IMPACT OF OPERATING CONDITIONS DURING BOTTLING AND OF TECHNICAL CORK PERMEABILITY ON THE OXYGEN CONTENT AND EVOLUTION OF BOTTLED SAUVIGNON BLANC WINE

Jean-Claude VIDAL*[1], Michel MOUTOUNET**
* UE999 Pech-Rouge, INRA, 11430 Gruissan, France
** UMR1083 Sciences pour l'œnologie, INRA, 34000 Montpellier, France

ABSTRACT
An experimental study conducted on a Sauvignon blanc wine showed that poor bottling conditions (high dissolved oxygen content, corking with no vacuum and no inert gas) have significantly greater impact than the cork permeability on total oxygen during the early months and hence on losses of free SO_2 and also on wine color and sensorial properties. During the first period following bottling, the kinetics of apparent oxygen consumption is a first order reaction. Under the usual conditions of wine storage, the greatest losses of free SO_2 take place during the first 15-30 days as they are correlated positively with the total oxygen trapped during bottling and released via the cork. The decrease in the concentration of free SO_2 is then smaller as it is related to cork permeability.
KEYWORDS: oxygen permeability, cork, bottling, total oxygen, polarographic probe, wine.

INTRODUCTION
Oxidative phenomena, depending on the presence of oxygen, affect the evolution of wines. Controlled oxidation contributes to the stabilization of color and the reduction of astringency in red wine, as during ageing in barrels *(1, 2)* or in micro-oxygenated vats *(3)*. In contrast, protection from oxygen would seem necessary for white wines that are to be drunk young *(4, 5)*. Finally, it is commonly accepted in oenology that marked oxidation has an adverse effect on wine quality. The various studies undertaken to characterize the appearance of dissolved oxygen during operations performed on wines show that bottling is one of the most critical phases *(6-10)*, especially as once the bottles have been sealed; and, the only remaining means for mastering the evolution of wines are the storage parameters (closure permeability, temperature, relative humidity, light, etc.).

It appears important to know the oxygen content in the headspace of a bottle of wine in order to complete this diagnosis. Using a device for taking a sample of the gas phase combined with gas chromatography (GC), Cook et al. *(11)* showed that the oxygen content of the headspace of 1.5 L bottles of Chablis decreased from 2.5 mL (3.57 mg) to 0.3 mL (0.43 mg) from the 15th to the 84th day after bottling and closing with corks. Vidal et al. *(12)* proposed an assay of oxygen in the headspace of a bottle by means of a polarographic probe that is easy to use *in situ*.

In parallel, other authors have addressed the determination of the permeability of stoppers using experimental air/stopper/gas set-ups (Oxtran, Mocon, Minneapolis, USA) based on the transfer of oxygen from outside to inside a container through a stopper *(13)*. Sanchez and Aracil developed a constant volume, variable pressure permeameter to determine the permeability of corks to oxygen *(14)*. Their results showed that the microagglomerate closure is less permeable to oxygen and more homogeneous than natural corks. Then, Rabiot et al. used the same apparatus to demonstrate that cork permeability increases if one of the faces of the cork is wet *(15)*.

Furthermore, analysis performed by Godden et al. *(16)* showed that 36 months after bottling, oxygen transmission measured in the same technological closure using a Mocon Oxtran unit was 0.0010 mL (1.4 µg)/day whereas the figure was 0.0179 mL (25.6 µg)/day, with greater variability, for the natural cork used as reference.

Valade and Tribaut-Sohier *(17)* estimated oxygen entry in capsuled bottles of Champagne, after bottle fermentation, using an air/capsule/bottle neck device sealed at the base and flushed with

1 *Corresponding author: phone +33 4 68 49 74 00; fax +33 4 68 49 44 02; email vidaljc@supagro.inra.fr

nitrogen and, the release of CO_2 from capsuled bottles whose necks were placed in a sealed chamber flushed with helium. These authors reported that their work showed that gas exchanges can be characterized by losses of CO_2 of the order of a milliliter per day that are thus larger than oxygen entry (10^{-2} mL/day) insofar as there was a good correlation between the two variables. They also demonstrated unequivocally that there is a positive correlation between capsule permeability to gases and the evolution of the Champagne. More recently, Squarzoni et al. *(18)* presented a set-up consisting of air/cork/pure nitrogen/synthetic solution to determine in time the variation of oxygen concentrations by sampling in the headspace and then GC. The authors also put forward the hypothesis that oxygen is released again via the cork after observing an accumulation from 1.14 to 4.29 mg oxygen in the headspace on the first few days after closure, depending on the type of cork.

Lopes et al. *(19)* adapted a method based on the reduction of an indigo carmine solution in a stopped bottle for the non-destructive determination of the release of oxygen through a cork or a cap. Whatever the type of closure, the rate of oxygen release peaked during the first month after bottling because of the release of the oxygen in the closure, according to the authors. The rate then stabilized between 0.33 and 28 µg/day during the next 11 months, according to the type of closure. In 2006, they showed that oxygen penetration in the bottle was smallest with screw caps, followed by technical corks, intermediate with natural corks and high with synthetic corks *(20)*.

Based on these findings, an experimental protocol was set up in order to answer the following questions. Is the impact of cork permeability more important in the evolution of wine than that of the amount of oxygen trapped in the bottle or that resulting from the pressure of sealing? Is it perceptible from the beginning of the storage period? Is oxygen released in the bottle?

MATERIALS AND METHODS

Experiment. A Sauvignon blanc wine was bottled so as to vary the initial dissolved oxygen content of bottled wine (target values: 0.3 and 3.0 mg/L), the cork permeability and the use or not of vacuum at sealing, as summarized in the experimental plan in **Table 1**. Analytical monitoring was performed with 12 dates of analysis after bottling, as shown in **Table 2**. Measurements of dissolved and gaseous oxygen (O_2), dissolved carbon dioxide (CO_2) and sulfur dioxide (SO_2) and assays of color **(Table 2)** and organoleptic characteristics were scheduled throughout bottle storage to characterize the evolution of the wine depending on the bottling-related parameters studied.

The bottles were stored upright in a cellar in which the average temperature and relative humidity during storage were 17.1 ± 1.1 °C and 75.6 ± 8% (1 measurement per 12 hours), respectively.

Table 1: Experimental plan for bottling a Sauvignon wine

Procedure	Initial dissolved O_2 mg/L/batch	Stopper	Closure pressure	Code	Total O_2 T0 mg/bottle	Storage conditions
1	2.64	Diam P1	Vacuum	O+P1vac	4.44 ± 0.20*	17.1°C upright
2		Diam P1	No vacuum	O+P1ap	6.29 ± 0.07	
3		Diam P10	Vacuum	O+P10vac	4.39 ± 0.19	
4		Diam P10	No vacuum	O+P10ap	6.26 ± 0.04	
5	0.23	Diam P1	Vacuum	O-P1vac	1.75 ± 0.20	
6		Diam P1	No vacuum	O-P1ap	3.44 ± 0.09	
7		Diam P10	Vacuum	O-P10vac	1.31 ± 0.10	
8		Diam P10	No vacuum	O-P10ap	3.34 ± 0.11	
5^{CH}	0.23	Diam P1	Vacuum	O-P1vac	1.75 ± 0.20	4°C horizontal

* ± *standard deviation.*

Table 2: Description of chemical analyses by date

Analysis	Code	Unit	Observations	Dates of analysis
dissolved O_2	O_2 wine	mg/L ➔ mg/bottle	Orbisphere polarographic sensor	Days T0, 3, 7, 14, 21, 28, 62, 90, 119, 188, 282, 556
O_2 in headspace	O_2 head	% v/v ➔ mg/bottle	Orbisphere polarographic sensor	
Aphrometric pressure	P_{aphr}	kPa	Ligapal aphrometer (Courmontreuil, France): shows the difference in pressure between the inside of the bottle and atmospheric pressure	
dissolved CO_2	CO_2	mg/L	Orbisphere thermal conductivity CO_2 sensor	
free SO_2	fSO_2	mg/L	Iodometry, polaro-amperometric titration by Oeno 20 (Oeno-bio)	Days T0, 14, 21, 28, 62, 90, 119, 188, 282, 556
total SO_2	tSO_2	mg/L		
L*, C*, h_{ab}	L*, C*, h_{ab}		Minolta chromameter (normal lamp intensity; illumination type D, sample container depth 1 cm, non-decarbonated wine). L* indicates clarity, C* saturation and h_{ab} hue angle	
Absorbance 420 nm	A_{420}		Spectrophotometry applied to decarbonated wine	
3-Sulfanylhexan-1-ol	3MH	ng/L	Gas phase chromatography (extraction of compounds) and mass spectrometry (separation of compounds) according to Schneider et al. (21)	Day 282 only

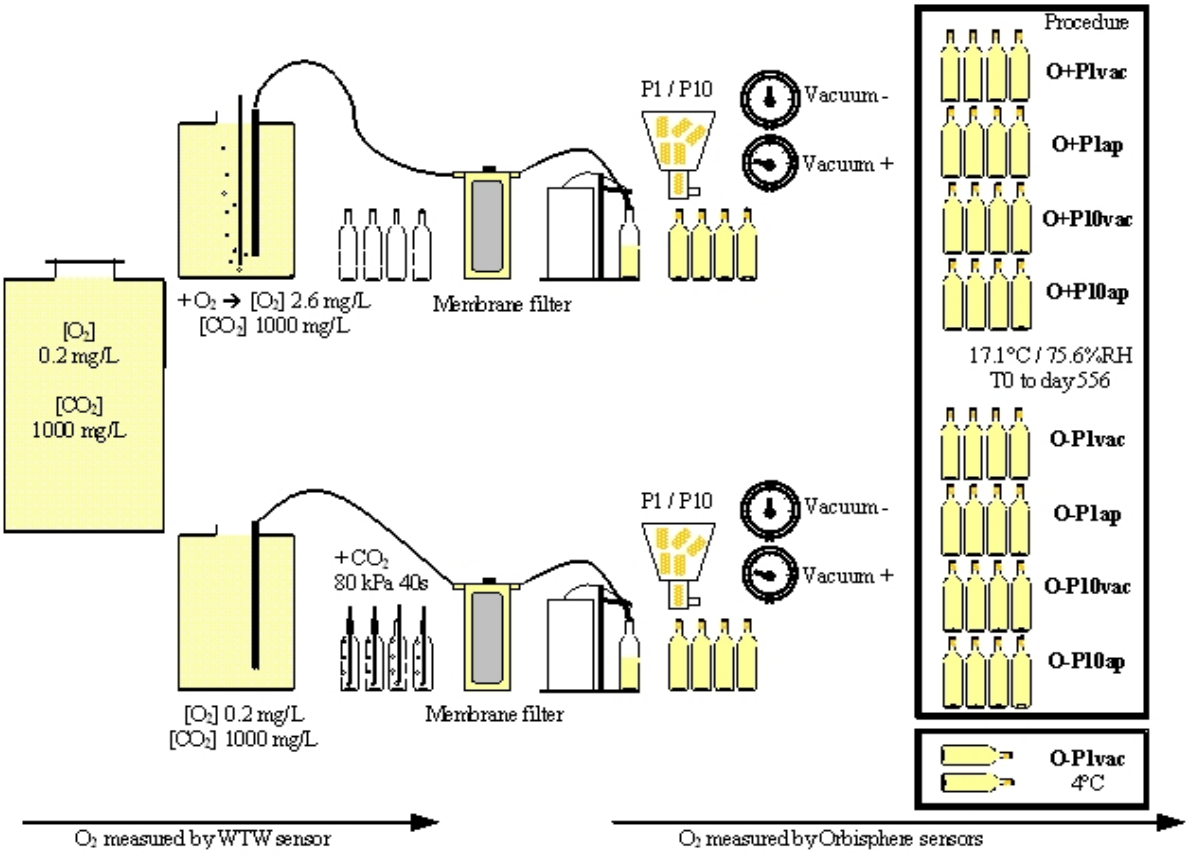
Bottling. **(Table 1, Figure 1)** The wine was divided into 2 batches and the dissolved oxygen content was kept at 0.2 mg/L by bubbling with nitrogen (Air Liquide, Paris, France; 99.95% pure) and monitored with a CellOx 325 oxygen sensor (WTW, Weilheim, Germany). The dissolved CO_2 was adjusted to 1000 mg/L by bubbling in both batches. The following day, one of the 2 batches was enriched with oxygen up to 2.6 mg/L by bubbling with oxygen (Air Liquide; 99.5% pure). 500 bottles were then filled using procedures 1 to 4. One day later, just before filling the second batch, the bottles were subjected to inert blanketing one by one using CO_2 (Air Liquide; 99.99995% pure; application pressure 80 kPa for 40 s) in order to achieve an oxygen content of < 2% saturation in comparison with air measured with CellOx 325 oxygen sensor directly in the bottle). 500bottles were thus filled in accordance with procedures 5 to 8.

For both batches, the headspace level was adjusted by micropipette according to the temperature of the wine (63 mm at 20°C) and the first bottles (approximately 20) were not kept as the dissolved oxygen content was not yet stabilized (measured by Orbisphere polarographic sensor, Geneva, Switzerland).

Chemical analyses performed. The material and methods used for measuring oxygen in the headspace and dissolved in the wine were identical to those described by Vidal et al. *(12)*.
A syringe was used to inject water into the wine while a 5-6 mL gas sample was taken from the headspace of the sealed bottle using a gas-proof syringe and then injected in the flow chamber in which the Orbisphere probe was inserted. To measure dissolved oxygen, after piercing the clossure of the bottle, the wine was forced under nitrogen pressure (≈ 200 kPa) at a 10 L/h flow into the stainless steel flow chamber in which the Orbisphère probe was fitted. The raw data were adjusted to 20 °C and 101.3 kPa to compare analyses performed under different conditions of temperature and pressure. The calculation method for converting results for gaseous oxygen into mg per bottle was that developed by Vidal and Moutounet *(22)* (based on the ideal gas law:

$m = \frac{pV}{rT}$ on condition that the aphrometric pressure and the headspace level in the bottle are known).

Figure 1: Synoptic diagram of bottling.



The dissolved CO_2 sensor was placed downstream of the dissolved oxygen sensor, allowing the assay of the two dissolved gases in the same bottle.
As O_2 and CO_2 assays were destructive, analysis of O_2 in the headspace was performed on other bottles that those used for measurement of aphrometric pressure and dissolved O_2 and CO_2.Three bottles were analyzed for each analytical parameter scheduled and for each procedure, except for 3-Sulfanylhexan-1-ol (n = 1). All the results presented in this article are based on the averages of these three replications.

Sensorial analyses. Descriptive analyses were performed 90, 188, 282 and 556 days after bottling by a jury made up of 14 to 23 professionals (experts) according to the date. The jury experts tasted three series of four procedures on each sensorial tasting date. The first series consisted of wines to which O_{2+} procedures were applied (Nos. 1 to 4, **Table 1**), the second of O_2- procedure wines (Nos. 5 to 8, **Table 1**) and the third of wines subjected to procedures Nos. 1, 4, 5 and 8. The experts have awarded scores to the wine using visual (hue), olfactory (intensity, fruit, oxidized) and taste (freshness, fruit) descriptors and overall quality. The wines were tasted at 10 °C. The wine service (order of service by expert, coding of samples) and statistical analysis were managed using Fizz version 2.30c software (Biosystèmes, Couternon, France). The raw data for the tastings was subjected to single factor analysis of variance (product = procedure). A Student-Newman-Keuls test of classification of averages at risk α = 5% was performed in case of significant difference.

The 5^{CH} procedure (O-P1vac Cold and Horizontal storage) was added to the tasting series on the 90^{th} and 282^{nd} days (**Table 1**).

Wine. A Sauvignon blanc wine of the year was treated with bentonite and filtered before bottling. The following analyses were carried out after bentonite treatment and before division into two batches for bottling: 11.8% alcohol by volume; pH = 3.40; TA = 3.80 gH_2SO_4/L; VA = 0.26 gH_2SO_4/L; iron =3.3 mg/L; copper = 0.24 mg/L

Bottling raw materials and equipment. Green 75 cL light Bordeaux bottles 77 PUTR rim, color CH., headspace level 63 mm at 20 °C (VOA, Albi, France). Technological corks: Oeneo Bouchage (Céret, France) Diam 44 x 24.2, made from ground cork treated with super critical CO_2 (patented by Oeneo Bouchage / CEA). Two permeability coefficients 1.97 10^{-14} (min/max: 0.8 10^{-14} / 4 10^{-14}, n = 6) and 15.5 10^{-14} (min/max: 9 10^{-14} / 37 10^{-14}, n = 6) mol/m.Pa.s, corresponding to a permeability of 33.3 and 261.8 µg/day at 0 °C, 1000 hPa under 100% O_2 atmosphere with the codes P1 and P10 respectively. Enolmatic Durfo (Calamandrana, Italy), vacuum filling machine with 0.65 µm membrane filter. Fimer (Canelli, Italy) single head corking machine (vacuum: 65 kPa).

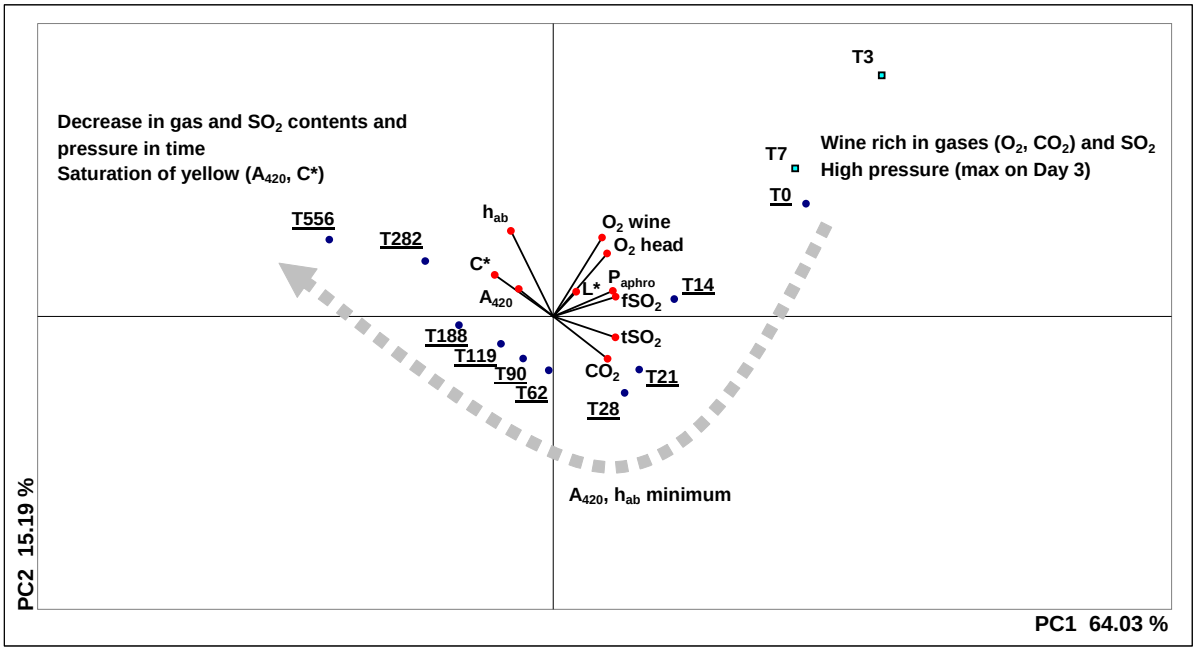
Data Analysis. Calculations of analytical data were made using Microsoft Excel 2002 software. Pearson's linear correlation test, principal component analysis (PCA) type Pearson (n), linear regression, ANOVA and tests for comparison of 2 averages were performed using Xlstat version 2007.1 program (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Average evolution of bottled wine in time

The main PCA plan which summarizes the overall average evolution of the wine from T0 to 18 months is shown in **Figure 2**. This analysis highlights the overall evolution of white wine used during storage in bottles.

Figure 2: Correlation biplot (coefficient n) plan (1, 2) of average PCA evolution by date of analysis. T0, T21, T28 ….T556 = average of all the procedures by dates of analysis (in days) and by analytical variable. T3 and T7 are supplementary individuals as the SO_2, color and CIELAB were not performed on Days 3 and 7.



Analytical variables: P_{aphro}, aphrometric pressure; O_2 wine, dissolved O_2 mg/bottle; O_2 head, headspace O_2 mg/bottle; dissolved CO_2, CO_2 mg/L; fSO_2, free SO_2; tSO_2, total SO_2; L*, lightness/darkness; C*, chroma; h_{ab}, hue angle; A_{420}.

At bottling, the bottles of wine were rich in gas (O_2 in the headspace, dissolved O_2 and CO_2) and SO_2 and are at high pressure (average aphrometric pressure 81 kPa).

After 3 and 7 days, the dissolved and gaseous oxygen levels and the pressure in the headspace were still higher. From 3rd to 28th day, the wine consumed on average 81% of oxygen in the headspace and 99% of dissolved oxygen. The wine has lost 6% of CO_2 on average, after 28 days. Free SO_2 losses were the largest at 30%. Color was characterized by an absorbance at 420 nm and a shade angle (h_{ab}) minimum.

Subsequently, the longer the storage period the more the oxygen levels (headspace O_2, dissolved O_2) in the bottle decreased, attaining very low values after 6 and 9 months (25 µg expressed as total O_2 per bottle). These two parameters rose significantly at 18 months with a large intra-procedure variability. The CO_2 content also fell with time, reaching its lowest value after 18 months (24% loss in comparison with T0). The higher pressure decreased over time but did not disappear completely, reaching figures around 36 kPa from the 119th day.

Affected by the quantity of oxygen trapped during bottling, the free and total SO_2 contents decrease respectively from 55% and 22% at 18 months after T0.

The yellow color of wines became increasingly saturated, with a net drop at 18 months, with in particular an increase of C* of 66% in comparison with color at T0. Intensity and hue are also evolving changed, as evidenced by the increase in A_{420} and the hue angle h_{ab}.

Changes in oxygen contents in time

The examination of changes in oxygen contents of gas and liquid phases presented in Figure 3 shows that these decreased over time but not linearly. It is faster in the first month, then slows down until concentrations are stabilized from the second month 2 according to the procedure at values below 170 µg/bt in the liquid phase (i.e. 227 µg/L) and in the gas phase (i.e. 1% v/v). When the oxygen contents are expressed as saturation percentage of gas and liquid phases, it can be seen that the two curves are positively correlated (p value < 0.0001, Pearson's linear correlation test). Whatever the date and procedure of analysis, the saturation percentage of the gas phase is always higher than that of the liquid phase. This means that dissolution always outweighs the deoxygenization of the wine as a result of the search towards equilibrium of the partial pressure between the two phases.

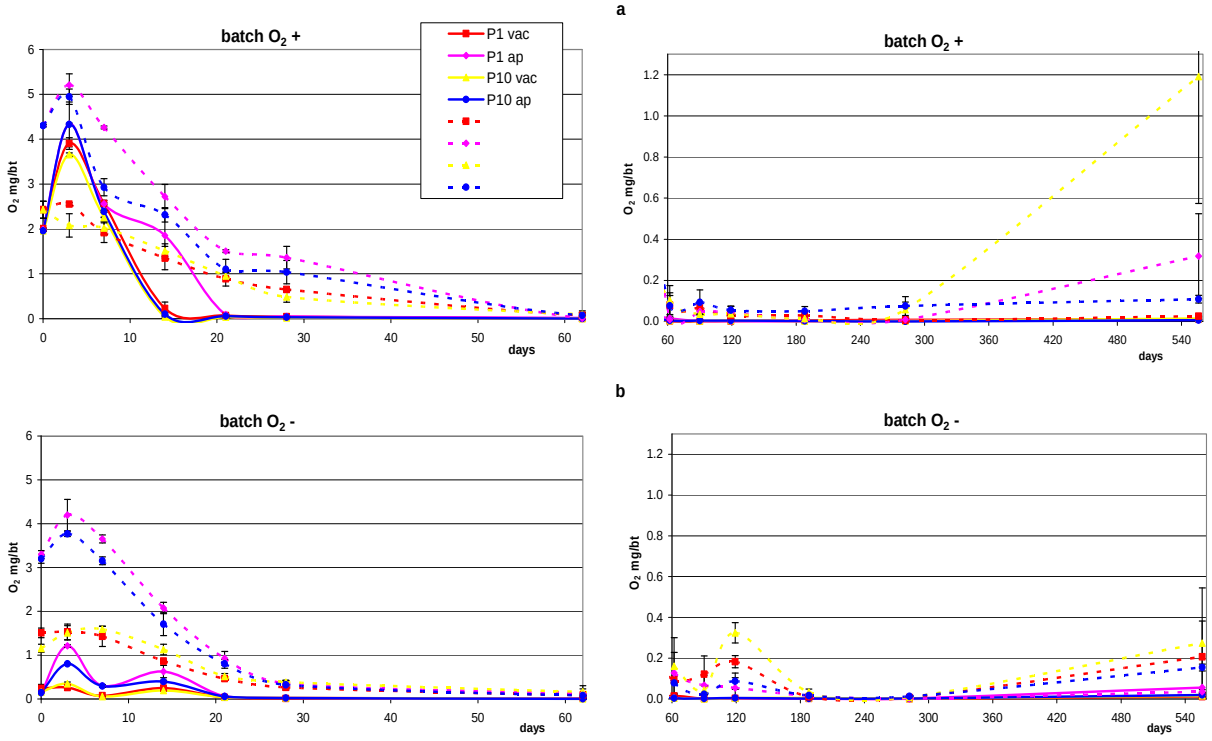
During this experimental bottling, a variable but non-negligible quantity of oxygen was trapped in the headspace (from 1.15 ± 0.09 to 4.30 ± 0.03 mg/bt according to the procedure) and dissolved in the wine (from 0.14 ± 0.01 to 2.01 ± 0.01 mg/bt according to the procedure). The sum of gaseous and dissolved oxygen ranged from 1.31 ± 0.10 to 6.29 ± 0.07 mg/bt according to the procedure.

Monitoring the kinetics of dissolved oxygen in the two phases revealed one oxygen dissolution peak after 3 days for both batches and another after 14 days for the O_2- batch only **(Figure 3)**. In addition to the increase in the dissolved oxygen content in wines, it can also be seen after 7 and especially 3 days that the sum of the gaseous and dissolved oxygen contents was higher than at T0 (O_2 grand total : 3.90 mg/bt at T0 and 5.58 mg/bt at T0 + 3 days, making an average increase of 43%).

The rise of dissolved oxygen content after 3 days is due to the dissolving of oxygen in the headspace, where saturation percentage is distinctly higher than in the liquid phase (74 instead of 35% sat. in average). This oxygen transfer is enhanced by the increase in pressure resulting from pressure at corking together with partial CO_2 release from the wine caused by the search for balance between the CO2 partial pressure in the two phases, but inversely with oxygen. These results have been confirmed on complementary experimental bottling of two other white wines sealed with or without vacuum and with CO_2 contents of 330 and 1075 mg/L (data not shown). It

seems to be reproduced more clearly after 7 days in O_2- procedure wines than in O_{2+} wines in accordance with Fick's law *(10)*. In the first phase lasting 1 - 2 months, the consumption of oxygen trapped during bottling is the main phenomenon, under the experimental temperature of 17.1 °C. After 28 days, the wine had consumed on average 83% of the total oxygen trapped at T0 and 96% after 62 days, or more if the increase on the third day is taken into account.

Figure 3: Evolution of dissolved and gaseous oxygen of the procedures with a) O_2 + and b) O_2 - batches. Unbroken lines: dissolved O_2 mg/bt, dotted lines: O_2 headspace mg/bt. Error bars represent the standard deviation of the three replicates



Beyond 2 months, balanced dissolved oxygen contents are attained. The average content of dissolved oxygen was between 2 and 16 µg/bt from the 62nd to the 282nd day, whatever the modality. For the same period, the average content of oxygen in the headspace in all procedures was between 2 and 180 µg/bt, with one exception (No. 7 O_2-P10 vac on Day 119: 320 µg/bt). In contrast with the dissolved phase, the oxygen concentration of the gas phase continued to decrease slightly from 82 µg/bt on average, from the 62nd to the 282nd day, to 11 µg/bt on average, from the 6th to the 9th month, in all procedures except Nos. 3 (O_2+ P10 vac) and 4 (O_2+ P10 ap).

The analyses performed after 18 months revealed substantial intra-procedure dispersion and average levels of gaseous and dissolved oxygen were higher 289 ± 164 µg/bt and 18 ± 11 µg/bt respectively, in contrast with the gaseous and dissolved oxygen levels of 60 and 13 µg/bt respectively measured by Vidal and Moutounet (*22*) in 5 batches of bottles older than 5 years. The interval between the last two dates of analysis and the lack of bottles for a later date of analysis does not allow us to affirm whether these high levels resulted from a steady increase or fluctuation that might have been caused by small variations in temperature and relative humidity (ditto for the peak on Day 119) or another reason.

The kinetics of apparent oxygen consumption in oenology

The oxygen content in the headspace is at equilibrium at the moment of measurement between the oxygen which has penetrated from the outside of the bottle, mainly the through the cork *(18, 23)* and the oxygen dissolved at the surface of contact with the wine. As for dissolved oxygen, its measurement is the result of the quantity dissolved in the contact surface with the headspace and

that which was consumed by the constituents of the wine between two measurements. Therefore, it is only possible to reason in terms of apparent consumption rate calculated from the oxygen contents.

Generalizing on the basis of the total oxygen content, given the strong positive linear correlation between dissolved and gaseous oxygen after discarding the values obtained at T0 + 556 days, in accordance with the evolution of oxygen levels described, calculation of the curves *f(t) = [totalO_2], Ln [totalO_2]* and *1/[totalO_2]* at 17.1 °C and then, modeling by linear regression provides the slope *k* (or rate constant) and the half reaction time $t_{1/2}$. Selection of the best model is based on the highest correlation coefficient and the verification of $t_{1/2}$ in relation to the plot of f(t) = [totalO_2]. From T0 to Day 62, the apparent consumption reaction of oxygen is of apparent order 1 **(Table 3)**. Unlike the rate, the value $t_{1/2}$ does not depend on the initial concentration. However, none of the three models is sufficiently significant from the 62nd to the 282nd day (average R^2: 0.431, 0.565 and 0.612 for orders 0, 1 and 2 respectively) as the plots of total O_2 oscillate around values most often below 100 µg/bt, even if it is the description of a model of apparent order 0.

Table 3: Modeling of total O_2 curves against time (0 to 62 days)

Procedure	Linear regression* best model *Ln(totalO_2 mg/bt) = cte – kt* (d)	R^2	$T_{1/2}$ model day *Ln2/k*
O+P1vac	Ln(totO_2)=1.690-6.967 time	0.967	9.9
O+P1ap	Ln(totO_2)=2.489-9.438 time	0.964	7.3
O+P10vac	Ln(totO_2)=1.578-6.544 time	0.952	10.6
O+P10ap	Ln(totO_2)=2.058-7.467 time	0.976	9.3
O-P1vac	Ln(totO_2)=0.580-4.920 time	0.935	14.1
O-P1ap	Ln(totO_2)=1.518-6.170 time	0.902	11.2
O-P10vac	Ln(totO_2)=0.539-4.001 time	0.904	17.3
O-P10ap	Ln(totO_2)=1.442-6.741 time	0.944	10.3
	Model average first order reaction	0.943 ± 0.026	11.3 ± 2.9

* Linear regression parameters: 95% confidence interval; model selection: best model/R^2 adjusted.

The results shown in **Table 4** are negative for the first three days because total oxygen was higher than at T0. As reported by Squarzoni et al. *(18)*, it can be supposed that this enrichment is mainly due to a part of the oxygen trapped in the cork and released into the headspace during the first three days and that the rate of penetration of oxygen into the bottle is greater than the rate of consumption by the wine. Analyses of oxygen in the gaseous zone and dissolved in ultrapure water (Millipore Q Pack) deoxygenated with nitrogen and then bottled in the same bottles (n = 16), closed with P10 corks under vacuum and then stored upright at 20 °C corroborate the hypothesis of oxygen penetration after closure resulting either from a release from the cork or from a passage at the cork/glass interface. Indeed, the total oxygen content increased from 0.98 ± 0.11 to 1.24 ± 0.26 mg/bottle in three days, corresponding to a significant of 26% increase (p value < 0.001, left-tailed Student's test).

The rate of oxygen disappearance and hence estimated oxygen consumption was even higher than initially trapped oxygen content was high. However, this content is significantly dependent first of all on the initial dissolved oxygen content and then the pressure (p value < 0.0001, ANOVA with stepwise model selection, 95% confidence interval, for the periods up to the 3rd day and from the 3rd to the 62nd day). Indeed, the estimated consumption rate (from the 3rd to the 62nd day) varies from 29 µg/bt/day in procedure 5 (O_2- P1 vac) to 164 µg/bt/day in procedure 2 (O_2+ P1 ap).

Table 4: Result for the consumption/penetration of apparent total oxygen in µg/bt/d by period and total oxygen content in µg/bt from Day 119 to Day 282

Result of[a]	Day 0 to 3	Day 3 to 62	Day 62 to 282	[total O_2]* Day 119 to 282
O+P1vac	-673	110	0.31	26.5
O+P1ap	-1086	164	0.06	23.0
O+P10vac	-451	97	0.21	40.1
O+P10ap	-1006	159	0.02	63.0
O-P1vac	-14	29	0.45	68.9
O-P1ap	-654	91	0.59	26.0
O-P10vac	-182	29	0.70	126.2
O-P10ap	-409	77	0.31	41.6
WaterP10vac	-85			

* Result: $[totalO_2]_j - [totalO_2]_i / j - i$, in which *i, j* = date of analysis in days elapsed.

Once the oxygen trapped in the packaging has been consumed, the average apparent rate is almost zero (0.33 µg/bt/day). Then, it is preferable to interpret the total oxygen content. For the period from the 119th to the 282nd day, the cork permeability is significantly the main factor influencing total oxygen content (p value < 0.0003, ANOVA with stepwise model selection, 95% confidence interval). During this period, the average total oxygen content for the procedures closed with P1 was 36 ± 11 µg/bt and 68 ± 21 µg/bt in bottles closed with P10 (p value < 0.03, significant left-tailed Mann-Whitney test), that is to say a P10/P1 permeability ratio of 1.9 rather than 7.9 theoretically. This means that the oxygen penetration rate is lower than the rate of consumption by the wine, especially as the impact of maintaining an overpressure (general average 36 ± 4 kPa during the period), through the significant interaction with the initial dissolved oxygen, accelerates the dissolution of the latter in the headspace; otherwise, the ratio would be closer to 7.9.

Relation between total oxygen and free SO_2 losses

Figure 4 summarizes the impact of bottling conditions on total oxygen and free SO_2 losses. The best conditions are: an high initial dissolved oxygen content, a closure without vacuum and P10 cork. Indeed, free SO_2 losses from bottles containing 6.26 mg total oxygen just after closure amounted to 44% after one month whereas such losses were only 12% from the bottles containing 1.75 mg (O_2-P1vac). These results corroborate those found in an equivalent experiment using 3-liter bag in box packaging stored at 20 °C, where free SO_2 losses were 19% after one month for an initial total oxygen content of 3.14 mg/L while they increased to 35% for an initial total oxygen content of 6.30 mg/L *(24)*. After 18 months of storage at 17.1 °C, the average free SO_2 content in bottles filled under good conditions (O_2-P1vac) was 20.5 ± 1.5 instead of 10.5 ± 0.5 mg/L for the other procedure (O_2+P10ap). In comparison, Godden et al. (25) measured on a Semillon wine containing 42 mg/L of ascorbic acid and closed with Altec stoppers (P1 type O_2 permeability) under operating conditions close to procedure O_2-P1vac , a free SO_2 content of 17 mg/L after 12 months of upright storage at room temperature.

From another point of view, the total oxygen content at T0 enables accurate prediction of the free SO_2 content on the 28th day, which corresponds to 83% of consumption of the initial total O_2, by linear regression (fSO_2 28th = 32.4 – 2.41 $totO_2$ T0; R^2 = 0.85). Next, the free SO_2 content on the 28th day gives the best prediction of free SO_2 on the 556th day (fSO_2 556th = -1,35 + 0.715 fSO_2 28D; R^2 = 0.80). By analogy, during the same experimental work (25), Godden et al. (26) have demonstrated that free SO_2 contents after 6 months did predict those after 24 months (R^2 = 0.89).The effect on the wine studied of increasing cork permeability by a factor of 7.9 (Diam® P1 and P10) seemed significantly lower during the first phase in comparison to the impact of the two other factors on trapped total oxygen and hence on the free SO_2 content after 28 days. Then, even if the initial dissolved oxygen remains the main factor, the cork permeability has a slight significant impact on free SO_2 content after 556 days (**Table 5**). Furthermore, from the 90th to the 556nd day,

when all the oxygen trapped in the packaging has been consumed, the average free SO_2 content in the bottles closed with P1 was only 1.1 mg/L higher than in bottles closed with P10.

Thus, as the consumption of oxygen trapped during bottling is the main factor affecting losses of free SO2, the free SO2 concentration after 1 month is a reference value to forecast whether the remaining concentration after 18 months will be sufficient to protect the wine from oxidation.

Figure 4: Evolution of total oxygen and free SO_2 in a Sauvignon blanc of procedures 4 (O+P10ap) and 5 (O-P1vac).
Unbroken lines: total O_2 mg/bt, dotted lines: free SO_2 mg/L. Error bars represent the standard deviation of the three replicates.

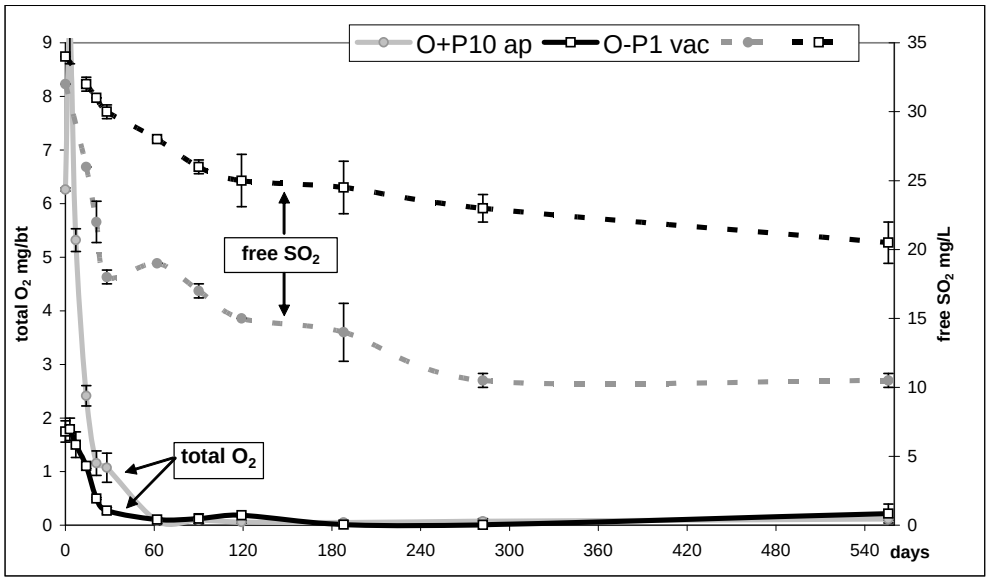


Table 5: ANOVA applied to free SO_2 contents after 28 and 556 days according to the three factors

Parameter	free SO_2 on Day 28	free SO_2 on Day 556
P value > F ANOVA[a]	0.002	0.001
Model	fSO_2 28D = 25.5 – 8 $dissO_2$ + 3 clos. press.[b]	fSO_2 556D = 17.3 – 6.6 $dissO_2$ + 2 perm

[a] ANOVA parameters: 95% confidence interval; model selection: stepwise (probability for entry/removal: 0.05/0.10).
[b] $dissO_2$: if high = 1, if low = 0; clos. press: if vac = 1, if ap = 0; perm: if P1 = 1, if P10 = 0.

Results of sensorial analyses

Only the sensorial analysis of the third series performed after 18 months confirms a significant negative impact, glimpsed in the sensorial analysis after 9 months (data not shown), of the high trapped oxygen content linked to the high cork permeability. Indeed, on this date the wines subjected to the two O_2- procedures (O-P1vac and O-P10ap) were better appreciated than the two others (O+P1vac and O+P10ap), certain criteria. The nose was considered to be less oxidized; they were fresher and fruitier at tasting and were of better overall quality **(Table 6)**. The difference between the verdicts for the extreme oxygen dissolution procedures, that is to say between procedure 5 (tank O_2-, cork P1, closure under vacuum) and procedure 4 (tank O_2+, cork P10, closure without vacuum) is even clearer for the last descriptors mentioned above. Regarding the color of wine, the comparison of the results of CIELAB analyses synthesized by the index ΔE^* **(Table 7)** and the results of sensorial analyses shows that the more developed color of the wines

bottled with cork P10 revealed by the chemical analyses was also perceived by the sensory panel at 18 months, and especially for bottling without vacuum **(Table 6)**.

At 636 ng/L, the 3-Sulfanylhexan-1-ol content of 5^{CH} procedure wines (O_2–P1vac, horizontal storage at 4°C from T0 onwards) was 2.3 times higher than the others after 9 months. This was also the only wine considered unanimously by the judges to have typically Sauvignon character as regards to the nose and taste, with the least developed color after 3 and 9 months. The judges also considered that in comparison with 5^{CH} the wines subjected to procedures 1 to 8 had lost their fruity nose and mouth when tasting after 3 months. As previously observed by several authors *(4, 25)*, this loss of characteristic aroma was perceived sensorially before the degradation of color (observed after 18 months).

Table 6: Synthesis of significant descriptors of sensorial analysis after 18 months (14 judges)

Procedure	Visual shade	Fruitiness (olfactory)	Oxidation (olfactory)	Freshness (taste)	Fruitiness (taste)	Overall quality
O+P1vac	3.68 B	4.30	2.14 B	4.71	3.95 AB	4.73 AB
O+P10ap	6.57	4.48	5.83 A	3.12	3.36 B	2.64 B
O-P1vac	3.01 B	3.70	1.91 B	5.00	5.06 AB	5.60 A
O-P10ap	5.43 A	5.85	2.36 B	6.21	6.09 A	5.13 AB
P value	0.0008 ***	0.1162	0.0007 ***	0.1287	0.0408 *	0.0488 *
Judge effect	ns	*	ns	ns	ns	ns

The procedures were sorted using the average scores awarded by the judges and then classified in significantly different groups referred to by letters (A, B, C, etc.). The averages that are not significantly different have the same letter(s). ns: not significant as level = 0.05; *: a descriptor significant as level = 0.05; *** significant as level = 0.001.

Table 7: Mean CIELAB ΔE* / T0 to Day 282 and 556 and A_{420} to Day 556

Procedure	ΔE^*_{282d} [a]	ΔE^*_{556d}	$A_{420\ T0}$	$A_{420\ 556d}$
O+P1vac	1.92 ± 0.10	3.29 ± 0.05	0.104 ± 0.001	0.127 ± 0.001
O+P1ap	2.08 ± 0.16	3.51 ± 0.02		0.128 ± 0.001
O+P10vac	1.83 ± 0.11	3.80 ± 0.11		0.135 ± 0.002
O+P10ap	1.84 ± 0.05	4.07 ± 0.30		0.138 ± 0.005
O-P1vac	1.00 ± 0.03	2.50 ± 0.05	0.108 ± 0.008	0.117 ± 0.001
O-P1ap	1.37 ± 0.03	2.90 ± 0.17		0.122 ± 0.000
O-P10vac	1.34 ± 0.06	2.99 ± 0.10		0.125 ± 0.002
O-P10ap	1.60 ± 0.08	3.30 ± 0.01		0.140 ± 0.001

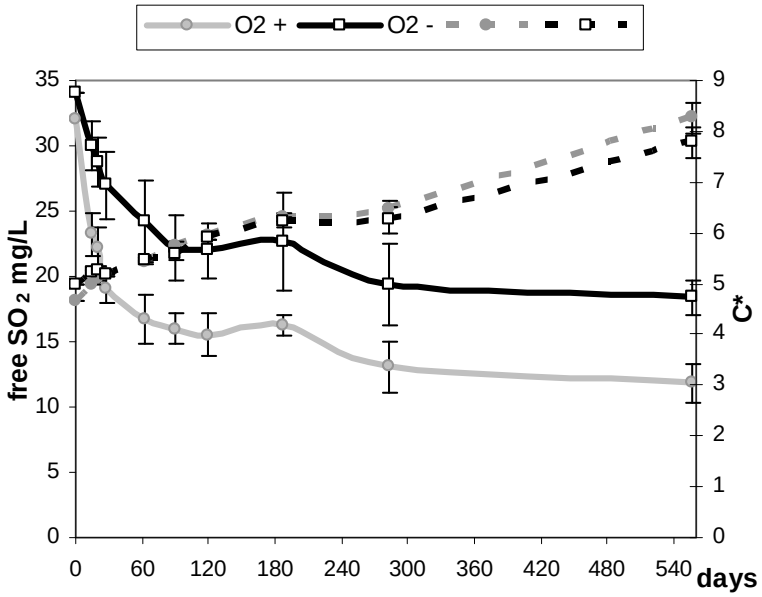
[a] $\Delta E^*_i = \sqrt{(L_i - L_{T0})^2 + (a_i - a_{T0})^2 + (b_i - b_{T0})^2}$

Impact of the initial dissolved oxygen content

Whatever the date of analysis, the wines of the O_2+ batch had lower free (and total) SO_2 contents and more saturated yellow color than those of the O_2- batch **(Figure 5)**. These analyses show that the wines subjected the O_2+ batch procedures were more oxidized than those of the O_2- batch as the wine consumed a larger amount of oxygen for a period similar to that of the batch bottled with a low oxygen content. Boulet et al. *(24)* made similar observations

It is important to stress the negative effect of high oxygen contents observed during sensorial analysis after 18 months of storage, all conditions closure and permeability being identical **(Table 6)**.

Figure 5: Evolution of free SO_2 and C* of the averages of procedures O_2 + (Nos. 1 to 4) and O_2 (Nos. 5 to 8).
Unbroken lines: free SO_2 mg/L, dotted lines: C*. Error bars represent the standard deviation of the three replicates.



Impact of closure under vacuum

The use of vacuum during closure (T0) acts on two levels. First, it reduces the oxygen percentage in the headspace, giving 16.93% v/v with vacuum against 19.63% v/v without vacuum, even though the corking machine used in this experiment was less effective than during the measurements made by Vidal and Moutounet *(22)* on other bottling lines. Secondly, it reduces aphrometric pressure, with an average of 25 ± 6 kPa with vacuum instead of 137 ± 2 kPa without. This explains the considerable variation in the amount of oxygen trapped in the gas phase (from 1.15 to 4.30 mg/bt according to the procedure) and also the great variation in the ratio of oxygen quantity between the headspace and the wine (from 1 to 23 depending on the procedure).

Corking pressure conditions play a major role in gas exchanges, especially during the first days of storage. They affect both the quantity of oxygen trapped in the headspace and the internal pressure. Absence of vacuum causes a positive internal pressure (generated when the cork is inserted); even stronger than the CO_2 content of the wine is high. During the first days after corking, high pressure causes more rapid dissolution in the wine of the oxygen in the headspace. The use or not of vacuum at corking thus had an impact on the amount of oxygen and the rate of oxygen consumption by the wine and hence on oxidative phenomena in the wine. This was confirmed by the evolution of free and total SO_2 and the chromatic characteristics of the wine. The wines bottled with corking without vacuum displayed lower free and total SO_2 concentrations and higher C* values (color saturation) than the wines closed under vacuum with an overall average of - 2.5 mg/L free SO_2, - 2.2 mg/L total SO_2 and + 0.1 C*, respectively.

Impact of cork permeability

The bottles closed with cork P1 had higher CO_2 contents and internal pressure than those closed with P10, all other conditions being the same during the 556 days of storage (average loss of CO_2 P1/P10 in 556 days: 21.2% / 26.8%; average pressure at 556 days for P1/P10: 44 ± 7 kPa / 22 ± 2 kPa). These differences lead to concluding that P1 (more dissolved CO_2 and higher internal pressure) forms a better seal to gases than P10.

In the second phase, that is to say when practically all the oxygen trapped during bottling has been consumed (from the 119th day), the bottles sealed with P1 tended to display a lower oxygen

content in the headspace than those sealed with P10 (respective averages of 33 ± 11 and 65 ± 21 µg/bt from the Day 119 to the Day 282). From 9 and especially 18 months onwards, the bottles closed with P10 corresponded to a more saturated color (C* at 18 months for the average of procedures using P1 and P10: 7.75 ± 0.07 and 8.31 ± 0.12) and a higher absorbance at 420 nm, as the average increase at 18 months after T0 was 17% with P1 corks and 27% with P10 corks **(Table 7)**.

CONCLUSIONS

Monitoring the kinetics of the bottles stored upright confirmed and refined the results described by Vidal and Moutounet (22).

A highly variable quantity of oxygen was trapped in the headspace and dissolved in the wine after bottling. This amount depended mainly on the dissolved oxygen content of the wine in the bottling tank, the quality of the bottling line regarding the protection against contamination by atmospheric oxygen throughout the procedure (inert blanketing of bottles, filling, closure) and the operating conditions *(10, 22)*. At the oenological temperature and pressure conditions (around 15-20°C and 101.3 kPa) during which the wine consumes oxygen, its transfer always takes place, whatever the observation date, from atmospheric air to the headspace and then to the wine, that is to say from the highest oxygen content (expressed in percentage saturation) to the lowest and vice versa for carbon dioxide for the same reason. At the moment of corking, the absence of vacuum without the use of blanketing gas causes an increase in the amount of oxygen trapped in the headspace. The increase in internal pressure depends on the corking conditions and causes a more rapid dissolving of oxygen via the headspace/wine contact surface. A high carbon dioxide concentration in a wine (bottled without CO_2 in the blanketing gas at corking), resulting from the search for equilibrium of the partial pressures between the liquid and gas phases, will slow down the fall in internal pressure and hence slow down the decrease in the rate of dissolution of oxygen.

During the first week of storage, the considerable difference in oxygen saturation percentages between the two phases causes a dissolution of headspace oxygen in the wine in such a way that the dissolved oxygen content is higher than the initial level after several days. During this same period and even during the first month according to Squarzoni et al. *(18)* and above all Lopes et al. (*23*), the total oxygen content often exceeds the initial level because of the intake of oxygen expelled from the cork when it is compressed in the neck of the bottle.

Subsequently, in the first and second months when the gas exchanges via the cork stabilize, the consumption of oxygen trapped during bottling is the main phenomenon. This depends on the storage temperature and content in the wine of oxygen reducing substances.

Once almost all the initial oxygen has disappeared, the gaseous and dissolved oxygen contents in the bottle stabilize at very low levels in the order of a few to several tens of micrograms according to the permeability of the closure of a given wine. However, given the monitoring performed, the hypotheses that these values may fluctuate under the influence of small variations in storage conditions (temperature, pressure and relative humidity) cannot be ruled out.

The kinetics of gas exchanges in the bottle affect the components of the wine. Indeed, the greatest losses of free SO_2 occur during the first 15-30 days as they are positively correlated with the oxygen trapped during bottling and released by the cork. Subsequently, the decrease in the concentration of free SO_2 is lower as it is related to cork permeability. Oxidative phenomena related to the consumption of oxygen by the wine cause a substantial loss of aromas that are sensitive to oxidation, such as compounds with a sulfanyl function, and then, deterioration of the color of the wine (color saturation and change of hue) as observed by several authors *(4, 27)*.

However, control of oxygen is only one answer to problems related to oxidation. As glimpsed in the samples stored at 4 °C, storage temperature has always been a key element in the evolution of wines, as put forward by Ferreira et al. *(5)* and Boulet et al. *(24)*. However, only detailed

understanding of the reaction mechanisms involved can generate reasoned solutions in different oenological situations.

ACKNOWLEDGMENTS

The authors thank Dominique Rabiot of the company Oeneo Bouchage for technical support, Alain Samson, head of the sensorial analysis platform at UE99 Pech-Rouge and Paul Flory for his participation in the experiments.

LITERATURE CITED

1. Glories, Y. Le bois et la qualité des vins et des eaux-de-vie. Guimberteau Ed., n° spécial *Connaiss. vigne vin.* **1987,** 81.
2. Feuillat, F. Contribution à l'étude des phénomènes d'échanges bois-vin-atmosphère à l'aide d'un «fût» modèle. Relations avec l'anatomie du bois de chêne (Quercus robur L, Quercus petraea Liebl.). Ph.D. Thesis, ENGREF: Nancy, France, 1996; 411 pp.
3. Boulet, J.C.; Moutounet, M. Micro-oxygénation des vins. In *Œnologie fondements scientifiques et technologiques,* Lavoisier TEC & DOC: Paris, France, 1998; 1311 pp.
4. Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **2002**, 77, 325-331.
5. Ferreira, A.C.S.; Guesdes De Pinho, P.; Rodrigues, P.; Hogg, T. Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. *J. Agric. Food Chem.* **2002**, 50, 5919-5924.
6. Allen, D.B. Blanketing during packaging. In *A manual on the effective use of inert gas to achieve wine quality. Wine technology and the pursuit of quality.* Air Liquide Australia Ltd: Elizabeth, Australia, 1994; 128 pp.
7. Berta, P.; Spertino, M.; Vallini, E. Ossigeno e imbottigliamento : determinazioni sperimentali del tenore di ossigeno nei vini e tecnologie di riempimento. In *Atti della Giornata di studio Ossigeno e vino*, Chiriotti editori SPA; Istituto di Enologia e Ingegneria Alimentare: Piacenza, Italy, 1999; 48 pp.
8. Ferrarini, R.; D'Andrea, E. Risultati delle misure dell'ossigeno durante la conservazione ed il condizionamento dei vini. *Industrie delle bevande* **2001**, 173(30), 259-261.
9. Castellari, M.; Simonato, B.; Tornielli, G.B.; Spinelli, P.; Ferrarini, R. Effects of different enological treatments on dissolved oxygen in wines. *Italian Journal of Food Science* **2004**, 16(3), 387-396.
10. Vidal, J.C.; Boulet, J.C.; Moutounet, M. Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site. 3ème partie. *Rev. Franç. Oen.* **2004**, 205, 25-33.
11. Cook, J.M.; Karelitz, R.L.; Dalsis, D.E. Measurement of oxygen, nitrogen, and carbon dioxide in beverage headspace. *J. Chromatogr. Sci*.**1985**, 23, 57-63.
12. Vidal, J.C.; Toitot, C.; Boulet, J.C.; Moutounet, M. Comparison of methods for measuring oxygen in the headspace of a bottle of wine. *J. Int. Sci. Vigne Vin.* **2004**, 38, 191-200.
13. Silva, A.; Lambri, M.; De Faveri, M.D. Evaluation of the performance of synthetic and cork stoppers up to 24 months post-bottling. *Eur. Food Res. Technol.* **2003**, 216, 529-534.
14. Sanchez, J.; Aracil, J.M. Perméabilité gazeuse de différents obturateurs. *Bulletin de l'O.I.V.* **1998**, 71, 805-806.
15. Rabiot, D.; Sanchez, J.; Aracil, J.M. Study of the oxygen transfer through synthetic corks for wine conservation. Récent Progrès en Génie des Procédés **1999**, 13 (71), 385-392.
16. Godden P.; Lattey, K.; Francis, L.; Gishen, M.; Cowey, G.; Holdstock, M.; Robinson, E.; Waters, E.; Skouroumounis, G.; Sefton, M.; Capone, D.; Kwiatkowski, M.; Field, J.; Coulter, A.; D'Costa, N.; Bramley, B. Maintien du vin en conditions optimales jusqu'au consommateur. Le vin, le bouchage et les autres variables de fonctionnement. 2nde partie. Infowine **2005**, 18, 1-11. www.infowine.com
17. Valade, M. ; Tribaut-Sohier, I. Capsules de tirage à joints synthétiques : des fournitures très importantes. *Vign. champen.* **2001**, 11, 50-77.
18. Squarzoni, M.; Limbo, S.; Luciano, P. Proprietà barriera all'ossigeno di differenti tipologie di tappi per vino. *Industrie delle bevande* **2004**, XXXII (Aprile), 113-116.
19. Lopes, P.; Saucier, C.; Glories, Y. Nondestructive colorimetric method to determine the oxygen diffusion rate through closures used in winemaking. *J. Agric. Food Chem.* **2005**, 53, 6967-6973.

20. Lopes, P.; Saucier, C.; Teissedre, P.L.; Glories, Y. Impact of storage position on oxygen ingress through different closures into wine bottles. *J. Agric. Food Chem.* **2006**, 54, 6741-6746.
21. Schneider, R.; Kotseridis, Y.; Ray, J.L.; Augier, C.; Baumes, R. Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.* **2003**, 51, 3243-3248.
22. Vidal, J.C.; Moutounet, M. Monitoring of oxygen in the gas and liquid phases of bottles of wine at bottling and during storage. *J. Int. Sci. Vigne Vin* **2006**, 40, 35-45.
23. Lopes, P.; Saucier, C.; Teissedre, P.L.; Glories, Y. Main routes of oxygen ingress through different closures into wines bottles. *J. Agric. Food Chem.* **2007**, 55, 5167-5170.
24. Boulet, J.C.; Vidal, J.C.; Deage, M. Présentation des résultats. In *Proceedings of the general meeting at Barossa Valley Resort*, South Australia. Performance BIB: Montpellier, France, 2004; 91 pp.
25. Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Høj, P.; Robinson, E. Wine bottle closures: physical characteristics and effect on composition and sensory properties of a Semillon wine 1. Performance up to 20 months post-bottling. *Australian Journal of Grape and Wine Research* **2001**, 7, 64-105.
26. Godden P.; Lattey, K.; Francis, L.; Gishen, M.; Cowey, G.; Holdstock, M.; Robinson, E.; Waters, E.; Skouroumounis, G.; Sefton, M.; Capone, D.; Kwiatkowski, M.; Field, J.; Coulter, A.; D'Costa, N.; Bramley, B. Maintien du vin en conditions optimales jusqu'au consommateur. Le vin, le bouchage et les autres variables de fonctionnement. 1st partie. Infowine **2005**, 17, 1-10. www.infowine.com
27. Singleton, V.L.; Trousdale, E.; Zaya, J. Oxidation of wines I. Young white wines periodically exposed to air. *Am. J. Enol. Vitic.* **1979**, 30, 49-54.