MONITORING OF OXYGEN IN THE GAS AND LIQUID PHASES OF BOTTLES OF WINE AT BOTTLING AND DURING STORAGE

SUIVI DE L'OXYGÈNE DES PHASES GAZEUSE ET LIQUIDE DE BOUTEILLES DE VIN À L'EMBOUTEILLAGE ET EN CONSERVATION

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Abstract: The assaying of oxygen in the headspace of a bottle combined with that of dissolved oxygen in the wine makes it possible to obtain the total oxygen per bottle. The first analyses performed at bottling show that 0.38 to 3.58 mg oxygen per bottle is trapped in the headspace. Operating conditions account for these substantial variations.

Monitoring the oxygen contents in the liquid and gas phases of three batches of wine over a period of several months and the analysis of old bottles show that the headspace functions as an oxygen reserve for the wine, that is to say that as the wine uses oxygen, there is passage of the gas from the headspace to the wine. This is related to a movement towards a balance between the two phases as the partial pressure of oxygen in the gas phase is always greater than that of the liquid phase. Finally, this gas exchange kinetics within the bottle outweighs the kinetics of penetration of the bottle by oxygen in the external atmosphere, at least while the total oxygen trapped at bottling has not been used up.

Résumé : L'objectif de cet article est de présenter les premières utilisations de la méthode de dosage de l'oxygène dans l'espace de tête de bouteilles de vins tranquilles par sonde polarographique (VIDAL *et al*., 2004b). Cette analyse, complétée par le dosage de l'oxygène dissous dans le vin, permet de déterminer l'oxygène total d'une bouteille de vin. Les premiers essais présentés dans cet article montrent que la quantité d'oxygène piégée dans l'espace de tête juste après l'obturation varie de 0.38 à 3.58 mg/bouteille, auquel il faut rajouter l'oxygène dissous dans le vin dont les valeurs mesurées varient de 1.3 à 6 mg/L. Les conditions opératoires expliquent ces variations importantes. Il s'agit principalement du type d'obturateur, du volume d'espace de tête et des techniques d'inertage.

Un suivi des teneurs en oxygène des phases liquide et gazeuse de trois lots de vin sur plusieurs mois montre qu'au cours des deux premiers mois, les teneurs en oxygène des phases gazeuse et liquide diminuent fortement. Ensuite, cette diminution ralentit avant que les concentrations se stabilisent progressivement vers des valeurs inférieures à 0.5 mg/L pour le vin et à 1 % v/v pour l'espace de tête. En outre, quel que soit le vin étudié et la date d'analyse, le pourcentage de saturation en oxygène de l'espace de tête est toujours supérieur à celui du vin. Cela signifie qu'à cause de la recherche d'équilibre, le transfert d'oxygène se fait toujours de l'espace de tête vers le vin. Ainsi, au fur et à mesure que le vin consomme l'oxygène qu'il contient, il y a une dissolution d'oxygène de l'espace de tête dans le vin par la surface de contact gaz-liquide.

Les vitesses expérimentales moyennes de consommation d'oxygène chutent dès le deuxième mois à des valeurs inférieures à 10 µg/bouteille/jour. Elles sont donc en cohérence avec des vitesses de diffusion à travers le bouchon de l'ordre du µg ou du dixième de µg/bouteille/jour, d'autant plus que sur des bouteilles âgées de 5 à 37 ans, on a constaté qu'il y a toujours de l'oxygène en très faible quantité (de 17 à 67 µg/bouteille), mais jamais d'accumulation. Ces premiers résultats témoignent que la cinétique de pénétration à travers l'obturant est inférieure aux cinétiques de dissolution-consommation à l'intérieur d'une bouteille.

L'intégration du suivi de l'oxygène total dans des études plus complètes ouvre des perspectives pour évaluer et améliorer les performances des protections contre l'oxygène mises en œuvre au conditionnement, pour comparer et évaluer l'impact réel sur les échanges gazeux des obturateurs, de la position de la bouteille durant le stockage et des paramètres de conservation.

Keywords: bottle, headspace, oxygen, polarographic probe, wine

Mots clés : bouteille, espace de tête, oxygène, sonde polarographique, vin

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INTRODUCTION

Oxidation phenomena dependant on the presence of oxygen affect the evolution of wines. Controlled oxidation contributes to stabilising colour and reducing the astringency of red wines as during ageing in barrels (GLORIES, 1987; FEUILLAT, 1996) or in micro-oxygenated tanks (BOULET and MOUTOUNET, 1998). In contrast, it seems necessary to protect white wines for drinking young from oxygen (FERREIRA *et al*., 2002; ESCUDERO *et al.*, 2002). Finally, it is commonly accepted in oenology that strong oxidation is not good for wine quality. Now, the different studies undertaken to characterise the amounts of oxygen dissolved during operations performed on wines show that bottling is one of the most critical phases (CASTELLARI *et al*., 2004; VIDAL *et al*, 2004 1; FERRARINI et D'ANDREA, 2001; BERTA *et al*, 1999; ALLEN, 1994), especially as once the bottles have been closed control of the evolution of wine can only be performed using storage parameters (temperature, relative humidity, light, etc.).

It seems important to know the oxygen content of the headspace in a bottle of wine in order to complete the diagnosis. Using a device for taking a sample of the gas phase combined with gas phase chromatography, COOK *et al.* (1985) 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.* (2004b) proposed a method of assaying the oxygen in the headspace of a bottle by means of a polarographic probe that is easy to use *in situ*.

Other authors have also addressed the determination of the permeability of stoppers using experimental air/cork/gas set-ups based on the transfer of oxygen from outside to inside a container through a cork (SILVA *et al*., 2003). VALADE and TRIBAUT-SOHIER, 2001 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 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 the gas exchanges can be characterised by losses of CO_2 of the order of a mL per day insofar as they observed good correlation with the entry of smaller quantities of oxygen (10-2 mL per day). 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. (*2004) 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 GPC assay. The authors also observed substantial oxygen accumulation in the headspace in the first 30 days and put forward the hypothesis that oxygen is released again *via* the cork.

This article presents the first use of the polarographic probe method for assaying oxygen in the headspace of bottles of still wine (VIDAL *et al.*, 2004b). Analyses were performed *in situ* just after corking to complete the measurement of oxygen dissolved in the wine and to calculate total oxygen at bottling. The evolution of the oxygen in the headspace and of that dissolved in the wine are described in the second part; this monitoring was conducted on 3 batches of wine over a period of time following bottling. Finally, bottles filled several years previously were analysed. The experiments sought to answer the following questions. How much oxygen is trapped at bottling? How does the oxygen in the gas and liquid phases over a period of time? Can measurement of total oxygen enable assessment of the quantity of oxygen entering the bottle over a period of time?

MATERIALS AND METHODS

I - POLAROGRAPHIC PROBES AND EXPRESSION OF THE RESULTS

The material and methodology used for assaying oxygen in the headspace and dissolved in the wine are identical to those described by VIDAL *et al.* (2004b).

The results of oxygen assay in the headspace are expressed initially as the percentage by volume of oxygen per sample volume (% v/v) and concentrations of oxygen dissolved in the wine in mg/L or µg/L.

The raw data were adjusted to normal conditions (20 °C and 101.3 KPa) to compare analyses performed under different conditions of temperature and pressure. The following formula was used for the oxygen in the liquid phase:

$$Ccn = k_l.Ci$$

In which Ccn = $[O_2]$ mg/L wine under normal conditions (20 °C, 101.3 KPa),

Ci = $[O_2]$ mg/L wine at its temperature and atmospheric pressure at the moment of analysis,

K_l= $[O_2]$ mg/L saturation in water under normal conditions / $[O_2]$ mg/L saturation in water at the temperature of the wine and at the atmospheric pressure at the moment of the analysis (ORBISPHÈRE, 2004).

$[O_2]$ mg/L saturation in water under normal conditions: 9.09 mg/L.

The correction formula used for the headspace is as follows:

$Ccn = k_g.Ci$

In which Ccn = [O_2] % v/v of the headspace under normal conditions,

Ci = [O_2] % v/v of the headspace at the temperature and atmospheric pressure at the moment of analysis,

kg = [O_2] % v/v saturation in water saturated air under normal conditions / [O_2] % v/v saturation in water saturated air at the temperature of the wine and at the atmospheric pressure on the day of analysis (ORBISPHERE, 2002).

[O_2] % v/v saturation in water saturated air under normal conditions = 20.72 % v/v.

An aphrometer was used to measure the pressure inside the bottle (pi) just before the assay. International pressure is the sum of atmospheric pressure and the pressure given by the aphrometer. After determination of the volume of the headspace, the mass of oxygen in the headspace of a bottle can be calculated using the perfect gases equation ($m = pV / rT$). Finally, the sum of the oxygen in the gas and liquid phases expressed in mg/volume of wine in the bottle, that is to say in mg/75 cL for all the bottles in this study, gives the total oxygen in the closed bottle.

II- MONITORING AT BOTTLING

Twelve batches were monitored from 10 bottling lines. Injection of water in the bottle to measure the oxygen in the headspace makes it impossible to measure the dissolved oxygen in the same bottle (except in bottles under pressure or closed with a screw cap). For this reason, four bottles were sampled just after the insertion of corks or closing of screw caps in order to double the measurements in the gaseous and liquid phases. The oxygen contents were quantified in the five minutes following the taking of the sample bottles just after closure. The main operating conditions of the 12 batches of bottles are summarised in table I. Batches 1b and 7b are identical to

Table I – Operating conditions for the bottling studied

Conditions opératoires des mises en bouteille étudiées

No.	75cL	Stopper	Operating conditions	Colour	CO_2 mg/L	Volume bottled, hl
1 1b	Burgundy type, unfill 55 mm	Natural 44x24	Bottle blanketing N_2 -65 KPa; gravity botting -88 KPa; closure -10 KPa	white	670	161
2	Bordeaux type, unfill 63 mm	Altec 44x24.5	Gravity bottling -60 KPa; closure -60 KPa	rosé	830	90
3	Burgundy type, unfill = 63 mm	Natural 44x24	Gravity bottling -15 KPa; closure -50 KPa	red	460	135
4	Bordeaux type, unfill 63 mm	Altec 38.5x24.5	Gravity bottling -15 KPa; closure -50 KPa	gris	800	111
5	Burgundy type, unfill 55 mm	Synthetic 44x22	Gravity bottling; closure -90 KPa	white	674	175
6	Bordeaux type, unfill 63 mm	Synthetic 43x22	Bottle blanketing 80 % N_2 / 20 % CO_2; Gravity bottling; closure -70 KPa	red	675	36
7 7b	Burgundy type, unfill 55 mm	Synthetic 43x22	Bottle blanketing N_2 -50 KPa; Gravity bottling -50 KPa; closure -90 KPa	white	1000	98
8	Burgundy type, unfill 50 mm	Screwcap	Bottle blanketing 80 %; N_2 / 20 % CO_2 Gravity bottling	white	920	84
9	Burgundy type, unfill 55 mm	Screwcap	Snow-drop^{TM} CO_2 (2); Gravity bottling -4 KPa; headspace flushed with CO_2	white	1000	55
10	Burgundy type, unfill 55 mm	Screwcap	Gravity bottling - 10 KPa	white	1000	98
11	Bordeaux type, unfill 55 mm	Screwcap	Gravity bottling - 10 KPa	red	350	102
12			Snow-drop^{TM} N_2 0,05 g (3)			

(1) Unfill : unfill height before closure (mm) ; (2) Snow-drop^{TM}: injection of liquid CO_2 in the bottom of the bottle (2 or 3 drops per bottle) before filling; (3) Snow-drop^{TM} N_2: injection of liquid N_2 after filling.

batches 1 and 7 respectively, except that the bottles sampled were filled using a defective filling nozzle causing failure of the vacuum. Batches 11 and 12 differ only in the injection of 0.05 g liquid nitrogen in batch 12 after filling.

III - EVOLUTION OF THE OXYGEN IN THE GAS AN LIQUID PHASES IN TIME

The oxygen contents of a white wine, a red and a rosé (batches 1, 2 and 3, table I) were determined at bottling and subsequently monitored for 74, 208 and 127 days respectively, that is to say until exhaustion of the stocks available. Six bottles of wine were used per assay date (t for headspace and 3 for wine). The temperature of the storage premises varied around 15 °C. The three wines were stored neck upwards.

The variable experimental conditions (storage temperature neither mastered nor controlled, bottles stored differently, different stoppers, 75 cl bottles only, insufficient duration, different dates of analysis, etc.) prevent the drawing up of a balanced experimental plan.

IV- ANALYSES OF OLDER BOTTLES OF WINE

The oxygen contents of two Chardonnay wines and three red wines were determined several years after bottling. All the bottles still had their original stoppers on the day of analysis.

The first three batches analysed and the Châteauneuf-du-Pape were stored at 15-17 °C (table II) while the Corbières (batch 4) was stored in a shipping premises.

RESULTS AND DISCUSSION

I - CONTROL AT BOTTLING

The headspace contained oxygen just after bottling in all the batches studied. The amount was nonetheless smaller than that of the air. The quantity varied from 0.38 to 3.58 mg per bottle depending on the batch. This broad range resulted from three types of parameter affecting the volume, the percentage of oxygen or the pressure within the headspace.

1) Factors affecting the volume

The headspace volume depends above all on the type of closure. Indeed, the bottles making up the first seven batches were closed with natural or synthetic corks and the average headspace volume was 7.1 mL. The bottles in the last five batches were closed with screw caps and the average headspace volume was 17.7 mL (tables I and III).

With identical bottle format and cork length, an increase of 8 mm in the unfilled space in the neck results in a 2.9 mL increase in headspace (tables I and III, batches 1 and 3).

With an identical bottle format and height above liquid, a cork 4.5-mm shorter increases the headspace by 1.2 mL (tables I and III, batches 4 and 6).

2) Factors influencing the percentage of oxygen

Prior inert blanketing of the bottles gives contents of 10 % v/v or less (tables I and III, batches 1 (6.01 %), 6 (9.94 %) and 8 (9.98 %).

These preliminary results do not reveal a distinct difference between the different headspace levels (tables I and III, batches 1 to 7).

In screw-cap bottles, the combination of Snow-drop™ treatment before filling and flushing the headspace with CO_2 appears to be less effective (tables I and III, batches 9 (12.86 %) and 10 (14.19 %)) than Snow-drop™ treatment after filling (tables I and III, batch 12: 3.06 %). Whatever the inert blanketing method chosen, the percentage of oxygen is always smaller than without such treatment (tables I and III, batch 11: 14.8 %).

It can also be seen in batches with more than five samplings that the oxygen content of the gas phase is logi-

Table II - Characteristics of the batches studied

Caractéristiques des lots étudiés

Batch No.	Wine	Date bottled	Date of analysis	75-cL bottle	Cork	Number of assays of headspace / wine
1	Merlot, red, 1998 13% vol.	June 1999	16 july 2004	Traditional Bordeaux	Natural 47x24	5/5
2	Chardonnay 1995 12.5 %vol.	June 1996		Light Burgundy bottle	Agglomerated 44x24	5/5
3	Chardonnay 1992 12.5% vol.	April 1994			Natural 47x24	5/5
4	Corbières, red, 1985 12% vol.	April 1986	4 april 2005	Light Bordeaux bottle	Natural 44x24	3/5
5	Châteauneuf-du-Pape, red, 1966 13.7% vol.	April 1968	31 May 2005	Châteauneuf-du-Pape, green, 73 cL	Natural 51x24	4/4

Table III - Total oxygen contents in a bottle of wine just after closure at 20 °C and 101.3 kPa

Bilan des teneurs en oxygène total dans une bouteille de vin juste après obturation à 20°C et 101.3 kPa

		Wine	Headspace			Total in bottle		
No.	Sampling (1)	Dissolved O_2 mg/L	Volume mL (3)	O_2 % v/v	Pressure KPa (4)	O_2 mg/bx (5)	O_2 total mg/ 75 cL vin	O_2 headspace/ O_2 vin
1	1	1,35	5.2	6.01	-10	0.38	1,39	0,38
1b	1	1,46	5,2	16,54	150	2,84	3,94	2,59
2	1	5,50	8,1	14,49	0	1,56	5,69	0,38
3	2	6,00	8,1	17,61	0	1,90	6,40	0,42
4	6	4,15 (0,34)	9,6	16,11 (0,69)	0	2,06	5,17	0,66
5	3	1,3 (0,02)	5,2	6,16 (0,4)	0	0,43	1,41	0,44
6	1	1,80	8,4	9,94	-27	0,82	2,17	0,61
7	1	2,03	5,4	12,76	0	0,92	2,44	0,60
7b	1	2,03	5,4	18,88	150	3,37	4,89	2,21
8	10	2,33 (0,39)	16,5	9,98 (2,64)	0	2,19	3,94	1,25
9	7	1,86 (0,38)	18,1	12,86 (0,92)	0	3,10	4,50	2,22
10	10	1,52 (0,23)	18,1	14,19 (0,82)	0	3,42	4,56	3,00
11	3	3,06	18	14,8 (0,4)	1	3,58	5,88	1,56
12	2	3,06	18	3,06	42	1,04	3,34	0,45
Means (6)	**2,83**			**11,5**		**1,91**	**3,91**	**1,00**

(1) No. samples: number of samples (2 analyses per sample); (2) σ O_2% : SD O_2 % v/v when >2 samplings; (3) Volume headspace (mL) = unfill volume (manufacturer's data) minus cork volume; (4) Pressure = aphrometer reading (KPa); (5) O_2 mg/bx =[P_{O2} (Pa) x volume (m^3)] / [r x temperature (°K)]; where P_{O2} = (Pi x O_2 % (Pa) and r = 2.59813 10^{-4} (J/mg); (6) The means do not include bottlings 1b and 7b; figures in brackets are standard deviations.

cally constant throughout the bottling operation (table III, batches 4, 9 and 10: standard deviation < 1 % v/v) except in batch 8 (standard deviation 2.64 % v/v) for unknown reasons. No beginning and end of bottling effect was observed in contrast with oxygen dissolved in the wine (VIDAL *et al.*, 2004a).

The conditions of batches 1b and 7b were identical to those of batches 1 and 7 respectively, except that the bottles sampled were filled with a nozzle with a defective seal. No fine air bubbles were observed in the wine in these bottles just after closing. The assays performed show that the dissolved oxygen content of the wine was very little affected, in contrast with that of oxygen in the headspace that increased by 10.53 % v/v in batch 1b in comparison with batch 1 and by 6.12 % v/v in batch 7b in comparison with batch 7 (table III).

3) Factors affecting internal pressure

A seal defect in the filling nozzle causes high excess pressure of 150 KPa (measured with an aphrometer) as in batches 1b and 7b mentioned above.

In batch 12, the excess pressure in the headspace was 42 Kpa as the time elapsing between the injection of liquid nitrogen and closure was not long enough for the nitrogen to expand completely.

These first analyses at bottling line output show the large amount of oxygen trapped in the headspace as there is a 1:1 ratio of O_2 in the headspace to O_2 in the wine. The smallest quantities were observed with bottling lines fitted with an inert blanketing device before filling and vacuum corking (> 70 KPa) and headspace < 6 mL.

In contrast, bottling with a defective vacuum system increases the internal pressure. Now, the higher the internal pressure, the greater the difference between the pressure of the gas in the headspace and that of the ambient atmosphere and the greater the mass of oxygen trapped in the headspace.

Unless protective measures are taken, screw cap closure has the double disadvantage in comparison with corks of resulting in a larger headspace and absence of vacuum, resulting in a higher oxygen content. The inert blanketing of empty bottles and flushing the neck before closure reduce this impact. However, Snow-drop^{TM} treatment after filling is the most effective way of reducing the amount of oxygen trapped in the headspace. The technique can be improved and refined by adjusting the mass of liquid nitrogen injected and the time elapsing between the injection of inert gas and the placing of the cap to ensure that the internal pressure never exceeds 300 KPa (whatever the storage temperature), beyond which the risk of loss of seal is great, especially as some glassmakers now supply bottle formats with 35 mm vertical headspace to achieve a substantial reduction of the volume of the latter.

II- THE EVOLUTION OF OXYGEN IN THE GAS AND LIQUID PHASES OVER A PERIOD OF TIME

1) Evolution of oxygen contents in time

Examination of the oxygen contents of the gas and liquid phases shown in table IV and illustrated in figure I shows that they decrease in time, once converted into % saturation. The decrease is not linear. It is rapid in the first two months and then slows before the concentrations stabilise after 2-3 months-according to the batch-at less than 0.5 mg/L (5.5 % sat.) in the liquid phase and less than 1 % v/v (4.83 % sat.) in the gas phase.

2) Correlation test

Pearson's linear correlation test was performed using the StatBoxPro version 5.0 program. The oxygen contents of the gas and liquid phases displayed positive correlation at the risk ± = 5 % for the white and 1 % for the two other batches of wines studied.

3) Modelling test

To be able to reason in terms of mass and average oxygen consumption rate, the results were converted into mg per bottle using the formula mentioned in table III for the gas phase and assuming that there is no difference between atmospheric pressure and internal pressure, knowing that just after closure the difference is -20 to +20 kPa (-0.2 to +0.2 bar) in closed bottles. However, this assumption remains to be checked as it is not certain that the internal pressure remains unchanged in time.

In this case, whatever the wine, table V shows that the relation between the oxygen in the liquid or gas phase and time can be modelled (TableCurve 2D program, Windows version 2.03) by a polynomial curve of the following type :

$$Y^{-1} = a + bx + cx^2$$

in which x = time in days and y = O2 in mg per bottle.

The model equations in table V can be used to estimate the average rate of reduction of total oxygen per batch of wine. This can be considered to be the rate of consumption by the wine if it is assumed that the gas exchanges are negligible from inside the bottle towards the outside (table VI).

The difference in saturation percentage between the two phases is at its maximum at bottling. It depends on the operating conditions. The gap closes gradually as time passes. Nevertheless, whatever the date of analysis and whatever the wine, the percentage saturation of the headspace is always greater than that of the liquid phase (table IV, figure I). Furthermore, the curves of the two phases per batch of wine are positively correlated. This means that because of the tendency towards equilibrium of the partial pressure between the two phases, oxygen transfer is always from the headspace into the wine. Thus as the dissolved oxygen is used by the wine, oxygen from the headspace is dissolved in the wine *via* the gas-liquid

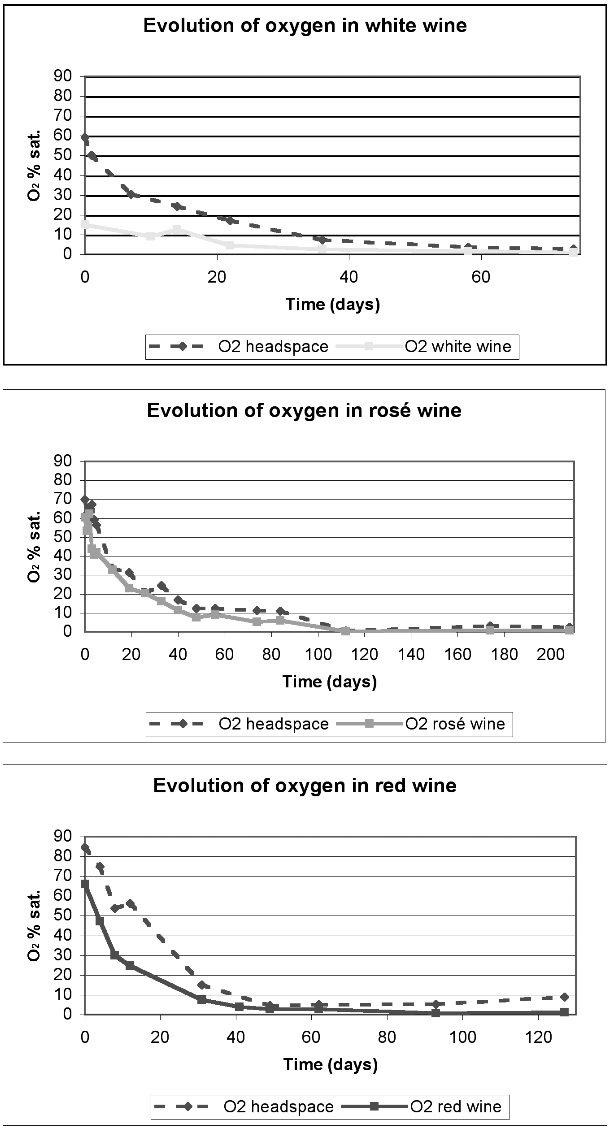

Figure I - Evolution in time of oxygen in % saturation of the gas and liquid phases of the three batches of wine

Évolution dans le temps de l'oxygène en % de saturation des phases gaz et liquide des trois lots de vin

Table IV - Evolution in time of the oxygen in the gas and liquid phases in three batches of wine

Évolution dans le temps de l'oxygène des phases gaz et liquide des trois lots de vin (20 °C et 101.3 kPa)

	White		Rosé		Red	
Time (days)	O_2 headspace % v/v	O_2 white wine mg/L	O_2 headspace % v/v	O_2 rosé wine mg/L	O_2 headspace % v/v	O_2 red wine mg/L
0	12,27	1,35	14,48	5,50	17,51	6,00
1	10,38		12,89	4,85		
2			13,39	5,66		
3			13,92	3,98		
4			12,25	3,70	15,48	4,28
5			11,70	3,80		
7	6,29					
8					11,13	2,71
10		0,82				
12			6,94	2,98	11,64	2,24
14	5,04	1,13				
19			6,47	2,08		
22	3,51	0,42				
26			4,31	1,85		
31					3,11	0,69
33			5,04	1,47		
36	1,49	0,23				
40			3,47	1,03		
41						0,35
48			2,56	0,68		
49					0,94	0,25
50						
56			2,54	0,82		
58	0,76	0,15				
62					1,03	0,24
74	0,58	0,08	2,29	0,48		
84			2,26	0,54		
93					1,09	0,06
112			0,09	0,02		
127					1,82	0,10
174			0,62	0,06		
208			0,49	0,07		

Table V - Characteristics in time of the model curves for oxygen in the gas and liquid phases of the three batches of wine

Caractéristiques des courbes modèles dans le temps de l'oxygène des phases gaz et liquide des trois lots de vin

Phase	Wine	Number of days	Model: $y^{-1}=a+bx+cx^2$			
			a	b	c	r^2
Headspace	White	74	1,206	0,128	6,226E-04	0,987
	Rosé	208	0,622	0,037	1,992E-04	0,979
	Red	127*	0,828	0,023	1,598E-03	0,976
Wine	White	74**	0,989	0,042	2,236E-03	0,999
	Rosé	208	0,237	0,018	1,213E-04	0,971
	Red	127	0,222	0,019	1,181E-03	0,997

* without the 127th day; ** without the 14th day

Table VI - Determination of the estimated average rates of total oxygen consumption of the three wines

Détermination des vitesses moyennes estimées de consommation d'oxygène total des trois vins

		O_2 mg/bx [(1)]		Consol. rate. O_2 µg/bx/j [(2)]			
Wine	Days	Headpsace	Vin	0 to 30 days	30 to 60 days	60 to 120 days	120 to 180 days
White	0	0,83	1,01	47,6	3,9		
	30	0,18	0,24				
	60	0,09	0,09				
Rosé	0	1,61	4,22	104,0	9,6	8,1	2,7
	30	0,53	1,14				
	60	0,282	0,57				
	120	0,126	0,24				
	180	0,073	0,14				
Red	0	1,21	4,51	133,4	6,2	3,0	
	30	0,34	0,54				
	60	0,13	0,18				
	120	0,04	0,09				

(1) values determined by model curves; (2) rate of O_2 consumption per bottle = sums of ΔO_2 mg/bx headspace and wine between two dates/number of days

Table VII - Average oxygen contents of the five batches of wine (20 °C and 101.3 kPa)

Teneurs moyennes en oxygène des cinq lots de vins (20°C et 101.3 kpa)

Batch No.	Type	Unfill mm (1)	Headspace mm	O_2 headspace % v/v	O_2 wine µg/L	O_2 headspace % sat.(2)	O_2 wine % sat. (3)
1	Merlot 1998	58,2	11,2	0,09 (0,01)	16,7 (5)	0,43	0,2
2	Chardonnay 1995	62,1	18,1	0,57 (0,32)	10,5 (3)	2,75	0,12
3	Chardonnay 1992	65,6	18,6	0,09 (0,04)	11,9 (3)	0,43	0,14
4	Corbières red 1985	73,3	29,3	0,37 (0,08)	14,2 (5)	1,85	0,15
5	Châteauneuf du Pape red 1966	70,6	19,6	0,32 (0,18)	5,3 (1)	1,54	0,06

(1) Unfill height before closure (mm); (2) [O2] % in relation to [O2] µg/L saturation in water under normal conditions; (3) [O2] % in relation to [O2] % v/v saturation in water vapour under normal conditions; SD in brackets

TableVIII - Average amounts of total oxygen in the seven batches of wine analysed at least three months after bottling (20 °C and 101.3 kPa)

Quantités moyennes en oxygène des sept lots de vins analysés au moins trois mois après leur mise en bouteille (20 °C et 101.3 kpa)

. Batch No	Type	Time after bottling (1)	Vol. headspace mL	Pressure KPa (3)	O2 µg/bx Headspace	Wine	Total
1	Merlot, red, 1998	5 years	3,8	18,4	5	12,5	17,5
2	Chardonnay, white, 1995	8 years	7,3	5,8	59	7,8	66,8
3	Chardonnay, white, 1992	10 years	7,5	46,8	11	8,9	19,9
4	Corbières, red,1985	19 years	10,6	0	52	10,5	62,5
5	Châteauneuf-du-Pape, red, 1966	37 years	6,4	0	27	3,9	30,9
6	Corbières, rosé, 2001	174-208th d (2)	8,1	0	67	45	112
7	Côtes du Rhône, red, 2001	93-127th d (2)	8,1	0	101	60	161

(1) Period of analysis after bottling; (2) Average O2 of the 2 dates given; (3) Pressure = aphrometer reading (KPa)

contact surface. This gaseous volume can be seen as a reserve of oxygen that empties gradually according to the rate with which oxygen reacts with the constituents of the wine.

The polynomial model put forward in table V seems pertinent above all for about 120 days according to the batch of wine studied, that is to say until the moment at which the oxygen content approaches zero. Average consumption rates vary from 47.6 to 133.4 µg per bottle per day from day 0 to day 30 and then fall to less than 10 µg per bottle per day in the following months (table VI). With an identical gas-wine contact surface (neck diameter 18.5 mm in the three batches), they logically depend on the initial levels and on the richness of the wines in substances involved in oxygen consumption.

It is then interesting to compare these experimental rates that take into account consumption by the wine and air/cork/headspace gas exchanges with various work on the intrinsic permeability of corks. RIBÉREAU-GAYON (1931) placed an indigo carmine solution bleached with sodium dithionite in 75 cL bottles closed with corks and estimated that average oxygen release through the cork was 0.43 mg per bottle during the first three weeks because of the phenomenon of the diffusion of the oxygen in the pores of the cork itself, and then 0.05 mg per bottle during the four following months; this corresponded to average diffusion rates T of 16 and then 0.4 µg per bottle per day.

More recently, SQUARZONI *et al.* (2004) determined the permeability of 12 synthetic and 4 natural corks. They also observed substantial accumulation of oxygen in the headspace, with 1.15 to 4.29 mg during the first 30 days, especially with natural corks that are more porous, with average penetration of 38 to 143 µg per bottle per day. Subsequently, during the 200 days of the test, they found that between 0.07 and 5 mg oxygen had penetrated via the cork, corresponding to an average penetration rate of 0.35 to 25 µg per bottle per day.

If the rate of diffusion through the cork were greater than the average consumption rate (table VI), the oxygen content of wines should increase. However, the opposite is observed. As a result, the lowest estimates put forward by SQUARZONI *et al.* and those of RIBÉREAU-GAYON seem more probable, especially as bottles are generally stored horizontally with a capsule covering the cork.

In contrast, the hypothesis mentioned above of release of oxygen via the cork might account for the temporary increase in the oxygen contents of the gas and liquid phases until about day 30 (e.g. the O_2 content of white wine of day 14, table IV). It can also be assumed that the partial release of CO_2 from the wine into non-blanketed headspace can increase the pressure in the bottle and thus cause increased dissolving of oxygen in the wine (e.g. O_2 dissolved in rosé wine on day 2, table IV).

More reserve is required when oxygen contents approach zero because of the shortage of bottles for continuing analytical monitoring for longer. Indeed, when practically all the oxygen trapped in the headspace and the wine at bottling has disappeared, does the amount of oxygen in the two phases finally stabilise, showing that oxygen penetration via the cork has been too small in comparison with the kinetics of consumption by the wine? Or does oxygen consumption by the wine, that reduces the oxygen consumption in the bottle, increase the difference between the oxygen concentration inside and outside, resulting in an increase in the rate of entry of oxygen through the cork from a certain oxygen threshold? Or if it had been possible to continue the analyses for longer, would the oxygen content have reached zero or, in contrast, would the oxygen levels increase slightly, as in rosé wine?

III - ANALYSES PERFORMED ON OLD BOTTLES OF WINE

The results show in table VII provide some answers to these questions. Indeed, the old bottles of wine analysed, in which the oxygen trapped during bottling had long been used up, displayed very low but distinct oxygen contents. These values are the results of oxygen that penetrates the headspace or the wine and that dissolved in the wine or that is consumed by it. It can be deduced that the quantity of oxygen entering via the cork does not compensate the quantity used by the wine. These equilibrium values show that maximum oxygen consumption capacity of the wines had not been attained (SINGLETON, 1985), even in the 1966 red wine, whatever the size of the headspace and thus whatever the state of the cork; the reaching of this capacity would be shown by an accumulation of dissolved oxygen in the wine. These analyses of old bottles of wine corroborate the preceding hypothesis that diffusion through the cork is some few tenths of a microgram or a microgram per bottle per day.

Furthermore, it was observed that the percentage saturation of the gas phase was always greater than that of the liquid phase, showing that exchange of gaseous oxygen still took place from the gas phase to the wine several years after bottling.

Finally, conversion of the oxygen contents into mass of oxygen per bottle using the formula provided in table III for the gas phase, it is seen, that in the five batches of old wines and rosé and red wines discussed in the preceding chapter the total oxygen contents are lower after several years (17 and 67 µg per bottle) compared to the results of analyses performed 3 and 7 months after bottling (batches 7 and 8, table VIII : 112 and 160 µg per bottle).

CONCLUSION

Measurement of the oxygen in the headspace of a bottle combined with that of the dissolved oxygen content of the wine makes it possible to reason in terms of the total quantity of oxygen per bottle.

Following the various aeration events to which wine is subjected before and during bottling, a bottle of wine contains a certain amount of oxygen in the headspace and a further quantity dissolved in the wine. During storage, the oxygen contents of the gas and liquid phases decrease strongly during the first two months. This decrease then slows before the concentrations stabilise gradually at less than 0.5 mg/L in the wine and 1 % v/v in the headspace. Furthermore, the percentage oxygen saturation of the headspace is always greater than that of the wine whatever the wine studied and the date of analysis. This means that the tendency towards equilibrium always results in transfer of oxygen from the headspace to the wine. Thus, as the wine uses up the oxygen that it contains, oxygen in the headspace dissolves in the wine *via* the gas-liquid contact surface.

The ratio of headspace/wine oxygen varied from 0.38 to 3 at bottling. This means that in certain cases there was three times as much oxygen in the headspace as dissolved in the wine. The operating conditions account for these substantial variations, and mainly the type of closure, the headspace volume, the technique used to protect against oxygen and the initial oxygen concentration in the wine before bottling.

Oxidation reactions become established in the first period when the oxygen had dissolved. It can be assumed that the rate of these reactions is governed by the oxygen present in the bottle at bottling. The kinetics of the reactions decreases as the oxygen content dwindles gradually. The average rate of oxygen consumption in the experiments was more than several tens of micrograms per bottle per day for the first 30 days and then fell to less than 10 µg per bottle per day in the second month. This result is in agreement with the rate of diffusion via the cork of some µg or tenth of a µg per bottle per day.

This period is characterised by low available oxygen levels and oxidation phenomena that are doubtless reduced. Assuming a closure that completely prevents gas exchanges, oxygen contents would tend towards zero and oxidation reactions to zero rates. However, various studies have shown that corks are permeable to atmospheric oxygen (RIBÉREAU-GAYON, 1931; SANCHEZ and ARACIL, 1998; SQUARZONI *et al.*, 2004) and our results show that measurable amounts of oxygen are always found, but in very small amounts (17 to 67 µg per bottle), as is shown by the analysis of samples kept for a number of years. It therefore seems that a system becomes established over a period of time in which dissolved oxygen amounts to less than some 20 µg per litre and the proportion of oxygen in the gas phase topping the wine is less than 0.6 % v/v, an equilibrium state that changes very slowly. Cork permeability means that atmospheric oxygen could enter the gas phase above the wine. The fact that the proportion of oxygen is higher in the gas phase than in the wine supports this idea. Given the very low rates of oxygen consumption after the first period of storage of wine in bottles, the permeability of the corks in position must be particularly low. If this were not the case, a gradual enrichment in oxygen should be observed.

This exploratory work helps to provide better understanding of the evolution of bottled wine and gas exchanges under real storage conditions. The hypotheses put forward should be investigated more closely in studies combining the monitoring of total oxygen and other analytical parameters such as the carbon dioxide content, internal pressure and the impact on wine quality by sensorial analysis and the monitoring of oxygen-sensitive substances. This type of investigation opens up prospects for the improvement of protection against oxygen during bottling, for comparing and evaluating the real impact of stoppers on gas exchanges, the position of the bottle during storage and storage parameters.

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