyphenol. Polyphenols having an ortho dihydroxy-or trihydroxy- substitution pattern on the aromatic ring, namely B ring of flavanols, or hydroxy-cinnamic acids (caftaric) or hydroxybenzoicacids (gallic or protocatechuic acids) are more prone to suffer nucleophilic addition. If there is no sulfur dioxide in the wine, hydrogen peroxide can take part in further oxidation steps, such as the Fenton reaction (Gambuti, Han, Peterson, & Waterhouse, 2015). In this case, iron and copper ions interact with hydrogen peroxide to form the hydroxyl radical HO$^{\bullet}$, a strong oxidant that reacts with many organic compounds in wine. The most important oxidation product of this reaction is acetaldehyde, in as much as ethanol is the most concentrated organic compound present in wine.

Acetaldehyde can further react with flavanols and anthocyanins forming pigments with a methylmethine bridge, usually referred as ethyl bridge, between the two units of flavanol and anthocyanin (Somers, 1971; Timberlake & Bridle, 1976). Pyranoanthocyanins are also indirect oxidation products of anthocyanins. In this case a new pyran ring is formed from the nucleophilic addition of alkenes rich in electrons to anthocyanins, such as the addition of acetaldehyde (Bakker & Timberlake, 1997), 4-vinylphenol (Fulcrand, dosSantos, SarniManchado, Cheynier, & FavreBonvin, 1996) or pyruvic acid (Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998).

Previous works on wine oxidation kinetics have shown that oxygen consumption rates are strongly wine-dependent (Vivas & Glories, 1993; Vivas, Vivas de Gaulejac, & Nonier, 2014). In wines, higher pH and added ellagitannins can increase oxygen consumption rates (Singleton, 1987; Vivas & Glories, 1996). In a previous report, initial OCRs were positively related to levels of copper, of tannins rich in epigallocatechin structural units and of absorbance at 620 nm (blue pigments) (Ferreira, Carrascón, Bueno, Ugliano, & Fernández-Zurbano, 2015). Studies in model systems reported a strong dependence of the rate on copper and iron concentrations (Danilewicz, 2007) and a positive effect of SO_2 in presence of catechin and epicatechin (Danilewicz, 2007; Danilewicz, Seccombe, & Whelan, 2008). In wines undergoing micro-oxygenation, OCR strongly increased when free SO_2 was exhausted, which was attributed to the Fenton reaction in which radicals are being continuously formed and reacting (Gambuti, Han, Peterson, & Waterhouse, 2015).

In a previous report two different OCRs were defined: a highly variable initial OCR and a rather stable average OCR. Initial OCRs were much higher and determined oxygen consumption in the first air saturation, notably in the first moments after contact with O_2; average OCRs remained constant in 3 to 4 consecutive cycles of air-saturation (Ferreira, et al., 2015). Unfortunately, in that experiment no specific analyses were performed after the first air-saturation, which made it difficult to assess the specific chemical changes linked to the fast oxygen uptake in the first saturation.

This work seeks to study in detail the kinetics of oxygen and sulfur dioxide consumption during a single air saturation, to determine the relationship between those kinetics and the wine chemical composition and also to evaluate their effects on the chemical changes taking place during the process. For that, commercial red wines have been extensively analytical characterized before and after the oxidation. Chemical analyses have included dissolved

oxygen, sulfur dioxide, acetaldehyde, color, total phenolic index (TPI), Folin-Ciocalteu index, metals, phenolics, tannins, amino acids and aroma compounds.

2. MATERIALS AND METHODS

2.1. Solvents and Chemicals

Dichloromethane, ethanol and methanol for gas chromatography analyses were purchased from Merk (Darmstadt, Germany). Methanol and acetonitrile of HPLC quality were obtained from Fluka Analytical (Buchs, Switzerland). Hydrochloric acid 37%, formic acid and ammonium formate high purity grade were purchased from VWR Prolabo (Fontenay sous Bois, France). Phloroglucinol, ascorbic acid (≥99%), acetaldehyde (≥99.5%), 2-chloroethanol (≥ 99.0%), methyl 2-methylbutyrate (≥ 99%), 2-buthanol (≥ 99%), Folin-Ciocalteu's phenol reagent, sodium carbonate (≥ 99%), gallic acid (≥ 99%) and (+)-catechin (≥ 99%) were supplied by Sigma-Aldrich (Madrid, Spain). Sodium metabisulfite 99% ($Na_2S_2O_5$), tartaric acid (99%), glycerol (99.5%), 1,2-propanediol (99.5%), sodium hydroxide (98%), ortho phosphoric acid (85%), hydrogen peroxide 3 % stabilized w/v VINIKIT, indicator 4,4, mixed (methyl red-methylene blue) VINIKIT, sodium hydroxide 0.01 mol/L VINIKIT were from Panreac (Barcelona, Spain). Standards and reagents for aroma compounds and amino acids determination were purchased from Sigma-Aldrich, Fluka, Panreac, Lancaster, PolyScience, Chemservice and Firmenich, and details of the chemicals have been already reported (Hernandez-Orte, Ibarz, Cacho, & Ferreira, 2003; Lopez, Aznar, Cacho, & Ferreira, 2002; Ortega, Lopez, Cacho, & Ferreira, 2001). Water was purified in a Milli-Q system from Millipore (Bedford, Germany) to get a resistance of 18.2 MΩ·cm at 25 °C.

2.2. Samples and oxidation procedure

For this study, eight Spanish wines of different vintages, between 2009 and 2014, and two different grape varieties, Garnacha (Grenache) and Tempranillo, were purchased at a local

store. Details of the samples together with some compositional parameters are shown in table 1.

Samples were oxidized in duplicate by saturating the wine with air. For each wine, two separate saturation replicates with 500 mL each, were carried out. This was done by gentle shaking 500 mL of wine in a 1 L closed flask for 10 seconds, after which the cap was opened to allow fresh air to enter, and the shaking operation was repeated 2 more times. Air-saturated wine was then distributed in screw capped 60 mL vials strictly avoiding any headspace, as reported by Ferreira *et al.* (Ferreira, et al., 2015). Dissolved oxygen was monitored at least twice a day with PSt3 sensors and an oxygen analyzer from Nomacorc SA (Thimister-Clermont, Belgium). Each saturation cycle was considered complete when the wine consumed 90% of the initial oxygen or after a week. At that time, the vials were opened inside a glove chamber from Jacomex (Dagneux, France) without oxygen (oxygen <0.002%) to get samples for analysis avoiding further oxidation of the wine. The rest of the wine was taken out of the chamber to undergo a new saturation. Initial and average oxygen consumption rates of each wine were determined in duplicate following the method formerly described (Ferreira, Carrascon, Bueno, Ugliano, & Fernandez-Zurbano, 2015).

Initial wines and samples after each saturation were analyzed for free and total SO_2 as well as for free and total acetaldehyde, color parameters, total polyphenol index and Folin-Ciocalteu index. Phenolic and tannin composition, aroma compounds, metals, and amino acids were determined in the initial wine and after the first saturation to study in depth this first oxidation stage.

2.3. Sulfur dioxide and acetaldehyde determination

Free sulfur dioxide and free acetaldehyde were determined by headspace gas chromatography with a mass spectrometer detector (HS-GC-MS) in a QP 2010 GC-MS from Shimadzu (Kyoto, Japan) with a DB-WAX ETR (30 m x 0.25 mm i.d. x 0.25 µm) capillary column from J&W

Scientific (Agilent Technologies, Santa Clara, CA, USA) following the procedure described in previous works (Carrascón, Ontañón, Bueno, & Ferreira, 2017). For the analysis of nominally free SO_2, 4.5 mL of sample acidified with 500 µL of orthophosphoric acid (85%) were incubated at 40 °C for 15 min. After this, 400 µL of the headspace were injected in a split/splitless injector with a 1:4 split ratio. External calibration curves in model wine (5 g/L tartaric acid, 12% ethanol, 1.5% propane-1,2-diol, 10 g/L glycerin, pH 3.5) containing known amounts of sulfur dioxide, obtained by dissolving sodium metabisulfite ($Na_2S_2O_5$) or acetaldehyde were prepared to quantify both compounds. Analyses were performed in duplicate.

For total sulfur dioxide determination, the aspiration/titration method recommended by the OIV (International Organization of Vine and Wine) was used (OIV, 2009b). All of the analyses were performed in duplicate.

Total acetaldehyde was determined by gas chromatography with flame ionization detection (GC-FID) by injection of 1 µL of wine sample spiked with 2-butanol as internal standard. A GC 8000 series from Fisons Instrument (Ipswich, United Kingdom) with a DB-WAX (30 m x 0.53 mm of i.d. x 2 µm) capillary column from J&W Scientific (Agilent Technologies, Santa Clara, CA) were used. The injector was kept at 250 °C and the split ratio was 1:4. Hydrogen was used as carried gas and the pressure was kept at 27.5 kPa. The temperature program was 50 °C for 5 min and then raised to 220 °C in 10 minutes. The FID temperature was 250 °C and the detector gases flows were 95 kPa for the make up gas, 35 kPa for hydrogen and 60 kPa for air. Analyses were performed in duplicate. External calibration in model wine (5 g/L tartaric acid, 12% ethanol, 1.5% propane-1,2-diol, 10 g/L glycerin, pH 3.5) containing known amounts of acetaldehyde was carried out.

2.4. Spectrophotometric measures

For color determination, absorbances at wavelengths 420, 520, and 620 nm of undiluted wine samples were measured using glass cells with optical paths of 1, 2 or 5 mm, taking the

measurement which provided absorbance readings between 0.3 and 0.7, as recommended by the OIV (OIV, 2009).

Total Phenolic Index (TPI) was determined as absorbance at 280 nm as described by Ribereau-Gayon *et al.* (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Folin-Ciocalteau assay was performed following the method described by Singleton *et al.* (Singleton, Orthofer, & Lamuela-Raventos, 1999) using 1 cm quartz cuvettes.

All the absorbance measurements were taken in duplicate, using a UV–vis spectrophotometer UV-17000 Pharma Spec from Shimadzu (Kyoto, Japan).

2.5. Metal analyses

A direct 5-fold aqueous dilution of wine was analyzed by inductively coupled plasma-mass spectrometry with collision/reaction cell (CCT-ICP-MS) as it was described by Grindlay et al. in 2014 (Grindlay et al., 2014) using rhodium as internal standard. Analyses were performed in duplicate. Metals quantified were iron, copper, zinc and manganese.

2.6. Amino acid analyses

For the determination of amino acids (valine, methionine, isoleucine, leucine, phenylalanine, cysteine), a derivatization procedure with aminoquinolyl-N-hydrosysuccinimidyl carbamate (AQC) followed by an analysis by high-performance liquid chromatography (HPLC) was carried out in duplicate, according to the method reported by Hernandez-Orte *et al*. (Hernandez-Orte, et al., 2003). A quaternary HPLC system Waters 2695 from Waters (Milford, MA) with a fluorescence detector ProStar 363 from Varian (Walnut Creek, CA) were used.

2.7. UHPLC–DAD-MS2 Analysis

Ultra-high performance liquid chromatography (UPLC) with diode array detector (DAD) and mass spectrometry (MS) was used to study the nonvolatile matter of wines before and after

oxidation in the first saturation. They were analyzed in triplicate in positive and negative mode. The analysis procedure was described by Vallverdú-Queralt *et al*. (Vallverdu-Queralt, Meudec, Ferreira-Lima, Sommerer, Dangles, Cheynier, et al., 2016). Samples were filtered (0.2 µm) and 0.5 µL of the filtered sample was injected. The equipment used was a Waters Acquity UPLC–DAD system (Waters, Milford, MA, USA), and the column was a reverse phase Acquity BEH C18 column (150 mm length, 1 mm internal diameter, 1.7 µm particle size) from Waters (Milford, MA, USA). The autosampler was kept at 8 °C and the column at 35 °C. Mobile phase consisted of water 1% formic acid (A) and methanol - 1% formic acid (B) and the flow was 0.08 mL/min. Gradient for elution was: isocratic with 2% B (1 min), 2–30% B (1–10 min), isocratic with 30% B (10–12 min), 30–75% B (12–25 min), 75–90% B (25–30 min), and isocratic with 90% B (30–35 min), followed by a column reconditioning. DAD detector recorded the UV–visible spectra from 200 to 650 nm.

The spectrometer hyphenated to the UPLC–DAD system was a Bruker Daltonics Amazon (Bruker, Darmstadt, Germany) mass spectrometer, equipped with an electrospray ion source (ESI) and an ion trap mass analyzer. It was operated in the negative ion mode with a capillary voltage of 4.5 kV and of 2.5 kV in the positive ion mode. The end plate off set was -500 V, temperature was kept at 200 °C, nebulizer gas was at 10 psi and dry gas at 5 L/min. For MS2 fragmentation experiments, collision energy was set at 35 V.

2.8. *Phloroglucinolysis*

Phloroglucinolysis reaction (acid-catalyzed depolymerization in the presence of a nucleophilic agent) was used for studying the composition of condensed tannins following the procedure described by Kennedy and Jones (Kennedy & Jones, 2001) with some modifications. To analyze wine samples, 400 µL of sample were brought to dryness in a centrifugal solvent evaporator (Genevac, Ipswich, UK) to obtain a pellet where the tannins were collected. The solid was dissolved in 600 µL of a solution of 50 g/L of phloroglucinol and 10 g/L of ascorbic acid in

methanol-HCl 0.2M. The mixture was incubated at 50 °C for 20 minutes to complete the reaction, cooled in an ice bath and finally, 600 µL ammonium formiate 200 mM were added to stop the reaction by increasing pH and stabilize the solution. Samples were centrifuged at 15000 rpm at 4 °C for 15 min and the supernatants were collected in 1.2 mL vials for analysis. The reaction was performed in triplicate for each sample. Depolymerized samples were analyzed by UPLC-MS. This analysis gives access to the nature and relative proportions of the terminal subunits and the extension subunits released as phloroglucinol derivatives by depolymerization. The total to terminal subunits molar ratio gives access to the mean degree of polymerization (mDP) (Ducasse, et al., 2010). The samples were analyzed using the same method described for the nonvolatile matter in positive and negative mode. Quantification was done in equivalents of catechin, epicatechin, epigallocatechin and epicatechin-3-*O*-gallate at 280 nm.

2.9. Aroma analyses

Major aroma compounds were determined as described by Ortega *et al.* (Ortega, et al., 2001) with some modifications reported recently (Herrero, 2015), by liquid–liquid microextraction of the wine with dichloromethane and further analysis in a gas chromatography with flame ionization detection in a CP-2800 GC from Varian (Walnut Creek,CA, USA). Analyses were performed in duplicate.

Minor and trace aroma compounds analysis were performed adapting the procedure described by Lopez *et al.* (Lopez, et al., 2002) but with lower sample and solvent volumes. Wine samples (15 mL) were extracted in a 65 mg LiChrolut EN cartridge, cleaned up with 1.5 mL of a 30% methanol in water at pH 3 rising solution and finally eluted with 0.6 mL of dichloromethane with 5% methanol (v/v). Extractions were performed in duplicate. Extracts were directly analyzed by gas chromatography with ion trap mass spectrometry detection

according to the conditions reported by Lopez *et al*. (Lopez, et al., 2002) in a 450-GC and Saturn 2200 GC/MS from Varian (Walnut Creek, CA, USA).

2.10. Data treatment and Statistical Analysis

Partial Least-Squares (PLS) regressions were performed using The Unscrambler 9.7 (CAMO Software AS, Oslo, Norway). Correlation studies and Student's t-test were carried out in Excel 2013 (Microsoft, WA).

3. RESULTS AND DISCUSSION

3.1. Initial oxygen consumption rates (OCRs) and initial wine composition

Wines were oxidized along three consecutive air saturation cycles in duplicate, which made it possible to determine initial and average oxygen consumption rates following a previously described strategy (Ferreira, et al., 2015). In such a procedure, wines are subjected to several consecutive air saturations with daily oxygen monitoring. The representation of the total accumulated amount of oxygen consumed versus time forms a plot in which the points corresponding to the accumulated oxygen consumed after the first, second and third (even fourth and fifth) saturations lie in a straight line. Taking advantage of this fact and applying linear regression analysis, two oxygen consumption rates, an initial and an average rate, can be estimated together with their uncertainty. These data are shown in Table 2. As can be seen, the range of initial OCRs varies from 1.95 ± 0.32 to 6.83 ± 0.87 mg/L/day, levels of O_2 consumed in the first saturation were fairly constant, as were also average OCRs.

In order to understand why wines consume O_2 at different initial rates, these were correlated to the wine chemical composition. Results, summarized in Table 3, revealed that only pH kept a non-significant positive correlation with initial OCRs, while several compounds were significantly and negatively correlated, in agreement with a previous report (Ferreira, et al., 2015). Compounds keeping negative correlations were total acetaldehyde, some constitutive subunits of the tannins determined by phloroglucinolysis and one flavanol monomer,

epicatechin-3-*O*-gallate. Moreover, a highly explicative and significant PLS model relating initial OCRs to initial wine composition could be obtained (model 1 in Table 4). Attending to the model, initial OCRs are negatively correlated to the wine initial contents in total acetaldehyde, gallic acid and to the content of catechin in terminal units of the tannins so that wines with higher levels of these compounds consume oxygen at slower rates. Conversely, wines with higher absorbance at 620 nm, and with higher levels of epigallocatechin and copper consume oxygen faster. The pattern of dependence expressed in the model has a strong resemblance to the model previously obtained (Ferreira, et al., 2015), which already recognized the essential positive role of copper, of the compounds with blue color (responsible for A 620 nm) and the negative contribution of gallic acid. The negative role of total acetaldehyde could not be identified in that work because only free acetaldehyde was measured.

It may be naively thought that the negative role of acetaldehyde on wine initial OCRs would be related to its ability to interact with SO_2. Attending to this, the presence of acetaldehyde, would displace the HSO_3^- + $CH_3CHO \leftrightarrow CH_3CH(OH)SO_3^-$ equilibrium to the right, decreasing levels of free SO_2 which would be less available, slowing down the reduction/reaction with quinones and hence the oxidation process. However, two objections can be made to this explanation. First, that this is valid for free acetaldehyde, rather than for total acetaldehyde. Second, that this explanation assumes that the availability of SO_2 is critical for the development of reaction, as found in synthetic wine (Danilewicz, 2007; Danilewicz, et al., 2008), while neither in the present work or in a previous report (Ferreira, et al., 2015) any model or relationship suggest a relevant role for free or total SO_2 on the kinetics of oxygen consumption in real wines. An alternative explanation should be sought.

A relevant observation in this sense is the fact that wines with highest amounts of acetaldehyde should be wines with smallest amounts of acetaldehyde-reactive polyphenols (ARPs) and, conversely, wines with smallest levels of acetaldehyde should be wines with

highest levels of ARPs. It should be noted that there is not a well-defined category of ARPs. Acetaldehyde is known to react to anthocyanins, flavanols and tannins to form ethyl-bridged polymeric pigments which have less astringency (Cheynier, Duenas-Paton, Salas, Maury, Souquet, Sarni-Manchado, et al., 2006; Vidal, Francis, Noble, Kwiatkowski, Cheynier, & Waters, 2004) and which become resistant to sulfite bleaching (Sheridan & Elias, 2015). According to recent data acetaldehyde may react not only to anthocyanidins, but to phenolic acids and flavonols (Aleixandre-Tudo, Lizama, Alvarez, Nieuwoudt, Garcia, Aleixandre, et al., 2016) or to catechin itself (Sheridan & Elias, 2016). The reaction of some of these ARPs with acetaldehyde is much faster than previously expected and takes places, albeit at lower rates, even in the presence of equimolar amounts of SO_2 (Sheridan & Elias, 2016). The hypothesis is that those ARPs, whose levels would be inversely related to the presence of acetaldehyde, would be very active in determining wine oxidation kinetics. Such hypothesis would be consistent with the fact that wines with highest initial OCRs tended to accumulate smallest levels of acetaldehyde during this first saturation, although in this case the relationship did not reach the statistical level of significance (table 5). It would be also consistent with the fact that copigments derived from ARPs become fairly more inert to SO_2 bleaching and oxidation (Boulton, 2001) and less astringent (Aleixandre-Tudo et al., 2016) after reaction with acetaldehyde. If astringency is mainly driven by the interaction with proline, primary through hydrogen bonds via hydroxyl groups, this could imply that those hydroxyl groups are less available in the ethylene bridged derivatives. Such smaller availability could also affect their interaction with metals catalyzing the oxidation to quinones. Additional experimental work is required to confirm this hypothesis.

It should be noted that for the second time, initial OCRs have not been found to be related to the wine contents in SO_2 or iron, while in model solutions these parameters were relevant (Danilewicz, 2007; Danilewicz, et al., 2008). This apparent discrepancy could be related to limitations of the analytical methodologies or simply to the fact that in real wine the other parameters are kinetically determinant.

3.2. Initial oxygen consumption rates (OCRs) and effects on wine composition

The chemical changes suffered by the wines in the first saturation are summarized in Table 5, together with their correlation with initial OCRs. As can be seen, none of the compounds more affected by oxidation were significantly correlated to OCR. Additionally, it should be noted that the positive correlation coefficients at the bottom of the table belong to species whose levels decreased during oxidation, and hence indicate that decreases are smaller in wines consuming oxygen faster. This means that simple correlation analysis does not provide any clue about the existence of any chemical being consumed at higher levels in wines with higher OCRs. This may be in part related to limitations of the analyses currently used to characterize the phenolic polymeric fraction of wines. Indeed, condensed tannins are only expressed with respect to units released by the depolymerization and it has been demonstrated in model solutions that the oxidation of tannins generates bonds which do not break under the conditions of the depolymerization reaction. The oxidation products remaining as oligomeric residues appear in the chromatographic profile as an unresolved and large broad peak more or less flat, under the individual peaks of the unoxidized monomers released from depolymerization. These oxidized products are not taken into account, to date, in the analysis of tannins. Other reaction products, such as ethyl bridged, direct reaction with anthocyanins or other molecules are detected with specific markers but are not quantified. Many publications about wine oxidation and tannins have addressed this issue in the last decade (Bindon, McCarthy, & Smith, 2014; Mouls & Fulcrand, 2012; Poncet-Legrand, Cabane, Bautista-Ortin, Carrillo, Fulcrand, Perez, et al., 2010; Vallverdú-Queralt, Meudec, Eder, Lamuela-Raventos, Sommerer, & Cheynier, 2017; Vernhet, Dubascoux, Cabane, Fulcrand, Dubreucq, & Poncet-Legrand, 2011). Nevertheless, data in the table confirm that wines with high initial OCRs have a different pattern of oxidation characterized by smaller decreases in some subunits released by depolymerization, particularly epicatechin and catechin as terminal units or epicatechin-3-o-gallate and catechin as extension units.

PLS modelling has made it possible to find a highly explicative and significant model relating initial OCRs to the chemical changes observed during the first saturation (model 2 in Table 4). The model indicates that wines with high initial OCRs produce smaller amounts of total acetaldehyde which, as earlier discussed, could be due to the fact that these wines contain large amounts of ARPs so that acetaldehyde would be quickly removed by reaction. The model also suggests that tannins containing epicatechin-3-o-gallate as terminal units are consumed in wines with high initial OCRs, while they are slightly produced in wines with low initial OCRs. Gallic acid and tannins rich in catechin as terminal units decrease at higher levels in wines with low OCRs, which suggests that they could be substrates consumed in the oxidation, likely by reaction with SO_2, and that such consumption is more intense at low rates. A final remark that can be extracted from the PLS model is that different OCRs in red wines involve different changes in the profile of units released from the depolymerization of tannins, especially in the terminal units: while slow O_2 consumptions are related to strong decreases in catechin units and increases in epicatechin-3-O-gallate as terminal units, fast OCRs are linked to higher decreases in epicatechin-3-O-gallate and increases in catechin as terminal units.

3.3. Consumption of sulfur dioxide and its relationship to the initial chemical composition

Data related to the consumption of SO_2 are summarized in Table 2. As can be seen, for an oxygen consumption in the first saturation around 7 mg/L, these wines consumed between 9.6 and 18.8 mg/L of SO_2, well below the 28 mg/L theoretically possible according to the expected 2:1 SO_2:O_2 stoichiometric molar ratio (Ferreira et al., 2015). These means that between 34 and 61% of the electrons taken by O_2 during its reduction proceeded from SO_2 while the rest, corresponding to between 3 and 4.7 mg/L of O_2, proceeded from the oxidation of other wine components, so that the actual molar ratios were between 0.69 and 1.22. All these parameters are listed in the table under the headings "SO_2 efficiency", "O_2 not SO_2" and "molar ratio

SO_2:O_2", respectively, while their correlation coefficients with the wine chemical composition are summarized in Table 6.

Most remarkably, the "SO_2 efficiency" in the second saturation which is given in the last column in Table 2, clearly increases in 5 out of the 8 samples, meaning that for a similar O_2 uptake, the wines consumed more SO_2 in the second saturation, even if there was less SO_2 available. Moreover, such increases were particularly high in the samples T2_12 and above all in T1_11. Such increase is in agreement with results from a previous publication (Carrascón et al., 2015) in which the existence of a "preSO_2 stage" was described. This phase takes place during the first saturation in some of the wines and is characterized by a very low SO_2 consumption, which increases in the second and subsequent saturations if there remains enough SO_2.

A low SO_2 consumption during the first saturation indicates that wine should contain other constituents able to compete with SO_2 for the Fe(III) primarily formed upon O_2 uptake, for the quinones or for the H_2O_2 generated during oxidation. Those constituents will be depleted in the first saturation, which explains why in the second saturation the consumption of SO_2 increases. The competition is obviously enhanced by the fact that some of the wines contained relatively low amounts of SO_2, as seen in Table 1. Nevertheless, it is noteworthy that SO_2 consumption is just weakly correlated to the levels of free or total SO_2 of the wine, as seen in Table 6, which suggests that there are other more influential factors determining SO_2 consumption. The list below enumerates some possibilities explaining a low SO_2 consumption together with the expected correlation with the SO_2 consumption parameters:

1. The existence of highly reactive nucleophiles able to react to quinones before SO_2 (positive correlation to O_2 not SO_2, negative to SO_2:O_2 molar ratio and SO_2 efficiency)
2. The existence of an antioxidant able to react to Fe(III) or to H_2O_2 before SO_2 (positive correlation to O_2 not SO_2, negative to SO_2:O_2 molar ratio and SO_2 efficiency)

3. The absence of reactive polyphenols whose quinones can easily be reduced back by SO_2 or react with it (negative correlation to O_2 not SO_2, positive to SO_2:O_2 molar ratio and SO_2 efficiency)
4. The presence of polyphenols forming stable quinones less reactive to SO_2 than to other nucleophiles (positive correlation to O_2 not SO_2, negative to SO_2:O_2 molar ratio and SO_2 efficiency)

On the basis of the correlation coefficients shown in Table 6, epigallocatechin and prodelphinidins seem to be the reactive polyphenols mentioned in third place and wines containing higher levels of these compounds will consume higher levels of SO_2 during oxidation. Conversely, tannins rich in catechin (terminal + extension units) would belong to the fourth category and, accordingly, wines richest in these tannins would consume little SO_2 during oxidation. Finally, methionine may act as one of the antioxidants mentioned in the second category. Methionine can easily form sulfoxides and sulfones by direct oxidation with Fe(III) (Firouzabadi, Iranpoor, & Zolfigol, 1998).

A quite simple and significant PLS model relating initial composition to the SO_2:O_2 molar ratio (or SO_2 efficiency) was obtained (Table 4 model 3) and indicates that the amount of SO_2 consumed during oxidation depends, essentially, on the wine content in epigallocatechin, either free or in tannins, and negatively to the anthocyanin/tannin ratio of the wine. This ratio was calculated as the ratio of anthocyanins determinated by UPLC-DAD and the total tannins resulted in the phloroglucinolysis essay, both of them in mg/L units, and which ranged from 0.03 to 1.30 in this experiment (table 1). The anthocyanin/tannin ratio is important in enology because critically determines the success of co-pigmentation (Boulton, 2001)), as probable indicator of the aptitude for wine ageing (Pérez-Magariño & González-San José, 2006) and as the model suggests, is could be also relevant for determining the efficiency in the use of SO_2. Its negative role could be due to the fact that higher levels of anthocyanins would be

responsible for a "de facto" smaller availability of free SO_2 because they form weak associations to SO_2. This result would be in agreement with recent estimations made by Coelho *et al.* (Coelho, Howe & Sacks, 2015) and would confirm that the reference aspiration-titration method does not give an accurate estimation of free SO_2.

3.4. Consumption of sulfur dioxide and its effects on the chemical composition

The magnitude of the compositional changes observed during the first saturation were also studied in relation to the efficiency in the use of SO_2. According to our previous reasoning, it can be argued that a poorer SO_2 consumption should concur with:

1. A stronger decrease of any antioxidant competing with SO_2 (negative correlation to O_2 not SO_2, positive to SO_2:O_2 molar ratio and SO_2 efficiency)
2. A stronger decrease of any nucleophile competing with SO_2 for reacting with quinones (negative correlation to O_2 not SO_2, positive to SO_2:O_2 molar ratio and SO_2 efficiency)
3. Smaller decreases of the polyphenols which oxidize strictly following the SO_2-centered oxidation cycle (positive correlation to O_2 not SO_2, negative to SO_2:O_2 molar ratio and SO_2 efficiency)
4. Higher increases of the reaction products derived from the compounds in 1) and 2) (positive correlation to O_2 not SO_2, negative to SO_2:O_2 molar ratio and SO_2 efficiency)
5. Smaller increases of the reaction products derived from the compounds in 3) (negative correlation to O_2 not SO_2, positive to SO_2:O_2 molar ratio and SO_2 efficiency)

Results of the correlation study are shown in table 6 and indicate, first, that methionine could belong to the first category, acting as antioxidant competing with SO_2, and reacting well with both Fe(III) and H_2O_2. This is supported in the significant increase of its sulfoxide, shown in table 5, in the fact that such increase is significantly correlated to the decrease of methionine ($P=0.018$) and in the weak negative correlation between its sulfone and the SO_2 efficiency shown in Table 6. The sulfone would belong to the fourth category in the previous list. It is also

worth mentioning, that although not correlated to SO_2 efficiency, the oxidation involved a significant increase in the sulfonate of cysteine (table 5), which suggests that cysteine may act also as antioxidant replacing SO_2. Second, phenylalanine seems to belong to the 2nd category in the list, competing with SO_2 for quinones. The addition products of this amino acid have not been identified so that different reaction mechanisms, including the Strecker degradation to form phenylacetaldehyde should be also possible. The formation of the aldehyde has been reported to take place when levels of free SO_2 drop below 5 mg/L (Bueno, et al., 2016), a threshold reached during the first saturation for most of the wines in the study. It is also worthmentioning that the adduct of cysteine with procyanidin increases significantly during oxidation (table 5), indicating that cysteine also acts as nucleophile. Third, epigallocatechin and prodelphinidins, together with procyanidins are the phenols in the third category: compounds which oxidize strictly following the SO_2–centered oxidation cycle with a large concomitant consumption of SO_2. Since these compounds decrease (table 5), their quinones should directly react to SO_2 rather than being reduced back to the o-diphenol form. Gallic acid would behave contrarily and since its decrease is positively correlated to the initial free SO_2 levels it seems that this phenol is preferably consumed whenever there is few free SO_2 available. Finally, the pigment guaiacylpyranopeonidin-3-*O*-glucoside, which is a pyranoanthocyanidin known to be formed by reaction of vinylguaiacol with peonidin (Vallverdú-Queralt, et al., 2016) would belong to the fifth category in the previous list.

The best PLS model explaining the effects of efficiency on the pattern of change of the wine is seen in Table 4 (model 4) and simply states that an efficient use of SO_2 is linked to larger decreases of epigallocatechin, prodelphinidins and procyanidin and to a smaller consumption of phenylalanine.

Finally, changes in aroma composition were not very intense (data not shown). It is worth mentioning the fact that *E*-isoeugenol increased significantly and that the increase was

negatively and significantly related to SO_2 efficiency. This could suggest that this volatile phenol is the ending point of an oxidative degradation of polyphenols which are preferably consumed through the SO_2-alternate mechanism for O_2 consumption.

4. CONCLUSIONS

Red wines can consume oxygen at quite different initial OCRs, which are critically dependent on wine content in copper, epigallocatechin and on A620 and are negatively related to wine content in gallic acid and in catechin as terminal units of depolymerisable tannins. The apparent role played by acetaldehyde lead to the hypothesis that Acetaldehyde Reactive Polyphenols (ARPs) may be key elements determining O_2 consumption kinetics. Different initial OCRs involve also different changes in the profile of tannins that are able to undergo depolymerization: low initial OCRs are related to strong decreases in catechin terminal subunits and increases in epicatechin-3-O-gallate as terminal units of tannins; fast OCRs are linked to higher decreases in epicatechin-3-O-gallate and increases in catechin as terminal units in tannins.

Regarding SO_2 consumption, it is confirmed that in many wines SO_2 is particularly poorly consumed in the first saturation. Altogether our results strongly suggest that some amino acids, notably methionine, phenylalanine and maybe, cysteine, are consumed in the first saturation replacing SO_2. Preliminary results indicate that cysteine could act as both reactive nucleophile and as antioxidant and methionine as antioxidant, while the mechanism of action of phenylalanine is not clear at present. A low SO_2 consumption, and hence an enhanced amino acid consumption, would be favoured by low levels of SO_2, by a low availability of free SO_2 caused by a high anthocyanin/tannin ratio, and by a polyphenolic profile poor in epigallocatechin and rich in tannins rich in catechin. On the contrary, wines consuming SO_2 efficiently tend to consume large amounts of epigallocatechin, prodelphinidins and procyanidins (particularly C type).

5. FUNDING SOURCES

This work has been funded by the Spanish MINECO (Project AGL2014-59840 and RTC-2015-3379) and partly cofunded by the European Union (FEDER). V.C. received a grant from the Spanish FPU program. Universidad de Zaragoza, Fundación Bancaria Ibercaja and Fundación CAI are acknowledged for the research stay grant CB3/15. Funding from Diputación General de Aragón (T53) and Fondo Social Europeo is acknowledged.

6. ACKNOWLEDGEMENTS

Hélène Fulcrand is acknowledged by providing fruitful discussion.

The authors declare no conflict of interest.

7. REFERENCES

Aleixandre-Tudo, J. L., Lizama, V., Alvarez, I., Nieuwoudt, H., Garcia, M. J., Aleixandre, J. L., & du Toit, W. J. (2016). Effect of acetaldehyde addition on the phenolic substances and volatile compounds of red Tempranillo wines. *Australian Journal of Grape and Wine Research, 22*(2), 205-214.

Atanasova, V., Fulcrand, H., Cheynier, W., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta, 458*(1), 15-27.

Bakker, J.; Timberlake, C. F. (1997). Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *Journal of Agricultural and Food Chemistry 45(1)*, 35-43.

Bindon, K. A., McCarthy, M. G., & Smith, P. A. (2014). Development of wine colour and non-bleachable pigments during the fermentation and ageing of (Vitis vinifera L. cv.) Cabernet Sauvignon wines differing in anthocyanin and tannin concentration. Lwt-Food Science and Technology, 59(2), 923-932.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture, 52*(2), 67-87.

Bueno, M., Carrascón, V., & Ferreira, V. (2016). Release and Formation of Oxidation-Related Aldehydes during Wine Oxidation. *Journal of Agricultural and Food Chemistry, 64*(3), 608-617.

Cano-Lopez, M., Pardo-Minguez, F., Schmauch, G., Saucier, C., Teissedre, P.-L., Lopez-Roca, J. M., & Gomez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *Journal of Agricultural and Food Chemistry, 56*(14), 5932-5941.

Carrascón, V., Ontañón, I., Bueno, M., & Ferreira, V. (2017). Gas chromatography-mass spectrometry strategies for the accurate and sensitive speciation of sulfur dioxide in wine. *Journal of Chromatography A*, *1504*, 27-34.

Cejudo-Bastante, M. J., Hermosin-Gutierrez, I., & Perez-Coello, M. S. (2011). Micro-oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics, volatile composition and sensory characteristics. Part I: Petit Verdot wines. *Food Chemistry, 124*(3), 727-737.

Cheynier, V., Duenas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture, 57*(3), 298-305.

Coelho, J.M., Howe, P.A., & Sacks, G.L. (2015). A Headspace Gas Detection Tube Method to Measure SO2 in Wine without Disrupting SO2 Equilibria, *American Journal of Enology and Viticulture*, *66*, 257-265.

Danilewicz, J. C. (2007). Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *American Journal of Enology and Viticulture, 58*(1), 53-60.

Danilewicz, J. C. (2011). Mechanism of Autoxidation of Polyphenols and Participation of Sulfite in Wine: Key Role of Iron. *American Journal of Enology and Viticulture, 62*(3), 319-328.

Danilewicz, J. C., Seccombe, J. T., & Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *American Journal of Enology and Viticulture, 59*(2), 128-136.

European Comission Regulation (EC) No 606/2009. (2009), vol. Annex I B.

Felipe Laurie, V., Zuniga, M. C., Carrasco-Sanchez, V., Santos, L. S., Canete, A., Olea-Azar, C., Ugliano, M., & Agosin, E. (2012). Reactivity of 3-sulfanyl-1-hexanol and catechol-containing phenolics in vitro. *Food Chemistry, 131*(4), 1510-1516.

Ferreira, V., Carrascón, V., Bueno, M., Ugliano, M., & Fernandez-Zurbano, P. (2015). Oxygen Consumption by Red Wines. Part I: Consumption Rates, Relationship with Chemical Composition, and Role of SO2. *Journal of agricultural and food chemistry, 63*(51), 10928-10937.

Firouzabadi, H., Iranpoor, N., & Zolfigol, M. A. (1998). Selective and efficient transformation of thioethers to their sulfoxides and catalytic conversions of thiols to the disulfides with hydrated iron(III) and copper(II) nitrates in aprotic organic solvents or under solvent free conditions. *Synthetic Communications, 28*(7), 1179-1187.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry, 47*(7), 1401-1407.

Fulcrand, H., dosSantos, P. J. C., SarniManchado, P., Cheynier, V., & FavreBonvin, J. (1996). Structure of new anthocyanin-derived wine pigments. *Journal of the Chemical Society-Perkin Transactions 1*(7), 735-739.

Gambuti, A., Han, G., Peterson, A. L., & Waterhouse, A. L. (2015). Sulfur Dioxide and Glutathione Alter the Outcome of Microoxygenation. *American Journal of Enology and Viticulture, 66*(4), 411-423.

Grindlay, G., Mora, J., de Loos-Vollebregt, M.T.C., & Vanhaecke, F. (2014). Evaluation of the multi-element capabilities of collision/reaction cell inductively coupled plasma-mass spectrometry in wine analysis. *Talanta, 128*, 379-385.

Guerrero, R. F., & Cantos-Villar, E. (2015). Demonstrating the efficiency of sulphur dioxide replacements in wine: A parameter review. *Trends in Food Science & Technology, 42*(1), 27-43.

Hernandez-Orte, P., Ibarz, M. J., Cacho, J., & Ferreira, V. (2003). Amino acid determination in grape juices and wines by HPLC using a modification of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method. *Chromatographia, 58*(1-2), 29-35.

Herrero P. (2015). Caracterización químico-sensorial del aroma de vinos procedentes de diferentes variedades tintas y blancas. Nuevas estrategias cuantitativas y quimio-sensoriales. Universidad de Zaragoza, Zaragoza, Spain.

Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. Journal of Agricultural and Food Chemistry, 49(4), 1740-1746.

Lopez, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A, 966*(1-2), 167-177.

Mouls, L., & Fulcrand, H. (2012). UPLC-ESI-MS study of the oxidation markers released from tannin depolymerization: toward a better characterization of the tannin evolution over food and beverage processing. Journal of Mass Spectrometry, 47(11), 1450-1457.

OIV. (2009). Compendium of International Methods of Analysis.

Ortega, C., Lopez, R., Cacho, J., & Ferreira, V. (2001). Fast analysis of important wine volatile compounds Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. *Journal of Chromatography A, 923*(1-2), 205-214.

Ortega Heras, M., Rivero-Perez, M.D., Perez-Magarino, S., Gonzalez-Huerta, C., & Gonzalez-Sanjose, M.L. (2008). Changes in the volatile composition of red wines during aging in oak barrels due to microoxygenation treatment applied before malolactic fermentation. *European Food Research and Technology, 226*(6).

Pérez-Magariño, S., & González-San José, M.L. (2006). Polyphenols and Colour Variability of Red Wines Made from Grapes harvested at different ripeness grade. *Food Chemistry*, 96 (2), 197-208.

Poncet-Legrand, C., Cabane, B., Bautista-Ortin, A.-B., Carrillo, S., Fulcrand, H., Perez, J., & Vernhet, A. (2010). Tannin Oxidation: Intra- versus Intermolecular Reactions. Biomacromolecules, 11(9), 2376-2386.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of Enology. Volume 2. The Chemistry of Wine Stabilization and Treatments* (New York ed. Vol. 2). Chichester: John Wiley & Sons.

Sheridan, M. K., & Elias, R. J. (2015). Exogenous acetaldehyde as a tool for modulating wine color and astringency during fermentation. *Food Chemistry, 177*, 17-22.

Sheridan, M. K., & Elias, R. J. (2016). Reaction of Acetaldehyde with Wine Flavonoids in the Presence of Sulfur Dioxide. *Journal of Agricultural and Food Chemistry, 64*(45), 8615-8624.

Singleton, V. L. (1987). Oxygen with Phenols and Related Reactions in Musts, Wines and Model Systems - Observations and Practical Implications. *American Journal of Enology and Viticulture, 38*(1), 69-77.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In L. Packer (Ed.), *Oxidants and Antioxidants, Pt A*, vol. 299 (pp. 152-178).

Somers, T. C. (1971). Polymeric nature of wine pigments. *Phytochemistry, 10*(9), 2175-&.

Timberlake, C. F., & Bridle, P. (1976). Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *American Journal of Enology and Viticulture, 27*(3), 97-105.

Ugliano, M. (2013). Oxygen Contribution to Wine Aroma Evolution during Bottle Aging. *Journal of Agricultural and Food Chemistry, 61*(26), 6125-6136.

Vallverdu-Queralt, A., Meudec, E., Ferreira-Lima, N., Sommerer, N., Dangles, O., Cheynier, V., & Le Guerneve, C. (2016). A comprehensive investigation of guaiacyl-pyranoanthocyanin synthesis by one-/two-dimensional NMR and UPLC-DAD-ESI-MSn. *Food Chemistry, 199*, 902-910.

Vallverdú-Queralt, A., Meudec, E., Eder, M., Lamuela-Raventos, R. M., Sommerer, N., & Cheynier, V. (2017). The Hidden Face of Wine Polyphenol Polymerization Highlighted by High-Resolution Mass Spectrometry. ChemistryOpen, 6(3), 336--339.

Vernhet, A., Dubascoux, S., Cabane, B., Fulcrand, H., Dubreucq, E., & Poncet-Legrand, C. (2011). Characterization of oxidized tannins: comparison of depolymerization methods, asymmetric flow field-flow fractionation and small-angle X-ray scattering. Analytical and Bioanalytical Chemistry, 401(5), 1559-1569.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta, 513*(1), 57-65.

Vivas, N., & Glories, Y. (1993). Les phénoménes d'oxydoreduction liés à l'élevage en barrique des vins rouges: Aspectd technologiques. *Rev. Fr. Oenol.*(33), 33-38.

Vivas, N., & Glories, Y. (1996). Role of oak wood ellagitannins in the oxidation process of red wines during aging. *American Journal of Enology and Viticulture, 47*(1), 103-107.

Vivas, N., Vivas de Gaulejac, N., & Nonier, M. F. (2014). Quelques aspect cinétiques de la consommation de l'oxygène et conséquences technologiqes des oxydations dans les vins rouges. *Rev. des Oenologues*(150), 27-31.

Table 1. Basic details about the wines used in the study together with chemical data relevant to the oxidation process

Wine Code	DO	Grape Variety	Vintage	Ethanol (v/v)	Total acetaldehyde (mg/L)	Total SO_2 (mg/L)	Free SO_2 (mg/L)	pH	Fe (mg/L)	Cu (µg/L)	Tannins (mg/L)	mDP	Anthocyanins (mg/L) / Tannins (mg/L)
G1_09	CB	G	2009	14.0	15.90	31	2.6	3.32	1.59	28.7	688	4.8	0.029
G2_13	CB	G	2013	15.0	17.63	31	10.5	3.26	1.25	97.8	729	5.2	1.155
G3_14	Cl	G	2014	14.5	20.33	43	14.8	3.29	1.37	55.2	1468	7.8	0.943
G4_14	CB	G	2014	13.5	16.38	37	14.4	3.31	1.50	55.4	1155	6.5	1.350
T1_11	Rj	T	2011	13.5	19.61	76	24.7	3.51	1.25	59.2	819	6.9	0.236
T2_12	Tr	T	2012	14.5	17.78	77	33.7	3.60	0.87	145.3	1363	9.5	0.416
T3_10	Rj	T	2010	13.5	13.90	40	13.3	3.61	1.18	44.3	757	7.6	0.164
T4_14	Rj	T	2014	13.5	17.25	24	10.1	3.58	2.17	239.7	1066	8.4	1.302

DO: Denomination of origin; CB: Campo de Borja; Cl: Calatayud, Rj: Rioja; Tr: Toro.
G: Garnacha; T: Tempranillo

Table 2. Initial and average oxygen consumption rates (OCRs) and data about the oxygen and sulfur dioxide consumed during the first saturation. Standard deviations shown in brackets.

	Initial rate (mg O_2/L/day)	Average rate (mg O_2/L/day)	Consumed O_2 (mg/L)	Consumed SO_2 (mg/L)	O_2 not SO_2 (mg/L)	Molar ratio SO_2:O_2	SO_2 efficiency (%)	SO_2 efficiency (%) (2^{nd} sat)
G1_09	3.70 (0.54)	0.98 (0.05)	7.22 (0.22)	11.2 (1.13)	4.42 (0.07)	0.78 (0.06)	39.1	40.0
G2_13	4.90 (0.19)	0.905 (0.02)	7.42 (0.13)	10.8 (3.96)	4.72 (0.86)	0.73 (0.25)	36.4	39.1
G3_14	1.95 (0.32)	0.93 (0.02)	7.32 (0.08)	14.8 (2.83)	3.62 (0.63)	1.01 (0.18)	50.6	57.2
G4_14	4.29 (0.25)	0.97 (0.02)	6.94 (0.09)	9.6 (2.26)	4.54 (0.66)	0.69 (0.17)	34.6	43.9
T1_11	4.40 (0.49)	0.88 (0.04)	7.69 (0.28)	18.8 (3.39)	2.99 (0.57)	1.22 (0.18)	61.1	91.8
T2_12	5.13 (1.11)	0.98 (0.10)	7.60 (0.12)	16.8 (3.39)	3.40 (0.97)	1.11 (0.24)	55.3	70.1
T3_10	6.83 (0.87)	1.16 (0.10)	7.45 (0.24)	16.0 (0.00)	3.45 (0.24)	1.07 (0.03)	53.7	48.2
T4_14	5.29 (0.11)	1.25 (0.01)	7.65 (0.06)	14.0 (1.13)	4.15 (0.22)	0.92 (0.07)	45.8	36.7

Table 3. Relevant correlation coefficients between initial oxygen consumption rates and wine initial compositional parameters.

	Correlation coefficients with initial OCRs
pH	0.689 #
Total Acetaldehyde	-0.708 *
Epicatechin-3-*O*-gallate	-0.710 *
Catechin terminal unit ‡	-0.649 #
Epicatechin terminal unit ‡	-0.757 *
Catechin-ethyl extension unit ‡	-0.713 *

ns: not significant; # $p(t) < 0.1$; *: $p(t) < 0.05$; ‡ released from tannin depolymerisation

Table 4. PLS regression models explaining: 1) the initial oxygen consumption rate in red wines as a function of the wine chemical composition 2) the relationship between initial OCRs and the chemical changes observed after the first saturation 3) SO_2 efficiency (as consumed SO_2:O_2 molar ratio) to the initial composition of the wines 4) relationships between SO_2 efficiency and the chemical changes observed during its oxidation

	PLS Regression Model	PCs	RMSE	R^2
1	Initial rate = 4.560 − 0.766 Total Acetaldehyde - 0.469 Gallic acid − 0.475 Catechin terminal unit (‡) + 0.195 Absorbance 620 nm + 0.310 Epigallocatechin + 0.306 Cu	5	0.046 (0.255)[a]	0.999 (0.971)[a]
2	Initial rate = 4.560 − 0.606 ΔTotal Acetaldehyde – 0.114 ΔFolin-Ciocalteu index – 0.762 ΔEpicatechin-3-*O*-gallate terminal unit (‡) + 0.729 ΔGallic acid + 0.494 ΔCatechin terminal unit (‡)	4	0.0713 (0.202)[a]	0.997 (0.982)[a]
3	SO_2:O_2 molar ratio = 0.940 - 0.085 Anthocyanins/Tannins + 0.053 % epigallocatechin in tannins + 0.121 Epigallocatechin	2	0.051 (0.071)[a]	0.921 (0.884)[a]
4	SO_2:O_2 molar ratio = 0.940 - 0.052 ΔProdelphinidins -0.061 ΔEpigallocatechin - 0.048 ΔProcyanidins + 0.053 ΔPhenylalanine	1	0.053 (0.076)[a]	0.915 (0.866)[a]

(‡): released from tannin depolymerization; [a] Values of the model by cross-validation.

Table 5. Average increments during oxidation and relevant correlation coefficients between initial oxygen consumption rates and increases of compounds measured in the first oxidation cycle. Except where indicated, increments are in mg/L.

	Average Increment	Correlation coefficients with initial OCRs
Total SO_2	-14.00 (-31.2 %) ***	ns
Free SO_2	-6.21 (-40.1%) ***	ns
Combined SO_2	-7.79 (-26.5 %) ***	ns
Total Acetaldehyde	1.63 (9.43 %) *	-0.625 #
Free Acetaldehyde	1.53 (100.3 %) *	ns
TPI [a]	-1.38 (-2.43 %) *	ns
Abs420 [a]	0.266 (7.01 %) ***	ns
Abs520 [a]	0.356 (7.77 %) *	ns
Abs620 [a]	0.089 (8.22 %) **	ns
Color total [a]	0.711 (7.51 %) **	ns
Catechin-ethyl extension units ‡ [b]	$3.40 \cdot 10^7$ (12.3 %) **	0.629 #
Catechin (terminal + extension) ‡	0.525 (6.78 %) *	ns
Epigallocatechin terminal unit ‡	-1.55 (-16.0 %) *	ns
Catechin-ethyl-Malvidin-3-O-Glucoside [b]	$1.30 \cdot 10^6$ (15.2 %) **	ns
Epigallocatechin	-1.37 (-12.7 %) **	ns
Catechin	-3.71 (-11.0 %) **	ns
Epicatechin	-1.39 (-8.93 %) *	ns
Epicatechin-3-O-gallate	-0.036 (-29.34 %) *	ns
Methionine	-2.54 (-15.0 %) *	ns
Methionine-sulfoxide [b]	$1.12 \cdot 10^6$ (14.0 %) **	ns
Cysteine-sulfonate [b]	$8.86 \cdot 10^5$ (13.6 %) *	ns
Procyanidin-cysteine [b]	$1.29 \cdot 10^6$ (37.0 %) **	ns
Compounds not reaching the level of significance but important for correlations and PLS models		
Folin-Ciocalteu [c]	2.61 (0.112 %) ns	
Total depolymerised units from tannins	-27.08 (-8%) ns	0.639 #
Catechin terminal unit ‡	-0.289 (-1.29%) ns	0.724 *
Epicatechin terminal unit ‡	-1.06 (-7.17%) ns	0.769 *
Epicatechin-3-o-gallate terminal unit ‡	-0.007 (-2.80 %) ns	ns
Epigallocatechin extension unit ‡	-6.67 (-9.52%) ns	0.663 #
Catechin extension unit ‡	-0.264 (-10.4 %) ns	0.731 *
Epicatechin-3-O-gallate extension unit ‡	-0.795 (-13.0 %) ns	0.813 *
Procyanidins	-10.8 (-3.38%) ns	0.632 #
Prodelphinidins	-3.22 (-2.72 %) ns	ns
Gallic acid	-3.22 (11.8 %) ns	ns

[a] Absorbance units. [b] Area units. [c] Concentration in Gallic acid equivalents (mg/L). ‡: released from tannin depolymerization. ns: not significant; # p(t) < 0.1*: p(t) < 0.05 ; **: p(t) < 0.01.

Table 6. Relevant correlation coefficients between the two parameters related to the efficiency in the consumption of sulphur dioxide during oxidation and the initial wine chemical composition and between SO_2 efficiency parameters and the compositional changes measured during the first oxidation cycle.

Initial composition	O_2 not SO_2	Molar ratio SO_2:O_2 or SO_2 efficiency	
Total SO_2	-0.785 *	0.759 *	
Free SO_2	-0.690 #	0.678 #	
pH	-0.643 #	0.693 #	
Abs 520 nm	0.675 #	ns	
Prodelphinidins	-0.721 *	0.692 #	
mDP	ns	0.629 #	
% extension units ‡	-0.641 #	0.646 #	
% terminal units ‡	0.641 #	-0.646 #	
Catechin ‡	0.876 **	-0.848 **	
Epigallocatechin	-0.867 **	0.902 **	
Methionine	0.638 #	-0.688 #	
Compounds decreasing in the first saturation	O_2 not SO_2	Molar ratio SO_2:O_2 or SO_2 efficiency	Initial Free SO_2
Δ Prodelphinidins	0.907 **	-0.918 **	ns
Δ Procyanidins	0.892 **	-0.912 **	ns

Δ Gallic acid	-0.627 #	0.663 #	0.748 *
Δ Epigallocatechin	0.943 ***	-0.937 ***	-0.713 *
Δ Epicatechin	0.663 #	-0.623 #	ns
Δ Methionine	-0.841 *	0.880 **	ns
Δ Phenylalanine	-0.821*	0.819 *	ns
Compounds increasing in the first saturation	O_2 not SO_2	Molar ratio SO_2:O_2 or SO_2 efficiency	Initial Free SO_2
Δ Guaiacylpyranopeonidin-3-o-glucoside	-0.811 *	0.777 *	ns
Δ Methionine-sulfone	0.670 #	-0.660 #	ns

‡: released from tannin depolymerization; Δ: increment; ns: not significant; # p(t) < 0.1; *: p(t) < 0.05 ; **: p(t) < 0.01; ***: p(t) < 0.001

Highlights:

1.- Wine Oxygen Consumption Rates (OCRs) perfectly modeled from chemical composition

2.- OCRs increase with Cu, epigallocatechin and A620, decrease with gallic acid

3.- Acetaldehyde Reactive Polyphenols (ARPs) maybe key determining OCRs

4.- SO_2 consumption related to epigallocatechin and to anthocyanin/tannin ratio

5.- Poor SO_2 consumers degrade amino acids and oxidize methionine into its sulfone