



**ORIGINAL RESEARCH ARTICLE**

# Migration of polyphenols from natural and microagglomerated cork stoppers to hydroalcoholic solutions and their sensory impact

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## ABSTRACT

During bottling aging, the wine comes into contact with the cork stopper due to the horizontal position of the bottle. The release of compounds, such as cork phenolic compounds, thus take place between the cork and the wine, depending on the type of cork stopper and the surface treatments applied. Many publications describe the extraction of these phenolic compounds in wine or hydroalcoholic solutions from natural corks, but few address microagglomerated corks, which are increasingly used by winemakers to seal their bottles. The aim of this study was therefore to compare the polyphenols, mainly hydrolysable tannins, transferred from natural and microagglomerated corks treated with supercritical CO<sub>2</sub> into hydroalcoholic solutions. For this purpose, polyphenols released in macerates of natural and microagglomerated cork stoppers were identified and quantified by HPLC-DAD-ESI-QQQ. Suberic acid was also quantified. In this study, despite the high intra-“natural cork stopper” variability, significant differences were found between both types of stoppers for all polyphenols, the agglomerated corks releasing significantly less polyphenols; i.e., 25 times less. In contrast, suberic acid was extracted from both types of corks in similar concentrations; therefore, its extractability was not impacted by the type of stopper. A sensory profile was also carried out on the macerates. Macerates of natural cork stoppers were perceived with notes of “cardboard, dust, plank, wood” and “cork taint” significantly higher than supercritical CO<sub>2</sub> treated microagglomerated cork stopper macerates. Moreover, the natural cork macerate with the highest content in polyphenol was perceived as being more bitter than that of microagglomerated cork stoppers.

**KEYWORDS:** natural cork stoppers, microagglomerated cork stoppers, polyphenols, ellagitannins, suberic acid, sensory impact, aging

## INTRODUCTION

Wine closure - in addition to grape quality, the winemaking process and storage conditions - plays an important role in the evolution and quality of wines after bottling (Echave *et al.*, 2021; Silva *et al.*, 2011). The wine closure industry is mainly divided into three categories: screw caps, synthetic closures and cork-based closures (Furtado *et al.*, 2021). Of the cork stoppers, three main types are industrially available for closing wine bottles: natural cork stoppers, colmated cork stoppers and technical stoppers, including agglomerated cork stoppers (0.25-8.00 mm granules), microagglomerated cork stoppers (0.25-3.00 mm granules) (ISO 633:2019) and stoppers with an agglomerated central part with two natural cork discs glued to one or both ends of the stopper (Azevedo *et al.*, 2022; Furtado *et al.*, 2021; Gil, 2009).

Natural cork stoppers are produced by punching the cork from the oak tree bark of *Quercus suber* L. Its production area corresponds to the western Mediterranean basin, Portugal and Spain being the main producers and suppliers of cork (Eriksson *et al.*, 2017). For example, natural cork stoppers led production within the cork stopper segment in 2020 in Portugal (APCOR., 2020). Cork is a protective tissue of the oak tree, which is highly structured due to an arrangement in regular layers of small watertight cells with a tangential honeycomb section filled with air (Azevedo *et al.*, 2022; Oliveira and Pereira, 2020). The main cell wall components of *Quercus suber* cork are suberin, lignin and polysaccharides (cellulose and hemicelluloses); according to Pereira (2007b), they represent  $\approx 50\%$ ,  $20\text{--}25\%$  and  $20\%$  of the total material respectively. Other compounds, such as extractives, are found in  $14\text{--}18\%$  of total cork material (Pereira, 2007b); these are defined as “low or medium molecular weight molecules that may be removed from the cells by solvent extraction without affecting the cellular structure of the material and its mechanical properties” (Pereira, 2007b). The structure and composition of cork give it mechanical and physical properties that make it an ideal material for the elaboration of wine corks. Indeed, cork is light and compressible, with an elastic memory (Anjos *et al.*, 2008; Anjos *et al.*, 2014; Pereira, 2013; Rosa and Fortes, 1988) that allows it to gradually regain its initial volume after compression, making it a suitable material for sealing wine bottles. Thanks to these characteristics, it is able to seal the space between the stopper and the bottleneck surface, thus preventing liquid percolation and limiting oxygen permeation (Oliveira and Pereira, 2020). In addition, it has a very low permeability to liquids (Fonseca *et al.*, 2013; González-Adrados *et al.*, 2008; Maga and Puech, 2005; Silva *et al.*, 2005) and low heat transfer properties (Silva *et al.*, 2005), whereas its oxygen permeability range is very wide (Azevedo *et al.*, 2022; Crouvisier-Urien *et al.*, 2018; Faria *et al.*, 2011; Lequin *et al.*, 2012). Regarding oxygen transfer, one of the most important parameters defining the quality of stoppers is the oxygen transmission rate (OTR) (Lopes *et al.*, 2006; Oliveira and Pereira, 2020; Pons *et al.*, 2021; Silva *et al.*, 2011). However, cork tissue is not totally homogenous due to lenticular channels crossing the cork layers.

These lenticular channels can be variable in number and dimension depending on the tree, and are responsible for the porosity of the cork (Pereira *et al.*, 1996). Thus, the occurrence of lenticels on the external surface of a natural cork stopper (i.e., its surface homogeneity/heterogeneity) determines its commercial quality grade (Oliveira *et al.*, 2012; Oliveira *et al.*, 2015). The quality of natural cork stoppers is directly correlated with the quality of the raw material (Pereira *et al.*, 1994). Before being used to close the wine bottle, the raw cork undergoes a series of different treatments. The first step consists in preparing the cork barks in a succession of processes: boiling, stabilisation, sorting according to their quality and gauging. After this preparation, the cork barks can be used for making natural cork stoppers which involves the following steps before packaging: plank slicing, punching, pre-sorting, drying, shape rectification, sorting, washing and draining/spinning, drying, sorting, marking or printing, surface lubrication and sterilisation (Pereira, 2007a; Pinho *et al.*, 2017). Colmated cork stoppers are made from natural cork whose lenticular channels have been filled with a mixture of cork powder and FDA-approved natural resin glues (FDA: US Food and Drug Administration). Agglomerated cork stoppers represent the second main segment of the cork stopper market in Portugal (APCOR., 2020). They are made of cork granules of varying sizes obtained from the cork offcuts from the punching process in the production of natural cork stoppers. As in the case of natural cork stoppers, the granules can be washed in a steam-cleaning process or treated with supercritical CO<sub>2</sub>; this has the advantage of eliminating traces of 2,4,6-trichloroanisole (TCA), which is susceptible of contaminating wine during storage (Hall *et al.*, 2004; Taylor *et al.*, 2000). The cleaned granules are then mixed with water and FDA-approved binder, and sometimes microspheres, before moulding. Then the agglomerated cork stoppers go through more or less the same steps as for natural cork stoppers: shape adjustments, sorting and marking before packaging. All types of cork stoppers undergo a surface treatment which consists in coating the stopper with a lubricant film, usually using paraffin and silicone of food grade quality (Pereira, 2007a). This facilitates the insertion and the extraction of the stopper from the bottleneck, as it reduces friction, and also improves the sealing capacity of the cork stopper (Gonzalez-Adrados *et al.*, 2012).

After bottling and capping, the horizontal positioning of the wine bottles results in the contact of the cork stopper with the wine and thus the transfer of volatile and non-volatile compounds from one medium to another. The desorption of volatile compounds from cork stoppers to wine model solutions and wine has been reported in several studies (Culleré *et al.*, 2009; Mislata *et al.*, 2020; Moreira *et al.*, 2016; Pinto *et al.*, 2019; Prat *et al.*, 2011; Slabizki *et al.*, 2016). Alcohols, aldehydes, ketones, esters and terpenes (Furtado *et al.*, 2021) can be transferred to wine. Some compounds can positively contribute to the aroma of the wine, such as esters or terpenes, bringing “fruity” and “flowery” notes (Culleré *et al.*, 2009; Furtado *et al.*, 2021). Meanwhile, other compounds are associated with unpleasant notes, as is the well-known case with TCA and other haloanisoles, which give wine a

“cork taint” (Alañón *et al.*, 2021; Chatonnet *et al.*, 2004; Juanola *et al.*, 2004; Prat *et al.*, 2011). Other off-flavours can be released, like “earthy” notes due to geosmin or “green, vegetative” notes due to 3-isobutyl-2-methoxypyrazine (IBMP) (Slabizki *et al.*, 2016). As previously mentioned, some cork treatments, such as steam-cleaning or supercritical CO<sub>2</sub> extraction, can contribute to getting rid of certain off-flavours (De Magalhães Nunes Da Ponte *et al.*, 2013; Hall *et al.*, 2004; Taylor *et al.*, 2000). Inversely to desorption, volatile compounds can also be transferred from the wine to the stopper. This phenomenon, called the “scalping phenomenon” (Blake *et al.*, 2009; Capone *et al.*, 2003; Oliveira *et al.*, 2020; Silva *et al.*, 2012), mainly observed with synthetic closures for which compounds having a low polarity, are able to be adsorbed at their surface (Blake *et al.*, 2009; Capone *et al.*, 2003; Furtado *et al.*, 2021).

Besides the transfer of volatile compounds, non-volatile compounds can also be extracted from cork during wine aging. These compounds belong to the “extractives” group, more specifically those that can be extracted with water and ethanol. These extractives include phenolic compounds of low molecular weight (MW < 300), like benzoic and cinnamic acid derivatives (vanillic, protocatechuic, gallic, ferulic and caffeic acids), phenolic and cinnamic aldehydes derivatives (vanillin, protocatechuic aldehyde, syringaldehyde, coniferaldehyde and sinapylaldehyde) and coumarine derivatives (aesculetin and escopoletin) (Azevedo *et al.*, 2014; Conde *et al.*, 1997; Fernandes *et al.*, 2011; Mazzoleni *et al.*, 1998; Peña-Neira *et al.*, 1999; Varea *et al.*, 2001). More complex polyphenols (MW > 300), such as condensed tannins (proanthocyanidins) and hydrolysable tannins, gallotannins and ellagitannins, can also be extracted. Proanthocyanidins have been found in cork from Spanish oak trees at  $0.8 \pm 0.4$  mg/g cyanidin equivalents of dry cork (Cadahía *et al.*, 1998) and from Algerian oak trees at  $2.99 \pm 0.29$  mg/g catechin equivalents of dry cork (Touati *et al.*, 2015). More recently, Reis *et al.* (2019) evaluated the content in condensed tannins of defatted and dried cork granulates at  $2.3 \pm 0.5$  mg proanthocyanidin fraction equivalents per gram. However, no identification of these compounds has been reported in the literature, probably due to the high polymerisation degree making their identification difficult (Reis *et al.*, 2020a). Gallotannins, which are gallic acid polymers, represent an important extractable group of phenolic compounds present in cork. Trigalloyl-glucose, tetragalloyl-glucose, pentagalloyl-glucose, HHDP-galloyl-glucose, HHDP-digalloyl-glucose, di-HHDP-galloyl-glucose, and trigalloyl-HHDP-glucose have been identified by Fernandes *et al.* (2011). Among the phenolic compounds, ellagitannins, ellagic acid polymers and other ellagic acid derivatives can also be easily extracted from cork using hydroalcoholic solutions. Several studies have highlighted the presence of roburins A, D and E, grandinin, vescalagin and castalagin (Azevedo *et al.*, 2014; Cadahía *et al.*, 1998; Fernandes *et al.*, 2011; Reis *et al.*, 2019; Reis *et al.*, 2020a), castalagin being the main ellagitannin to have been quantified in cork (Cadahía *et al.*, 1998; Varea *et al.*, 2001). Varea *et al.* (2001) did not find any significant differences in the migration

of castalagin, vescalagin and grandinin in a model wine solution between different types of cork stoppers (uncoated and coated natural cork stoppers, two- or four-piece cork stoppers, agglomerated cork stoppers with or without two disks), although natural cork stoppers yielded, in general, higher amounts of polyphenols. Other ellagitannins, such as vescalin, castalin, vescavalonic and castavalonic acids, HHDP-glucose, di-HHDP-glucose, mongolicain A/B, ellagic acid-pentose and ellagic acid-deoxyhexose, have also been identified (Fernandes *et al.*, 2011; Reis *et al.*, 2020a). Using HPLC-DAD/ESI-MS, total ellagitannins in cork granulates was estimated to be between 15 and 32 mg/g by Reis *et al.* (2020b). Some others acid gallic/ellagic derivatives have also been extracted from cork, such as valoneic acid and sanguisorbic acid dilactones and their derivatives, isomers of dehydrated tergallic-C-glucoside (Fernandes *et al.*, 2011; Reis *et al.*, 2020a). Isomers of the latter have also been found by Reis *et al.* (2020b) in cork stoppers originating from different areas of Spain and Portugal at a total concentration of between  $2.6 \pm 0.8$  and  $8.3 \pm 1.0$  mg/g of cork granulates. Free ellagic acid has also been found in wine model solution at a concentration of between 0 and 0.3 mg/L gallic acid eq. depending on stopper type and cork surface treatment after 18 months of bottling (Azevedo *et al.*, 2014). The release of phenolic compounds from cork seems to depend on the type of cork (Azevedo *et al.*, 2014; Gabrielli *et al.*, 2016). Low quality and/or uncoated cork stoppers are both factors that cause a higher release of phenolic compounds, which can lead to protein haze in white wines (Gabrielli *et al.*, 2016). Finally, suberic acid, an octanedioic acid, can also be extracted from cork, as it is a component of suberin, which is highly present in cork (Velez Marques *et al.*, 2016).

The migration of phenolic compounds - mainly hydrolysable tannins, including ellagitannins - from the cork stopper to a hydroalcoholic solution, such as wine, can have an organoleptic impact on the latter. Indeed, these compounds are known to contribute to bitterness and astringency (Glabasnia and Hofmann, 2006). The sensory perception of ellagitannins have been particularly studied during wine barrel aging, when these compounds were released from wood to wine. Chira and Teissedre (2015) showed that wine matured in lightly toasted barrels, which had the highest ellagitannin content compared to wines aged in more toasted barrels, was perceived as less sweet and at the same time bitter and more astringent. In their study on wines from different countries matured in oak barrels subjected to different toasting methods, González-Centeno *et al.*, (2016) also found that astringency and bitterness were significantly linked to ellagitannin levels. The migration of such compounds from cork stoppers to wine could thus have a sensory impact.

A lot of publications have dealt with the composition of cork from *Quercus suber* L. or the migration of phenolic compounds from natural cork stoppers to wine or hydroalcoholic solution, but only a few have addressed the migration of these compounds from agglomerated cork stoppers. Therefore, this study aimed to compare commercial natural cork stoppers with commercial microagglomerated cork stoppers treated



with supercritical CO<sub>2</sub> for the extraction of polyphenols (mainly hydrolysable tannins) into hydroalcoholic solution; this was done by identifying and quantifying the polyphenols using HPLC-DAD-ESI-QQQ. A sensory profile was also performed to identify any sensory differences between the macerates obtained from the two types of cork stoppers.

## MATERIALS AND METHODS

### 1. Chemicals

Water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). Ethanol of high-performance liquid chromatography (HPLC) grade were purchased from VWR International (Pessac, France). Suberic acid, gallic acid and ellagic acid were purchased from Merck (Darmstadt, Germany). Vescalagin was extracted from oak wood powder and previously purified in our laboratory (Gadrat *et al.*, 2022). Methanol and formic acid of LC-MS grade was purchased from Fisher Chemical (Illkirch, France).

### 2. Cork macerates

#### 2.1. Corks

Batches of eight different commercial natural cork stoppers (N1 to N8, 44 mm x 24 mm) with different quality grades (Fleur, Extra, Super) from different cork producers, and eleven different microagglomerated cork stoppers (A1 to A11, 44 mm x 24 mm) produced by Diam bouchage (Céret, France) treated with supercritical CO<sub>2</sub> were used in this study. Natural and microagglomerated cork stoppers received the same surface treatment consisting of a commercial paraffin/silicone (60/40) emulsion applied at 40 mg/cork.

#### 2.2. Hydroalcoholic macerations

Hydroalcoholic macerates to determine the polyphenol composition of both types of cork stoppers and the inter- and intra-“type of cork stopper” variability:

For each eight natural (N1 to N8) and eleven microagglomerated (A1 to A11) cork stopper batches, six cork stoppers were kept in 500 mL Schott (borosilicated glass) with 400 mL of 12 % (v/v) hydroalcoholic solution (ethanol) for 10 days at 40 °C to perform an accelerated extraction of the soluble compounds. The nineteen macerates were then stored at 4 °C before sample preparation.

Hydroalcoholic macerates to evaluate the intra-batch variability:

In order to evaluate the intra-batch variability, an additional series of macerates were prepared as described above from batches of cork stoppers: A1, A4, A5, N2, N4, N5, N6, and N7. Regarding batch N3, two additional macerates were prepared. These new macerates were renamed as follows: a “bis” suffix was added when they were prepared for a second time and a “ter” suffix if it was for the third time.

### 3. Phenolic compounds analysis by HPLC-DAD-QQQ

#### 3.1. Sample preparation

One mL of homogenised hydroalcoholic macerates was evaporated to dryness under reduced pressure to remove the ethanol. The dry extract was dissolved in 1 mL of Milli-Q water filtered through 0.45 µm PTFE syringe filter before HPLC injection.

#### 3.2. HPLC-DAD analyses

The samples were analysed by HPLC with an Agilent 1200 Infinity series system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector (DAD) according to Gadrat *et al.* (2021). The used column was a Kinetex column (150 × 3 mm, 2.6 µm particle size, Phenomenex, Le Pecq Cedex, France). Two solvents were used for elution: acidified water at 0.1 % formic acid (eluent A) and acidified methanol at 0.1 % formic acid (eluent B). The flow rate was set at 400 µL/min, and the injection volume was 10 µL. The gradient of eluent B was adapted as follows: 8 % from 0 to 13 min; 8 to 25 % from 13 to 15 min, 25 to 60 % from 15 to 30 min, 60 to 98 % from 30 to 31 min, 98 % from 31 to 36 min, 98 to 2 % from 36 to 37 min, 2 % from 37 to 40 min; then the HPLC column was equilibrated for 4 min using the initial conditions before the next injection. The DAD signals were carried out at 280 nm and 250 nm wavelengths.

#### 3.3. QQQ analyses

MS analyses were performed using a 6460 Triple Quadrupole mass spectrometer equipped with a heated electrospray ionisation probe (both from Agilent Technologies, Waldbronn, Germany) connected to the HPLC system via the DAD cell outlet. The calibration of the mass analyser was realised each week using an ESI-L Low Concentration Tuning Mix (Agilent Technologies, Waldbronn, Germany). The targeted screening was performed with ionisation and optimised spectrometric parameters in negative mode, as described by (Gadrat *et al.*, 2021): gas temperature and flow were 350 °C and 5 L/min respectively; sheath gas temperature and flow were 250 °C and 10 L/min respectively; and capillary voltage was 4500 V.

For the quantification of the different compounds present in the cork extracts, mass acquisitions were performed and optimised in negative ionisation mode, and the cell accelerator voltage was 8 V. All data were processed using MassHunter Qualitative Analysis software (Agilent Technologies, Waldbronn, Germany).

#### 3.4. Quantification of compounds

Quantification was carried out using external standards. Response of ellagic acid at UV 250 nm was used to quantify ellagic acid and its derivatives (0.05-200 mg/L; R<sup>2</sup>=0.999; LOQ=0.07 mg/L; LOD=0.02 mg/L). For other compounds, quantification was done using SIM mode with gallic acid (*m/z* 169; 0.01-50 mg/L; R<sup>2</sup> = 0.993; LOQ = 0.03 mg/L; LOD = 0.01 mg/L), vescalagin (*m/z* 933.1; 0.05-200 mg/L;

$R^2 = 0.999$ ; LOQ = 0.14 mg/L; LOD = 0.04 mg/L) and suberic acid ( $m/z$  173.1; 0.01-50 mg/L;  $R^2 = 0.998$ ; LOQ = 0.012 mg/L; LOD = 0.004 mg/L) as standard for gallic acid derivatives, ellagitannins and suberic acid respectively. Selected ions  $[M - H]^-$  were 169, 173.1, 301, 469, 481.1, 550 (for 1101), 602.2 (for 1205), 613.1, 631.1, 783.1, 924.2, 933.1, 961.1, 990.2, 1065.1 and 1101.1  $m/z$ .

#### 4. Sensory analysis

The sensory analysis session was carried out by experienced assessors from the Oenology Research Unit at ISVV (University of Bordeaux). The session took place in a thermo-regulated room at 20 °C with controlled hygrometry (ISO 8589:2007) in individual booths. For each test, 20 mL of solution was presented in black glasses (NF V 09 110).

The different macerates were pooled in equivalent volumes according to the results of an Ascending Hierarchical Classification (ACH) that was based on a Principal Component Analysis (PCA) of the polyphenol content of the eight natural and eleven agglomerated cork stopper macerates. Thus, only four samples were numbered with 3-digit codes and presented randomly in a balanced manner. Thirteen judges were present during the session. They were asked to score the intensity of different descriptors on a continuous 8 cm scale. Zero cm corresponded to the lowest intensity and 8 cm to the maximum intensity for the considered descriptor. The following olfactory, mouthfeel and taste descriptors were evaluated: "vanilla", "spicy", "cardboard, dust, plank, damp wood" notes, "cork taint" odour for the olfactory descriptors and "sweetness", "acidity", "bitterness" and "astringency" for the taste and mouthfeel descriptors.

For each descriptor, the collected data were then processed in PCA analyses using samples as individuals and judges as variables to highlight the judge consensus (Supplementary Figure 1). The data were then centered and reduced according to each judge to avoid different use of the scoring scale by the judges. The recalculated data were processed by a one-way ANOVA to highlight any differences between the samples evaluated for the descriptors. For each descriptor and each sample, results were presented as the mean score with error bars according to confidence interval.

#### 5. Statistical data analyses

All data were statistically processed using RStudio software (Version 1.1.463 - © 2009-2018 RStudio, Inc.) for ANOVA and the RCMR package with FactmineR plugin for PCA analysis.

ANOVAs were performed on polyphenol and suberic acid contents to highlight possible differences between natural and microagglomerated cork stoppers. When compounds were detected as traces (tr) or not detected (nd) in a sample, the ANOVA was performed by taking the LOQ/2 and LOD/2 values respectively for these compounds. Different letters show significant difference between natural and microagglomerated cork stoppers for a compound with  $\alpha = 0.05$ .

The PCA was performed on the polyphenol and suberic acid contents of the hydroalcoholic macerates obtained from the nineteen batches of cork stoppers; i.e., eight from natural cork stoppers (N1 to N8) and eleven from microagglomerated cork stoppers (A1 to A11). In order to evaluate the intra-batch variability, the additional macerates, A1bis, A4bis, A5bis, N2bis, N3bis, N3ter, N4bis, N5bis, N6bis, and N7bis were included in the previous PCA as additional individuals (illustrative individuals) not contributing to the correlation circle.

## RESULTS AND DISCUSSION

### 1. Identification and quantification of polyphenol and suberic acid from natural and microagglomerated cork stopper macerates

#### 1.1. Identification of compounds

The macerates of eight natural (N1 to N8) and eleven agglomerated cork stoppers treated with supercritical CO<sub>2</sub> (A1 to A11) were analysed by HPLC-MS in order to identify and quantify their polyphenol content. Example of chromatograms of one of each type of stopper were presented in Figure 1. Thirty compounds were identified in the natural cork stopper macerate, whereas only nine of them were detected in the microagglomerated cork stopper macerate.

All the compounds were identified on the basis of their  $[M-H]^-$  mass, their retention time, literature references (Gadrat *et al.*, 2021; Reis *et al.*, 2020a; Saucier *et al.*, 2006) and standards when available. The labels of the identified peaks from Figure 1 are shown in Table 1. The compounds gallic acid (9) and its dimeric form, free ellagic acid (30), were identified. Both were present in the natural and microagglomerated cork stopper macerates. Simple ellagic acid derivatives were also identified as esters of hexahydroxydiphenic acid (HHDP): HHDP-glucose isomer 1 (3) and 2 (4) and bis-HHDP-glucose isomers 1 (5), 2 (15), 3 (17), and 4 (20) at  $m/z$  481 and  $m/z$  783 respectively. HHDP-glucose isomers and bis-HHDP-glucose isomers 3 and 4 were found in the macerates of both types of stoppers. A large number of C-glycosidic ellagitannins were identified in the natural cork extracts, of which the following monomeric ellagitannins: vescalalin (1), castalin (2), vescalagin (14), castalagin (19), vescalalonin and castavalonin acids (12 and 16 respectively), and the lyxose/xylose-bearing monomers, grandinin (10) and roburin E (13). Two peaks at  $m/z$  961 (18 and 23) were found in the natural cork macerates, with a retention time of 12.7 and 19.0 respectively. Peak 18 was identified as ethylvescalagin, probably formed from vescalagin and ethanol of the hydroalcoholic solution (Gadrat *et al.*, 2022; Saucier *et al.*, 2006). On the other hand, peak 23 remains unknown to date. Dimeric ellagitannins were also found in the natural cork stopper macerates: roburins A (7) and D (11) and the lyxose/xylose-bearing dimers, roburins B (6) and C (8), the latter two being, to our knowledge, identified for the first time in natural cork. More complex ellagitannins were also present in the natural cork macerates: the flavano-ellagitannins

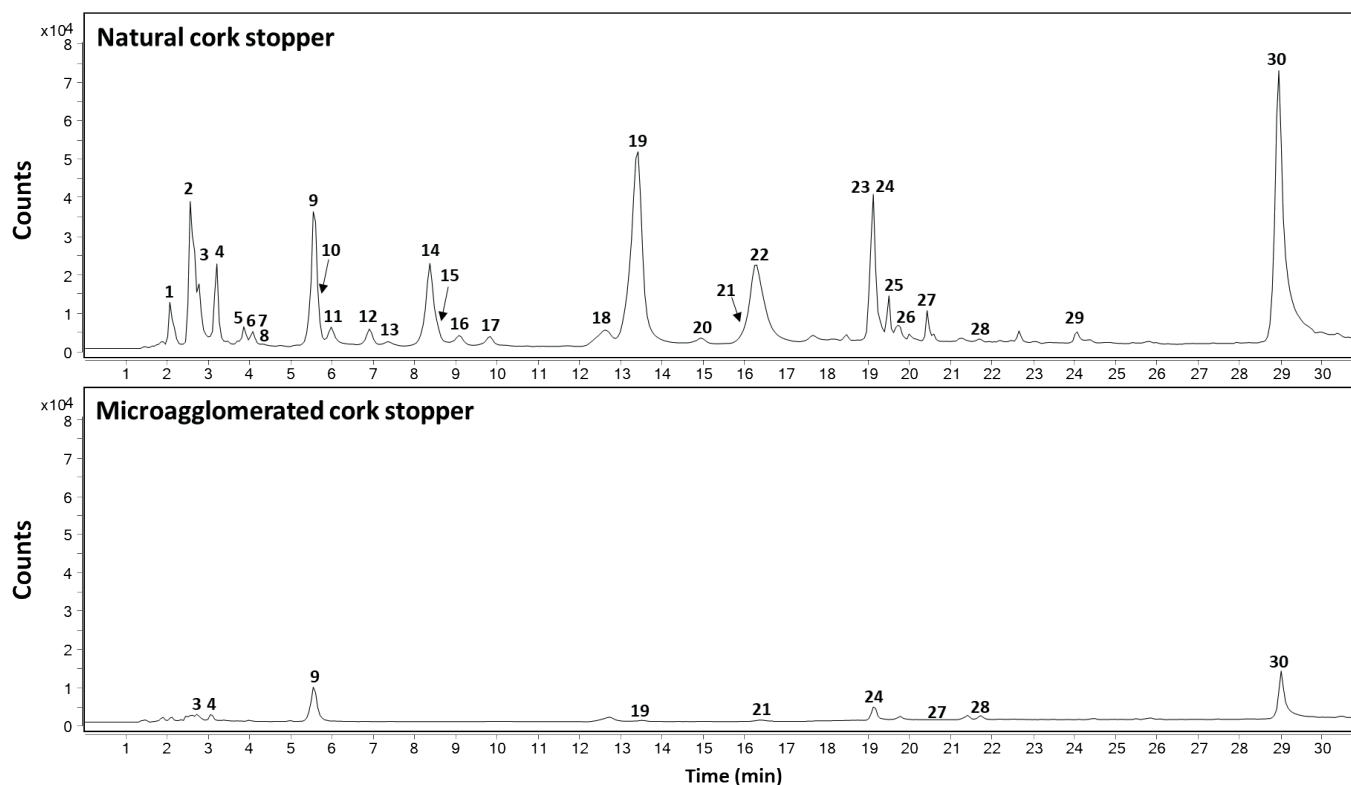
acutissimin A/B (25) and mongolicain A/B (27). The latter was also found in the agglomerated cork extracts. Others gallic and ellagic acid derivatives were observed in the macerates of both types of cork: the valoneic acid dilactone (22) and the three isomers of dehydrated tergallic-*C*-glucose (21, 24 and 26). The sanguisorbic acid lactone (29), an isomer of valoneic acid dilactone, was found only in the natural cork stopper extract. Finally, although it was not a polyphenol, suberic acid (28), extracted from suberin present in cork, was also identified in both types of cork macerates.

## 1.2. Quantification of phenolic compounds and suberic acid

All identified polyphenols were quantified in the nineteen macerates. The mean content of each compound in the eight natural and the eleven microagglomerated cork stoppers is given in Table 1.

The mean total polyphenol content of the macerates from the microagglomerated cork stoppers was found to be about 25 times lower than that from natural ones after 10 days at 40°C. The majority of the identified polyphenol compounds were either present in very low concentrations (< 0.40 mg/L) or absent in the extracts of agglomerated cork treated with supercritical CO<sub>2</sub>, compared to the macerates from the natural corks. Indeed, although castalagin and vescalagin were present at mean concentrations of 50.53 ± 28.13 mg/L and 11.98 ± 8.56 mg/L respectively in the natural cork macerates,

very little was extracted from the microagglomerated corks, with concentrations of 0.37 ± 0.61 mg/L and trace amounts respectively. Castalagin was found to be the most abundant of the *C*-glycosidic ellagitannins extracted from the natural corks. These results are in agreement with those of Varea *et al.* (2001) and Reis *et al.* (2019), who found castalagin in higher concentrations than other *C*-glycosidic ellagitannins. For Reis, *et al.* (2020b), castalagin was not the most abundant ellagitannin, but it was one of the most present compounds in cork stoppers from different geographical origins in Spain and Portugal. The differences in the observations between these authors may also be due to the standards used for the quantification of the compounds. Varea *et al.* (2001) also compared *C*-glycosidic ellagitannins, which are susceptible to migrating from different cork stoppers to wine, including four natural cork stoppers and one agglomerated cork stopper. In contrast to the present study, they found no significant differences in terms of castalagin, vescalagin, grandinin and roburin E concentrations between natural and agglomerated cork stoppers. These contradictory results could be explained by the fact that the agglomerated corks in these studies did not undergo the same treatments during their manufacture. In the present study, the agglomerated cork stoppers were treated with supercritical CO<sub>2</sub>, but no information was given about this by Varea *et al.* (2001). Indeed, the supercritical CO<sub>2</sub> treatment was found to extract phenolic compounds from vegetal material, such as low molecular phenolic compounds, proanthocyanidins and hydrolysable tannins



**FIGURE 1.** Example of total ion chromatograms (TIC) obtained after HPLC-MS analysis of hydroalcoholic maceration samples (10 days at 40°C) of natural (N1) and microagglomerated (A3) cork stoppers.

Label of peak numbers is shown in Table 1.

**TABLE 1.** Quantification methodology and polyphenol content (mg/L) of hydroalcoholic macerates from natural and microagglomerated corks.

N <sup>o1</sup>	Compounds	M-H	RT <sup>2</sup>	SIM/UV <sup>3</sup>	Quant. <sup>4</sup>	Natural corks <sup>5</sup>	Microagglomerated corks <sup>6</sup>
9	gallic acid	169	5.6	169	gall	4.67 <sup>a7</sup> ± 1.15	0.70 <sup>b</sup> ± 0.45
30	ellagic acid	301	29.0	UV <sub>250nm</sub>	ella	5.32 <sup>a</sup> ± 2.48	1.39 <sup>b</sup> ± 1.02
22	valoneic acid dilactone	469	16.5	UV <sub>250nm</sub>	ella	3.06 <sup>a</sup> ± 2.44	tr <sup>8</sup> <sup>b</sup>
29	sanguisorbic acid dilactone	469	24.1	UV <sub>250nm</sub>	ella	0.18 <sup>a</sup> ± 0.12	nd <sup>10</sup> <sup>b</sup>
3	4,6-HHDP-Glc <sup>9</sup> isomer 1	481	2.6	481	vesc	7.34 <sup>a</sup> ± 3.24	0.95 <sup>b</sup> ± 0.98
4	4,6-HHDP-Glc isomer 2	481	3.1	481	vesc	6.70 <sup>a</sup> ± 3.21	0.64 <sup>b</sup> ± 0.43
21	dehydrated tergallic-C-Glc isomer 1	613	16.3	613	gall	3.47 <sup>a</sup> ± 2.70	0.04 <sup>b</sup> ± 0.04
24	dehydrated tergallic-C-Glc isomer 2	613	19.2	613	gall	2.65 <sup>a</sup> ± 1.77	0.44 <sup>b</sup> ± 0.08
26	dehydrated tergallic-C-Glc isomer 3	613	19.9	613	gall	0.24 <sup>a</sup> ± 0.13	0.13 <sup>b</sup> ± 0.02
1	vescalin	631	2.1	631	vesc	2.57 <sup>a</sup> ± 1.50	tr <sup>b</sup>
2	castalin	631	2.5	631	vesc	8.10 <sup>a</sup> ± 4.32	tr <sup>b</sup>
5	bis-HHDP-Glc isomer 1	783	3.9	783	vesc	1.37 <sup>a</sup> ± 0.97	nd <sup>b</sup>
15	bis-HHDP-Glc isomer 2	783	8.6	783	vesc	2.26 <sup>a</sup> ± 1.47	nd <sup>b</sup>
17	bis-HHDP-Glc isomer 3	783	9.9	783	vesc	1.29 <sup>a</sup> ± 0.84	tr <sup>b</sup>
20	bis-HHDP-Glc isomer 4	783	15.0	783	vesc	1.03 <sup>a</sup> ± 0.69	tr <sup>b</sup>
14	vescalagin	933	8.4	933	vesc	11.98 <sup>a</sup> ± 8.56	tr <sup>b</sup>
19	castalagin	933	13.4	933	vesc	50.53 <sup>a</sup> ± 28.13	0.37 <sup>b</sup> ± 0.61
18	peak961	961	12.7	961	vesc	1.34 <sup>a</sup> ± 0.87	tr <sup>b</sup>
23	ethylvescalagin	961	19.0	961	vesc	1.12 <sup>a</sup> ± 0.79	nd <sup>b</sup>
10	grandinin	1065	5.7	1065	vesc	1.64 <sup>a</sup> ± 1.05	nd <sup>b</sup>
13	roburin E	1065	7.4	1065	vesc	0.78 <sup>a</sup> ± 0.55	nd <sup>b</sup>
12	vescavalonic acid	1101	6.9	550	vesc	1.57 <sup>a</sup> ± 1.25	nd <sup>b</sup>
16	castavalonic acid	1101	9.1	550.1	vesc	0.91 <sup>a</sup> ± 0.76	nd <sup>b</sup>
27	mongolicain A/B	1175	20.5	587	vesc	2.32 <sup>a</sup> ± 0.87	tr <sup>b</sup>
25	acutissimin A/B	1205	19.5	602.2	vesc	2.13 <sup>a</sup> ± 1.84	nd <sup>b</sup>
7	roburin A	1850	4.1	924.2	vesc	1.64 <sup>a</sup> ± 1.36	nd <sup>b</sup>
11	roburin D	1850	6.0	924.2	vesc	3.58 <sup>a</sup> ± 2.37	nd <sup>b</sup>
6	roburin B	1982	4.0	990.2	vesc	0.23 <sup>a</sup> ± 0.19	nd <sup>b</sup>
8	roburin C	1982	4.4	990.2	vesc	0.24 <sup>a</sup> ± 0.22	nd <sup>b</sup>
<b>Total polyphenols</b>						<b>130.23<sup>a</sup> ± 71.12</b>	<b>5.00<sup>b</sup> ± 3.47</b>
28	suberic acid	173	21.7	173	sub	0.04 <sup>a</sup> ± 0.01	0.04 <sup>a</sup> ± 0.02

<sup>1</sup> Peak numbers are reported in Figure 1. <sup>2</sup> Retention time. <sup>3</sup> Mode of quantification. <sup>4</sup> Used standard for quantification (gall: gallic acid; ella: ellagic acid; vesc: vescalagin; sub: suberic acid). <sup>5</sup> Mean of eight different natural cork stoppers batches. <sup>6</sup> Mean of eleven different agglomerated cork stoppers batches. <sup>7</sup> Different letters show significant difference between natural and microagglomerated cork stoppers for a compound with  $\alpha = 0.05$ . <sup>8</sup> Traces (<LOQ). <sup>9</sup> Glucose. <sup>10</sup> Not detected.



(Markom *et al.*, 2010; Murga *et al.*, 2000; Murga *et al.*, 2002; Pereira and Meireles, 2010).

Castalin and vescalin were also present in trace amounts in microagglomerated cork macerates compared to natural cork ones. Moreover, C-glycosidic ellagitannins of higher molecular weight than 934 were not extracted at all from the microagglomerated cork stoppers. Only traces of the flavanellagittannin, mongolicain (MW = 1176), were found in the macerates of agglomerated cork closures, in contrast to being one of the most present compound of high molecular weight in the natural cork macerates ( $2.32 \pm 0.87$  mg/L).

The most extracted compounds found in the microagglomerated cork extracts were ellagic and gallic acids, HHDP-glucose isomers and dehydrated tergallic acid-C-glucose isomer 2, with concentrations of between  $0.44 \pm 0.08$  and  $1.39 \pm 1.02$  mg/L; i.e., compounds with a molecular weight below 614. However, these compounds were present in the microagglomerated cork stopper extracts in significantly lower concentrations than in the natural cork macerates, in which ellagic acid and 4,6-HHDP-Glc isomer 2 were four times and ten times more present respectively. Azevedo *et al.* (2014) reported the concentration of gallic acid to be 2 to 3 times higher in the uncoated natural cork stoppers than in the microagglomerated cork stopper (not treated with supercritical CO<sub>2</sub>) after 27 months of bottling at 30°C. Varea *et al.* (2001) found a significant difference in terms of gallic and ellagic acids between the agglomerated and the natural 2nd class cork stoppers (lower concentrations in the agglomerated cork stoppers), but not between the agglomerated cork stoppers and each of the uncoated, special class and 4th class (pore-filled) natural stoppers respectively; however, the released concentrations of gallic and ellagic acids by the agglomerated cork stoppers were systematically lower than those released by the natural cork stoppers.

Finally, suberic acid was also quantified in the extracts and was found at a concentration of 0.4 mg/L regardless of the type of stopper. No differences were highlighted between the

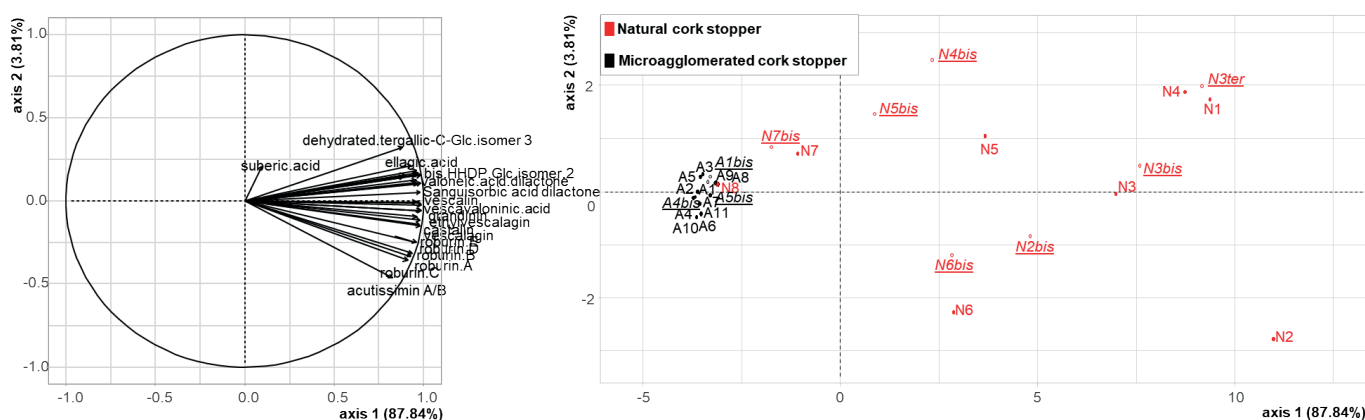
natural and microagglomerated cork stoppers, unlike in the case of polyphenols.

## 2. Overall distribution of natural and agglomerated cork stopper in terms of polyphenol content

### 2.1. Inter- and intra-“type of cork stopper” variability

The data obtained regarding the polyphenol content of the eight natural and eleven microagglomerated cork stoppers were processed in a Principal Component Analysis (Figure 2).

The two-dimensional PCA representation through Axis 1 and Axis 2 explains more than 91 % of the variability of all individuals, with Axis 1 contributing over 87 %. The latter Axis defined all the identified polyphenols, as these variables are close to the circle (Figure 2A). Only the variable “suberic acid” was not well represented by Axis 1. Thus, Axis 1 accounted for polyphenol content from low (on the left) to high (on the right). In the individual plot (Figure 2B), the macerates from the natural and microagglomerated cork stoppers were distributed in a very distinct way: all the agglomerated cork stopper extracts were closely clustered on the left-hand side of the graph, their respective global polyphenol contents being very low and very similar to each other. This result shows a low intra-group variability within the agglomerated cork stoppers treated with supercritical CO<sub>2</sub> for polyphenol release. In contrast, the macerates from the natural cork stoppers were widely dispersed, with coordinates of -1.5 to more than +10 on Axis 1 (Figure 2B). This dispersion reveals the natural cork stoppers to be very heterogeneous from one batch to another in terms of the migration of polyphenol within hydroalcoholic solution. This highlights that there is a high intra-“type of stopper” variability, which could already be observed in Table 1, showing high standard deviations for natural cork stoppers for almost every compound. The difference in terms of polyphenol migration in hydroalcoholic solution between natural and agglomerated cork stopper can be explained by the differences between the cork manufacturing processes.



**FIGURE 2.** Plots of principal component analysis (PCA, axes 1 and 2) of the polyphenol content of 8 natural cork stoppers and 11 microagglomerated cork stoppers. A: correlation circle of PCA. B: plot of individuals.

In the plot of individuals, underlined individuals represent additional individuals and do not contribute to the correlation circle. A = agglomerated cork stopper and N = natural cork stopper.



Moreover, the wide variability of the natural cork stoppers is probably the result of their varying quality. For example, the presence of lenticular channels, varying in number and dimension at the surface of the stoppers, could explain the greater or lesser extractability of polyphenol, as the surface contact between hydroalcoholic solution and cork differs. Finally, it should be noted that the macerate from N8 corks was the only one present within the group of agglomerated cork stoppers (Figure 2B).

## 2.2. Intra-batch variability

An additional series of macerates was prepared from batches of previously analysed cork stoppers to determine variability within the same batch of stoppers. A greater number of natural corks were chosen, since the type of closure shows the greatest intra-group variability. The polyphenol content of these extracts was also determined by HPLC-MS. These individuals were added in the PCA plot as additional (or illustrative) individuals without contributing to the correlation circle (Figure 2B). All the re-done macerates from both types of cork stopper were located in their respective group. Moreover, A1bis, A4bis and A5bis were close to A1, A4 and A5 respectively, meaning that the agglomerated corks in each batch were homogeneous. This was also the case for N6bis and N7bis. However, the other replicates of macerates from the natural cork, N5bis, N4bis, N2bis and N3ter, were farther from the first extract. This result may reflect the high variability of the natural cork within the same batch.

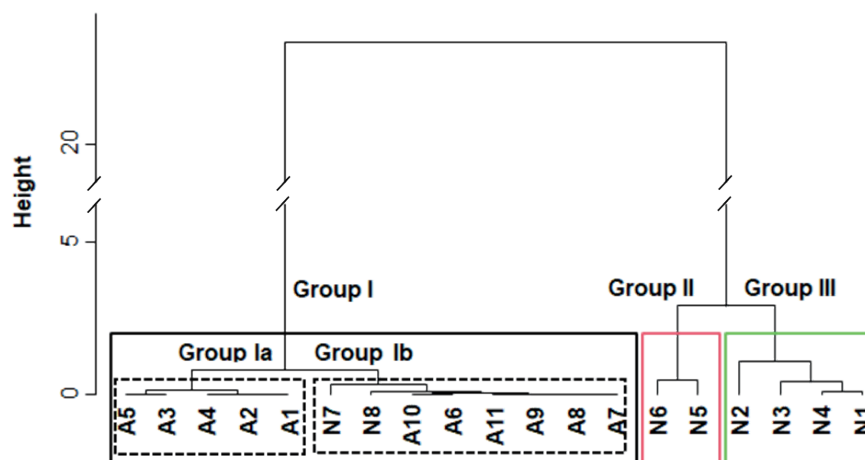
In general, there were three levels of variability in the extractability of polyphenols from the cork stopper into the hydroalcoholic solution. The first depended on the type of closure: the migration of the polyphenols in hydroalcoholic solution was significantly lower from the microagglomerated cork than from the natural cork. This could be directly related to the manufacturing processes. The agglomerated cork stoppers used in this study were produced using a treatment involving supercritical CO<sub>2</sub>, which is known to remove contaminants, such as TCA (Taylor *et al.*, 2000),

as well as phenolic compounds, such as low molecular phenolic compounds, proanthocyanidins and hydrolysable tannins (Markom *et al.*, 2010; Murga *et al.*, 2000, 2002; Pereira and Meireles, 2010). Thus, the cork granulates used to manufacture microagglomerated corks, which have already undergone an initial extraction with supercritical CO<sub>2</sub>, have less phenolic compounds to release than natural corks. Moreover, this type of stopper, which does not have lenticels, is more homogeneous both on the surface and internally. The second and third levels of variability highlighted in this study concerns only natural corks, with the second level of variability referring to the heterogeneity of natural cork depending on its quality grade. As previously described by Oliveira *et al.*, (2012, 2015) and Pereira *et al.* (1994), the quality of cork correlates with its surface porosity, which in turn is linked to the number and dimension of the lenticular channels; this could explain the more or less high extractability of polyphenols from one batch of natural corks to another. Finally, natural cork can be heterogenous within one batch of the same quality grade. Oliveira *et al.* (2012) demonstrated that the standard deviation of surface parameters, such as porosity coefficient or number of pores, for three quality classes of natural cork stoppers varied highly between stoppers in the same quality class.

## 3. Sensory impact of natural and agglomerated cork stoppers

A sensory profile was carried out on cork macerates to identify any olfactory and gustative differences between them. In order to limit the number of samples to be submitted to the panel of judges, a hierarchical ascendant classification (HAC; Figure 3) was carried out using the PCA data (Figure 2).

Three main groups of cork macerates were identified. Group I was divided into two sub-groups, Ia and Ib. Sub-group Ia comprised only macerates from agglomerated cork (A1 to A5). All the other agglomerated cork macerates were found in sub-group Ib, along with two macerates obtained from the natural cork stoppers (N7 and N8), which were



**FIGURE 3.** Hierarchical ascendant classification based on the PCA of the polyphenol content of 8 natural cork stoppers and 11 microagglomerated cork stoppers.

A = microagglomerated cork stopper and N = natural cork stopper.

closer to the agglomerated cork macerates according to the PCA (Figure 2). Groups II and III, which were closer to each other than to Group I, contained macerates obtained only from natural corks. Thus, based on the results of the HAC, two of the most distant macerates from sub-group Ia (i.e., A1 and A5), sub-group Ib (i.e., N7 and A7) and all the macerates from group II and then group III were pooled in equal volume. These four groups represent four categories of cork stoppers based on the amount of polyphenols they release: very low for Macerate Ia ( $1.24 \pm 0.18$  mg/L), low for Macerate Ib ( $31.02 \pm 28.29$  mg/L), medium for Macerate II ( $118.01 \pm 9.88$  mg/L) and high for Macerate III ( $186.77 \pm 19.50$  mg/L) (Table 2). The four samples thus obtained were presented to a panel of judges for olfactory and gustative evaluation. The descriptors selected and submitted to the panel were those usually used to describe the aroma and taste of corks and the perception of hydrolysable tannins (Chatonnet *et al.*, 2004; Culleré *et al.*, 2009; Simpson *et al.*, 2004; Soares *et al.*, 2020): "vanilla" notes, "spicy" notes, "cardboard, dust, plank, damp wood" notes, and "cork taint" odour as olfactory descriptors, and "sweetness", "acidity", "bitterness" and "astringency" as gustative descriptors.

Regarding the proposed olfactory descriptors, the PCA revealed that the judges did not show a consensus on "vanilla" notes only (Supplementary Figure 1), but significant differences were still found (Figure 4). The judges showed a consensus on the other olfactory descriptors, "spicy" notes, "cardboard, dust, plank, damp wood" notes and "cork taint", and significant differences were found (Figure 4). In terms of all four descriptors, the Macerate Ia, namely that obtained exclusively with microagglomerated corks, was generally perceived as being different from the other samples. It was significantly perceived as being less "spicy", with less "vanilla" and "cardboard, dust, plank, damp wood" notes and as having the lightest "cork taint" odour compared to all three of the other macerates, which were obtained with at least one macerate or exclusively from natural corks. The latter three macerates were perceived as being similar to each other in terms of olfactory notes, but those obtained from natural corks

only (Macerates II and III) tended to be perceived as having more intense negative notes of "cardboard, dust, plank, damp wood" notes and a more pronounced "cork taint" odour. These results are in agreement with those obtained by Bobé and Loisel (2006) and Culleré *et al.* (2009). In their comparative study of the aromatic profile of different kinds of wine cork stoppers, Culleré *et al.* (2009) showed that "spicy" notes (generated by guaiacol) were perceived in natural corks, but not in agglomerated cork cleaned with supercritical CO<sub>2</sub>. Moreover, while "mushroom" notes (generated by octen-3-one) were not perceived in agglomerated cork macerates, they were perceived in natural cork ones. More generally, the authors found that, of the cork stopper macerates, the agglomerated cork ones (cleaned with supercritical CO<sub>2</sub>) had the fewest aromas and the lowest sensory scores for most olfactory descriptors.

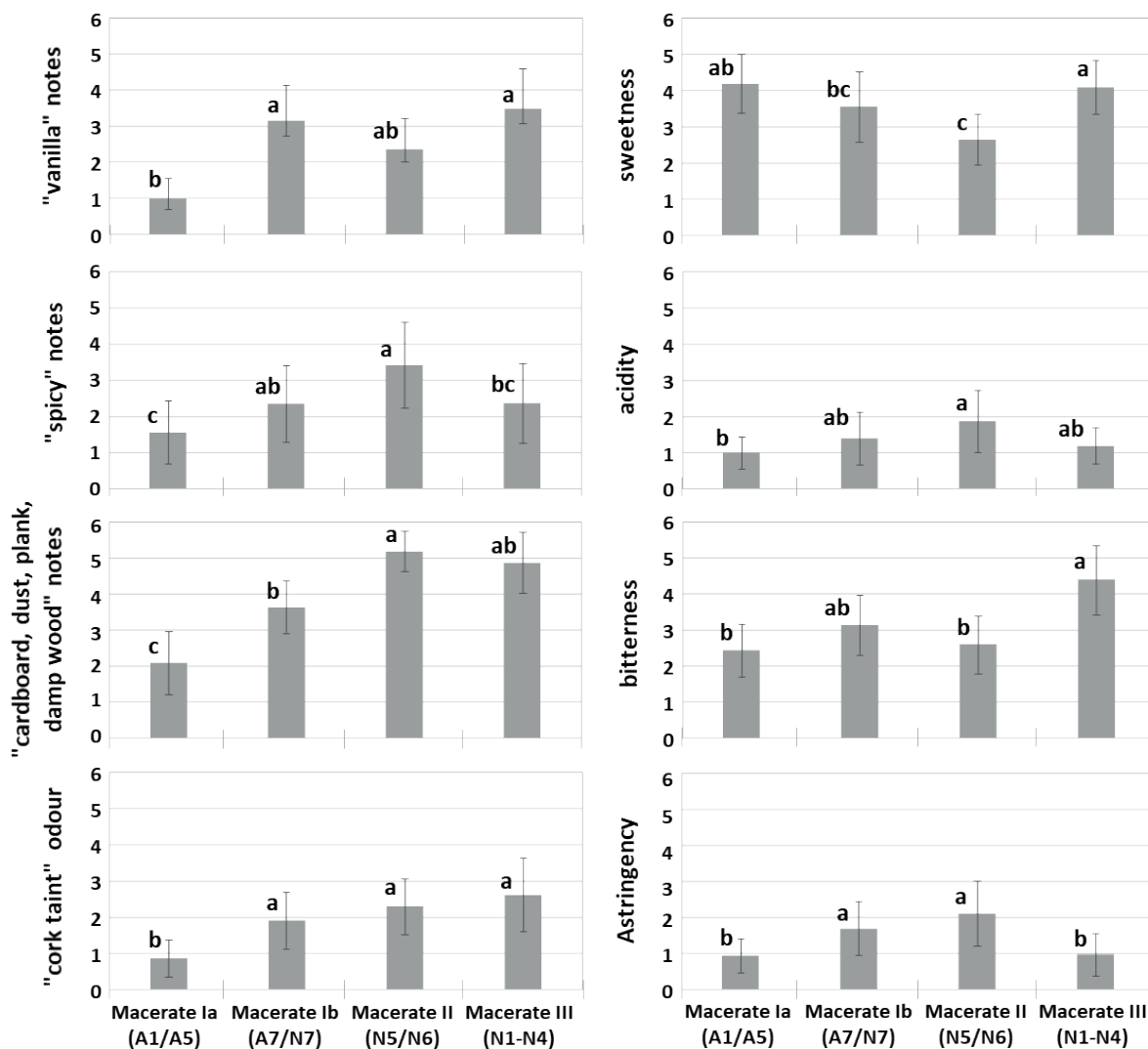
This difference of perception between agglomerated and natural cork samples is therefore the result of the treatments applied during the cork manufacturing process. As mentioned previously, supercritical CO<sub>2</sub> treatment performed on agglomerated cork stoppers removes traces of compounds related to "cork taint" (De Magalhães Nunes Da Ponte *et al.*, 2013; Taylor *et al.*, 2000), as well as other volatile molecules (Lack *et al.*, 2009).

Regarding taste and mouthfeel descriptors, the judges showed a consensus on all the chosen descriptors. Concerning the sweetness of the four samples, Macerate II was perceived as being significantly less sweet than the Macerate Ia and Macerate III samples, but as having a similar intensity to Macerate Ib. It should be noted that of the four assessed samples, Macerates II and III were perceived as being the least sweet and the sweetest respectively, despite both having been obtained from natural cork stoppers. This confirms the variability that can exist within the natural cork stopper group. Concerning "acidity", the scores were inversely related to the "sweetness" ones; the sweeter the sample was perceived, the less acidic it was perceived, and *vice versa*. As regards the "bitterness" descriptor, Macerate III was perceived as being significantly more bitter than Macerate Ia

**TABLE 2.** Polyphenol and suberic acid contents (mg/L) of the four group of cork stopper macerates obtained by HAC1.

	Macerate Ia <sup>2</sup>		Macerate Ib		Macerate II		Macerate III	
gallic acid	0.34 <b>b</b> <sup>3</sup>	± 0.33	2.18 <b>ab</b>	± 1.78	5.30 <b>a</b>	± 1.17	5.17 <b>a</b>	± 0.38
ellagic acid	0.24 <b>b</b>	± 0.18	2.64 <b>b</b>	± 1.00	3.78 <b>b</b>	± 1.85	7.40 <b>a</b>	± 0.94
hydrolysable tannins	0.65 <b>c</b>	± 0.13	26.20 <b>c</b>	± 25.51	108.93 <b>b</b>	± 6.86	174.20 <b>a</b>	± 19.42
<b>total polyphenols</b>	<b>1.24c</b>	<b>± 0.65</b>	<b>31.02c</b>	<b>± 28.29</b>	<b>118.01b</b>	<b>± 9.88</b>	<b>186.77a</b>	<b>± 19.50</b>
suberic acid	0.03 <b>a</b>	± 0.01	0.04 <b>a</b>	± 0.00	0.05 <b>a</b>	± 0.00	0.04 <b>a</b>	± 0.01

<sup>1</sup> Hierarchical ascendant classification based on the PCA of the polyphenol content of eight natural cork stoppers and eleven microagglomerated cork stoppers (Figure 3). <sup>2</sup> Macerate Ia was obtained from macerates A1 and A5, Macerate Ib from macerates N7 and A7, Macerate II from macerates N5 and N6 and Macerate III from macerates N1 to N4, all in equal volume. <sup>3</sup> Different letters show significant difference between groups with  $\alpha = 0.05$ .



**FIGURE 4.** Mean score of eight sensory descriptors for four groups of hydroalcoholic macerates from natural and microagglomerated cork stoppers obtained by thirteen judges.

Macerates Ia, Ib, II and III were obtained by pooling cork stopper macerates A1/A5, A7/N7, N5/N6 and N1/N2/N3/N4 respectively in equivalent volume and according to their proximity based on an ACH (Figure 3). Different letters show significant difference between cork groups with  $\alpha = 0.05$  based on centered-reduced scores.

and Macerate II, but not different from Macerate Ib, although the latter was also scored lower. Macerate III was possibly perceived as being the most bitter due to its higher content in polyphenols (Table 2) - mainly ellagitannins. Indeed, Chira and Teissedre (2015) and González-Centeno *et al.* (2016) have previously found that more bitterness was perceived in wines with higher content of ellagitannin that had come from the oak barrels during aging. They found the same trend for astringency, but this was not the case in the present study, in which significantly higher “astringency” was perceived in Macerates Ib and II. Macerates Ia and III were found to be the least astringent, meaning that, in this study, the perception of astringency did not seem to be related to polyphenol content (Table 2).

Thus, in general, in comparison to natural cork stoppers, commercial microagglomerated cork stoppers seemed to have a very small impact on sensory perception, especially

on olfactory perception, with regards to the proposed descriptors.

## CONCLUSIONS

After bottling, wine undergoes further modifications related not only to its own chemistry, but also to the type of stopper used to seal off the bottle. In addition to possible oxygen transfer through the closure, volatile and non-volatile compounds can migrate from the cork into the wine. This is particularly the case for polyphenols, which are intrinsically present in corks made from natural cork. However, this migration of phenolic compounds depends on the type of cork stopper used. In the present study, very few polyphenols migrated from microagglomerated cork stoppers cleaned with supercritical CO<sub>2</sub> into hydroalcoholic solutions compared to the natural ones. Moreover, the variability in polyphenol

migration from microagglomerated cork stoppers was found to be very low compared to that of the natural cork stoppers, probably due to the differing quality grades of the latter. Only one compound, suberic acid, which was weakly extracted, was found in similar concentrations, regardless of stopper type. From a sensory point of view, microagglomerated stoppers had the least overall impact on the olfactory perception of hydroalcoholic solutions. Further research should be carried out on other types of cork stoppers to study the migration of polyphenols both in hydroalcoholic solutions and in wines. It would also be of interest to identify the compounds present in cork that may be individually responsible for any changes in the sensory perception of wines.

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