SEALING EFFECTIVENESS OF DIFFERENT TYPES OF CLOSURES TOWARDS VOLATILE PHENOLS AND HALOANISOLES

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Abstract

Aim: To determine if aerial contamination can induce the migration of volatile compounds through wine closures after bottling.

Methods and results: Bottled white wines sealed with cork stoppers (natural and microagglomerate), synthetic closures and screw caps were stored under an environment contaminated with three deuterium-labeled compounds: (d_5)-2,4,6-trichloroanisole (d_5-TCA), (d_4)-4-ethylphenol (d_4-E4P) and (d_5)-4-ethylguaiacol (d_5-E4G). Wines, closure sections (outer, middle and inner) and screw cap liner were assessed over time for the concentration of different compounds by solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). The results collected during 30 months of storage showed that large amounts of all compounds were essentially retained in the outer portion of cylindrical closures, both cork and synthetic. However, these compounds were able to penetrate through synthetics and screw caps and contaminate the wine.

Conclusion: Cork stoppers have proven to be an effective barrier to the migration of aerial volatile compounds such as d_5-TCA, d_4-E4P and d_5-E4G, whereas permeable closures such as synthetic and screwcap saranex did allow the migration of those compounds into bottled wines.

Significance and impact of the study: This study provides practical information about the sealing properties of different closures for a sound decision-making with regard to packaging. To the best of our knowledge, this is the first report of post-bottling aerial contamination via migration of volatile compounds through wine closures.

Key words: permeation, barrier, synthetic closures, screw caps, cork stoppers, wine

Résumé

Objectif: Déterminer si une exposition à une contamination aérienne peut induire la migration de composés volatils exogènes au travers des obturateurs après embouteillage du vin.

Méthodes et résultats: Des vins blancs bouchés avec des bouchons en liège (naturel et aggloméré), des bouchons synthétiques et des capsules à vis ont été stockés dans un environnement contaminé avec trois composés deutériés, soit le (d_5)-2,4,6-trichloroanisole (d_5-TCA), le (d_4)-4-éthylphénol (d_4-E4P) et le (d_5)-4-éthylguaiacol (d_5-E4G). Au cours des 30 mois de stockage, la concentration des différents composés a été analysée par microextraction en phase solide-chromatographie en phase gazeuse-spectrométrie de masse (SPME-GC-MS) dans les vins ainsi que dans les différentes parties des bouchons (externe, moyenne et interne) et les joints des capsules à vis. Les résultats obtenus ont montré que de grandes quantités de tous les composés ont été essentiellement retenues dans les parties externes des bouchons en liège et synthétiques. Toutefois, ces composés ont pu migrer au travers des bouchons synthétiques et capsules à vis, et par conséquent contaminer le vin.

Conclusion: Les bouchons en liège ont été de vraies barrières à la migration aérienne des composés volatils tels que d_5-TCA, d_4-E4P et d_5-E4G, alors que les obturateurs perméables tels que les synthétiques et la capsule à vis saranex ont permis la migration de ces composés dans les vins embouteillés.

Signification et impact de l'étude: Cette étude fournit des informations pratiques sur les propriétés barrière aux gaz des différents obturateurs qui sont fondamentales pour une prise de décision éclairée vis-à-vis du conditionnement du vin. À notre connaissance, c'est la première fois que la contamination aérienne du vin après l'embouteillage par la migration de composés volatils à travers certains obturateurs a été mise en évidence.

Mots clés: perméabilité, barrière, bouchons synthétiques, capsules à vis, bouchons en liège, vin

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INTRODUCTION

Packaging plays an important role in the quality of food products as it enables a great number of functions: it protects the food from contamination and spoilage; simplifies the transport and storage process; and provides uniform measurement of contents (Risch, 2009). Thus, food packaging is essential and pervasive; almost all food is in some way packaged, otherwise its safety and quality would be compromised (Robertson, 2009).

In the wine industry, the glass bottle is the most used and consolidated packaging, representing 87 % of packaging units sold, with the remaining 13 % including liquid cartons, bag-in-box, beverage cans and polyethylene terephthalate (PET) bottles. Closures, another important element of wine packaging, sell ~17 billion units per year and are one of the most important factors that may influence wine quality after bottling (Godden *et al.*, 2005). The primary function of a wine closure as a part of wine packaging is to ensure a good seal in order to prevent any organoleptic deterioration of the wine during storage. However, some types of closures may allow the mass transfer of various small molecules and gases, such as oxygen, into bottled wines (Skurray *et al.*, 2000; Capone *et al.*, 2002; Lopes *et al.*, 2011).

Robertson (2009) proposed two mechanisms by which gases can permeate through polymeric materials, including closures: pore effect and solution-diffusion effect. The pore effect is the gas transfer through microscopic pores, pinholes and cracks in the material, whereas the solution-diffusion effect involves gas dissolution in a material, diffusion through it under the influence of a concentration gradient, and evaporation at the other end. This latter process is considered "true permeability" and varies inversely with the thickness of the materials (Robertson, 2009). There is also a third process in which diffusion might occur through the interface between materials, *e.g.*, the interface between the glass bottleneck and the closure. However, Lopes *et al.* (2007) reported that this process is negligible in both synthetic and cork stoppers, at least during the first 38 months of storage (Lopes *et al.*, 2007). The extent of this effect is controlled by the individual closure characteristics such as closure diameter, elasticity, dimensional stability with time, and ability to adhere to the glass surface.

The mass transfer of different compounds through the closure greatly depends on their chemical and mechanical properties, resulting in closures with different barrier properties and therefore different ranges of oxygen transfer rates (OTR) (Poças *et al.*, 2009). Cork stoppers are essentially impermeable to atmospheric gases, although the cork itself can provide, under compression, a certain amount of oxygen that is trapped inside its structure (Lopes *et al.*, 2007). Synthetic closures have high permeability to oxygen and in contrast, screw caps are essentially airtight, although their sealing effectiveness is dependent on the liner's barrier properties (Lopes, 2006).

Mass permeation through closures is not exclusive to oxygen; other exogenous gases and volatile compounds are also able to permeate through closures into bottled wines. Lopes *et al.* (2009) and Lopes *et al.* (2011) showed that small volatile compounds, namely 2,4,6-trichloroanisole (TCA) and 2,4,6-trichlorophenol, were able to permeate through synthetic stoppers and contaminate bottled wine model solutions. Skurray *et al.* (2000) showed that the sealing effectiveness of natural cork to vanillin was better than with synthetic polymers. In addition, Capone *et al.* (2002) and Lopes *et al.* (2011) indicated that microagglomerate and natural cork stoppers are effective barriers to the transmission of exogenous d_5-TCA into wine bottles. Recently, the Winemaking and Extension Services of the Australian Wine Research Institute (AWRI) obtained evidence that exposure to the aerial contamination over a period of several months can allow the migration of the taint compounds such as tetrachloroanisole (TeCA) through the crown seals and into the wine (AWRI, 2011). These studies showed that aerial contamination followed by migration into the wine is indeed a mode of tainting after bottling. However, aerial contamination followed by migration into the wine through packaging remains little studied, namely in terms of primary packaging elements that are permeable to gases, *i.e.*, closures.

The commercial offer of closures with different levels of OTR have been studied, with the objective of giving winemakers a tool to produce different styles of wine after bottling, according to the shelf-life (Vidal and Aagaard, 2008; Ugliano *et al.*, 2009). However, this argument seems to go against the concept of wine packaging and closure, which should provide an effective protection against moisture, oxygen, carbon dioxide and other gases, and volatile compounds. Therefore, the assessment of closure barrier properties to compounds other than oxygen is essential in order to highlight the potential risks associated with the use of permeable

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wine closures and clarify the closure's contribution to the wine aerial contamination after bottling.

This study aimed to investigate the permeation of aerial volatile compounds such as (d_5)-2,4,6-trichloroanisole (d_5-TCA), (d_4)-4-ethylphenol (d_4-E4P) and (d_5)-4-ethylguaiacol (d_5-E4G) through five different types of commercially-available closures (natural and microagglomerate cork, synthetic 1 and synthetic 2, and screw cap closures) after bottling. The mechanism by which permeation occurs through the above-mentioned closures is discussed. This experiment seeks to determine if aerial contamination can induce the migration of volatile compounds through wine closures after bottling.

MATERIALS AND METHODS

1. Chemicals

Deuterium-labelled 2,4,6-trichloroanisole (d_5-TCA), 2,4,6-tribromoanisole (d_5-TBA), 4-ethylphenol (d_4-E4P, d_{10}-E4P) and 4-ethylguaiacol (d_5-E4G) were purchased from CDN Isotopes Inc (Pointe-Claire, Quebec, Canada). Ethanol 96 % v/v was purchased from Manuel Vieira & Co (Torres Novas, Portugal). Deionised water was purified with a Milli-Q water system (Millipore, Bedford, MA) before use.

2. Bottles and closures

"Cantil" green colour bottles (375 mL) were supplied by Barbosa & Almeida (Avintes, Portugal). The bottlenecks had the following specifications: diameter of 15.5-16 mm at 3 mm and 18-19 mm at 44 mm from the bottle top.

The five types of wine closures used in this experiment were: natural cork (visual grade "superior", 38 mm length, 24 mm diameter), microagglomerate cork (Neutrocork®, 38 mm length, 24 mm diameter), synthetic closures, 1 and 2 (Nomacorc Light®, 40 mm length, 22 mm diameter, and Nomacorc Premium®, 44 mm length, 22 mm diameter, respectively) and Stelvin® screw cap with saranex liner (30 mm length and 22 mm diameter). Cork stoppers were supplied by Amorim & Irmãos, S.A. (Santa Maria de Lamas, Portugal) and synthetic closures and screw caps were taken from stocks held by Austrian and Portuguese wine producers, who had purchased them from Nomacorc S.A. and Alcan Packaging, respectively. Cork stoppers were coated with paraffin/silicone. Synthetic closures were coated with an unknown Food and Drug Administration (FDA)-approved coating.

3. Bottling and storage

Bottling took place at Sogrape facilities (Avintes, Portugal) in January 2009 using a Portuguese white wine (pH = 3.1; alcohol content = 10% v/v; total acidity = 5.7 g/L as tartaric acid; volatile acidity = 0.12 g/L as acetic acid). The wine was filled to 62 ± 3 mm from the top of the bottle. The bottles were then sealed without application of inert gases or vacuum prior to the insertion of the different cylindrical closures. At the same time, on a different bottling line, screwed bottles were filled with the same wine at 62 ± 2 mm from the top of the bottle and then sealed with screw caps without application of inert gases or vacuum in the bottleneck. A total of 75 bottles were sealed: 15 bottles per closure type.

An aluminium bag (883 mm length, 1 167 mm diameter) was inserted into a cardboard box (597 mm length, 375 mm width and 466 mm height) and adjusted to its dimensions. Five bottles sealed with each closure type were then inserted horizontally in an aluminium bag/cardboard, which was then artificially contaminated with the insertion of 3 filter papers, each containing 183 µg of d_5-TCA, 180 mg of d_4-E4P and 16 mg of d_5-E4G. For that purpose 1 ml of d_5-TCA (61 mg/L), 1 mL of d_4-E4P (60,1 g/L) and 1 mL of d_5-E4G (5.3 g/L) dissolved in ethanol were added to each filter paper. Thus, each storage atmosphere had an approximate contamination of 1.75 µg/L_{air}, 1.73 mg/L_{air} and 0.15 mg/L_{air} of d_5-TCA, d_4-E4P and d_5-E4G, respectively. This assumes that all volatile compounds had evaporated from the filter papers, which was certainly not the case. Finally, the aluminium bag was sealed by heat and the cardboard was closed. The levels of contamination used were totally random due to the lack of data in the literature on the amounts of volatile compounds, namely TCA, likely to be detected in storage atmospheres.

Three boxes were made up, each containing 25 bottles, corresponding to 1, 12 and 30 months of storage. All bottles were stored for up to 30 months under room conditions (temperature and moisture were not recorded).

4. Analysis of TCA and volatile phenols in wine and closures

After 1, 12 and 30 months of storage, the deuterium-labelled compounds were quantified in

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both wine and closures. At each time point, the bottles were removed from the aluminium bags and the bottlenecks were covered with aluminium foil (to minimize the loss of contaminants from the closure surface to the atmosphere). After 24 hours, the bottlenecks were cut from the bottle at 50 ± 4 mm from the top of the bottle using a common glass/metal cutting machine.

Four samples (10 mL each) of wine were collected from each bottle and placed in 20-mL solid phase microextraction (SPME) vials containing 3.0 g of NaCl. 100 µL of internal standard d_5-TBA (2 µg/L) was added to two vials and 50 µL of d_{10}-E4P (0.1 g/L) was added to the other two vials in order to carry out the chloroanisole and volatile phenol analyses, respectively.

The bottlenecks were totally broken using a hammer and the closures were collected. Each closure was then cut parallel to the closure ends into three identical cylindrical sections, representing the inner section of the closure (in contact with the wine), the central section, and the outer section (in contact with the contaminated atmosphere). The screw cap liners were removed from the aluminium cap using a knife. Each closure section and screw cap liner was then soaked in 60-mL glass flasks with 40 mL of hydroalcoholic solution (12 % ethanol) for 24 hours at room conditions.

Four 10-mL samples of each soak were collected into 4 SPME vials : two vials each received 100 µL of d_5-TBA (2 µg/L) for the analysis of TCA and the other two each received 50 µL of d_{10}-E4P (0.1 g/L) for the analysis of volatile phenols.

All the wines and closure section soaks were then analyzed for TCA and volatile phenols by SPME-gas chromatography-mass spectrometry (SPME-GC-MS) (Evans *et al.*, 1997; Boutou and Chatonnet, 2007). A scheme of the experimental protocol is shown in Figure 1.

5. Analysis of TCA by GC-MS

A QP-2010 Plus gas chromatograph mass spectrometer (GC-MS) (Shimadzu, Kyoto, Japan) equipped with a Multipurpose Autosampler (MPS2) (Gerstel, Mülheim an der Ruhr, Germany) in SPME operation mode was used for the analysis of TCA. The vials were transported from the tray to the agitator held at 55 ºC. The incubation time was 3 minutes and the extraction time was 11 minutes under agitation (250 rpm). The 100-µm

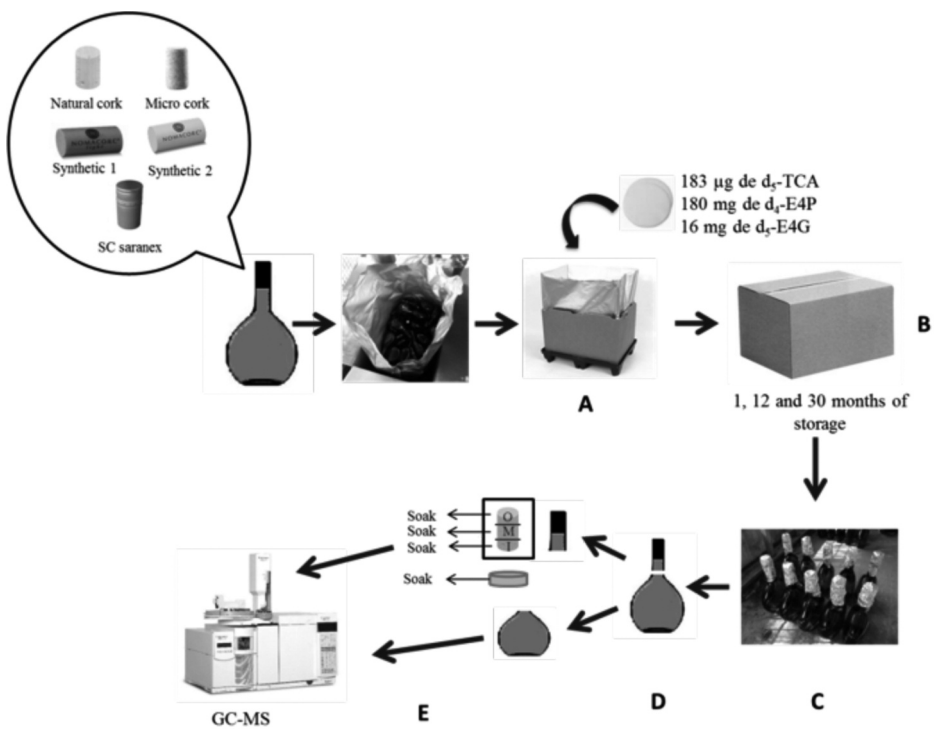


Figure 1 - Diagram of the contaminated storage atmosphere with deuterium-labelled 2,4,6-trichloroanisole (d_5-TCA), 4-ethylphenol (d_4-E4P) and 4-ethylguaiacol (d_5-E4G) in the aluminium bag/cardboard box (A); bottle storage inside the aluminium bag (B); collection procedure and analyses of closure sections and wine samples by GC-MS (C, D and E).

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polydimethylsiloxane (PDMS) fiber was desorbed into the injector held at 270 ºC for 4 minutes in splitless mode. The compounds were then separated on a RTX-5MS capillary column (30 m x 0.25 mm i.d.) (Restek, Bellefonte, PA) with 0.25 µm film thickness. Helium was used as the carrier gas, which was programmed to flow at a constant speed of 47 cm/sec during the flow run (flow 1.61 mL/min). The temperature program started at 90 ºC and then increased at a rate of 10 ºC/min to 205 ºC and finally at 30 ºC/min to 280 ºC.

The MS operated with electron impact ionization in selected ion monitoring mode. The quantification ions were 215 m/z for d_5-TCA (qualifier ion 217 m/z) and 349 m/z for d_5-TBA (qualifier ions 347 and 351 m/z) (internal standard). GC-MS interface and ion source were at 280 ºC and 200 ºC. The limits of detection and quantification of the method were 0.3 and 1 ng/L for d_5-TCA, respectively.

6. Analysis of volatile phenols by GC-MS-MS

A Varian CP – 3800 GC (Agilent Technologies, Santa Clara, CA) equipped with a Multipurpose Autosampler (MPS2) (Gerstel, Mülheim an der Ruhr, Germany) in SPME operation mode was used for the analysis of volatile phenols. The incubation temperature was 55 ºC for 3 minutes. The extraction time was 15 minutes under agitation (250 rpm) with a 100-µm PDMS fiber. The compounds were desorbed into the injector held at 270 ºC for 4 minutes in splitless mode. The compounds were separated on a TRB-5MS capillary column (30 m x 0.25 mm x 0.25 µm) (Teknokroma, Barcelona, Spain). Helium was used as carrier gas at a flow of 1.0 mL/min. The temperature program started at 90 ºC and then increased at a rate of 0.5 ºC/min to 100 ºC and finally at 50 ºC/min to 320 ºC.

The MS-MS detector used as single MS was a Saturn 2200 (Agilent Technologies, Santa Clara, CA). The quantification ions selected were 111 m/z for d_4-E4P (qualifier ion 127 m/z), 139 m/z for d_5-E4G (qualifier ion 157 m/z) and 113 m/z for d_{10}-E4P (qualifier ion 131 m/z) (internal standard). The ion trap temperature was 160 ºC, and the manifold and transfer line temperatures were set at 60 ºC and 220 ºC, respectively. The limits of detection and quantification were 3.3 and 10 µg/L for d_4-E4P and 1.7 and 5 µg/L for d_5-E4G, respectively.

7. Data analysis

All data were treated using Microsoft Excel 2010 software. Analysis of variance (ANOVA), Fisher's least significant difference was carried out with XLStat software (Addinsoft, Paris, France).

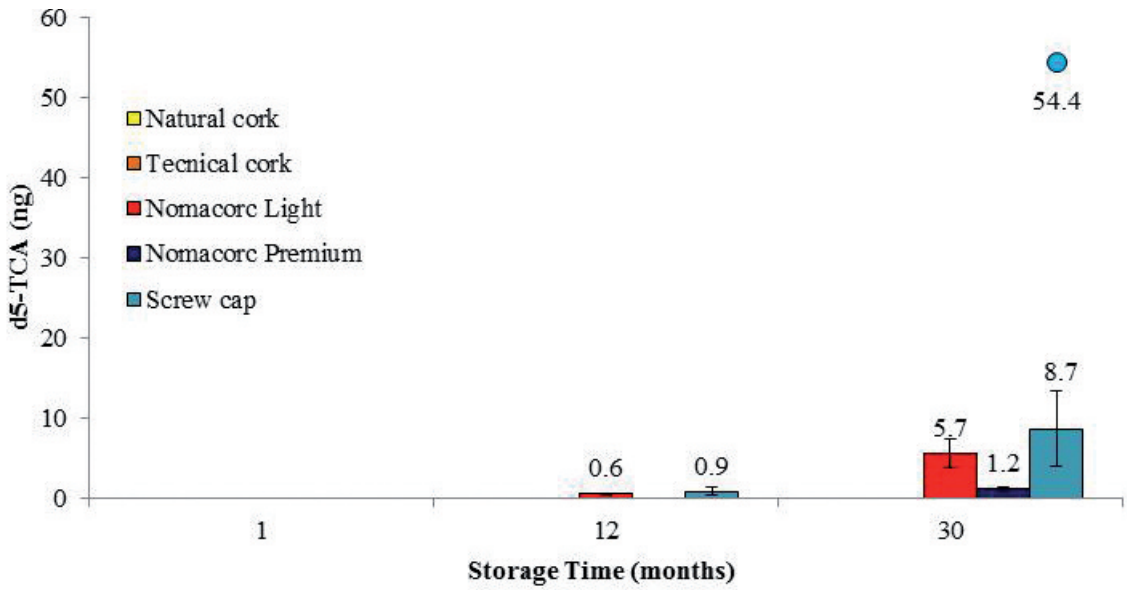


Figure 2 - Amount of (d_5)-2,4,6-trichloroanisole (d_5-TCA) in bottled wine sealed with different closures obtained after 1, 12 and 30 months. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation. The dot corresponds to an outlier value which was not taken into account for the calculation of the average and standard deviation and in the statistical treatment. At 1-month storage time, no contaminant was detected.

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RESULTS

The amount of deuterium-labelled 2,4,6-trichloroanisole (d_5-TCA), 4-ethylphenol (d_4-E4P) and 4-ethylguaiacol (d_5-E4G) was determined in the wines (Figures 2, 4 and 6) and closures (Figures 3, 5 and 7) at three time points over a 30-month period under the contaminated atmosphere.

1. d_5-TCA in wines and closure sections

The exogenous d_5-TCA was not detected in wines sealed with natural and microagglomerate corks (Figure 2). In contrast, 0.9 ng (2.5 ng/L) and 8.7 ng (23.5 ng/L) of d_5-TCA were quantified in wines sealed with screw caps after 12 and 30 months, respectively. These contaminations represented

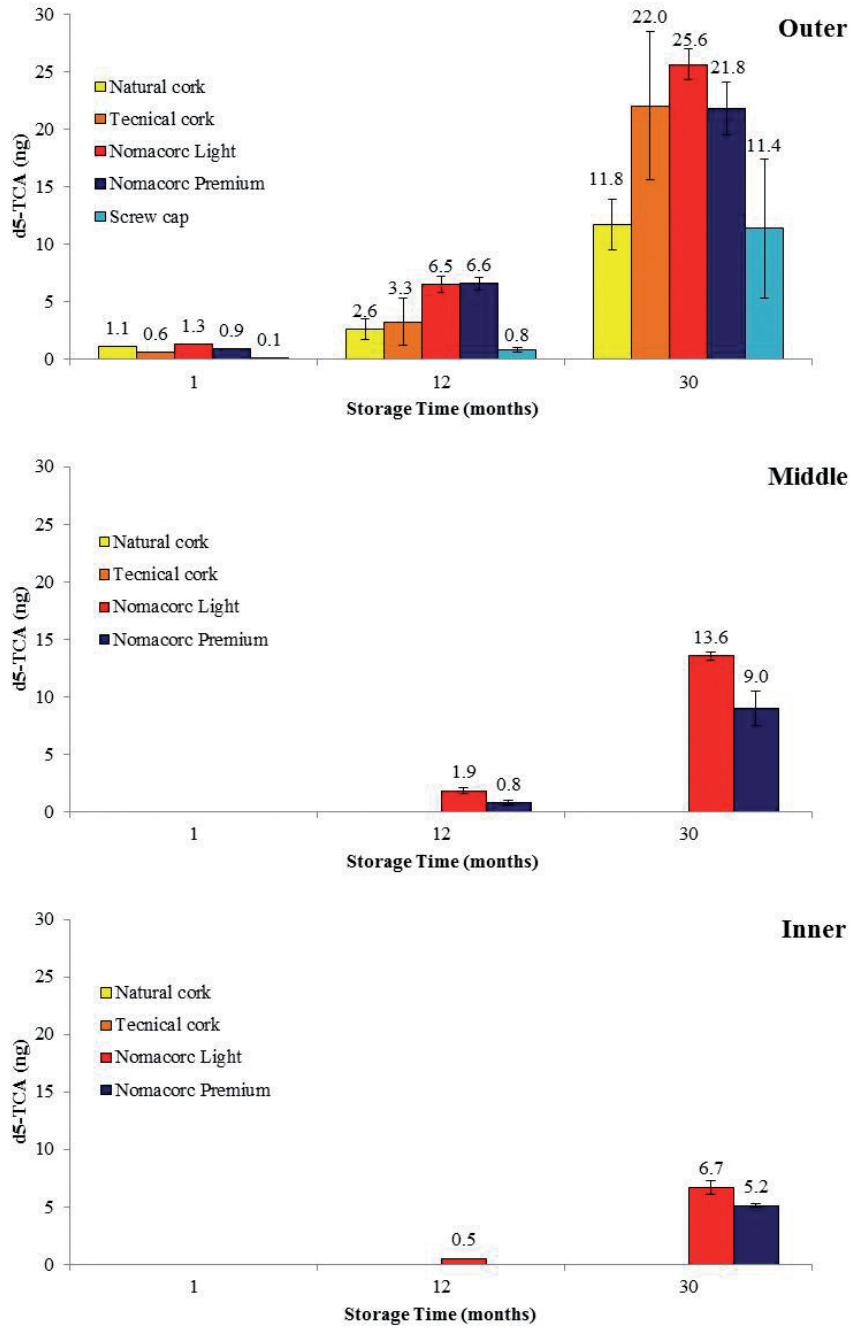


Figure 3 - Amount of (d_5)-2,4,6-trichloroanisole (d_5-TCA) in the different closure sections (outer, middle and inner) after 1, 12 and 30 months in bottle. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation. At 1-month storage time, no contaminant was detected in the middle and inner sections of the closures.

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0.0005 % and 0.005 % of the theoretical total amount of d_5-TCA (183 µg) added to each box. The blue dot represents a screw cap outlier at 30 months; this value corresponded to 54.4 ng (146.9 µg/L) of TCA and was not considered for the mean and statistical data analysis; however, it is shown since it is part of a real bottling trial. Likewise, the wines sealed with synthetic 1 presented 0.6 ng (1.7 ng/L) and 5.7 ng (15.3 ng/L) of d_5-TCA after 12 and 30 months storage, respectively (0.0003 % and 0.003 % of the total), while those sealed with synthetic 2 presented 1.2 ng (3.4 ng/L) of d_5-TCA only after 30 months storage (0.0007 % of the total). The levels of d_5-TCA in wines sealed under screw caps and synthetic 1 were significantly higher than those found in wines sealed with synthetic 2 ($p < 0.0001$).

The distribution of d_5-TCA in the different closure sections varied according to the type of closure (Figure 3). The results showed that d_5-TCA was mainly confined to the outer section of microagglomerate and natural corks, increasing significantly throughout the storage period from 0.6 ng and 1.1 ng at 1 month and 3.3 ng and 2.6 ng at 12 months to 22.0 ng and 11.8 ng at 30 months, respectively ($p < 0.0001$). The middle and inner sections of both types of corks did not present any d_5-TCA. Conversely, the screw cap liner had 0.1 ng, 0.8 ng and 11.4 ng of d_5-TCA at 1, 12 and 30 months, respectively.

The outer sections of synthetic 1 and synthetic 2 displayed 1.3 ng and 0.9 ng, 6.5 ng and 6.6 ng, and 25.6 ng and 21.8 ng of d_5-TCA at 1, 12 and 30 months, respectively (Figure 3). In the middle sections, 1.9 ng and 0.8 ng of d_5-TCA at 12 month and 13.6 ng and 9.0 ng at 30 months were detected. In the inner sections, 0.5 ng of d_5-TCA was only detected in synthetic 1 at 12 months. However, at 30 months, 6.7 ng and 5.2 ng of d_5-TCA were detected in synthetic 1 and synthetic 2, respectively. In both the middle and inner sections, the levels of d_5-TCA in synthetic 1 were statistically higher than those observed in synthetic 2 ($p < 0.0001$).

2. d_4-E4P in wines and closure portions

No d_4-E4P was detected in the wine sealed with natural and microagglomerate cork during the 30 months of storage (Figure 4). Conversely, wines sealed with screw cap saranex had 4.4 µg (11.8 µg/L) and 11.1 µg (29.9 µg/L) of d_4-E4P after 12 and 30 months, respectively. This contamination represented 0.002 % and 0.006 % of the theoretical total amount of d_4-E4P (180 mg) added to each box. Likewise, wines sealed with synthetic 1 had 4.8 µg (12.9 µg/L) and 12.5 µg (33.9 µg/L) of d_4-E4P at 12 and 30 months, respectively (0.003 % and 0.007 % of the total in the box), while for those sealed with synthetic 2, d_4-E4P was only detected at 30 months: 9.9 µg (26.8 µg/L, 0.006 % of the total in the box). The levels of d_4-E4P in wines sealed under synthetic 1 were slightly higher, but statistically significant, than those found in wines sealed with the screw cap and synthetic 2 ($p < 0.0001$).

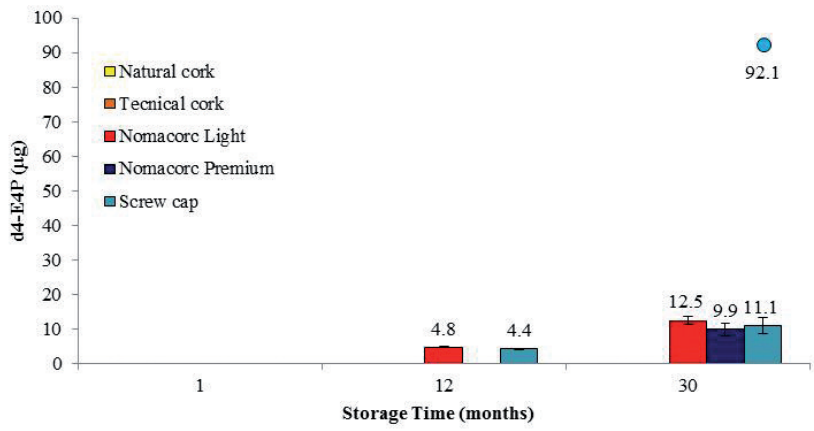


Figure 4 -Amount of (d_4)-4-ethylphenol (d_4-E4P) in bottled wine sealed with different closures obtained after 1, 12 and 30 months. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation. The dot corresponds to an outlier value which was not taken into account for the calculation of the average and standard deviation and in the statistical treatment. At 1-month storage time, no contaminant was detected.

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The distribution of d_4-E4P throughout different closure sections is represented in Figure 5. The outer sections contained the highest amount, with the microagglomerate cork displaying the highest values: 15.0 µg, 29.0 µg and 862.4 µg of d_4-E4P at 1, 12 and 30 months, respectively ($p < 0.0001$). The natural corks also displayed significantly high amounts of d_4-E4P in the outer sections, with 16.8 µg, 8.5 µg and 183.0 µg after 1, 12 and 30 months of storage, respectively. Likewise, d_4-E4P was also detected in screw cap with saranex liner, with 0.1 µg and 17.2 µg at 12 and 30 months, respectively.

d_4-E4P was found in the outer sections of both synthetic closures (synthetic 1 and synthetic 2),

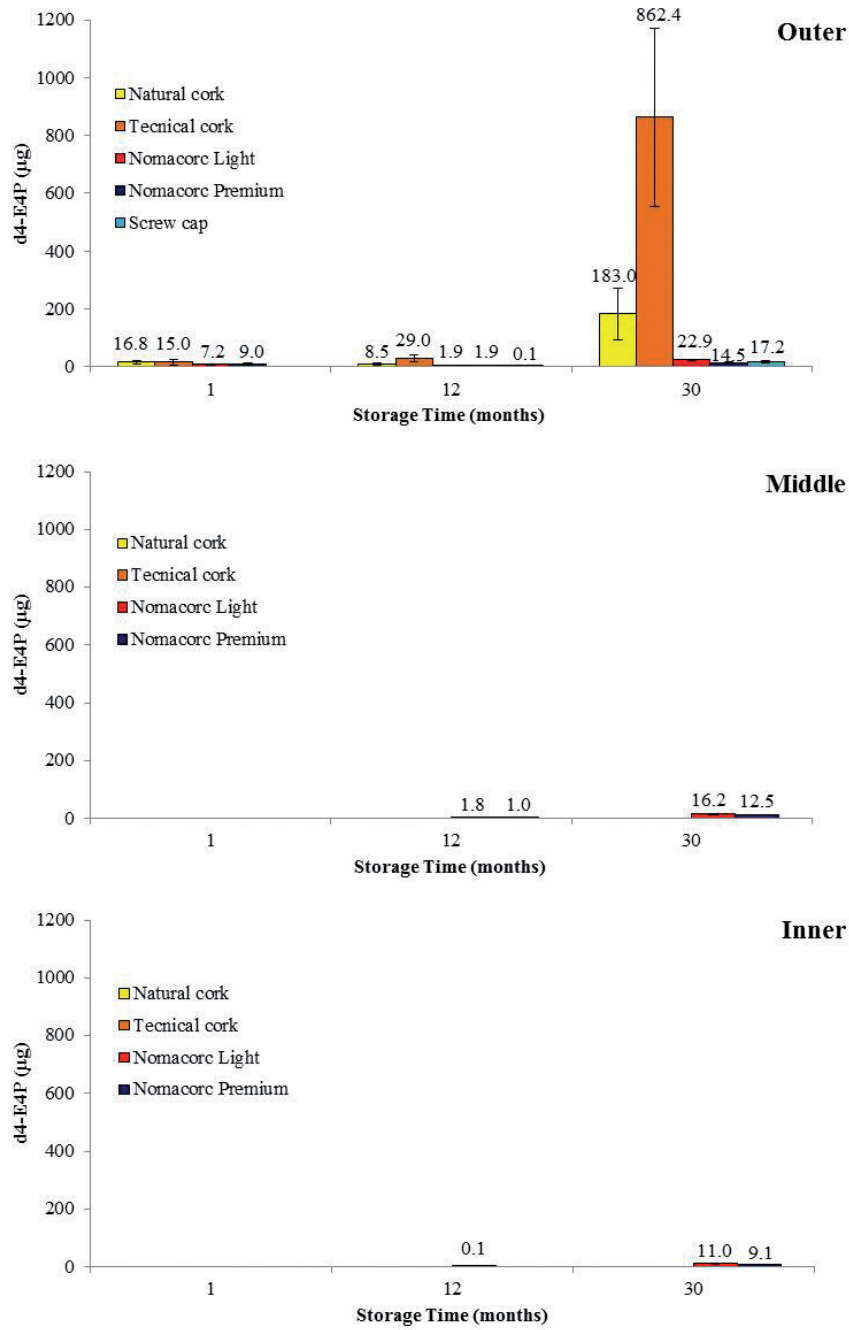


Figure 5 - Amount of (d_4)-4-ethylphenol (d_4-E4P) in the different closure sections (outer, middle and inner) after 1, 12 and 30 months in bottle. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation. At 1-month storage time, no contaminant was detected in the middle and inner sections of the closures.

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reaching 22.9 µg and 14.5 µg at 30 months, respectively. In the middle sections of synthetic 1 and synthetic 2, d_4-E4P was quantified at 1.8 µg and 1.0 µg, and 16.2 µg and 12.5 µg after 12 and 30 months, respectively. In the inner sections, residual amounts of d_4-E4P (0.1 µg) were detected in synthetic 1 at 12 months. However, after 30 months similar amounts of d_4-E4P (11.0 µg and 9.1 µg) were detected in synthetic 1 and synthetic 2, respectively ($p = 0.05$).

3. d_5-E4G in wines and closure sections

d_5-E4G was not detected in wines sealed with natural and microagglomerate cork during the 30 months of storage (Figure 6. At 12 months, no amount of d_5-E4G was detected in the wines. In contrast, at 30 months, wines sealed with synthetic 1 and synthetic 2 were contaminated with 6.7 µg (18.2 µg/L) and 4.0 µg (10.9 µg/L) of d_5-E4G, respectively. These concentrations represented 0.04 % and 0.03 % of the theoretical total amount of d_5-E4G (16 mg) added to each box. Likewise, wine sealed with the screw cap saranex presented 2.6 µg (9.3 µg/L) of d_5-E4G only after 30 months of storage (0.02 % of the d_5-E4G initially added to the box). As was observed with d_4-E4P, the levels of d_5-E4G in wines sealed with synthetic 1 were significantly higher than those found in wines sealed with screw cap and synthetic 2 ($p < 0.0001$).

As was observed with previous compounds, the outer section of the closures contained the greatest spike concentrations of the compounds (Figure 7). In natural cork the amount of d_5-E4G was 1.8 µg and 12.4 µg at 12 and 30 months, respectively, while microagglomerate cork recorded the highest values, with 4.5 µg and 42.4 µg at 12 and 30 months, respectively ($p < 0.0001$). Synthetic 1 had 2.7 µg and 14.0 µg of d_5-E4G at 12 and 30 months, while synthetic 2 had 2.3 µg and 12.8 µg at 12 and 30 months, respectively. Although some residual amounts of this compound were detected in screw cap liners at 12 months, they were below the limit of quantification of the method; however, at 30 months 3.1 µg of d_5-E4G was quantified. In the middle section of cork closures, d_5-E4G was not detected. In contrast, synthetic 1 and synthetic 2 closures presented 2.2 µg and 7.1 µg, and 0.8 µg and 6.2 µg at 12 and 30 months, respectively. The inner cork sections were not contaminated with d_5-E4G, as had already been observed with the middle sections. Synthetic 1 had 0.6 µg and 3.7 µg of d_5-E4G at 12 and 30 months, while synthetic 2 had a detectable amount of d_5-E4G (3.7 µg) only after 30 months storage.

4. Diffusion coefficients

The diffusion of the three exogenous compounds through synthetic and screw cap closures can be represented by the transmission rate. The transmission rate through a membrane of L thickness, with uniform initial distribution and different surface concentrations (assuming no boundary layers) can be obtained by the Fick's law (Karbowiak *et al.*, 2010a). Considering unidirectional mass transfer, the first Fick's law in

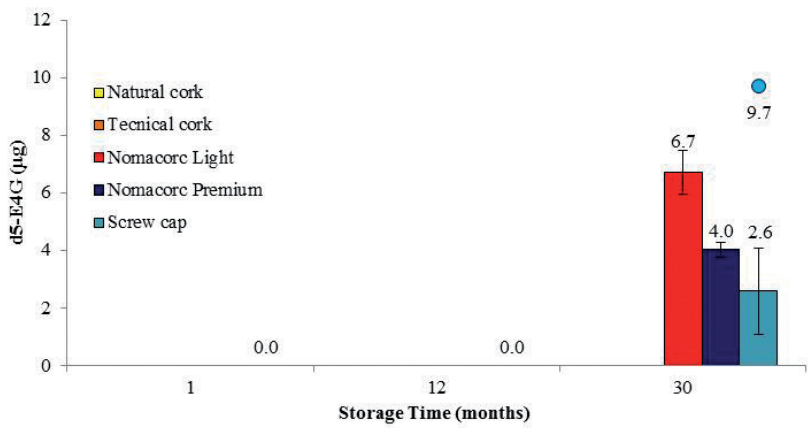


Figure 6 - Amount of (d_5)-4-ethylguaiacol (d_5-E4G) in bottled wine sealed with different closures obtained after 1, 12 and 30 months. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation. The dot corresponds to an outlier value which was not taken into account for the calculation of the average and standard deviation and in the statistical treatment. At 1- and 12-month storage time, no contaminant was detected.

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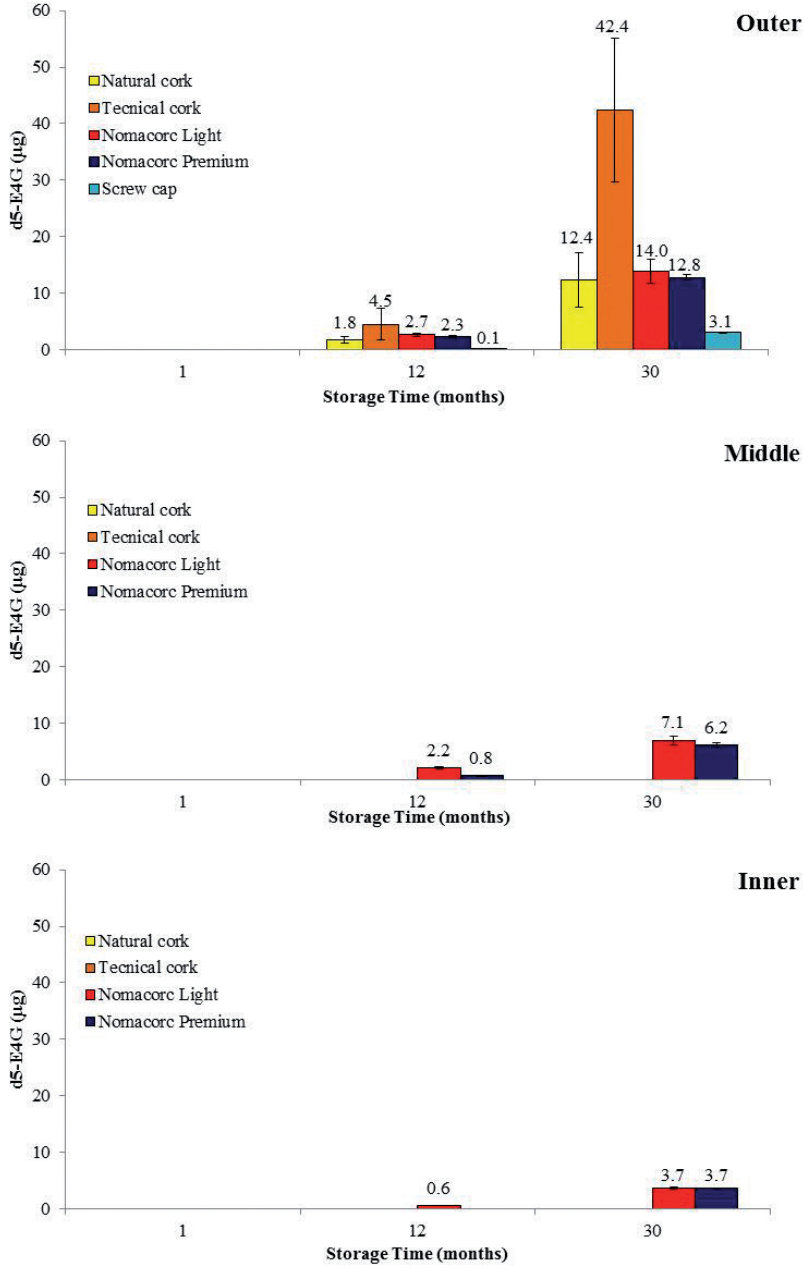


Figure 7 - Amount of (d_5)-4-ethylguaiacol (d_5-E4G) in the different closure sections (outer, middle and inner) after 1, 12 and 30 months in bottle. Values per time point and closure are the mean of 5 replicates and the error bars represent the standard deviation.
At 1-month storage time, no contaminant was detected in the middle and inner sections of the closures.

transient state ($\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$) can be described by an analytical solution (Crank, 1975):

$$\frac{m_t}{m_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} exp\left[\frac{-D(2n+1)^2\pi^2 t}{4L^2}\right] \quad (1)$$

where m_t (g) is the mass of the diffusing substance at time t (s), m_∞ is the mass of diffusing substance at equilibrium, L is the half-thickness of the closure sample (m) and D is the apparent diffusion coefficient (m^2/s).

For the application of the Fick's law to the mass transfer obtained in the experiments described in this study, the mass value of the contaminant present in the outer surface (m_∞) of the closures was obtained after soaking the outer parts of the cylindrical closures or the entire liner of the screw

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caps. Conversely, the mass of diffusion substance at time t (m_t) was defined as the mass of contaminant in wine. To calculate the diffusion coefficient it was assumed that equilibrium was reached at 30 months; thus, the m_∞ values correspond to this period of analysis.

Synthetic 1 displayed higher transmission rates to all compounds than synthetic 2 (Table 1) ($p < 0.0001$). This fact can be either related to the different chemical properties of these closures and/or their length differences. Synthetic 2 was 4 mm longer than synthetic 1 and therefore, diffusion takes longer to reach the bottom of the closure and the wine. Regarding volatile phenols, the diffusion coefficients of synthetic 1 and synthetic 2 were identical, while for TCA the diffusion coefficient in synthetic 2 was 10 times lower than in synthetic 1.

One of the wines sealed with screw cap had an abnormally high amount of contaminants at 30months; this amount was higher than those found on the respective screw cap liner. Mathematically, m_∞ was lower than m_t, which is impossible since m_∞ is the maximum equilibrium amount of contaminant on the material and so $m_\infty \geq m_t$. Taking this fact into consideration, the amount detected in this wine did not all come from diffusion through the liner but from another path, possibly a small defect or fracture in the liner or a leak that was not macroscopically visible. The highest abnormal sample value was nullified for the calculation of the diffusion coefficient. As can be observed in table 1, screw cap with saranex liner had lower diffusion coefficients than synthetic closures for all volatile compounds, which means that its chemical/structural composition is less permeable than synthetic closures.

Natural and microagglomerate corks have no diffusion coefficient since they did not allow the permeation of exogenous compounds into bottled wines (Table 1).

DISCUSSION

All compounds used in this study were confined and sorbed by the outer portions of microagglomerate and natural cork stoppers, which is consistent with previous studies showing that cork is an effective barrier against the permeation of exogenous compounds into wines. Capone *et al.* (2002), Lopes *et al.* (2009) and Lopes *et al.* (2011) also observed that volatile non-polar compounds such as 2,4,6-trichloroanisole and 2,4,6-trichlorophenol, confined to the outer portion of cork stoppers, were unable to migrate beyond this point. Likewise, d_4-E4P and d_5-E4G were also retained in the outer portions of natural and microagglomerate corks. Therefore, aerial contamination followed by migration into the wine after bottling is highly unlikely to occur when bottled wines are sealed with cork stoppers. Nevertheless, cork has strong sorption properties towards volatile phenolic compounds, as has already been observed by Karbowiak *et al.* (2010b). The highest amounts of these compounds detected in the outer section of microagglomerate corks may be related to the presence of polyurethane glue, which enhances volatile phenol sorption (Anjaneyulu *et al.*, 1990). Nevertheless, it cannot be excluded that the difference observed between these two different types of cork stoppers may also be related to an easier desorption of

Table 1 - Diffusion coefficients of the different contaminants through cork stoppers and synthetic and screw cap closures.

	Diffusion Coefficient D (m^2/s)*		
	TCA	E4P	E4G
Natural cork	0	0	0
Microagglomerate cork	0	0	0
Synthetic 1	1.06×10^{-13} (3.23×10^{-14})	8.41×10^{-13} (8.04×10^{-14})	4.78×10^{-13} (5.34×10^{-14})
Synthetic 2	1.11×10^{-14} (2.70×10^{-15})	5.88×10^{-13} (1.11×10^{-13})	1.23×10^{-13} (7.62×10^{-15})
Screw cap	1.56×10^{-15} (1.87×10^{-15})	1.11×10^{-15} (2.27×10^{-14})	2.18×10^{-15} (6.53×10^{-16})

*This calculation considered m_∞ as the mass of contaminant in the outer section of the closure or the screw cap liner at 30 months of storage; mt as the mass of contaminant in wine at 12 or 30 months; and the diffusion coefficient as the mean of the diffusion coefficient value obtained at 12 and 30 months.
The standards deviations are presented in parentheses.

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volatile phenols from cork when compared with synthetic materials.

Synthetic closures such as synthetic 1 and synthetic 2 also sorbed high amounts of d_5-TCA, d_4-E4P and d_5-E4G in their outer sections. However, these compounds diffused continuously beyond the outer section of these closures to the middle and inner sections, reaching the wine at different periods of time. These results agree with those obtained by Lopes *et al.* (2009) and Lopes *et al.* (2011), who also observed that exogenous 2,4,6-trichloroanisole and 2,4,6-trichlorophenol was able to migrate through synthetic closures such as Nomacorc Classic. Screw cap saranex liner also sorbed some amounts of those compounds; however, it did not prevent the permeation into wines. The amount of contaminants found in wines sealed with screw caps was highly variable and strongly affected by the performance of one screw cap at 30 months, which allowed an abnormal permeation of all three compounds. These results seem to be consistent with some empirical and scientific evidence that screw cap saranex displays variable barrier properties when compared with screw cap saran tin (Lopes, 2005). This poor performance may either be due to the sealing operation, which was unable to redraw properly the liner over the bottleneck and hence form a proper seal, or to the intrinsic compositional properties of the saranex liner. Additional experiments should be conducted in order to provide a deeper understanding of the performance of this type of screw cap.

The results obtained in this study suggest that there is a relationship between the gas barrier properties of the different closures and the permeation of volatile compounds. Synthetic closures, permeable to atmospheric oxygen, are also more permeable to volatile phenols and TCA than screw cap saranex. Likewise, synthetic 1 is also more permeable to oxygen and the above-mentioned compounds than synthetic 2. Therefore, conditions favouring oxygen and air permeation through closures can also allow the ingress of other exogenous volatile compounds, which can be detrimental to the quality of bottled wines.

It is proposed that synthetic closures and screw caps are “permeable closures”, allowing the permeation of volatile compounds into bottled wines through the solution-diffusion effect mechanism. According to this mechanism, contaminants sorb to the outer portion of the closure, diffuse through it in response to the concentration gradient, desorb into the headspace, and finally dissolve into the wine (Robertson, 2009). The results obtained in this study suggest that the pore effect mechanism occurred in one screw cap at 30 months, which strongly affected the average value of the group. This screw cap could have had some cracks or pores that allowed the gas transfer.

This experiment has proven the importance of permeation of contaminants through different types of wine closures. This study showed that aerial contamination followed by migration into bottled wine after bottling is possible in the wine industry via migration of volatile taints through permeable closures.

CONCLUSION

This work aimed to determine if exposure to aerial contamination can induce the migration of volatile compounds through different wine closures after bottling. The study focused on the migration of volatile phenols and TCA through natural and microagglomerate cork stoppers and synthetic and screw cap closures used to seal bottled wines stored in a contaminated atmosphere. Cork stoppers have proven to be an effective barrier to the migration of exogenous d_5-TCA, d_4-E4P and d_5-E4G, which were confined to their outer portions. In contrast, those compounds were able to migrate through synthetic and screw cap closures and into the wine.

The current study provides useful insights on the gas barrier properties of different commercial closures, which may affect the chemical and sensory properties of wines after bottling. Exposure of the bottled wines to tainted environments over a period of several months can allow the migration of tainting compounds through permeable closures into the wine and therefore negatively affect its quality. Therefore, the barrier effectiveness of each closure is a parameter that should be taken into account by closure users during the purchase process in order to guarantee the full protection of the bottled wine and thus prevent its contamination after bottling.

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