Release and Formation of Oxidation Related Aldehydes during Wine Oxidation

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ABSTRACT

Twenty-four Spanish wines were subjected to five consecutive cycles of air saturation at 25ºC. Free and bound forms of carbonyls were measured in the initial samples and after each saturation. Non-oxidized commercial wines contain important and sensory relevant amounts of oxidation-related carbonyls under the form of odorless bound forms. Models relating the contents in total aldehydes to the wine chemical composition suggest that fermentation can be a major origin for Strecker aldehydes: methional, phenylacetaldehyde, isobutyraldehyde, 2-methylbutanal and isovaleraldehyde. Bound forms are further cleaved releasing free aldehydes during the first steps of wine oxidation, as a consequence of equilibrium shifts caused by the depletion of SO_2. At low levels of free SO_2, *de novo* formation and aldehyde degradation are both observed. The relative importance of these phenomena depends on both the aldehyde and the wine. Models relating aldehyde formation rates to wine chemical composition, suggest that amino acids are in most cases the most important precursors for *de novo* formation.

KEYWORDS

Methional, phenylacetaldehyde, sulfur dioxide, Strecker aldehydes, bound forms.

INTRODUCTION

It is undeniable that some oxidation during wine making and aging is required in order to reach wine optimum quality.[1] Positive effects of controlled oxidation are the decrease of wine astringency[2] and the stabilization of wine color.[3, 4] However, oxidation can also lead to major negative modifications in wine composition and sensory properties, such as the development of yellow and brown colors[5] and wine aroma deterioration.[1, 6] Oxidative spoilage of wine aroma comprises the loss of citric and fresh aromas by reaction between polyfunctional mercaptans and quinones formed in the oxidation[7, 8] and the development of powerful oxidation related odorants such as phenylacetaldehyde (honeylike)[9] and methional (boiled potato odor).[10] At low concentrations these aldehydes may add to the complexity of a wine, but at higher levels, they are responsible for the loss of freshness[11] and for the development of specific oxidation-related off-odors.[12] In those wines in which polyfunctional mercaptans are not key aroma compounds, the formation of these aldehydes is the main cause of wine aroma deterioration.

On the other hand, carbonyls in general, and aldehydes in particular, are highly reactive molecules. They are able to react to wine polyphenols[13] and they can also form strong reversible intermolecular interactions with many molecules such as SO_2, amino acids and proteins and other chemical species.[14 - 16] The adducts that wine carbonyls form with SO_2 (chemically α-hydroxyalkylsulfonates), may play a particularly outstanding role on the development of oxidation related-off odors in wines. Their existence would imply in fact that wine may contain a pool of powerful oxidation related odorants under the form of non-volatile and hence non-odorous complexes. At least theoretically, such a pool could release back into the wine the free odorants, as SO_2 disappears by

oxidation or by reaction with other wine components. This possibility has been recently suggested when the aldehyde formation rates of wines exposed to different levels of oxygen were found to be strongly correlated to the wine levels in combined SO_2.[6] Previous observations about the strong differences in volatility of wine aldehydes[17] would be also consistent with the relevance of their bound forms. The documented existence of those adducts[18, 19] and the likely reversibility of the equilibrium, makes that, without the ability to discern free from bound forms, it is not possible to make a correct diagnose about the nature of the problem. The observed increments of aldehydes during wine bottle storage might be the simple consequence of the release of bound forms once SO_2 is depleted[20 - 23] – in this case bound forms should decrease -, but they could also be formed by direct oxidation of precursors – in this case total forms should increase -. Preventive and remedial actions would be completely different in each case.

Recently, an analytical procedure specifically designed to measure free forms of aldehydes and to estimate bound forms has been developed and validated.[19] Such a procedure will be herein used in order to get more precise insights into the chemical processes involved in the development of oxidation-related aldehydes during wine oxidation. Specific goals of the present research are: 1) to assess the presence of bound forms of aldehydes in non-oxidized commercial wines; 2) to assess which changes in levels of free forms of aldehydes should be attributed to release from adducts and which ones to *de novo* formation or to other chemical processes; and 3), to obtain clues about the potential origin of both, adducts and of aldehydes formed *de novo*.

MATERIALS AND METHODS

Chemicals

Ethanol, dichloromethane and methanol were supplied by Merck (Darmstadt, Germany), tartaric acid 99%, glycerol 99.5%, 1,2-propanediol 99.5% and sodium metabisulfite 97% were from Panreac (Barcelona, Spain), acetonitrile and sodium hydroxide 99% were from Scharlau (Barcelona, Spain). Water was purified in a Milli-Q system from Millipore (Bedford, Germany). Chemicals used for the analytical characterization were analytical grade and were supplied by Aldrich (Madrid, Spain), Fluka (Madrid, Spain), Chem Service (West Chester, PA, USA) and Firmenich (Switzerland). Purity of chemical standards is over 95% in all cases and most of them are over 99%. Specific details can be obtained from method references [19, 24 - 35].

Analytical Characterization

Analysis carried out in the original wines and in sample taken after each one of the saturation cycles included absorbance at 280, 420, 520 and 620 nm, free and total sulfur dioxide, free carbonyls and free acetaldehyde. Exhaustive analyses performed at the beginning of the experiment included total carbonyls, pH, metal cations, different aldehydes precursors such as amino acids or alcohols, trolox equivalent antioxidant capacity (TEAC) and Folin-Ciocalteu, polyphenols (21 anthocyanins, 12 hydroxycinnamic acids, 9 benzoic acids, *trans* and *cis*-aconitic acids, ellagic acid, 2 stilbenes, 8 flavanols, 21 flavonols, 3 proanthocyanidins, average polymerization degree and other parameters of polymeric polyphenols), protein precipitable proanthocyanidins and polymeric pigments.

The quantitative determination of free sulfur dioxide was carried out by direct GC-MS analysis of the headspace in equilibrium over the acidified wine sample. HS-GC-MS

88 analyses were performed using a GCMS-QP2010 from Shimadzu (Kyoto, Japan) with a
89 DB-WAX (30 m x 0.25 mm i.d. x 0.25 µm film thickness) column from J&W Scientific
90 (Agilent Technologies, Santa Clara, CA, USA). 4.5 mL of wine were transferred to a 10
91 mL standard headspace vial, to which 20 µL of 2-chloroethanol was added as internal
92 standard, capped, and further acidified with 500 µL of orthofosforic acid (85%) just
93 before the analysis. Samples were incubated at 40^{o}C for 15 minutes and 400 µL of the
94 headspace were injected in a split/splitless injector at 200^{o}C in split mode with a 1:4
95 split ratio. Linear velocity was kept at 44.2 cm/s. The temperature program was 50 °C
96 for 4 min, then raised at 50°C/min to 220°C keeping this temperature for 5 min. The
97 mass spectrometer was used in single ion monitoring (SIM) mode. Sulfur dioxide
98 (retention time (tr) 1.870 min) was monitored at m/z 48 and 64 and 2-chloroethanol (tr
99 = 6.626) with m/z 44, 49 and 80. Quantitative data were obtained by interpolation of
100 relative peak areas in the calibration curves made with synthetic wine (5g/L tartaric
101 acid, 12% ethanol, 1.5 % propane-1,2-diol, 10 g/L glycerin, pH 3.5) containing known
102 amounts of sulfur dioxide, obtained by dissolving sodium metabisulfite ($Na_2S_2O_5$) from
103 Panreac (Barcelona, Spain). This calibration solution was freshly prepared from the
104 solid just before the analysis. A validation study carried out with more than 20 wines
105 demonstrated that results were comparable to those provided by the aspiration/titration
106 method, but precision (RSD(%)<5% for free SO2 above 5 mg/L) and sensitivity (1
107 mg/L) were better.
108 Total sulfur dioxide was determined by the aspiration/titration method (Rankine method
109 recommended by the OIV, International Organization of Vine and Wine).[28] Combined
110 sulfur dioxide levels were calculated as the difference between total and free sulfur
111 dioxide.

The determination of free forms and the simultaneous estimation of bound forms of 14 odor-active carbonyls in wine is described in the method proposed by Bueno et al.[19] The wines were spiked with surrogates, other carbonyls not present in the original wine and with chemical and SO_2 bonding properties very similar to those of wine natural carbonyls. Carbonyls in the headspace were preconcentrated on a PDMS/DVB fiber and are further analyzed on a GC–MS equipped with a quadrupole in SIM mode.

Metals analyzed were copper, iron, manganese and zinc. Microwave assisted digestion was used as sample treatment. Samples were further analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES), as described by Gonzalvez et al.[26]

A precolumn derivatization procedure with aminoquinolyl-N-hydrosysuccinimidyl carbamate (AQC) for the determination of amino acids levels (valine, methionine, isoleucine, leucine, phenylalanine) in wines using a quaternary high-performance liquid chromatography (HPLC) eluent system was followed as described by Hernandez-Orte et al.[27]

The determination of different major aroma compounds such as isobutanol, isoamyl alcohol, benzyl alcohol, methionol, β-phenylethanol were carried out using a variation of the method published by Ortega et al.[29] as described elsewhere.[35] The strategy followed a liquid−liquid microextraction with dichloromethane and uses several internal standards to correct for matrix effects (recoveries above 95% in all cases). 2-butanol was used as internal standard for isobutanol, 4-methyl-2-pentanol for isoamyl alcohol and benzyl alcohol and 4-hydroxy-4-methyl-2-pentanone for methionol and β-phenylethanol, all of them spiked at 1.5 mg/L to the wine. Analyses were carried out using a GC-3800 from Varian (Walnut Creek, CA) equipped with a flame ionization detector (FID). The column used was a DB-WAX from J&W (Folsom, CA) 30 m × 0.32 mm × 0.5 mm film thickness, preceded by a silica precolumn from Agilent

137 Technologies (Santa Clara, CA) 3m × 0.32 mm i.d. The carried gas was He at 2.2
138 mL/min. Two microliters were injected in split mode (1:20). Injector and detector were
139 both kept at 250 ºC. The temperature program: 40 ºC for 5 min, then raised at 4 ºC/min
140 up to 102 ºC, 2 ºC/min up to 112 ºC, 3 ºC/min up to 125 ºC, this temperature was kept
141 for 5 min, 3 ºC/min up to 160 ºC, 6 ºC/min up to 200 ºC and this temperature was kept
142 for 30 min.
143 TEAC and Folin-Ciocalteau assays were adapted from procedures described by Rivero-
144 Perez et al.[30] and Singleton et al.[33] respectively. Absorbance measurements were taken
145 by duplicate using 1 cm quartz cuvettes.
146 For all absorbance measurements, the UV-vis spectrophotometer UV-17000 Pharma
147 Spec from Shimadzu (Duisbug, Germany) was used.
148 Protein-precipitable proanthocyanidins (PPAs) were estimated using ovalbumin as the
149 precipitation agent and tannic acid solutions as standards. The analysis was performed
150 using the method published by Saenz-Navajas et al.[31] in duplicate at room temperature.
151 The procedure for polymeric pigments determination was carried out as described
152 elsewhere.[32] Monomeric pigments (MP), small polymeric pigments (SPP), and large
153 polymeric pigments (LPP) were determined in a UNICAM UV2 Spectrophotometer
154 (Burladingen, Germany) in duplicate.
155 Analyses of the polyphenolic matter was performed following the method described by
156 Gonzalez-Hernandez et al.[24] Two mL of wine were filtered by 0.45 μm and fractionated
157 by Gel Permeation Chromatography (GPC) in an automated fraction collector from
158 Gilson (Middleton, WI, USA) with a Vantage L column (120 mm × 12 mm) from
159 Millipore (Bedforf, Ma, USA) packed with TSK Toyopearl gel HW-50F (Tosohaas,
160 Montgomery Ville, PA, USA). Two fractions were collected and brought to dryness
161 under vacuum. Fraction 1 was dissolved in 2 mL of formic acid/water (5:95, v/v) and it

was further analyzed by UPLC–DAD-MS for quantifying anthocyanins and by UPLC–MS for quantifying flavonols, flavanols, hydroxycinnamic acids, phenolic acids, aconitic acid and resveratrol. Fraction 2 was dissolved in 2 mL of methanol. The vanillin (4-hydroxy-3-methoxybenzaldehyde) assay was performed according to the method described by Sun et al.[34] in the second fraction obtained from the GPC to determine proanthocyanidins (PAs) in catechin equivalents units. To study the polymeric matter of the samples, acid-catalyzed degradation of the second fraction in the presence of toluene-α-thiol was performed according to the method described by Gonzalo-Diago et al.[25]

Wines and oxidation process

Twenty-four different Spanish wines (16 reds, 5 whites and 3 rosés), from different wine making areas, were used in the present study (Associated content, Table S1). Samples were selected to cover a wide range of different characteristics associated to the oxidation phenomena.

The oxidation experiment consisted of five consecutive air-saturation cycles. The chemical composition of wines before the oxidation was extensively characterized by duplicate. In addition, at the end of each one of the cycles, some basic parameters were also determined (see analyses details below). Two bottles of each wine were opened inside a glove chamber from Jacomex (Dagneux, France) in which oxygen in the gas phase was below 0.002 % (v/v). The content of 2 bottles was mixed in a beaker and after ensuring that dissolved O_2 was non-detectable (< 1 μg/L, measured with a fluorescence probe –OptiOx SG-9 from Mettler Toledo-España, Barcelona) samples for analysis were taken in different hermetic vials. Then 500 mL were spiked with standards and surrogates as is described in Bueno et al.[19] Then the spiked wine was taken out of the chamber, saturated with air by gentle shaking in a 1 L closed pyrex

bottle for 10 seconds, after which the cap was opened to let fresh air get into, and the shaking operation was repeated 2 more times until the oxygen level of the wine reached 6 mg/L. The air-saturated wine was then distributed into eight 60 mL tightly screw capped clear glass vials supplied by WIT-France (Bordeaux, France), three of them containing PSt3 oxygen sensors (Nomacorc S.A., Thimister-Clermont, Belgium). The tubes were filled up completely, and were carefully closed avoiding any headspace. Post-hoc studies revealed that with this procedure headspace ranged from nothing to a bubble of air with not higher than 120 μL. Previous studies had confirmed that the amount of oxygen passing through those closures was negligible for the purposes of the experiment (< 0.5 mg/L per week). Wines were stored in an incubator in the dark at 25 °C and dissolved oxygen level was daily monitored with a Nomasense oxygen analyzer from Nomacorc S.A. The oxidation cycle was considered finished once O_2 levels dropped to 10% of the initial concentration or after a week. Then the vials were opened and mixed inside the glove chamber within a 500 mL pyrex bottle and 58 mL of wine for intermediate analyses were taken. The remaining wine was taken out for a new saturation cycle in n-1 tubes, 2 of which at least contained oxygen sensors (n being the number of WIT tubes used in the previous cycle). Therefore, at the end of the experiment 144 samples have been generated (24 different commercial wines + 24 × 5 different oxidation states).

Statistical analysis and data treatment

Simple statistical calculations were carried out with Excel 2013 (Microsoft, WA, USA). Partial Least-Squares (PLS) regression were performed using The Unscrambler 9.7 (CAMO Software AS, Oslo, Norway). The quality parameters studies to evaluate the prediction ability of the model were the slope pf the regression curve between real and

211 predicted Y variables (*m*), the root-mean-square error (RMSE) for the prediction and the
212 percentage of variance explained by the model (%EV).

213 # RESULTS AND DISCUSSION

214 **Free and bound forms of carbonyls in commercial non-oxidized wines**

215 A novel method specifically designed to quantify free forms of carbonyls and to estimate
216 bound forms of these compounds[19] has been applied to determine 14 carbonyls in 24
217 Spanish commercial wines. Results of the analyses are summarized in Table 1 while the
218 relative distribution of bound forms estimated for each analyte or group of analytes
219 from its corresponding surrogate is summarized in Table 2. The complete set of results
220 is given in the Associated content, Table S2. As seen in the tables, only aldehydes and
221 2,3-diketones were found to be present both under free and bound forms while ketones
222 such as acetovanillone, β-damascenone or β-ionone were exclusively found as free
223 forms. This is not incompatible with the known ability of β-damascenone to irreversibly
224 bind to sulfur dioxide.[36]

225 The estimations have a reasonable accuracy as determined during method validation[19]
226 and make it possible to confirm that normal, commercial non-oxidized wines contain
227 relevant amounts of aldehydes and diketones under bound forms. Methional,
228 isovaleraldehyde and phenylacetaldehyde are found mostly under bound forms in most
229 wines (average levels between 78 and 91%). Isobutyraldehyde, 2-methylbutanal,
230 benzaldehyde, diacetyl and 2,3-pentanedione are also majorly found under bound forms
231 (> 60% in average), while furfural and 5-methylfurfural are mostly as free forms but up
232 to 45% of the total wine content can be under bound forms. Deep sensory consequences
233 would be expected if these bound forms were released, since some of the bound
234 components are present at concentrations well above odor thresholds. In fact, the wine

contents in isovaleraldehyde would increase by factors as high as 20, those of methional and phenylacetaldehyde by factors as high as 10 and those of diacetyl by factors as high as 4. In the cases of decanal and acetaldehyde, the total fraction was estimated using the apparent equilibrium constant with SO_2 published elsewhere.[15] In accordance to those estimations, more than 99% of these compounds can be under bound forms in wines containing high levels of free SO_2, indicating that total levels of these aliphatic aldehydes can be very high and that their release may also have strong sensory consequences.

Modelling the total aldehyde content of wine from its present chemical composition

The estimated total amounts of Strecker aldehydes found in the set of wines have been related to the wine chemical composition (summarized in Associated content, Table S3) by PLS modeling. Metal cations and the potential precursors of aldehydes: higher alcohols and Strecker amino acids, were included in the models which are summarized in Table 3.

The models have in all cases highly satisfactory prediction abilities with explained variances over 88% (by cross-validation) and have a quite consistent structure in all cases, regardless of wine type. The models suggest that the actual wine content in total aldehyde can be satisfactorily predicted from the wine content in precursor amino acid, precursor alcohol, Zn, combined or total SO_2 and to other components specific to each aldehyde and wine type. In all cases, wine aldehyde levels are positively related to the precursor alcohol and leaving aside isobutyraldehyde, also to combined or total SO_2. Aldehyde levels are also in all cases (except methional in white and rosés) negatively related to the wine level of Zn. The amino acid precursors seem to be also essential in most models, but in these cases coefficients can be either positive or negative.

Although the models are not definitive evidence and further specific experimental material should be produced, the observed patterns seem to favor the hypothesis that the main origin of Strecker aldehydes is alcoholic fermentation. Strecker aldehydes are in fact normal intermediates in the yeast amino acid synthesis and are further reduced to the corresponding alcohols by dehydrogenase-class enzymes, which would explain the positive weight of the alcohol in the models. The presence of free SO_2 during fermentation could trap the aldehyde under bound forms avoiding its enzymatic reduction, which would be consistent with the positive coefficients found for combined and/or total SO_2. The negative role of Zn would be consistent with the known role played by this cation in alcohol dehydrogenases from saccharomyces and other fungus.[37, 38] Finally, the erratic correlation coefficient of the amino acid precursor, mostly negative except for isobutyraldehyde and for isovaleraldehyde in reds, is difficult to explain since not much is really known about the relationship between yeast fermentation and the presence of residues of amino acids in wine. It should be noted that only in one of the cases (isovaleraldehyde in reds) free SO_2 appears with a negative correlation coefficient, suggesting that the direct chemical oxidation of the alcohol or the amino acid cannot be completely excluded as a formation route, although data suggest that it is not the main formation path.

Evolution of carbonyl surrogates during oxidation

Wines were oxidized following a forced oxidation procedure consisting of five consecutive air-saturation cycles. Such a procedure provides a reasonable way to obtain samples with a controlled consumption of oxygen. Although it is apparently different to the slow oxidation suffered by the wine in the bottle, it is not that different to the oxidation suffered in the winey by the accidental exposure of the wine to oxygen. In addition, there are no obvious reasons to think that the relative ability of different wines to form or release aldehydes is going to be altered.

In the study, wines were spiked with surrogates representing structurally different aldehydes and ketones at the beginning of the forced oxidation procedure. Surrogates are non-naturally occurring wine carbonyls with chemical (including SO_2 bonding) properties very similar to those of wine native carbonyls, and their presence makes it possible to assess some of the chemical reactions taking place along wine oxidation. A first statement is that, at least concerning aldehyde formation, the most relevant variable in wine oxidation was found to be the free SO_2 level, and only when levels of aldehydes were plotted versus this variable, some meaningful relationship emerged. More precisely, and taking into account that free SO_2 levels measured in this work include "molecular" SO_2 and HSO_3^-, whose relative distribution is pH dependent, the most meaningful relationships emerge when free aldehyde levels are plotted either to 'molecular' SO_2, or to its complementary, HSO_3^- form.

For instance, the levels of free 3,5,5-trimethylhexanal (surrogate for isovaleraldehyde) in the 144 samples generated in the forced oxidation protocol (24 different commercial wines + 24 × 5 different oxidation states) are plotted in Figure 1 versus the molecular sulfur dioxide level of the wines. As can be seen, there is a close relationship between both variables, so that the lower the molecular SO_2 level, the higher the level of free surrogate. In addition, the solid and dashed lines represent the expected free aldehyde level attending to the molecular SO_2 level of the sample; to the known spiked amount of surrogate; and to its apparent complex formation constant measured both in synthetic (dashed line[19]) or real (solid line) wines. Taking into account that the surrogate is not naturally formed in wine, we must unequivocally conclude that the increase is due to the release of the surrogate complexed with SO_2 once this molecule is oxidized.

The figure also reveals (see the zoomed area) that at very low levels of molecular SO_2 the measured levels of free surrogate of some wines become consistently below

309 expected values with a trend towards progressively smaller values as molecular SO_2
310 levels further drop. Such a decrease should be attributed to the oxidative degradation of
311 surrogates at those low SO_2 levels, which would be in agreement with the expected
312 generalization of the Fenton reaction once the levels of free SO_2 are no longer able to
313 trap the H_2O_2 formed in the wine oxidation cycle.[39, 40] In the case of 3,5,5-
314 trimethylhexanal such decrease is observed when levels of molecular SO_2 fall below
315 0.10 mg/L. The same general pattern, with a less marked but yet obvious degradation
316 trend at very low levels of molecular SO_2, was observed for the surrogates 3-
317 (methylthio)butanal and hydrocinnamaldehyde. Since there is no reason to think that
318 native wine aldehydes behave differently to their surrogates, it can be concluded that the
319 levels of free aldehydes during wine oxidation are determined at least by the three
320 following factors: 1) the previous existence of bound forms; 2) the cleavage of those
321 bound forms to release free forms attending to the chemical equilibrium sulfite +
322 carbonyl ↔ alkylhydroxysulfonate; and 3) the oxidative degradation of the aldehydes
323 taking place at very low levels of molecular SO_2. A fourth factor, namely the "de novo"
324 formation of aldehydes, will be considered in the following section.

325 The plot shown in Figure 1 and its analogues for hydrocinnamaldehyde and 3-
326 (methylthio)butanal (Associated content, Figure S1), make it possible to estimate the
327 average apparent formation constants (K_a) for the three surrogates following the same
328 behavior. This was done by excluding from the representation those data points at very
329 low levels of molecular SO_2 affected by degradation and representing the inverse of the
330 molar concentration of complexed aldehyde versus the inverse of the molar
331 concentration of molecular SO_2. Since the adducts aldehyde-SO_2 have a 1:1
332 stoichiometry,[41] such representation should yield a straight line whose slope is $1/K_a$,
333 attending to Equation 1,

$$\frac{1}{\mathrm{B}} = 1 + \frac{1}{K_a} \times \frac{1}{[\text{molecular } SO_2]} \qquad [1]$$

where *B* represents the molar concentration of complexed aldehyde, obtained as the difference between the concentration of surrogate added and its free measured concentration in each sample. These plots in the three cases showed good straight lines with intercepts not significantly differing from 1, as expected from Equation 1 (see Associated content Figure S2). The constants obtained were similar, although smaller than those measured in synthetic wine.[19] The values obtained were (17.1± 0.6) × 10^5, (6.20 ± 0.04) × 10^5 and (9.30 ± 0.51) × 10^5 for 3,5,5-trimethylhexanal, hydrocinnamaldehyde and 3-(methylthio)butanal respectively.

Evolution of native carbonyls during wine oxidation

The previous observations can help to understand the observed evolutions of free native aldehydes during wine oxidation. A plot free-methional vs. molecular SO_2 for one of the wine samples is given in Figure 2. The solid line represents the evolution of measured free methional in the wine during oxidation and the horizontal dashed line corresponds to the estimated levels of total methional present originally in wine. The dotted line, partially concealed by the solid line, represents the levels of expected free methional estimated from the total methional originally present in the wine, the apparent formation constant for the adduct and the molecular SO_2 level of the sample. It is evident from the plot, that estimated and measured free amounts of methional are totally coincident in the two first samples, those taken at levels of molecular SO_2 above 0.1 mg/L, meaning that the observed increases in free methional in this region can be attributed to the cleavage of its hydroxyalkylsulfonate, so that in this phase of oxidation increases are really the

357 result of release. In the last three sampling points, however, the estimated levels fall
358 well below the measured levels, strongly suggesting that at those low levels of
359 molecular SO_2, strong *de novo* formation of methional from different precursors is
360 actively taking place. As aforementioned, such *de novo* formation at those low levels of
361 molecular SO_2 would be consistent with the development of Fenton reaction once SO_2
362 cannot prevent the accumulation of H_2O_2.[39]

363 In order to get better insights of all the phenomena affecting free levels of aldehyde
364 during wine oxidation, a different type of plot has been produced. For each aldehyde,
365 wine and sampling point, the difference between the measured free aldehyde level and
366 the estimated free aldehyde (for that particular wine at that particular molecular SO_2
367 concentration using the corresponding apparent formation constant) has been calculated
368 and plotted versus the molecular level of SO_2 in the sample. Three of these plots are
369 given as examples in Figure 3 (methional, decanal and 2-methylbutanal).

370 Figure 3a shows that the finding exemplified in Figure 2 about the coincidence between
371 measured and expected free aldehyde levels, extends to most wines and sampling points
372 with molecular SO_2 levels above 0.15 mg/L. Above this level, differences between
373 measured and expected values are close to 0, and only in few cases a decreasing trend is
374 observed. Below this region, however, the points scatter above and below 0 in Figure
375 3a. A point above 0 means that the free aldehyde found in wine is above expected,
376 suggesting *de novo* formation, while a point below 0 means that it is below expected,
377 suggesting oxidative degradation. The random pattern of scatter is an artifact, since each
378 wine shows in general a well-defined trend. For instance, the solid and dashed lines
379 represented in Figure 3a group the sampling points of two specific wines. In the case of
380 the wine represented by the dashed line, it is apparent that there is a strong *de novo*
381 formation of methional at low SO_2 levels, while in the wine represented by the solid

382 line, there is a neat degradation of methional and only at very low levels of molecular
383 SO_2 some *de novo* formation becomes apparent. For decanal, represented in Figure 3b,
384 and for which there is no known precursor in wine (natural 1-decanol levels are very
385 low), only the degradation pattern is observed, and becomes apparent in some wines at
386 levels of molecular SO_2 below 0.6 mg/L. The case of 2-methylbutanal, shown in Figure
387 3c, is rather the contrary, since *de novo* formation prevails over degradation. For this
388 compound, *de novo* formation took principally place also at low levels of molecular
389 SO_2, although in one particular white wine (solid line), *de novo* formation was observed
390 at levels between 0.4 and 0.5 mg/L molecular SO_2. Exactly the same trend was observed
391 for 2-methylbutanal, including the premature *de novo* formation for the same white
392 wine (Associated content Fig. S3a.). The plot for isovaleraldehyde showed also mostly
393 de novo formation and no degradation (Associated content Fig. S3b.), while for
394 phenylacetaldehyde *de novo* formation was evident only at very low SO_2 levels (less
395 than 0.1 mg/L), while some degradation is apparent at levels as high as 0.5 mg/L
396 (Associated content Fig. S3c.).

397 **Modelling aldehyde formation rates (AFRs)**

398 Data in Figures 2 and 3 reveal that the release of bound forms explains quite
399 satisfactorily the observed increases in free aldehyde as long as the levels of molecular
400 SO_2 are above 0.1-0.2 mg/L. The design of the present experiment, however, in which
401 the wines were forced to 5 consecutive oxygen-saturation cycles regardless of their
402 initial SO_2 content, does not make it possible to build satisfactory models for the
403 production of aldehydes, mostly *de novo*, at low SO_2 levels. Fortunately, we do have at
404 hand data from a previous experiment[6] in which wines were subject to a wide range of
405 levels of oxygen during months of storage. In such a case, aldehydes were found to
406 increase in an approximately linear way with the oxygen consumed. Such linear

407 relationships made it possible to determine the aldehyde formation rates (AFRs) of each
408 wine.[6] Those AFRs were found to be significantly correlated to the amino acid
409 precursor (in case of Strecker aldehydes) and combined SO_2 (in most cases), but were
410 not further modelled because at that moment it was not possible to correctly discern
411 between free and bound forms. However, with the apparent equilibrium constants
412 determined in ref.[19] and in the present study, it is possible to estimate for those wines
413 the bound fraction of each aldehyde present at the beginning of the experiment. With
414 such estimations at hand together with the chemical composition of the unoxidized
415 wines it has been possible to build some PLS models which give further insights on the
416 formation and release of Strecker aldehydes along wine oxidation. The models are
417 summarized in Table 4 and reveal a quite consistent structure in all cases. All models
418 bear positive correlation coefficients to the three different types of precursors: amino
419 acids, alcohols and the initial amount of aldehyde under bound forms, suggesting that in
420 fact the three phenomena concur to form or release these aldehydes. The models have a
421 relatively satisfactory prediction power and provide a preliminary estimation about the
422 contribution of each formation/release route to the AFR of each aldehyde. Attending to
423 such estimations, the amino acids would be the most relevant source of these
424 compounds, in accordance with results from Grant-Preece et al.[18] The alcohol would be
425 also important in the case of isovaleraldehyde, which comes from the major wine
426 alcohol, isoamyl alcohol, and would have null influence in the case of methional, which
427 comes from the minor methionol, in apparent disagreement with previous
428 observations.[42] The levels of bound aldehydes have a higher weight in the cases of
429 isovaleraldehyde and phenylacetaldehyde, those aldehydes whose alcohols were formed
430 at higher levels along the alcoholic fermentation. Nevertheless, apart from the fact that
431 release takes place in the first phase of wine oxidation, not much is yet known about the

mechanisms and time periods in which *de novo* formation of aldehydes takes place along wine oxidation. These questions will have to be specifically addressed in future research.

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ASSOCIATED CONTENT

Wines analyzed in the experiment including origin, age, varietal composition and some basic compositional parameters. Free SO_2 (mg/L) and free (determined) and total (estimated) forms of wine carbonyls (μg/L) in the 24 wines. Concentration ranges and average concentrations in the initial wines of amino acids and alcohols potentially precursors for oxidation aldehydes and some trace mineral elements with potential catalytic activity upon the oxidation processes. Measured levels of different surrogates as a function of wine molecular SO_2 content. Relationship between the inverse of the molar concentration of bound forms (1/B) and molecular SO_2 for 144 samples (24 different commercial wines + 24 × 5 different oxidation states). Differences between the measured and estimated free levels of some aldehydes along wine oxidation as a function of the molecular SO_2 level of the wine.

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FIGURE CAPTIONS

Figure 1. Measured levels of free 3,5,5-trimethylhexanal as a function of wine molecular SO_2 content. Solid and dashed lines give the expected free level estimated using the 3,5,5-trimethylhexanal-SO_2 adduct dissociation constant measured in synthetic (dashed) and real (solid) wines. The zoomed area gives the details of two wines in which a strong degradation of the surrogate at low molecular SO_2 levels is observed.

Figure 2. Levels of methional of a red wine measured during its oxidation as a function of its molecular SO_2 content. Dashed line represents the estimated levels of total methional of the unoxidized wine sample. Dotted line represents the free levels estimated using the buthional-SO_2 adduct dissociation constant measured in real wine.

Figure 3. Differences between the measured and estimated free levels of some aldehydes during wine oxidation as a function of the molecular SO_2 level of the wine. The data from the 24 wines after 5 different oxidation levels are represented: (a) methional, lines group points from specific wines; (b) decanal; (c) 2-methylbutanal. For methional and 2-methylbutanal, the apparent formation constant (K_a) for the corresponding surrogate calculated in real wine was taken. In case of decanal the K_a reported in synthetic wine by de Azevedo et al. 2007 was used.