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Impact of Oxygen Dissolved at Bottling and Transmitted through Closures on the Composition and Sensory Properties of a Sauvignon Blanc Wine during Bottle Storage

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This work outlines the results from an investigation to determine the effect of the oxygen dissolved at bottling and the specific oxygen barrier properties of commercially available closures on the composition, color and sensory properties of a Bordeaux Sauvignon Blanc wine during two years of storage. The importance of oxygen for wine development after bottling was also assessed using an airtight bottle ampule. Wines were assessed for the antioxidants (SO_2 and ascorbic acid), varietal thiols (4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol), hydrogen sulfide and sotolon content, and color throughout 24 months of storage. In addition, the aroma and palate properties of wines were also assessed. The combination of oxygen dissolved at bottling and the oxygen transferred through closures has a significant effect on Sauvignon Blanc development after bottling. Wines highly exposed to oxygen at bottling and those sealed with a synthetic, Nomacorc classic closure, highly permeable to oxygen, were relatively oxidized in aroma, brown in color, and low in antioxidants and volatile compounds compared to wines sealed with other closures. Conversely, wines sealed under more airtight conditions, bottle ampule and screw cap Saran-tin, have the slowest rate of browning, and displayed the greatest contents of antioxidants and varietal thiols, but also high levels of H_2S, which were responsible for the reduced dominating character found in these wines, while wines sealed with cork stoppers and screw cap Saranex presented negligible reduced and oxidized characters.

KEYWORDS: Cork stoppers; synthetic closures; screw caps; reduction; oxidation; Sauvignon Blanc

INTRODUCTION

Wine development is extremely dependent on the amount of oxygen that wine receives during winemaking and aging (*1*, *2*). Some of the opportunities for picking up oxygen occur during transfer operations, the wood barrel stage, filtration, and the bottling process. After bottling, oxygen exposure depends on the sealing effectiveness of closures, which differ in their oxygen barrier properties (*2*−*5*). In general, synthetic stoppers allow oxygen to enter into the bottle at a relatively high rate, while screw caps and technical corks let in relatively little oxygen (*3*−*5*). In natural cork stoppers, most of the oxygen diffuses out of the cell structure into the bottle at a relatively low rate (*6*).

The discussion of the impact of different closures on wine development after bottling leads to the commonly asked question of whether wines require oxygen to age or develop. This question has generated controversial answers and theses over time. Pasteur, in studies conducted in 1873, was the first to study the effect of oxygen on wine development. According to him "oxygen is the greatest enemy of wine", but also, "oxygen makes the wine, which ages under its influence" (*7*). In contrast, Emile Peynaud considered that "it is the opposite of oxidation, a process of reduction, or asphyxia, by which wines develop in the bottle" (*8*). This statement was based on the studies of Jean Ribéreau-Gayon, who considered that negligible amounts of oxygen diffused from natural cork stoppers into bottled wines (*1*). Nowadays, it is admitted that some degree of continuous and controlled oxygenation seems to be beneficial for red wine maturation as the oxidative phenolic reactions lead to color enhancement and the reduction of astringency (*9*). For white wine production and storage, any exposure to oxygen, apart from oxidative juice handling, is considered negative. This detrimental development is often related to the loss of fruit and fermentation derived flavors, and the development of oxidized characters, accompanied by an accelerated browning of color (*10*, *11*). Although it appears possible for white wines to develop in the bottle in the total absence of oxygen, recent studies have suggested that undesirable reduced characters can develop if the wine's redox potential is too low as a result of too little oxygen exposure after bottling (*12*−*14*).

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Some good progress has been made in determining the reasons for the importance of oxygen, and identifying the factors that can enhance or dilute its impact, including the closure. There have been several studies assessing the influence of different closures on wine development after bottling (*12*−*16*). Most of them have shown that wines sealed with synthetic closures have a tendency to lose fruit attributes and develop oxidized characters over short periods of storage (*12*−*15*). On the other hand, screw capped bottles scored highest for fruity aromas, maintaining the highest levels of antioxidant compounds while showing the least color development. However, undesirable reduced characters were far more prevalent in screw cap sealed wines (*12*−*14*). Recently, Kwiatkowski et al. suggested that the development of these characters after bottling is more related to the low diffusion of oxygen through closures than to the oxygen levels at bottling (*14*).

Volatile sulfur compounds play an important role in the aroma of wines, even at low levels, often being responsible for reduced "off-flavor" characters, but also for typifying scents (*17*, *18*). Long chain polyfunctional thiols display a remarkable effect on the typical box-tree and tropical fruit aroma of different varietal wines, like Sauvignon Blanc (*19*, *20*). In contrast, short-chain thiols, sulfides, disulfides, thioesters and heterocyclic compounds can spoil the wines (*17*, *18*, *21*). The sensory attributes of these compounds change with their concentration. At low levels, a particular thiol may smell of peas or vegetal, and at high levels, may smell of onion, garlic, cooked cabbage, rotten eggs, rubber or putrefaction. Hydrogen sulfide is perhaps the most important volatile sulfur compound, often being responsible for reduced "off-flavors", mainly with those related to "rotten egg" and sewage-like characters (*17*, *18*, *21*). This compound can be formed metabolically by yeast from inorganic sulfur compounds and sulfite, or organic sulfur compounds, cysteine and glutathione during alcoholic fermentation (*18*). However, little is known about its formation after bottling and contribution to postbottling reductive character.

This study was focused on the effect of the oxygen dissolved at bottling and the specific oxygen barrier properties of closures on the aromatic composition, color and sensory properties of a Bordeaux Sauvignon Blanc wine during two years of storage. The wine development after bottling under anaerobic conditions was assessed using an airtight bottle ampule. The main purpose of this study was to highlight the importance of the oxygen management at bottling, but also of oxygen transmission rates of closures as predictable tools for the optimization of wine shelf life. It is hoped that the results of this study help to elucidate the role of oxygen on wine development during the postbottling period.

MATERIALS AND METHODS

Chemicals. Pure reference compounds for 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl-acetate (3MHA) were purchased from Interchim (Montlucon, France). L-Ascorbic acid (≥99%), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (≥99%) and sodium hydrosulfide hydrate were obtained from Sigma-Aldrich (St Quentin Fallavier, France). Hydrogen sulfide was obtained by dissolving hydrosulfide hydrate in deionized water at pH 3.2.

Wine. Wine used for the trial was produced during 2004 vintage from Sauvignon Blanc grapes grown in the Côtes de Duras (France). Fermentation was carried out in stainless steel tanks under 18 °C during 20 days. Tartaric precipitation was carried out in isotherm tanks under constant temperature of 3 ± 1 °C during 7 days. The wine was filtered and bottled at Domaine d'Amblard (Saint Sernin de Duras, France) in March 2005. Ascorbic acid was added to wine four days prior to bottling in order to obtain a concentration of 100 mg/L.

Bottles. Bottles were supplied by Saint-Gobain Glass Packaging (Cognac, France). The bottles used for cylindrical closures (cork stoppers and synthetic closures) were of Antique green color and 750 mL of capacity (manufacture's code 8005568), produced to the following CETIE 35-100 TR specifications: a diameter of 18−19 mm at a depth of 3 mm and a diameter of 19−21 mm at a depth of 45 mm from the bottle entrance.

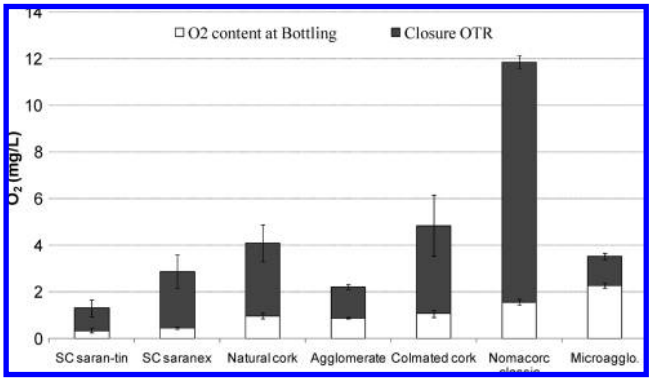

Figure 1. Oxygen dissolved at bottling and oxygen transmission through different closures during 24 months. The order of bottling is represented from left to the right. Values of oxygen at bottling are the mean of 3 bottles. Oxygen transfer rates (OTR) of closures were obtained from colorimetric measurements of 10 replicates, which were taken from the same bale of the closures used in the bottling trial. SC saran-tin = screw cap Saran-tin; SC saranex = screw cap Saranex; Microagglo. = microagglomerate cork.

For screw cap closures, 750 mL Antique green color bottles with a screw thread (manufacture's code 8005574) were used.

Hermetic bottles named "all-in-glass bottles" were supplied by Rudolf Gantenbrink (Limburg, Germany). These bottles were of Antique green color and 750 mL of capacity. The airtightness of these bottles was confirmed in previous studies (*6*, *7*).

Closures. Eight sealing systems were tested in the trial: two Stelvin screw cap closures (60 mm length and 30 mm of diameter) with different liners, Saran tinfoil and Saranex 38, respectively; a natural cork stopper (reference "flor", 44 mm length and 24 mm diameter), a colmated cork stopper (reference 3, 44 mm length and 24 mm diameter); two "technical" cork stoppers, an agglomerated cork (45 mm length and 24 mm diameter) and a microagglomerate Neutrocork (44 mm length and 24 mm diameter); a synthetic closure, Nomacorc classic (43 mm length and 22 mm diameter). A hermetic bottle ampule sealed with glass closure tubes (40 mm length and 10 mm of diameter) as it is described in Bottling and Storage was also used.

Both Stelvin screw caps were supplied by Pechiney Capsules (Chalon sur Saône, France). Natural, colmated and "technical" cork stoppers were supplied by Amorim & Irmãos, S.A. (Santa Maria de Lamas, Portugal). Nomacorc closures, manufactured by coextrusion processes, were supplied by Nomacorc S.A. (Thimister-Clermont, Belgium).

The cork stoppers were hydrogen peroxide bleached, however, no peroxide residue was detected. Cork stoppers and Nomacorc closures were coated with silicone and an unknown FDA approved coating, respectively.

Oxygen barrier properties of each closure were characterized using a colorimetric method as described elsewhere (*6*). Oxygen transmissions through each type of closure during 24 months are presented in **Figure 1**.

Bottling and Storage. The bottling line comprised a filler (CIFOM, Italy), a single headed corker (La Girondine, Bordeaux, France) and an Eagle Closys AROL single head screw capper (AROL S.A., Canelli, Italy).

The bottling run was initiated with the screw cap, Saran-tin and Saranex, closures, respectively. The cylindrical closures were then applied in the following order: natural cork, agglomerate, colmated cork, Nomacorc classic and microagglomerate. Simultaneously, the hermetic bottle ampules were filled directly from the filter. The order in which the closures were applied is represented in **Figure 1**. A total of 40 bottles for each type of sealing system were sealed over a period of 2 h. The temperature of wine during bottling varied from 11.5 to 14.2 °C

The wine was filled into screwed bottles at 45 ± 1 mm from the top of the bottle under a cadence of 500 bottles/hour. The bottles were then sealed under a flush of nitrogen (0.1 bar), which was applied immediately prior to the insertion of screw caps.

All bottles sealed with cylindrical closures were filled to 63 ± 1 mm from the top, under similar conditions described above. The cylindrical closures were compressed to a diameter of 16 mm before insertion under vacuum into bottles. Five bottles were tested prior to the bottling run to evaluate the efficiency of the sealing machine. The internal pressure displayed an overpressure of ~0.3 bar, which indicated that the vacuum was not achieved.

The hermetic bottles were filled directly from the filter under nitrogen flux (Glasshütte Limburg, Limburg, Germany) at 55 ± 1 mm from the top of the bottle. The bottles were then sealed with glass closures by flame welding (1200 °C) to bottleneck using a sealing glass prototype (Glasshütte Limburg, Limburg, Germany).

Bottles sealed with cylindrical closures were left upright for 1 h, and then stored horizontally in stainless steel pallets. The bottles sealed with glass (bottle ampule) and screw caps were stored vertically in cartons. All bottles were stored over 24 months under cellar conditions.

Standard Chemical Analysis for a White Wine. Wines were analyzed for free and total sulfur dioxide by amperometric titration corrected with acetaldehyde. Glucose, fructose, L-malic acid and acetaldehyde were determined by enzymatic assays (Boehringer, Mannheim, Germany). The pH was measured using a pH-meter CG825 (Schott-Geräte, Germany). The concentration of ethanol, titratable and volatile acidity and the concentration of tartaric acid were determined by near-infrared reflectance using WineScan FT 120 (Foss France S.A., Nanterre, France). The laccase activity was measured using the enzymatic assay described by Grassin and Dubourdieu (*22*). Analysis of iron, copper and potassium were performed before bottling using inductively coupled plasma atomic emission spectroscopy. Wine submitted to sensory analysis were analyzed for chloroanisoles and bromoansioles by SPME−GC−MS as described by Evans et al. (*23*). All the analyses were performed before bottling. The pH, volatile acidity and the concentration of free and total sulfur dioxide were measured at 48 h, 2, 12, and 24 months. Unless otherwise indicated, five replicate bottles per type of closure were analyzed at each time point after bottling.

Measurements of dissolved oxygen (3 measurements for each closure run) in wine were made using an Orbisphere 29971 (Trappes, France) sampler for bottles. The closure seal was pierced by a needle and the wine was fed to the 31120A measuring probe using polyurethane tubing under a nitrogen pressure of 1 bar. For oxygen measurements, the solution flowed over a PFA 2956A Teflon membrane in a 32007B circulation chamber connected to an Orbisphere Moca 3650 single channel microprocessor analyzer.

Ascorbic Acid Measurements. The concentration of ascorbic acid was determined according to the high performance liquid chromatography method described by Lopes et al. (*24*). The white wine samples were filtered through GHP Acrodisc 25 mm, 0.45 μm filters (Pall Life Sciences, Ann Arbor, MI), immediately prior to injection into the HPLC system. The column was a PLRP-S 100 Å (5 μm) column (150 × 4.6 mm) (Polymer Laboratories, Shropshire, U.K.). Elution conditions were as followed: flow rate 1 mL/min at room temperature, 20 μL sample loop; solvent A, water/trifluoroacetic acid (99:1 v/v); solvent B, acetonitrile/solvent A (80:20 v/v). The gradient elution profile was 0 to 5 min 100% A, 5 to 6 min 100% B, 6 to 10 min 100% B, 10 to 11 min 100% A, 11−12 min 100% A. The concentration of ascorbic acid in five bottles sealed with each type of closure was measured after 48 h, 2, 12, and 24 months of storage.

Color Measurements. The wine color was analyzed by 2 methods:

The absorbance at 420 nm was measured using a Unikon 922 spectrophotometer (Kontron Instruments, Milan, Italy) in 10 mm quartz cuvette.

Wines were also submitted to Tristimulus CIELab measurements of the parameters L^* (lightness/darkness), a^* (red/green chromaticity), b^* (yellow/blue chromaticity) and the derived values C^* (chroma) and h_{ab} (hue angle) using a Minolta series CM-508i spectrocolorimeter equipped with a transmittance accessory CM-A76 (Osaka, Japan). These measurements were carried out at room temperature in a 10 mm quartz cuvette using an illuminant D65 and a 10° observer angle according to the CIELab76.

Determination of Volatile Thiols. The concentration of 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexan-1-ol (3MH) was determined according to the method described by Tominaga et al. (*19*). 500 mL of wine, spiked with 2.5 nmol of 4-methoxy-2-methylbutane-2-thiol, was extracted with two successive additions of dichloromethane (100 mL). After separation from the aqueous phase, organic phase was extracted with a sodium *p*-hydroxymercuribenzoate solution, which was then fixed onto an anion exchange column before the thiols were eluted with cysteine and extracted into dichloromethane. The extract was dried using anhydrous sodium sulfate and exposed to a nitrogen stream up to 250 μL. Manual injection of 2 μL was performed in an Agilent 6890N GC with an 5973 MS detector. The thiols were separated on a 50 m BP20 capillary column (220 × 0.25 μm) using helium carrier gas at 28 cm/s and an oven temperature ramping from 40 to 220 °C during 71 min. Five replicates of each type of closure were analyzed at 24 months of storage.

Determination of Hydrogen Sulfide. The concentration of hydrogen sulfide was determined according to the method described by Lavigne et al. (*25*). A volume of 150 mL of wine was removed from each bottle, and then bottles were hermetically sealed with silicone stoppers. After 24 h in the dark at room temperature, 1 mL of the gas phase was injected according to the headspace technique.

Chromatographic experiments were performed using a Hewlett-Packard 5980-I coupled with a HP 19256-A flame photometric detector at λ = 393 nm. The column was Chromosorb WHP (4 m × 3 mm). The oven temperature was kept at 65 °C for 5 min and programmed at a rate of 6 °C min/L to 110 °C. The carrier gas was hydrogen (15.5 mL/min). Its flow rate in the flame was 93 mL/min, and a mixture of nitrogen/oxygen (80/20) at 100 mL/min was used. The makeup gas was nitrogen at 55 mL/min. The wine was assessed at bottling and after 24 months of storage by analysis of five replicates of each type of closure.

Determination of Sotolon (3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone). The concentration of sotolon was determined according to the method described by Lavigne et al. (*26*). 100 mL of wine, spiked with 77 nmol of the internal standard 3-octanol, was extracted successively with 10, 5, and 5 mL of dichloromethane. Extracts were then blended, dried over anhydrous sodium sulfate and concentrated up to 0.5 mL under nitrogen stream. Two microliters of the obtained extract was injected in a Star 3400 CX gas chromatograph fitted to a Varian Saturn 2000 electronic ion trap mass spectrometer. The resolution of sotolon was obtained with a fused silica column coated with SPB1 (60 m × 0.25 mm × 1 μm). Helium was used as carrier gas. Five replicates of each type of closure were analyzed at 24 months of storage.

Sensory Analysis. Descriptive sensory analyses were performed at 2, 12, and 24 months postbottling by a panel of 11 judges recruited from the staff of the Faculty of Enology of Bordeaux (France). All panelists had extensive experience in wine tasting and regular participations in sensory panels with Sauvignon Blanc wines.

All the assessments were performed at room temperature 18 ± 1 °C in individual booths under daylight lighting. 50 mL of wine was presented in standard ISO 3591 "XL5-type" tasting glasses with glass covers identified by three digit random codes and assessed within one hour of pouring.

The sensory attributes scored were aroma intensity, overall fruitiness and aroma freshness, reduced and oxidized characters. Wine defects were also rated, when perceived by the panelists. Panelists were instructed to assess first the aroma and then palate of wines, scoring each attribute on a scale of 0 to 5, where 0 indicated that the attribute was not perceived and 5 high intensity of the attribute. Eight samples, one per closure type, were presented to each panelist per session. At each time point, 4 sessions were carried out over two days (10 to 12 a.m.). Thus each panelist assessed 32 samples.

Data Analysis. All data were treated using Microsoft Excel 2000 software. Analysis of variance (ANOVA), Fisher's least significant difference, correlation and regression analyses, and PCA (principal component analysis) were carried out with XLSAT software (Addsinsoft, Paris, France).

RESULTS AND DISCUSSION

Wine Composition and Bottling. The general wine composition before and immediately after bottling is presented in **Table 1**. These compositional parameters are typical for a Sauvignon Blanc wine, confirming that wine preparation for bottling was appropriate (*27*).

The pH before bottling was 3.25 and remained relatively stable after storage, varying from 3.18 to 3.22, independently of the type

Table 1. Sauvignon Blanc Wine Composition before and Immediately after Bottling

compositional variable	value
Measurements before Bottling[a]	
alcoholic strength	12.1% v/v
pH	3.25
total acidity	4.27 g/L as tartaric acid
volatile acidity	0.29 g/L as acetic acid
tartaric acid	1.40 g/L
malic acid	3.02 g/L
glucose plus fructose	0.40 g/L
laccase activity	none detected
acetaldehyde	42 mg/L
iron	3.5 mg/L
copper	0.4 mg/L
potassium	5.2 g/L
2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole, 2,3,4,5,6-pentachloroanisole, 2,4,6-tribromoanisole	none detected
Measurements Made after Bottling[b]	
total SO_2	132 mg/L
free SO_2	41 mg/L
ascorbic acid	85 mg/L
color parameters	
A_{420nm}	0.057
L^*	99.29
a^*	−0.70
b^*	3.83
C^*	3.89
h_{ab}	100.3

[a] Analysis carried out from a tank sample one day prior to bottling. [b] Analysis carried out on 3 control bottles (i.e., bottle ampules).

of closure. Likewise, the level of volatile acidity was low and remained stable throughout the study (ranging from 0.27 to 0.30 g/L as acetic acid).

In this trial, most of the parameters that might have influenced the closures' subsequent performance were carefully controlled during bottling; however, some variations were observed due to practical constraints of bottling. The most important variation was the concentration of dissolved oxygen in wine, which varied from 0.19 to 2.4 mg/L throughout the bottling run (**Figure 1**). The bottling was interrupted after screw cap insertion in order to change the type of bottles and to do the necessary bottling line modifications required by cylindrical closures. As result of these interruptions, the level of dissolved oxygen increased significantly toward the end of the bottling as the level of wine in the tank decreased. The possible implications of this constraint on the development of white wine after bottling are discussed below.

Ascorbic Acid. Ascorbic acid is a powerful oxygen scavenger, which is purposely added to wines to prevent oxidation and prolong its shelf life. The impact of bottling conditions and closure type on the levels of ascorbic acid were observed immediately after bottling. Forty-eight hours after bottling, the concentration of ascorbic acid was similar, with the exception of the wine in the bottle ampule. The wine sealed under bottle ampule, presented levels of ascorbic acid 6 to 7 mg/L higher than those sealed with other closures (**Figure 2a**). This difference was likely related to the bottling procedure, as wine under bottle ampule was filled directly from the filter under nitrogen, preventing some contact with oxygen, which was more marked in wines sealed with screw caps and cylindrical closures.

At 2 months of storage, the concentration of ascorbic acid dropped significantly, being different among bottles sealed with different sealing systems ($p < 0.001$). The level of ascorbic acid in

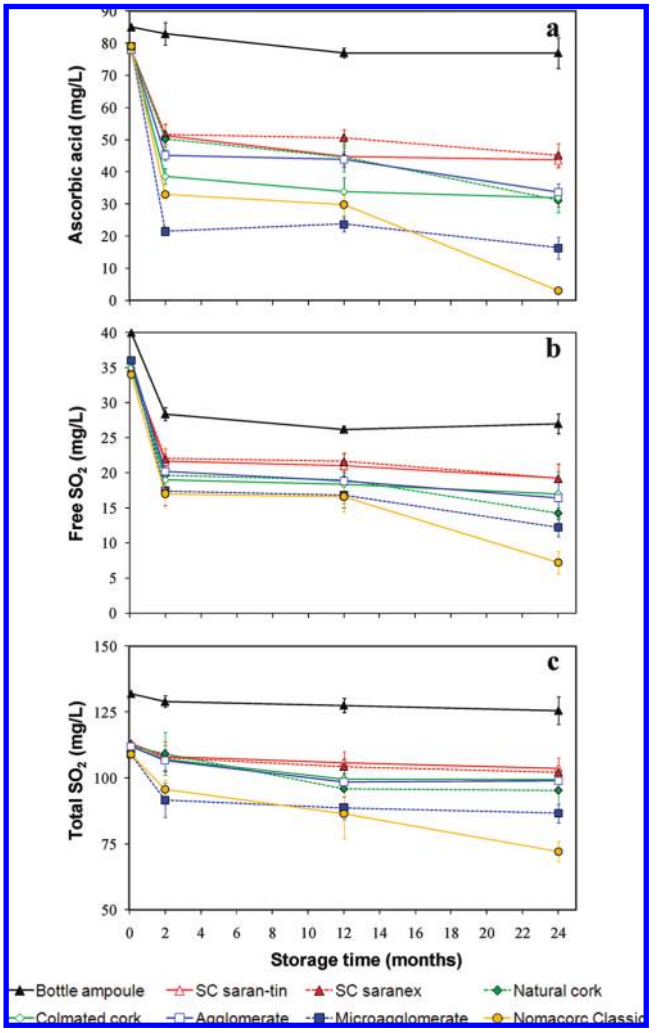

Figure 2. Impact of storage time and closure on ascorbic acid (**a**), free (**b**) and total SO_2 (**c**) concentrations in Sauvignon Blanc wine.

the wine sealed under hermetic conditions (bottle ampule) only dropped 2 mg/L. On other hand, the concentration of ascorbic acid dropped to 50 and to 52 mg/L for natural cork and screw cap sealed wines, respectively; while those sealed under agglomerate and colmated corks presented 45 and 39 mg/L of ascorbic acid, respectively. The lowest concentrations of ascorbic acid were found in wines sealed with Nomacorc classic and microagglomerate closures, 33 and 22 mg/L, respectively. This effect is probably related to the greater amount of oxygen dissolved in these wines at bottling when compared to the other wines (**Figure 1**).

At 12 and 24 months of storage, the ampule bottle contained the highest concentrations of ascorbic acid, 77 mg/L. At 24 months, wines under screw caps presented significantly higher amounts of ascorbic acid than those sealed with natural, colmated and agglomerate corks ($p < 0.001$). In wines sealed with Nomacorc closures, the ascorbic acid was completely depleted after 24 months of storage.

The ascorbic acid loss mainly occurred in the first two months of storage, even though after this period all wines continued to lose ascorbic acid but at different rates. For the wines in bottle ampules and sealed with screw caps, colmated and microagglomerate closures the rate of ascorbic acid losses from two months onward seemed to be identical, even if the absolute concentrations were different. In wines sealed under natural and agglomerate cork the rate of loss of ascorbic acid seemed to be slightly higher than the precedent wines, but significantly lower than those sealed

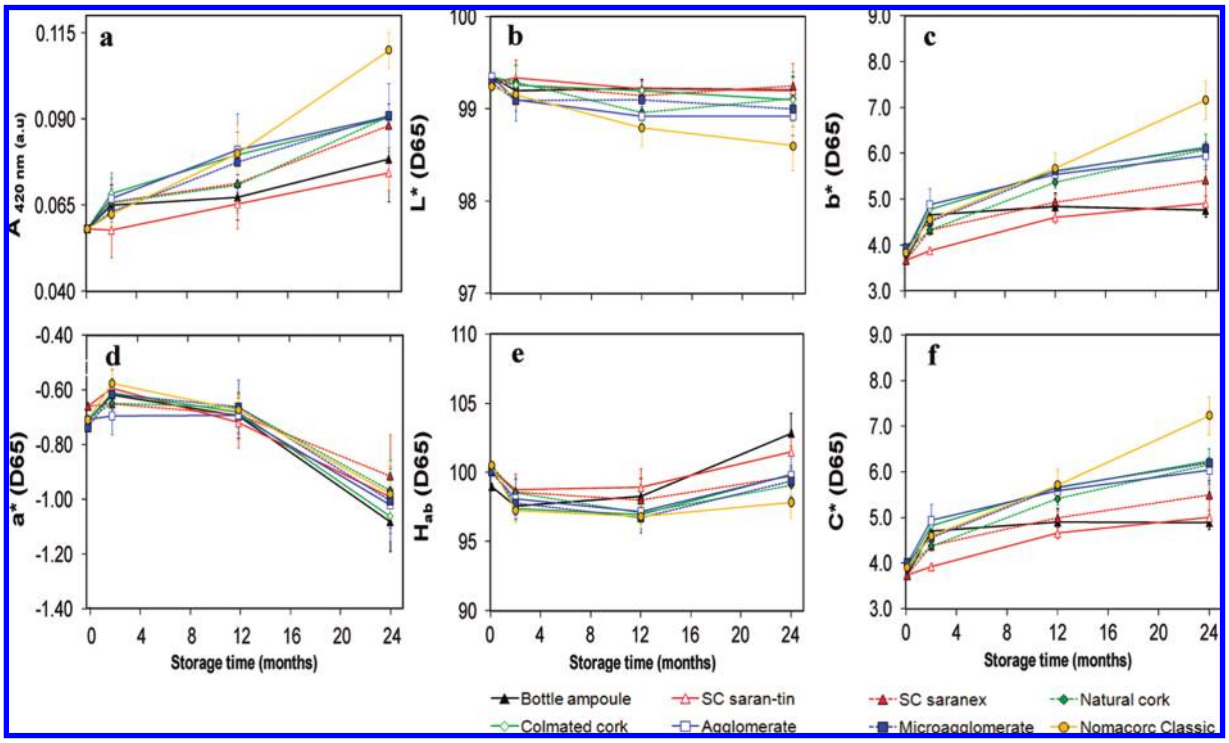

Figure 3. Impact of storage time and closure on A_{420nm} (**a**) and CIELAB color values of a Sauvignon Blanc wine: (**b**) L^* (lightness/darkness), (**c**) b^* (yellow/blue chromaticity), (**d**) a^* (red/green chromaticity), (**e**) h_{ab} (hue angle), (**f**) C^*(chroma).

under Nomacorc closure. Under anaerobic conditions (i.e., bottle ampule), almost all ascorbic acid added was retained, which shows, in contrast to Skouroumounis et al. (*13*), that the depletion of ascorbic acid in wines only occurs due to oxidative reactions (*28*).

A theoretical maximum consumption of ascorbic acid by oxygen can be calculated assuming a direct reaction, where 1 mol of oxygen consumes ~1 mol of ascorbic acid (*11*). Assuming this relationship, the estimated loss of ascorbic acid in wines due to oxygen dissolved at bottling and transmitted through closures is substantially lower than those really observed after 24 months. However, if the consumption of ascorbic acid due to the estimated volume of oxygen in the headspace is included, the total estimated loss of ascorbic acid in wines sealed with screw caps Saran and Saranex would be 37 and 45 mg/L, levels closer to those observed. Conversely, for cylindrical closures, the theoretical losses of ascorbic acid are still lower than those observed. This seems to indicate that the oxygen amount in the headspace was underestimated, once the loss of ascorbic acid from 2 months onward is closer to the observed values. The amount of oxygen retained in the headspace increases with its internal pressure, especially when the vacuum equipment does not work properly, as it was observed in this trial (*29*). The oxygen in the headspace after bottling was not determined; however, it is recognized that most of the oxygen entrained in the bottle during bottling resides in the headspace (*30*, *31*).

Sulfur Dioxide. The effects of bottling conditions and closure type on the levels of free and total sulfur dioxide were observed soon after bottling and during bottle storage. Forty-eight hours after bottling, the concentrations of free sulfur dioxide in wines were very similar among the different cylindrical closures (**Figure 2b**). Likewise, the levels of total sulfur dioxide were identical, being slightly lower in those sealed under Nomacorc and microagglomerate closures (**Figure 2c**). The wine in bottle ampules presented levels of free and total sulfur dioxide 5 and 20 mg/L higher than those sealed with other closures. Again, this difference was likely related to the bottling, once bottle ampules were filled directly from the filter.

At 2 months postbottling, the level of free and total sulfur dioxide in ampule dropped to 28 and 129 mg/L, respectively. In addition, the level of free and total sulfur dioxide dropped to 22 and 108 mg/L for screw cap sealed wines; while those sealed under natural, agglomerate and colmated corks presented 20 and 107 to 109 mg/L of free and total sulfur dioxide. Wines sealed with microagglomerate and Nomacorc closures presented the lowest levels of free and total sulfur dioxide (**Figure 2b**,**c**). The effect of bottling appears to be significant once the levels of free and total sulfur dioxide decreased significantly at this stage, being more important in those bottles sealed with Nomacorc classic and microagglomerate, which contained the highest levels of dissolved oxygen at bottling.

At 12 and 24 month, the ampule bottle retained the highest concentrations of free and total sulfur dioxide, 26 and 126 mg/L, respectively. The wines sealed under screw caps presented also high amounts of free and total sulfur dioxide, which dropped from 22 to 19 mg/L and from 108 to 104 and 102 mg/L, respectively. Wines sealed with Nomacorc presented the lowest levels of sulfur dioxide, which decreased significantly throughout storage, the level of free sulfur dioxide after 24 months being lower than 10 mg/L, which is consider to be the limit of protection of white wine (*15*, *19*). Wines sealed with cork stoppers (natural, colmated, agglomerate and microagglomerate) presented intermediate levels of free and total sulfur dioxide, the rate of loss of sulfur dioxide from two months onward being similar, even though the absolute concentrations varied (**Figure 2b**,**c**).

The results of sulfur dioxide presented a similar trend to that found with ascorbic acid. In the first two months of storage, the levels of sulfur dioxide strongly decreased due to the oxygen introduced at bottling and then continued to drop in the 22 months thereafter, being particularly important in wines sealed with Nomacorc, which allows continuous oxygen entry into

Table 2. Concentrations of Some Volatile Compounds in Sauvignon Blanc Wine Sealed with Different Closures after 24 Months of Storage[a]

		after 24 months							
			screw cap						
compounds	bottling	ampule	Saran-tin	Saranex	natural cork	colmated cork	agglomerate	microagglomerate	Nomacorc classic
4MMP (ng/L)	na[b]	19.3 (4.4) a	15.1 (6.5) ab	5.8 (2.9) bc	14.3 (0.9) a	17.3 (10.4) a	15.5 (2.1) a	6.6 (4.6) c	5.1 (1.2) c
3MH (ng/L)	na	821 (110) a	647 (138) ab	396 (68) bc	454 (14) bc	361 (146) c	599 (255) ab	436 (132) bc	114 (41) d
H_2S (μg/L)	1.4	29.6 (4.7) a	21.1 (3.6) b	15.0 (3.7) c	6.9 (3.6) d	6.6 (2.6) d	6.5 (5.5) d	2.5 (1.7) d	3.5 (1.9) d
sotolon (μg/L)	na	nd[c]	0.2 (0.2)	0.1 (0.0)	0.3 (0.0)	0.6 (0.6)	0.3 (0.3)	0.9 (0.4)	1.1 (0.6)

[a] The same letters in the same row indicate no significant difference between the corresponding values ($p = 0.05$). Standard deviations of 5 replicates are given in parentheses. [b] Not analyzed. [c] Below detection limit.

bottles at high rates (*4*−*6*). However, the direct reaction of sulfur dioxide with oxygen under wine conditions is very slow and essentially irrelevant (*32*). Thus, the sulfur dioxide probably reacted with hydrogen peroxide, aldehydes and ketones (*33*).

Color Measurements. *A_{420nm}.* The wine absorbance at 420 nm (A_{420nm}) is a measure of the level of yellow/brown color of white wine, being considered as a useful indicator of wine development and degree of oxidation. The values of A_{420nm} for the wines during the storage period are given in **Figure 3a**. Forty-eight hours after bottling, the values of A_{420nm} of the wines were 0.058 au, which was very similar to those obtained at bottling. The yellow color of wines (A_{420nm}) increased and became more pronounced over time. At 2 months, the wine sealed under different sealing systems presented similar A_{420nm} values ($p = 0.05$). By 12 months the A_{420nm} values in bottles sealed under Nomacorc classic, agglomerate, colmated and microagglomerate stoppers were slightly higher, but statistically significant, when compared to bottles sealed under screw caps, natural cork and ampule ($p = 0.006$). After 24 months of storage, the trends became more pronounced; the bottles sealed with Nomacorc classic displayed significantly higher A_{420nm} values than bottles sealed with other closures ($p < 0.001$). The wines sealed under screw cap Saran-tin and ampule bottles presented the lowest A_{420nm} values ($p < 0.001$).

CIELab. Wine color was also assessed throughout 24 months of storage using the CIELab coordinates (**Figure 3b**−**f**). It was observed that the wine color became more yellow (higher b^* and C^* values) and more intense (lower L^*) over the 24 months of storage. The L^* (lightness) values of wines were not significantly affected during storage, with the exception of wines sealed with Nomacorc classic, where L^* values decreased significantly from two months onward ($p < 0.001$). At 24 months, the lightest wines were those sealed under ampule and screw cap Saran-tin and the darkest were those sealed with Nomacorc classic ($p < 0.001$) (**Figure 3b**).

The b^* and C^* increased throughout the trial; the highest values were observed for wines sealed with Nomacorc closures at 24 months, the lowest for those sealed under ampule and screw caps, and intermediate for other wines (**Figure 3c**,**f**).

The a^* values changed throughout the trial; the slight increase during the first two months of storage was followed by a decrease in the 22 months thereafter. The type of sealing did not affect significantly the a^* values over the duration of the trial ($p = 0.05$) (**Figure 3d**).

The h_{ab} values decreased slightly during the first two months of storage, followed by an increase in the 22 months thereafter, which mainly occurred between 12 and 24 months. After 24 months, the highest values h_{ab} were observed for wines sealed under ampule and screw cap Saran-tin and the lowest for those sealed with Nomacorc classic ($p < 0.001$) (**Figure 3e**).

The value of the parameter ΔE^*_{ab}, a measure of color differences between samples, was also calculated (data not shown). At 24 months the ΔE^*_{ab} values, between either ampule or screw cap Saran-tin and Nomacorc classic, were greater than 1, indicating that the color of these bottled wines would be perceived as different from each other by the human eye.

These findings indicate that wine color changed throughout storage, being particularly distinctive at 24 months, when the levels of ascorbic acid and sulfur dioxide were almost depleted, such as Nomacorc sealed wines. Conversely, under anaerobic environment (bottle ampule), the wine color changes were residual when compared with other wines. Therefore, color development after bottling depends on the contact of wine with oxygen throughout storage (*12*, *13*, *15*). Thus, the oxygen management at bottling and the choice of wine closure type is likely to have a considerable impact on the wine color after bottling.

Volatile Thiols. 3-Mercaptohexan-1-ol (3MH), 3-mercaptohexylacetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP) are key volatile thiols responsible for the distinctive varietal grapefruit, passion fruit and box tree aroma of Sauvignon Blanc wines. These thiols play a key role in the aromatic quality of Sauvignon Blanc perceived by the consumers (*34*).

The concentrations of volatile thiols 3MH and 4MMP after 24 months in bottle are given in **Table 2**. The concentrations obtained are well above the perception thresholds in wines, 60 ng/L for 3MH and 0.8 ng/L for 4MMP (*19*, *20*). After 24 months, the highest concentrations of 4MMP were found in wines sealed under bottle ampule, but without significant differences from those sealed with screw cap Saran-tin, natural, colmated and agglomerated stoppers ($p = 0.05$). Conversely, the lowest concentrations of 4MMP were observed in wines sealed with microagglomerate, screw cap Saranex and Nomacorc classic closures.

The highest concentrations of 3MH were also found for wines sealed under bottle ampule followed by those sealed with screw cap Saran-tin and agglomerated stoppers, the lowest for those sealed Nomacorc classic and intermediate for the other wines. The 3MHA was not found in any of the samples analyzed.

These results suggest that both thiols degraded over time via oxidative reactions once the lowest concentrations were found in wines that had high oxygen exposure either during bottling or during the storage due to high oxygen permeability of closures (*35*). This situation was particularly evident in wines sealed with Nomacorc, where the levels of sulfur dioxide and ascorbic acid after 24 months were very low. It is therefore possible that, under these conditions, electrophilic oxidation products, as quinones, would preferentially react with thiols (3MH and 4MMP), once the levels of sulfur dioxide were very low (*33*).

Surprisingly, the concentrations of 3MH and particularly 4MMP were relatively low in wines sealed with screw cap Saranex, although the levels of ascorbic acid and sulfur dioxide and color parameters did not indicate that the oxidation level was more pronounced than in wines sealed with screw cap Saran-tin and cork stoppers. This observation suggests that 3MH and

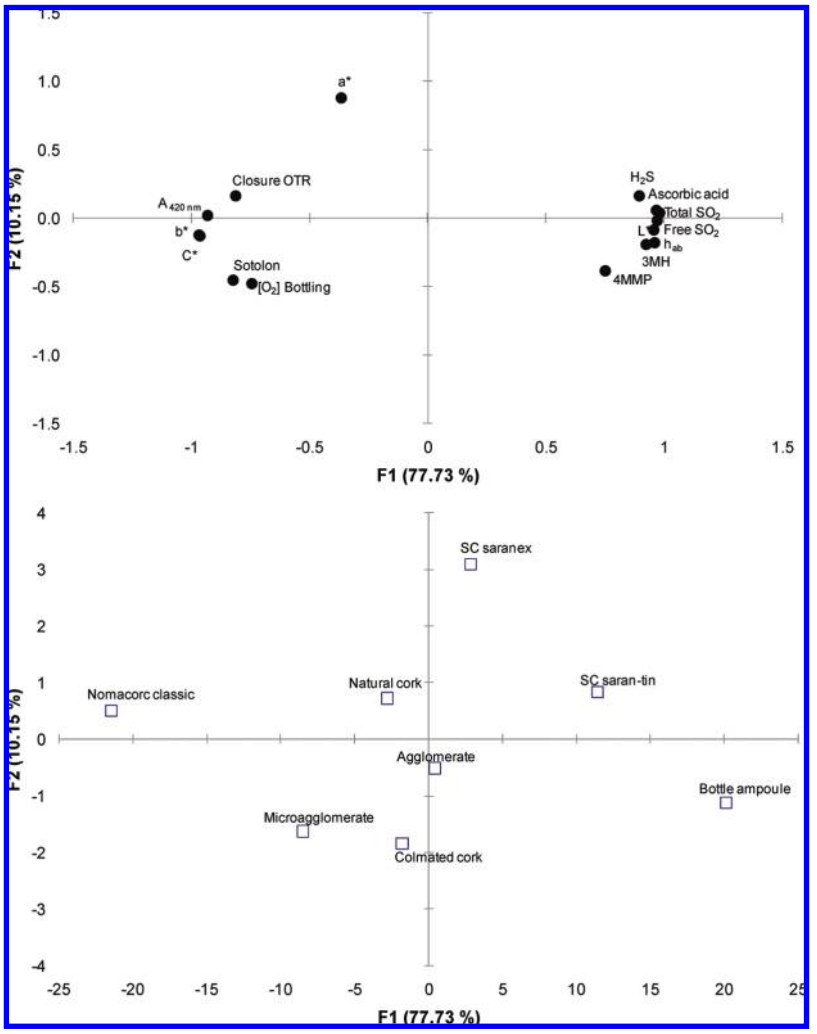

Figure 4. Biplot of principal components 1 and 2 for compositional data for the Sauvignon Blanc bottled wine sealed with different closures after 24 months of storage. Compositional attributes: 3MH = 3-mercaptohexan-1-ol; 4MMP = 4-mercapto-4-methylpentan-2-one; H_2S = hydrogen sulfide; [O_2] bottling = oxygen dissolved at bottling; Closure OTR = oxygen transfer rates.

4MMP could also have been sorbed by the Saranex liner of screw cap. This liner is formed by different polyethylene layers, which are well-known to remove volatile compounds through flavor scalping (*36*). Recent studies have shown that flavor scalping is particularly noted in wines sealed under Tetrapack and "bag-in-box" containers, which have a strong sorption capacity of nonpolar compounds (*37*, *38*). Additionally, closures also display different sorptive capacities, which are more marked with synthetic closures than with natural corks and screw caps (*37*, *38*). Therefore, it seems possible that the loss of these volatile flavor compounds after bottling could also occur by flavor scalping of closures. However, additional studies will be required to fully understand the changes due to sorption capacities of closures and those related with their oxygen barriers properties.

Hydrogen Sulfide. The concentration of hydrogen sulfide (H_2S) was determined at bottling and after 24 months of storage (**Table 2**). Immediately after bottling, the concentration of H_2S was 1.4 μg/L. By 24 months the concentrations of H_2S were highest in screw cap sealed wines, but particularly in those under bottle ampule, while those sealed under cork stoppers and Nomacorc classic closures presented the lowest H_2S content. These findings indicate that the H_2S content increased throughout storage for all wines; however, it was far more pronounced in wines sealed under hermetic conditions and with low oxygen permeation closures, as screw caps. These findings are in agreement with the observations that "struck flint/rubber" reductive characters were far more prevalent in screw caps and ampule sealed wines (*12*−*14*). Moreover, the levels of H_2S in wines sealed under bottle ampule and screw cap Saran-tin are similar to those found in wines presenting reduced "off-flavor" characters (*21*).

The formation of this compound after bottling is not completely understood; however, we can speculate that H_2S could be formed from the reduction of sulfate or sulfite catalyzed by transition metals (iron or copper), phenols or ascorbic acid, when oxygen levels in bottle are near nil (*18*, *39*). Alternatively, the H_2S produced during fermentation could remain in wine reversibly bound to some electrophilic oxidation products (aldehydes, ketones, quinones), being slowly released during storage (*32*). Then, the H_2S could either accumulate in wines under anaerobic conditions or be readily oxidized when in contact with oxygen introduced at bottling or permeating through the closure.

Sotolon. Sotolon (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone) is a volatile compound with an intense odor of curry and *rancio* that could contribute to the oxidation aromas of prematurely aged dry white wines.

The concentrations of sotolon were determined 24 months after bottling, and the results obtained are given in **Table 2**. All these concentrations remain below its wine perception threshold, 2 μg/L. Under anaerobic conditions, bottle ampule, this

compound was not detected. The wines sealed with microagglomerate and Nomacorc closures exhibited slightly higher contents of sotolon than other closures; however, these differences were not statistically significant. Nevertheless, these findings seems to be consistent with recent evidence that the formation of sotolon after bottling is related with the ability of closures to exclude oxygen, being highest in wines sealed with synthetic closures when compared to those sealed under cork stoppers (*26*).

Principal Components and Correlation Analyses. To facilitate the visualization of the differences and similarities between wines in all compositional parameters after 24 months and their relationship with oxygen content at bottling and oxygen transfer rates of closures, principal component analysis was applied to the pooled data (**Figure 4**). The first two principal components account for 87.9% for the variation. The first axis, representing 77.7% of the total variance, H_2S, 3MH, 4MMP, free and total SO_2, ascorbic acid, L^* and h_{ab} were positively correlated between them and negatively correlated with sotolon content, b^* and C^*, A_{420nm}, oxygen content at bottling and closure OTR. The a^* parameter displays the larger contribution to the second axis, which represents 10.1%. The sealing systems were well separated in the plane defined by the first two components, with wines sealed under Nomacorc classic discriminated on the basis of its high values of A_{420nm}, b^* and C^* parameters, and low values of volatile and antioxidant compounds, L^* and h_{ab} parameters. The bottle ampule and screw caps were primarily separated by the high concentration of antioxidant compounds, H_2S, 3MH and 4MMP content; whereas the majority of cork stoppers were primarily discriminated from screw caps on the basis of the lower content of H_2S and to a lesser extent by 3MH, 4MMP, free and total SO_2, ascorbic acid, L^* and h_{ab}. The microagglomerate cork was further discriminated on the basis of its oxygen content at bottling and sotolon level.

Wines were clearly discriminated in function of closures along the first axis, which can thus be interpreted as an "oxidation-reduction" axis, with reductive and oxidized wines being plotted on the extreme right and left in **Figure 4**, respectively. From this experiment, it is clear that wine development after bottling is extremely dependent on oxygen content at bottling and from the oxygen barrier properties of different closures.

Sensory Analyses. The results of the descriptive analysis carried out at 2 months showed that differences between the closures were not statistically significant (data not shown). The ANOVA undertaken for the data obtained at twelve months after bottling showed that there were significant differences among the closure samples for each of the attributes scored (**Figure 5a**). There were no significant bottle replicate differences for any attribute. The Nomacorc closure was distinctly differentiated from the other closures, being scored substantially higher in oxidized and lower in aromatic intensity and freshness than the other samples. The ampule and screw cap Saran-tin samples were rated as highest in reduced and conversely less intense in overall fruit and oxidized attributes ($p < 0.001$ and $p = 0.007$). The microagglomerate and agglomerate sealed wines were rated lower significantly in overall fruit than those sealed with natural and colmated corks, and screw cap Saranex, which were rated as the highest in this attribute ($p < 0.001$).

When sensory analyses were undertaken 24 months after bottling, significant differences were found among the closure samples for each of the attributes scored ($p = 0.05$). The ANOVA carried out from the data obtained showed that there were no significant bottle replicates for any attribute. A similar trend to that found at 12 months was observed, but with more pronounced differences. Again, the wines sealed under ampule and screw cap Saran-tin were rated highest in reduced aroma compared to the other closures. For the oxidized attribute, the Nomacorc classic sealed wines were rated highest, which negatively affected the aroma intensity, freshness and overall fruit attributes. For overall fruit character, the wines sealed under colmated, natural corks and screw cap Saranex were rated highest, those sealed under microagglomerate cork rated as intermediary and the other wines rated lowest (**Figure 5b**).

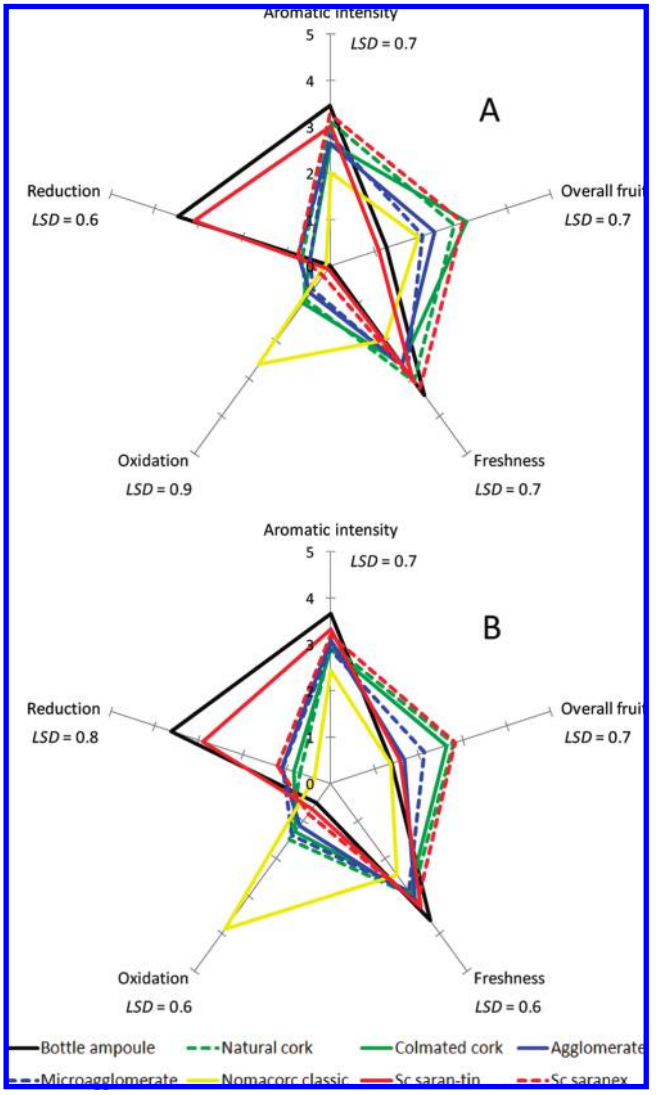

Figure 5. The effect of closure treatment on selected sensory attributes for a Sauvignon Blanc wine after (**a**) 12 months, (**b**) 24 months of storage. Values at 12 and 24 months are the means of 4 replicates. Least significant differences (LSD) at the 5% level are indicated.

Wine defects were detected at 12 and 24 months in wines sealed with agglomerate corks, which were considered "muted". The analysis of anisoles by GC−MS confirmed that 2,4,6-trichoroanisole was indeed present, at concentrations from 1 to 3 ng/L. At these thresholds, the TCA masks the fruitiness of wine, reducing its aromatic quality (*2*). None of the other wines presented TCA or other haloanisoles above 0.5 ng/L.

The sensory results confirm the compositional and color analyses, showing that closures play a major impact on the wine development after bottling, as observed in other studies (*15*−*22*). Wines sealed hermetically as bottle ampule or under very poor oxygen environment as exhibited by those sealed under screw caps displayed a "rotten egg" and "putrefaction" dominating character, which completely masked the fruity flavors. However, the screw cap Saranex was able to minimize these

reduced-like aromas, which means that the levels of H_2S presented by these in wines were not high enough to spoil the wine. Conversely, wines sealed under Nomacorc closures lose their fruity attributes and develop oxidized aromas. Cork stoppers seem to have an intermediate role, minimizing both reductive and oxidative characters; however, the agglomerate corks affected negatively the wine aroma by transmission of taint compounds.

The combination of bottling conditions and oxygen transfer rates through closures had a significant effect on Sauvignon Blanc wine development after bottling. In the first two months of storage, the amount of oxygen dissolved in wine and those introduced in the headspace at bottling played a key role for the important loss of ascorbic acid and sulfur dioxide. However, wine development from two months onward seems to be rather related with oxygen barrier properties of the different sealing systems used. High oxygen transfer rates, as shown by the synthetic closure, caused irreversible damage to the wine and its postbottling development. Due to the continuous entry of oxygen through this closure, ascorbic acid, sulfur dioxide and varietal thiol (3MH and 4MMP) contents were largely depleted, which led to the consequent development of oxidized characters during the 24 months of storage. Conversely, wines sealed hermetically as bottle ampule or with closures with very low oxygen transfer rates as exhibited by screw caps, displayed the greatest concentrations of sulfur dioxide and ascorbic acid, and varietal thiols, but also high levels of H_2S, which completely masked the fruitiness of wine, being responsible for its defective reduced dominating character. However, the screw cap Saranex was able to minimize the sensory perception of "rotten egg" and "putrefaction" reduced like aromas in wines, in spite of the level of H_2S being significantly higher when compared to those sealed with cork stoppers and Nomacorc. Therefore, it can be concluded that, under the conditions of this study, an oxygen sensitive variety such as Sauvignon Blanc wine benefits from some low oxygen exposure after bottling, at the levels provided by cork stoppers. These wines retained high enough amounts of varietal thiols to maintain the typical box-tree and tropical fruit aroma of Sauvignon Blanc but, at the same time, kept the deleterious sulfides at very low levels.

This work outlines the importance of the oxygen management at bottling, but also of oxygen transmission rates of closures as predictable tools of wine shelf life. Further research is still needed to fully understand the mechanism of reactions involving oxygen and ascorbic acid, sulfur dioxide, phenol and aromatic compounds in wines. Additionally, it is important to understand the factors regulating the production of sulfur volatiles, which are responsible for the appearance of reduced character after bottling and their relationship with wine composition before bottling.

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