JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 6967−6973 **6967**

Nondestructive Colorimetric Method To Determine the Oxygen Diffusion Rate through Closures Used in Winemaking

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Oxygen is one of the most important factors determining the aging potential of bottled wine, and oxygen diffusion into bottled wine is extremely dependent on the sealing effectiveness of the closure. A nondestructive colorimetric method was developed to measure oxygen diffusion from 1 to 9.8 mg/L during the postbottling period. This method was used to study oxygen diffusion through different closures available on the market. After 365 days of horizontal storage, only the control bottle was impermeable to atmospheric oxygen; all other closures exhibited variable rates of oxygen diffusion, which were much greater in the first month than in the following months. It was shown that the rate of diffusion was clearly influenced by the type of closure material used.

KEYWORDS: Indigo carmine; sodium dithionite; oxygen diffusion; closures; bottles

INTRODUCTION

The contact between wine and oxygen is critical for winemaking and bottle-aging processes. Some degree of oxygenation may be beneficial for red wine development as the oxidative phenolic reactions will lead to color enhancement and the reduction of astringency (*1*−*3*). On the other hand, the quality of white wines is generally impaired by air exposure due to a change in sensorial and chromatic qualities (*4*). Excessive oxygen exposure can induce some typical aldehydic and maderized off-flavors and also lead to the development of a brownish color (*5*, *6*).

During bottle aging, the closure displays a vital role for the evolution of the wine. In 1933, Ribéreau-Gayon found little oxygen diffused into wine after bottling (*7*). However, it is now generally recognized that natural cork seals are permeable to oxygen, and it has been estimated that microquantities of oxygen diffuse into bottled wine during aging (*8*). This oxygen permeability varies widely from cork to cork, and this heterogeneity is one of the main factors that may contribute to bottle variation among wines (*8*, *9*). It has recently been shown that sealing systems can influence the evolution of white and red wines (*10*−*13*). It has also been shown that oxygen diffusion through corks may contribute to the sporadic oxidation of white wine during commercial storage (*14*, *15*). There is however, little published information about oxygen diffusion during bottle aging or the influence of the different sealing systems in this regard.

Various methods have been developed to measure oxygen or the oxidation level of beverages after bottling. These include measurement of the oxidation−reduction potential (*8*), measurement of dissolved oxygen by polarographic probe, measurement of total oxygen in bottled beer using an oximeter, based on Henry's law (*16*), and measurement of gas composition in the headspace of a bottle by gas chromatography (*9*, *17*) or even for white wines by simple absorbance measurement at 420 nm (*10*). However, the problem with most of these techniques is that they are destructive toward the closures (i.e., a single bottle cannot be analyzed without compromising the closure seal). Recently, some nondestructive methods have been reported, for example, the determination of white wine oxidation by measuring the absorbance in clear or colored bottles (*18*) and also the measurement of acetic acid in wine bottles by high-resolution ^{1}H NMR spectroscopy (*19*). However, these techniques do not determine the precise oxygen exposure. In 1933, Ribéreau-Gayon developed a colorimetric method to estimate the amounts of oxygen that diffused into the bottles during aging. This was based on the color change from the oxidation−reduction reaction of indigo carmine (*7*). In our laboratory, preliminary trials showed that this method could be improved by the use of colorimetry (*20*).

The colorimeter systems, using uniform color space (CIELAB), are of great value for the characterization and determination of food chromatic properties. For example, tristimulus colorimetry has been widely used, to determine color changes during food processing (*21*, *22*) or wine development (*23*−*26*).

The aim of our work was to develop a nondestructive colorimetric method to determine the diffusion of oxygen into bottles after bottling. This development was primarily done to investigate the importance of the several sealing systems available on the market.

MATERIALS AND METHODS

Reagents. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA) prior to use. Indigo carmine was purchased from Acros (ref 157440250, Noisy-le-Grand, France). Sodium dithionite

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10.1021/jf0404849 CCC: $30.25
Published on Web 08/06/2005

Table 1. Mean Characteristics, Moisture, and Coefficient of Porosity of the Closures Prior to Bottling[a]

closure		code	length (mm)	diameter (mm)	mean (g)	density (cm^3)	moisture content (%)	coefficient of porosity (%)	oxygen peroxide (mg/L)
natural cork	first grade	N24	44.9 (0.29)	24.0 (0.11)	3.76 (0.18)	0.18 (0.02)	4.8 (0.60)	8.0 (1.51)	nd
	first grade	N22	44.2 (0.32)	21.8 (0.05)	3.10 (0.24)	0.19 (0.01)	2.3 (0.26)	7.7 (3.8)	nd
	first grade	N26	45.2 (0.32)	26.1 (0.18)	3.70 (0.47)	0.15 (0.01)	5.7 (0.36)	5.2 (2.2)	nd
	third-grade colmated	N3c	44.6 (0.17)	24 (0.03)	3.99 (0.53)	0.10 (0.02)	6.6 (0.27)		nd
technical cork	Agglomerate	A	45.1 (0.24)	23.8 (0.09)	6.10 (0.24)	0.30 (0.01)	4.6 (0.07)		nd
	Twin Top	TT	44.3 (0.08)	23.4 (0.06)	5.47 (0.09)	0.29 (0.00)	3.6 (0.16)		nd
	Neutrocork	Nt	43.5 (0.08)	23.7 (0.08)	5.50 (0.39)	0.29 (0.02)	3.8 (0.17)		nd
synthetic	Supremecorq	S	44.7 (0.29)	21.4 (0.07)	8.71 (0.20)	0.54 (0.0)			nd
	Nomacorc	No	42.8 (0.07)	22.0 (0.01)	6.14 (0.06)	0.38 (0.00)			nd

[a] Parentheses enclose standard deviations. nd, not detected.

and sodium benzoate were obtained from Prolabo (ref 27 888.293, Fontenay S/Bois, France).

Syringes and Needles. One milliliter tuberculin syringes were purchased from CODAN Medical ApS (Rodby, Denmark), and the 0.6 mm needles (length of 25 mm) were purchased from TERUMO Europe N.V. (Leuven, Belgium). They were both used in the calibration procedure to inject oxygen into the calibration bottle and to outlet the gas bottle content.

Sixty milliliter sterile syringes were purchased from TERUMO Europe N.V. as were needles with a diameter of 0.8 mm and a length of 50 mm. They were used for sodium dithionite solution injection in calibration and commercial bottles.

Closures. In this trial, nine different closures were studied. All natural and "technical" cork closures were supplied by Amorim & Irmãos (Santa Maria de Lamas, Portugal). The synthetic closures were supplied by Supremecorq, Inc. (Kent, WA) and Nomacorc S.A. (Thimister-Clermont, Belgium). The selected closures were a first-grade natural cork stopper with three different diameters; a natural colmated cork stopper; three "technical cork" closures (cork-based closures with synthetic composite), namely, Twin Top, Agglomerate, and Neutrocork; a Nomacorc closure produced by an extrusion process; and, finally, a Supremecorq closure produced by a molding process. The natural and technical corks were covered with a silicone coating. The Supremecorq closures were coated with food grade silicone. The Nomacorc closures were covered with an unknown FDA-approved coating. All closures were analyzed prior to bottling for physical characteristics, moisture, and oxygen peroxide content using a previously procedure (*27*) (**Table 1**).

Surface Image Analysis of Natural Cork Closures. The surface image of the cork body (cylindrical lateral surface) was obtained with a CCD-IRIS/RGB camera that was mounted to a Kaiser RS1 board that captured the image with controlled illumination. The images that were obtained were processed using Analysis software.

For the image acquisition, eight rectangular frames of the body of each cork stopper were captured. The first image was taken perpendicular to the cork's growth rings, whereas the other seven were taken successively by rotating the cork stopper 45° to the right. The surface images of the two flat ends of the cork stoppers were acquired using one circular frame for each top.

The observed images corresponded to 89 and 86% of the total surface of the lateral body and of the top, respectively.

The thresholds for each cork stopper were adjusted and ranged between 143 and 168, between 131 and 161, and between 108 and 143 for RGB (red, green, blue), respectively.

For each cork stopper (body and top) two variables were calculated: total pore area (ap_total, mm^2) and porosity coefficient [(cP, %) as the quotient between total pore area and total area].

Bottles. All bottles (375 mL) ["extra-white" (colorless) and "bordelaise" classic bottles (manufacturer's code 1.31.152.01)] used for cylindrical closures in this trial were supplied by Saint-Gobain Glass Packaging (Cognac, France). Different diameter measurements from the inside of the bottleneck were taken at various depths (3, 10, 20, 30, and 45 mm) by using an automatic caliper. The measurements complied with the CETIE 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.

The white glass bottles (375 mL) used for the control were supplied by Gantenbrink Corp. (Limburg, Germany).

The bottle used for calibration and the Pyrex bottles were purchased from Atelier Jean Prémon (Bordeaux, France). The calibration bottle had the following specification: a 375 mL clear Pyrex bottle with no bore neck containing two openings in the body of the bottle, which were closed by closures with silicone rings of 5 mm in thickness (**Figure 1**).

Preparation of Indigo Carmine Solution. The oxidation solution was prepared by dissolving 250 mg of indigo carmine (oxidized compound) and 5 g of sodium benzoate (antimicrobial properties) in 1 L of purified water. The obtained solution displayed an indigo blue color.

Procedure of Reduction of Indigo Carmine Solution in the Calibration Bottle. The calibration bottle was filled with 350 mL of indigo carmine solution, and both openings were sealed. The bottle was then flushed for 15 min with nitrogen gas (Air Liquide) at a pressure of 0.2 bar to remove all of the oxygen present (**Figure 1A**). For the purpose of flushing, a needle of 0.6 × 25 mm, which was fitted to the nitrogen system, was inserted into the bottle through the lower silicone closure. Another needle inserted into the upper silicone closure acted as a gas outlet. After 15 min of nitrogen flushing, an aqueous sodium dithionite solution (2.25 g/L) was injected into the bottle. The sodium dithionite solution had to be prepared just before use to avoid the loss of reducing properties once in aqueous solution. A 60 mL syringe was filled with 20 mL of sodium dithionite solution, and the needle (0.6 × 25 mm) was inserted into the lower silicone bottle ring. The solution's color changed from indigo blue to yellow. After injection, the three needles (sodium dithionite, nitrogen, and oxygen outlet) were removed from the silicone rings in the openings. After the indigo carmine reduction, the bottle was subjected to the tristimulus measurements (L^*, a^*, b^*, CIELAB76). This was performed by taking four measurements, doing 90° rotations with the bottle in the upright position. The measurements were made in the dark at room temperature (22 ± 2 °C). The average of the four collected values was used.

Calibration of Indigo Carmine Solution. A syringe was filled with 0.6 mL of air (0.13 mL of oxygen/0.18 mg of oxygen) at 20 °C and fitted with a needle (0.6 × 25 mm) prior to injection of the air into the upper silicone bottle opening (**Figure 1B**). After the removal of the syringe and needle, the bottle was shaken vigorously by hand for 1 min to homogenize the indigo carmine solution prior to the tristimulus measurements (CIELAB76). The same procedures were followed as described previously. The colorimetric measurements were taken until the values were stable (5 min). During this period the bottle was stored in a nitrogen atmosphere. This reoxidation procedure was repeated until the indigo carmine solution returned to its original indigo blue color. The calibration was optimized after five measurements.

The hypothetical chemical pathway of the reduction of indigo carmine by the sodium dithionite and reoxidation of the reduced indigo carmine by atmospheric oxygen is described in **Figure 2**.

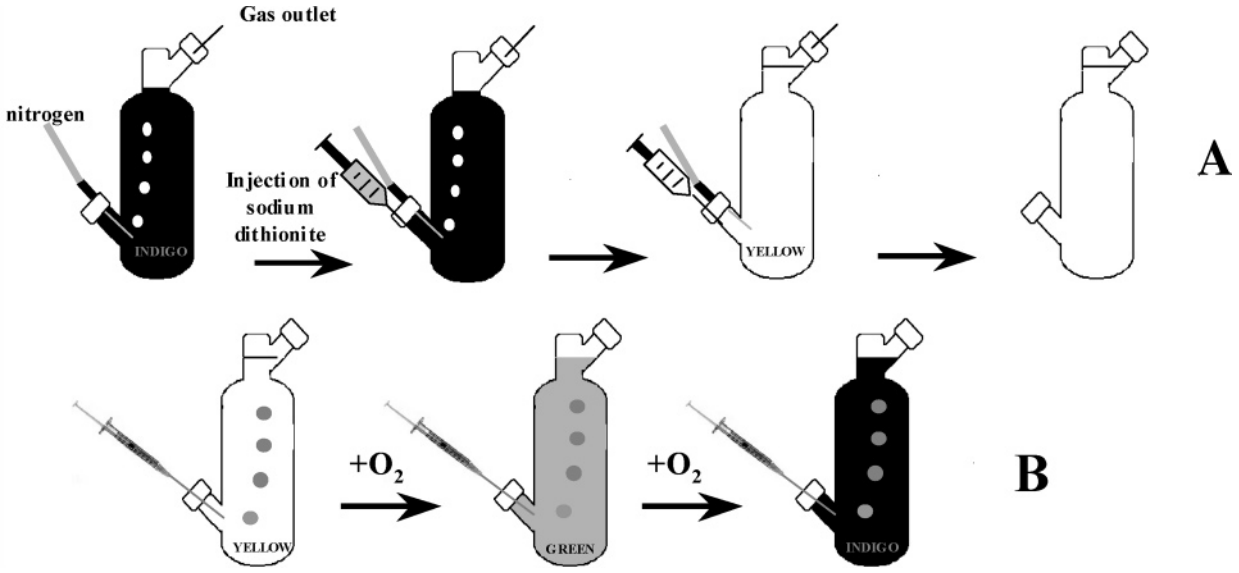

Figure 1. Diagram of the calibration procedure: reduction of indigo carmine in the bottle (**A**) and bottle-controlled oxidation of reduced indigo carmine by injection of microquantities atmospheric oxygen (**B**).

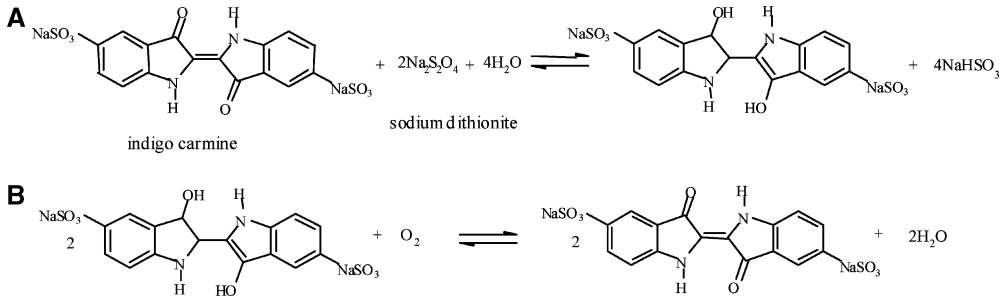

Figure 2. Hypothetical reduction of indigo carmine by sodium dithionite (**A**) and reoxidation of reduced indigo carmine by atmospheric oxygen (**B**).

Comparing Tristimulus Measurements for Commercial and Pyrex Bottles. To assess the effect of glass bottles in the *L**, *a**, *b** values, 15 commercial and Pyrex bottles were filled with either purified water or a solution of indigo carmine (reduced or oxidized). In total, there were 45 bottles of each type sealed with a Neutrocork closure (airtight for atmospheric oxygen in previous tests). All bottles were submitted to tristimulus measurements (*L**, *a**, *b**, CIELAB76), following the same procedure as described previously.

Measurement of Dissolved Oxygen by Polarographic Probe. Fifteen commercial bottles were used, which displayed different *L**, *a**, *b** values that corresponded to the different oxygen amounts (according to our method). These bottles were subjected to dissolved oxygen measurements using a polarographic probe to evaluate the residual amounts of dissolved oxygen in the indigo carmine solution. The measurements were performed before and after reduction.

The bottles were shaken vigorously (agitator 74404, Bioblock Scientific, Illkirch, France) at 100 rpm for 5 min to homogenize the gas (headspace) and liquid phase prior to the oxygen measurements.

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

Bottling Procedure and Storage. For the application of the indigo carmine method to the commercial bottles, some changes had to be made. The sterilized commercial bottles were filled with 350 mL of indigo carmine solution and were closed with a silicone closure containing one hole into which polyurethane tubes were inserted (**Figure 3**). The first tube (minimum length at 250 mm) was connected to the nitrogen system on the one side, and on the other side it was fitted with a glass frit to reach the bottom of the bottle. The other tube (40 mm) allowed for oxygen elimination. All bottles were flushed for 15 min with nitrogen gas (Air Liquid) at a pressure of 0.2 bar to remove all oxygen present prior to sodium dithionite solution injection. In this case a 60 mL syringe fitted with a longer needle (0.8 × 50 mm) was used to pass through the silicone closure. Once the bottled solution was reduced, the bottles were placed in the head corking machine under a continuous flush of nitrogen (0.5 bar) just before the closure was inserted to avoid oxygen recontamination of bottle. The bottling procedure was done on a single-headed corker (La Girondine, Bordeaux, France) that was equipped with a gas flusher. All closures were compressed to a diameter of 15.8 mm before insertion, which is in accordance with manufacturer specification. The final filling level for each bottle was 65 ± 2 mm from the top of the bottle. The pressure in the headspace after cork insertion varied from 0 to 10 kPa. The temperature of the indigo carmine solution ranged from 18.1 to 22.4 °C during the bottling procedure.

The control bottles were prepared using the same procedure describe earlier except that they were sealed with a glass stopper by flame welding at 1200 °C. The sealing machine releases a flame that is digitally controlled by air, oxygen, and clean propane for 5 s while the bottle is rotated at 300 rpm (Gantenbrink Corp., Limburg, Germany).

In total there were nine closure types and the control bottle with four replicates of each type (40 bottles). All bottles were left upright for 24 h after bottling and then stored horizontally (20 ± 1 °C, 65 ± 1% relative humidity).

Bottle Colorimetric Measurements. The *L**, *a**, *b** measurements (CIELAB76) were obtained by directly scanning the bottled solutions with a Minolta series CM-508i spectrocolorimeter equipped with a transmittance accessory CM-A76 (Osaka, Japan). *L**, *a**, *b** values were collected using illuminant D65 and a 10° observer according to the CIELAB76 (*28*). Hue angle (*H**) was calculated from arctan-(*b**/*a**).

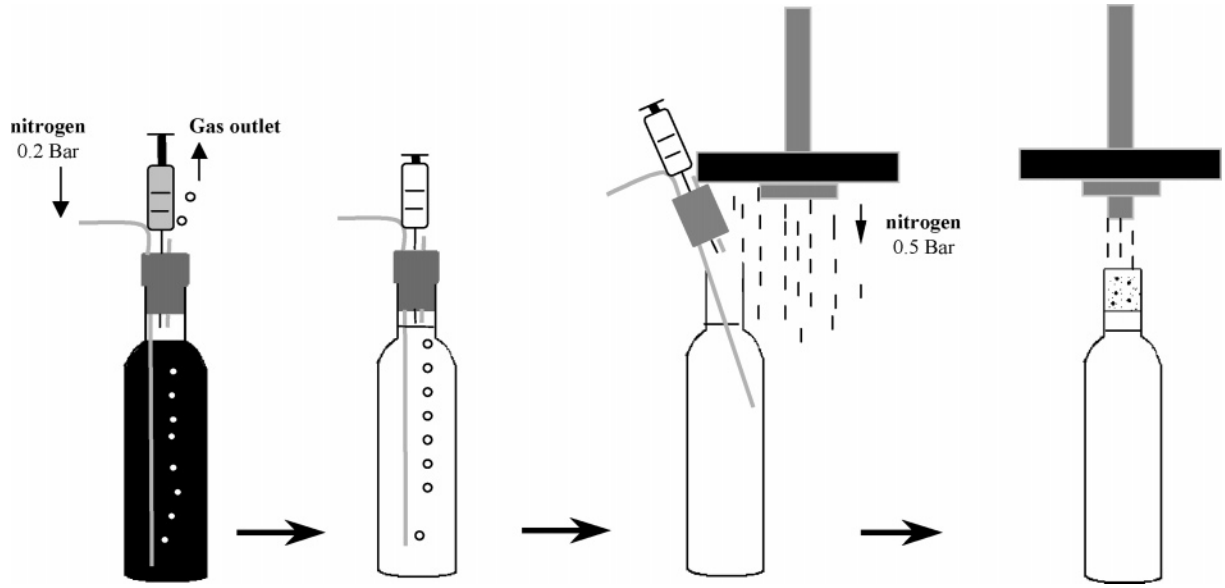

Figure 3. Diagram of the reduction of indigo carmine in commercial bottles and the sealing procedure of the closures.

A clean Pyrex bottle filled with water was used to do autozero calibration (blank). All bottles were cleaned with ethanol and dried before spectrocolorimeter measurements. The chromatic measurements were carried out in the upright position at 5 cm from the base of the bottle. Four body measurements were collected by rotating each bottle 90° on its vertical axis. All positions were marked on the bottleneck to allow for consistent measurement over time. All measurements were made in the dark at room temperature (22 ± 2 °C).

Data Analysis. All chromatic data were analyzed by using Microsoft Excel 2000 software. Analysis of variance (ANOVA), correlation, and regression analyses were carried out with Statistica 6 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The purpose of this investigation was to develop a noninvasive colorimetric method that could measure the amount of oxygen diffusion into bottles during the postbottling period, with the goal of evaluating different closures.

Measurement of Dissolved Oxygen Using a Polarographic Probe. During the oxidation of the indigo carmine solution, the oxygen present in the medium should be consumed by the oxidation reaction. To test this assumption, 15 commercial bottles were prepared containing different oxygen levels (1−9.8 mg/L). These were all quantified using the calibration curve. These bottles were subjected to dissolved oxygen measurements with a polarographic probe. The analytical data obtained revealed that the amounts of dissolved oxygen in the bottles were <9 μg/L (data not shown). These data indicated that the oxygen that diffused into the bottle was essentially consumed by the indigo carmine solution. We will, however, express the data found by our method as an equivalent amount of dissolved oxygen, representing the oxygen that was consumed by the 370 mL of indigo carmine solution. It should be noted that the values of oxygen diffusion are proportional to the volume of solution (e.g., the values should be divided by ∼2 in the case of a 750 mL bottle).

Calibration Procedures and Application to Commercial Bottles. According to our calibration procedure, an aqueous solution of indigo carmine that was previously reduced (**Figure 1A**) was reoxidized by injecting controlled oxygen volumes, which led to a color change from yellow to indigo blue (**Figure 1B**). With each oxygen injection, the bottle was submitted to the L^*, a^*, b^* colorimetric measurements (CIELAB76), which were converted into hue angle (H^*). Thus, it was possible to establish a relationship between the oxygen content of the bottle and the L^* and H^* parameters (**Figure 4**). An exponential relationship was obtained between the oxygen content of the bottle versus the L^* values ($R^2 = 0.9991$), and a logarithmic relationship was obtained for the equivalent oxygen concentrations versus the H^* ($R^2 = 0.9897$), between 1 and 9.8 mg/L (again, values based upon a 370 mL bottle configuration). The measurement is not possible below 1 mg/L due to the small variation of L^* and H^* when the injected amounts of oxygen were lower than 1 mg/L. The small variability may be due to residual amounts of sodium dithionite, which scavenged the injected oxygen in the place of the reduced indigo carmine. Therefore, our calibration curve is valid only in the range of 1−9.8 mg/L. The maximum equivalent concentration of oxygen (6 mL/L or 8.4 mg/L at room temperature and atmospheric pressure) in a 12% ethanol solution falls within this range of oxygen (*29*).

Effect of Bottle Type on Colorimetric Measurement. One of our goals was to have a suitable method that could be applied to commercial wine glass bottles. To assess the effect of glass on our measurements, Pyrex and clear glass commercial bottles were filled with different solutions (water, oxidized and reduced indigo carmine) before colorimetric measurements were made. Pyrex and commercial bottles induced significantly different H values for two of the three solutions. In this instance, the H^* parameter seemed to be insufficient and was therefore not used for our calibration (**Table 2**).

With regard to the L^* measurements, the Pyrex and commercial bottles showed very similar values for each solution. There was, in particular, no statistically significant effect on the L^* values measured between the Pyrex and commercial glass bottles (**Table 2**). Thus, the obtained L^* calibration curve could be used to estimate the oxygen content in colorless commercial glass bottles. There was no statistically significant effect on the L^* values measured among the 15 commercial glass bottles. The error level caused by the use of different commercial bottles was evaluated in different solutions (water, oxidized and reduced indigo carmine). The error level was <20 μg/L.

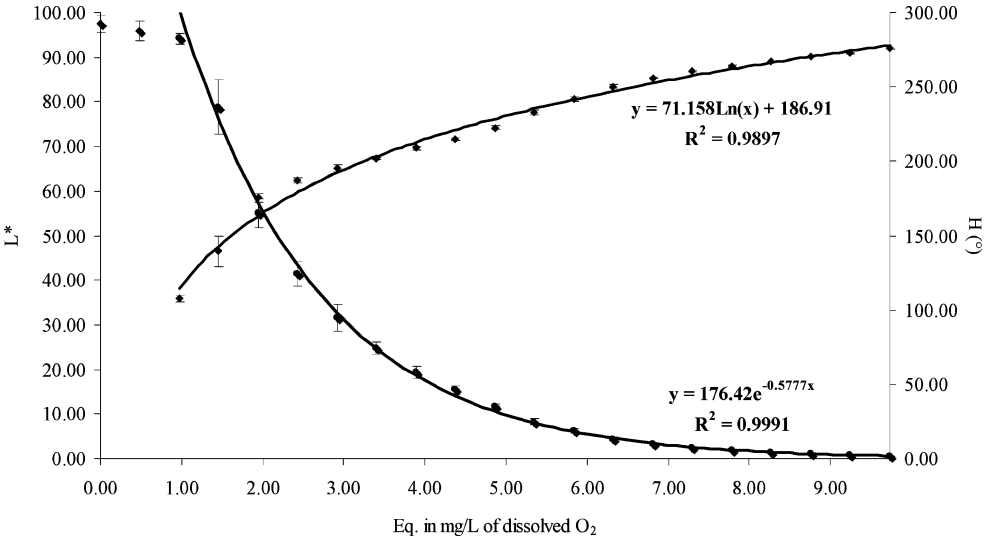

Figure 4. Relationship between *L** (black symbols) and *H* (gray symbols) colorimetric parameters and the microquantities of atmospheric oxygen injected ($n = 5$).

Table 2. Mean *L** and *H** Values of Commercial and Pyrex Bottles for Three Different Color Solutions (Water, Indigo Carmine, and Indigo Carmine Reduced)[a]

		*L**			*H**		
		bottle type		differences between	bottle type		differences between
solution	color	Pyrex	commercial	bottle types	Pyrex	commercial	bottle types
water	white	100.4 (0.34)	100.1 (0.43)	ns	73.9 (1.8)	75.6 (4.2)	ns
indigo carmine reduced	yellow	96.4 (0.85)	95.8 (0.64)	ns	106.3 (1.1)	104.4 (1.1)	***
indigo carmine	indigo	0.18 (0.00)	0.18 (0.00)	ns	294.1 (3.6)	286.2 (5.0)	***

[a] Fifteen bottles of each solution were analyzed. Parentheses enclose standard deviations. ns, not significant at $p = 0.05$. ***, significant at $p < 0.001$.

The oxygen content was calculated from the following equation:

$$\text{oxygen concentration} = \ln(L^*/176.42)/-0.5777$$

Application to Oxygen Diffusion in Commercial Bottles. Our method was applied to determine oxygen diffusion through the different closure types in commercial bottles. The bottles were sealed with the different types of closures available on the market including natural cork stoppers, technical cork stoppers, synthetic stoppers, and a glass stopper as control.

Kinetics of Oxygen Diffusion through the Closures. The analytical data of oxygen diffusion showed that all of the closures and the control exhibited equivalent concentrations of oxygen of <1 mg/L (0.26 mL) immediately after corking ($t = 0$) (data not shown). As previously mentioned, our method is not sensitive enough, and therefore we decided to set the data values of dissolved oxygen below 1 mg/L to an arbitrary value of 1 mg/L.

The analytical data for the oxygen diffusion over a storage period of 365 days (horizontal position) indicated significant differences between all closures ($p = 0.05$).

The control did not display an increase in oxygen concentration over a storage period of 365 days. This indicated that our control bottle was essentially airtight, whereas the other closures exhibited permeability to atmospheric oxygen.

The technical cork closures (A, Nt, and TT) (**Table 1**) exhibited low oxygen permeability (below 3 mg/L or 0.78 mL of oxygen after 365 days of storage) with no significant differences between them (**Figure 5A**).

The synthetic closures, particularly the Supremecorq closure (S), had substantial oxygen permeability and reached the limit of oxygen quantification (9.8 mg/L or 2.5 mL of oxygen) for our method within 140 days of storage (**Figure 5B**). The Nomacorc closure (No) as well as two N24 natural cork stoppers also exhibited high levels of oxygen diffusion, which reached the limit of oxygen quantification for our method after 290 days of storage.

All closures that reached the limit of oxygen quantification were removed from the bottleneck and weighed to determine solution uptake by the closure during the storage period (difference from the closure at $t = 0$). The synthetic closures absorbed negligible amounts of the indigo carmine solution. On the other hand, the natural cork stoppers increased in weight by 0.21−0.33% after 290 days of storage. This mass increase represents an error in the amount of oxygen of 20−32 μg/L, with natural cork stoppers, and can be considered to be negligible.

The natural cork closures displayed lots of variation among the four replicates of each closure type with regard to oxygen diffusion and ranged from 8.3 mg/L (2.1 mL) to 5.9 mg/L (1.5 mL) for N24 and N26, respectively, over 365 days of storage (**Figure 5C**). Despite the different diameter and surface treatment of the cork stoppers, there were no significant differences in overall oxygen diffusion observed ($p = 0.05$). These results showed that no significant correlation could be established between the dimensions and coefficient of porosity of the natural cork and the amount of oxygen diffusion over 365 days.

The moisture content of N22 was lower (2.3%) than that recommended (4−8%). However, despite this lower moisture content, no significant differences in oxygen diffusion were observed compared other natural cork stoppers.

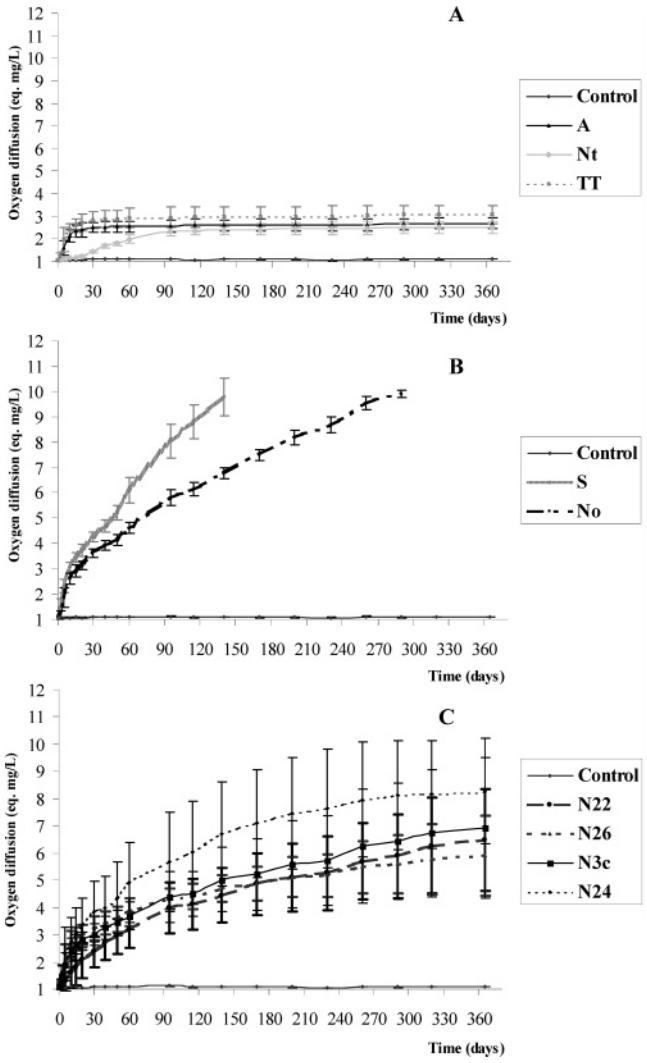

Figure 5. Kinetics of oxygen diffusion through each closure type: technical cork closures (**A**); synthetic closures (**B**); natural cork closures (**C**). Commercial bottles were stored horizontally over a period of 365 days. A, Agglomerate cork closure; TT, Twin Top cork closure; Nt, Neutrocork cork closure; No, Nomacorc closure; S, Supremecorq closure; N22, natural cork, first-grade closure, diameter = 22 mm; N24, natural cork, first-grade closure, diameter = 24 mm; N26, natural cork, first-grade closure, diameter = 26 mm; N3c, natural cork, third-grade colmated.

The kinetics profiles and amounts of diffused oxygen observed with this study are in agreement with those described by Squarzoni et al. (*17*). These authors showed that the oxygen diffusion after 400 days of storage was ~0.8 mL for technical cork stoppers (one plus one) and 1.8 mL for natural cork stoppers. The synthetic closures displayed a large range of oxygen diffusion, which cannot be compared with our data because of a lack of information on specific closure.

Rates of Oxygen Diffusion through the Closures. The oxygen diffusion kinetics differed for each closure. The rates of oxygen diffusion through the different closures were greater in the first month of storage than in the following months (**Table 3**). This corresponds to previous findings reported by Ribéreau-Gayon, who estimated the amount of oxygen that diffused through different closures into bottles during horizontal storage (*7*).

In the first month, the majority of closures displayed an oxygen diffusion rate that varied between 2 and 4 mg/L/month (0.52 and 1 mL/month). The exceptions were the Neutrocork closure (Nt), which displayed a rate of oxygen diffusion of 1.4 mg/L/month (0.36 mL/month), and the Supremecorq closure (S), which showed a rate of oxygen diffusion of >4 mg/L/month (1 mL/month) (**Table 3**).

Table 3. Mean Rate of Oxygen Diffusion through the Closure Seals into Commercial Bottles during Different Storage Periods ($n = 4$)[a]

	1 month	2–12 months	
closure	mg of O_2/L/month	mg of O_2/L/month)	cluster analysis (tree clustering)
Agglomerate (A)	2.5 (0.23)	0.01 (0.02)	**A**
Neutrocork (Nt)	1.4 (0.05)	0.10 (0.02)	
Twin Top (TT)	2.8 (0.37)	0.02 (0.01)	
natural cork			
first grade (N22)	2.3 (0.60)	0.37 (0.22)	**B**
first grade (N24)	3.8 (1.10)	0.50 (0.30)	
first grade (N26)	3.2 (0.40)	0.24 (0.16)	
third grade, colmated (N3c)	3.0 (0.40)	0.35 (0.20)	
Supremecorq (S)	4.3 (0.17)	1.5 (0.34)	**C**
Nomacorc (No)	3.6 (0.17)	0.85 (0.25)	

[a] Parentheses enclose standard deviations.

The rate of oxygen diffusion after the first month was extremely variable among the closures. A cluster analysis allowed the different closures that had similar rates of oxygen diffusion between 2 and 12 months to be grouped (**Table 3**). The A group is formed by the technical cork closures (A, Nt, and TT), which had very low rates of oxygen diffusion after the first month. The natural cork closures of the B group had very variable rates of oxygen diffusion, displaying values of 0.24 mg/L/month (0.06 mL/month) (N22) and 0.50 mg/L/month (0.13 mL/month) (N24). The synthetic closures of the C group had rates of oxygen diffusion close to 1 mg/L/month (0.26 mL/month) and more (S). These data suggest that the material and the production process of the closures that were evaluated might be responsible for the different rates of oxygen diffusion obtained after the first month of storage.

These findings indicate that the rate of oxygen diffusion through the natural cork stoppers into commercial wine bottles during postbottling storage is more important than do the previous findings reported by Ribéreau-Gayon (*7*). By using a similar colorimetric method, he estimated that the oxygen amount that diffused through a natural cork closure into a 750 mL bottle during horizontal storage was between 0.18 and 0.72 mg/L (0.10−0.38 mL) over the first 3 weeks and between 0 and 0.13 mg/L (0−0.07 mL) 4 months thereafter (*7*). However, Ribéreau-Gayon did not refer to the characteristics of the natural cork closures or the bottles (bore neck, etc.) used. Moreover, no reference to the conditions of storage were described. It is likely that the closures, bottle, and conditions of storage were not the same., This may partially explain some of the differences between our data and Ribéreau-Gayon's data.

These results agree with those obtained by Godden et al. (*10*), who showed that the SO_2 loss in white wine was most evident with synthetic closures. The SO_2 losses were intermediate for the natural cork stoppers and least evident for the technical cork closures. These results were also confirmed by Francis et al. , who showed that wine bottled mostly with synthetic closures exhibited undesirably high oxidized aroma scores in comparison with other closures after a period of 24 months (*13*).

Our findings are also in agreement with those obtained by Chatonnet and Labadie (*12*). They showed that wines corked with synthetic stoppers were much more susceptible to oxidation when compared to those corked with natural and technical cork stoppers.

The exact mechanism of oxygen diffusion into the bottle remains unknown. However, it is possible that, initially, the oxygen within the closure diffuses out of the closure and into the bottle. This phenomenon would explain the high rate of oxygen diffusion during the first month for almost all closures. As a second step, we observed a slow diffusion process, which may be due to atmospheric oxygen diffusion through the closure. This phenomenon will certainly be affected by the material characteristics of the closure.

Further research efforts are still needed to fully understand the mechanism of oxygen diffusion through the closures into the bottle. In addition, this method could be applied to other types of closures available (screw caps and other synthetic closures) on the market and can also be used to evaluate the effect of the bottle storage (horizontal or vertical). Moreover, it will be interesting in the future to perform the same type of experiments with red and white wines. The influence of microquantities of oxygen during bottle aging could then be studied together with the development of wine sensorial properties.

ACKNOWLEDGMENT

We acknowledge Saint-Gobain Glass Packaging (Cognac, France) for donating the bottles; Rudolf Gantenbrink (Gantenbrink Corp., Limburg, Germany) for the control bottle's sealing; Dr. Helena Pereira and Augusta Costa (Universidade Técnica de Lisboa, Lisbon, Portugal) for the image surface analysis of the natural cork stoppers; and Adriaan Oelofse and James Kennedy for revising the manuscript.

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Received for review December 16, 2004. Revised manuscript received June 14, 2005. Accepted June 17, 2005. We thank Amorim France (Eysines, France) and ANRT [Association Nationale pour la Recherche Technologique (Paris) Cifre Grant 097/2004] for their financial support of this research.

JF0404849