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Abstract

Aims: The management of O_2, CO_2 and SO_2 at bottling and the choice of closure are two key factors of the shelf life of bottled wines before bringing them to market. The impact of eight closures (four screw caps, two synthetic stoppers and two technical stoppers) was evaluated on a red Merlot/Tannat wine. The results of a rosé wine are also discussed.

Methods and results: Analytical monitoring (O_2, CO_2, SO_2, aphrometric pressure, L*, a*, b*) was carried out over 538 days of storage at 20°C, along with two sensory analyses at 10 and 17 months. The average wine total O_2 content at the time of bottling was 2 mg/L. Intra- and inter-procedure variability was controlled, including for dissolved CO_2 content.

Conclusion: Unlike closures with the highest Oxygen Transmission Rate (OTR), the two technical stoppers and the two screw caps with Saranex seal, harboring the lowest OTR, matched with the wines exhibiting a low total O_2 content at equilibrium (from 4 to 18 months after bottling), with more free SO_2 and less color change. However, the OTR gradient (5 to 67 µg/d) observed through the physicochemical analyses was not necessarily confirmed by the two sensory analyses.

Significance and impact of the study: This study puts into perspective the impact of closure OTR on the sensory characteristics evolution of wine consumed within the first two years, especially when total O_2 at bottling exceeds 1.5 mg/L.

Key words: bottling, wine, screw cap, stopper, closure, shelf life, oxygen, carbon dioxide

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Introduction

Oxygen is one of the main factors for wine's evolution. At bottling, the level of oxygen captured in the headspace (HSO) and dissolved in the wine (DO) must be reduced as much as possible. The Oxygen Transmission Rate (OTR) of closures then regulates the transfer of oxygen inside the bottle after bottling. Thus, the management of O_2, CO_2 and SO_2 at filling and the choice of closure act as key factors of the shelf life of bottled wines.

Oxygen ingress during and post bottling leads to a loss of sulfites. In wine, the reaction between O_2 and SO_2 is extremely slow (Waterhouse and Laurie, 2006). Sulfites react with the products of wine oxidation and in particular with hydrogen peroxide, produced from the oxidation of phenolic compounds (Danilewicz *et al.*, 2008; Danilewicz and Wallbridge, 2010). The wine becomes more sensitive to oxidation and ages faster. Godden *et al.* (2001) highlighted a critical concentration of free SO_2 of 10 mg/L below which a Semillon wine is perceived as substantially affected by oxidized aromas. For red wines, controlled oxygen ingress is necessary and variable according to the expected quality before and after bottling, especially to avoid reduction (Caillé *et al.*, 2010; Ugliano *et al.*, 2012).

The commercial choice between stopper (natural, technical or synthetic) and screw cap has a direct impact on the volume and inerting process of the headspace as well as the OTR of closure. The volume and technical management of the headspace (vacuum, gas sparging, snowdrop) explain that the quantity of oxygen trapped in the headspace can vary from 0.4 to 3.6 mg/bottle (bt) (Vidal and Moutounet, 2006). The bottling line audits outlined by O'Brien *et al.* (2009) confirm this broad range of oxygen amount. Kontoudakis *et al.* (2008) showed that stopper type significantly affected the HSO content. The headspace volume of a corked bottle is significantly lower than that of a capped bottle, but on the other hand the cork releases part of the oxygen trapped in its own structure due to the compression of the stopper in the bottleneck (Squarzoni *et al.*, 2004).

Regarding bottle storage position, there is no consensus to date on an effect on oxygen mass transfer through the closure and wine aging over time, even if, in theory, the oxygen diffusion coefficient through the closure into the wine is smaller than into the headspace. Mas *et al.* (2002) concluded that white and red wines were best preserved when bottles were stored horizontally rather than vertically. Puech *et al.* (2006) on rosé and red wines and Skouroumounis *et al.* (2005) on white wines found no significant difference. Godden *et al.* (2001) concluded that upright storage tended to accelerate SO_2 loss from a Semillon wine, but in many cases this effect was marginal.

The principal methods for determination of wine closure OTR are the coulometric method by Mocon Oxtran with nitrogen flushing of the inner face of the cell (ASTM F1927; ASTM International, 2014), the differential permeability method with pressure difference between both ends of the stopper (Sanchez and Aracil, 1998), the luminescence method on corked or capped bottles filled with nitrogen or deoxygenated acid water (Diéval *et al.*, 2011; Vidal *et al.*, 2011), and the colorimetric method with indigo carmine (Lopés *et al.*, 2005). The Mocon Oxtran technology is by far the most commonly used in the packaging industry. But when applied to the bottle/closure system, it cannot exactly mimic the storage conditions where the closure is in contact with the wine (horizontal storage) or in contact with the water vapor-saturated headspace (vertical storage). Another major drawback of this method is the long time required to reach the steady state of oxygen ingress through the closure when 40-mm-long stoppers are tested (Poças *et al.*, 2010). These reasons explain why manufacturers use also methods with operating conditions closer to enological reality and which better integrate the desorption of oxygen by the stopper mainly during the first month, such as luminescence and colorimetric methods (Diéval *et al.*, 2011; Lopés *et al.*, 2006).

However, whatever the used method, the range of OTR of natural corks is roughly intermediate between that of screw caps/technical stoppers and synthetic stoppers, but with greater heterogeneity by comparison with industrial stoppers (Karbowiak *et al.*, 2009; Lopés *et al.*, 2005; Macku and Reed, 2011; Sanchez and Aracil, 1998).

Many studies have examined the impact of OTR on wine quality, showing that higher oxygen permeability was associated with a higher decrease in SO_2 level, a higher increase in absorbance at 420 nm and premature emergence of oxidized aromas in white wines (Brajkovich *et al.*, 2005; Chatonnet and Labadie, 2003; Godden *et al.*, 2001; Lopés *et al.*, 2009; Skouroumounis *et al.*, 2005). Conversely, screw caps are cited by the majority of these articles as closures for which reductive notes are most frequent due to their low OTR.

Generally speaking, red wine behaves in a similar way to white wine, but thanks to its higher phenolic compounds content, it is much more resistant to oxidation (Mas *et al.*, 2002) but also more sensitive to reduction when oxygen ingress post-bottling is insufficient (Caillé *et al.*, 2010; Ugliano *et al.*, 2012), in particular with screw caps (Kwiatkowski *et al.*, 2007). On two rosé Grenache wines, higher OTR wines were also perceived as more floral and fruity and less animal than those stored under lower oxygen exposure after 10 months, in agreement with previous observations on red Grenache wines (Wirth *et al.*, 2012).

Thus, for entry and mid-range wines, synthetic or technical stoppers and aluminum caps usually supersede natural corks.

Based on these findings, an experimental protocol was set up in order to answer the following questions: What is the impact of these closures on the shelf life of a red wine bottled at an industrially achievable Total Oxygen content (TO_2) and intended to be drunk within two years? Are there differences between stoppers and screw caps? Finally, which physicochemical and sensory characteristics are most affected by the oxygen permeability of the closure? The results of the same experimentation on an AOC Coteaux Varois rosé wine are also discussed. To the best of our knowledge, this is one of the first studies based on the relationship between OTR and the consumptions of oxygen and sulfites under industrial conditions controlled by dissolved gases and sulfites.

Materials and methods

1. Experiment

A 2013 IGP Côtes de Gascogne red wine (70% Merlot + 30% Tannat) was bottled on 26 June 2015 at INRA Pech-Rouge bottling facility (Gruissan, France) in 75-cL flint glass Bordeaux bottles at targeted levels of O_2, CO_2 and SO_2 and with weak dispersion. Two synthetic stoppers (B1, B4), two technical stoppers (B2, B3), two screw caps with Saranex seal (C1, C2) and two screw caps (C3, C4) with seal without polyvinylidene chloride (PVDC) were tested (Table 1). The two types of closure and the different length of stoppers (42 or 44 mm) led to different headspace volumes and inerting processes. The target TO_2 in bottle was set at 1.5 mg/bt (2 mg/L), a value reasonably achievable at the industrial level whatever the closure used.

After bottling, bottles were stored upright in the dark in a thermostatically controlled room at 19.9 ± 0.5°C, with 67.2 ± 15.8 %HR (monitored, but not controlled). Both destructive and non-destructive physicochemical analyses were carried out on several dates spread out over 538 days after bottling. An expert panel performed sensory analyses at 10 and 17 months.

2. Methods of OTR measurement

Given the small thickness of the seal, the oxygen release of screw caps is negligible, as shown by Vidal *et al.* (2011). For stoppers, as discussed in the introduction, manufacturers generally prefer the luminescence and colorimetric methods of OTR measurement to better quantify the higher release of oxygen by stopper at the beginning of storage, which significantly increases the OTR. The coulometric method tends to undervalue this phenomenon and gives an OTR 0.009 mg/d lower than the luminescence method for B1 (0.014 mg/d). The OTR of B2 by coulometry is enhanced by an estimated release of 1.5 mg. For stoppers B3 and B4,

Table 1 - Description of the eight closures.

Code	Type	Length mm	Diameter mm	Weight g	Density kg/m³	OTR[2] mg/d	Method of OTR measurement
B1	Synthetic stopper	42	22.5	4.8	285	0.014[3]	Luminescence
B2	Technical stopper	44	24.5	5.6	270	0.005[4]	Coulometry
B3	Technical stopper	44	24.0	5.7	280	0.005[3]	Colorimetry
B4	Synthetic stopper	42	22.0	7.6	488	0.047	
C1	Screw cap + Saranex seal		31.5			0.005	
C2	Screw cap + Saranex seal		31.5			0.005	Coulometry
C3	Screw cap + seal without PVDC[1]		31.5			0.067	
C4	Screw cap + seal without PVDC		31.5			0.007	

[1] PVDC: Polyvinylidene chloride.
[2] OTR: Oxygen transmission rate in mg/d: mg/day/closure, given by the manufacturer.
[3] OTR, included release, calculated on 1 year.
[4] OTR, included estimated release of 1.5 mg, calculated on 1 year.

manufacturers provided OTR value obtained by a single method presented in Table 1.

3. Bottles

Cork bottles: OI, standard 75-cL BD CAR II LG, unfilled level 63 mm; screw cap bottles: OI, standard 75-cL BD CAR II LG BVS, unfilled level 45 mm.

4. Wine analysis just after filling

12.9 %vol.; sugar 2.6 g/L; TA 3.33 g H_2SO_4/L; VA 0.43 g H_2SO_4/L; pH 3.49; free SO_2 27 ± 0 mg/L; total SO_2 68 ± 0 mg/L; CO_2 325 ± 15 mg/L; L* 67.21; a* 33.89; b* 8.91; A_{420} 2.532; A_{520} 3.379; A_{620} 0.633; Total Phenol Index 49.

5. Bottling

The INRA Pech-Rouge bottling line for experimental wines allowed the control and management of dissolved gases on the three elements of the chain (Figure 1):

- a filtration skid (RS IW, Tübingen, Germany) with preparation tank (105 L), prefiltration (1 µm) and final filtration (0.65 µm);

- a single head filler MTB 1/1 (Perrier, Le Cheylard, France) with or without neutral gas flushing of filler tank (46 L) and bottles before filling,

- a single head corking machine Gemini R (Arol, Canelli, Italy) with coupling vacuum and inert gas (CO_2 for this study) in several cycles before corking in order to reduce the oxygen amount of the headspace.

This line achieves homogeneous bottling of small volumes of wines with very low variations in TO_2 and dissolved CO_2 (DCO_2) (Vidal, 2015; Vidal *et al.*, 2012).

Four batches of 100 L were required for the filling of the 450 bottles of the study. The preparation tank was filled by gravity with the starting tank of red wine. The wine was sparged with N_2 gas using a porous injector bolted to the bottom of the preparation tank until DO reached 0.15 mg/L then was adjusted to 300 mg/L of DCO_2 by sparging with CO_2 gas.

The circuit was purged with N_2 from the outlet of the preparation tank to the head filler machine. The wine was forced into the circuit by N_2 to the filler tank through the filtration skid using overpressure of 100 **Figure 1. The three elements of INRA Pech-Rouge bottling line for experimental wines: filtration skid + filling and corking machines.**kPa applied to the top of the preparation tank. Bottles were blanketed before filling. A slight depression of 8 kPa assisted the filling height adjustment.

Filled cork bottles were sealed by the single head corking machine. Two combined cycles of CO_2 (1 s) followed by vacuum 75 kPa (1 s) were performed. Filled screw cap bottles were crimped by a single head capping machine Galaxy (Costral, Riquewihr, France) without inerting of headspace and cap. The unscrewing torque was checked for the four kinds of screw cap bottles with Orbis 6 Nm digital torque tester (Mecmesin, Slinfold, England).

Since the DO was set to a low level for all procedures, the TO_2 target value of 1.5 mg/bt was reached thanks to the management of the headspace according to the type of bottle and its unfilled level.

6. Physicochemical analyses

On line O_2 monitoring was performed using a PreSens luminescent probe and PSt3 O_2-sensitive optical spots (PreSens Precision Sensing GmbH, Regensburg, Germany) integrated at four checkpoints on the bottling line and at the top and

Figure 1. The three elements of INRA Pech-Rouge bottling line for experimental wines: filtration skid + filling and corking machines.

bottom of the preparation and filler tanks. DCO_2 was monitored by sampling in the preparation tank using a Carbodoseur (Dujardin-Salleron laboratories, Noizay, France).

The following destructive analyses were performed at T0, 1, 4, 8, 12 and 18 months: aphrometric pressure (simplified aphrometer for still wines, Ligapal, Cormontreuil, France); free and total SO_2 (potentiometric titration, Titromatic, Crison Instruments, Alella, Spain); and ΔEab* (spectrophotometer CM3600d, Konica Minolta, Roissy CDG, France, standard illuminant D65, 10° standard observer). Non-destructive monitoring was conducted every month from T0 to 18 months for unfilled level (set square for wine bottle): calculation of the headspace volume; dissolved and gaseous O_2 (luminescence with PreSens PSt3 glued spots inside bottles); and dissolved CO_2 (laser spectroscopy, Lsensor CO_2, FT System, Padova, Italy). For destructive and non-destructive chemical analyses, three repetitions were performed by parameter / procedure / date.

Twelve bottles capped with C4 screw cap were stored at 7°C (C4 7°C). These bottles were used for SO_2 analyses at 243, 370 and 532 days.

7. Sensory analyses

Descriptive quantitative analysis was conducted by an expert sensory panel (22 judges), selected on the basis of their sensory performances and interest (ISO 8586-2, 1994), and trained to descriptive sensory analysis of wines. As a first step, the expert panel selected attributes by consensus to describe the samples. Table 2 presents the final selected attributes. Then the panelists were trained to understand and consistently use these attributes and familiarized with the sensory space of the product. Finally, they rated each attribute on an unstructured linear scale from "low" to "high". For olfactory and taste analyses, wines were evaluated in duplicate, in monadic service, according to a random order (Latin square) minimizing carry-over effects, in black tulip-shaped glasses (to ensure that visual perceptions did not influence olfactory and taste analyses), between 17 and 19°C. Then for visual attributes, samples were evaluated in comparative service, in 215-mL wine glasses, in "normal daylight".

Sensory data were converted to a 10-point scale by Fizz Software version 2.40 A (Biosystemes, Couternon, France).

8. Statistical analysis

Statistical analyses were performed using XLSTAT software version 2014 (Addinsoft, Paris, France).

After confirming the panel's good performance (repeatability, consensus and discrimination), the sensory results were analyzed by analysis of variance (two factors: judge and wine). When significant differences were revealed ($p < 0.05$), mean intensities were compared using the Tukey (HSD) multiple comparison test.

The results of free - total SO_2 and consumption ratios of SO_2/O_2 were analyzed by analysis of variance (one factor: closure). When significant differences were revealed ($p < 0.05$), analytical parameters were compared using the Tukey (HSD) multiple comparison test.

Table 2 - Selected sensory attributes and composition of their reference standards.

Sensory cluster	Attribute	Reference standard
Visual	Color intensity	
Olfactory	Amylic	Isoamyl acetate
	Animal (leather)	4-Ethylphenol
	Cooked red fruits	Red fruits jam
	Dry wood (dust)[1]	Unheated wood powder
	Pastry (vanilla, caramel)	Caramel syrup
	Pepper	Black pepper
Taste	Astringency	Grape stem tannin extract
	Bitterness	Caffeine
	Sourness	Tartaric acid
	Sweetness[1]	Grape sugar
	Alcohol[1]	Absolute ethanol

[1] Attributes added at 17 months of storage.

The treatment allowed to classify the different wines in several distinguished groups (A, B, C, D, E).

To summarize the impact of the closures on the physicochemical parameters of the rosé and the red wines, a principal component analysis (PCA) was carried out with the same software. The analytical data were centered and normalized before being treated by PCA.

Results and discussion

1. Technical parameters (unscrewing torque, aphrometric pressure, unfilled level)

The unscrewing torque of capped bottles tested after crimping was on average 16 ± 1 lbf/inch for all four screw cap procedures. The unfilled level which determines the headspace volume was on average 63 ± 2 mm for stoppers and 45 ± 2 mm for screw caps (net of seal thickness) from T0 to 538 d. Aphrometric pressure fluctuated between -160 and 200 kPa from T0 to 538 d. The monitoring of these three parameters was in accordance with usual technical recommendations.

2. Dissolved gases

Just after bottling, 86% of TO_2 (TO_2 = HSO + DO) was located in the headspace of bottles. The average TO_2 was 1.5 mg/bt (2 mg/L). Heterogeneity remained limited because the highest intra-procedure standard deviation was 0.28 mg/bt (Table 3) and the maximum intra-procedure deviation was 0.53 mg/bt (between B1 and C3). As previously described (Dombre *et al.*, 2016; Toussaint *et al.*, 2014; Vidal and Moutounet, 2011), both oxygen in the headspace and dissolved in the wine were consumed. This decrease was not linear because 90% of initial TO_2 was consumed after 35 days. From the 48th day, TO_2 was less than 0.10 mg/bt, except for B4 and C3. These procedures had the most variable and highest TO_2 content, mainly due to HSO level (higher than 0.1 mg/bt at 532 d), while their DO level was equivalent to the other procedures (around 0.01-0.02 mg/bt). Beyond the 90th day, we could consider that all closures reached their steady state (Figure 2). TO_2 stabilized at an equilibrium value which was the resultant of O_2 ingress by the closure and O_2 consumption by the wine, for the same wine and in the same storage conditions. Thus, we could link the closure OTR to the average TO_2 between the 90th and 532nd days (Tables 1 and 3).

The CO_2 concentration remained stable up to 532 days, except for the B4 procedure for which the losses represented 18% (corresponding to a loss of 60 mg/L), below the sensory perception threshold.

3. Color

The distance between two colors (ΔEab*) was used to summarize the evolution of wine color between T0 and 538 d.

$$\Delta Eab^* = \sqrt{(L_0 - L_{538d})^2 + (a_0 - a_{538d})^2 + (b_0 - b_{538d})^2}$$

Over time, b* increased and a* decreased, color gradually changed to tile color. After 538 d, the ΔEab* varied between 6.6 and 8.3 regardless of the procedure. C4, B1, B3 and C2 were the procedures

Table 3 - Contents of O_2 at bottling and TO_2 at equilibrium.

Type	Code	T0 HSO mg/bt	T0 DO mg/bt	T0 TO_2 mg/bt	TO_2 equilibrium mg/bt[1]
Stopper	B1	1.08 ± 0.00	0.15 ± 0.06	1.22 ± 0.06	0.06 ± 0.02
	B2	1.19 ± 0.12	0.19 ± 0.05	1.38 ± 0.09	0.04 ± 0.01
	B3	1.03 ± 0.07	0.30 ± 0.19	1.34 ± 0.17	0.05 ± 0.01
	B4	0.99 ± 0.07	0.25 ± 0.05	1.24 ± 0.12	0.11 ± 0.06
Screw cap	C1	1.43 ± 0.21	0.19 ± 0.03	1.62 ± 0.18	0.04 ± 0.01
	C2	1.46 ± 0.17	0.18 ± 0.03	1.64 ± 0.17	0.05 ± 0.01
	C3	1.61 ± 0.25	0.15 ± 0.03	1.75 ± 0.28	0.23 ± 0.03
	C4	1.54 ± 0.07	0.20 ± 0.09	1.74 ± 0.13	0.07 ± 0.02
Stoppers averages		1.07 ± 0.06 (10.4 ± 0.6 %v/v)	0.25 ± 0.09	**1.30** ± 0.11	
Screw caps averages		1.51 ± 0.17 (7.9 ± 0.9 %v/v)	0.18 ± 0.05	**1.69** ± 0.19	

HSO: Headspace oxygen; DO: Dissolved oxygen; TO_2: Total oxygen; mg/bt: mg/bottle.
[1] TO_2 at equilibrium: average TO_2 between 90 and 532 days of storage.
Averages and standard deviations are based on three bottles per procedure.

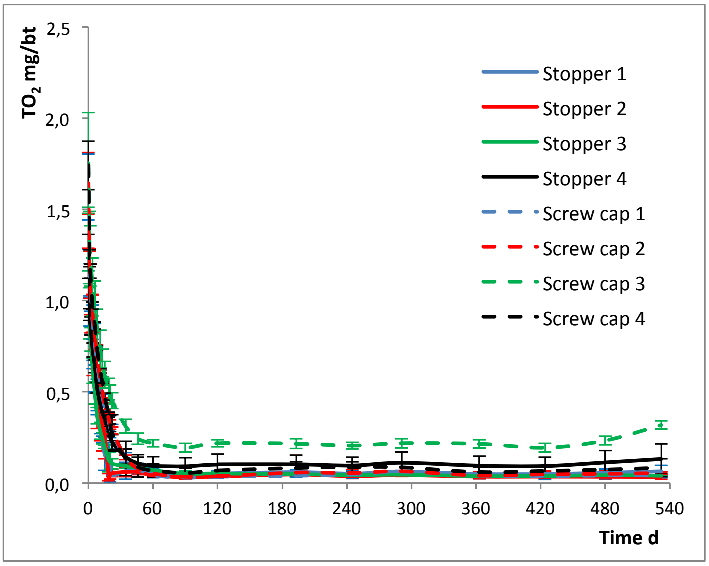

Figure 2 - TO_2 = f(t) for each procedure.
TO_2 mg/bt: Total oxygen in mg/bottle.
Averages and standard deviations are based on three bottles per procedure.

whose color changed the least, unlike B4 and C3. However, even if the ΔEab* between T0 and 370 d (538 d) were at least 4.8 (6.6), the maximum inter-procedure deviation was 1.7 (ΔEab* = 8.3 - 6.6 respectively for B4 - C4 at 538 d). The evolution of wine color until 538 d is mainly due to the aging of wine. By comparison, the ΔEab* after 18 months of storage is less than 1 between wines of Cabernet-Sauvignon sealed by natural cork, synthetic closure and screw cap with 16 mL of headspace volume (Kwiatkowski *et al.*, 2007).

Meanwhile, the color of the red wine stored at 7 °C changed very little (ΔEab* = 0.6 and 1.1 respectively at 370 and 538 d).

Therefore, until 18 months of storage, the impact of temperature on color is clearly greater than that of stopper.

4. Sulfites

From 370 d, the dispersion stayed or extended between the C1, C2 and B2 procedures (for which the free SO_2 was at least 11 mg/L) and the B4 and C3 procedures (for which the free SO_2 toggled below the 10 mg/L threshold); the B3, C4 and B1 procedures exhibited an intermediate position (Figure 3a). The distribution of stoppers was the same for total SO_2 (Figure 3b).

However, it should be mentioned that the impact of the stopper on free SO_2 conservation reached a maximum of 5 mg/L between procedures at 538 d (Figure 3a), that is to say below the value of 7 mg/L

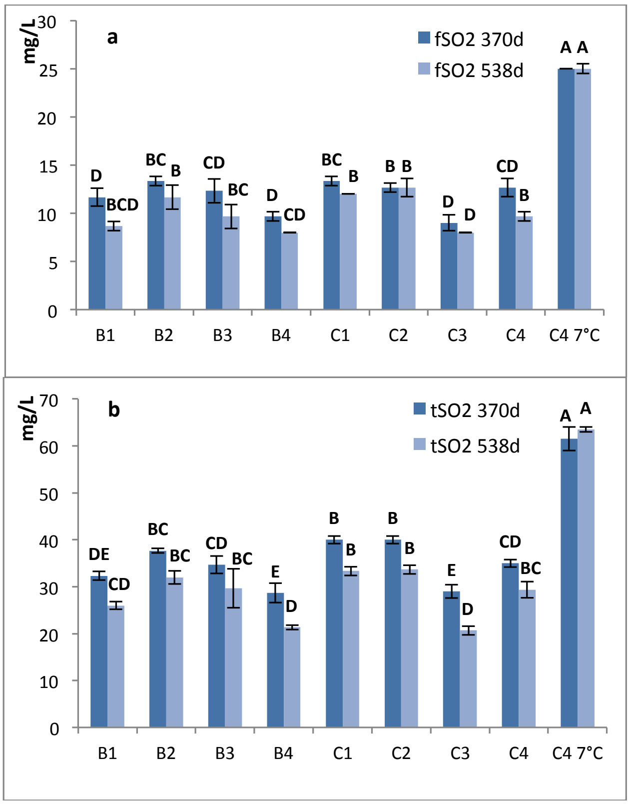

Figure 3 - Free SO_2 (a) and total SO_2 (b) at 370 and 538 days.
Stoppers B1 to B4; screw caps C1 to C4.
Different labels (A, B, C, D, E) indicate means that significantly differ at $p < 0.0001$.
Averages and standard deviations are based on three bottles per procedure.
fSO_2: free SO_2; tSO_2: total SO_2 in mg/L; d: day.
Free and total SO_2 at T0 = 27 ± 0 / 68 ± 0 mg/L.

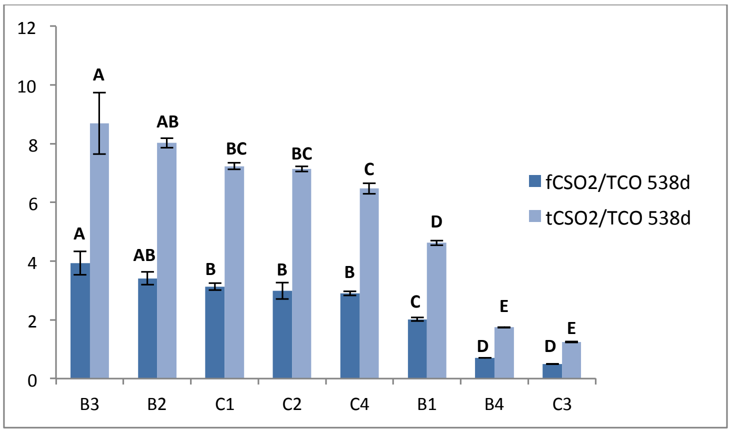

Figure 4 - Ratios of free/total consumed SO_2/TCO at 538 days.

Stoppers B1 to B4; screw caps C1 to C4.

f(t)CSO_2/TCO: Free (total) consumed SO_2 / Total consumed oxygen. $fCSO_2$, $tCSO_2$ and TCO expressed in mg/L.

Ratios ranked in descending order of $fCSO_2$/TCO at 538 d.

Different labels (A, B, C, D, E) indicate means that significantly differ at $p < 0.0001$.

Averages and standard deviations are based on three bottles per procedure.

$$TCO_i \frac{mg}{L} = (TO_{2\,T0} - TO_{2\,i}) \frac{mg}{bt} / 0{,}75 + (OTR \times i\ days)$$

$$\text{Free consumed } SO_2/TCO = \frac{f\ C\ SO_2\ \frac{mg}{L}}{TCO \frac{mg}{L}}\ i\ days = \frac{fSO_2\ T0 - fSO_2\ i}{TCO_i}$$

$$\text{Total consumed } SO_2/TCO = \frac{t\ C\ SO_2\ \frac{mg}{L}}{TCO \frac{mg}{L}}\ i\ days = \frac{tSO_2\ T0 - tfSO_2\ i}{TCO_i}$$

of the expanded uncertainty for free SO_2 content of 10 to 30 mg/L (EURL Œnologues de France, 2016). Only the cold treatment had a clear effect on the preservation of free SO_2, as SO_2 consumption reactions slowed (free SO_2 at 538 d = 10/25 mg/L for C4/C4 7°C).

5. Sulfite versus oxygen consumption

The oxidation of phenolic compounds leads to the production of quinones and hydrogen peroxide. SO_2 reacts with the latter, thus preventing the oxidation of ethanol according to the Fenton reaction, and reduces quinones towards their initial phenolic form. Under ideal experimental conditions, the O_2:SO_2 molar ratio of the reaction is 1:2 (Danilewicz, 2016), corresponding to a maximum theoretical consumption of 4 mg SO_2/ mg consumed O_2. During wine bottle storage, a mass ratio below 4 or a molar ratio of 1:<2 means that part of the oxygen that enters the bottle does not directly reacts with SO_2 but with other wine constituents (Danilewicz, 2016; Han *et al.*, 2015). Nucleophilic compounds come into competition with sulfites to react with the quinones. Waterhouse *et al.* (2016) used the mass ratios with free SO_2 consumption ($fCSO_2$) and total SO_2 consumed ($tCSO_2$) to evidence this phenomenon: the more the mass ratio is below 4, the more the oxidation of other wine constituents is important.

The ranking of stoppers in descending order of $fCSO_2$/TCO at 538 d led to a classification similar to that of OTR: B3 > B2 > C1, C2 > C4 > B1 >>> B4 > C3 (Figure 4). This classification was identical to the ratio calculated with $tCSO_2$ at 370 and 538 days. The only difference was the slightly inverted order between C1 and C2 at 370 days for the ratio calculated with $fCSO_2$. As expected, a decline was observed for all values between these two dates, highlighting more intense oxidation over time. As illustrated in Figure 4, the $tCSO_2$/TCO ratio was greater than 4 for the six least permeable stoppers. This result has already been observed in previous studies on tannin-rich red wine after 12 and 15 months of storage, evidencing oxygen-independent SO_2 consumption reactions (Gambuti *et al.*, 2017; Ugliano *et al.*, 2012).

TO_2 at T0 was between 1.22 and 1.75 mg/bt according to procedures, and the average TO_2 was 1.30 mg/bt for corked bottles and 1.69 mg/bt for capped bottles (Table 3). Therefore, the screw cap procedures started with an average handicap of 0.39 mg/bt compared to the stopper procedures, which was linked to the bottling conditions but independent of the closure type. This bias arbitrarily increased the TCO of capped bottles and impacted their sulfite content without any possibility to truly quantify it afterwards.

In addition, the OTR of B2 included an estimated (but unmeasured) release of 1.5 mg/stopper. If we considered a release of 2 mg/stopper, the mass ratios with fSO_2 and tSO_2 at 538 d decreased respectively to 3.07 and 7.22, bringing B2 behind C2, and even behind C4 if we considered the handicap on TO_2 at T0 (but excluding the unquantifiable impact on sulfites).

To the more or less accurate estimate of the release of stoppers (particularly for B2), we must add the high uncertainty on the measurement of free and total SO_2, regardless of the analytical method (EURL Œnologues de France, 2016).

Finally, all these sources of variation and uncertainty influenced the ratio values and stoppers ranking.

6. Impact of the OTR on physicochemical parameters

TO_2 at equilibrium, fSO_2 losses and ΔEab* are physicochemical parameters which are not statistically related to the OTR but whose evolution is influenced by the diffusion of oxygen through the stopper. Table 4 collects these parameters by comparison to OTR and $fCSO_2$/TCO ratio at 538 d.

OTR expressed in mg/day/closure; TO_2 at equilibrium in mg/bottle; $fCSO_2$ and TCO in mg/L; d: day.

Table 4 shows that the impact of oxygen exposure on red wine followed the rise of closure OTR. But more than a ranking, it evidenced an opposition between B2, B3, C1, C2 and B4, C3, with C4 and B1 having an intermediate position.

As a matter of fact, 2 mg/L of TO_2 at bottling corresponds to one year of oxygen ingress by B3, B2, C1 or C2 closures and 5 mg/L corresponds to one year of oxygen ingress by B1 stopper. In addition, previous studies (Ugliano, 2013; Vidal *et al.*, 2014) on wines stored at ambient temperature have shown that oxidized characters begin to appear around 12 mg/L (9 mg/75 cL) of oxygen ingress in the bottle. Thus, given the concerned quantities of oxygen, it is essential to control and manage oxygen at packaging by reducing the targeted amount of total O_2 trapped in bottle (TO_2 at T0) but also its heterogeneity (standard deviation of each lot), so that stopper fully plays its role of oxygen diffuser, especially for wines aimed to be drunk within 18 months.

The same study was made on an AOC Coteaux Varois rosé wine 12.5 %vol. To extend the analysis of the impact of OTR, the averages of the two wines (rosé and red) were calculated for each physicochemical parameter at 370 and 538 days and processed by principal component analysis. The first and second principal components explained 95.27% (PC1 80.03% and PC2 15.25%) of the total variance (Figure 5). Projection of the wines on these first two PCs showed that stoppers and screw caps were separated along the second axis because of a higher TO_2 content at T0 for the latter, while the first axis appeared related to OTR. Figure 5 shows the same opposition between closures according to their OTR as Table 4.

7. Sensory analyses

Panel performance was checked for each analysis time point. Panel repeatability and consensus were good.

At 10 months, wines were significantly discriminated by the *color intensity* attribute ($p < 0.0001$; Figure 6). No olfactory and taste differences were observed between the eight procedures. C2 was significantly different from all other procedures by displaying a lighter color. Between the seven other wines, C3 had a significantly darker color than B2.

At 17 months, two olfactory attributes (*animal*, $p < 0.030$ and *pepper*, $p < 0.032$) and the visual attribute

Table 4 - Ranking comparison of OTR vs physicochemical parameters at 538 days.

Parameter	OTR and impact of oxygen exposure			
	range	low	medium	high
OTR mg/d	0.005 to 0.067	B2, B3, C1, C2	< C4 < B1	<< B4 << C3
TO_2 at equilibrium mg/bt	0.04 to 0.23	B2, C1 < B3, C2	< B1 < C4	<< B4 << C3
% losses free SO_2 538 d	53 to 70	C2, C1, B2	<B3, C4 < B1	< B4, C3
ΔEab* 538 d	6.6 to 8.2	C4 < B1 < C2, B3	< C1, B2	< C3 < B4
$fCSO_2$/TCO 538 d	3.9 to 0.5	B3, C1, C2, B2	> C4 > B1	>> B4, C3

Stoppers B1 to B4; screw caps C1 to C4.

OTR expressed in mg/day/closure; TO2 at equilibrium in mg/bottle; $fCSO_2$ and TCO in mg/L; d: day.

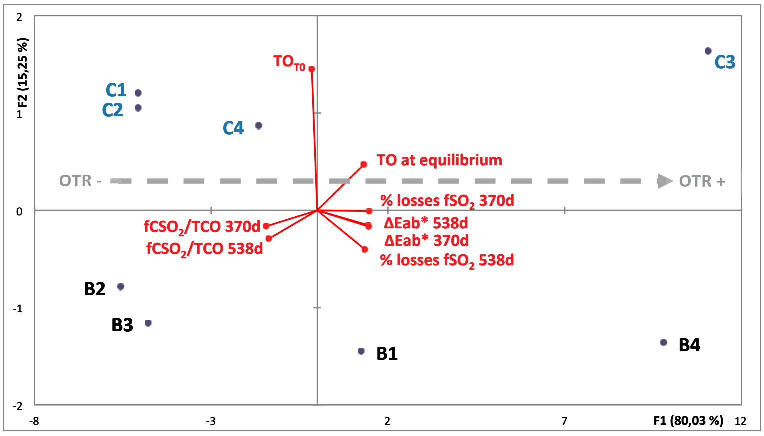

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Figure 5 - PCA on averages of the rosé and red wines by closure at 370 and 538 days.

Stoppers B1 to B4; screw caps C1 to C4.

Projection of the eight closure procedures on the first two principal components and contribution of the variables.

TO_{T0}, TO at equilibrium; % losses fSO_2, ΔEab*, $fCSO_2$/TCO at 370 and 538 d.

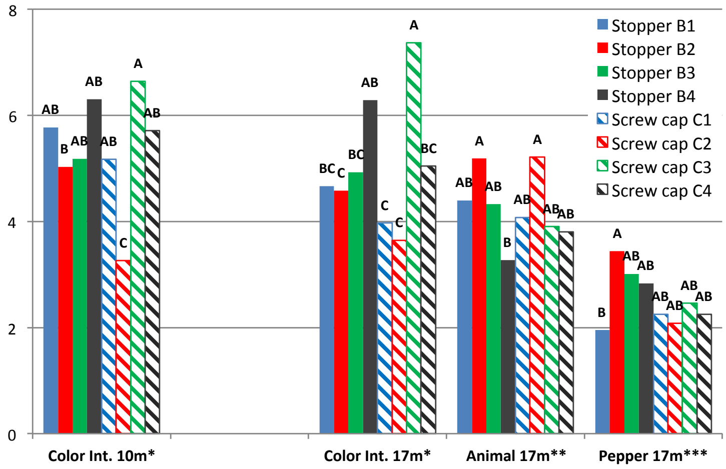

Figure 6 - Means of the significant attributes at 10 and 17 months of storage.

Color Int.: Color intensity; m: month.

Different labels (A, B, C) indicate means that significantly differ at $^*p < 0.0001$; $^{**}p < 0.030$; $^{***}p < 0.032$.

(*color intensity*, $p < 0.0001$) allowed to discriminate wines (Figure 6). No taste difference was observed. At the olfactory level, the C2 and B2 procedures were perceived as significantly more animal than B4. The B2 procedure also had more intense peppery aromas than B1. As at 10 months, the C3 procedure had a significantly darker color, particularly compared to C2, B2 and C1.

The sensory evolution of wines in the 17 months of storage differed according to closures. The evolution of wines closed by the four screw cap procedures was important: wines became less bitter and the intensity of pepper aromas decreased. However, the four stopper procedures had different developments. For the B1 and B4 procedures, animal aromas decreased, whereas for B3 they became more intense. For the B2 procedure, the color became lighter and the intensity of *cooked red fruit* and *pastry* aromas decreased.

A graduation of OTR was observable based upon *animal* and *color intensity*. Wines with low OTR closures had a more intense animal odor and a lighter color; those with high OTR had a darker color. This observation was made in a previous study on a Grenache wine where visual and olfactory differences were observed according to the OTR levels (max. OTR difference: 4.05 mg/year /37.5 cL of wine) but with little impact on taste attributes (Caillé *et al.*, 2010).

The results obtained with a synthetic cork and a small headspace corroborated our conclusions: a Cabernet Sauvignon wine evolved towards significantly reduced notes, even if these descriptors were not the dominant characteristics of the wines (Kwiatkowski *et al.*, 2007). However, for Ugliano *et al.* (2015), an intake of 1 mg/O_2/year was enough to modify the olfactory characteristics from reduced to fruity; but this conclusion was very dependent on the type of red wine.

For the rosé wine, the expert panel performed a sensory analysis at 7 and 12 months. No visual difference was observed between the eight procedures on both dates. The most important fact is the appearance of a graduation of OTR at 12 months based upon the attributes *sulfur* ($p = 0.002$) and *fresh fruits* ($p = 0.017$). Wines with low OTR closures had a more intense sulfur odor, while those with high OTR closures exhibited more fresh fruits aromas. These results are in accordance with those of Wirth *et al.* (2012).

Conclusion

As regards the physicochemical analyses until 18 months of storage, the important points were:

- the average CO_2 losses were negligible,

- the free SO_2 was always above 10 mg/L, except for B4 and C3 closures,

- the HSO of B4 and C3 closures stabilized at higher content, while there was no significant difference in DO between all the closures,

- the color became lighter with time ($\Delta Eab^* \geq 4.8$ as from 370 d), but it was more due to the aging of wine than to the impact of closure,

- the beneficial effect of storage at 7°C for C4 screw cap was clear on SO_2 conservation and protection of the initial color.

Finally, the ranking of closures from the overall experiment was similar to that of the manufacturers, namely from less to more permeable:

C1, C2, B2, B3 > C4 > B1 >>> B4 > C3

The C1, C2, B2 and B3 closures were difficult to differentiate, as the oxygen ingress of the first year is around 1.8-1.9 mg for these four closures. The difficulty of finding a link between physicochemical and sensory results mostly came from the fact that between 10 and 18 months, differences in oxygen intake were low between the stoppers (except for B4 and C3) and wines remained covered by free SO_2. But even with the most permeable closures (C3 and B4), the wines were not systematically characterized by oxidation or aging attributes.

The physicochemical analyses of the rosé wine also highlighted the outlined OTR gradient, but it was on Merlot/Tannat that the sensory analyses were the most affected by OTR gradient at 17-18 months.

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