Can aldehyde accumulation rates of red wines undergoing oxidation be predicted in accelerated conditions? The controverted role of aldehyde–polyphenol reactivity

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Abstract

BACKGROUND: The accumulation of acetaldehyde and Strecker aldehydes during wine oxidation is detrimental to quality and often determines wine shelf-life. Knowing in advance the specific tendency of a wine to accumulate these compounds would help decision making during winemaking. An accelerated test based on a forced oxidation procedure at 45 °C (5 days) to measure aldehyde accumulation rates (AARs) is proposed and assessed by comparing results with those obtained by oxidation at 25 °C (36 days). Reactivities of aldehydes in those same wines stored in anoxia at both temperatures were also measured.

RESULTS: Wine oxygen consumption rates at 25 °C are poorly correlated with those observed at 45 °C. By contrast, AARs of methional and of 2- and 3-methylbutanals measured during wine oxidation at 25 °C are equivalent to those measured at 45 °C. AARs from isobutanal and acetaldehyde are also correlated, while AARs from phenylacetaldehyde are not. Partial least squares models explaining AARs show intriguing differences regarding the apparent limiting role played by wine anthocyanins and other polyphenols in the ability of wines to accumulate aldehydes. Measured differences in aldehyde pattern are similar to those of the other Strecker aldehydes.

CONCLUSION: The proposed assay makes it possible to obtain a reasonable estimate of a wine's tendency to accumulate aldehydes, with the exception phenylacetaldehyde, in 5 days. Neither differences in aldehyde reactivity between wines nor the change in reactivities with temperature support a major role for reactivity in differentially limiting AARs during wine oxidation. © 2021 The Authors. *Journal of The Science of Food and Agriculture* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: acetaldehyde; oxidation; Strecker aldehydes; anthocyanins; polyphenols; sulfur dioxide

INTRODUCTION

Oxygen is necessary for winemaking. Winemakers agree that an adequate dosage of O_2 reduces astringency and bitterness, reduces green and vegetal character and stabilizes color.[1,2] However, excessive exposure to O_2 can have a dramatic impact on wine quality.[3] The amount of O_2 which is excessive is wine dependent, since the ability of wine to develop oxidation-related symptoms is strongly dependent on its polyphenolic content, levels of antioxidants and metal content.[4,5]

The most obvious symptoms of oxidative deterioration are the development of yellow and brown pigments[6] and the appearance of oxidation-related off-odors. The latter is particularly worrying, because it often happens without any clear visual change. Oxidation-related off-odors are mainly attributed to the accumulation of acetaldehyde and Strecker aldehydes, particularly methional and phenylacetaldehyde,[7,8] during oxidation. The

accumulation is the result of the combination of two opposed processes: one the formation of the aldehyde, and a second a reaction of the aldehyde with different wine components.

Acetaldehyde has its origin in the oxidation of ethanol by means of the hydroxyl radical generated in the Fenton reaction present in wine.[9] Strecker aldehydes (isobutanal, 3-methylbutanal, 2-methylbutanal, methional and phenylacetaldehyde) are principally produced from the degradation of the corresponding amino acids – valine, leucine, isoleucine, methionine, and phenylalanine,

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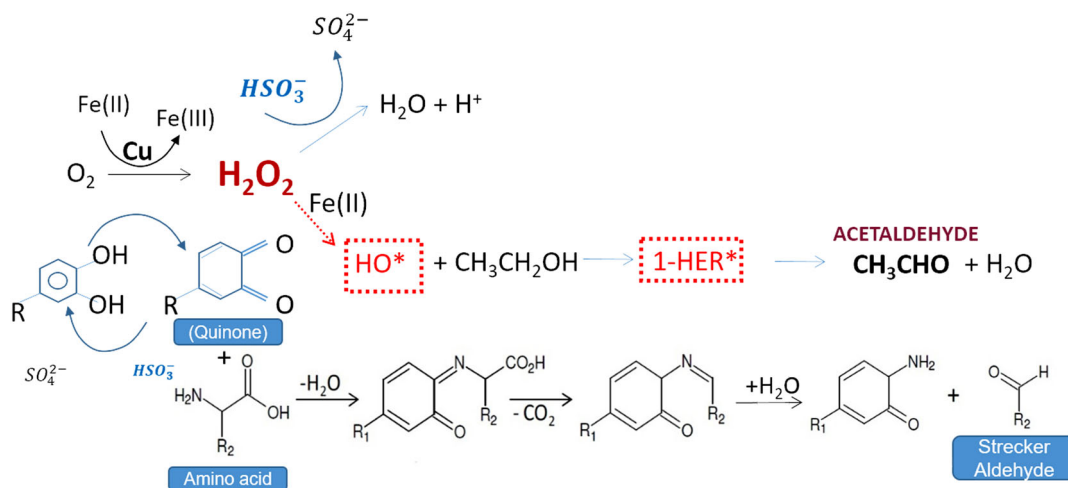
Scheme 1. Chemical scheme for oxidation processes: formation of acetaldehyde and Strecker aldehydes.

respectively – by reaction with α-dicarbonyls (as quinones) via the Strecker reaction.[8,10] Scheme 1 shows the chemical scheme for oxidation processes.

The reactivity between acetaldehyde and wine polyphenols has been well described. The reaction takes place through a nucleophilic attack of polyphenols on the protonated form of the aldehyde, resulting in an ethyl bridge between two different units of polyphenolic material,[11,12] or of a vitisin-type pigment.[13] Strecker aldehydes also react with polyphenols following the same mechanism as recently demonstrated.[14] Other researchers have suggested, by means of partial least squares (PLS) models, that aldehydes react mainly with anthocyanins and small tannins,[15] and that these aldehyde-reactive polyphenols could have a crucial role in determining the differential ability of young and aged wines to accumulate aldehydes, and also in determining the specific accumulation pattern followed by phenylacetaldehyde. In any case, reactions between aldehydes and polyphenols are mostly irreversible, and reaction products evolve to xanthene forms and pyroanthocyanins, among others.[16,17]

In spite of its large influence on wine longevity, there are no commonly accepted tests to assess wine resistance to oxidation, particularly for assessing the wine's tendency to develop oxidation-related off-odors. Researchers often use forced oxidation procedures at temperatures as high as 35,[18] 45,[19,20] or 55 °C,[21,22] but the target has been limited to assess O_2 consumption rates (OCRs), identify oxidation markers or predict browning tendency. Owing to the interest for industry and research of a practical assay for measuring wine's tendency to develop oxidation-related off-odors, the main goal of the present paper was to assess whether aldehyde accumulation rates (AARs) of red wines undergoing oxidation at 25 °C can be well predicted by an assay under accelerated conditions. A second goal of the paper was to assess whether differences in AARs, under both conditions, could be attributed to a different pattern of reactivity between aldehydes and wine polyphenols.

MATERIALS AND METHODS
Solvents and chemicals
Solvents and chemicals have been previously described by Bueno et al.[22] and are detailed in Supporting Information Appendix S1.

Samples
For this study, eight Spanish red wines of different vintages (Supporting Information, Table S1) made mainly with Grenache and Tempranillo, were purchased at a local store. Four of them had been aged in oak barrels for more than 12 months. All samples were filtered through amicrobic filters (0.22 μm) (ref. SCGP U02 RE, Merck (Darmstadt, Germany)) before the oxidation procedures.[23]

Oxidation procedures
The forced oxidation procedures were similar to that described by Marrufo-Curtido et al.[24] Briefly, duplicate samples were oxidized in screw-capped 60 mL volume-calibrated vials containing a known volume of O_2-saturated wine and a defined headspace. Volume calibration is necessary to have an accurate estimation of the total amount of O_2 contained in the tube (in wine and headspace). The amount of total O_2 given to each sample was 32 mg L^{-1} plus the stoichiometrically required amount to oxidize all its SO_2. The prepared vials were left in a thermostatic bath (model OLS23, Grant Instruments Ltd, Cambridge, UK) with orbital shaking (90 rpm) to ensure equilibrium between liquid and gas phases, at the selected temperature. At 45 °C, dissolved O_2 was monitored every 30 min during the first 4 h, then every 6 h approximately until the second day and finally every 12 h until 95% of the O_2 present in the tube was consumed, at 45 °C, using PSt3 sensors and an O_2 analyzer (Nomacorc SA, Thimister-Clermont, Belgium). At 25 °C, O_2 measurements were taken every day. Oxidation was considered to be completed after the samples had consumed 95% of the O_2 present in the vial. At that point, acetaldehyde and Strecker aldehydes were determined.

Anoxia procedure
This experiment was carried out with the same eight commercial wine samples of the oxidation procedures 8 months after the previous experiment. Samples were reanalyzed to determine levels of acetaldehyde and Strecker aldehydes, and were then spiked with these molecules to increase their levels to the maximum level found in the oxidation experiment at 25 °C. These levels were 91 μg L^{-1} isobutanal, 112.3 μg L^{-1} 3-methylbutanal, 45.3 μg L^{-1} 2-methylbutanal, 102 μg L^{-1} methional, 154 μg L^{-1}

phenylacetaldehyde and 38 mg L^{-1} total acetaldehyde. Wines were then carefully closed within an anoxic chamber, bagged with O_2-resistant plastic bags containing O_2 scavengers, and were left to incubate in complete anoxic conditions for 5 days at 45 °C and 36 days at 25 °C, to emulate the previous oxidation experiments. After this time, total acetaldehyde and Strecker aldehydes in all samples were measured. The experiment was carried out in duplicate.

Wine chemical characterization

Initial wines were analyzed for total and free SO_2, color parameters, total polyphenol index and Folin–Ciocalteu index, phenolic and tannin composition, metals, major aroma compounds and amino acids. Initial and final samples were analyzed for total Strecker aldehydes and total acetaldehyde.

Sulfur dioxide determination

For total and free sulfur dioxide determination, the aspiration/titration method recommended by the OIV (International Organization of Vine and Wine) was used. Methods are described in detail in Supporting Information Appendix S1.[25]

Spectrophotometric measurements

For color determination, as recommended by the OIV, total phenolic index (TPI) was determined, as described by Ribéreau-Gayon et al.[26] Folin–Ciocalteu assay was performed following the method described by Singleton et al.[27,28]

Metal analyses

Samples were treated and analyzed as described Gonzalvez et al.[29] Metals quantified were iron, copper, zinc and manganese.

Amino acid analyses

The determination of valine, methionine, isoleucine, leucine and phenylalanine was carried out according to the method reported by Hernández-Orte et al.[30]

Polyphenol analyses

Phenolic acids, flavanols and anthocyanins were determined in triplicate following the procedure described by Vallverdu-Queralt et al.[31]

The phloroglucinolysis reaction was used for studying in triplicate the composition of condensed tannins, following the procedure described by Ducasse et al.[32]

Major aroma compounds

Major aroma compounds were determined using a variation of the method published by Ortega et al.[33]

Total acetaldehyde determination

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.

Strecker aldehyde determination

The determination of total Strecker aldehydes (isobutanal, 2-methylbutanal, 3-metylbutanal, methional and phenylacetaldehyde) in wine is described in the method proposed by Bueno et al.[34]

O_2 consumption kinetics

The O_2 consumed at each time point was directly determined from the measured dissolved O_2. The O_2 consumption kinetics at 25 °C was studied by Marrufo-Curtido et al.[24]

At 45 °C, in the first 60 min the amount of O_2 consumed was directly measured, compared to initial concentration and standardized to 1 day for first-hour kinetic coefficient (per day; Table 1). For the rest of the oxidation experiment at 45 °C, a pseudo-first-order kinetic model was assumed to explain the consumption of O_2 by the wine. Two different consecutive time segments were found (kinetic coefficient second and third periods; Table 1).

Attending to the model, for one unit of time (expressed in days), it holds that

$$\frac{[O_2]_t}{[O_2]_0} = e^{-k} \tag{1}$$

Table 1. Average O_2 consumption rates (OCR, mg L^{-1} d^{-1}) at 25 and 45 °C and kinetic coefficients describing the three differential kinetic periods observed at 45 °C

	25 °C			45 °C			Kinetic coefficients (d^{-1})		
	Time (days)	O_2 consumed (mg L^{-1})	Average OCR	Time (days)	O_2 consumed (mg L^{-1})	Average OCR	1st hour[a]	2nd period[b]	3rd period[b]
SL-A	52.7	48.7	0.92	6.60	50.0	7.58	3.89	0.930	0.977
TS-A	52.7	53.0	1.01	4.60	46.7	10.15	4.31	0.954	0.986
BL-A	27	42.4	1.57	3.10	39.7	12.81	4.65	0.985	0.990
CH-A	52.6	42.0	0.80	4.60	38.3	8.33	4.63	0.948	0.966
MF-Y	19.5	35.0	1.79	3.60	38.9	10.81	5.46	0.984	1.000
TP-Y	29.4	37.8	1.29	3.60	36.2	10.06	5.30	0.934	0.987
HV-Y	29.4	35.4	1.20	6.60	38.0	5.76	4.50	0.902	0.996
BS-Y	30.6	40.0	1.31	3.60	38.7	10.75	5.37	0.928	0.993
Average	36.7	41.8	1.2	4.54	40.8	9.5	4.8	0.946	0.987

-A represents aged wines and -Y refers to young wines.
[a] The fraction of O_2 consumed in the 1st hour, per day, for making it comparable to the other coefficients.
[b] The coefficient is the parameter $1 - e^{-k}$, where k is the kinetic constant of the pseudo-first-order process and represents the percent O_2 consumed in 1 day.

where e^{-k} represents the fraction of dissolved O_2 not consumed by the wine in 1 day, and hence $1 - e^{-k}$ represents the fraction of dissolved O_2 consumed by the wine in 1 day.

Data treatment and statistical analysis

Correlation studies and simple Student t-test were directly carried out with Excel 2013 (Microsoft, Redmond, WA, USA). PLS regression analysis was carried out using The Unscrambler 9.7 (CAMO Software AS, Oslo, Norway).

RESULTS AND DISCUSSION

General description of the oxidation

Oxygen consumption kinetics at 45 °C for the eight wines in the study are summarized in Table 1. The forced oxidation procedure was very reproducible, as it was at room temperature, and the plots of O_2 concentration *versus* time of replicated samples were completely identical; in relative terms, differences between replicates were below 0.5%. All the wines consumed between 36 and 50 mg L^{-1} O_2 in less than 1 week, the average time being 4.5 days. This is, on average, 8.5× less than the time required at 25 °C, which was considered satisfactory for an assay.

In all cases, the O_2 consumption plots were segmented in three periods: the first hour, and two time segments in which pseudo-first-order kinetics could be satisfactorily applied. The second segment lasted between 1 and 2 days (1.7 on average), depending on the wine, and the third segment extended for the rest of the oxidation. OCRs in the first hour were of greater magnitude, so that 10% of all the O_2 supplied to the wine was consumed in this time. In the two following sections, O_2 consumption followed pseudo-first-order kinetics, whose constants were on average 0.946 and 0.987, respectively. This indicates a strong consumption, since attending to first-order kinetics this means that the wines consume 94.6% or 98.7% of the total O_2 present in a day. It should also be observed that OCRs in the last time segment are higher than those observed in the second one.

Regarding the comparison between average OCRs at 45 and 25 °C, the first observation that should be made is that average OCRs at both temperatures are not significantly correlated

($R^2 = 0.35$; n.s.). Even though the average OCR at 25 °C was well correlated with the kinetic constants measured in the second kinetic period at 45 °C ($R^2 = 0.88$; $P < 0.0005$), the differences between both temperatures become obvious when plots of consumed temperature *versus* normalized time are compared (Supporting Information, Fig. S1). While there are samples in which both plots were completely overlapping, there were others where the OCR consumption was faster at 45 °C and others for which the opposite was observed (Fig. S1). Average OCRs at 45 °C are, on average, 7.7 times (between 4.8 and 10.4) faster than those measured at 25 °C.[24]

The changes measured during oxidation are summarized in Table 2. The complete dataset is given in Supporting Information, Table S2. As can be seen, levels of O_2 not SO_2 (consumed O_2 not invested in the oxidation of SO_2) consumed were very similar in all samples: between 27 and 35 mg L^{-1}. This was expected, since the amount of O_2 delivered to each wine was 35 mg L^{-1} plus the stoichiometrically necessary amount to consume all its SO_2. Levels of acetaldehyde accumulated ranged from zero to 17 mg L^{-1} at 25 °C and from 2 to 9 mg L^{-1} at 45 °C – 6.3 and 6.0 mg L^{-1} on average, respectively. The highest accumulation of acetaldehyde occurs at both temperatures in older wine. Considering that 1 mol O_2 can oxidize 1 mol ethanol to form acetaldehyde, the average 31 mg L^{-1} O_2 not SO_2 could form up to 42.6 mg L^{-1} acetaldehyde; i.e., acetaldehyde accumulated is just 14% of that maximum value. This strongly implies that large amounts of H_2O_2 have been invested in oxidizing wine products other than ethanol, and that large amounts of the acetaldehyde formed have reacted with wine polyphenols and other wine molecules.

Levels of Strecker aldehydes accumulated were very diverse between wines. They ranged from the negative values of 3-methylbutanal measured in one of the samples oxidized at 25 °C to 144 μg L^{-1} of phenylacetaldehyde measured in another sample oxidized at 45 °C. On average, the Strecker aldehyde least accumulated was 2-methylbutanal, while the most accumulated was phenylacetaldehyde. As can be seen in Table 3, minimum and maximum levels of Strecker aldehydes accumulated at both temperatures were relatively similar, except in the case of

Table 2. General description of the measured changes during oxidation at the two temperatures

Parameter	Temperature (°C)	min	max	Average	SD	RSD (%)
'O$_2$ not SO$_2$' (mg L^{-1})	25	28.6	34.2	31.7	1.91	6.0%
	45	27.4	34.8	30.7	2.22	7.2%
Acetaldehyde (mg L^{-1})	25	−0.9	17.7	6.28	6.52	104%
	45	2.4	9.4	5.95	2.63	44%
Isobutanal (μg L^{-1})	25	7.6	41.0	16.9	11.0	65%
	45	12.1	34.1	22.4	7.75	35%
2-methylbutanal (μg L^{-1})	25	3.4	25.6	8.90	7.58	85%
	45	4.5	26.1	11.0	7.62	69%
3-methylbutanal (μg L^{-1})	25	−9.2	74.9	16.6	26.5	160%
	45	13.9	79.0	31.9	23.1	72%
Methional (μg L^{-1})	25	7.8	69.9	24.0	21.6	90%
	45	8.2	67.5	24.5	19.9	81%
Phenylacetaldehyde (μg L^{-1})	25	18.3	103	66.3	30.9	47%
	45	9.5	144	52.8	42.4	80%

Data are minimum (min), maximum (max), average, standard deviation (SD) and relative standard deviation (RSD (%)) values of the 'O$_2$ not SO$_2$' consumed and of the aldehydes accumulated during oxidation. O$_2$ not SO$_2$ is obtained simply by subtracting from the total amount of O$_2$ consumed the amount of O$_2$ equivalent to the measured decrease in total SO$_2$.

phenylacetaldehyde. Differences between wines were of large magnitude, as previously described,[8,15] and would be responsible for relevant sensory differences. Notwithstanding such sensory relevance, it should be noted that in molar terms the summation of the maximum levels of Strecker aldehydes accumulated were just 3.5 µmol L^{-1}, for the formation of which just 0.11 mg L^{-1} O$_2$ were required – less than 0.3% of the O$_2$ not SO$_2$ consumed in the experiment.

Differences in the accumulation of aldehydes at the two temperatures will be better discussed in terms of AARs (see below).

Aldehyde accumulation rates
Aldehyde accumulation rates, normalized by the amount of O$_2$ not SO$_2$ (AARs), or by that parameter and also by time (t-AARs), can be seen in Table 3 and Supporting Information, Table S3, respectively.

The comparison between both temperatures is summarized in the last rows of the tables, which give the equations and basic statistics of the regression lines relating accumulation rates at 45 °C with those at 25 °C. It can be seen that regression models are very good for methional, and 2-methyl and 3-methylbutanals, relatively acceptable for acetaldehyde and isobutanal, and nonexistent for the case of phenylacetaldehyde. As AARs in Table 3 are normalized only by O$_2$ not SO$_2$, the magnitudes at both temperatures are directly comparable. In this sense, it is quite remarkable that in the cases of methional and 2-methylbutanal slopes and intercepts do not significantly differ from 1 and 0, respectively, indicating that these aldehydes accumulate, essentially, in similar magnitudes in both experiments. For 3-methylbutanal, the slope does not differ from 1, but there is a significant positive intercept, which indicates that at 45 °C all rates are slightly higher than those at 25 °C. Therefore, it can be concluded that the relative tendencies of red wines to

accumulate these three Strecker aldehydes during oxidation can be predicted by the accelerated assay at 45 °C.

The predictive abilities of the models for isobutanal and acetaldehyde are insufficient. In the case of isobutanal, accumulation at 45 °C is higher than that at 25 °C in all wines, except in the case of the sample showing maxima accumulation (CH), for which the AAR at 25 °C is slightly higher than that at 45 °C. This has a major effect on the slope of the regression line, which becomes smaller than 1. In the case of acetaldehyde, strong differences between young and aged samples are observed. In the four aged samples (SL, TS, BL and CH) (first four rows in the table), the AARs for acetaldehyde are higher at 25 °C, while for the four young ones (MF, TP, HV and BS) (last four rows) the opposite is observed, because young wines hardly accumulated any acetaldehyde at 25 °C, and accumulated small amounts at 45 °C. In young wines, at 45 °C the degradation of nucleophilic flavanols could occur[35,36] and hence the greater accumulation of acetaldehyde.

Those divergences are still more evident in the case of phenylacetaldehyde, because the lack of correlation can be completely attributed to the radical difference in accumulation patterns between young and aged red wines. For aged wines, the AARs at 45 °C were related to those measured at 25 °C by the straight line AR$_{45}$ = −0.22 + 1.82AR$_{25}$ ($R = 0.99996$, significant $P = 5.3 \times 10^{-5}$), indicating that this aldehyde is accumulated at 45 °C nearly twice as much as it was at 25 °C. In strong contrast, as can be seen in Table 3 that AARs of young wines at 45 °C were between two and six times smaller than those at 25 °C.

To compare accumulation kinetics, t-AARs, which are AARs normalized by both O$_2$ not SO$_2$ and by time, are better used (Supporting Information, Table S3). The regression lines between these parameters at both temperatures make it possible to state that:

Table 3. Aldehyde accumulation rates (AARs) of eight different red wines (mg or µg L^{-1} per unit of O$_2$ not SO$_2$ consumed) at two temperatures

	Acetaldehyde		Isobutanal		2-Methylbutanal		3-Methylbutanal		Methional		Phenylacetaldehyde	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
SL-A	0.295	0.251	0.658	0.977	0.336	0.518	0.540	1.296	1.107	0.923	1.037	1.685
TS-A	0.249	0.168	0.358	0.442	0.166	0.230	0.269	0.547	0.376	0.515	0.541	0.755
BL-A	0.546	0.316	0.667	0.842	0.407	0.529	1.006	1.663	1.117	1.256	1.617	2.700
CH-A	0.316	0.256	1.199	1.118	0.749	0.856	2.190	2.590	2.044	2.213	2.705	4.705
MF-Y	0.203	0.240	0.503	0.745	0.206	0.240	0.210	0.748	0.381	0.431	3.615	1.394
TP-Y	0.006	0.178	0.247	0.473	0.110	0.154	−0.299	0.476	0.344	0.466	2.058	0.325
HV-Y	−0.028	0.075	0.242	0.638	0.109	0.184	0.096	0.463	0.242	0.236	2.929	0.908
BS-Y	−0.019	0.078	0.344	0.554	0.125	0.166	0.013	0.570	0.331	0.401	2.338	1.280
max	0.546	0.316	1.199	1.118	0.749	0.856	2.190	2.590	2.044	2.213	3.615	4.705
min	−0.028	0.075	0.242	0.442	0.109	0.154	−0.299	0.463	0.242	0.236	0.541	0.325
max/min	−19.55	4.24	4.95	2.53	6.89	5.55	−7.33	5.60	8.44	9.39	6.68	14.46
Average	0.196	0.195	0.527	0.723	0.276	0.360	0.503	1.044	0.743	0.805	2.105	1.719
Regression between 25 and 45 °C	AR$_{45}$ = 0.12 + 0.333 AR$_{25}$ $R = 0.898$ ($P = 0.0024$)		AR$_{45}$ = 0.36 + 0.687 AR$_{25}$ $R = 0.900$ ($P = 0.0023$)		AR$_{45}$ = 0.049[a] + 1.12[b] AR$_{25}$ $R = 0.985$ ($P = 7.7\ 10^{-6}$) AR$_{45}$ = 1.24AR$_{25}$ R = 0.993 ($P = 4.8\ 10^{-7}$)		AR$_{45}$ = 0.568 + 0.946[c] AR$_{25}$ $R = 0.972$ ($P = 5.2\ 10^{-5}$)		AR$_{45}$ = 0.046[a] + 1.02[b] AR$_{25}$ $R = 0.985$ ($P = 8.6\ 10^{-6}$) AR$_{45}$ = 1.06 AR$_{25}$ $R = 0.994$ ($P = 3.3\ 10^{-7}$)		R = 0.167 n.s. Aged: AR$_{45}$ = -0.22 + 1.82 AR$_{25}$ $R = 0.99995$ ($P = 5.3\ 10^{-5}$)	

[a] Not significantly different from 0.
[b] Not significantly different from 1. -A represents aged wines and -Y refers to young wines.

(1) Methional and 2-methylbutanal accumulate at 45 °C at rates 10.1 and 11.0 times faster than at 25 °C in a strictly proportional way (intercept = 0), respectively ($P < 0.0001$).

(2) For 3-methylbutanal, $t\text{-AARs}_{45} = 124 + 10\ t\text{-AARs}_{25}$ ($P = 0.0004$).

(3) For isobutanal, $t\text{-AARs}_{45} = 53 + 7.6\ t\text{-AARs}_{25}$ ($P = 0.0007$).

(4) For acetaldehyde, $t\text{-AARs}_{45} = 26 + 3.7\ t\text{-AARs}_{25}$ ($P = 0.0010$).

(5) Phenylacetaldehyde in aged wines accumulates at 45 °C, 16.6 times faster than at 25 °C in a strictly proportional way (intercept = 0); ($P = 0.0087$); in young wines $t\text{-AARs}_{45}$ are between 1.3 and 4.7 times higher than those at 25 °C.

PLS models of AARs

To gain additional insights into the reasons for differences between AARs at 45 and 25 °C, PLS regression models relating AARs with wine compositional parameters were carried out. Models at 45 °C are given in Table 4 and should be compared with the models previously published (also included in Supporting Information, Table S4). Models at 45 °C explain by cross-validation between 80.6% and 97.5% of the original Y variance. As can be seen, models for Strecker aldehydes give positive coefficients for the corresponding amino acid precursors and positive coefficients for iron (except phenylacetaldehyde), at both temperatures. There is one more coincidence between the models found at both temperatures: the greater the amount of initial catechin in tannins, the greater the accumulation of phenylacetaldehyde. This may suggest that quinones derived from catechin in tannins are particularly reactive towards phenylalanine, yielding phenylacetaldehyde at both 25° and 45°.

Aside from this, there is no other similarity between the models that explains the accumulation of acetaldehyde and Strecker aldehydes during oxidation at different temperatures. Models for the three aliphatic Strecker aldehydes at 45 °C show remarkable parallelisms between them, while the models for methional and phenylacetaldehyde have several specificities, as detailed below. The model for methional is less robust and has some interesting features, such as the negative coefficients of methionol,

Table 4. PLS models relating the increase in total Strecker aldehyde and acetaldehyde at 45 °C, normalized by the consumed O_2 not invested in the oxidation of SO_2 in each wine, to the initial composition of the wines

	Isobutanal	3-Methylbutanal	2-Methylbutanal	Methional	Phenylacetaldehyde	Acetaldehyde
R^2	0.978	0.991	0.9807	0.960	0.996	0.963
R^2 cross-validation	0.928	0.872	0.882	0.806	0.975	0.926
RMSE	0.888	0.654	0.231	0.484	2.612	0.473
RMSE cross-validation	1.864	2.827	0.654	1.220	7.358	0.763
PCs	3	3	3	3	3	1
B0	4.855	15.285	2.182	0.523	−12.313	5.950
Diacetyl		3.198				
Valine	0.179					
Leucine		0.326				
Isoleucine			0.134			
Methionine				0.115		
Phenylalanine					4.365	
Initial isovaleraldehyde		4.618				
Methionol				−0.888		
Prodelphinidine (egc-egc)				−0.143		
Procyanidin (cat-cat)						−0.812
Catechin in tannins					5.821	
Epicatechin-3-O-gallate	−1.522					
Prodelphinidine B3	2.206		0.353	−0.200		
cat-cat-egc	1.726				−16.189	−0.680
(epi)cat-vyn	0.934		1.145			
Pirano-malv-vinylguaiacol			−0.237			
Petunidine-3-O-glucoside					−14.709	
Delphinidine-3-O-glucoside					−14.125	
Peonidine-3-O-glucoside-4-vinylguaiacol					−7.970	
Terminal units (without ethyl) in tannins						−0.702
Total extension in tannins						−0.855
%egc in tannins	2.565	3.860	1.367			
Methionine sulfoxide				−0.666		
Gallic acid				−0.651		
Combined SO_2				−1.437		
Mn					1.914	
Cu				0.398		
Fe	0.753	1.245	0.011	0.057		

egc: epigallocatechin; cat: catechin; epi: epicatechin; malv: malvidin; vyn: vinylguaiacol.

methionine sulfoxide and combined SO_2. The model for phenylacetaldehyde is completely different, since it is the only one in which anthocyanin derivatives have negative coefficients in the model.

The most relevant and consistent difference between models in Table 4 and those at 25 °C (Supporting Information, Table S4) is that all models at 25 °C, except that for phenylacetaldehyde, were characterized by the presence of a number of anthocyanins with negative coefficients, while in models at 45 °C exactly the opposite was observed. At this temperature, it appears that anthocyanins only limit the accumulation of phenylacetaldehyde.

The apparent role of anthocyanins in limiting AARs was attributed to the known reactivity of these molecules and other wine polyphenols towards aldehydes,[11,37-41] so it was thought that wine could contain a category of wine polyphenols particularly reactive to aldehydes. These polyphenols, called ARPs or 'aldehyde reactive polyphenols', would play a key role in wine oxidation by limiting the accumulation of acetaldehyde and Strecker aldehydes and in determining OCRs.[15,42] Attending to those previous works, such reactivity should be responsible for two major differences in the accumulation pattern of aldehydes: the poor ability of young wines to accumulate acetaldehyde and Strecker aldehydes other than phenylacetaldehyde, and the particular accumulation pattern followed by phenylacetaldehyde. However, models in Table 4 challenge this interpretation since, to hold true, the reactivity of phenylacetaldehyde towards wine polyphenols should increase with temperature, while that of the other aldehydes should decrease. This is further examined below.

Reactivity of aldehydes in wines at 25 and 45 °C

Wines equivalent to those studied in the first part of the study were spiked with acetaldehyde and Strecker aldehydes, so that their final concentration was similar and equal to that observed in the sample containing maximum levels of that aldehyde after oxidation at 25 °C. Spiked wines were incubated in anoxia at 25 and 45 °C for time periods equivalent to those of the oxidation experiment (36 and 5 days, respectively). Initial and final contents in acetaldehyde are given in Table 5 and the fraction consumed of each Strecker aldehyde is given in Table 6. Precipitates were

not found in any vial, contrary to what was obtained in experiments for a longer time (120 days) and stronger additions of acetaldehyde.[43,44]

Results in Table 5 reveal that wines hardly consume any acetaldehyde at 45 °C, regardless of their age, while at 25 °C all the wines consumed between 8 and 28 mg L^{-1} of acetaldehyde. The poor aldehyde consumption at 45 °C is consistent with the observation that the rate of formation of ethyl bridges between polyphenol units only doubles for an increase of 20 °C (from 22 to 42 °C) while, as shown in Table 1, OCRs multiply by a factor close to 8 for an equivalent temperature increase. In other words, the data in Table 5 support that at 45 °C the consumption of acetaldehyde by wine is too small to be significantly measured after just 5 days. While this result seems to be consistent with the tested hypothesis, since the relative reactivity at 45 °C is smaller than that at 25 °C, there are several observations that do not fit with such a hypothesis. First, reactivity at 25 °C is similar between young and aged wines; second, as reactivity at 45 °C is almost null, a much higher accumulation of acetaldehyde should be expected at this temperature, which is not the case, as seen in Tables 2 and 3.

Moreover, acetaldehyde consumption at 25 °C was essentially related to the wine initial free SO_2 level, as expected. Leaving aside the sample with smallest SO_2, which consumed abnormally high levels of acetaldehyde, the amounts consumed can be estimated by the following equation:

$$Acetaldehyde_{reacted} = 18.5 - 0.331 - C_{freeSO_2}$$

with $R = 0.83$, significant $P = 0.02$. Residuals of the regression are given in the last column of Table 5. These residuals are very small, ranging from -2.9 to 1.8, with a standard deviation of just 1.6 mg L^{-1}, and do not maintain any correlation with AARs. This means that differences between wines in reactivity towards acetaldehyde at 25 °C are small and not related to wine age. All these results suggest that even if a significant part of the acetaldehyde formed during oxidation at 25 °C reacts with wine polyphenols, such reactivity does not seem to have a major influence on the observed differences in AARs between different wines.

Table 5. Consumption of acetaldehyde in anoxia at two different temperatures

	Initial data			After 5 days at 45 °C			After 36 days at 25 °C			
	Free SO_2 (mg L^{-1})	Native acetaldehyde	Spiked acetaldehyde	Remaining	Consumed	Consumed (%)	Remaining	Consumed	Consumed (%)	Regression residual
SL-A	9.6	29.4	8.6	36.7	1.30 ± 1.0	3.4%	22.2	15.8 ± 0.8	41.6%	0.47
TS-A	7.2	19.6	18.4	38.6	−0.60 ± 0.4	−1.6%	22.6	15.4 ± 0.4	40.5%	−0.73
BL-A	8.8	19.9	18.1	43.4	−5.40 ± 0.8	−14.2%	21.6	16.4 ± 0.4	43.2%	0.80
CH-A	22.4	37.7	0.3	38.4	−0.40 ± 0.6	−1.1%	29.8	8.20 ± 0.6	21.6%	−2.89
MF-Y	2.4	6.8	31.2	33.7	4.30 ± 0.5	11.3%	9.6	28.4 ± 1.6	74.7%	—
TP-Y	7.2	11.1	26.9	39	−1.00 ± 1.7	−2.6%	22.7	15.3 ± 1.1	40.3%	−0.83
HV-Y	17.6	8.22	29.8	34.1	3.90 ± 0.4	10.3%	23.5	14.5 ± 0.7	38.2%	1.84
BS-Y	24	10.2	27.8	36.5	1.50 ± 0.4	3.9%	26.1	11.9 ± 0.3	31.3%	1.34

All samples were spiked with variable amounts of acetaldehyde to make their final levels equal to the initial maximum level (38 mg L^{-1}). Data are expressed in mg L^{-1}. Residuals refers to the differences between measured values and values of acetaldehyde consumed, estimated by the regression model

$$Acetaldehyde_{consumed\ 25°C} = 18.5 - 0.331 - C_{free\ SO_2}$$

-A represents aged wines and -Y refers to young wines.

Table 6. Fraction (%) of Strecker aldehyde reacted during incubation (36 days at 25 °C and 5 days at 45 °C) in anoxia of wine samples spiked with aldehydes so that all samples contained similar levels of Strecker aldehydes

	Isobutanal		2-Methylbutanal		3-Methylbutanal		Methional		Phenylacetaldehyde	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
SL-A	71	29.6	41.7	33.3	77.3	51	21.6	16.9	50.5	29.8
TS-A	75.3	42.7	49.5	42.6	77.9	53.3	31.5	24.5	65.2	44.9
BL-A	73.9	40	47.2	30	76.9	51.3	35.2	26.2	65	41.7
CH-A	72.7	26.1	42.2	24.8	76.1	44.5	24.9	16.4	54	24.4
MF-Y	77.4	37.5	44.1	25.9	83.2	48.8	63.5	26.6	67	48.6
TP-Y	73.1	31.8	62.7	45.2	76.3	49.4	42.7	31.7	72.2	49.7
HV-Y	75.2	40.1	56.8	30	78.6	54.5	34.7	27.3	66.3	45.8
BS-Y	72.1	45	44.1	37	75.5	54.8	25.6	24.3	54.3	43.2
Mean	**73.8**	**36.6**	**48.5**	**33.6**	**77.7**	**51**	**35**	**24.2**	**61.8**	**41.0**
SD	2.1	6.7	7.5	7.5	2.4	3.4	13.4	5.2	7.8	5.2
RSD (%)	2.8%	18.3%	15.5%	22.2%	3.1%	6.7%	38.3%	21.5%	12.6%	22.2%
*R*1	0.392		0.571		−0.076		0.615		0.823*	

*R*1: Pearson correlation coefficients between consumption at both temperatures.-A represents aged wines and -Y refers to young wines.
*Significant at *P* < 0.05.

The reactivity of Strecker aldehydes is given in Table 6. As can be seen, reactivity is higher at 25 °C, although in this case the reactivity at 45 °C was important too. The average reactivity of isobutanal decreases by a factor 2, while those of the other aldehydes, including phenylacetaldehyde, decrease by a factor of 1.5, so that the decrease in reactivity is quite homogeneous and certainly cannot explain the particularities observed in the PLS models at 45 °C for phenylacetaldehyde. It should be also mentioned that the average percentages of Strecker aldehyde reacted at 25 °C, given in Table 6, have a linear relationship with the formation constants of their hydroxysulfonates with SO_2[34,44] measured at this temperature:

$$\%Reacted = 77.6 - 8.99 \times 10^{-3} \times K_f$$

where $R = 0.991$, significant at $P < 0.001$. This relationship suggests that the five Strecker aldehydes react equally with wine components at 25 °C, which contrast with the particular pattern followed by phenylacetaldehyde, supporting again that reactivity is not the major reason for differences in AARs between Strecker aldehydes. Finally, results also show an amazing homogeneity in the amounts of aldehyde reacted among wines, particularly evident for isobutanal and 3-methylbutanal at 25 °C, which, studies have shown, are those forming the weakest complexes with SO_2. For the other aldehydes, relative standard deviation for reactivity is below 22.2%, except for methional, suggesting in any case that reactivity is not a dominant factor explaining the variability in Strecker aldehyde accumulation seen in Table 2.

2-methylbutanal; it is acceptable for isobutyraldehyde and acetaldehyde, and nonexistent in the case of phenylacetaldehyde.

(1) On average, red wines consume O_2 at 45 °C around 8 times faster than at 25 °C, accumulate acetaldehyde 3.7 times faster, and aliphatic Strecker aldehydes and methional between 7.6 and 11 times faster. Accumulation of phenylacetaldehyde at 45 °C is 1.3–5 times faster for young wines, and 17 times faster for aged wines than at 25 °C, respectively.

(2) While OCRs at both temperatures are not correlated, AARs, except for those of phenylacetaldehyde in young wines, are well correlated. In the cases of methional, 2-methylbutanal and 3-methylbutanal, AARs at 25 °C measured in a 36-day forced oxidation procedure are equivalent to those measured at 45 °C in a 5-day assay.

(3) Anthocyanins, which in PLS models explaining AARs at 25 °C were found to have negative coefficients except for phenylacetaldehyde, play opposite roles in models at 45 °C. The question is whether those negative coefficients could be attributed to a key role of aldehyde–polyphenol reactivity in limiting AARs, responsible for observed differences between young and aged wines and between phenylacetaldehyde and the other aldehydes.

(4) The study of reactivities of acetaldehyde and Strecker aldehydes in anoxia reveals that, although reactivity can effectively limit the amount of aldehyde accumulated during oxidation, it cannot explain the differences in AARs observed between wines, between young and aged wines, or those observed between phenylacetaldehyde and the other Strecker aldehydes.

CONCLUSIONS

The proposed accelerated oxidation procedure at 45 °C does not directly provide kinetics information about the consumption of O_2 in a wine at 25 °C.

The potential for oxidation at 45 °C as a predictor of the generation of aldehydes is very good for methional, 3-methylbutanal and

3876

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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