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***Demonstration of cork stoppers impact  
in sensorial quality of bottled wine***

Dissertação do 2º Ciclo de Estudos Conducente ao Grau de Mestre  
em Controlo de Qualidade

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**Julho 2019**

É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA DISSERTAÇÃO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.



This work was supported by the CorkPlus 3310 project – “Contribution of cork stoppers to the chemical and sensory properties of bottled wine” co-financed by the European Regional Development Fund (FEDER) through the Operational Programme for Competitiveness and Internationalisation (COMPETE 2020), Portugal, and UID/MULTI/04378/2013 - POCI/01/0145/FEDER/07728.

## ***Acknowledgements***

First of all, I would like to thank my supervisor Dr. Joana Monteiro Pinto for all her dedication and help along this journey. Her guidance was essential for the execution of this thesis. I would like also to thank my co-supervisors Dr. Paula Guedes de Pinho and Professor Maria de Lourdes Bastos for all their support and shared knowledge. At last, a special acknowledgement to Ana Sofia Oliveira, without whom this journey would not be the same. I really appreciated all her patience and kindness in teaching me all the tools I needed to perform this work and for that I am forever grateful.

I would also like to show my gratitude to everyone that participated directly or indirectly in this journey. A huge and special acknowledgement to my parents and remaining family members that, even at a long distance, supported me and all my choices. Lastly, but not least, I want to thank my friends, the ones near and far, for all their support during these years.



## ***Abstract***

The bottle closures can highly impact the volatile composition of wines due to different permeability to oxygen and the absorption and/or migration of volatile organic compounds (VOCs) from and into the wine. The present thesis aimed to assess the effect of VOCs extracted from cork in the volatile composition of bottled wines. In this work, a headspace solid-phase microextraction with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS) approach was used to characterize the VOCs extracted from natural cork stoppers into wine model solution. The results showed that 34 VOCs were extracted from cork into wine model solution, comprising aldehydes, alcohols, benzenoids, furans, monoterpenes and one phenol. In a second study, a HS-SPME-GC-MS/MS approach was developed in order to evaluate if some VOCs were able to divide several grades of natural cork stoppers into two different subclasses of quality. “Flor” was divided into two subclasses based on the levels of camphor and eucalyptol, whereas “Superior” was divided based on the levels of eucalyptol and “Terceiro” based on furfural. For “Extra” cork stoppers, the results were inconclusive. The volatile profile extracted from two different qualities of natural cork stoppers, namely “Terceiro” and “Flor”, by wine model solution during 32 months post-bottling was assessed by HS-SPME-GC-MS. Furthermore, the effect of a washing process (Clean 2000) on VOCs composition of wine model solution sealed with “Terceiro” and “Flor” natural cork stoppers was also studied and the results showed that the VOCs extraction by wine model solutions was, overall, independent of the natural cork grade sealing the bottle or of the disinfection treatment. In this experiment, a study of the migration behaviour of VOCs into wine model solution over time (from 6 to 32 months post-bottling) was made. The results unveiled that several esters, alcohols, aldehydes, ketones and terpenes were extracted from cork into wine model solution, revealing migration behaviour patterns following three different trends. Finally, the effect of different cork stoppers and storage conditions on VOCs composition of a Porto wine from 6 to 26 months post-bottling was evaluated and unveiled statistically significant alterations in the levels of 3-hexen-1-ol, furfural, isoamyl alcohol and  $\beta$ -damascenone, at 15 months. Furthermore, the behaviour of the 34 VOCs quantified at 6, 15 and 26 months post-bottling showed alterations on VOCs levels over time that followed three different trends. The main results suggested that the wine suffered some grade of oxidation, which is supported by the increase of benzaldehyde, furfural and diethyl succinate levels over time.

## **Keywords**

Volatile organic compounds, cork, wine, GC-MS.

## **Resumo**

Os vedantes podem afetar extensivamente a composição volátil dos vinhos engarrafados devido à diferente permeabilidade ao oxigênio e à absorção e/ou migração de compostos orgânicos voláteis (VOCs) do vinho. A presente tese teve como objetivo avaliar o efeito dos VOCs extraídos da cortiça na composição volátil dos vinhos engarrafados. Neste trabalho, a técnica de microextração em fase sólida no *headspace* seguida de cromatografia gasosa acoplada à espectrometria de massa (HS-SPME-GC-MS) foi utilizada para caracterizar os VOCs extraídos de rolhas de cortiça naturais para a solução modelo de vinho. Os resultados demonstraram que 34 VOCs foram extraídos das rolhas cortiça naturais para a solução modelo de vinho, constituindo aldeídos, álcoois, benzenoides, furanos, monoterpenos e um fenol. Num segundo estudo, a técnica de HS-SPME-GC-MS/MS foi utilizada de forma a avaliar a capacidade de diferentes VOCs para dividir diversas classes de rolhas de cortiça naturais (Flor, Superior, Terceiro, Extra) em duas subclasses de qualidade. A classe Flor foi dividida em duas subclasses com base nos níveis de cânfora e eucaliptol, enquanto a classe “Superior” foi dividida apenas com base nos níveis de eucaliptol e a classe Terceiro com base no furfural. Nas rolhas de cortiça Extra, os resultados mostraram-se inconclusivos. O perfil volátil extraído de duas diferentes qualidades de rolhas de cortiça naturais, nomeadamente Terceiro e Flor por solução modelo de vinho durante 32 meses de engarrafamento foi obtido por HS-SPME-GC-MS. Para além disto, o efeito de um processo de lavagem (“Clean 2000”) na composição de VOCs nas soluções modelo de vinho vedadas com rolhas de cortiça naturais Terceiro e Flor também foi estudado e os resultados mostraram que a extração de VOCs pelas soluções modelo de vinho foi, de um modo geral, independente da classe de rolha de cortiça natural a vedar a garrafa ou do tratamento de desinfecção. Nesta experiência, foi também realizado um estudo do comportamento de migração destes compostos ao longo do tempo (de 6 até 32 meses). As soluções modelo de vinho mostraram potencial para extração de ésteres, álcoois, aldeídos, cetonas e terpenos, revelando um comportamento que segue um de três tendências diferentes. Por último, o efeito de diferentes rolhas de cortiça e condições de armazenamento na composição volátil de um vinho do Porto foi verificado e os resultados mostraram alterações estatisticamente significativas nos níveis de 3-hexen-1-ol, furfural, isoamyl alcohol e  $\beta$ -damascenone, aos 15 meses. Para além disto, o comportamento dos 34 VOCs quantificados aos 6, 15 e 26 meses mostraram alterações dos níveis dos VOCs ao longo do tempo a seguirem três tendências diferentes. Os resultados principais sugerem que o vinho possa ter sofrido algum grau de oxidação, o que é suportado pelo aumento dos níveis de benzaldeído, furfural e succinato de dietilo ao longo do tempo.



**Palavras-Chave**

Compostos orgânicos voláteis, cortiça, vinho, GC-MS.

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## ***List of Abbreviations***

**FDA** – Food and Drug Administration

**HS-SPME-GC-MS** – Headspace solid-phase microextraction coupled with Gas Chromatography coupled to Mass Spectrometry.

**IBMP** – 3-Isobutyl-2-methoxypyrazine

**IPMP** – 3-Isopropyl-2-methoxypyrazine

**MPs** – Methoxypyrazines

**MS/MS** – Tandem Mass Spectrometry

**SBMP** – 3-sec-Butyl-2-methoxypyrazine

**3SHA** – 3-Sulfanylhexyl acetate

**TCH** – 2,2,6-Trimethylcyclohexane

**TDN** – 1,1,6-Trimethyl-1,2-dihydronaphthalene

**VOCs** – Volatile organic compounds

# 1. Introduction

## 1.1. The History of Cork

The word “cork” is believed to be derived from the Latin word *cortex*, which means bark [1]. In fact, cork is the outer layer of the oak tree *Quercus suber* L. [1]. Throughout the centuries, cork was used in several applications (e.g., fishing nets, anchors and footwear [2]) for the ancient civilizations [1, 3]. However, the major application that lingered and grew over all these years was certainly their use as wine closures [1]. From what is known, their use as wine closures started with Greek civilization in the fifth century BC (Figure 1), followed by the Roman civilization [1, 4]. However, the cork stoppers started being used more commonly after the fifteenth century, when the glass wine bottles emerged [1]. In the mid-eighteenth century, the first cork stopper handmade industry opened in Spain, but eventually almost all cork stoppers manufacturing industry migrated to Portugal [1]. In 1970s, there were approximately 500 commercial cork manufacturers in Portugal, representing a huge economic factor for the country and half of the cork closures’ world production [1]. Nowadays, the cork properties continue to be studied and explored for all sorts of applications, such as decoration, fashion or everyday objects, due to its unique properties [3]. Portugal continues to be the global leader of cork manufacturing with 50% of the world’s total cork production, providing annually, approximately, 100 000 tons of cork [5].



**Figure 1** – Amphora’s Greek wine from the fifth century BC sealed with cork (reprinted from [4]).

## 1.2. Cork from *Quercus suber* L.

*Quercus suber* L. (Figure 2) is native mainly to the Western Mediterranean, specifically Portugal, Spain, Italy and Morocco [6-8]. This tree grows at temperatures between -5 °C and 40 °C [4] and normally reaches a height of 12-18 m and a width of 2-3 m, presenting a life expectancy between 100 to 300 years [1, 9].



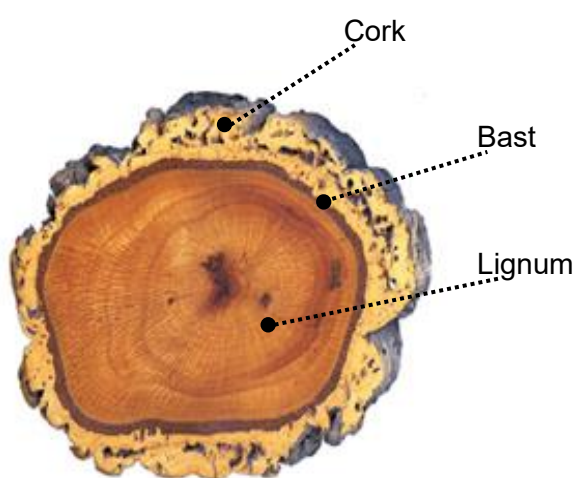
**Figure 2** – *Quercus suber* L. representation (reprinted from [10]).

This evergreen oak possesses an unique feature, namely the ability to continually grow an outer layer in its bark with properties that are valuable for the industry [11]. This means this layer, i.e., cork, can be periodically removed from the tree and that a new layer formation takes place [11]. Cork is botanically designated as phellem and exerts a role of protection of the *Q. suber* L. tree (e.g., protection to temperature variation, against fire, water loss or biological attacks [12]) [13]. Phellem is composed by dead cells that were originated from the phellogen tissue, which comprises cell generation capacity (meristematic) during the spring and summer [13].



### 1.3. Cork harvesting

Cork harvesting consists in removing the outside layer of the oak's stem and branches manually (Figure 3) [14]. This removal step is executed periodically and considering the maintenance of the good physiological condition of the tree, for which experienced hands are needed [15]. The harvesting is performed between the end of the spring and the beginning of the summer as a result of the cork being physiologically active [15].



**Figure 3** – Inner view of *Quercus suber* L. (adapted from [16]).

After the stripping process, the oak is marked with the last digit of the year in which the cork was harvested [14]. The tree is ready to be harvested for the first time only when it reaches a specified width and height (70 cm of circumference when measured at 1.3 m from the ground), which usually occurs at approximately 25 years old [17]. The resultant cork, virgin cork, is hard, irregular and round, making the natural cork stoppers' production impossible [17]. After the first harvesting, the oak is ready to be harvested again in periods of 9-15 years, depending on the geographical region for attaining an increase of 3-3.5 cm in the cork plank's thickness, between the months of May and August [15, 18]. In the second stripping, the cork is less irregular and less hard, though only the third stripping begin to provide cork with the ideal quality for cork stoppers production, which is called amadia cork [17].



**Figure 4** – Cork harvesting (reprinted from [4, 18]).

#### **1.4. Cork stoppers**

Since cork stoppers are the most common product assembled from natural cork [19], in this chapter the main focus is to understand and describe the steps by which they are manufactured.

##### **1.4.1. Production**

After harvesting, the cork planks are stored outdoors for approximately 6 months for stabilization of the texture and for the oxidation of polyphenols (Figure 5) [13, 15, 18]. The next step consists in boiling the planks in water at 95 °C during one hour in order to stimulate their flexibility and expand the lenticels, leading to the increase in volume in about 15% [15], the increase of softness and straightening of the planks [13, 17]. This step also contributes for the reduction of microorganisms' population [13]. The next step consists in drying the planks by stacking them to rest in a well ventilated and sterile area for two or three days, followed by their storage in adequate conditions. Then, the planks are separated accordingly to their quality aspects (thickness, porosity, absence of defects) into categories, being used to different final products [18]. For example, the thickest planks are used for natural cork stoppers production, while thinner planks could be used for the discs production in technical cork stoppers [18].

Cork planks are stored for 6 months in the outdoors after harvesting



Then, they are boiled for cell expansion and microflora's reduction.



Layed to rest in a well-ventilated sterile area for 2 or 3 days



The planks are separated into quality categories

**Figure 5** – Representation of the steps involved in the production of cork stoppers (adapted from [20-22]).

Cork stoppers production is different accordingly to the type. For example, natural cork stoppers are punched out of a whole cork plank using a cylindrical drill. Then, the stoppers are cleaned and disinfected by washing in an aqueous solution of hydrogen peroxide, or by other disinfection techniques using microwave or ozone [13]. The washing step using hydrogen peroxide (or paracetic acid) aims to clean, remove dust and disinfect the cork stoppers and this treatment must not leave a residue higher than 0.2 mg per stopper [23]. Furthermore, the cork stoppers can undergo a treatment in order to reduce its 2,4,6-trichloroanisole (TCA) and other contaminants content. TCA is a chloroanisole mainly responsible for the “cork taint”, a “mouldy” and “musty” off-flavour that is supposedly originated by the presence of fungi [24]. The Amorim group developed the ROSA® process, which is based on a pressurized steam distillation for extracting TCA (70 – 80%) and other contaminants from cork granules for technical stoppers production [15]. Another example is the INOS II process, which is applied in the cork discs used for technical cork stoppers production [15]. This treatment is based on immersing the discs in water and, with varying pressures, extract water-soluble compounds from cork and cleaning the lenticels from small particles, leading also to the reduction of TCA [15]. At last, the cork stoppers are dried in special ovens, selected and then printed [13]. They go through quality control selection tests

and, at the end, before sale, they are sterilized with sulfur dioxide gas and sealed in gas-barrier bags [13].

#### 1.4.2. Type

There are several types of cork stoppers that are used accordingly to the wine's quality, namely:

##### a) Natural

These stoppers are produced from high quality cork planks with no defects (Figure 6). The planks are cut into strips and then perforated with a drill. Then, the stoppers are washed with hydrogen peroxide or paracetic acid (or microwaves and ozone) for washing and disinfection and separated according to their quality. At the end, they are printed (food-quality ink, heat marking or laser marketing [21]), lubricated (silicon or paraffin) and packaged in plastic bags in an inert atmosphere (sulphur dioxide) to impede microorganisms' development [15].



a)



b)

**Figure 6** – a) Natural cork stoppers being punched out from a cork plank (reprinted from [25]); b) example of natural cork stoppers (reprinted from [26]).

Natural cork stoppers are categorized according to their quality in a descending order as “Flor”, “Extra”, “Superior”, “Primeiro”, “Segundo”, “Terceiro”, “Quarto”, “Quinto” and “Sexto” [27]. This classification is done based on the porosity (percentage of lenticels) of the cork stopper [15].

### **b) Colmated**

The colmated cork stoppers (Figure 7) are made by using natural cork stoppers of lower quality (“Quinto” and “Sexto”) whose pores (lenticels) are sealed with cork powder associated with a natural resin or water-based glue approved by Food and Drug Administration (FDA), for a more homogenous stopper surface [15].



**Figure 7** – Example of colmated cork stoppers (reprinted from [28]).

### **c) Agglomerated**

The agglomerated cork stoppers (Figure 8) are the result of high quality natural cork production by-products [29], meaning that it is converted into granules (2 – 8 mm) and glued using FDA-approved glues (e.g., polyurethane glue [30]) [29-31]. These stoppers are either molded or extruded, resulting in rods that are cut in the desired length [30].



**Figure 8** – Example of agglomerated cork stoppers (reprinted from [32]).



#### d) Technical

Technical cork stoppers are composed of a compact agglomerated cork body with two discs of natural cork glued in one (“2+0” technical stopper) or both ends (“1+1” technical stoppers) (Figure 9) [15]. The agglomerated cork body is the same as the agglomerated cork stoppers and the discs of natural cork can be produced from the rougher sides of the cork planks and are washed in pure water at 70 °C, dried and then stored until their use is needed [15]. Then, they are glued to the agglomerated body using FDA-approved glue [15].



**Figure 9** – Examples of technical cork stoppers: “1+1” technical cork stoppers (left) and “2+0” technical cork stoppers (right) (reprinted from [33]).

#### e) Bartop

These closures consist in capsulated natural cork, which means that the body of this closure is composed by a natural cork stopper with a capsule in the top (Figure 10) [34, 35].

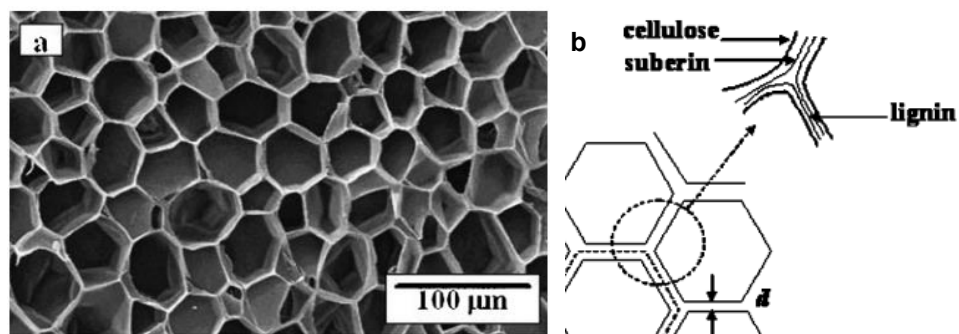


**Figure 10** – Example of bartop stoppers (reprinted from [36]).

It is particularly used for spirit drinks [35]. These closures surpass the same steps that natural cork with the addition of the application of capsules after a treatment. These closures are then sorted again, packaged and dispatched [37].

### 1.5. Cork properties

Cork has been used over the centuries as a bottle closure due to its physical properties: high flexibility, elasticity, compressibility and recovery, very low permeability to liquids and low density [38, 39]. These characteristics are the result of cork's cellular structure and composition, i.e., the highly ordered arrangement of small, hollow and closed cells, usually referred as "honeycomb" and suberin as the main component of the cell's wall, associated with lignin (Figure 11) [38, 40]. The cork cells are usually described as thin-walled hexagonal prisms packed in columns parallel to the tree's radial direction [11]. The cell's wall is composed by suberin, lignin, polysaccharides (cellulose and hemicellulose), and extractives, i.e., compounds susceptible of being extracted from cork, as represented in Table 1 [13]. There is no communication between cells or intercellular space, with the exception of lenticular channels. Lenticular channels, which are responsible for cork's porosity, are channels crossing the cork planks along a radial direction from the inside living tissues to the outside, allowing gas exchanges, such as the oxygen [13, 38-40].



**Figure 11** – a) Scanning electron microscopy (SEM) micrograph of radial section of natural cork after boiling (reprinted from [13]); b) cork's cellular walls main components: a lignin-rich lamella, a suberin and wax wall and a polysaccharide wall (reprinted from [13]).

**Table 1** – Chemical composition of cork from *Quercus suber* L. (adapted from [15]).

Component	Average % from the total material	Description
Suberin	40 - 50 %	Most important structural component of cork cells' wall; confers protection against harming factors
Lignin	20 - 25 %	Responsible for mechanical support and rigidity of cork cells; confers cork's hydrophobicity
Polysaccharides	20 %	Mainly cellulose and hemicellulose, structural components of the cell wall
Extractives	20 %	Molecules susceptible of being extracted from cork by a solvent. ex: triterpenes, polyphenols, sterols,...

Suberin is an aliphatic polymeric molecule that is composed by glycerol, long chain fatty acids and alcohols linked by hydroxyl and carboxyl groups [12]. This is the major and most important structural cork cell wall constituent [12, 41]. This biopolymer is responsible for the protection of the tree from pathogens, environmental aggressions, temperature and water loss [42]. Besides, its unique structure is what confers cork its special physical properties described above [42]. Lignin, the second one, has an aromatic nature and is composed by three phenylpropane monomers [12]. In cork, this polymer functions as mechanical support to the cell, conferring rigidity to its walls [15]. Besides, lignin is extremely hydrophobic, meaning that its water absorption is very low [15].

In summary, these particularities combined make this natural material suitable to successfully preserve the organoleptic characteristics of wine [29, 43].

## 1.6. Wine composition

Wine is a hydroalcoholic beverage produced from grape juice through sugar fermentation lead by yeasts [44, 45]. It is consumed due to its sensorial properties and the pleasure of the drinking moment, hence it is important that the flavour sensations correspond to the consumer's expectancy [44]. The wine's organoleptic characteristics vary due to the combination of different factors: geographic origin, the grape's chemical profile, the fermentation microflora, the substances formed or altered during yeast fermentation,



the compounds extracted during wine storage (e.g., oak barrels), the wine ageing during bottle storage, and the cultural traditions, the local know-how and technological processes used for its production [46-49].

The complex composition of wine comprises, approximately, 80-85% water, 9-15% ethanol, which is produced during yeast fermentation [50], and several minor constituents (~3%) that are responsible for its particularities [51]. These minor constituents comprise sugars, vitamins, tannins in red wines, lipids, nitrogenous compounds, phenols, organic acids (tartaric (2 - 5 g/L), malic (0 - 4 g/L), citric (0 - 4 g/L) and acetic ( $\leq$  2 g/L) acids) and volatile compounds, among others [51, 52]. The organic acids are responsible for wine's acidity and for a sour flavour that is counterbalanced by the presence of sugars and enriched by the astringent taste conferred by the polyphenols [51]. The wine's aroma is also characterized by the myriad of volatile organic compounds (VOCs) comprised in several chemical classes, namely alcohols, organic esters, fatty acids, aldehydes, lactones, phenols, sulphur containing compounds, methoxypyrazines, norisoprenoids, ketones and terpenes [51].

Higher **alcohols** (e.g., benzyl alcohol, 1-octen-3-ol, 2-ethyl-1-hexanol, 2-methyl-1-butanol, 1-propanol [53]), i.e., volatile alcohols with two carbon atoms or more, represent nearly 50% of the aromatic constituents of wine, excluding ethanol [53]. At low concentrations ( $\leq$  0.3 g/L), these compounds confer an interesting complexity to the wine's *bouquet* [53]. At higher quantities, their strong pungent scent gradually overlaps the wine's fragrance [53]. These alcohols can be naturally present in the grapes and survive the fermentation process or they can be originated from grape-derived aldehydes, by denitrification of amino acids or produced during the fermentation process [53]. In the last case, their synthesis is promoted by the presence of oxygen and high temperatures during the fermentation [53]. Sulphur dioxide, which acts as an antioxidant in wine, represses their production [53]. Other important alcohols are, for example, **diols**, and the most outstanding diol present in wine is 2,3-butanediol with a smooth bittersweet taste. The most prominent **polyol** in wine is glycerol, which has a slightly sweet taste associated [53]. Alcohol changes during wine storage are usually intimately linked with their corresponding ester [54]. For example, if an alcohol increases due to aging, it may be related to its ester hydrolysis [54]. On the other hand, a decrease may be due to esterification phenomenon, in which the alcohol is converted into an ester, or due to oxidation into their corresponding aldehydes [54, 55].

Organic **esters** are usually present at trace amounts in fermented beverages like wine, though, they are the major group found in wine, after water, ethanol and higher alcohols [52]. Usually, each ester individually does not surpass the aroma threshold,

however, they possess synergistic effects between all the esters present, impacting the wine aroma [52]. Normally, they are considered the primary fruity aromas source [52]. These compounds can be formed enzymatically (e.g., ethyl acetate, ethyl butanoate, ethyl hexanoate and ethyl octanoate [52]) or non-enzymatically (e.g., diethyl succinate [56]) [52]. In the first case, esters are synthesized with the assistance of esterases, lipases or alcohol acetyltransferases [52]. In the second case, esters are formed during wine ageing by chemical esterification between alcohol and acids at low pH [52]. These compounds suffer changes overtime after bottling due to hydrolysis, oxidation and esterification phenomena [52]. Ethyl esters of straight-chain fatty acids and acetates are the result of lipid metabolism of yeasts during fermentation, being produced at high quantities [57]. These compounds result from a chemical condensation between alcohols and organic acids, tending to reach an equilibrium with their correspondent alcohols and acids over time [57].

Organic **acids** are very important constituents of wine [55]. They are determinant to the wine pH, which leads to direct effects on its appearance and on its microbial and chemical stability [55]. Moreover, they are particularly important to its taste, conferring mostly the sense of sourness [55]. This sense is specially conferred by the non-volatile acids, such as citric, lactic, malic, succinic and tartaric acids, which are usually originated from grapes' ripening and during fermentation [55]. The concentration of volatile organic acids is intimately related to the corresponded ester [55]. The main volatile acid is acetic acid, although others can be found, such as butyric acid [53]. These compounds confer unpleasant aromas to wine at high concentrations and detectable amounts are usually related with wine spoilage by microorganisms [53].

The majority of **aldehydes** encountered in wine are originated during fermentation or extracted from the oak barrels [53]. The most abundant aldehyde is acetaldehyde (> 90% of total aldehyde content), which is an intermediate in ethanol production and is associated with "bruised apple" and "nutty" aromas when present in concentrations above the sensory threshold in wine (10 mg/L [58]) [53, 59]. High amounts of acetaldehyde are normally associated with wine oxidation and this issue can be surpassed by the addition of sulphite that binds to acetaldehyde forming a non-volatile adduct of acetaldehyde hydroxysulfonate [59]. The more oxidative damage a wine receives, the higher amount of acetaldehyde the wine presents, the higher quantity of sulphur dioxide (SO<sub>2</sub>) is bounded, which is undesirable since bound SO<sub>2</sub> provides limited protective antioxidant and antimicrobial activity [59]. Furfural is another sensorial important aldehyde and, which is formed from sugars and its formation is accelerated by high temperatures [53]. Benzaldehyde, a phenolic aldehyde with a "bitter-almond" scent, may have different origins, namely from the grape itself, from the oxidation of benzyl alcohol or from metabolic action of some yeast strains [53]. Hexanals

and hexenals are important aldehydes originated from grapes that are not metabolized during fermentation and portray “grassy” or “herbaceous” odors in some grape varieties, such as Grenache and Sauvignon blanc [53]. Aldehydes can be formed from alcohols’ oxidation during storage [57]. Hence, these aldehydes are usually related with deteriorative “oxidized” aromas [55]. The major examples are methional, acetaldehyde and phenylacetaldehyde, which are formed through wine oxidation [55]. Furthermore, aldehydes are highly reactive molecules [60] and can form reversible adducts with bisulfite [55].

**Ketones** present in wines are usually originated from grapes and are not transformed during fermentation. The norisoprenoid ketones  $\beta$ -damascenone,  $\alpha$ -ionone and  $\beta$ -ionone are some examples of ketones and they confer pleasant aromas of “exotic flower”, “rose” and “violet-raspberry”, being apparently great contributors for the aroma of several red grape varieties such as Riesling and Chardonnay [53]. Other ketones are originated through fungi metabolism, namely 1-octen-3-one and 1,5-octadien-3-one, with unpleasant mushroomy flavours [53]. Others are produced during fermentation and the only one that appears to have sensory significance is 2,3-butanedione, with “buttery” and “nutty” taste [53]. The increase of ketones in wine during storage is usually related to Fenton oxidation of alcohols, Strecker reaction of an amino acid with  $\alpha$ -dicarbonyl containing compounds and/or direct oxidation of fatty acids [55].

**Lactones** present in wine may have multiple origins once they can already be present in the grapes, be produced during wine fermentation and aging or be extracted from oak barrels [53]. One of the major impacting lactones formed during wine oxidative maturation is sotolon (3-hydroxy-4,5-dimethyl-2[5H] furanone), which is associated with “nutty”, “sweet”, “curry” or “burnt” odors [53, 61]. Sotolon has a low perception threshold of 1 – 5  $\mu\text{g/L}$ , which consequently means that its aroma is easily perceived [61]. When present at concentrations above the perception threshold, this compound confers a premature-aging flavour in dry white wines [61]. In other wines, such as Madeira, Port and Sherry, sotolon is associated as a characteristic flavour [62]. The sotolon formation occurs from the aldol condensation of acetaldehyde and  $\alpha$ -ketobutyric acid or by the reaction between ethanol and ascorbic acid [61, 62]. Furanol and homofuranol also have an important impact on wine aroma, conferring “fruity” and “caramel” notes [63].

**Volatile phenolic compounds** are low molecular weight aromatic alcohols that confer to wines odors such as “smoke”, attributed mainly to guaiacol, 4-methylguaiacol, 4-vinylguaiacol and syringol, “medicinal/cleaning products”, attributed to phenol, 4-vinylphenol and syringol, “vanilla”, attributed to vanillin, “spice”, attributed to 4-ethylguaiacol and eugenol, and “leather”, attributed to *m*-cresol and 4-ethylphenol [55]. Several volatile phenols (e.g., guaiacol, vanillin and eugenol) are extracted during wine storage in oak

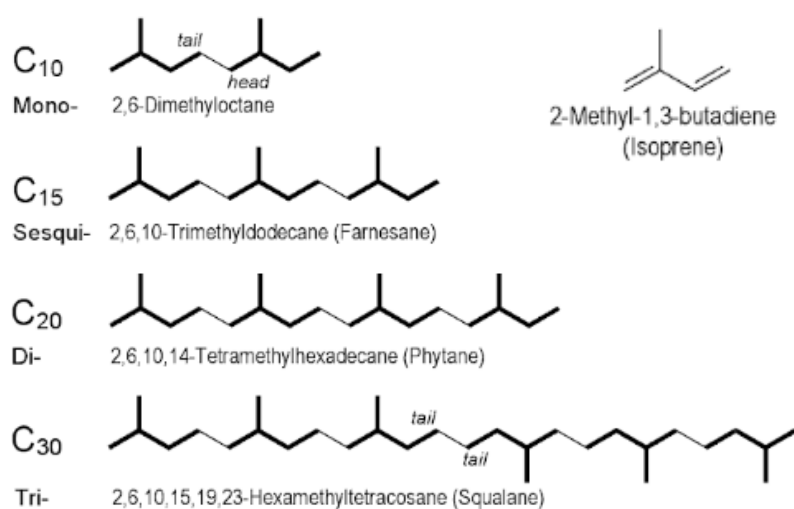
barrels and are formed due to thermal degradation of lignin, contributing with positive aromas to wine when present at appropriate concentrations [55]. These compounds can be found in grapes as non-volatile glycosylated conjugates that can be hydrolysed by acids or enzymes during wine fermentation or storage, subsequently releasing the volatile compounds into wine [53]. Volatile phenols can also be originated through yeast metabolism of hydroxycinnamic acids present in grapes, arising unpleasant aromas conferred by 4-ethylphenol and 4-ethylguaiacol [55].

**Volatile sulphur-containing compounds** in wine are originated by yeast metabolism or by the addition of SO<sub>2</sub> for antimicrobial and antioxidant purposes [53, 59]. They are usually associated with unpleasant “rotten egg”, “cooked cabbage”, “onion” and “rubber” odors due to molecules such as hydrogen sulphide (H<sub>2</sub>S), methanethiol, ethanethiol and methionol [49]. However, there are several volatile thiols that confer pleasant aromas to wine, such as “herbaceous”, “fruity”, “mineral”, “smoky” and “toasty” [49]. For example, 3-sulfanylhhexyl acetate (3SHA) is described as “fruity” and reminiscent of “passion fruit” in Sauvignon blanc wines, with a perception threshold of 4 ng/L, being present in concentrations until several hundred ng/L [59]. 3SHA is usually hydrolysed during wine ageing into 3-sulfanylhhexan-1-ol (3SH) that presents “grapefruit” and “tropical fruit” descriptors [59]. Its sensory threshold is, approximately, 60 ng/L and is usually present at higher amounts [59]. These volatile thiols are also present in wines made from other *Vitis vinifera* grape varieties, conferring similar characters to wine [59]. In red wines, 3SH is highly susceptible to oxidation and very reactive with quinones, meaning that this compound suffers a drastic reduction over time [59]. In fact, volatile thiol compounds are, in general, susceptible to oxidation.

**Methoxypyrazines** (MPs) are nitrogen-containing heterocyclic products of amino acid metabolism [64]. These volatile compounds are highly odorant and are present in some grape varieties such as Sauvignon blanc and Cabernet Sauvignon [65]. They impart sensory characters such as “grass”, “vegetative” and “herbaceous” to wine [66]. MPs have small perception thresholds (2-16 ng/L in wine [67]) and, when present at low concentrations, they have a positive impact on the aroma profile of these varieties, whereas at higher amounts ( $\geq 20$  ng/L [68]), they confer unpleasant characters to wine [69]. The most abundant MP in grapes is 3-isobutyl-2-methoxypyrazine (IBMP) [66], along with 3-isopropyl-2-methoxypyrazine (IPMP) and 3-sec-butyl-2-methoxypyrazine (SBMP) [68]. The sensory threshold above which IBMP is recognizable in red wine is 15 ng/L and it is present in higher concentrations than IPMP and SBMP in both grapes and wines, being the most impacting MP in wine [67, 68]. There are several factors that affect the grape IBMP concentrations, such as maturity, sunlight exposure, temperature and yield [68].

**Norisoprenoids** are the products of carotenoid's breakdown found in grapes [70]. The major grape carotenoids, representing 85% of the total, are  $\beta$ -carotene and lutein (in the range of mg/kg) [70]. The carotenoids are naturally very unstable molecules, which means that some chemical and enzymatic reactions occur in order to produce a more stable molecule [70]. In this case, the product molecules are the highly aromatic norisoprenoids [70-74]. Many norisoprenoids have been found in wine, such as 2,2,6-trimethylcyclohexanone (TCH),  $\beta$ -damascenone,  $\beta$ -ionone, actinidiol, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). TCH is only found in Port wines and is associated to a "rock-rose"-like aroma [70].  $\beta$ -Damascenone is characterized by "cooked apple" and "floral" aromas, presenting a very low odor threshold (2 ng/L in water [71] and 50 ng/L in wine model solution [75]) [70].  $\beta$ -Ionone presents "violet", "woody" and "raspberry" odors and low odor threshold (7 ng/L in water [71] and 90 ng/L in model wine solution [76]). TDN has been one of the norisoprenoids that has aroused more interest in wine, because of its unpleasant "kerosene" and "petrol"-like aromas associated with an odor threshold of 20  $\mu$ g/L [70]. This compound is highly associated to aged Riesling wines, whose concentrations can reach 200  $\mu$ g/L [70].

**Terpenes** are an important group of aromatic compounds characterizing the odor of many flowers, fruits, seeds, leaves, woods and roots [53]. Relatively to their chemical structure, terpenes have a basic five carbon isoprene unit (2-methyl-1,3-butadiene) [53]. Generally, they are composed of two (**monoterpenes**,  $C_{10}$ ), three (**sesquiterpenes**,  $C_{15}$ ), four (**diterpenes**,  $C_{20}$ ) or six (**triterpenes**,  $C_{30}$ ) isoprene units (Figure 12) [53]. For this reason, terpenes are also called isoprenoids [77].



**Figure 12** – Terpenes chemical structure (reprinted from [77]).

These compounds can possess diversified functional groups constituting alcohols, ketones, aldehydes, esters or hydrocarbons [53, 64]. In wine, terpenes are usually derived from grapes and are only sensorially detected as free terpenes [64]. Besides, they have been shown to be synthesized by *Saccharomyces cerevisiae* without grape precursors [78]. This family of compounds is largely associated to “fruity” (citric) and “floral” aromas, although some present “resin”-like odors ( $\alpha$ -terpinene, *p*-cymene, myrcene and farnesol [64]) [64]. Monoterpenes (linalool,  $\alpha$ -terpineol, nerol, geraniol, citronellol and hotrienol [64]) are the most odoriferous terpenes with “floral”, “fruity” and “citrus” characters [59, 64]. Monoterpenes can be present at free and bound forms in grapes and the matured berries show more bound forms than free forms [47]. The bound form implies glycosidic bonds and is an odourless form of monoterpenes [47]. During fermentation, yeasts release glucosidases, which are enzymes that can hydrolyse these bonds, which will result in the release of the monoterpenes that were bound and will contribute to wine’s aroma [47]. These compounds with their floral and sweet notes are typical of the aromatic grape varieties such as Muscat, Riesling and Gewürztraminer [79]. The most commonly found monoterpenes in wines are geraniol, nerol, rose oxides (“floral”, “rose”-like), linalool (“coriander”), linalool oxides (“camphoraceous”, “green”) and nerol oxide (“herbaceous”). Other monoterpenes that could also be found in some wines are  $\beta$ -pinene (“pine”), limonene (“green”, “citrus”), terpinolene (“fresh”, “pine”), citronellal (“citrus”, “green”), linalyl acetate (“fruity”, “citrus”) and neryl acetate (“floral”, “fruity”, “rose”) [80]. 1,8-Cineole, a monoterpene also known as eucalyptol for being the major aroma compound present in *Eucalyptus* leaves, is considered important for conferring pleasant “eucalypt” and “minty fresh” attributes to wine and for its low sensory threshold ranging from 1.1 – 1.3  $\mu\text{g/L}$  in some wines [46]. Consumers tend to prefer wines that contain this compound above its sensory threshold and below its rejection threshold (27.5  $\mu\text{g/L}$ ) [81]. Its origin in wine is thought to be due to the proximity of grapes to *Eucalyptus* leaves, especially when in contact with harvested grapes, being absorbed by their skin [81].

**Sesquiterpenes** (e.g.,  $\alpha$ -muurolene, farnesol, nerolidol [80]) are much less volatile and aroma-active than monoterpenes [81]. In grapes and wines, they are usually present at low amounts, making difficult their detection and quantification [80]. Sesquiterpenes are believed to bring health benefits such as anti-inflammatory and antioxidant effects [80]. Rotundone is a grape-derived sesquiterpene ketone that accumulates in the exocarp (monoterpenes accumulate in the mesocarp) and was first discovered in the Australian cool climate Shiraz grapes and wine [81]. This compound confers a strong “black pepper” scent to wine, being easily perceived due to its low perception threshold of 16 ng/L in red wines, constituting a prevalent aroma compound in Shiraz wines [81]. Throughout the process of

fermentation, rotundone is extracted from the must, but it is believed that it binds to the grape solids and only approximately 10% (w/v) of total rotundone in the grape berries is present in the final wine [81]. During wine bottle storage, this sesquiterpene remains stable along time [81].

All these groups of compounds are important to understand the complexity of wine chemical composition and how it affects its aroma, perception and mouthfeel. Hence, it is important to study the alterations conferred to the levels of these volatile aroma compounds during storage and understand how these alterations occur in order to continuously control and improve wine's organoleptic characteristics.

### **1.7. Effect of bottle closures on wine evolution**

The bottle storage conditions, such as temperature, light, humidity and bottle position, can lead to the development of different wine characters. Besides this, one of the main aspects that affect the wine evolution in the bottle is the closure chosen to seal the bottle [82]. The closure used will highly affect the organoleptic properties of wine, since they influence the oxygen permeability, promote the migration of several compounds and possess the capacity to absorb important aroma substances from wine [82]. This subchapter will focus on the extraction of VOCs from cork stoppers into wine and the effect of this phenomenon on wine aroma.

#### **1.7.1. Oxygen transmission rate through cork stoppers**

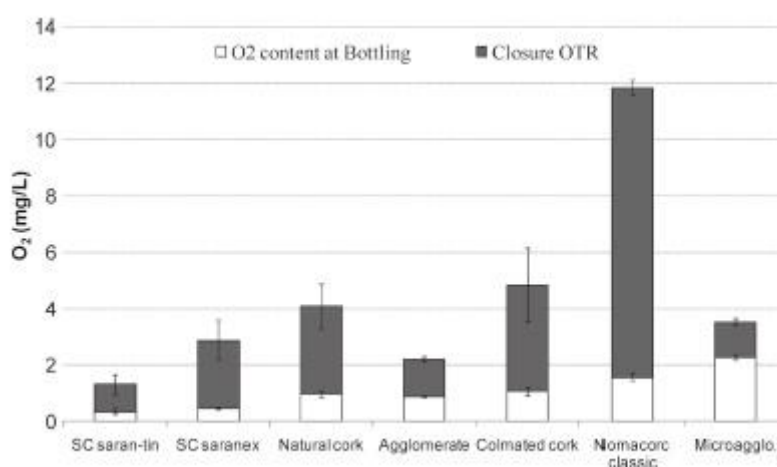
The oxygen exposure of bottled wine will depend on the oxygen present in the headspace at bottling, on the oxygen entering in the interface stopper/bottle, on the oxygen entering through the stopper and on the oxygen released by the stopper due to compression [82, 83]. Natural cork stoppers are permeable to oxygen and this permeability is widely variable from cork to cork due to cork stoppers' heterogeneity as a natural product [84].

The wine oxidation usually occurs through a cascade mechanism where oxygen and metals (e.g., iron and copper) lead to the formation of free radicals of oxygen and/or peroxides [85]. These products will react with chemical compounds in wine, leading to changes in wine aroma and colour (browning in white wines and brick-yellow in red wines) [85, 86]. The aroma associated with oxidation is usually described as "cooked vegetable", "hay", "cider", "woody" and "honey-like" [86]. According to some studies, the main VOCs responsible for these oxidized flavours are Strecker aldehydes (acetaldehyde, 3-methylbutanal, methional and 2-phenylacetaldehyde), other aldehydes (benzaldehyde,

furfural, 5-methyl-2-furfural and hexanal), some alcohols (1-octen-3-ol and eugenol), 1,2-dihydro-1,1,6-trimethylnaphthalene (TDN) and sotolon [58, 87]. These compounds were found in higher concentrations in wines exposed to oxygen when compared to wines not exposed to oxygen [87]. Wine possesses antioxidants that are used to modulate the extent of the oxidative damage, from which sulphur dioxide (SO<sub>2</sub>) is usually added [88].

For all these reasons, the cork stoppers will have a determinant impact in the wine evolution post-bottling, since different cork stoppers will have a specific oxygen transmission rate (OTR) and will lead to different oxygen exposure, which results in different extents of aroma alteration [89]. However, the presence of continuous and controlled quantities of oxygen is usually beneficial for red wine maturation due to colour enhancement and astringency reduction [89]. Relatively to the white wines, the exposure to oxygen is usually related with loss of fruity characters, as well as the emergence of oxidized flavours [89]. It should be noted that little oxygen exposure could also lead to the development of unpleasant reduced characters, such as “rubber” and “struck-flint” [89].

Taking into account the previous statements, it is important to choose the ideal sealant for wine. In regard to cork stoppers' OTR, a study performed by Lopes *et al.*, 2009, showed that the agglomerated and microagglomerated cork stoppers were the least permeable to oxygen, whilst colmated and natural cork stoppers the highest (Figure 13) [89]. The sensory descriptive analysis revealed that, although with lower OTR, white wines sealed with agglomerated and microagglomerated cork stoppers experienced a higher loss of fruity characters when compared with the other cork stoppers [89]. Another study also stated that technical and agglomerated cork stoppers presented lower OTR when comparing to natural and colmated cork stoppers [84]. Besides, both natural and colmated cork stoppers presented highly variable OTR [84].



**Figure 13** – Comparison between the OTR of different sealants (reprinted from [89]).



It is important to comprehend how the several types of cork stoppers may influence wine's aroma through oxidation due to different OTR. Natural and colmated cork stoppers have shown the highest and most variable OTR amongst cork stoppers due to their heterogeneity, however no significant effect on wine's sensorial properties due to oxidation has been proven.

### 1.7.2. Extraction of volatile organic compounds (VOCs) from cork stoppers into wine

Some cork components can be extracted from cork stoppers into wine, affecting positively or negatively its quality [90]. VOCs that can be extracted from cork into wine include hydrocarbons, alcohols, acids, aldehydes, ketones and low molecular weight polyphenols [91]. In Table 2, several compounds identified as being extracted from cork by wine or wine model solution in several studies are listed.

**Table 2** – List of VOCs reported to be extracted from cork into wine or wine model solution.

Compound	CAS	Odor descriptor	Reference
<b><i>Alcohols</i></b>			
3-Methyl-1-butanol	123-51-3	Whisky <sup>a</sup>	[92]
1-Octen-3-ol	3391-86-4	Mushrooms <sup>a</sup>	[92]
1-Octanol	111-87-5	Wax <sup>a</sup>	[92]
Geosmin	19700-21-1	Earth, musty	[92, 93]
Isobutanol	78-83-1	Flowery, anise	[94]
<b><i>Aldehydes</i></b>			
Propanal	123-38-6	Fruity, fresh green <sup>b</sup>	[95]
Butanal	123-72-8	Fruity, burnt, sweet <sup>b</sup>	[95]
Pentanal	110-62-3	Dry fruit, nutty	[95]
Hexanal	66-25-1	Grass, herbaceous <sup>b</sup>	[95, 96]
Heptanal	111-71-7	Fatty, rancid <sup>b</sup>	[95, 96]
Octanal	124-13-0	Lemon	[94-96]
Nonanal	124-19-6	Herbal, citrus <sup>b</sup>	[95, 96]
Decanal	112-31-2	Fruity, citrus <sup>b</sup>	[95, 96]
Undecanal	112-44-7	Citrus, floral <sup>c</sup>	[95]
2-Propenal	107-02-8	Almond, cherry <sup>c</sup>	[95]
( <i>E</i> )-2-Butenal	123-73-9	Flower <sup>c</sup>	[95]
( <i>E</i> )-2-Pentenal	1576-87-0	Fruity, green <sup>c</sup>	[95]
( <i>E</i> )-2-Hexenal	6728-26-3	Almond, fruity <sup>c</sup>	[95]
( <i>E</i> )-2-Heptenal	18829-55-5	Fatty, green <sup>c</sup>	[95]

Compound	CAS	Odor descriptor	Reference
( <i>E</i> )-2-Octenal	2548-87-0	Fatty, herbal <sup>c</sup>	[95]
( <i>E</i> )-2-Nonenal	18829-56-6	Green, cucumber <sup>c</sup>	[95]
( <i>E</i> )-2-Decenal	3913-81-3	Fatty, oily <sup>c</sup>	[95]
2-Methyl-1-propanal	78-84-2	Fruity, malty <sup>b</sup>	[95]
2-Methyl-1-butanal	96-17-3	Almond, nutty <sup>c</sup>	[95]
3-Methyl-1-butanal	590-86-3	Fruity, cheesy <sup>b</sup>	[95]
Benzaldehyde	100-52-7	Bitter almonds <sup>b</sup>	[95, 96]
Phenylacetaldehyde	122-78-1	Floral, honey <sup>b</sup>	[95]
<b>Benzenoids</b>			
<i>o</i> -Cymene	527-84-4	-	[96]
Naphthalene	91-20-3	Pungent, tarry <sup>c</sup>	[96]
Guaiacol	90-05-1	Phenolic, spicy	[43, 92, 94]
Methyl guaiacol	91-16-7	Leather, spicy <sup>d</sup>	[43]
2,4,6-Trichloroanisole	87-40-1	Musty, earthy, mouldy	[43, 92, 93, 97-99]
<i>m</i> -Cresol	108-39-4	Leather	[94]
Vanillin	121-33-5	Vanillin	[94]
Methyl vanillate	3943-74-6	Vanillin	[94]
<b>Pyrazines</b>			
MDMP	-	Musty, dusty	[93]
IPMP	25773-40-4	Green, vegetative	[93]
IBMP	24683-00-9	Green bell pepper	[93]
<b>Dicarbonyls</b>			
Diacetyl	431-03-8	Buttery, cream	[94, 95]
Glyoxal	107-22-2	-	[95]
Methylglyoxal	78-98-8	-	[95]
<b>Ketones</b>			
Propan-2-one	67-64-1	Apple, ethereal <sup>c</sup>	[95]
2-Butanone	78-93-3	Fruity, acetone <sup>b</sup>	[95]
3-Methyl-2-butanone	563-80-4	Camphor <sup>c</sup>	[95]
2-Pentanone	107-87-9	Fruity <sup>b</sup>	[95]
2-Hexanone	591-78-6	Ether <sup>c</sup>	[95]
2-Heptanone	110-43-0	Fruity, herbal <sup>c</sup>	[95]
3-Penten-2-one	625-33-2	Fishy, phenolic <sup>c</sup>	[95]
4-Heptanone	123-19-3	Fruity, sweet <sup>c</sup>	[95]
2-Cyclohexen-1-one	930-68-7	Green, roasted <sup>c</sup>	[95]
6-Methyl-5-heptanone	13019-20-0	Fruity, green <sup>c</sup>	[95]
2-Octanone	111-13-7	Bitter, earthy <sup>c</sup>	[95]
2-Nonanone	821-55-6	Fresh, herbal <sup>c</sup>	[95]

Compound	CAS	Odor descriptor	Reference
2-Decanone	693-54-9	Fatty, floral <sup>c</sup>	[95]
2-Undecanone	112-12-9	Fresh, floral <sup>c</sup>	[95]
1-Octen-3-one	4312-99-6	Mushroom	[94]
<b>Furans</b>			
Furfural	98-01-1	Toasty, caramel <sup>b</sup>	[95]
5-Methyl-2-furfural	620-02-0	Spicy, toasty <sup>b</sup>	[95]
<i>p</i> -Fluorobenzaldehyde	459-57-4	-	[95]
<b>Esters</b>			
Ethyl hexanoate	123-66-0	Fruity, brandy <sup>b</sup>	[96]
Ethyl heptanoate	106-30-9	Fruity, nutty <sup>b</sup>	[96]
Ethyl nonanoate	123-29-5	Fruity, waxy <sup>c</sup>	[96]
Fenchyl acetate	13851-11-1	Citrus, herbal <sup>c</sup>	[96]
Isobornyl acetate	125-12-2	Herbal, woody <sup>c</sup>	[96]
Ethyl isobutyrate	97-62-1	Fruity, strawberry	[94]
Ethyl 2-methylbutyrate	7452-79-1	Fruity, green apple	[94]
Ethyl isovalerate	108-64-5	Fruity, anise	[94]
3-Methylbutyl acetate	123-92-2	Fruity, anise	[94]
Ethyl butyrate	105-54-4	Fruity	[94]
Butyl acetate	123-86-4	Grass	[94]
<b>Monoterpenes</b>			
$\alpha$ -Pinene	80-56-8	Minty <sup>c</sup>	[95, 96]
Camphene	79-92-5	Herbal, woody <sup>c</sup>	[96]
$\beta$ -Pinene	80-56-8	Green, hay <sup>c</sup>	[96]
1,4-Cineole	470-67-7	Minty, pine <sup>c</sup>	[95, 96]
Citronellol	106-22-9	Citrus, floral <sup>c</sup>	[43]
$\alpha$ -Terpinene	99-86-5	Citrus, herbal <sup>c</sup>	[95, 96]
Limonene	5989-54-8	Lemon, orange <sup>c</sup>	[43, 95, 96]
Eucalyptol	470-82-6	Mint, herbal <sup>c</sup>	[95, 96]
Terpinolene	586-62-9	Pine, woody <sup>c</sup>	[43, 96]
Fenchone	1195-79-5	Earthy, herbal <sup>c</sup>	[95, 96]
Fenchol	1632-73-1	Lemon, pine <sup>c</sup>	[43, 95, 96]
$\alpha$ -Campholenal	4501-58-1	Green, leafy <sup>c</sup>	[96]
L-Camphor	464-49-3	Camphor <sup>c</sup>	[43, 95, 96]
<i>trans</i> - $\beta$ -Terpineol	7299-40-3	-	[96]
<i>trans</i> -3-Pinanone	547-60-4	Spicy <sup>c</sup>	[96]
Isoborneol	124-76-5	Herbal, woody <sup>c</sup>	[95, 96]
2-Methylisoborneol	2371-42-8	Musty, muddy	[93]
L-Borneol	464-45-9	Camphor, anise	[43, 94-96]
2-Methylisoborneol	2371-42-8	Earth, musty <sup>a</sup>	[92]

Compound	CAS	Odor descriptor	Reference
<i>cis</i> -3-Pinanone	15358-88-0	Camphoreous, cedar <sup>c</sup>	[95, 96]
$\alpha$ -Terpineol	98-55-5	Floral, mint <sup>c</sup>	[94-96]
1-Terpineol	7785-53-7	Floral, lilac <sup>c</sup>	[95]
4-Terpineol	562-74-3	Earth, musty <sup>c</sup>	[95, 96]
Linalool	78-70-6	Flowery, muscat	[43, 94]
<i>cis</i> -Linalool oxide	11063-77-7	Earthy, sweet <sup>c</sup>	[95]
L-(-)-Menthol	2216-51-5	Minty, peppermint <sup>c</sup>	[95]
2-Pinen-4-one	18309-32-5	Menthol <sup>c</sup>	[95]
<b>Sesquiterpenes</b>			
$\alpha$ -Copaene	3856-25-5	Spice, woody <sup>c</sup>	[96]
D-Longifolene	475-20-7	Rose, sweet <sup>c</sup>	[96]
$\beta$ -Cadinene	523-47-7	Green, woody <sup>c</sup>	[96]
L-Calamenene	483-77-2	Herb, spice <sup>c</sup>	[96]
Eremophila ketone	158930-41-7	-	[96]

<sup>a, b, c, d</sup> Extracted from [92], [100], [101] and [102], respectively.

### 1.7.3. Scalping of VOCs from wine by cork stoppers

The scalping phenomenon is defined as the direct sorption of VOCs and other food constituents by the package materials [103, 104]. The scalping of VOCs present in wine composition by cork stoppers has been reported, which can have a negative or positive impact on wines' aroma [105]. Table 3 lists the VOCs reported to be scalped by cork stoppers from wine. These compounds comprise several chemical classes, namely esters, benzenoids, methoxypyrazines and volatile phenolic compounds.

A negative impact of scalping in wine composition is related to the sorption of VOCs that confer positive characters, namely the capacity to sorb ethyl octanoate and ethyl decanoate, which confer pleasant "fruity" aromas to wine [106]. However, the scalping capacity of cork stoppers can have a positive impact on wine's aroma, particularly by sorption of compounds that confer unpleasant characters to wine, such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), a grape derived C<sub>13</sub> norisoprenoid responsible for "kerosene" and "petrol"-like characters in aged white wines [107], and TCA, for which cork stoppers have shown an even higher ability to scalp than to transmit the taint compound to wine [108].

**Table 3** – List of VOCs reported to be scalped by cork stoppers from wine. IBMP – 3-isobutyl-2-methoxypyrazine; IPMP – isopropyl-2-methoxypyrazine and SBMP – 3-sec-butyl-2-methoxypyrazine.

Compound	CAS	Odor descriptor	Reference
<b>Esters</b>			
Ethyl octanoate	106-32-1	Fruity, sweet <sup>a</sup>	[106]
Ethyl decanoate	110-38-3	Fruity, apple <sup>a</sup>	[106]
<b>Benzenoids</b>			
Naphthalene	91-20-3	Pungent, tarry <sup>a</sup>	[106]
TCA	87-40-1	Mouldy, musty	[108]
TDN	30364-38-6	Kerosene, petrol	[106, 109]
<b>Methoxypyrazines</b>			
IBMP	24683-00-9	Green bell pepper	[105, 110]
IPMP	25773-40-4	Green, vegetative	[105, 110]
SBMP	24168-70-5	Green, vegetative	[105, 110]
<b>Volatile phenolic compounds</b>			
Guaiacol	90-05-1	Phenolic <sup>a</sup>	[102]
4-Methylguaiacol	93-51-6	Phenolic, leather <sup>a</sup>	[102]
4-Ethylguaiacol	2785-89-9	Spicy, smoky <sup>a</sup>	[102, 111]
4-Propylguaiacol	2785-87-7	Spicy, phenolic <sup>a</sup>	[102]
4-Vinylguaiacol	7786-61-0	Smoky <sup>a</sup>	[102]
4-Ethylphenol	123-07-9	Smoky, animal <sup>a</sup>	[102, 111]
Eugenol	97-53-0	Phenolic <sup>a</sup>	[102]

<sup>a</sup> Extracted from [101].

The understanding of scalping processes might be an important tool for wine producers to select and modify the wine properties for an optimal condition. Hence, more studies should be made to achieve a better understanding [107].

## 1.8. Scope of the thesis

In order to assess the effect of cork stoppers on wine's volatile composition, the present work aimed to:

- 1) Characterize the volatile profile of natural cork stoppers;
- 2) Develop and apply a HS-SPME-GC-MS/MS approach to discriminate four different qualities of natural cork stoppers based on the levels of some VOCs (targeted approach);

- 3) Evaluate the volatile profile extracted from different qualities of natural cork stoppers, with and without a washing process, into wine model solution at 6, 12 and 32 months post-bottling;
- 4) Study the impact of different closures and cellar conditions in the volatile profile of Porto wine, at 6, 15 and 26 months post-bottling.

## **2. Materials and Methods**

### **2.1. Chemicals**

All standard compounds (1,4-cineole (98%), 1-decanol (99,9%), 1-hexanol (99,9%), 1-octanol (100%), 2-heptanone (99%), 2-nonanone (97%), 2-pentylfuran, 2-undecanone (97%), 3-hexen-1-ol (98%),  $\alpha$ -ionone (90%),  $\alpha$ -pinene (99%),  $\alpha$ -terpinene (95%),  $\beta$ -cyclocitral (90%),  $\beta$ -damascenone (100%),  $\beta$ -linalool (80%), benzaldehyde (98%), L-borneol (99%), camphor (99%), camphene, decanal (95%), ethyl butanoate (98%), ethyl decanoate (98%), ethyl heptanoate (98%), ethyl hexanoate (99%), ethyl isobutyrate (98%), ethyl isovalerate (100%), ethyl 2-methylbutanoate (98%), ethyl nonanoate (98%), ethyl octanoate (99%), eucalyptol (99%), furfural (99%), hexyl acetate (98%), isoamyl acetate (97%), isoamyl alcohol (98%), limonene (99%), cis-linalool oxide (97%), nonanal (95%), octanal (99%), phenylacetaldehyde (90%), phenylethyl alcohol (99%)) and reagents were supplied by Sigma-Aldrich, Inc. (Steinheim, Germany). Ultrapure water was obtained from a Milli-Q Millipore purification system (Millipore, Billerica, Massachusetts, USA).

### **2.2. Sample preparation**

#### **2.2.1. Extraction of VOCs from natural cork stoppers into wine model solution**

For the analysis of VOCs extracted from natural cork stoppers into wine model solution, five natural cork stoppers (without treatment) were macerated into wine model solution (12% ethanol, 5 g/L tartaric acid and pH 3.2) in a shot of 250 mL ( $n=3$ ) during 48 hours in the dark. After this period, 8 mL from each shot were transferred into 20 mL GC vials to be analysed by HS-SPME-GC-MS.

#### **2.2.2. Extraction of VOCs from natural cork tops for discrimination of different grades of natural cork stoppers**

Firstly, cork tops of three different grades "Extra" ( $n=93$ ), "Flor" ( $n=92$ ) and "Superior" ( $n=95$ ) were macerated into of wine model solution for 4 hours. For the HS-SPME-GC-MS/MS analysis, 5 mL of each sample was placed in a 20 mL vial. Due to the limited time for VOCs extraction from the cork tops, a second approach was adopted, consisting of cork tops maceration with 7 mL of wine model solution in falcons of 50 mL, during 2 days in the dark. Then, 5 mL of wine model solution was transferred into 20 mL GC vials and analysed by HS-SPME-GC-MS/MS.

### **2.2.3. Wine model solution sealed with “Terceiro” and “Flor” natural cork stoppers with and without washing**

Wine model solution (12% ethanol, 5g/L tartaric acid and pH 3.2) was sealed with two different grades of natural cork stoppers (“Terceiro” and “Flor”) subjected to a washing step, Clean 2000 (aqueous solution containing 2% H<sub>2</sub>O<sub>2</sub>) and no washed, in 0.375 L bottles. After 6, 12 and 32 months ( $n=5$  for each group at each time), the bottles were opened and 5 mL of each solution was transferred into a 20 mL GC vial for HS-SPME-GC-MS analysis.

### **2.2.4. Porto wine sealed with natural cork stoppers and stored under different conditions**

A Vintage Symington Porto wine (20% alcohol content), 2014, was stored under different conditions in June 16 of 2016 (164 bottles). Twenty five bottles were opened at each time point (6, 15 and 26 months) comprising five distinct groups: 1) wine sealed with “Flor” natural cork (49 x 24 mm) stored in vertical position in “Garrafeira 5” (**Flor V (G5),  $n=5$** ); 2) wine sealed with “Flor” natural cork (49 x 24 mm) stored in horizontal position in “Garrafeira 5” (**Flor H (G5),  $n=5$** ); 3) wine sealed with “Flor” natural cork (49 x 24 mm) stored in horizontal position in “Garrafeira Castelo” (**Flor H (Gcast),  $n=5$** ), characterized by different and oscillating room temperature (up to 10 °C higher than “Garrafeira 5”) and humidity conditions; 4) wine sealed with “Flor” Bartop (27 x 19.5 mm) stored in vertical position in “Garrafeira 5” (**Bartop V (G5),  $n=5$** ) and 5) wine sealed with “Flor” Bartop (27 x 19.5 mm) stored in horizontal position in “Garrafeira 5” (**Bartop H (G5),  $n=5$** ). The wine was originated from the same vat and the dark glass bottles and cork stoppers were from the same batch. These measures aimed to reduce the variability. Wine bottles were opened at three time points, namely 6, 15 and 26 months. For analysis by HS-SPME-GC-MS, 250  $\mu$ L of each sample was placed into a 20 mL vial.

## **2.3. Analysis of VOCs by HS-SPME-GC-MS/MS for discrimination of different grades of natural cork**

The HS-SPME method for VOCs extraction was carried out in automated mode using a Bruker CTC PAL-xt (Varian Pal Autosampler, Switzerland) interfaced to the GC-MS. HS-SPME was performed after 5 min of sample incubation at 60 °C, under continuous stirring (500 rpm). Then, a 50/30  $\mu$ m divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco Inc., Bellefonte, Pennsylvania) was exposed to the headspace of the vial for 30 min at the same temperature and continuous stirring (250 rpm).



Once the extraction step was completed, the VOCs adsorbed to the fiber were desorbed during 6 min in the GC injection port (at 250 °C). A 436-GC model (Bruker Daltonics) coupled to a EVOQ tripe quadrupole (TQ) mass spectrometer (Bruker Daltonics) was used to analyse VOCs with a fused silica (Rxi-5Sil MS) capillary column (30 m x 0.25 mm internal diameter x 0.25 µm; Restek Corporation, U.S., Bellefonte, Pennsylvania) and high purity helium C-60 (Gasin, Portugal) as carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was held at 60 °C for 1 min, followed by increasing in an increment of 2.5 °C/min from 60 to 100 °C (1 min) and from 100 to 250 °C (0 min). The first ionization using TQ mass spectrometry was conducted in the electron impact (EI) mode at 70 eV. Data acquisition was performed in full scan mode and a 40-250 *m/z* mass range with a scan time of 500 ms. Then, a second ionization was conducted and the quantifier ions chosen were fragmented using different energies of EI. The GC-MS/MS conditions are listed in Table 4. These parameters were implemented to this work specifically, in order to improve the results obtained.

**Table 4** – Analytical parameters validated during the GC-MS/MS method development for each compound. The quantified ions are represented in bold.

	Furfural	Camphene	2PF	Limonene	Eucalyptol	Camphor
RT (min)	3.90	6.46	8.04	9.58	9.68	15.14
Scan type	MRM	MRM	MRM	MRM	MRM	MRM
Scan time (ms)	1400	467	350	350	350	1400
Precursor ion mass ( <i>m/z</i> )	<b>96</b>	<b>121</b>	<b>138</b>	<b>107</b>	<b>154</b>	<b>152</b>
Product ion mass ( <i>m/z</i> )	<b>39</b> 67	<b>93</b> 79	<b>81</b> 94	<b>91</b> 65	<b>85</b> 69	<b>108</b> 81
Collision energy (eV)	35	10	6	15	10	5

2PF – 2-Pentylfuran; MRM – Multiple reaction monitoring.

Multiple reaction monitoring (MRM) was the scan type used in this experiment, since it is a highly specific and sensitive mass spectrometry technique that can selectively quantify compounds within complex mixtures [112]. In quadrupole 1 (Q1) of the GC-TQ, only a small chosen ion population with the *m/z* fragments of interest were transmitted, augmenting the sensitivity of the method [112].

#### **2.4. Analysis of VOCs by HS-SPME-GC-MS for the study of wine model solutions and Porto wine**

VOCs were extracted from wine model solution and Porto wine by HS-SPME method using a 50/30  $\mu\text{m}$  DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, Pennsylvania), in a Combi-PAL autosampler (Varian Pal Autosampler, Switzerland). First, the sample was incubated at 45 °C for 5 min, followed by extraction at the same temperature for 30 min, under continuous stirring (250 rpm). After extraction, the analytes were thermally desorbed into the GC injector during 6 min at 250 °C. A 436-GC model (Bruker Daltonics) coupled to a SCION single quadrupole (SQ) mass spectrometer (Bruker Daltonics) was used to analyse VOCs with a fused silica (Rxi-5Sil MS) capillary column (30 m x 0.25 mm internal diameter x 0.25  $\mu\text{m}$ ; Restek Corporation, U.S., Bellefonte, Pennsylvania) and high purity helium C-60 (Gasin, Portugal) as carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was held at 40 °C for 1 min, followed by consistent increments of 5.0 °C/min from 40 to 250 °C (5 min) and from 250 to 300 °C (0 min). Single quadrupole mass spectrometry was conducted in the EI mode at 70 eV. Data acquisition was performed in full scan mode considering a 40-250  $m/z$  mass range with a scan time of 500 ms.

The VOCs present in wine model solution and Porto wine were identified by comparing the retention indices (RI), determined using a commercial hydrocarbon mixture (C6-C20), and the mass spectra fragmentation patterns of each compound with the RI and the mass spectra present in the National Institute of Standards and Technology (NIST) standard reference database version 14 (Gaithersburg, Maryland, USA). The identification was confirmed through analysis of standard compounds at the same conditions, when commercially available.

#### **2.5. Quantification of VOCs in wine model solution and Porto wine**

For VOCs quantification, standards were dissolved in wine model solution and analysed in the same conditions by HS-SPME-GC-MS, in order to obtain a calibration curve for each compound of interest. These calibration curves were obtained by injecting several levels of known concentrations of each compound and established by the area of the peak against the concentration.

#### **2.6. Statistical analysis**

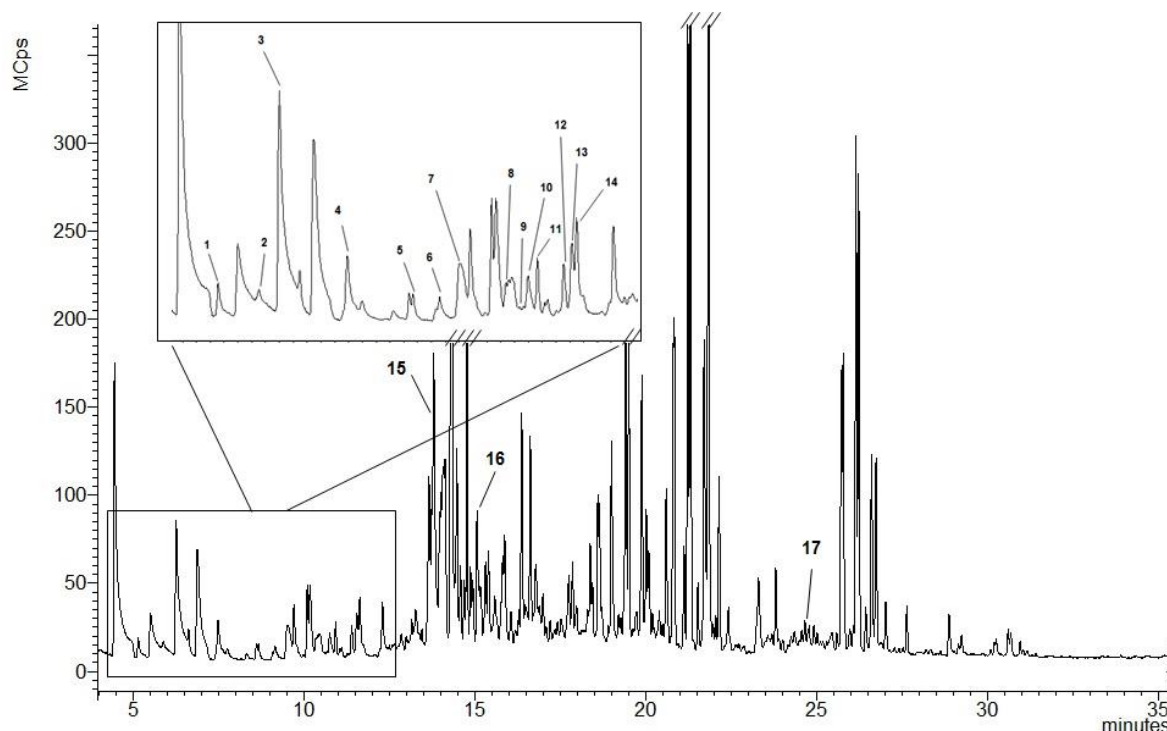
The statistical analysis in the present thesis was assessed using the software Graphpad Prism 6 (USA). The statistical significance between groups of samples was

assessed using the ordinary one-way Analysis of Variance (ANOVA) and Kruskal-Wallis test for multiple comparisons and Unpaired t-test and Mann-Whitney test for single comparisons. The results were considered significant when  $p$  values were below than 0.05. Heatmap plot, which is a hierarchical clustering statistical method, was obtained using MetaboAnalyst 4.0 [113].

### 3. Results and discussion

#### 3.1. Characterization of the VOCs profile extracted from cork stoppers by wine model solution

The VOCs profile extracted from natural cork stoppers by wine model solution is represented in Figure 14 and Table 5. Overall, 34 compounds were detected, from which 7 were putatively identified and 14 were formally identified using standard compounds. Comparing with the list of VOCs reported to be extracted from cork by wine model solution and wine (Table 2, 1.7.2.), 9 VOCs were identified in this work for the first time, namely 2-methyl-1-octanol, chlorobenzene, ethylbenzene, *m*-xylene and *o*-xylene, ethyl octanoate, 2-pentylfuran, (-)-3-carene and 2,4-di-*tert*-butylphenol.



**Figure 14** – HS-SPME-GC-MS chromatogram of VOCs extracted from cork by wine model solution. 1- Hexanal, 2- furfural, 3- chlorobenzene, 4- *o*-xylene, 5-  $\alpha$ -pinene, 6- camphene, 7- benzaldehyde, 8- 2-pentylfuran, 9- ethyl hexanoate, 10- octanal, 11- (-)-carene, 12- *o*-cymene, 13- limonene, 14- eucalyptol, 15- nonanal, 16- camphor and 17- 2,4-di-*tert*-butylphenol.

**Table 5** – List of VOCs extracted from cork by wine model solution based on NIST 14 and standard compounds.

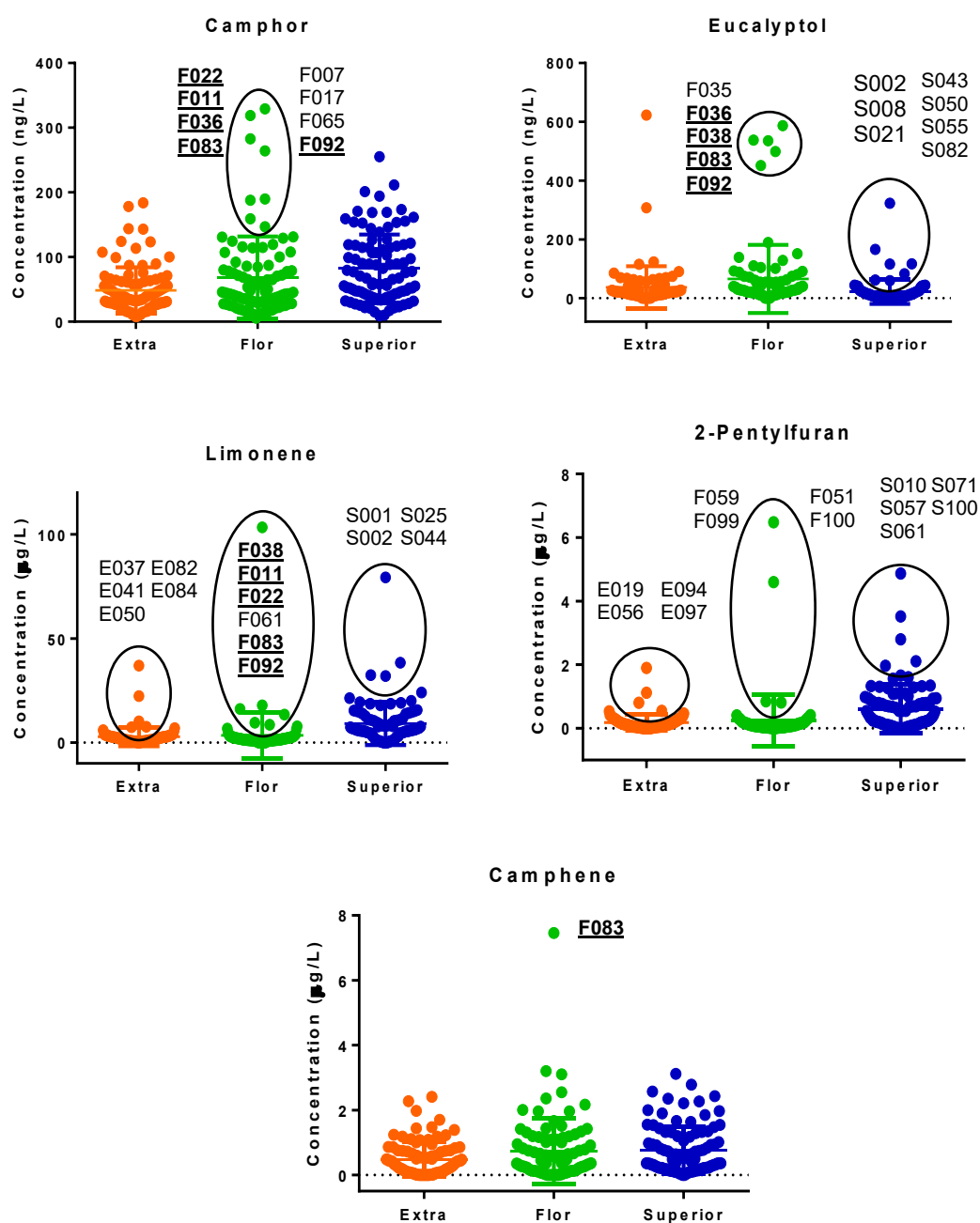
Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
<b>Aldehydes</b>						
Hexanal	66-25-1	5.12	802	800	41/56/72/82	STD
Octanal	124-13-0	10.72	1002	1003	41/56/57/69/84	STD
Nonanal	124-19-6	13.78	1103	1104	41/55/70/81/95	STD
Benzaldehyde	100-52-7	9.51	962	962	51/77/105	STD
<b>Alcohols</b>						
2-Methyl-1-octanol	818-81-5	14.46	1126	1119	41/57/71/83/112	NIST 14
<b>Benzenoids</b>						
Chlorobenzene	50-29-3	6.23	845	849	51/77/112	NIST 14
Ethylbenzene	100-41-4	6.60	859	855	51/65/77/91/106/ 112	NIST 14
<i>m</i> -Xylene	108-38-3	6.85	869	866	51/65/77/91/106	NIST 14
<i>o</i> -Xylene	106-42-3	7.46	892	887	51/65/77/91/106	NIST 14
<i>o</i> -Cymene	527-84-4	11.40	1025	1022	58/77/91/103/119 /134	NIST 14
<b>Esters</b>						
Ethyl hexanoate	123-66-0	10.58	998	1000	43/60/70/88/99	STD
Ethyl octanoate	106-32-1	16.45	1194	1196	57/70/88/101/127	STD
<b>Furans</b>						
Furfural	98-01-1	5.89	832	833	41/69/95/96/207	STD
2-Pentylfuran	3777-69-3	10.31	989	993	53/81/93/138	STD
<b>Monoterpenes</b>						
$\alpha$ -Pinene	7785-26-4	8.65	933	937	65/77/93/105/121	STD
Camphene	79-92-5	9.14	949	952	53/67/79/93/121/ 163	STD
Limonene	138-86-3	11.53	1029	1031	53/67/79/93/107	STD
Eucalyptol	470-82-6	11.62	1032	1032	43/55/71/81/111/ 139	STD
L-Camphor	464-48-2	15.05	1146	1142	55/67/81/95/108/ 152	STD
(-)-3-Carene	20296-50- 8	10.90	1008	1011	77/79/93/105/121 /136	NIST 14
<b>Phenols</b>						
2,4-Di- <i>tert</i> - butylphenol	96-76-4	24.65	1503	1519	57/191	STD
<b>Unknowns</b>						
Un 1	-	9.06	947	-	41/57/69/83/98	-
Un 2	-	13.95	1109	-	43/55/70/83/112	-
					41/55/67/82/95/1	-
Un 3	-	16.75	1204	-	10	-
Un 4	-	17.72	1239	-	41/57/70/83/97	-
Un 5	-	18.29	1259	-	41/57/69/83/97	-
Un 6	-	18.36	1262	-	41/55/69/83/97	-
Un 7	-	18.42	1264	-	41/55/69/83/98	-
Un 8	-	23.22	1446	-	43/95/137	-

Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
Un 9	-	24.92	1514	-	55/69/83/111	-
Un 10	-	27.64	1630	-	73/137/221	-
Un 11	-	28.88	1684	-	41/57/70/83/115	-
Un 12	-	30.10	1740	-	43/57/71/85/91	-
Un 13	-	31.41	1801	-	41/55/70/105	-

<sup>a</sup> Kovats retention indices (RI) determined using a commercial hydrocarbon mixture (C6-C20). <sup>b</sup> RI reported in literature. RT - retention time; STD - standard compound; NIST - National Institute of Standards and Technology database version 14; Un - Unknown.

### 3.2. Discrimination of different grades of natural cork stoppers based on levels of VOCs determined by HS-SPME-GC-MS/MS

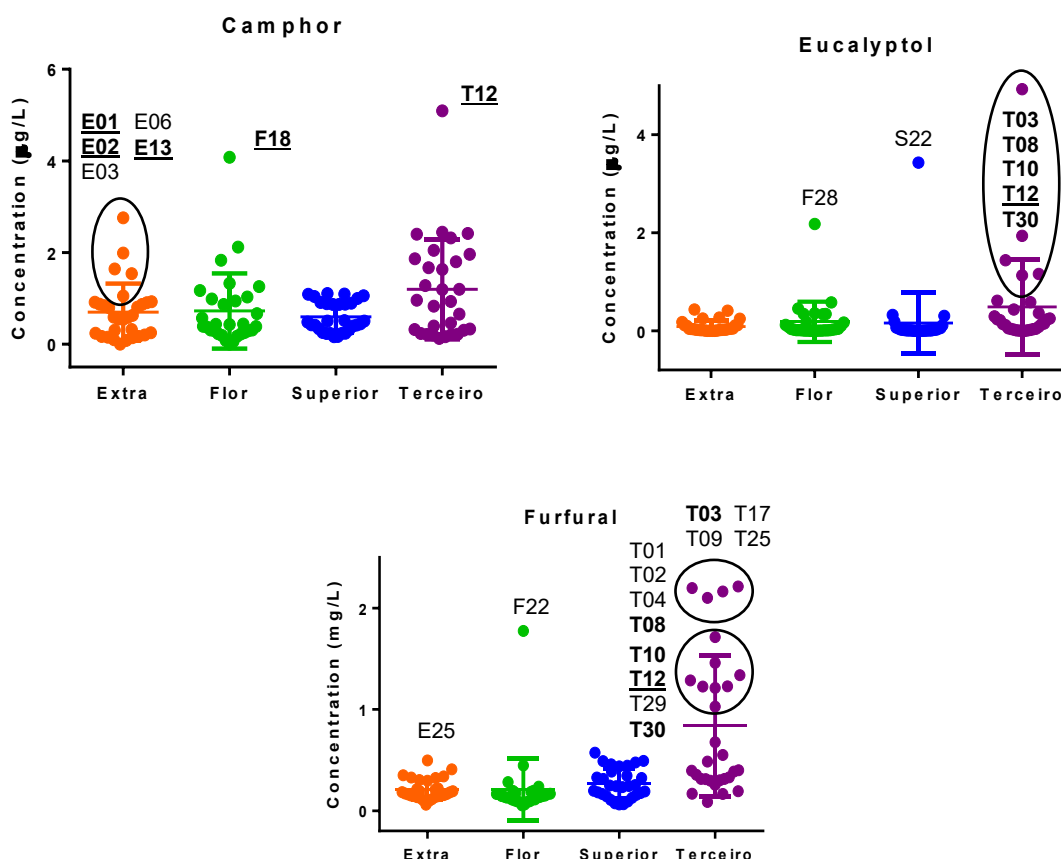
In order to define more homogenous classes of natural cork stoppers for wine bottling, the profile of VOCs extracted from cork to wine model solution was used to divide several grades of natural cork into two subclasses. In a pilot study, performed previously out of the scope of this thesis, “Flor”, “Superior” and “Terceiro” natural cork tops were directly analysed by HS-SPME-GC-MS. The results showed that “Flor” cork tops were discriminated into two subclasses based on the levels of several VOCs, namely 2-pentylfuran, cyclene, camphene, camphor, limonene and eucalyptol, whilst “Superior” and “Terceiro” subclasses were discriminated only by furfural and 5-methyl-2-furfural (data not shown). In the first study performed in the scope of this thesis (*Assay 1*), a HS-SPME-GC-MS/MS method was developed for quantification of the previously identified discriminant VOCs in “Flor” ( $n=92$ ), “Superior” ( $n=95$ ) and “Extra” ( $n=93$ ) cork stoppers, after compound extraction from cork tops by wine model solution. The results are represented in Figure 15 and showed that camphor was responsible for separating “Flor” into two subclasses, while eucalyptol was responsible for discriminating “Flor” and “Superior” subclasses. 2-Pentylfuran and limonene discriminated all grades of natural cork into two subclasses, while camphene showed no potential to discriminate any of the classes of natural cork studied. These results are summarized in Table 6.



**Figure 15** – Results of Assay 1 representing the concentration levels of camphor, eucalyptol, camphene, limonene and 2-pentylfuran extracted from “Extra”, “Flor” and “Superior” cork tops into wine model solution. In bold are represented the samples grouped in subclass 2 by more than one compound.

In Assay 1, the cork tops were macerated in wine model solution during 4 hours, which was thought to be insufficient for the extraction of quantifiable amounts of furfural and 5-methyl-2-furfural, the compounds previously found to discriminate “Terceiro” and “Superior”. For this reason, a second assay (Assay 2) was performed, where “Flor”, “Superior”, “Extra” and “Terceiro” ( $n=30$ ) natural cork tops were macerated for 2 days in wine model solution. At this time, only furfural, eucalyptol and camphor were present at

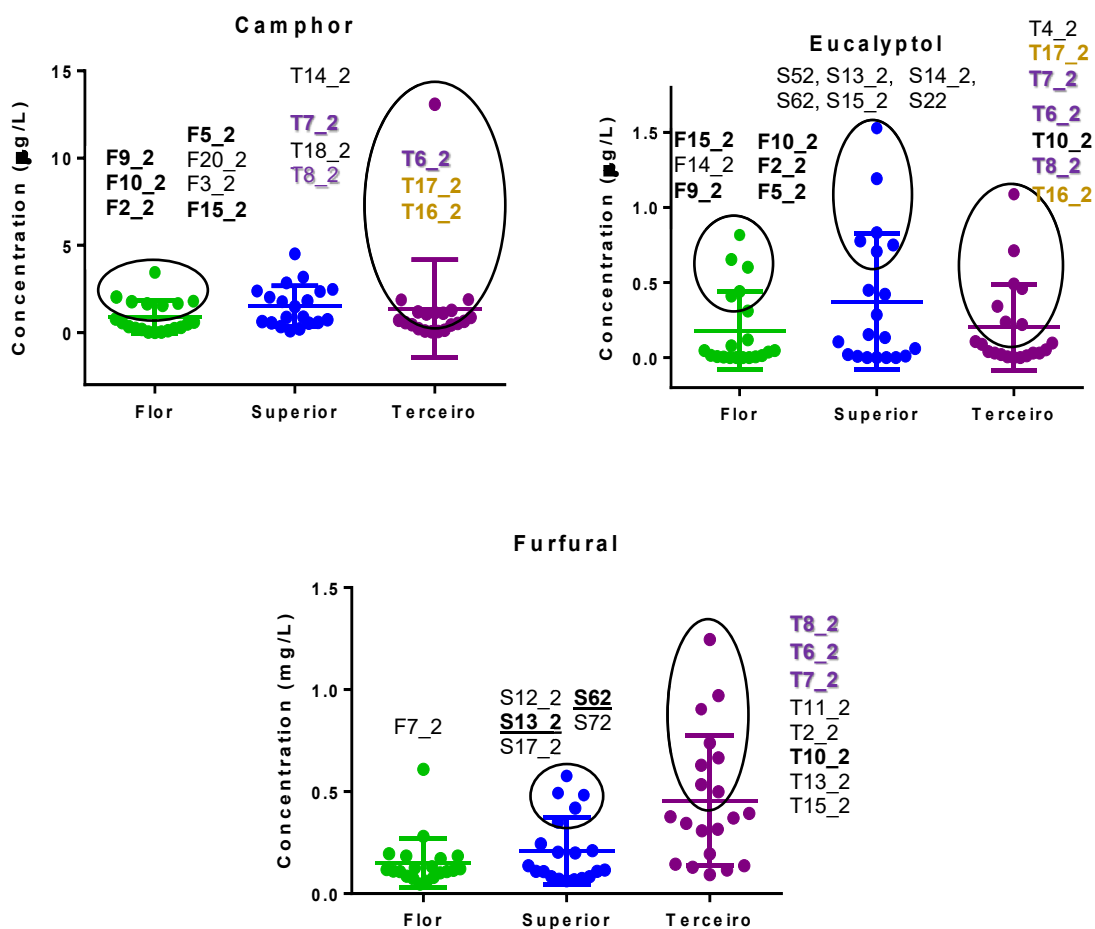
quantifiable amounts in the samples. The results (Figure 16) showed that camphor enabled the discrimination of “Extra” cork stoppers into two different subclasses of quality, while eucalyptol and furfural only subdivided “Terceiro”.



**Figure 16** – Results of Assay 2 representing the concentration levels of camphor, eucalyptol and furfural extracted from “Extra”, “Flor”, “Superior” and “Terceiro” cork tops into wine model solution. In bold are represented the samples grouped in subclass 2 by more than one compound.

Finally, the other top of the cork stoppers used on pilot study ( $n=20$ ) (data not shown), which was referred on the beginning of this chapter, was macerated in wine model solution during 2 days and analysed by HS-SPME-GC-MS/MS (Assay 3). The results obtained (Figure 17) unveiled that camphor was responsible for subdividing “Flor” and “Terceiro” into two subclasses, while eucalyptol subdivided all classes of natural cork into 2 subclasses and furfural subdivided “Superior” and “Terceiro”. The results related to camphor and eucalyptol are substantially different from the results obtained from the pilot study, since both compounds were only found to discriminate “Flor” natural cork stoppers into two subclasses.





**Figure 17** – Results of Assay 3 representing the concentration levels of camphor, eucalyptol and furfural extracted from “Flor”, “Superior” and “Terceiro” cork tops into wine model solution. In bold are represented the samples grouped in subclass 2 by more than one compound.

Overall, the results summarized in Table 6 unveiled that “Flor” cork stoppers can potentially be subdivided into two subclasses based on the levels of eucalyptol and camphor, since both compounds were found to discriminate two subclasses of “Flor” in two of the three studies. In addition, “Superior” can also be subdivided based on the levels of eucalyptol, as shown in Assay 1 and Assay 3 (not confirmed in Assay 2). “Terceiro” cork stoppers were only studied in Assay 2 and Assay 3 and the results unveiled that furfural was able to subdivide this class into two subclasses. Finally, the results obtained for “Extra” cork stoppers were inconclusive, since no compounds were found in common in both assays (Assay 1 and Assay 2).

**Table 6** – Summary of the percentage of samples in subclass 1 and subclass 2 for all compounds found to discriminate different subclasses of natural cork stoppers. Statistical significance assessed using Mann-Whitney test (\*\*\*\* -  $p < 0.0001$ ).

Class	Assay	VOC	% Samples Subclass 1	% Samples Subclass 2	p-value
“Flor”	1	Camphor	91%	9%	****
		Eucalyptol	94%	6%	****
		Limonene	93%	7%	****
		2-Pentylfuran	96%	4%	****
	2	-	-	-	-
	3	Camphor	65%	35%	****
		Eucalyptol	70%	30%	****
	1	Eucalyptol	92%	8%	****
		Limonene	96%	4%	****
		2-Pentylfuran	95%	5%	****
“Superior”	2	-	-	-	-
	3	Eucalyptol	70%	30%	****
		Furfural	75%	25%	****
	2	Furfural	60%	40%	****
		Eucalyptol	83%	17%	****
		Camphor	65%	35%	****
	3	Eucalyptol	65%	35%	****
		Furfural	60%	40%	****
	1	Limonene	95%	5%	****
		2-Pentylfuran	96%	4%	****
“Extra”	2	Camphor	83%	17%	****

These findings suggested that natural cork stoppers can be divided into two subclasses of more homogenous quality based on the profile of VOCs that can be extracted from cork by wine model solution, although more studies should be made to confirm these results.

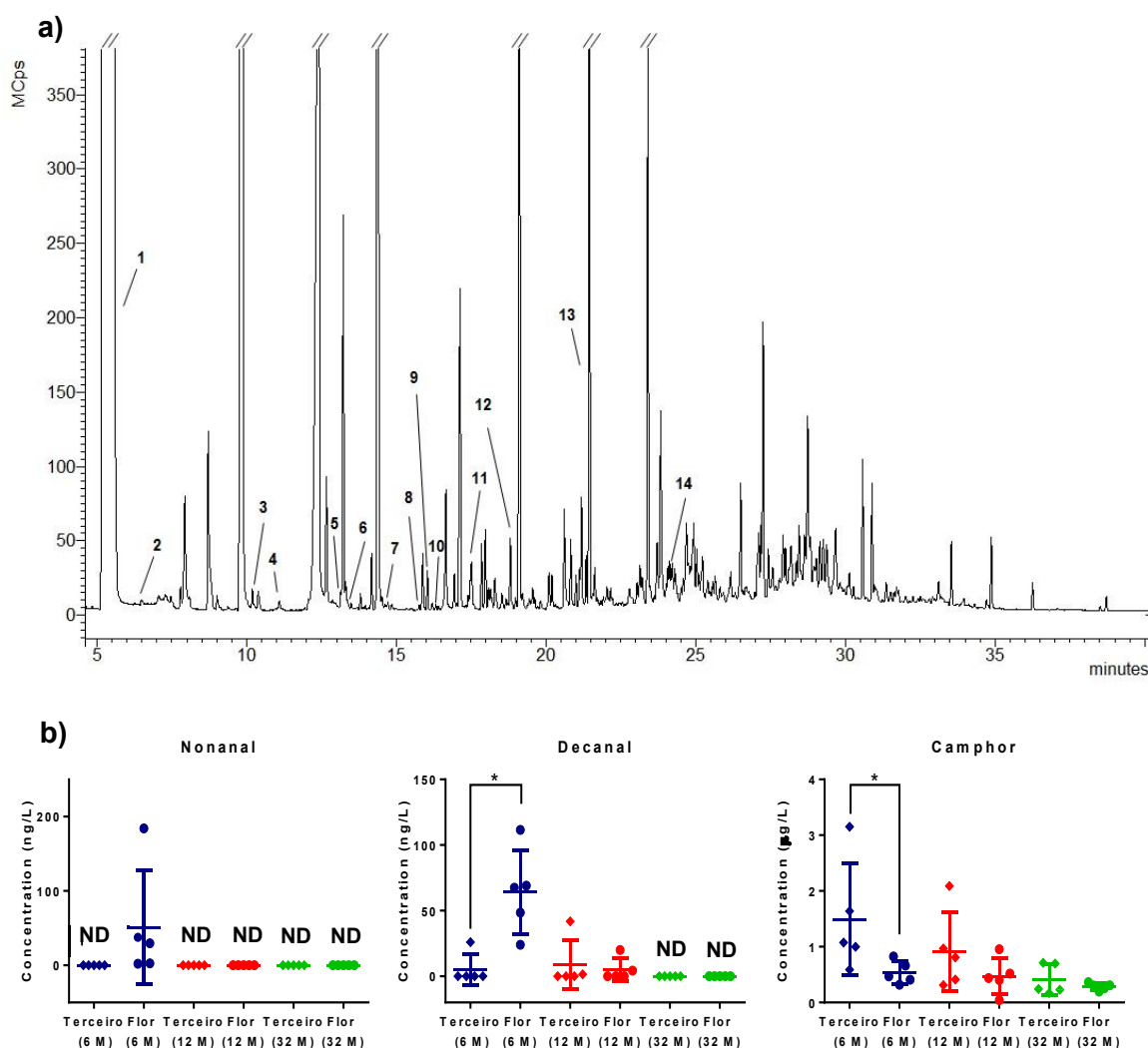
### 3.3. Volatile profiling of wine model solution bottled with “Flor” and “Terceiro” natural cork stoppers with and without washing

The aim of this study was to assess the VOCs profile extracted from two different qualities of natural cork stoppers, namely “Flor” and “Terceiro” by wine model solution.

Furthermore, the effect of washing on VOCs profile was also studied, including a process called Clean 2000, which consisted in disinfection using an aqueous solution containing 2% of H<sub>2</sub>O<sub>2</sub>. For this purpose, wine model solution was sealed with “Flor” and “Terceiro” cork stoppers without washing and with “Clean 2000” (*n*=5 per group) in bottles of 0.375 L. Three time points were considered, namely 6, 12 and 32 months, in order to study the migration behaviour of VOCs from cork into wine model solution.

### 3.3.1. VOCs composition of wine model solution sealed with “Flor” versus “Terceiro”

The VOCs profile of wine model solution sealed with “Flor” at 6 months post-bottling is represented in Figure 18a and Table 7.



**Figure 18** – a) Representative HS-SPME-GC-MS chromatogram of VOCs extracted by wine model solution from “Flor” cork stopper at 6 months post-bottling. 1- Furfural, 2- *p*-

xylene, 3- ethyl hexanoate, 4- *o*-cymene, 5- ethyl heptanoate, 6- nonanal, 7- camphor, 8- ethyl 7-octenoate, 9 – ethyl octanoate, 10- decanal, 11- *m*-di-*tert*-butylbenzene, 12- ethyl nonanoate, 13- ethyl decanoate and 14- 2,4-di-*tert*-butylphenol; b) Compounds changing between “Flor” and “Terceiro” natural cork stoppers at 6 months of analysis. Statistical significance assessed using Kruskal-Wallis test. ND- not detected.

Overall, 35 compounds were detected, from which 6 were putatively identified and 15 were formally identified using standard compounds. Comparing with the list of VOCs obtained previously (Table 4, 3.1.), ethyl octanoate and 2,4-di-*tert*-butylphenol were also found being extracted from cork for the first time. Other VOCs were also detected in this study for the first time, to our knowledge, namely ethyl 7-octenoate, ethyl decanoate, *p*-xylene, *m*-di-*tert*-butylbenzene and 2-carene.

**Table 7** – List of VOCs extracted from cork by wine model solution based on NIST 14 and standard compounds.

Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
<b>Aldehydes</b>						
Nonanal	124-19-6	13,37	1089	1104	57/70/82/95	STD
Decanal	112-31-2	16,32	1189	1206	55/67/82/95	STD
<b>Esters</b>						
Ethyl hexanoate	123-66-0	10,19	985	1000	60/70/88/99	STD
Ethyl heptanoate	106-30-9	13,15	1082	1097	60/70/88/113	STD
Ethyl 7-octenoate	35194-38-8	15,77	1171	1187	55/70/88/96	NIST
Ethyl octanoate	106-32-1	16,05	1180	1196	57/70/88	STD
Ethyl nonanoate	123-29-5	18,82	1278	1296	55/70/88	STD
Ethyl decanoate	110-38-3	21,44	1376	1396	55/70/88/101	STD
<b>Benzenoids</b>						
<i>p</i> -Xylene	106-42-3	6,49	855	865	63/77/91/106	NIST 14
<i>o</i> -Cymene	527-84-4	10,96	1010	1022	91/119/191	NIST 14
<i>m</i> -Di- <i>tert</i> -butylbenzene	1014-60-4	17,51	1232	-	57/73/175	NIST 14
<b>Furans</b>						
Furfural	98-01-1	5,55	819	833	75/96/133	STD
<b>Monoterpenes</b>						
1,4-Cineole	470-67-7	10,68	1001	1016	71/111/125	STD
$\alpha$ -Terpinene	99-86-5	10,73	1003	1017	93/105/121/136	
2-Carene	554-61-0	10,77	1004	1001	77/93/121	NIST 14
Limonene	138-86-3	11,10	1015	1031	68/79/93/107	STD
Eucalyptol	470-82-6	11,21	1018	1032	57/69/81/108/117	STD
Camphor	464-48-2	14,62	1132	1145	55/73/81/95	STD
L-Borneol	464-45-9	15,36	1157	1160	57/70/95/112	STD
L-Menthol	2216-51-5	15,53	1163	1175	71/101/129	NIST 14
<b>Phenol</b>						

Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
2,4-Di- <i>tert</i> -butylphenol	96-76-4	24,22	1486	1519	57/71/86/191	STD
<b>Unknowns</b>						
Un 1	-	7,29	885	-	77/133/151	-
Un 2	-	12,37	1057	-	96/133/193/207	-
Un 3	-	13,22	1085	-	75/126/193	-
Un 4	-	14,04	1112	-	57/69/83/98	-
Un 5	-	17,01	1214	-	57/111/125	-
Un 6	-	19,12	1289	-	59/73/147	-
Un 7	-	19,45	1301	-	57/69/85/111	-
Un 8	-	20,20	1329	-	59/76/104	-
Un 9	-	21,83	1391	-	57/71/85/96	-
Un 10	-	22,33	1410	-	59/69/85/99	-
Un 11	-	23,14	1442	-	57/71/85/177	-
Un 12	-	23,71	1465	-	57/71/83	-
Un 13	-	24,87	1512	-	55/73/83	-
Un 14	-	26,58	1585	-	55/69/83/97	-

<sup>a</sup> Kovats retention indices (RI) determined using a commercial hydrocarbon mixture (C6-C20). <sup>b</sup> RI reported in literature. RT - retention time; STD - standard compound; NIST - National Institute of Standards and Technology database version 14; Un – Unknown.

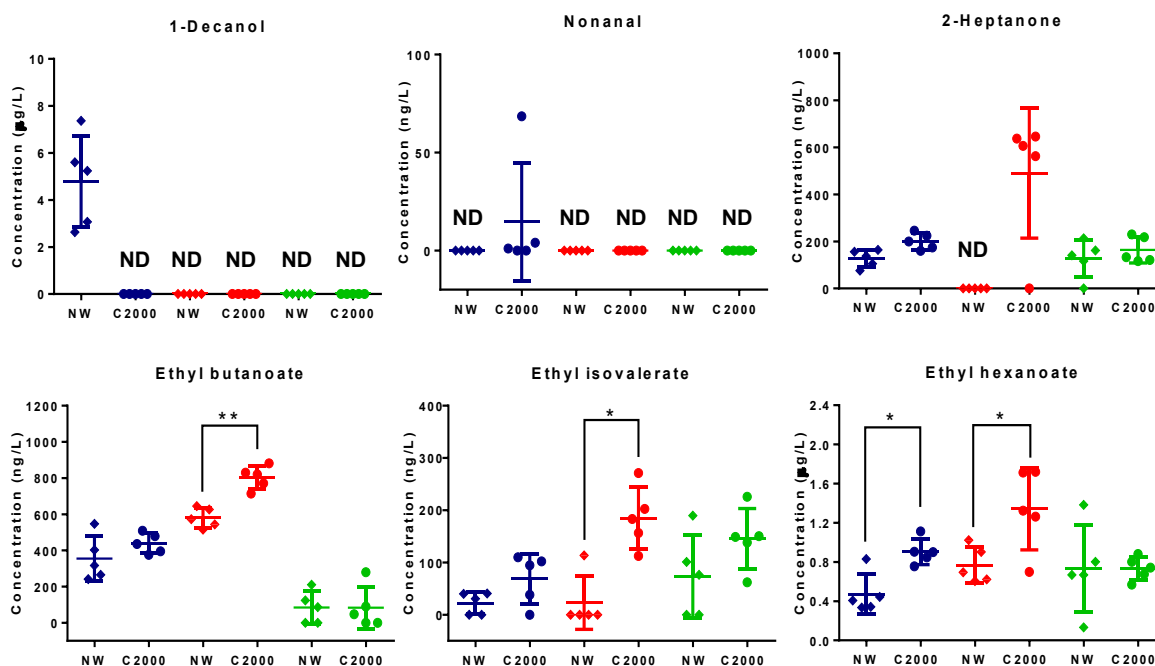
The results for quantification of 22 VOCs in wine model solution sealed with “Flor” and “Terceiro” over time are represented in Supplementary Table S1. The statistical analysis showed that, at 6 months, 3 of the 22 VOCs quantified were responsible for differentiating these two natural cork grades (Figure 18b, Table 8), though these differences disappeared over time. This could mean that, for wine model solutions, and for the compounds quantified, these two different qualities of natural cork, lead to very similar changes in wines over time.

**Table 8** – Compounds discriminating “Terceiro” and “Flor” cork stoppers. Statistical significance assessed using Kruskal-Wallis and Mann-Whitney tests (\* -  $p < 0.05$ ).

Compound	Groups	% variation (p-value)
<b>Nonanal</b>	“Flor” vs. “Terceiro” (6M)	Only detected in Flor
<b>Decanal</b>	“Flor” vs. “Terceiro” (6M)	91.9 ± 47.5 (*)
<b>Camphor</b>	“Flor” vs. “Terceiro” (6M)	-46.3 ± 28.9 (*)

### 3.3.2. VOCs composition of wine model solution sealed with “Terceiro” with and without washing

The comparison of wine model solution sealed with “Terceiro” with (Clean 2000) and without washing (No Wash) is represented in Figure 19 and Table 9. The concentration values obtained for the 22 VOCs present in each class are in Supplementary Table S2. The results unveiled that 1-Decanol, nonanal and ethyl hexanoate were present at different concentrations in wine model solution sealed with “Terceiro” with (Clean 2000) and without washing at 6 months post-bottling. 1-Decanol was only present at 6 months in wine model solution sealed with “Terceiro” cork stoppers without washing. On the other hand, nonanal and ethyl hexanoate were increased in wine model solution sealed with Clean 2000 compared with No Wash. This suggests that, in the first months, the washing step may have a significant effect on wine characteristics.



**Figure 19** – Compounds changing between wine model solution sealed with “Terceiro” with (C2000 – Clean 2000) and without (NW – No Wash) washing (6 months – blue; 12 months – red; 32 months – green). Statistical significance assessed using Kruskal-Wallis test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ). ND – not detected.

At 12 months post-bottling, ethyl hexanoate was still increased in wine model solution sealed with Clean 2000 compared with No Wash. In addition, ethyl butanoate and ethyl isovalerate were also present in higher amounts in wine model solution sealed with Clean 2000 vs. No Wash at 12 months post-bottling. Finally, 2-heptanone was present at

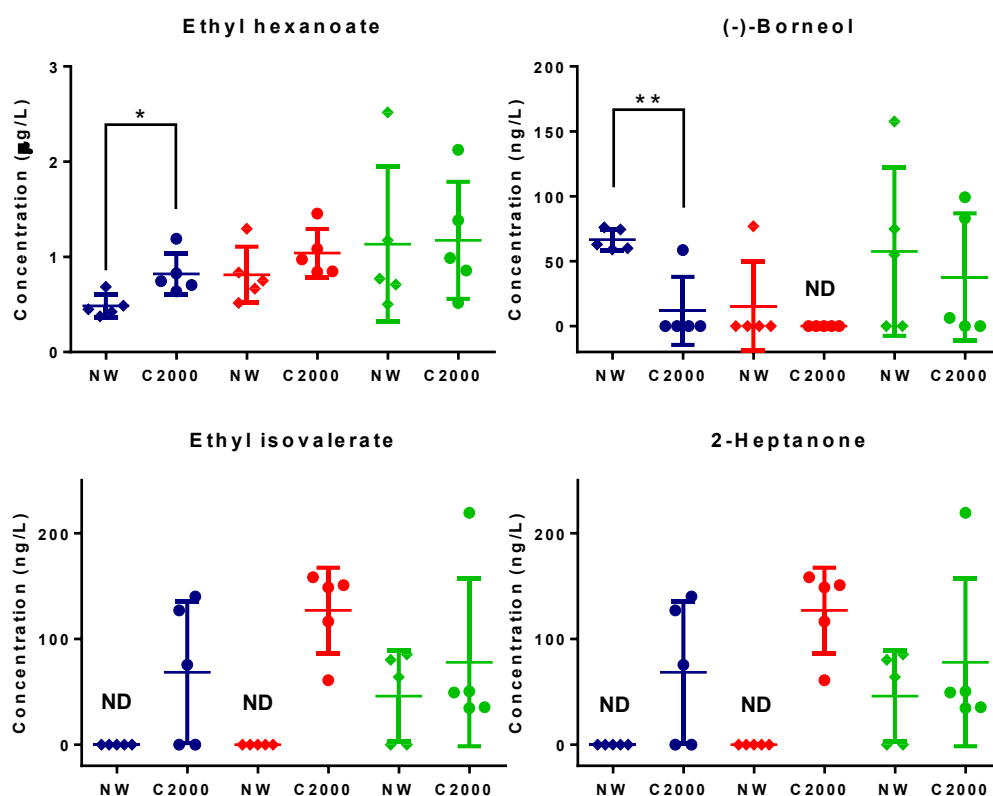
significant higher amounts in wine model solution sealed with Clean 2000 vs. No Wash at 12 months. These results showed that the differences found in VOC levels tended to disappear over time, which suggests that wine bottling with “Terceiro” with or without washing will lead to similar changes for bottling periods above 12 months.

**Table 9** – List of compounds changing between “Terceiro” with (Clean 2000) and without washing. Statistical significance assessed using Kruskal-Wallis test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ).

Compound	Groups	% variation (p-value)
<b>1-Decanol</b>	Clean 2000 vs. No Wash (6M)	Only detected in No Wash
<b>Nonanal</b>	Clean 2000 vs. No Wash (6M)	Only detected in Clean 2000
<b>2-Heptanone</b>	Clean 2000 vs. No Wash (12M)	Only detected in Clean 2000
<b>Ethyl butanoate</b>	Clean 2000 vs. No Wash (12M)	$38.4 \pm 58.2$ (**)
<b>Ethyl isovalerate</b>	Clean 2000 vs. No Wash (12M)	$87.7 \pm 67.2$ (*)
<b>Ethyl hexanoate</b>	Clean 2000 vs. No Wash (6M)	$91.5 \pm 68.5$ (*)
	Clean 2000 vs. No Wash (12M)	$42.7 \pm 1.8$ (*)

### 3.3.3. VOCs composition of wine model solution sealed with “Flor” with and without washing

The comparison of wine model solution sealed with “Flor” with (Clean 2000) and without washing (No Wash) is represented in Figure 20 and Table 10. The concentration values obtained for the 22 VOCs present in each class are in Supplementary Table S3. The results showed that ethyl hexanoate was also increased in wine model solutions sealed with Clean 2000 vs. No Wash, similarly to “Terceiro”. However, this difference is only observed at 6 months of storage. The terpene (-)-borneol was also found to change at 6 months post-bottling, being present at considerably lower amounts in wine model solution sealed with Clean 2000 vs. No Wash, suggesting that the washing step may partially deplete the (-)-borneol content of “Flor” cork stoppers.



**Figure 20** – Compounds changing between wine model solution sealed with “Flor” with (C2000 – Clean 2000) and without (NW- No Wash) washing. (6 months – blue; 12 months – red; 32 months – green). Statistical significance assessed using Kruskal-Wallis test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ). ND – not detected.

Furthermore, ethyl isovalerate and 2-heptanone were also found statistically different at 12 months, being present at higher concentrations in wine model solution sealed with Clean 2000 vs. No Wash. However, these differences also tended to disappear over time, as found for “Terceiro” cork stoppers.

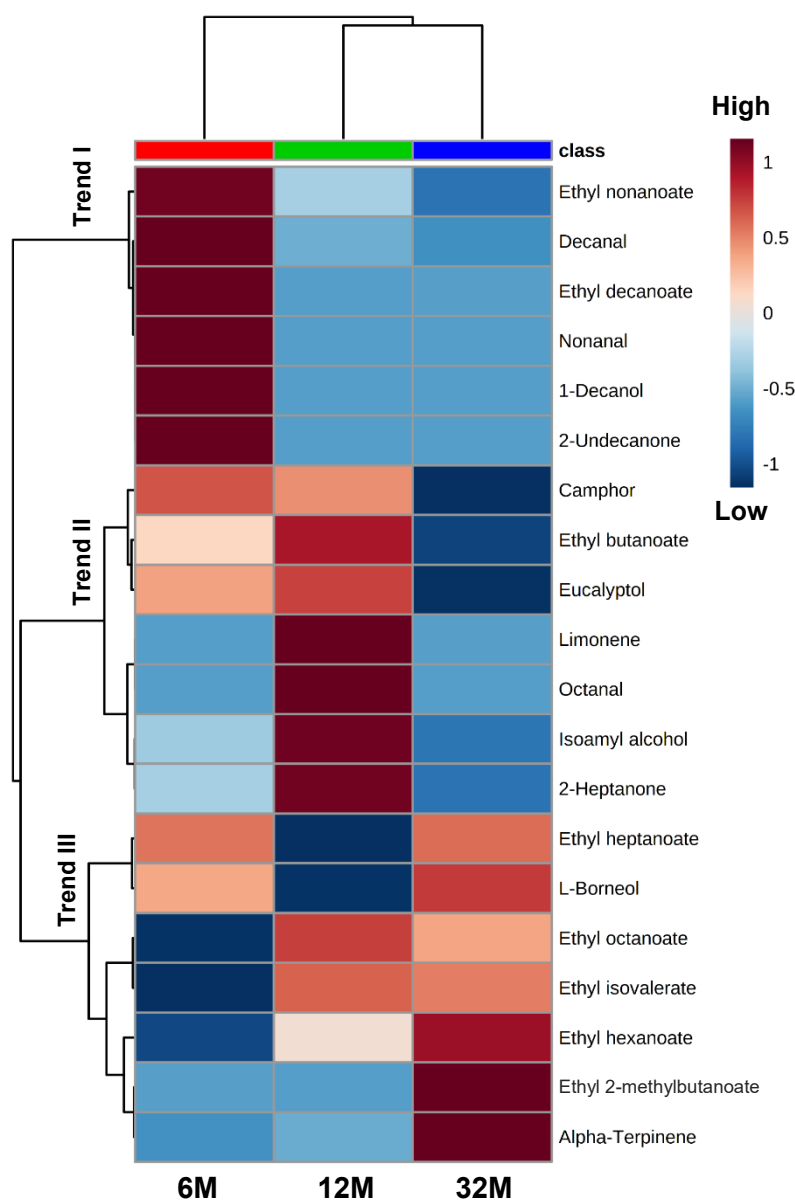
**Table 10** – List of compounds changing between “Flor” with (Clean 2000) and without washing (No Wash). Statistical significance assessed using Kruskal-Wallis test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ).

Compound	Groups	% variation (p-value)
<b>Ethyl hexanoate</b>	Clean 2000 vs. No Wash (6M)	$69.4 \pm 12.3$ (*)
<b>(-)-Borneol</b>	Clean 2000 vs. No Wash (6M)	$-82.4 \pm 87.6$ (**)
<b>Ethyl isovalerate</b>	Clean 2000 vs. No Wash (12M)	Only detected in Clean 2000
<b>2-Heptanone</b>	Clean 2000 vs. No Wash (12M)	Only detected in Clean 2000



### 3.3.4. Migration behaviour of VOCs extracted from cork stoppers into wine model solution over time

The migration behaviour of VOCs from cork stoppers into wine model solution can be important to understand wine evolution in bottle. In Figure 21, a Heatmap representing the average concentration of VOCs over time is shown.



**Figure 21** – Heatmap representing the migration kinetics of VOCs extracted from “Flor” and “Terceiro” natural cork stoppers by wine model solution at 6, 12 and 32 months. Dark red colours are associated with higher concentrations and darker blue colours with lower concentrations.

A hierarchical cluster analysis was performed to cluster the VOCs with similar behaviour, thus resulting in three main trends. In *trend I*, six VOCs decreased from 6 to 32 months, namely 1-decanol, nonanal, decanal, ethyl nonanoate, ethyl decanoate and 2-undecanone. The decrease in the levels of 1-decanol over time may be related to oxidation [55], while the decrease of nonanal, decanal and 2-undecanone may be related with their very reactive nature [55]. The hydrolysis of esters is a well-known process occurring over time, thus the decrease in the levels of ethyl nonanoate and ethyl decanoate may be attributed to hydrolysis [57], oxidation [55] and/or scalping.

In *trend II*, three VOCs showed a tendency to decrease from 12 to 32 months (constant levels from 6 to 12 months), namely L-camphor, ethyl butanoate and eucalyptol, and other four VOCs unveiled a tendency to increase from 6 to 12 months and then decrease from 12 to 32 months, namely limonene, octanal, isoamyl alcohol and 2-heptanone. In general, this behaviour may be explained due to the heterogeneity of cork stoppers, since independent samples were used. The decrease in the levels of ethyl butanoate may be related with hydrolysis or oxidation processes [55].

In *trend III*, three different tendencies were observed: 1) decrease from 6 to 12 months and then increase from 12 to 32 months (ethyl heptanoate and L-borneol), 2) increase from 6 to 12 months and then remain constant from 12 to 32 months (ethyl octanoate and ethyl isovalerate), and 3) constant concentrations from 6 to 12 months and then increase from 12 to 32 months (ethyl hexanoate, ethyl 2-methylbutanoate and  $\alpha$ -terpinene). No explanation was found for the tendency observed for ethyl heptanoate and L-borneol, besides the heterogeneity of cork stoppers. Regarding the increase of ethyl esters and  $\alpha$ -terpinene, this behaviour may be related with a possible extraction of these compounds from cork stoppers into wine model solution.

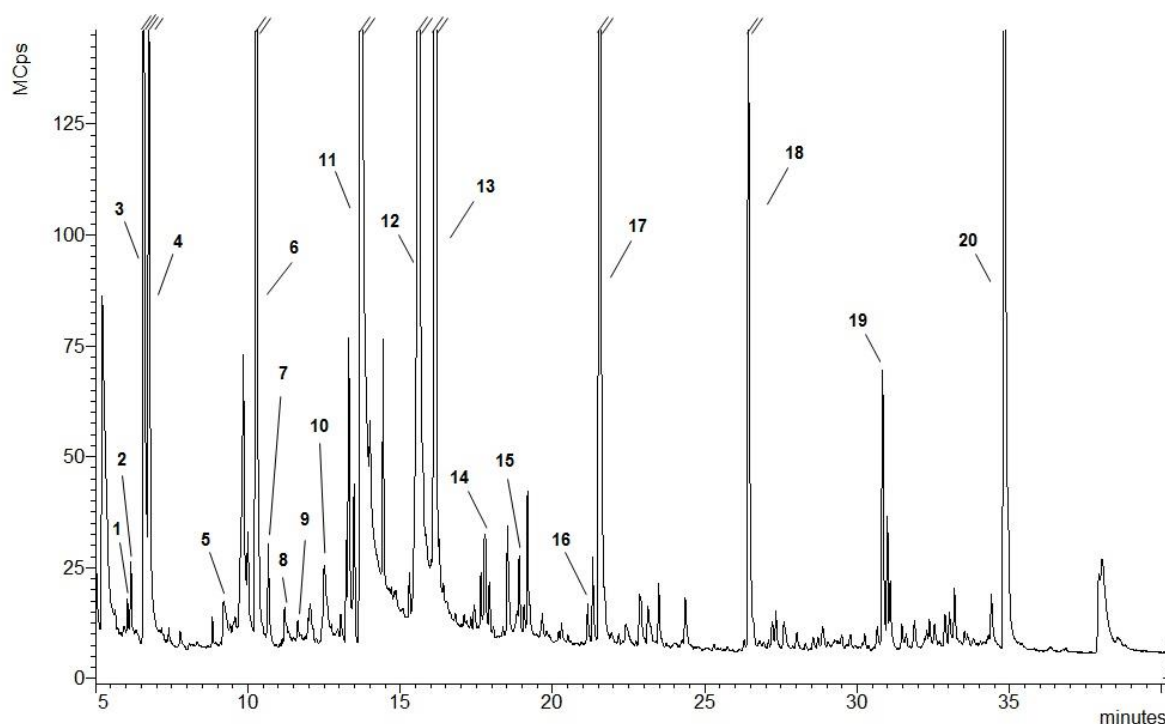
### **3.4. Volatile profiling of Porto wine sealed with cork stoppers and stored under different conditions**

The VOCs profile of Porto wine sealed with “Flor” natural cork stoppers and bartop under different cellar conditions (bottle position and cellar type) was studied over time (6, 15 and 26 months) by HS-SPME-GC-MS. Five group classes were defined: 1) “Flor” cork stoppers stored in a vertical position in a cellar called “Garrafeira 5”; 2) “Flor” cork stoppers stored in a horizontal position in “Garrafeira 5”; 3) “Flor” cork stoppers stored in a horizontal position in “Garrafeira Castelo”; 4) bartop stoppers stored in a vertical position in “Garrafeira 5”; and 5) bartop stoppers stored in a horizontal position in “Garrafeira 5”. “Garrafeira Castelo” differed from “Garrafeira 5” in atmospheric temperature, which was, approximately,

10 °C higher. In this experiment, the main goals were to evaluate the effect of different types of stoppers (“Flor” natural cork and bartop), different bottle positions (vertical and horizontal) and different cellars (“Garrafeira 5” and “Garrafeira castelo”). The other goal was to evaluate the evolution of Porto wine over time.

### 3.4.1. Volatile profile of Porto wine

The VOCs profile of Porto wine at 6 months of storage is represented in Figure 22 and the compounds detected are listed in Table 11. Overall, 52 compounds were detected, from which 13 were putatively identified and 25 were formally identified using standard compounds. The main VOCs group identified were esters, as expected since they are the major group found in wine after higher alcohols and are mainly responsible for “fruity” characters in wines [52].



**Figure 22** – Representative HS-SPME-GC-MS chromatogram of a Porto wine at 6 months post-bottling. 1- Ethyl 2-methylbutanoate, 2- ethyl isovalerate, 3- 1-hexanol, 4- isoamyl acetate, 5- benzaldehyde, 6- ethyl hexanoate, 7- hexyl acetate, 8- limonene, 9- phenylacetaldehyde, 10- 1-octanol, 11-  $\beta$ -linalool, 12- diethyl succinate, 13- ethyl octanoate, 14- phenylethyl acetate, 15- ethyl nonanoate, 16-  $\beta$ -damascenone, 17- ethyl decanoate, 18- ethyl dodecanoate, 19- ethyl tetradecanoate and 20- ethyl hexadecanoate.

**Table 11** – List of VOCs detected in Porto wine samples at 6 months post-bottling, based on NIST 14 and standard compounds.

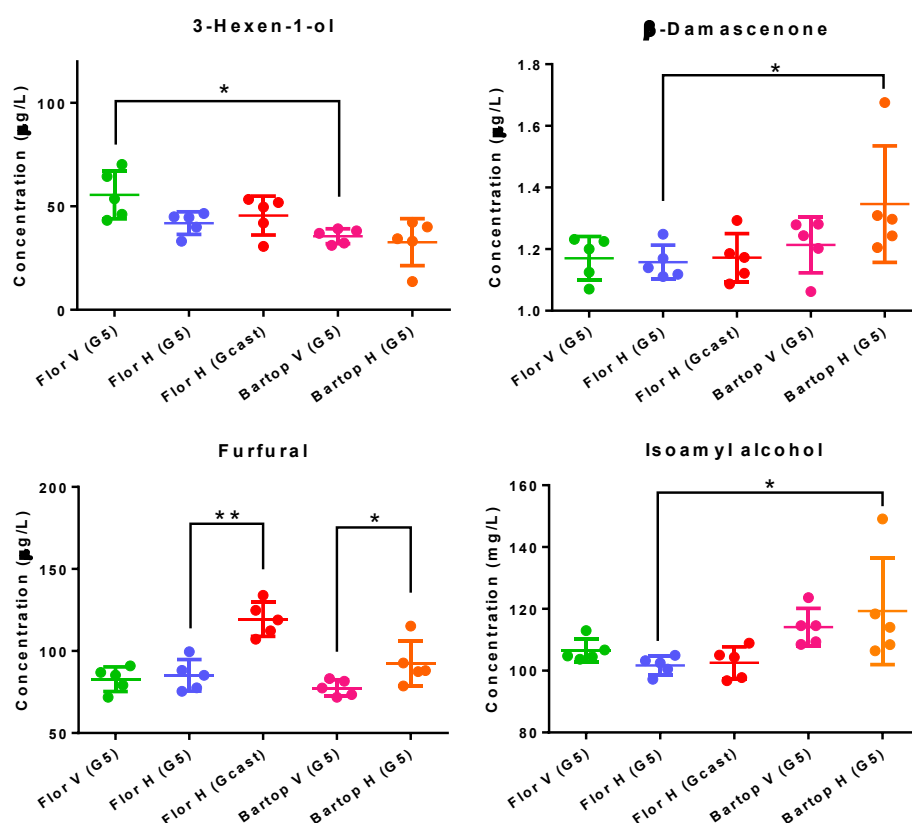
Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
<b>Aldehydes</b>						
Benzaldehyde	100-52-7	9.20	951	962	51/77/105	STD
Phenylacetaldehyde	122-78-1	11.66	1033	1045	55/91/97/120	STD
Nonanal	124-19-6	13.49	1093	1104	57/70/85/103	STD
β-Cyclocitral	432-25-7	16.81	1206	1220	67/81/109/137	STD
<b>Alcohols</b>						
3-Hexen-1-ol	928-96-1	6.34	849	856	55/67/91/106	STD
1-Hexanol	111-27-3	6.58	858	868	56/69/84	STD
1-Heptanol	111-70-6	9.58	964	970	55/70/101	NIST 14
1-Octanol	111-87-5	12.5	1061	1071	55/70/83	STD
Phenylethyl alcohol	98-85-1	13.68	1100	1116	65/91/103/122	STD
<b>Esters</b>						
Ethyl isovalerate	108-64-5	6.15	842	854	57/70/85/88	STD
Isoamyl acetate	123-92-2	6.75	865	876	55/70/87	STD
Ethyl valerate	539-82-2	7.41	890	900	57/85/101	NIST 14
Ethyl 2-methylbutanoate	7452-79-1	6.05	838	849	57/74/85/102	STD
Ethyl hexanoate	123-66-0	10.25	987	1000	43/70/88/99/115	STD
Hexyl acetate	142-92-7	19.67	1001	1011	56/61/69	STD
Isoamyl hexanoate	123-92-2	17.64	1236	1252	55/70/99	NIST 14
Ethyl heptanoate	106-30-9	13.23	1085	1097	60/70/88/113	STD
Ethyl octanoate	106-32-1	16.12	1183	1196	57/70/88/101	STD
Ethyl decanoate	110-38-3	21.57	1381	1396	55/70/88/101	STD
Diethyl succinate	123-25-1	15.59	1165	1182	55/73/101/129	NIST 14
Phenylethyl acetate	103-45-7	17.78	1241	1258	59/91/104	NIST 14
Ethyl nonanoate	123-29-5	18.91	1282	1296	55/70/88/101	STD
Methyl decanoate	110-42-9	19.67	1309	1325	55/74/87/143	NIST 14
Ethyl 9-decenoate	67233-91-4	21.32	1371	1387	55/69/88/101	NIST 14
Ethyl dodecanoate	106-33-2	26.43	1578	1595	55/70/88/101	NIST 14
Isopentyl decanoate	2306-91-4	27.60	1629	1646	55/70/85/155	NIST 14
Ethyl tetradecanoate	124-06-1	30.86	1775	1794	55/70/88/101	NIST 14
Ethyl pentadecanoate	41114-00-5	32.87	1875	1894	57/70/88/101	NIST 14
Ethyl hexadecanoate	628-97-7	34.82	1975	1993	55/70/88/101	NIST 14
<b>Furans</b>						
Furfural	98-01-1	5.64	822	833	95/96	STD
<b>Ketones</b>						
2-Heptanone	110-43-0	7.11	879	891	43/56/69	STD
2-Nonanone	821-55-6	13.05	1079	1092	58/73/115	STD
β-Damascenone	23726-93-4	21.15	1365	1386	69/77/195/121	STD
4-Butanolide	96-48-0	7.78	904	915	56/86/95	NIST 14

Compound	CAS	RT (min)	RI <sup>a</sup>	Reported RI <sup>b</sup>	Most abundant ions (m/z)	Identification method
<b>Monoterpenes</b>						
Limonene	138-86-3	11.2	1018	1031	68/79/93/107	STD
β-Linalool	78-70-6	13.31	1088	1099	55/71/80/93	STD
α-Pinene	7785-26-4	8.34	922	937	79/93/133	STD
<b>Phenols</b>						
2,4-Di-tert-butylphenol	96-76-4	24.37	1492	1519	57/191/206	STD
<b>Unknowns</b>						
Un 1	-	17.10	1217	-	79/93/121	-
Un 2	-	17.32	1224	-	79/93/136	-
Un 3	-	17.94	1247	-	69/79/93/121	-
Un 4	-	18.52	1268	-	77/93/107/121	-
Un 5	-	19.24	1294	-	73/123/138	-
Un 6	-	19.80	1314	-	57/101/129	-
Un 7	-	20.20	1329	-	73/83/95/109	-
Un 8	-	22.18	1404	-	69/93/119	-
Un 9	-	22.41	1413	-	55/71/101/129	-
Un 10	-	22.89	1432	-	57/70/127	-
Un 11	-	23.13	1442	-	73/115/127	-
Un 12	-	25.30	1531	-	57/73/101/155	-
Un 13	-	26.30	1573	-	71/87/111	-
Un 14	-	27.22	1612	-	55/69/83	-

<sup>a</sup> Kovats retention indices (RI) determined using a commercial hydrocarbon mixture (C6-C20). <sup>b</sup> RI reported in literature. RT - retention time; STD – standard compound; NIST – National Institute of Standards and Technology database version 14; Un – Unknown.

#### 3.4.2. Comparison of the different storage conditions at the same time point

The 34 VOCs quantified at 6, 15 and 26 months of Porto wine post-bottling are shown in Supplementary Tables S4, S5 and S6. The results showed no statistically significant differences in the VOCs levels between the conditions tested at 6 months post-bottling, which suggested that the evolution of Porto wine is similar. However, at 15 months post-bottling, several differences in the VOC concentrations between the group classes were observed (Figure 23, Table 12).



**Figure 23** – Compounds differing between Flor V (G5) vs. Bartop V (G5), Flor H (G5) vs. Bartop H (G5), Flor H (G5) vs. Flor H (Gcast) and Bartop H (G5) vs. Bartop V (G5). Statistical significance assessed using Mann-Whitney test (\* -  $p < 0.05$ ; \*\*  $p < 0.01$ ).

3-Hexen-1-ol was present at statistically significant higher amounts in Flor V (G5) compared with Bartop V (G5).  $\beta$ -Damascenone and isoamyl alcohol were present at statistically significant lower amounts in wine sealed with Flor H (G5) than Bartop H (G5). Furfural was present in statistically significant lower amounts in Flor H (G5) when compared to Flor H (Gcast), which may be explained by higher temperatures in “Garrafeira Castelo” thus accelerating wine oxidation or by the Maillard reaction responsible for furfural formation through carbohydrates reduction [61]. At last, furfural was also observed at statistically significant higher amounts in wine sealed with Bartop H (G5) when compared with Bartop V (G5), which may indicate that the horizontal position favoured the furfural extraction from the stopper [95].

**Table 12** – Compounds discriminating the sample groups at 15 months of storage. Statistical significance assessed using Kruskal-Wallis and Mann-Whitney tests (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ).

Compound	Groups	% variation (p-value)
3-Hexen-1-ol	Flor V (G5) vs. Bartop V (G5)	$36.0 \pm 9.7$ (*)

<b>β-Damascenone</b>	Flor H (G5) vs. Bartop H (G5)	-16.4 ± 4.8 (*)
<b>Isoamyl alcohol</b>	Flor H (G5) vs. Bartop H (G5)	-6.8 ± 5.6 (*)
<b>Furfural</b>	Flor H (G5) vs. Flor H (Gcast)	-37.5 ± 6.9 (**)
	Bartop H (G5) vs. Bartop V (G5)	14.7 ± 9.4 (*)

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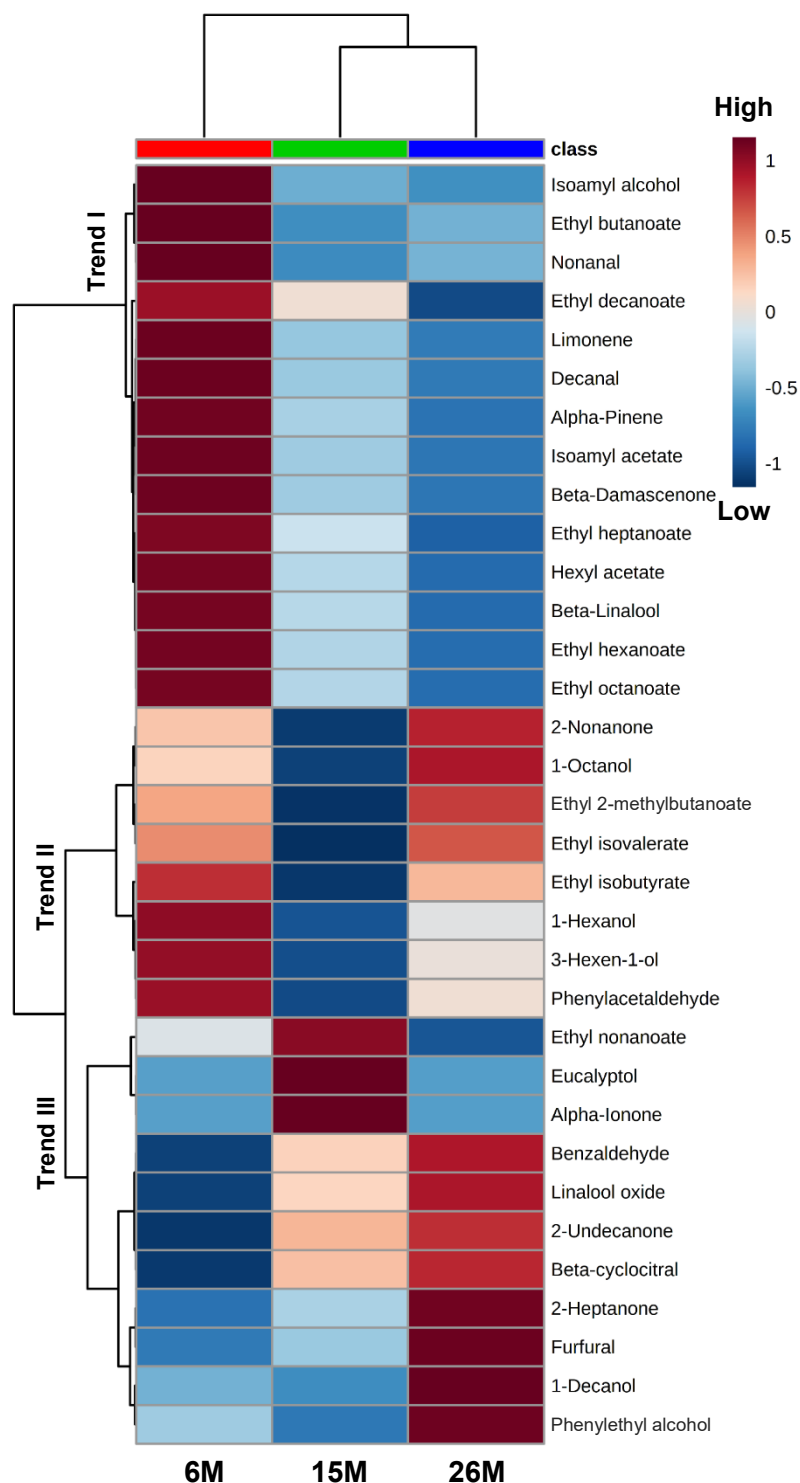
At 26 months post-bottling, no statistically significant differences in VOC levels were observed among the different conditions studied, suggesting that these conditions only affected Porto wine volatile composition until 15 months of storage.

### 3.4.3. Evolution of Porto wine during the 26 months of storage

The evolution of VOCs in Porto wine from 6 to 26 months was also studied. The Heatmap representing the average concentrations at each time points is shown in Figure 24, where it is possible to recognise three main trends. Diethyl succinate, phenylethyl acetate and camphor were only quantified at 15 and 26 months and hence their levels are represented separately in Figure 25.

In *trend I*, fourteen compounds unveiled a tendency to decrease over time, namely isoamyl alcohol, nonanal, decanal, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, hexyl acetate, limonene, α-pinene, β-linalool and β-damascenone. Amyl alcohols have been reported to decrease over time [56] due to esterification phenomena or oxidation, being converted into aldehydes [57]. The decrease in the levels of nonanal and decanal may be associated with their highly reactive nature [60]. Regarding esters, their decrease may be related to hydrolysis into correspondent alcohols and acids [61]. Ethyl octanoate and ethyl decanoate decrease could also be associated with scalping phenomenon by cork stoppers, as referred in subchapter 1.7.2 [106]. Relatively to β-Linalool is susceptible to oxidation originating the correspondent terpene oxide, linalool oxide, which levels increased in this experiment [57]. β-Damascenone has been associated with a large decrease during wine storage, which was corroborated by this study [57]. Its decrease could be associated with binding SO<sub>2</sub>, creating sulfonate adducts [55].

In *trend II*, eight compounds unveiled a tendency to decrease from 6 to 15 months and then increase from 15 to 26 months, namely 2-nonanone, 1-octanol, ethyl 2-methylbutanoate, ethyl isovalerate, ethyl isobutyrate, 1-hexanol, 3-hexen-1-ol, phenylacetaldehyde. 1-Octanol, 1-hexanol and 3-hexen-1-ol level changes may be linked with their corresponding ester hydrolysis and esterification, due to equilibrium changes [54]. Phenylacetaldehyde usually increases during wine storage due to oxidation [55], hence its increase over time was expected in this work.



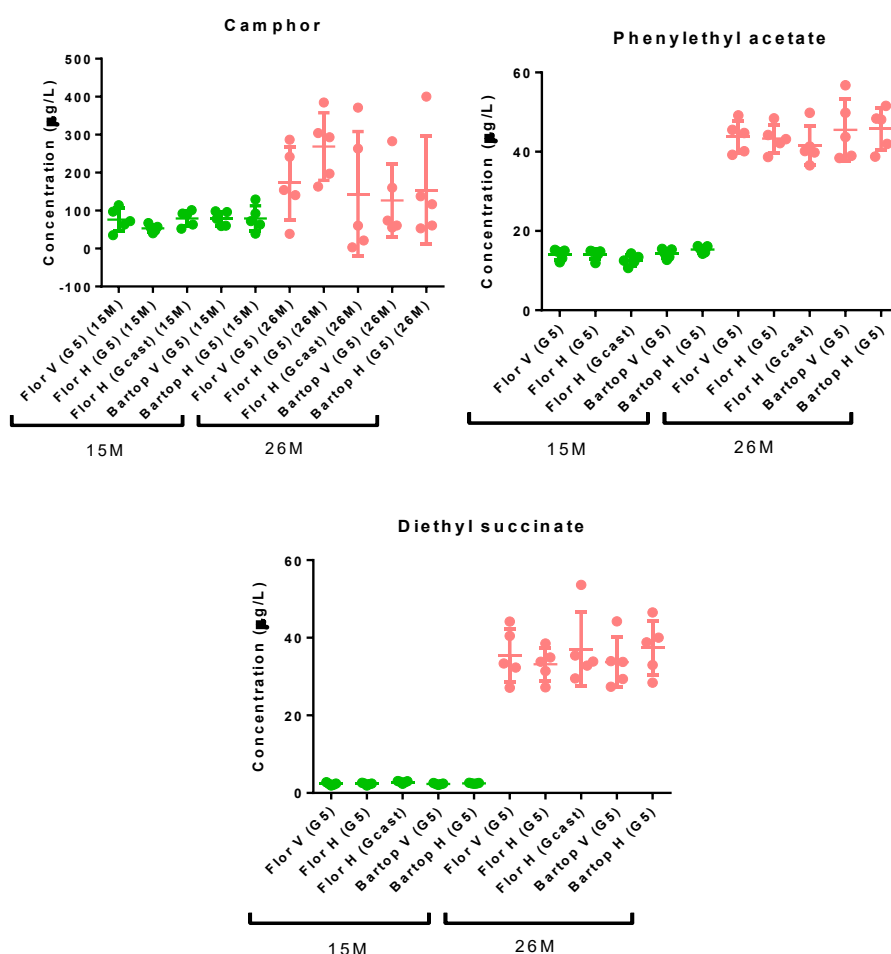
**Figure 24** – Heatmap representing the average concentration levels of VOCs in Porto wine at 6, 15 and 26 months. Dark red colours are associated with higher concentrations and darker blue colours with lower concentrations.

Regarding 2-methylbutanoate, ethyl isovalerate and ethyl isobutyrate, their variation over time may be due to equilibrium changes with their correspondent alcohols and acids. Finally, 2-nonanone increase from 15 to 26 months post-bottling may indicate that it was



formed in wine probably due to Fenton oxidation of alcohols or direct oxidation of fatty acids [55].

*Trend III* showed two main clusters: 1) compounds increasing from 6 to 15 months and then decreasing from 15 to 26 months (ethyl nonanoate, eucalyptol and  $\alpha$ -ionone); 2) compounds increasing over time (benzaldehyde, linalool oxide, 2-undecanone,  $\beta$ -cyclocitral, 2-heptanone, furfural, 1-decanol and phenylethyl alcohol). The tendency observed for ethyl nonanoate may be related to equilibrium changes between the ester and its correspondent alcohol and acid in wine [54]. Regarding eucalyptol and  $\alpha$ -ionone, Wine monoterpenes are usually present as non-volatile bound form glycosides and these bound forms are cleaved over time leading to an increase in free monoterpenes [57]. However, the tendency observed for eucalyptol and  $\alpha$ -ionone were not corroborated with literature. 1-Decanol and phenylethyl alcohol increase over time may be related to its correspondent ester hydrolysis.  $\beta$ -Cyclocitral and benzaldehyde increasing over time may be due to formation through alcohols oxidation [57]. Benzaldehyde is known for being an oxidation marker that increases with wine oxidation [58]. Furfural augmented over time, which was expected since it is formed during wine ageing through Amadori (or Heyns) rearrangement, which is a specific mechanism of Maillard reactions that reduce sugars and an amino group-containing substance producing cyclic compounds such as furfurals, pyrazines or thiazoles [57]. 2-Heptanone and 2-undecanone increase in wine is usually related to Fenton oxidation of alcohols, Strecker reaction of an amino acid with  $\alpha$ -dicarbonyl containing compounds or a direct oxidation of fatty acids [55].



**Figure 25** – Concentration levels of camphor, phenylethyl acetate and diethyl succinate in Porto wine at 15 and 26 months post-bottling.

Camphor, phenylethyl acetate and diethyl succinate increased from 15 to 26 months of storage. Camphor increase may be related to extraction from natural cork, since it has been reported as being extracted from natural cork by wine and wine model solution (Table 1, subchapter 1.7.1). Phenylethyl acetate has been reported to decrease in wine over time due to hydrolysis [57]. However this ester increased over time in the present work, which may be related to extraction from the cork stopper by Porto wine. Finally, diethyl succinate increased during storage, which may be attributed to esterification between succinic acid and ethanol [57, 114].

## 4. Conclusions

The VOCs profile extracted from natural cork stoppers by wine model solution comprised a total of 21 VOCs, from which 9 were identified for the first time in this work as capable of being extracted from cork by wine model solution, namely 2-methyl-1-octanol, chlorobenzene, ethylbenzene, *m*-xylene, *o*-xylene, ethyl octanoate, 2-pentylfuran, (-)-3-carene and 2,4-di-*tert*-butylphenol.

A HS-SPME-GC-MS/MS preliminary approach showed that it is possible to define more homogenous subclasses of quality for natural cork stoppers. “Flor” can potentially be discriminated based on the levels of eucalyptol and camphor, while “Superior” by the levels of eucalyptol. “Terceiro” natural cork stoppers could be discriminated based on the levels of furfural, whereas the results for “Extra” were inconclusive. More studies should be made to confirm these results.

“Terceiro” and “Flor” natural cork stoppers potential effect in wine model solution VOCs profile was studied during 32 months. From the 22 VOCs quantified, only 3 were found altered at 6 months, indicating that “Terceiro” and “Flor” may only affect wines at early stages of storage. Furthermore, the effect of a washing process, Clean 2000, on VOCs profile extracted from “Terceiro” and “Flor” was observed only at 6 and 12 months post-bottling, suggesting that the washing step may only be relevant for wines expected to be consumed in less than a year. At last, the behaviour of the 22 VOCs over time was studied and followed three main trends. The majority of VOCs levels oscillated over time possibly due to cork heterogeneity or the balance between desorption/adsorption from the cork stopper.

Finally, a Symington Porto wine was stored under different storage conditions and analysed at 6, 15 and 26 months post-bottling. 4 VOCs were found altered only at 15 months post-bottling, suggesting that these conditions only affected Porto wine volatile composition until 15 months of storage. The evolution of the 34 VOCs over time unveiled that, similarly to wine model solutions, VOCs followed one of three main trends. The majority of ester levels decreased over time due to hydrolysis, whereas some VOC levels oscillated possibly as a result to chemical equilibrium changes occurring in wine or to cork heterogeneity. The benzaldehyde, furfural and linalool oxide increase over time, suggested the occurrence of wine oxidation.

These studies are important to comprehend the impact of natural cork stoppers in wine’s chemical composition and the effect that they potentially have on its sensorial properties. An interesting future approach would be to associate these methodologies with sensorial descriptive analysis, in order to correlate the presence of certain VOCs with sensorial alterations.

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## 6. Appendix

**Supplementary Table S1** – Quantification of 22 volatile compounds present in wine model solutions sealed with “Terceiro” and “Flor” natural cork stoppers for 32 months.

Compound	RT	Quantifier ion	Concentration						Units	Descriptor	Olfactory perception threshold
			6 months		12 months		32 months				
			Terceiro	Flor	Terceiro	Flor	Terceiro	Flor			
<b>Alcohols</b>											
Isoamyl alcohol	3.66	55.2	6.38 ± 3.02	4.40 ± 2.51	17.6 ± 6.6	18.5 ± 2.3	ND	ND	µg/L	Fusel, cheese	30 mg/L
1-Decanol	18.24	70.1	4.78 ± 1.74	2.97 ± 2.69	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	floral, fruity, waxy	400 µg/L
<b>Aldehydes</b>											
Nonanal	13.43	57.2	BLOQ	51.1 ± 68.0	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Green, slightly, pungent	1 µg/L
Decanal	16.38	57.2	5.20 ± 10.40	64.1 ± 28.8	8.65 ± 16.60	4.88 ± 7.78	ND	ND	ng/L	Grassy, orange skin	1 mg/L
Octanal	10.71	56.2	BLOQ	BLOQ	329 ± 391	394 ± 362	BLOQ	BLOQ	ng/L	Citrus	2.5 µg/L
<b>Esters</b>											
Ethyl butanoate	4.82	71.1	354 ± 111	359 ± 52	580 ± 49	591 ± 126	83.8 ± 79.4	174 ± 348	ng/L	Strawbetty, apple, banana	20 µg/L
Ethyl 2-methylbutanoate	5.97	57.2	0.160 ± 0.237	0.157 ± 0.124	0.683 ± 0.470	0.449 ± 0.350	1.80 ± 1.37	3.35 ± 3.93	µg/L	Apple, fruity	18 µg/L
Ethyl isovalerate	6.08	88.1	22.2 ± 18.5	BLOQ	22.7 ± 45.4	BLOQ	73.4 ± 70.9	45.9 ± 38.2	ng/L	Apple, sweet, fruity	3 µg/L
Ethyl hexanoate	10.2	88.1	0.472 ± 0.184	0.484 ± 0.107	0.770 ± 0.165	0.812 ± 0.262	0.731 ± 0.399	1.13 ± 0.73	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.18	88.1	0.304 ± 0.059	0.319 ± 0.126	0.419 ± 0.152	0.310 ± 0.083	0.314 ± 0.182	0.376 ± 0.111	µg/L	Fruity	-
Ethyl octanoate	16.08	88.1	0.689 ± 0.194	0.789 ± 0.235	2.30 ± 0.67	1.79 ± 0.77	1.33 ± 0.93	1.92 ± 1.71	µg/L	Pineapple, pear, fruity, sweet	600 µg/L
Ethyl nonanoate	18.91	88.1	146 ± 69.8	546 ± 579	163 ± 10.2	123 ± 63	36.9 ± 22.4	25.2 ± 7.0	ng/L	Rose, fruity	-
Ethyl decanoate	21.5	88.1	3.27 ± 2.19	4.65 ± 0.90	0.0500 ± 0.0309	0.0273 ± 0.0285	0.0300 ± 0.0276	0.0366 ± 0.0561	µg/L	Brandy, fruity, grape	200 µg/L
<b>Ketones</b>											

Compound	RT	Quantifier ion	6 months		Concentration 12 months		6 months		Units	Descriptor	Olfactory perception threshold
			Terceiro	Flor	Terceiro	Flor	Terceiro	Flor			
2-Heptanone	7.0	58.1	128 ± 33	189 ± 62	BLOQ	341 ± 279	127 ± 71	146 ± 33	ng/L	Sweet, fruity, woody	-
2-Undecanone	18.8	58.2	77.2 ± 51.3	100 ± 82	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Fruity, green, floral	-
<b>Terpenes</b>											
1,4-Cineole	10.68	111.1	47.0 ± 47.8	BLOQ	BLOQ	BLOQ	0.596 ± 1.191	BLOQ	ng/L	Fresh, mint, eucalyptus	-
Eucalyptol	11.2	81.1	264 ± 193	48.4 ± 45.4	37.7 ± 47.6	19.3 ± 38.6	BLOQ	BLOQ	ng/L		1.1 µg/L
Camphor	14.61	95.1	1.49 ± 0.90	0.537 ± 0.183	0.918 ± 0.633	0.471 ± 0.292	0.410 ± 0.243	0.286 ± 0.057	µg/L	Herbal, camphor	-
Limonene	11.15	68.1	BLOQ	BLOQ	15.1 ± 13.5	8.71 ± 9.21	BLOQ	BLOQ	ng/L	Flowery, green, citrus	200 µg/L
α-Pinene	8.27	93.1	ND	ND	ND	ND	ND	ND	ng/L	Pine	62 µg/L
α-Terpinene	10.73	93.1	38.8 ± 25.7	BLOQ	28.3 ± 38.2	1.93 ± 3.86	90.2 ± 33.4	74.2 ± 9.0	ng/L	Resin	-
(-)-Borneol	15.36	95.1	75.7 ± 113.0	66.5 ± 7.3	68.1 ± 86.3	15.4 ± 30.8	99.5 ± 129.4	57.5 ± 58.2	ng/L	Camphor, anise	-

**Supplementary Table S2** – Quantification of 22 volatile compounds present in wine model solutions sealed with “Terceiro” natural cork stoppers with and without “Clean 2000” for 32 months. NW – No Wash and C2000 – “Clean 2000”.

Compound	RT	Quantifier ion	Concentration						Units	Descriptor	Olfactory perception threshold
			Terceiro 6 months		Terceiro 12 months		Terceiro 32 months				
			NW	C2000	NW	C2000	NW	C2000			
Alcohols											
Isoamyl alcohol	3.66	55.2	6.38 ± 3.02	5.27 ± 3.08	17.6 ± 6.6	20.2 ± 2.2	ND	ND	µg/L	Fusel, cheese floral, fruity, waxy	30 mg/L
1-Decanol	18.24	70.1	4.78 ± 1.74	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L		400 µg/L
Aldehydes											
Nonanal	13.43	57.2	BLOQ	14.7 ± 26.9	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Green, slightly pungent	1 µg/L
Decanal	16.38	57.2	5.2 ± 10.4	24.2 ± 15.0	8.65 ± 16.60	BLOQ	ND	ND	ng/L	Grassy, orange skin	1 mg/L
Octanal	10.71	56.2	BLOQ	BLOQ	330 ± 391	69.9 ± 58.9	BLOQ	BLOQ	ng/L	Citrus	2.5 µg/L
Esters											
Ethyl butanoate	4.82	71.1	354 ± 111	439 ± 50	580 ± 49	803 ± 57	83.8 ± 79.4	83.2 ± 103.8	ng/L	Strawberry, apple, banana	20 µg/L
Ethyl 2-methylbutanoate	5.97	57.2	0.160 ± 0.237	0.266 ± 0.250	0.683 ± 0.450	1.28 ± 1.85	1.80 ± 1.37	1.25 ± 1.80	µg/L	Apple, fruity Apple, sweet, fruity	18 µg/L
Ethyl isovalerate	6.08	88.1	22.2 ± 18.5	68.9 ± 42.8	22.7 ± 45.4	185 ± 53	73.4 ± 70.9	145 ± 52	ng/L		3 µg/L
Ethyl hexanoate	10.20	88.1	0.472 ± 0.184	0.904 ± 0.117	0.770 ± 0.165	1.344 ±0.375	0.731 ± 0.400	0.734 ± 0.107	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.18	88.1	0.304 ± 0.059	0.448 ± 0.155	0.419 ± 0.152	0.506 ± 0.260	0.314 ± 0.182	0.239 ± 0.067	µg/L	Fruity Pineapple, pear, fruity, sweet	-
Ethyl octanoate	16.08	88.1	0.689 ± 0.195	0.965 ± 0.365	2.30 ± 0.67	2.45 ± 1.07	1.33 ± 0.93	0.716 ± 0.475	µg/L		600 µg/L
Ethyl nonanoate	18.91	88.1	146 ± 70	251 ± 204	163 ± 10	181 ± 40	36.9 ± 22.4	22.1 ± 3.32	ng/L	Rose, fruity	

Compound	RT	Quantifier ion	Concentration						Units	Descriptor	Olfactory perception threshold
			Terceiro 6 months		Terceiro 12 months		Terceiro 32 months				
			NW	C2000	NW	C2000	NW	C2000			
Ethyl decanoate	21.50	88.1	3.27 ± 2.19	2.98 ± 2.66	0.0500 ± 0.0309	4.75 ± 9.46	0.0300 ± 0.0276	0.00660 ± 0.01320	µg/L	Brandy, fruity, grape	200 µg/L
<b>Ketones</b>											
2-Heptanone	7.00	58.1	128 ± 33	201 ± 31	BLOQ	490 ± 247	127 ± 71	164 ± 50	ng/L	Sweet, fruity, woody	-
2-Undecanone	18.80	58.2	77.2 ± 51.3	76.7 ± 70.8	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Fruity, green, floral	-
<b>Terpenes</b>											
1,4-Cineole	10.68	111.1	47.0 ± 47.8	BLOQ	BLOQ	BLOQ	0.596 ± 1.191	5.25 ± 10.49	ng/L	-	-
Eucalyptol	11.20	81.1	264 ± 193	368 ± 414	37.7 ± 47.6	18.5 ± 16.6	BLOQ	BLOQ	ng/L	Fresh, mint, eucalyptus	1.1 µg/L
Camphor	14.61	95.1	1.49 ± 0.90	1.43 ± 0.19	0.918 ± 0.634	0.372 ± 0.156	0.410 ± 0.243	0.434 ± 0.227	µg/L	Herbal, camphor	-
Limonene	11.15	68.1	BLOQ	BLOQ	15.1 ± 13.5	4.22 ± 5.61	BLOQ	0.789 ± 1.579	ng/L	Flowery, green, citrus	200 µg/L
α-Pinene	8.27	93.1	ND	ND	ND	ND	ND	ND	ng/L	Pine	62 µg/L
α-Terpinene	10.73	93.1	38.8 ± 25.7	67.1 ± 50.4	28.3 ± 38.2	4.03 ± 5.63	90.2 ± 33.4	98.8 ± 53.5	ng/L	Resin	-
(-)-Borneol	15.36	95.1	75.7 ± 113.0	129 ± 109	68.1 ± 86.3	48.5 ± 41.3	99.5 ± 129.3	98.0 ± 106	ng/L	Camphor, anise	-

**Supplementary Table S3** – Quantification of 22 volatile compounds present in wine model solutions sealed with “Flor” natural cork stoppers with and without “Clean 2000” for 32 months. NW – No Wash and C2000 – “Clean 2000”.

Compound	RT	Quantifier ion	Concentration						Units	Descriptor	Olfactory perception threshold
			Flor 6 months		Flor 12 months		Flor 32 months				
			NW	C2000	NW	C2000	NW	C2000			
<b>Alcohols</b>											
Isoamyl alcohol	3.7	55.2	4.40 ± 2.51	5.10 ± 4.97	18.5 ± 2.3	20.2 ± 1.4	ND	ND	µg/L	Fusel, cheese floral, fruity, waxy	30 mg/L
1-Decanol	18.2	70.1	2.97 ± 2.69	2.20 ± 2.15	BLOQ	BLOQ	BLOQ	BLOQ	µg/L		400 µg/L
<b>Aldehydes</b>											
Nonanal	13.4	57.2	51.1 ± 68.0	0.100 ± 0.200	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Green, slightly pungent	1 µg/L
Decanal	16.4	57.2	64.1 ± 28.8	20.4 ± 20.8	4.88 ± 7.78	2.32 ± 3.35	ND	ND	ng/L	Grassy, orange skin	1 mg/L
Octanal	10.7	56.2	BLOQ	BLOQ	394 ± 362	116 ± 205	BLOQ	BLOQ	ng/L	Citrus	2.5 µg/L
<b>Esters</b>											
Ethyl butanoate	4.8	71.1	359 ± 52	419 ± 57	591 ± 126	540 ± 28	174 ± 348	60.2 ± 56.7	ng/L	Strawbetty, apple, banana	20 µg/L
Ethyl 2-methylbutanoate	6.0	57.2	0.157 ± 0.124	0.339 ± 0.383	0.449 ± 0.350	BLOQ	3.35 ± 3.93	1.80 ± 3.29	µg/L	Apple, fruity	18 µg/L
Ethyl isovalerate	6.1	88.1	BLOQ	68.5 ± 60.0	BLOQ	127 ± 36	45.9 ± 38.2	77.8 ± 71.0	ng/L	Apple, sweet, fruity	3 µg/L
Ethyl hexanoate	10.2	88.1	0.484 ± 0.107	0.820 ± 0.195	0.812 ± 0.263	1.04 ± 0.23	1.13 ± 0.73	1.17 ± 0.55	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.2	88.1	0.319 ± 0.126	0.392 ± 0.141	0.310 ± 0.083	0.344 ± 0.095	0.376 ± 0.111	0.336 ± 0.160	µg/L	Fruity	-
Ethyl octanoate	16.1	88.1	0.789 ± 0.235	0.804 ± 0.160	1.80 ± 0.78	1.19 ± 0.20	1.92 ± 1.71	0.789 ± 0.507	µg/L	Pineapple, pear, fruity, sweet	600 µg/L



Compound	RT	Quantifier ion	Concentration						Units	Descriptor	Olfactory perception threshold
			Flor 6 months		Flor 12 months		Flor 32 months				
			NW	C2000	NW	C2000	NW	C2000			
Ethyl nonanoate	18.9	88.1	546 ± 579	132 ± 73	123 ± 63	88.4 ± 72.4	25.2 ± 7.0	19.2 ± 8.8	ng/L	Rose, fruity Brandy, fruity, grape	-
Ethyl decanoate	21.5	88.1	4.65 ± 0.90	2.65 ± 2.37	0.0273 ± 0.0285	0.0103 ± 0.0121	0.0366 ± 0.0561	0.0390 ± 0.00780	µg/L		200 µg/L
<b>Ketones</b>											
2-Heptanone	7.0	58.1	189 ± 61	266 ±156	341 ± 279	465 ± 234	146 ± 33	182 ± 31	ng/L	Sweet, fruity, woody Fruity, green, floral	-
2-Undecanone	18.8	58.2	100 ± 82	112 ± 67	BLOQ	BLOQ	BLOQ	BLOQ	ng/L		-
<b>Terpenes</b>											
1,4-Cineole	10.7	111.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	-	-
Eucalyptol	11.2	81.1	48.4 ± 45.4	58.8 ± 88.6	19.3 ± 38.6	113 ± 166	BLOQ	BLOQ	ng/L	Fresh, mint, eucalyptus	1.1 µg/L
Camphor	14.6	95.1	537 ± 183	393 ± 179	471 ± 292	421 ± 277	286 ± 57	320 ± 106	ng/L	Herbal, camphor	
Limonene	11.2	68.1	BLOQ	BLOQ	8.71 ± 9.22	52.8 ± 96.0	BLOQ	0.218 ± 0.436	ng/L	Flowery, green, citrus	200 µg/L
α-Pinene	8.3	93.1	ND	ND	ND	ND	ND	ND	ng/L	Pine	62 µg/L
α-Terpinene	10.7	93.1	BLOQ	23.8 ± 29.2	1.93 ± 3.86	43.7 ± 64.4	74.2 ± 9.0	253 ± 366	ng/L	Resin	-
(-)-Borneol	15.4	95.1	66.5 ± 7.3	11.7 ± 23.4	15.4 ± 30.8	BLOQ	57.5 ± 58.2	37.7 ± 44.0	ng/L	Camphor, anise	-

**Supplementary Table S4** – Quantification of 36 volatile compounds present in Porto wine at 6 months after bottling.

Compound	RT	Quantifier Ion	T0	Concentration 6 months					Units	Descriptor	Olfactory perception threshold
				Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
Alcohols											
Isoamyl alcohol	3.74	55.1	297	372 ± 97 78.8 ±	448 ± 136	373 ± 132	413 ± 124	446 ± 210 55.5 ±	mg/L	Fusel, cheese	30 mg/L
3-Hexen-1-ol	6.30	67.1	0	69.4	87.5 ± 73.3	79.1 ± 67.5	103 ± 84	71.8	µg/L	green, bitter, fatty	400 µg/L
1-Hexanol	6.57	56.2	0.032	0.740 ±	0.967 ±	0.950 ±	1.15 ±	0.686 ±	mg/L	Grass, resin, flower	8 mg/L
1-Octanol	12.47	56.2	8	0.721	0.862	0.601	0.63	0.635	mg/L	Intense citrus, roses	120 µg/L
Phenylethyl alcohol	13.71	91.1	0	10.4 ±	13.9 ± 12.2	16.1 ± 11.0	17.0 ±	14.3 ± 9.1	µg/L		
				13.0			14.3	13.6 ±			
				19.2 ±			29.1 ±				
				12.8	16.5 ± 14.1	19.9 ± 11.2	13.0	13.3	mg/L	Rose, honey	14 mg/L
				7.97 ±				4.12 ±			
1-Decanol	18.32	55.1	0	7.56	5.34 ± 10.68	12.2 ± 11.7	11.6 ± 9.5	8.24	µg/L	floral, fruity, waxy	400 µg/L
Aldehydes											
				3.68 ±			4.23 ±	3.96 ±		Almond, burnt sugar	
Benzaldehyde	9.16	77.1	3.66	1.07	3.60 ± 0.70	3.46 ± 0.83	1.28	1.18	µg/L		2 mg/L
Phenylacetaldehyde	11.64	91.0	27.0	29.6 ± 8.2	32.5 ± 7.6	28.2 ± 4.4	33.1 ± 6.9	33.2 ± 7.7	µg/L	Green, honey	1 µg/L
Nonanal	12.19	98.1	6.04	12.2 ± 5.2	10.6 ± 3.9	8.47 ± 4.40	11.0 ± 6.4	10.8 ± 5.4	µg/L	Green, slightly pungent	1 µg/L
				15.7 ±			17.4 ±	9.14 ±		Grassy, orange	
Decanal	16.45	57.1	0	27.3	2.95 ± 3.86	8.88 ± 8.50	23.3	16.18	µg/L	skin	1 mg/L
β-Cyclocitral	16.84	137.1	86.5	252 ± 179	251 ± 217	211 ± 196	194 ± 279	306 ± 428	ng/L	Floral, sweet, mint	-
Octanal	10.46	56.2	0	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Citrus	2.5 µg/L
Esters											
Ethyl isobutyrate	4.05	56.2	81.6	178 ± 52	195 ± 74	183 ± 69	181 ± 85	190 ± 87	µg/L	Fruity	15 µg/L
Ethyl butanoate	4.89	71.1	107	156 ± 44	173 ± 66	146 ± 55	159 ± 73	166 ± 71	µg/L	Strawbetty, apple, banana	20 µg/L
Ethyl 2-methylbutanoate	6.05	57.1	8.50	16.5 ± 5.2	17.7 ± 6.5	17.2 ± 6.2	16.5 ± 7.7	17.7 ± 7.2	µg/L	Apple, fruity	18 µg/L

Compound	RT	Quantifier Ion	T0	Concentration 6 months					Units	Descriptor	Olfactory perception threshold
				Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
Ethyl isovalerate	6.16	57.2	15.7	27.5 ± 6.0	30.9 ± 9.0	28.4 ± 8.2	26.6 ± 10.4	28.2 ± 12.3	µg/L	Apple, sweet, fruity Banana, sweet, fruity	3 µg/L
Isoamyl acetate	6.75	55.1	671	635 ± 164 6.33 ±	702 ± 252	572 ± 263	617 ± 158 6.03 ±	718 ± 472 7.00 ±	µg/L		30 µg/L
Hexyl acetate	10.66	56.2	6.08	1.32	7.34 ± 2.79	4.94 ± 1.58	2.69	2.82	µg/L	Cherry, pear	1.5 mg/L
Ethyl hexanoate	10.26	88.1	572	687 ± 110 5.22 ±	756 ± 282	643 ± 335	682 ± 213 5.42 ±	888 ± 504 5.81 ±	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.22	88.1	3.64	1.80 1.34 ±	5.94 ± 2.62	4.48 ± 2.09	3.30 1.42 ±	2.93 1.64 ±	µg/L	Fruity Pineapple, pear, fruity, sweet	- 600 µg/L
Ethyl octanoate	16.12	88.1	0.909	0.48 2.96 ±	1.34 ± 0.73	1.21 ± 0.66	0.74 3.21 ±	0.95 3.86 ±	mg/L		
Ethyl nonanoate	18.91	88.1	1.78	1.78 0.570 ±	3.32 ± 1.86	2.73 ± 2.01	2.39 0.763 ±	3.05 1.12 ±	µg/L	Rose, fruity Brandy, fruity, grape	- 200 µg/L
Ethyl decanoate	21.54	88.1	0	0.527	0.580	0.433	0.648	1.31	mg/L		
<b>Furans</b>											
Furfural	5.66	95.0	112	134 ± 29	98.4 ± 14.0	124 ± 35	114 ± 30	96.4 ± 13.2	µg/L	Cooked vegetables, pungent	14.1 mg/L
<b>Ketones</b>											
2-Heptanone	7.09	58.1	1.41	1.71 ± 0.19 1.11 ±	1.85 ± 0.43	1.63 ± 0.27	1.75 ± 0.35 1.21 ±	1.75 ± 0.41 1.23 ±	ng/L	Sweet, fruity, woody Sweet, herbal, green	- -
2-Nonanone	13.05	58.1	0.815	0.54 2.72 ±	1.20 ± 0.43	1.03 ± 0.33	0.56 4.69 ±	0.50 5.33 ±	µg/L		
2-Undecanone	18.84	58.1	0.249	3.95 3.02 ±	1.17 ± 1.41	4.83 ± 5.36	5.33 3.95 ±	2.45 3.35 ±	ng/L	Fruity, green, floral	-
β-Damascenone	21.18	69.1	2.07	1.57	3.00 ± 1.32	2.86 ± 1.20	2.16	2.09	µg/L	Fruit, mulberry	50 ng/L
<b>Terpenes</b>											
α-Pinene	8.35	93.1	0.249	1.91 ± 1.84	1.17 ± 1.00	0.408 ± 0.774	0.651 ± 0.830	1.68 ± 1.77	ng/L	Pine	62 µg/L

Limonene	11.21	67.1	4.32	6.30 ± 2.98	8.88 ± 3.91	4.77 ± 1.00	5.49 ± 1.68	8.10 ± 5.43	ng/L	Flowery, green, citrus	200 µg/L
Eucalyptol	11.29	81.1	0	0.137 ± 0.274	0.278 ± 0.158	0.216 ± 0.198	0.296 ± 0.243	0.312 ± 0.135	ng/L	Fresh, mint, eucalyptus	1.1 µg/L
cis-Linalool oxide	12.95	59.1	0	3.00 ± 4.12	2.97 ± 4.04	6.62 ± 5.43	5.54 ± 2.39	2.39 ± 2.92	µg/L	Citrus, green	25 µg/L
β-Linalool	13.34	93.1	6.19	24.5 ± 2.6	24.0 ± 7.1	18.3 ± 6.0	22.2 ± 6.7	24.2 ± 8.4	µg/L	Flower, muscat, lavender	25 µg/L
α-Ionone	22.27	121.0	0	0.599 ± 0.827	0.0871 ± 0.1167	1.03 ± 1.07	1.00 ± 1.03	0.646 ± 1.026	ng/L	Floral, fruity, violet	90 ng/L
1,4-Cineole	10.83	111.1	0	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	Minty, pine	-

**Supplementary Table S5** – Quantification of 38 volatile compounds present in Porto wine at 15 months after bottling.

Compound	RT	Quantifier Ion	Concentration 15 months					Units	Descriptor	Olfactory perception threshold
			Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
<b>Alcohols</b>										
Isoamyl alcohol	3.57	55.1	211 ± 15	207 ± 14	204 ± 4	206 ± 8	221 ± 23	mg/L	Fusel, cheese	30 mg/L
3-Hexen-1-ol	6.07	67.1	55.5 ± 9.4	41.9 ± 4.2	45.5 ± 7.4	35.5 ± 3.1	32.7 ± 7.6	µg/L	green, bitter, fatty	400 µg/L
1-Hexanol	6.43	56.2	82.8 ± 5.9	85.2 ± 7.0	119 ± 8	77.5 ± 3.9	92.4 ± 9.2	µg/L	Grass, resin, flower	8 mg/L
1-Octanol	12.51	56.2	12.4 ± 0.9	11.5 ± 0.6	12.1 ± 0.8	11.8 ± 0.4	12.5 ± 1.6	µg/L	Intense citrus, roses	120 µg/L
Phenylethyl alcohol	13.76	91.1	10.9 ± 1.3	9.89 ± 1.37	10.6 ± 1.0	10.7 ± 0.9	11.2 ± 1.0	mg/L	Rose, honey	14 mg/L
1-Decanol	18.41	55.1	5.39 ± 0.18	5.27 ± 0.15	5.14 ± 0.12	5.37 ± 0.23	5.26 ± 0.44	µg/L	floral, fruity, waxy	400 µg/L
<b>Aldehydes</b>										
Benzaldehyde	9.12	77.1	13.2 ± 0.9	12.8 ± 0.6	12.4 ± 0.5	13.0 ± 0.5	13.8 ± 0.7	µg/L	Almond, burnt sugar	2 mg/L
Phenylacetaldehyde	11.63	91.0	16.8 ± 1.2	17.0 ± 1.0	16.5 ± 1.7	14.5 ± 2.5	16.4 ± 1.4	µg/L	Green, honey	1 µg/L
Nonanal	13.52	98.1	2.13 ± 1.27	1.24 ± 0.30	2.14 ± 0.65	1.49 ± 0.21	1.62 ± 0.20	µg/L	Green, slightly pungent	1 µg/L
Decanal	16.50	57.1	2.86 ± 1.50	1.82 ± 0.28	2.21 ± 0.30	2.61 ± 0.66	2.33 ± 0.19	µg/L	Grassy, orange skin	1 mg/L
β-Cyclocitral	16.92	137.1	427 ± 8	457 ± 25	469 ± 14	456 ± 24	460 ± 29	ng/L	Floral, sweet, mint	-
Octanal	10.46	56.2	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	Citrus	2.5 µg/L
<b>Esters</b>										
Ethyl isobutyrate	3.88	56.2	63.6 ± 15.2	74.9 ± 32.2	60.5 ± 15.1	69.0 ± 23.9	91.8 ± 15.2	µg/L	Fruity	15 µg/L
Ethyl butanoate	4.75	71.1	79.9 ± 9.0	83.6 ± 18.2	65.8 ± 15.4	83.5 ± 24.5	96.2 ± 19.3	µg/L	Strawbetty, apple, banana	20 µg/L
Ethyl 2- methylbutanoate	5.89	57.2	10.1 ± 2.2	10.7 ± 2.5	2.48 ± 9.68	10.8 ± 3.17	12.9 ± 3.42	µg/L	Apple, fruity	18 µg/L
Ethyl isovalerate	6.01	57.2	12.8 ± 2.9	14.4 ± 3.4	13.5 ± 3.65	14.6 ± 4.0	17.0 ± 6.1	µg/L	Apple, sweet, fruity	3 µg/L
Isoamyl acetate	6.66	55.1	107 ± 18	109 ± 28	82.4 ± 19	108 ± 23	125 ± 27	µg/L	Banana, sweet, fruity	30 µg/L
Hexyl acetate	10.75	56.2	2.33 ± 0.41	2.45 ± 0.50	1.78 ± 0.35	2.42 ± 0.37	2.88 ± 0.64	µg/L	Cherry, pear	1.5 mg/L

Compound	RT	Quantifier Ion	Concentration 15 months					Units	Descriptor	Olfactory perception threshold
			Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
Ethyl hexanoate	10.33	88.1	200 ± 30	198 ± 48	175 ± 34	202 ± 36	234 ± 51	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.35	88.1	2.77 ± 0.36	2.73 ± 0.54	2.50 ± 0.37	2.83 ± 0.37	3.23 ± 0.67	µg/L	Fruity	-
Diethyl succinate	15.69	101.0	2.33 ± 0.25	2.33 ± 0.19	2.83 ± 0.20	2.34 ± 0.14	2.49 ± 0.08	mg/L	Fruity, melon	200 mg/L
Ethyl octanoate	16.27	88.1	507 ± 72	479 ± 106	443 ± 67	529 ± 72	629 ± 189	µg/L	Pineapple, pear, fruity, sweet	600 µg/L
Phenylethyl acetate	17.84	104.1	14.1 ± 1.1	14.1 ± 0.9	12.6 ± 1.0	14.3 ± 1.0	15.3 ± 0.7	µg/L	Rose, violet	250 µg/L
Ethyl nonanoate	19.04	88.1	5.14 ± 0.29	5.06 ± 0.31	4.87 ± 0.11	5.33 ± 0.18	5.65 ± 0.79	µg/L	Rose, fruity	-
Ethyl decanoate	21.65	88.1	251 ± 39	196 ± 35	195 ± 12	244 ± 20	347 ± 202	µg/L	Brandy, fruity, grape	200 µg/L
<b>Furans</b>										
Furfural	5.44	95.0	143 ± 10	152 ± 14	209 ± 24	143 ± 5	164 ± 32	µg/L	Cooked vegetables, pungent	14.1 mg/L
<b>Ketones</b>										
2-Heptanone	7.03	58.1	290 ± 26	315 ± 47	326 ± 39	332 ± 33	354 ± 69	ng/L	Sweet, fruity, woody	-
2-Nonanone	13.10	58.1	709 ± 31	685 ± 67	683 ± 36	717 ± 29	792 ± 112	ng/L	Sweet, herbal, green	-
2-Undecanone	18.95	58.1	217 ± 23.7	203 ± 17	195 ± 13	229 ± 15	256 ± 43	ng/L	Fruity, green, floral	-
β-Damascenone	21.28	69.1	1.17 ± 0.06	1.16 ± 0.04	1.17 ± 0.05	1.21 ± 0.07	1.35 ± 0.13	µg/L	Fruit, mulberry	50 ng/L
<b>Terpenes</b>										
α-Pinene	8.36	93.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	Pine	62 µg/L
Limonene	11.20	67.1	ND	ND	ND	ND	ND	µg/L	Flowery, green, citrus	200 µg/L
Eucalyptol	11.37	81.1	54.3 ± 5.4	52.9 ± 9.6	57.5 ± 7.9	63.1 ± 3.8	59.8 ± 4.4	ng/L	Fresh, mint, eucalyptus	1.1 µg/L
cis-Linalool oxide	12.50	59.1	24.2 ± 2.6	24.2 ± 1.5	26.8 ± 1.8	25.6 ± 2.1	28.0 ± 5.6	µg/L	Citrus, green	25 µg/L
β-Linalool	13.39	93.1	9.19 ± 0.44	8.61 ± 0.48	7.97 ± 0.25	9.02 ± 0.47	10.5 ± 2.07	µg/L	Flower, muscat, lavender	25 µg/L
Camphor	14.77	95.1	76.2 ± 23.4	53.2 ± 7.8	79.4 ± 17.5	78.2 ± 15.0	79.9 ± 25.2	ng/L	Herbal, camphor	-
α-Ionone	22.36	121.0	86.7 ± 2.0	87.2 ± 1.4	90.1 ± 1.8	87.9 ± 1.1	88.8 ± 3.1	ng/L	Floral, fruity, violet	90 ng/L
1,4-Cineole	10.83	111.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	Minty, pine	-

**Supplementary Table S6** – Quantification of 38 volatile compounds present in Porto wine at 26 months after bottling.

Compound	RT	Quantifier Ion	Concentration 26 months					Units	Descriptor	Olfactory perception threshold
			Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
<b>Alcohols</b>										
Isoamyl alcohol	3.88	55.1	451 ± 86.4	445 ± 90.8	413 ± 143	485 ± 94.1	489 ± 108	mg/L	Fusel, cheese	30 mg/L
3-Hexen-1-ol	6.50	67.1	65.9 ± 11.1	58.9 ± 7.32	58.7 ± 8.10	70.8 ± 16.8	68.8 ± 7.46	µg/L	green, bitter, fatty	400 µg/L
1-Hexanol	6.85	56.2	868 ± 142	762 ± 113.9	874 ± 171	981 ± 254	981 ± 192	µg/L	Grass, resin, flower	8 mg/L
1-Octanol	12.81	56.2	15.8 ± 1.18	15.0 ± 1.03	15.8 ± 1.89	16.7 ± 2.71	16.5 ± 1.65	µg/L	Intense citrus, roses	120 µg/L
Phenylethyl alcohol	14.04	91.1	54.9 ± 8.57	52.7 ± 4.96	49.8 ± 8.25	50.8 ± 5.83	55.6 ± 7.30	mg/L	Rose, honey	14 mg/L
1-Decanol	18.65	55.1	49.6 ± 0.240	49.4 ± 0.110	49.4 ± 0.192	29.8 ± 23.8	49.8 ± 0.305	µg/L	floral, fruity, waxy	400 µg/L
<b>Aldehydes</b>										
Benzaldehyde	9.46	77.1	15.4 ± 1.31	14.3 ± 1.23	13.9 ± 2.35	17.4 ± 3.87	17.8 ± 3.05	µg/L	Almond, burnt sugar	2 mg/L
Phenylacetaldehyde	11.92	91.0	25.2 ± 1.25	25.2 ± 0.511	25.5 ± 0.847	25.6 ± 1.26	25.7 ± 1.10	µg/L	Green, honey	1 µg/L
Nonanal	13.82	98.1	4.45 ± 0.325	4.07 ± 0.223	4.34 ± 1.40	4.54 ± 0.540	3.67 ± 1.47	µg/L	Green, slightly pungent	1 µg/L
Decanal	16.78	57.1	1.21 ± 0.683	0.406 ± 0.450	0.767 ± 0.389	2.32 ± 2.30	1.12 ± 0.51	µg/L	Grassy, orange skin	1 mg/L
β-Cyclocitral	17.15	137.1	0.507 ± 0.0354	0.498 ± 0.0189	0.503 ± 0.0570	0.532 ± 0.0685	0.517 ± 0.0483	µg/L	Floral, sweet, mint	-
Octanal	10.72	56.2	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	µg/L	Citrus	2.5 µg/L
<b>Esters</b>										
Ethyl isobutyrate	4.27	56.2	169 ± 27.2	153 ± 21.6	162 ± 70.6	194 ± 54.8	125 ± 68.8	µg/L	Fruity	15 µg/L
Ethyl butanoate	4.79	71.1	102 ± 20.5	101 ± 12.3	82.0 ± 46.6	127 ± 40.6	89.3 ± 54.9	µg/L	Strawbetty, apple, banana	20 µg/L
Ethyl 2- methylbutanoate	6.31	57.2	18.5 ± 3.06	16.5 ± 1.65	18.7 ± 7.24	21.6 ± 6.69	15.1 ± 7.99	µg/L	Apple, fruity	18 µg/L
Ethyl isovalerate	6.43	57.2	31.0 ± 5.77	28.0 ± 2.73	32.1 ± 13.0	34.5 ± 11.4	25.1 ± 13.7	µg/L	Apple, sweet, fruity	3 µg/L
Isoamyl acetate	7.03	55.1	98.8 ± 18.1	91.3 ± 9.55	73.9 ± 30.4	107 ± 29.7	86.7 ± 38.8	µg/L	Banana, sweet, fruity	30 µg/L
Hexyl acetate	10.98	56.2	1.81 ± 0.223	1.77 ± 0.104	1.48 ± 0.346	1.99 ± 0.426	1.64 ± 0.532	µg/L	Cherry, pear	1.5 mg/L

Compound	RT	Quantifier Ion	Concentration 26 months					Units	Descriptor	Olfactory perception threshold
			Flor V (G5)	Flor H (G5)	Flor H (Gcast)	Bartop V (G5)	Bartop H (G5)			
Ethyl hexanoate	10.58	88.1	157 ± 27.8	139 ± 10.6	131 ± 55.3	181 ± 57.9	134 ±68.4	µg/L	Apple, banana	14 µg/L
Ethyl heptanoate	13.57	88.1	2.12 ± 0.337	1.94 ± 0.165	1.98 ± 0.617	2.42 ± 0.677	1.92 ± 0.801	µg/L	Fruity	-
Diethyl succinate	15.95	101.0	35.5 ± 5.46	33.8 ± 3.09	33.9 ± 6.62	33.7 ± 4.29	37.4 ± 5.32	mg/L	Fruity, melon	200 mg/L
Ethyl octanoate	16.48	88.1	317 ± 184	400 ± 88.8	390 ± 152	449 ± 115	505 ± 177	µg/L	Pineapple, pear, fruity, sweet	600 µg/L
Phenylethyl acetate	18.06	104.1	43.8 ± 3.26	43.2 ± 2.40	40.2 ± 3.31	45.5 ± 6.20	45.8 ± 4.31	µg/L	Rose, violet	250 µg/L
Ethyl nonanoate	19.28	88.1	1.44 ± 0.254	1.42 ± 0.0993	1.13 ± 0.503	1.76 ± 0.636	1.36 ± 0.491	µg/L	Rose, fruity	-
Ethyl decanoate	21.89	88.1	132 ± 46.1	143 ± 14.0	109 ± 78.0	179 ± 73.0	146 ± 73.8	µg/L	Brandy, fruity, grape	200 µg/L
<b>Furans</b>										
Furfural	5.88	95.0	239 ± 42.6	228 ± 27.9	272 ± 60.0	270 ± 74.2	288 ± 64.2	µg/L	Cooked vegetables, pungent	14.1 mg/L
<b>Ketones</b>										
2-Heptanone	7.39	58.1	0.808 ± 0.323	0.948 ± 0.0484	0.984 ± 0.0754	1.07 ± 0.107	0.990 ± 0.111	µg/L	Sweet, fruity, woody	-
2-Nonanone	13.37	58.1	1.31 ± 0.0838	1.27 ± 0.0360	1.22 ± 0.134	1.36 ± 0.164	1.28 ±0.141	µg/L	Sweet, herbal, green	-
2-Undecanone	19.16	58.1	328 ± 81.7	261 ± 22.8	223 ± 77.2	353 ± 95.1	281 ± 76.7	ng/L	Fruity, green, floral	-
β-Damascenone	21.52	69.1	1.02 ± 0.105	0.950 ± 0.0582	0.944 ± 0.138	1.10 ± 0.215	1.08 ± 0.136	µg/L	Fruit, mulberry	50 ng/L
<b>Terpenes</b>										
α-Pinene	8.66	93.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Pine	62 µg/L
Limonene	11.53	67.1	ND	ND	ND	ND	ND	ng/L	Flowery, green, citrus	200 µg/L
Eucalyptol	11.61	81.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Fresh, mint, eucalyptus	1.1 µg/L
cis-Linalool oxide	12.78	59.1	31.8 ± 5.39	28.1 ± 3.79	32.8 ± 9.28	32.4 ± 8.07	35.7 ± 6.37	µg/L	Citrus, green	25 µg/L
β-Linalool	13.65	93.1	6.48 ± 1.00	5.69 ± 0.852	5.16 ± 1.18	7.33 ± 2.31	7.09 ± 1.39	µg/L	Flower, muscat, lavender	25 µg/L
Camphor	15.06	95.1	155 ± 87.7	293 ± 70.7	15.1 ± 149	127 ± 76	140 ± 104	ng/L	Herbal, camphor	-



$\alpha$ -Ionone	22.59	121.0	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Floral, fruity, violet	90 ng/L
1,4-Cineole	11.09	111.1	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	ng/L	Minty pine	-