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Main Routes of Oxygen Ingress through Different Closures into Wine Bottles

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The main routes of oxygen ingress into wine bottles through "technical" cork stoppers (Neutrocork), natural cork stoppers, and synthetic closures (Nomacorc) were investigated. A comparison was made among closures left uncovered (controls), closures with the closure−glass interface covered, and closures completely covered with a polyurethane impermeable varnish. The oxygen ingress into the bottles was measured by a nondestructive colorimetric method. Technical cork stoppers were essentially impermeable to atmospheric oxygen during the first 24 months of storage. Oxygen within natural corks diffused slowly but continuously into the bottles over the first 12 months of storage and in very tiny amounts through the cork−glass interface the 12 months thereafter. Nomacorc synthetic closures were permeable to atmospheric oxygen, mainly after the first month of storage.

KEYWORDS: Cork stoppers; synthetic closures; oxygen permeation

INTRODUCTION

The factors affecting the impact of oxygen on the development of wine during bottle aging are not completely understood. Closure is "perhaps the most obvious variable that might influence wine development in the bottle, but it is only one factor" (*1*). Other contributing factors include oxygenation during wine-making, bottling line conditions, headspace volume and gas composition, storage conditions, and the composition of the wine itself.

During bottle aging, oxygen ingress into wine bottles depends on the sealing effectiveness of the closures, which differ in their oxygen barrier properties. Recently, we have shown that only a bottle sealed with a glass closure by flame (bottle ampule) is completely airtight, while more commercial closures are permeable to oxygen (*2*, *3*). Generally, oxygen ingress through closures into wine bottles is much more important during bottling and in the first month than in the following months of storage. For natural corks, this is followed by a gradual decline in oxygen ingress rates for the remainder of the first year (2−6 μL/day) and a very low rate of oxygenation in the 24 months thereafter (0.1−2 μL/day). Screw caps and "technical" cork stoppers display a consistently low level of oxygen permeation during storage (less than 1 μL/day). In contrast, synthetic closures, Nomacorc and Supremecorq, continue to exhibit high oxygen permeation rates, 6 and 13 μL/day, respectively.

Given their high oxygen permeation rates, the use of synthetic closures resulted in wines with a tendency to lose fruit attributes and develop oxidized, "wet wool", and toasty aromas prematurely (*4*−*8*). In contrast, too little oxygen has been linked with the presence of undesirable struck flint/rubber (reduced) aroma characters, more noticeable in screw-cap-sealed wines (*1*, *7*, *8*). Generally, wine sealed with natural corks displayed intermediate performances (*7*).

The mechanisms and main routes of oxygen ingress through closures have not been studied extensively. Jean Ribéreau-Gayon was the first to report the natural cork stopper permeability: 0.10−0.38 mL of oxygen over the first three weeks and between 0 and 0.07 mL over the four following months (*9*). He suggests that, primarily, oxygen diffuses out of the cork into wine due to the high air pressure in the cork cells. A negligible volume of atmospheric oxygen permeates through the cork−glass interface (0.12 mL of oxygen per year). In addition, Caloghiris et al. (1997) reported that air contained within the corks might account for their oxidative capacities (*10*). It was recently reported that the main route for oxygen entry into wine bottles through natural cork was the cork−glass interface (*11*). In contrast, for synthetic closures most of the oxygen diffuses throughout the closure body. The elucidation of the main routes by which oxygen diffuses through different types of closures into bottles might allow a complete understanding of their role in bottled wine development and lead to the development of improved closures.

This study complements and extends the results of previous research on oxygen barrier properties of closures using a nondestructive (i.e., without opening the bottles) colorimetric method (*2*, *3*). This method screens oxygen ingress through closures into indigo carmine bottled solutions that gradually changes color from yellow to indigo as oxygen reacts with the reduced indigo carmine. Our purpose was to investigate the different routes of oxygen ingress through different cork stoppers and synthetic closures during bottle aging.

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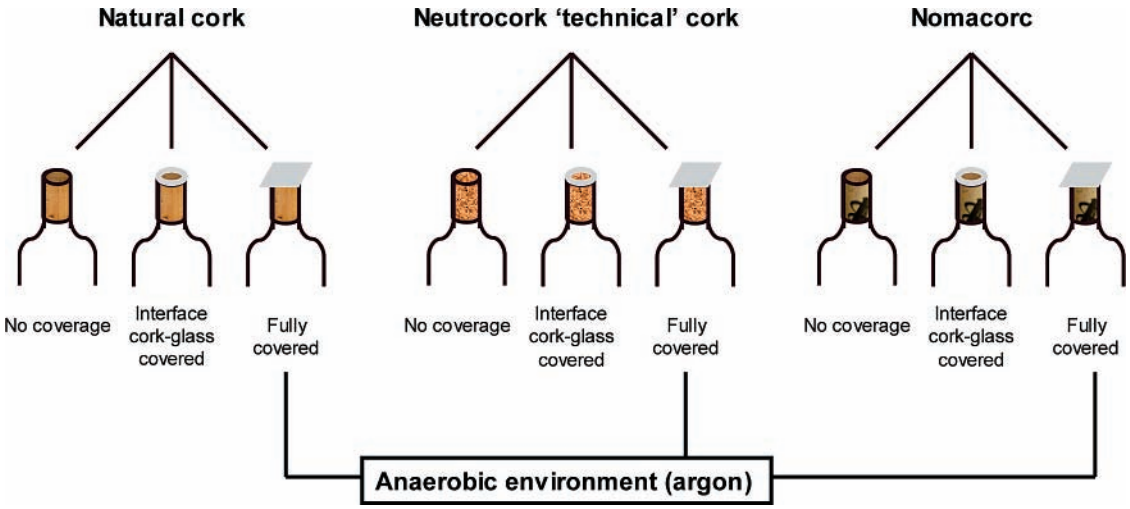

Figure 1. Diagram of the experimental protocol.

MATERIALS AND METHODS

Chemicals. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA) prior to use. Indigo carmine was purchased from Acros (Noisy-le-Grand, France). Sodium dithionite and sodium benzoate were obtained from Prolabo (Fontenay S/Bois, France).

Polyurethane varnish, Duracin Cera, used to cover the closure−glass interface and closure top surface was obtained from CIN, S.A. (Maia, Portugal).

Closures. Three different closures were tested: third-grade natural cork stoppers (44 mm length and 24 mm diameter), Neutrocork technical corks (44 mm length and 24 mm diameter), and Nomacorc synthetic closures (43 mm length and 22 mm diameter). Cork stoppers and Nomacorc closures were supplied by Amorim & Irmãos, S.A. (Santa Maria de Lamas, Portugal) and Nomacorc S.A. (Thimister-Clermont, Belgium), respectively.

The cork stoppers were silicone coated. The Nomacorc closures were covered with a nondescribed coating. The moisture content of the cork stoppers ranged from 4% to 6%. The corks were hydrogen peroxide bleached; however, no peroxide residue was detected.

Calibration Procedure. The calibration procedure is fully described by Lopes et al. (*2*). Sodium dithionite solution (3.9 g/L) was used to reduce 350 mL of indigo carmine solution (250 mg/L) in a calibration bottle. Reduced indigo carmine solution was then oxidized by injecting 20 controlled oxygen volumes. Each oxygen injection led to a gradual color change, which was measured by a CIELAB colorimeter. Thus, a calibration curve with an exponential relationship between the L^* valves and the oxygen injected into the bottle between 0.25 and 2.5 mL was established.

Bottling and Storage Procedures. A 350 mL portion of indigo carmine solution was reduced in 375 mL colorless "bordelaise" bottles (Saint-Gobain Glass Packaging, Cognac, France) as described previously (*2*, *3*). The bottled indigo carmine reduced solutions were then sealed with different cork stoppers and synthetic closures under a continuous flush of nitrogen (0.5 bar). Further details of the head-corking machine and closure compression diameter before insertion have been described by Lopes et al. (*2*). Prerun checks of the fill height, closure insertion depth, and headspace pressure were done to optimize the bottling procedure. The final filling level for each bottle was 65 ± 2 mm from the top of the bottle. The internal pressure in the headspace immediately after cork insertion ranged from 0 to 10 kPa. The temperature of the indigo carmine solution ranged from 19.3 to 21.2 °C.

This method was used to investigate the contribution of the different routes of oxygen ingress through closures into bottled indigo carmine solutions. For each closure type three different "treatments" were set up. Indigo carmine bottled solutions were sealed normally (i.e., uncovered). For another set of closures the closure−glass interface was covered with an impermeable polyurethane varnish (data not shown). A thin layer of varnish was applied to the internal surface of the bottleneck before closure insertion; the external closure−glass interface was then also covered with varnish. In this way, oxygen ingress between the closure and the bottleneck was prevented and only the permeation throughout the closure's body was measured. Finally, another set of closures were completely covered with polyurethane varnish and glass (20 × 20 mm). Therefore, only oxygen within the closures able to ingress into the bottles was measured. Four replicates for the three closure types and three treatments were performed. During each measurement period, the adhesion of the varnish to the closure−glass interface and to fully covered closures was visually controlled. No signs of lack of adhesion were observed during the experiment. A diagram of the experiment protocol is shown in **Figure 1**.

All 36 bottles were left upright for 24 h and then stored horizontally for 24 months under a constant temperature of 20 ± 1 °C and a relative humidity of 65 ± 1%. The bottles fully covered were stored in a chamber of 27 L capacity under constant argon Alphagaz 2 (Air Liquide, Floirac, France) flow.

Bottle Colorimetric Measurements. The CIELAB measurements (L^*, a^*, b^*) were obtained by direct scanning of the bottled solutions with a Minolta series CM-508i spectrocolorimeter equipped with a transmittance accessory, CM-A76 (Osaka, Japan). The colorimeter parameters were illuminant D65 and 10° observer.

A blank was performed before each set of analysis using a clean Pyrex bottle filled with water. The bottles were cleaned with ethanol and dried before CIELAB measurements. These measurements were performed in an upright position 5 cm from the base of the bottle. Four body measurements were collected by rotating each bottle 90° on its vertical axis.

Data Analysis. Analytical data were obtained with Microsoft Excel 2000. Analysis of variance (ANOVA) and Tuckey tests were carried out with STATISTICA 6 (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

The analytical data obtained for oxygen ingress into wine bottles during 24 months of horizontal storage showed significant differences among the natural corks, technical corks, and synthetic closures tested ($p = 0.05$) (**Figure 2**). These data agree with those previously reported that show similar oxygen ingress patterns for identical closures considering similar storage periods (*3*).

After 24 months of horizontal storage, oxygen ingress amounts through uncovered, interface-covered, and fully covered Neutrocork technical corks were 0.7, 0.7, and 0.6 mL of oxygen, respectively, without significant differences between the three treatments ($p = 0.05$) (**Figure 2A**). The rates of oxygen ingress through Neutrocork corks with different levels of coverage

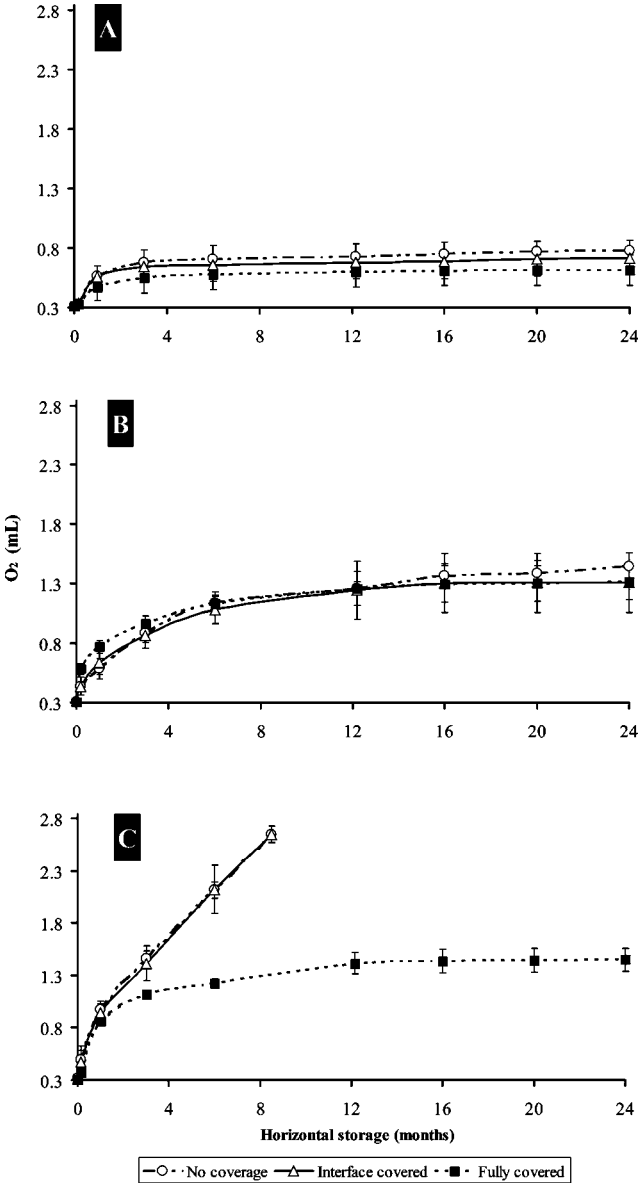

Figure 2. Kinetics of oxygen ingress into commercial bottles through Neutrocork stoppers (**A**), natural cork stoppers (**B**), and Nomacorc synthetic closures (**C**): indigo carmine bottled solutions sealed normally (i.e., uncovered) (circles), with the closure–glass interface covered with polyurethane varnish (triangles), and with the closure completely covered with polyurethane varnish and glass (squares). All bottles were stored horizontally for 24 months. Error bars represent the standard deviation of four replicates.

varied from 14 to 17 μL/day during the first month of storage (**Table 1**). From the 2nd to 24th month, oxygen ingress rates through Neutrocork with different levels of coverage were 0.3, 0.2, and 0.2 μL/day, respectively. These results confirm the reproducibility of our method, since the oxygen ingress rates obtained for uncovered Neutrocork stoppers, 0.3 ± 0.1 μL of O_2/day from the 2nd to 24th month, are similar to those reported in our previous paper, 0.4 ± 0.1 μL of O_2/day, considering a similar period of measurement (*3*). Uncovered Neutrocork corks were slightly more permeable than the covered ones; however, these differences were not statistically significant. The results obtained in this study seem to show that oxygen diffuses from the Neutrocork internal structure due to the compression in the bottleneck, mainly during the first month. After this period, very tiny amounts of oxygen diffuse into the wine bottles. It seems

Table 1. Oxygen Ingress Rates (μL/day) through "Technical" Cork (Neutrocork), Natural Cork Stoppers, and Synthetic Closures (Nomacorc) with Different Levels of Coverage during 24 Months of Horizontal Storage[a]

closure	storage period	no coverage	interface-covered	completely covered	ANOVA
Neutrocork	first month	17(3)[b]	17(3)	14(4)	ns
	2nd to 24th month	0.3(0.1)	0.2(0.1)	0.2(0.1)	ns
natural cork 3rd grade	first month	20(3)	20(3)	24(2)	ns
	2nd to 12th month	2.0(0.2)	1.8(0.5)	1.6(0.5)	ns
	12th to 24th month	0.5(0.2) a	0.2(0.1) b	0.2(0.1) b	*
Nomacorc	first month	30(3)	30(3)	27(1)	ns
	2nd to 24th month	6.5(1.0) a	6.9(0.3) a	1.3(0.3) b	***

[a] The on-line letters "a" and "b" indicate statistically different groups (Tuckey test). [b] Standard deviations are given in parentheses.

that this tiny oxygenation could take place by the cork−glass interface. However, the main route of oxygen ingress into the wine bottles over this period cannot be fully determined since our method could not precisely measure oxygen amounts lower than 0.1 μL/day.

Uncovered, interface-covered, and fully covered natural corks displayed oxygen ingress of 1.4, 1.3, and 1.3 mL over 24 months of storage, respectively. There were no significant differences ($p = 0.05$) among the three curves (**Figure 2B**). The rates of oxygen ingress through natural corks, independent of the level of coverage, were statistically identical for the first month of storage, ranging from 20 to 24 μL/day. For the remainder of the first year, oxygen ingress rates through uncovered, interface-covered, and fully covered natural corks were 2.0, 1.8, and 1.6 μL/day, respectively. No statistically significant differences ($p = 0.05$) among the three treatments were detected. After 12 months, oxygen diffuses through uncovered natural corks at 0.5 μL/day, which is slightly but statistically higher than through partially and fully covered natural corks (0.2 μL/day) (**Table 1**). The oxygen ingress rates for ref *3* uncovered natural corks ranged from 1.8 to 2.3 μL of O_2/day from the 2nd to 12th month and from 0.4 to 0.8 μL of O_2/day from the 12th to 24th month. These values fit perfectly within the range of oxygen ingress rates for natural corks reported in our previous paper, 1.7−6.1 μL of O_2/day from the 2nd to 12th months and 0.1−2.3 μL of O_2/day from the 12th to 36th month (*3*).

When natural cork stoppers are compressed in the bottleneck immediately after bottling, the air pressure in the cells ranges from 0.6 to 0.9 MPa (*12*, *13*). Therefore, air at atmospheric pressure (0.101 MPa) is unable to enter into the bottles through the cork or between the cork−bottleneck interface. However, the extremely high internal pressure could force air out of the cork, which occurs preferentially during the first 12 months, being facilitated by the plasmodema, small openings of 60 nm diameter in the walls separating each individual cork cell (*13*). Theoretically, natural corks (44 mm length and 24 mm diameter) contain 3.4−3.6 mL of oxygen within their structure, assuming a volume of 20 mL, 80−85% of which is air (*13*, *14*). We have shown that 1.3 mL of oxygen diffuses from the natural corks into the bottles, which represents 36−38% of the theoretical total oxygen within their cell structure. It is possible that, after the first 12 months of storage, as air contained in the cork cells diffuses out, the cork stopper's internal pressure is diminished, allowing ingress of tiny amounts of atmospheric oxygen through the cork−glass interface into the bottles (*12*).

With regard to the Nomacorc synthetic closures, the results showed that uncovered and interface-covered closures exhibited high oxygen permeation, reaching 2.5 mL of oxygen (limit of quantification for the method) within approximately 8 months. In contrast, closures fully covered with impermeable polyurethane varnish allowed ingress of 1.4 mL of oxygen during 24 months, which was consistently lower than those of the other two treatments (**Figure 2C**). The rates of oxygen ingress through Nomacorc closures, independent of the level of coverage, were statistically identical for the first month of storage, ranging from 27 to 30 μL/day. After the first month of storage, oxygen ingress rates through uncovered, interface-covered, and fully covered Nomacorc were 6.5, 6.9, and 1.3 μL/day until the completion of this study. As was observed with Neutrocork and natural cork stoppers, the oxygen ingress rates obtained for uncovered Nomacorc closures, 6.5 $\pm$ 1 μL of O_2/day, agree with those reported in our previous paper, 6.3 $\pm$ 0.1 μL of O_2/day (*3*). Uncovered and interface-covered Nomacorc closures were clearly much more permeable than those fully covered. These data clearly indicate that atmospheric oxygen can ingress throughout Nomacorc synthetic closures, mainly after the first month in the bottle (*11*). It is likely that, at constant temperature, when Nomacorc synthetic closures are compressed in the bottleneck, their internal pressure is higher than the atmospheric pressure, preventing the atmospheric oxygen from entering the bottle. Probably, air contained within the foam core of Nomacorc closures diffuses out during the first month due to their open "cell" structure, leading to a gradual loss of internal pressure. When the internal pressure is lower than the atmospheric pressure, oxygen permeates throughout the body of the Nomacorc closure, which explains the different kinetics shown in **Figure 2C**.

In summary, this study has demonstrated that in natural and technical cork stoppers, most of the oxygen diffuses out of the cork into the bottles during the first 12 and 24 months of storage, respectively, although some permeation through the cork−glass interface is possible, mainly after 12 months of storage in natural cork stoppers. Conversely, Nomacorc synthetic closures were clearly permeable to atmospheric oxygen under the conditions of this study.

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