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# Study of the impact of the wine closure on the sensory and chemical characteristics of red wines, in particular mouthfeel sensations.

by

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# STUDY OF THE IMPACT OF THE WINE CLOSURE ON THE SENSORY AND CHEMICAL CHARACTERISTICS OF RED WINES, IN PARTICULAR MOUTHFEEL SENSATIONS

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# *ABSTRACT*

Mouthfeel sensations such as astringency, texture, acidity, heat and body, influence the sensory character of wines, in particular red wines. Several studies indicate that the type of closures used in bottling wine may influence the sensory profile of the wine, through oxygen permeability, which influences oxidation reactions, and/or through the reaction of the closure material itself with the wine during storage.

The present study was carried out in the context of a collaboration between Amorim Cork, SA, and Escola Superior de Biotecnologia-UCP. The main objective of this collaboration, held between March and December 2023, was to study the impact of different cork stoppers mainly in mouthfeel sensations of bottled red wines from different countries and regions. To this end a sensory panel was selected and trained, and sensory tests (duo-trio discrimination tests and descriptive sensory tests) were carried out to detect sensory perceptible differences between commercial wines bottled with different closures and to determine their in-mouth sensory profile.

Additionally, the same wines were subjected to physical-chemical analysis performed in external laboratories. The measured physical-chemical parameters were free and total sulfur dioxide, the total polyphenol index (TPI), color intensity (CI), wine color tonality (CT) and volatile organic compounds (VOCs): aldehydes, furans, ketones and some esters. These parameters were previously described in the literature as indicators of the oxidation state of the wine and may be influenced by packaging conditions, namely the closures used in this study. The results thus obtained were statistically analyzed using a Kruskal-Wallis test.

The sensory discrimination tests performed uncovered the existence of perceptible differences between the wines bottled with different stoppers in six of the eight sensory sessions held, mainly between the wine bottled with natural cork stoppers and with the technical cork stoppers (microagglomerated or discs). These differences were related to differences in the intensity of sensory mouthfeel attributes of these wines, as shown by uni and multidimensional analysis performed of the sensory profile results.

For most wines bottled with different stoppers no significant differences regarding free and total $SO_2$, total polyphenol index and colour parameters (CI and CT) were observed. Significant differences were found for some wines bottled with different stoppers regarding some VOCs (phenylacetaldehyde, decanal, β-cyclocitral, furfural, hexyl acetate, diethyl succinate, ethyl octanoate and ethyl decanoate). In both cases, however, none of the physical-chemical parameters analyzed allowed the identification of any trend relating the differences in the wine composition with the cork type.

**Key Words:** wine; astringency; sensory analysis; cork stopper; oxidation.

# *RESUMO*

Caraterísticas sensoriais percebidas na boca, como adstringência, textura, acidez, “calor” e “corpo”, influenciam o caráter sensorial dos vinhos, em particular dos vinhos tintos.

Vários estudos indicam que o tipo de vedante usado no engarrafamento do vinho pode influenciar o seu perfil sensorial, através da permeabilidade ao oxigénio, que influencia as reações de oxidação, e/ou através da reação do material do vedante com o vinho durante o armazenamento.

O presente estudo foi realizado no contexto de uma colaboração entre a Amorim Cork, SA, e a Escola Superior de Biotecnologia-UCP. O principal objetivo deste trabalho, realizada entre março e dezembro de 2023, foi estudar o impacto de diferentes rolhas principalmente nas sensações na boca de vinhos tintos engarrafados de diferentes países e regiões. Para isso, um painel sensorial foi selecionado e treinado, e testes sensoriais (testes de discriminação duo-trio e testes descritivos) foram realizados para detetar diferenças percetíveis entre vinhos comerciais engarrafados com diferentes vedantes e para determinar seu perfil sensorial na boca.

Adicionalmente, os mesmos vinhos foram submetidos a análises físico-químicas realizadas em laboratórios externos. Os parâmetros físico-químicos medidos foram, o dióxido de enxofre livre e total, o índice total de polifenóis (TPI), a intensidade de cor (CI), a tonalidade de cor do vinho (CT) e os compostos orgânicos voláteis (VOCs): aldeídos, furanos, cetonas e alguns ésteres. Esses parâmetros foram descritos anteriormente na literatura como indicadores do estado de oxidação do vinho e podem ser influenciados pelas condições de embalagem, nomeadamente pelos vedantes usados neste estudo. Os resultados obtidos foram analisados estatisticamente usando um teste de Kruskal-Wallis.

Os testes de discriminação sensorial realizados revelaram a existência de diferenças percetíveis entre os vinhos engarrafados com diferentes vedantes em seis das oito sessões sensoriais realizadas, principalmente entre o vinho engarrafado com rolhas de cortiça natural e com rolhas técnicas (microaglomeradas ou discos). Essas diferenças estavam relacionadas com diferenças na intensidade dos atributos sensoriais de sensação na boca desses vinhos, como mostrado pela análise uni e multidimensional realizada dos resultados do perfil sensorial.

Para a maioria dos vinhos engarrafados com diferentes vedantes, não foram observadas diferenças significativas em relação ao dióxido de enxofre livre e total, índice total de polifenóis e parâmetros de cor (CI e CT). Foram encontradas diferenças significativas para alguns vinhos engarrafados com diferentes vedantes em relação a alguns VOCs (fenilacetaldeído, decanal, β-ciclocitral, furfural, acetato de hexilo, succinato de dietilo, octanoato de etilo e decanoato de etilo). Em ambos os casos, no entanto, nenhum dos parâmetros físico-químicos analisados permitiu a identificação de qualquer tendência relacionando as diferenças na composição do vinho com o tipo de rolha.

**Palavras Chave**: vinho, adstringência, análise sensorial, rolhas de cortiça, oxidação.

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## CONTENTS

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## Abbreviations List

TPI - Total Polyphenol Index

OD – Optical Density

CI – Colour Intensity

CT - Colour Tone

OTR - Oxygen transmission rate

OIR - Oxygen initial release

PRPs - Proline-rich proteins

HRPs - Histidine-rich proteins

ASTM - American Society for Testing and Materials

MRs - Mechanoreceptors

FDA - Food and drug administration

VOCs - Volatile organic compounds

OPT - Olfactory Perception Threshold

GPA - Generalized Procrustes Analysis

PCA – Principal Component Analysis

MFA – Multifactorial Analysis

*“A real connoisseur does not drink wine but tastes of its secrets.”*

*Salvador Dali*

# *Chapter1- GENERAL INTRODUCTION AND OBJECTIVES*

Cork stoppers have been used to seal wine bottles for several centuries and their properties can affect the wine characteristics over time.

It is well-established that factors like astringency, texture, acidity, heat, and body play a significant role in determining the overall sensory profile of wines, mainly red wines. Sensory evaluation of wine mouthfeel is a complex process that involves many factors, including the wine's chemical composition and the array of oral sensations it produces. Mouthfeel encompasses the tactile, chemosensory, and taste attributes of perceived viscosity, astringency, hotness, and bitterness. Astringency and mouthfeel perception are relevant to the overall quality of the wine; however, their origin and description are still uncertain and are constantly updated. (Paissoni *et al.*,2023).

The type of closure used for wine bottles can impact the wine's sensory profile through oxygen permeability, which influences oxidation reactions, and/or through the reaction of the closure material itself with the wine during storage.

This study was conducted in collaboration between Amorim Cork, SA, the world's largest producer and supplier of cork stoppers, and the Escola Superior de Biotecnologia-UCP, and focused on the influence of different types of cork stoppers on the mouthfeel sensory characteristics of bottled red wines. This partnership likely provides the expertise and resources required for a comprehensive study.

Another objective of the study was to investigate the correlation of the sensory mouthfeel differences observed in the wines bottled with different closures with specific physical and chemical parameters related the with oxidation phenomena such as free and total sulfur dioxide, the total polyphenol index (TPI), color intensity (CI), wine tonality and volatile organic compounds (VOCs): aldehydes, furans, ketones and some esters.

***Chapter 2*** of the thesis contains a detailed bibliographic review on the topic studied. Material and methods are described at ***Chapter.3*** The results and discussion from this study are presented in ***Chapter 4.***

# *Chapter 2 - BIBLIOGRAPHIC REVIEW*

## 2.1 Available stoppers and their effects on wine quality

### *2.1.1 Types of Stoppers*

In this short review focused on wine stoppers, only those applied for the closure of wine in bottles will be discussed.

#### 2.1.1.1 Cork based stoppers.

Cork-based stoppers are the most used as wine bottle closures. Cork is a secondary plant tissue stripped from the outer bark of cork oak (*Quercus suber* L.). The cork tissue consists in a close cellular arrangement like a honeycomb structure, forming a foam-like compact matrix with some porosity (lenticels). The cells have hollow, air-filled interiors and are basically dead parenchymatous cells. The main constituents include biopolymers such as lignin, suberin, cellulose and hemicelluloses. Other compounds are also found, more easily extracted and with impact to wine-to-cork interactions, including nonpolar compounds (e.g., lipids and some terpenes) and polar compounds like phenolic and polyphenolics (Costa *et al.*, 2019; Pereira, 2013).

Cork shows several advantageous properties that are essential for its application as a sealing closure in wine bottles (Oliveira & Pereira, 2020): low density, very low permeability to liquids and gases, low conductivity, chemical stability and durability, and high compressibility with dimensional recovery.

During the last decades different cork-based materials have been developed. The different cork stoppers commercially available include those obtained from natural cork, colmated cork, agglomerated and a blend of these materials originating the so-called technical closures (Figure 2.1).

**Figure 2.1**. Types of cork closures that can be used for wine bottling (**1**) - Natural cork stoppers; (**2**) - Colmated cork stoppers; (**3**,**4**) - Technical cork stoppers: closures made by cork granulate (agglomerated). (Azevedo *et al.*, 2022).

Natural cork stoppers are produced by drilling small holes through planks of cork wood to obtain the stopper with the desirable cylindrical shape. Therefore, they are one-piece obtained directly from the natural cork tissue. The stoppers are then cleaned, sterilized, bleached with hydrogen peroxide, and sorted according to their porosity (Furtado *et al*., 2021). The cork plank thickness is a main parameter to establish the technological quality required to produce natural cork stoppers (Oliveira & Pereira, 2020). The commercial grade and quality classes of natural cork stoppers are then determined mainly by their external surface homogeneity, closely related to porosity, which is classically quantified by surface image analysis (Oliveira *et al*., 2015). The lack of voids or defects (the existence of lenticular channels) on the cork surface determines the surface homogeneity. It is considered that the more lenticels there are, related to the cork macroporosity, the lower the quality of the stopper (Lagorce-Tachon *et al*., 2015; Azevedo *et al*., 2022).

Colmated cork stoppers are also made from natural cork, but they undergo to an additional treatment with glue and cork powder that covers the lenticels, reducing porosity and to enhancing their mechanical and aesthetic qualities (Silva *et al*., 2011). The type of glue and the cork power characteristics are expected to have a significant influence on the cork stopper properties (Azevedo *et al*., 2022).

Agglomerated cork stoppers are made with granulated cork of different dimensions (e.g., 2-8 mm size particles for macroagglomerated and ≤ 2 mm particles for microagglomerated stoppers), using by-products obtained in the manufacturing of natural cork stoppers. They are produced either by the method of extrusion or via individual moulding, using FDA-approved glues (polyurethanes and isocyanates) and granulated cork that was previously subjected to a variety of cleaning procedures (Crouvisier-Urion *et al*., 2018; Silva *et al*., 2011). The judicious choice of the binder is particularly relevant due to potential migration of compounds into wine from these synthetic products.

The so-called technical cork stoppers are constituted by an agglomerated cork in the body and natural cork discs on the tops. The main objective is to obtain a cork stopper with the advantages of agglomerates and at the same time to avoid the wine coming into contact with synthetic constituents (Azevedo *et al*., 2022). Typically, the natural cork discs are made by punching thin cork planks that are insufficiently thick to make natural cork stoppers, and they are attached to the agglomerated body using a FDA-approved binder (Furtado *et al*., 2021). Technical stoppers designated by 2+0 and 1+1 are commercially available, corresponding to stoppers with two natural cork discs placed into one or both ends, respectively, of an agglomerated cork body. In addition, for an improved control over the gas transference from and to the wine, the stopper for sparkling wines is typically a multilayered cylinder with two microagglomerated disks at either end and a centre body made of natural cork or macroagglomerated cork (Rives *et al*., 2012).

Despite the diversity of available stoppers, the main purpose is to act as a sealing closure in bottles, thus avoiding any leakage of the liquid, either through the stopper itself or at the interface between stopper and bottle. On the other hand, the stopper must be easily removed from the bottle for consumption.

Cork shows useful physical-chemical and mechanical properties to achieve this purpose. It has a very low permeability to liquids preventing the wine to migrate through the stopper. Regarding its mechanical properties, cork is a viscoelastic material that can withstand large deformations under compression without suffering damage with an extensive dimensional recovery when stress is relieved (Mano, 2002). These characteristics allow for an appropriate compression of the stopper against the bottleneck and a tight contact between the bottle surface and the stopper (Oliveira & Pereira, 2020) being a perfect closure even if the glass expands or contracts due to a change in storage or transport temperature (Azevedo *et al.*, 2022).

As discussed in more detail below (§2.1.2.1), the oxygen transmission rate (OTR) and the oxygen initial release (OIR) are important parameters regarding stopper quality and can affect wine chemical and sensorial characteristics. Since cork is a natural material and the cellular microstructure varies greatly, the cork stoppers usually show a broad range of OTR and OIR. The main purpose of the use of agglomerated cork or technical stoppers is to achieve a tighter range of OTR due to their more homogeneous structure (Chanut *et al.*, 2021; Echave *et al.*, 2021).

#### 2.1.1.2 Synthetic stoppers

Besides the cork stoppers, other materials have been used for bottled wines.

Synthetic stoppers entered the market with the main objective to assure wines without the so-called “cork taint”, *i.e.*, the presence in cork of 2,4,6-trichloroanisole (TCA), a compound produced by fungi that when present in cork stoppers can migrate into wine and impart undesirable musty and moldy flavours (Silva *et al.*, 2011; Furtado *et al*, 2021). They are essentially compressed plastics produced by injection moulding or co-extrusion, using polymers like low density polyethylene (LDPE) (co-extruded stoppers) and styrene-butadiene-styrene (SBS) or styrene-ethylene-butylene-styrene (SEBS) (injection moulded stoppers) (Azevedo *et al.*, 2022). Besides the absence of off-flavour compounds, they are also cheaper than natural cork. However, some disadvantages are also associated to synthetic stoppers: the fact of being non-biodegradable materials, the higher permeability to oxygen when compared to cork stoppers, making them not suitable for long aging, difficulties in removing the stopper from the bottle, removing volatiles from wine and assisting some chemicals' desorption into the wine (Dwivedi *et al.*, 2019; Furtado *et al.*, 2021). For instance, due to their high porosity, synthetic stoppers are typically associated to high oxidation rates in wine (He *et al.*, 2013; Oliveira *et al.*, 2020; Silva *et al.*, 2003).

Additionally, synthetic stoppers have a propensity to harden over time, loosening tightly at the stopper-glass interface, also associated to premature oxidation processes (Echave *et al.*, 2021), contributing to their disadvantageous use for long periods of wine aging when compared to cork stoppers. However, synthetic stoppers might be useful for young wines or those that only require brief maturing times. Therefore, synthetic stoppers are mainly considered for bottling young wines or for those intended for short aging periods.

#### 2.1.1.3 Screwcaps

Screwcaps are metal caps, usually made with aluminium, screwing onto threads on the neck of a bottle, with an inner thermoplastic layer to allow for proper barrier properties. They also appeared in the market mainly to prevent the “cork taint” issue.

One of the advantages of their use is the ease of removal from the bottle and replacement (Furtado *et al*., 2021). However, the contact between the wine and the metal can cause problems related to the migration of metal ions into the wine, so this contact should be minimized using the most inert materials possible, inside the screw cap, to establish a barrier between the wine and the metallic cap.
Another important characteristic is their very low, almost null, oxygen permeation, so they are most suitable for wines sensitive to undesirable oxidation (Lopes *et al*., 2009; Vidal *et al*., 2011). On the other hand, since screwcaps allow for very little oxygen ingress, they may also cause the development of reductive aromas (Coelho *et al*., 2018; Hopfer *et al*., 2012).

### ***2.1.2 Influence on wine characteristics***

Despite the uncertainties remain regarding the factors influencing oxygen permeability that affect the development of wine during bottle aging, some important are well-recognised. Here, we will focus on the impact of the stopper as well as other factors, including the contact of wine with oxygen during the wine-making stages, bottling conditions, the concentration of free sulphur dioxide at the time of bottling, the volume and gas composition of the headspace, storage conditions (temperature, light, humidity, bottle position during storage), and the composition of the wine itself (Lopes *et al*., 2006; Karbowiak *et al*., 2010).

Numerous studies have found that stopper characteristics have a significant impact on the wine's preservation and bottle aging (Hopfer *et al*., 2012, 2013; Oliveira *et al*., 2013; Oliveira *et al*., 2020; Silva *et al*., 2011), strongly affecting the organoleptic properties of bottled wines.

The influence of the stopper on wine characteristics is mainly related to transport phenomena through the stopper material and to exchange of compounds from the stopper to the wine and vice-versa, closely related to two of main stoppers’ properties: gas permeability and chemical inertness. The potential effects on wine quality are expected to be more significant for relatively long aging periods (Furtado *et al*., 2021).

During wine aging, the entry of oxygen through the bottle is highly dependent, not only on the effectiveness of the closures, which have different oxygen barrier properties, but also on how it is sealed (Lopes *et al*., 2007). The careful selection of the stopper, considering the different characteristics of the available stoppers discussed above (§1.1), is thus important to achieve the desired wine flavour and taste. Due to the impact of oxidation in wine quality, permeability to oxygen is one of the main factors to take into consideration. Desorption of compounds from the stopper and migration into the wine, and adsorption of wine compounds by the stopper (scalping) are also worth to mention. These aspects will be shortly discussed in the next sections.

#### 2.1.2.1 Permeability to oxygen

The influence of oxygen in wine composition during bottle storage is widely known. On this subject, several recent reviews are available that discuss in detail the main factors and oxidation reactions involved, impacting the characteristics of the wine (e.g., Gabrielli *et al*., 2021; Karbowiak *et al*., 2009; Oliveira *et al*., 2011). Here, we are interested in discussing the role of cork stoppers in the bottled wine regarding the oxygen permeability and the consequences for the chemical and organoleptic properties of wine.

Oxigen ingress into the wine bottle depends on the sealing effectiveness of the closure and on its ability to oxygen permeability. In fact, the closure used for bottling is a key factor during wine storage and aging since it is the only permeable barrier between the wine and the environment. This semi-permeable barrier regulates the amount of oxygen that enters the bottle and the migration of some volatile compounds (Gutiérrez-Escobar *et al*., 2021). As expected, the different commercial stoppers discussed above also show different oxygen barrier properties due to their different composition and microstructure. Screw caps and technical cork stoppers typically show low oxygen migration rates into bottles, while natural cork stoppers show moderate rates and synthetic closures the higher rates (Crouvisier-Urion *et al*., 2018). Also, due to their natural characteristics, natural cork stoppers have typically a variable OTR that can be homogenized by microagglomeration (Echave *et al*., 2021), as discussed above.

Using stoppers with intermediate gas barrier properties generally result in balanced wines (Chatonnet *et al.*, 2000; Godden *et al.*, 2001; Mas *et al.*, 2002), preventing premature oxidation without developing unpleasant reduction aromas. Both in cork stoppers and in synthetic stoppers, contrarily to what may happen with screw caps closures, there is enough gas exchange that prevents the formation of sulphurous compounds responsible to unpleasant smells. Wines bottled with synthetic closures are usually more oxidized and had a lower sulphur dioxide content when compared to wines sealed with other closures (Skouroumounis *et al*., 2005).

In addition to the OTR, the oxygen released from the cork during bottling is also an important parameter. The oxygen released from inside the cork (OIR) is followed by oxygen transfer through the cork over time (OTR). The cork is compressed when the closure is inserted into the bottle reducing its diameter by about 25% to fit the bottleneck internal bore. The cellular air entrapped in the matrix contains around 4 mg of oxygen for a cork with height 44 mm and 24 mm diameter, and gradually permeates out at each end of the cork. Thus, in the early stages of storage, the wine is essentially exposed to the oxygen released from the stopper (OIR) besides to the one already in the headspace. Different oxygen release patterns may occur depending on the bottling techniques (Cardoso *et al.,* 2022)

In general, oxygen permeation through closures is higher during bottling and in the first month of storage than in later months. Oxygen entrance into the bottle seems to occur mainly throughout the cork stopper's macroscopic and cellular structure, rather than through the interface between the cork and the bottle glass (Lopes *et al*., 2007). The cork itself has a very low permeability to oxygen (Lopes *et al*., 2005, 2007), but as discussed above, the cork tissue consists in a honeycomb structure of cells and lenticels, forming a porous structure.

Typically, the kinetics of oxygen entrance into the bottle can be adjusted to logarithmic models, with an initial high permeation rate, followed by a decreasing ingress rate during the 1st month and ahead, until steadying at a low and rather constant ingress rate from the 3rd to the 12th month and afterward (Oliveira *et al*.,2013).

The oxidative deterioration of bottled wines differs from the aging process which is a consequence of reactions promoted by oxygen. These reactions originate changes in colour, aroma and flavour different from those that occur during the wine maturation. For this reason, the judicious choice of the stopper, depending on the wine chemical composition, bottling conditions, and storage, is essential to achieve a stable balance that promotes the desired organoleptic evolution of bottled wines (Skouroumounis *et al*., 2005). Currently, it is recognized that the quality of a wine is impaired by excessive exposure to oxygen, but a continuous and slow oxygenation can be beneficial for its evolution, particularly in the early stages of maturation (Castelari *et al*., 2002; Atanasova *et al*., 2002). Moderate oxygenation may be positive since the oxidative reactions to which phenolic compounds are subject can enhance colour and reduce astringency (Castelari *et al*., 2002; Moutounet & Mazauric., 2000). On the other hand, an excessive exposure to oxygen may lead to the appearance of some undesirable aromas and browning (Singleton *et al*., 1979; Escudero *et al*., 2002), causing an acceleration of wine' maturation.

Compounds involved in oxidative processes include oxygen (initiator of the process), phenolic compounds (oxidizable substrates that presumably are the precursors of pigments formed during browning reactions) and metal ions such as $Fe^{3+}$, $Cu^{2+}$ and $Mn^{2+}$, which act as catalysts. Other factors such as temperature, pH and light can also contribute to these reactions (Macias *et al*., 2001; Silva Ferreira *et al*., 2002).

Closures with higher permeability are expected to favour colour stabilization but also promote the oxidation of phenolic and aroma compounds (Capone *et al*., 2003). Different studies also showed that the use of more gas permeable stoppers, like natural cork and synthetic closures, results in more intense colours and caramel and red fruit attributes, and less vegetative and animal (reductive) aromas (Caillé *et al*., 2010; Silva *et al*., 2011).

Among the phenolic compounds (§2.2), the anthocyanins are especially prone to oxidation and thus highly affected by exposure to oxygen. The conversion of anthocyanins into other pigments was shown to increase with the oxygen rate, and thus with stoppers' OTR, leading also to the loss of free $SO_2$ and favan-3-ol monomers (de Esteban *et al*., 2019; Wirth *et al*., 2010). These studies also revealed a progressive decrease in proanthocyanidin and hydroxycinnamic acid concentrations without a clear influence of the kind of stopper used (Gutiérrez-Escobar *et al*., 2021).

The influence of the different types of stoppers is highly dependent on wine characteristics. For example, St. Magdalener wines showed a lower concentration of anthocyanin glucosides and acetyl-glucosides after six months of bottle storage when blend stoppers (natural cork microgranules) were used, while Merlot wines the anthocyanin acetyl-glucosides were significantly higher when bottled with the same blend stoppers, compared to other kinds of stoppers (natural cork, agglomerated natural cork, and technical cork) (Rossetti *et al.*, 2020)

### 2.1.2.2 Migration of compounds

Despite the main advantages of cork as a closure material for bottling wines, cork is not an inert material and it is currently accepted that certain cork constituents can be transferred into the wine when the cork is in direct contact with this matrix which is essentially a constitute by ethanol and water (Azevedo *et al.*, 2014; Varea *et al.*, 2001). It has been demonstrated that cork stoppers transfer hydrocarbons, alcohols, ketones, phenolic compounds, including tannins, and other soluble in ethanol/water chemicals constituents to wine, which may have a potential impact on wine's organoleptic properties such as colour, aroma, astringency, and bitterness (Azevedo *et al.*, 2021; Furtado *et al.*, 2021; Pinto *et al.*, 2019). Anyway, worth to mention that it is general accepted that cork meet the general safety criteria applicable to food contact materials (Corona *et al.*, 2014).

The migration of phenolic compounds into wine was higher for natural cork stoppers, especially for those with lower quality, when compared with agglomerated cork stoppers without surface treatment, at least in the case of model wine solutions, as studied by Azevedo *et al.*, 2014. The highest levels of phenolic compounds migration were related to the highest level of porosity/defects of the analysed stoppers.

Natural cork stoppers with lower porosity showed higher amounts of volatile compounds (e.g., limonene, eucalyptol, camphor, 2-pentylfuran, camphene, cyclene, furfural, and 5-methyl-2-furfural), comparing with those with higher porosity, also resulting in higher migration levels into model wine solutions (Furtado *et al.*, 2020). Worth to mention that any extrapolation to what may happen with real wine systems must be carefully done. Probably the presence of the same or similar compounds in wine will affect the mass transfer rate and the significance of these volatile amounts for the general wine aroma still needs further evaluation.

Some studies also demonstrated the potential migration of compounds from agglomerated cork stoppers, associated with the binder material, into wine. Six and Feigenbaum (2003) identified aromatic amines as potential migrants into wine, originated from polyurethane binders. Canellas *et al.* (2021) also proposed the migration into wine of several compounds (amides, amines, and urethane), also originated from polyurethane adhesives, resulting from reactions between isocyanates and acetic acid and ethanol.

For the first time, 2,4-di-*tert*-butylphenol and *trans*-4-*tert*-butylcyclohexanol were described in the volatile composition of wines sealed with synthetic closures and microagglomerated cork stoppers, respectively. Both compounds are widely used in plastics, cosmetics, and fragrance industries but more studies should be performed to understand their sensory impact on wine chemical and sensory properties (Oliveira., *et al.* 2020).

On the other side, stoppers may also adsorb several wine compounds, thus resulting in changes in wine quality, namely on the wine aroma profiling (Furtado *et al.*, 2021).

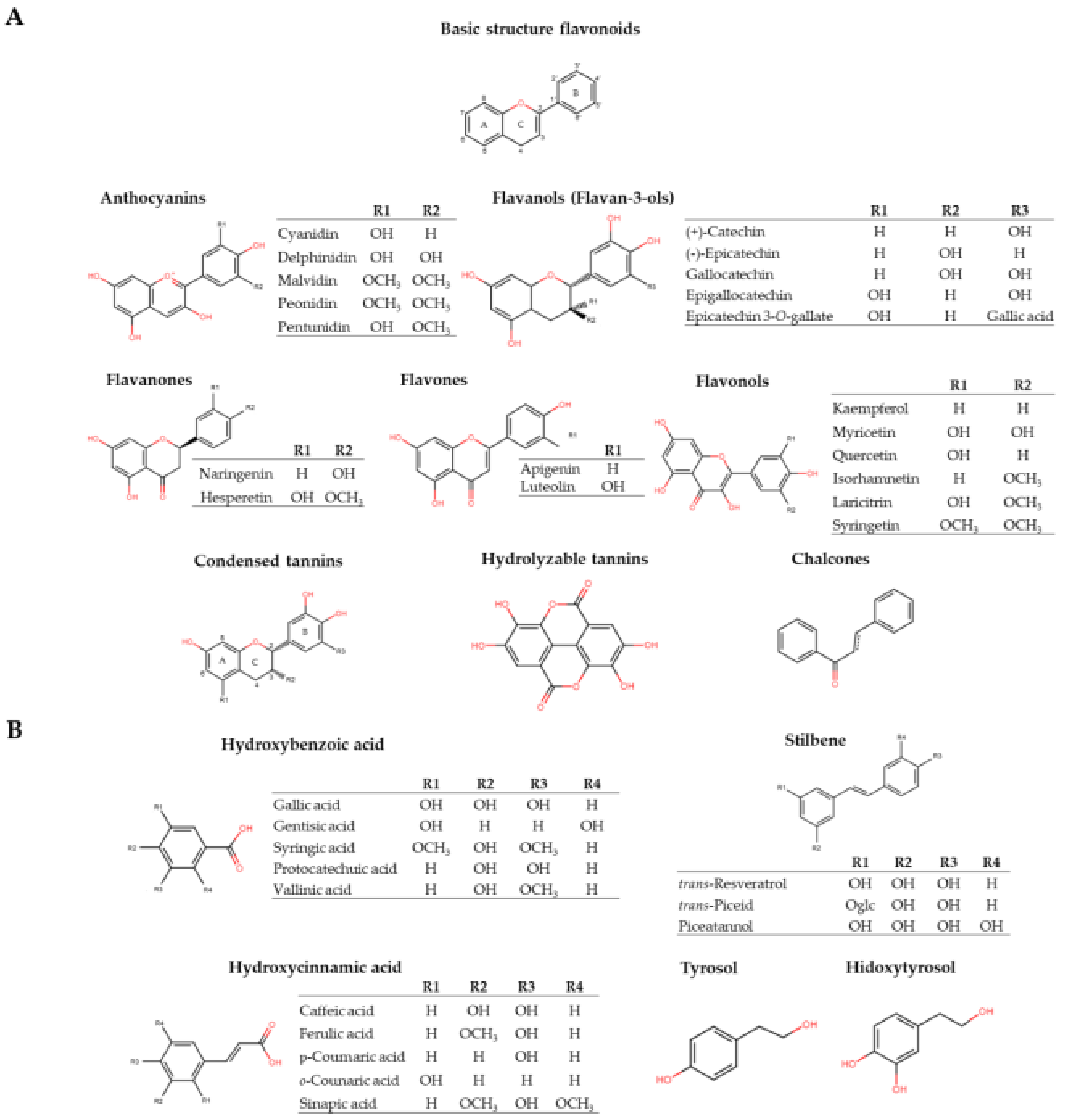
## 2.2 Wine phenolic compounds

Wine is mostly composed by ethanol and water and by other minor compounds namely, alcohols, sugars, acids, minerals, proteins, and other substances like organic acids, volatiles and phenolic compounds also known as polyphenols.

The term “polyphenols” or “phenolics” is used to define a group of plant secondary metabolites that presents one or more hydroxyl (-OH) groups attached to one or more benzene rings, which can be methylated or glycosylated. Polyphenols are usually found in conjugated forms with sugar residues by $\beta$-glycosidic bonds (*O*-glycosylated) or by direct bonds of the sugar to a carbon atom of the aromatic ring (C-glycosides) (Visioli *et al*., 2020). Glucose is the main sugar in fruit, many phenolic compounds are bonded to it.

Polyphenols are synthetized by the phenylpropanoid pathway, being the amino acid phenylalanine (a shikimate pathway product) its common precursor (Ortega *et al*., 2020). From straightforward phenolic acids to high molecular mass polymeric forms like hydrolysable and condensed tannins, polyphenols exhibit extremely varied chemical structures (Ribéreau-Gayon *et al.*, 2000). They can be divided into flavonoid and non-flavonoid compounds where flavonoids are most prevalent (Adams, 2006). Flavonoids include flavanols, flavonols, flavanonols, flavones, anthocyanins, and hydrolysable and condensed tannins. The second group, the non-flavonoids, includes hydroxybenzoic and hydroxycinnamic acids and others phenolic derivatives such as stilbenes (e.g., resveratrol). (Gutiérrez-Escobar *et al*., 2021) (Figure 2.2).

Phenolic compounds are of great importance in oenology since they are related to the quality of wines. Some of these compounds are responsible for the colour, body, flavour and astringency wine characteristics. They are also responsible for the differences between red and white grapes, due to the presence or absence of anthocyanins (Ribéreau-Gayon, *et al*., 2006, Ribéreau-Gayon., *et al*., 2000; Paixão *et al*., 2007).



|  | R1 | R2 |
|---|---|---|
| Cyanidin | OH | H |
| Delphinidin | OH | OH |
| Malvidin | $OCH_3$ | $OCH_3$ |
| Peonidin | $OCH_3$ | $OCH_3$ |
| Pentunidin | OH | $OCH_3$ |

|  | R1 | R2 | R3 |
|---|---|---|---|
| (+)-Catechin | H | H | OH |
| (-)-Epicatechin | H | OH | H |
| Gallocatechin | H | OH | OH |
| Epigallocatechin | OH | H | OH |
| Epicatechin 3-*O*-gallate | OH | H | Gallic acid |

|  | R1 | R2 |
|---|---|---|
| Naringenin | H | OH |
| Hesperetin | OH | $OCH_3$ |

|  | R1 |
|---|---|
| Apigenin | H |
| Luteolin | OH |

|  | R1 | R2 |
|---|---|---|
| Kaempferol | H | H |
| Myricetin | OH | OH |
| Quercetin | OH | H |
| Isorhamnetin | H | $OCH_3$ |
| Laricitrin | OH | $OCH_3$ |
| Syringetin | $OCH_3$ | $OCH_3$ |

|  | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Gallic acid | OH | OH | OH | H |
| Gentisic acid | OH | H | H | OH |
| Syringic acid | $OCH_3$ | OH | $OCH_3$ | H |
| Protocatechuic acid | H | OH | OH | H |
| Vallinic acid | H | OH | $OCH_3$ | H |

|  | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| *trans*-Resveratrol | OH | OH | OH | H |
| *trans*-Piceid | Oglc | OH | OH | H |
| Piceatannol | OH | OH | OH | OH |

|  | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Caffeic acid | H | OH | OH | H |
| Ferulic acid | H | $OCH_3$ | OH | H |
| p-Coumaric acid | H | H | OH | H |
| *o*-Counaric acid | OH | H | H | H |
| Sinapic acid | H | $OCH_3$ | OH | $OCH_3$ |

**Figure 2.2** (**A**) Structure of representative flavonoid compounds found in wine; (**B**) Structure of the non-flavonoid compounds found in wine (Gutiérrez-Escobar *et al*., 2021)

Phenolic compounds are unevenly present in the various parts of the grape. In the seeds, there is a greater amount of flavanols and gallic acid, in the pulp, hydroxycinnamyl tartaric acids are usually the most abundant, in fibrovascular vessels the flavanols and acids phenolics and in the skins flavonols are found in higher quantity, as well as proanthocyanidins (condensed tannins) and anthocyanins. The phenolic compounds are found dissolved in the vacuoles of pulp cells, adsorbed, or bound to polysaccharides, and free in the vascular juice of the pellicle cells (Paixão *et al*., 2007). In fact, polyphenolic compounds are among the main macromolecules present in red wine.

### ***2.2.1. Flavonoid compounds***

Flavonoids are phenolic compounds that are characterized by a basic and common C6–C3–C6 skeleton, the basic structure consisting of aromatic rings connected by a pyran ring (Ribéreau-Gayon *et al.*, 2006). This class of phenolic compounds can be divided in different families that are distinguished by the degree of oxidation of the pyran ring.

Anthocyanins, flavan-3-ol and proanthocyanidins, polymers of flavan-3-ol monomeric units, are quantitatively the most important flavonoids, typically present in the pips, pulp and skin of grapes. The colour and astringency of wines are mainly due to this class of compounds. Flavonoids can be found in the free form or polymerized with other flavonoids, sugars, non-flavonoid compounds, or even combinations of the above (Cabrita and Laureano., 2003).

#### 2.2.1.1 Anthocyanins

Anthocyanins are responsible for the colour of grapes and red wines and are located mainly in the skins but can also be found in the pulp in the case of dye grapes (Núñez *et al.,* 2004). Structurally they are polyhydroxy or polymethoxy glucosides of the flavilium cation (2-phenyl-benzopyryl). Anthocyanins are distinguished by the number of hydroxyl and methyl groups present on the lateral ring, the number and nature of the sugars attached to the molecule, and the number and nature of the aliphatic or aromatic chains esterified with sugars.

The concentration of anthocyanins in red grapes varies with grape variety, degree of maturation and viticulture practices (Ribéreau-Gayon *et a.l*, 2006).

The aglycone forms of anthocyanins are called anthocyanidins, which are unstable in water and much less soluble than anthocyanins, glycosylation is thought to provide stability and solubility to these pigments.

In grapes of the *Vitis vinifera*, a glucose molecule is attached to the anthocyanin group in the position 3 (Ribéreau-Gayon *et al.*, 2006). The anthocyanins commonly found in *Vitis vinifera* are cyanidin 3-D-glucoside, delphinidin, peonidin, petunidin and malvidin. Their relative amounts vary according to the grape variety, but malvidin 3-D-glucoside is always present in higher levels. The main acids that can esterify glucose at position 6 are caffeic, p-coumaric and acetic (Núñez *et al.*, 2004).

#### 2.2.1.2 Flavanols

Flavanols are located mainly in seeds and stalks and in lesser amounts in skins. These compounds are found in the form of monomers, oligomers, or polymers (condensed tannins). The most important flavanols are flavan-3-ol and proanthocyanidins.

Flavan-3-ols are characterized by having a saturated heterocyclic ring. The main flavan-3-ols found in grapes and wines are (+)-catechin and (-)-epicatechin, which are epimers on carbon 3, and small amounts of epicatechin gallate (Rinaldi and Moio 2020).

Proanthocyanidins are compounds that release anthocyanidins when heated in a strongly acidic and alcoholic environment, due to the rupture of bonds between monomeric units (da Silva, 1995).

Depending on whether cyanidin or delphinidin are released, these molecules are called procyanidins or prodelphinidins. The first are polymers of catechin and epicatechin and the second are constituted by gallocatechins and epigallocatechins.
As mentioned above, proanthocyanidins are constituted by flavan-3-ol monomeric units and, depending on the number of times this unit is repeated, proanthocyanidins can be dimers, trimers, oligomers or polymers. Thus, proanthocyanidins from grapes and wines are mainly procyanidins, *i.e.* oligomers and polymers of (+)-catechin and (-)-epicatechin linked by C4-C8 and C4-C6 bonds (da Silva 1995).
Procyanidins, also known as condensed tannins, are phenolic compounds that have the ability to combine with proteins and other polymers such as polysaccharides, causing the sensation of astringency, which is the loss of saliva lubrication due to the precipitation of proteins (Ribéreau-Gayon *et al*., 2000)

#### 2.2.1.2.1 Tannins

Astringent wines are commonly defined as “tannic” because tannins are the main polyphenolic compounds involved in the sensation of astringency. The first helpful phytochemical definition of tannin was given by Swain and Bate-Smith (1962), who described it as "water-soluble phenolic compounds with molecular weights ranging from 500 to 3000, which have the ability to precipitate alkaloids, gelatine, and other proteins”.

Tannins are a group of chemical compounds found in grapes, particularly in the skins, stems, and seeds, as well as in oak barrels used for wine aging. They are responsible for the distinctive bitterness and mouth-drying sensation associated to many red wines.
Grape seed and skin tannins are felt as more astringent as the mean degree of polymerisation (mDP), and galloylation increase (Vidal *et al*., 2003), related to the fact that their ability to precipitate proteins also increases with mDP up to a given degree of polymerisation (Bajec and Pickering, 2008; Sun *et al*., 2013).

Considering the structural diversity of tannins from red grapes, it is well established that the average size of skin tannins is much larger than that of seed tannins. Tannins extracted from skins have degrees of polymerization around 30 compared with mDP around 10 for those extracted from seeds (Prieur *et al*., 1994; Bindon *et al*., 2010). Seed tannins are also characterized for having a greater amount of galloylated units (13– 29%) compared with skin tannins (3–6%) (Cheynier 2006 a; Riou *et al* 2002). The smaller molecular weight of seed tannins may be the reason for the reported bitterness of these compounds, and this may explain why seed tannins are considered undesirable in wine.

Grape stem tannins can contribute to the final phenolic composition of wine and potentially increase the tannin concentration. The mDP of stem tannins ranges from 4 to 28, with a lower proportion of epigallocatechin compared with epicatechin gallate subunits (Souquet *et al.,* 2000; Vivas *et al.,* 2004).
Skin tannins are linked to polysaccharides and protein and contribute to the softness and roundness but can impart herbaceous notes if fruit is not ripe. Seed tannins give structure to the wine but can also impart excessive astringency (Soares *et al*., 2017).

Tannins are typically classified as condensed or hydrolysable tannins. Red wine tannins consist mainly on condensed tannins extracted earlier from grape skins during the fermentation process. As fermentation continues, tannins begin to be extracted from grape seeds and pulp, increasing with maceration time (Peyrot and Kennedy, 2003; Herderich and Smith, 2005) and subsequently structurally modified during winemaking and ageing. Salivary proteins seem to have a higher affinity for condensed tannins than for hydrolysable tannins because of different structural flexibility, size, polarity, affinity constants, and presence of free galloyl groups (Charlton *et al*., 2002; Hofmann *et al*., 2006; Rinaldi *et al*., 2010; Dobreva *et al*., 2014).

Following fermentation, wine components continue to go through chemical changes. Wine's gradual oxidation and acidity trigger bond-breaking and rearrangement processes (Zanchi 2008; Haslam 1980) are hypothesized to be the catalysts for tannin polymerization and the synthesis of various colours and coloured polymers (Mateus *et al.,* 2006; Salas *et al.,* 2005; Mateus *et al*., 2003).

Hydrolysable tannins comprise three subclasses such as simple gallic acid, poly-galloyl esters of glucose (gallotannins), and esters of ellagic acid (ellagitannins). Hydrolysable tannins are not naturally found in grapes. On the other hand, they are the main commercial tannins legally authorized as wine additives. A small percentage of hydrolysable tannins are extracted from oak barrels or chips during ageing or can be added during winemaking (Sarneckis *et al*., 2006). However, due to their low amounts in red wine, these tannins are not the major contributors to the astringency sensation.

Oakwood tannins were mainly associated with smooth and mouth-drying sensations at low concentrations (Stark *et al*., 2010). Astringency subqualities such as mouth-coat, full-body, persistent were mainly associated with oak-derived tannin, whilst the velvet, soft, and satin terms were associated with the exotic wood-derived tannin (Rinaldi *et al*., 2020).

### *2.2.2 Non-flavonoid compounds*

Non-flavonoid compounds include phenolic acids, namely from the benzoic and cinnamic groups. In grapes, they are mainly hydroxycinnamic acids found in the vacuoles of skin and pulp cells, in the form of tartaric esters. These compounds play an important role in the oxidation processes that lead to the browning of musts and wines.

#### 2.2.2.1. Benzoic and Hydroxycinnamyl Tartaric Acids

The most important derivatives of benzoic acid are vanillic, syringic and salicylic, which appear attached to cell walls, and gallic acid which is found in the form of ester of flavanols. Other benzoic acids that exist in smaller amounts are protocatechin and p-hydroxybenzoic acid. These acids are found in the grapes mainly in the form of esters; during wine production and aging they undergo hydrolysis and thus can be found in the wine either in free or combined form (Ribéreau-Gayon *et al*., 2006).

The most important phenolic acids of the cinnamic series are ferulic, p-coumaric and caffeic acids, found in grapes mainly as esters of tartaric acid.

Unlike other phenols, the importance of hydroxycinnamyl tartaric acids is not linked to their contribution to astringency, but to the oxidative browning phenomena that musts or white wines can undergo. These compounds, rich in hydroxyl groups, are the first phenolic substances to be oxidized into their respective quinones by phenoloxidase enzymes. These quinones can react with other compounds present in musts, leading to the appearance of compounds with colours that can vary from yellow to brown.

In addition to impacting taste characteristics, polyphenols also affect stability through oxidative processes that follow (browning in white wines and oxidation in red wines) (Gutiérrez-Escobar., 2021).

Besides the influence of all these factors regarding red wine polyphenols, it is also important to refer that tannins and anthocyanins are both relatively unstable compounds that can undergo through various types of chemical reactions such as spontaneous cleavage of interflavan bonds in acidic medium, as well as acetylation and oxidation (Fulcrand *et al*., 2006). The complex interactions between these compounds and other molecules are a challenge for red wine composition analysis and consequently for the study of red wine quality sensorial parameters such as astringency.

These compounds are removed throughout the wine-making process from distinct grape bunch sections.

According to the circumstances, their structural differences are significant when wine ages in a barrel, tank, or bottle (Ribéreau-Gayon *et al*.,2000).

### *2.2.3. Reactions affecting phenolic compounds in wines.*

After crushing the grapes and before fermentation starts, several chemical reactions (condensation, oxidation, reactions between phenolic compounds and metabolites resulting from the secondary metabolism of yeasts) can occur involving this phenolic compounds, especially anthocyanins, catechins and procyanidins, resulting in the formation of new polymeric pigments responsible for colour changes in wine, bluish-red in young wines and orange-brown in mature wines (Paixão *et al*., 2017; Gutiérrez *et al*., 2005).

During wine aging many reactions can occur leading to changes in phenolic compounds, producing new polymeric and oligomeric compounds strongly coloured contributing to the reduction of astringency, affecting the colour and flavour of wines. Anthocyanins play a role in some of these reactions and are converted into more stable oligomeric and polymeric pigments, which has a significant impact on colour and astringency properties of wines. Condensation reactions also involve flavanols. For this reason, wine aging is often necessary to achieve the desired wine quality. However, and sometimes, longer aging periods may cause undesirable oxidation reactions.

Thus, knowing factors that may affect these reactions are important to help winemakers to select appropriate oenological technologies to tailor-make wine with desired organoleptic properties.

Younger wines typically have higher levels of anthocyanins and other phenolic compounds, whereas older wines have higher concentrations of polymeric pigments. The decrease in anthocyanins concentration in wine during aging has been associated with their polymerization, originating tannins with higher molecular weights (Fulcrand *et al*., 2004; Mateus, 2001; McRae *et al*., 2010).

As discussed above, wine tannins and salivary proteins interact forming protein-tannin complexes, which are responsible for wine astringency. Wine astringency is a crucial sensory characteristic of red wine and is directly related to the phenolic compounds group, and particularly to concentration of tannins. However, monomeric, and dimeric flavan-3-ols can also induce astringent and bitter sensations (Hufnagel and Hofman 2008). Galloylation of monomers/oligomers and polymers enhances protein precipitation, and its extent depends on the grape variety (Rinaldi *et al* 2014). The presence of high galloylation seems to be responsible for the rough perception (Vidal *et al* 2003), which in turn can be decreased by a high content of epigallocatechin units linked to tannin molecules. On the contrary, it seems that the hydroxylation of the B-ring seems to decrease velvety astringency and increase the perception of puckering and drying astringency of wine fractions (Gonzalo-Diago *et al*., 2013)

Oxidized tannins have been shown to feature greater intramolecular interactions, altering their conformation in solution to more condensed or folded structures rather than the extended forms of pristine grape tannins (Poncet-Legrand 2010). The changes in tannin structures during fermentation and wine aging have an impact upon the binding of the tannins with salivary proteins and thus on the astringency of the wine.

The processes that may occur, during aging, are complex and dependent on a diversity of factors (Figure 2.3). The initial concentration of phenolic and volatile compounds, additives, oxygen, pH, acidity, minerals, and microorganisms, among other internal and external elements, affect these chemical processes. External parameters include temperature, barrels, closure, humidity, and luminosity (Gutiérrez-Escobar *et al*., 2021; Paixão *et al*., 2007).

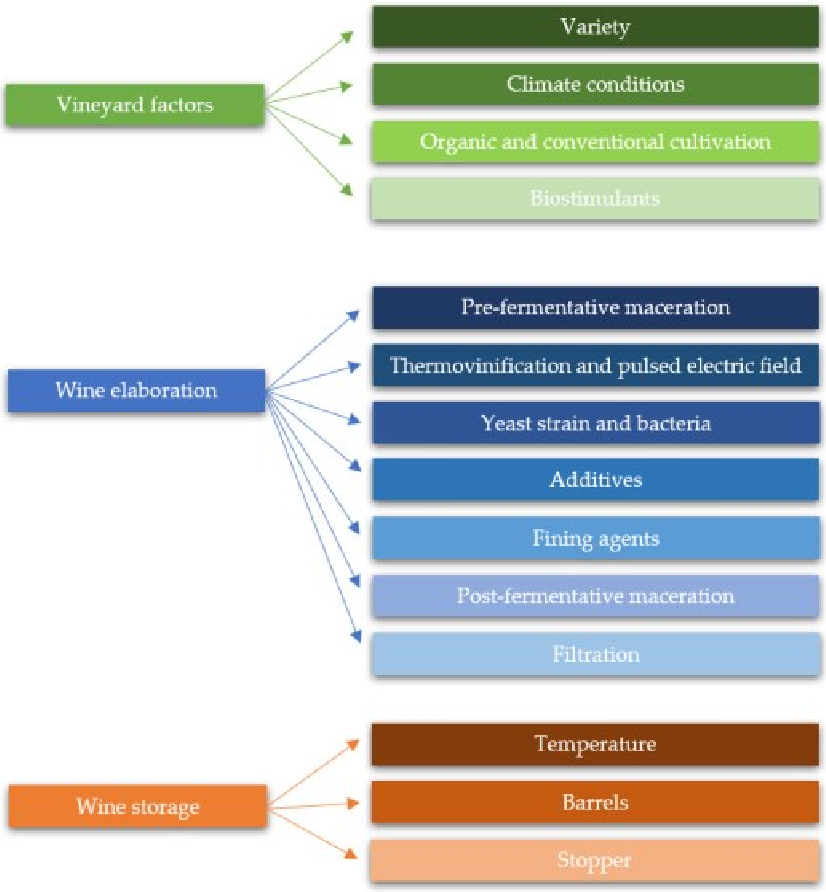


**Figure 2.3.** Diagram showing main external factors that influence phenolic compounds present in grapes, must and wine (Gutiérrez-Escobar *et al*., 2021).

## 2.3 Wine astringency – human perception mechanisms

### *2.3.1 – Perception of astringency*

The human being consciously interacts with the environment through five senses, which determines the sensorial perception. Perception is defined in the Oxford dictionary as "awareness through the senses interpreted in the light of experience". In other words, sensory perception is awareness that arises through a single sense or a combination of multiple senses and personal factors.

The oral perception of food is the result of the interaction of food characteristics in the mouth and immediately interpreted by the brain (Engelen, *et al.,* 2007). Sensory responses to the taste, aroma, colour and texture also determine consumers' food preferences and eating habits.

Mouthfeels such as astringency, texture, acidity, heat, and body play an important role in defining the sensory character of wine. One of the most important sensations and a quality attribute of red wine is astringency, but a balanced level of astringency is needed to make it a desirable product. For winemakers, astringency adds flavours to red wines and prolongs their aftertaste. In fact, the famous winemaker Emile Peynaud 1996 states that the effects of harmony, balance and elegance of astringency correspond to the better red wines.

Sensory perception of astringency is related to “dry mouth” sensation, although it is a very complex perception with several definitions proposed over time. The American Society for Testing and Materials (ASTM) defines astringency as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM 2004).

The word astringency comes from the Latin as “ad *stringere”*, which means "to bind".

Bate-Smith *et al*., (1954) suggested that astringency is a sensation rather than a taste. They opposed the explanation of astringency as an additional flavour to the five accepted taste sensations (ie, sweet, sour, salty, bitter, and umami). They referred to it as an event induced by the interaction of tannins and the precipitation of proline-rich salivary proteins (PRPs) in the oral cavity. Joslyn and Goldstein (1962) also supported the tactile theory of astringency, stating that "precipitation of tissue proteins is accompanied by tissue contraction due to water loss and a decrease in tissue permeability to water and solutes". On the other hand, Lawless and Corrigan (1995) also defined astringency as a more physical event, referring to the sensations of tightness and traction felt in the mouth musculature and the sensations of dryness and roughness when there is contact and movement in the mouth. This general conceptual view has been maintained until today. However, it was unclear whether the astringent compounds trigger mechano-sensation, chemo-sensation, or a combination of both. Later, in 1998, Peleg *et al*., referred to astringency as a complex phenomenon that causes a series of sensations, triggered by different types of substances, and explained by different mechanisms.

It is possible to ascertain that over time research regarding astringency has had several important gaps and its mechanisms have been subject of debate. Most studies argues that the basis of astringency is mainly mechanical (tactile) rather than chemo-sensorial (gustatory) (Lyman and Green, 1990, Soares *et al*., 2017).

In fact, ancient Indian culture considered astringency a basic taste. However, since then, it has been understood as a tactile sensation due to the mechanical effect of decreased salivary lubrication (Kallithraka *et al.,* 2001; Rossetti *et al*., 2009), as shortly discussed in the previous section about phenolic compounds.

The oral developmental mechanisms of astringency have received increasing attention, given the recent trend to supplement food products with beneficial substances that may also have undesirable astringency, then enabling to simultaneously minimizing their potential undesirable mouthfeel (Bajec and Pickering, 2008).

The perception of astringency results from the interaction of astringents compounds with the oral cavity, soft palate, gums, lips, cell membrane proteins, epithelial cells, mechano-receptors (MRs) and chemoreceptors (Rinaldi and Moio 2021).

As already mentioned in the previous section, phenolic compounds, and in particular tannins, play an important role in the perceived astringency in wines. Despite the complex mechanisms involved, astringency is generally accepted as resulting from interactions of polyphenols with proteins, from the oral epithelial tissues (Payne *et al* 2009), and/or salivary proteins (Gawel, 1998; Rinaldi & Moio, 2021). Tannins may also interact with taste receptors, particularly bitter receptors in the case of small, condensed tannins (Kallithraka *et al.,* 1997, 2001; Brossaud *et al.,* 2001), aspects described in more detail in the review of Bajec and Pickering (2008). An important consequence is the increased friction at the oral cavity (Rossetti *et al* 2009), triggering neuronal stimuli that lead to the perception of astringency. It is now generally accepted that not only mechanoreceptors are involved, but also chemosensory detection mechanisms (Canon *et al.,* 2018; Kurogi *et al.,* 2015; Schöbel *et al.,* 2014). Multiple-modal system which implicates several possible astringency mechanisms.is represented in (Figure 2.4) (Ma, W,,*et al* 2014).

Interactions between tannins and salivary proteins, associated with astringency, mainly involve hydrogen bonding and hydrophobic interactions (Rinaldi and Moio, 2020). The hydrophobic interactions formed between the phenolic rings of procyanidins and proline residues, and the hydrogen bonding between the hydroxyl groups on the phenolic B ring and the hydrogen-accepting sites of the peptide bond are among the most important (Poncet *et al.,* 2007). Covalent bonds may also occur during oxidation (Parish *et al.*, 2000) and nucleophilic addition processes (Beart *et a.l*, 1985).

It is generally accepted that these processes occur in different phases (McRae and Kennedy, 2011), originating the protein/peptide coated by polyphenols, which provides cross-linking between two or more peptides to a critical point, finally leading to the precipitation of salivary proteins with tannins (Rinaldi and Moio, 2020).

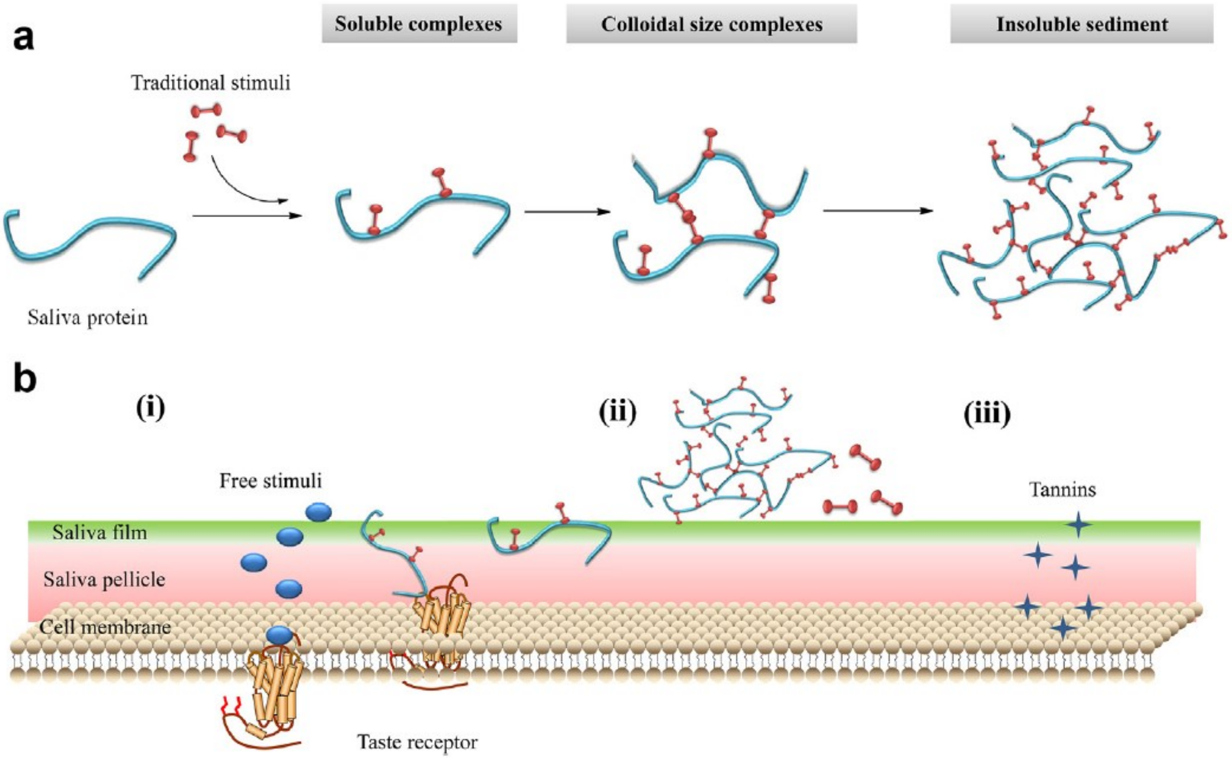


**Figure 2.4.** Schematic representation of possible astringency mechanisms: (a) A 3-stage model of the interaction between stimuli and proteins; (b) Astringency stimulation: (i) “Free” stimuli and soluble

stimuli–protein complexes deplete the protective salivary film and eventually bind to the pellicle or even to the receptors exposed; (ii) Insoluble stimuli–protein complex and traditional stimuli are rejected against salivary film. Insoluble stimuli–protein complexes trigger astringency sensation via increasing friction. (iii) Tannins interact with oral cavity membrane (From Ma, *et al.,* 2014).

The complexation between tannins and salivary proteins and the stability of these complexes depends on the characteristics of tannins and proteins (Rinaldi and Moio, 2020).

Despite the innumerous studies already performed on the subject, it is still difficult to relate astringent sensations in the mouth with specific phenolic compounds in wine, due to the reactivity of different polyphenols. Therefore, the relationship between the specific chemical components of the wine and the specific property of the taste remains unclear.

Nevertheless, it is well-known that different types of tannins and proteins can have distinct affinities with each other. For example, condensed tannins (proanthocyanidins) tend to form stronger complexes with proteins than do hydrolysable tannins such as gallotannins. Likewise, different types of proteins, such as albumins, globulins and glutelins, may have different binding affinities for tannins (Rinaldi and Moio, 2020). The hydrophobicity of tannins also influences the effectiveness of protein binding, thus affecting astringency, as demonstrated for artificially oxidized tannins (Zanchi *et al.*, 2008).

Differences in tannin structures have been shown to have a significant impact on protein binding efficiency as well as the perception of astringency (Gawel *et al.,* 2007). Higher concentrations of tannins and the number of free phenolic groups are directly correlated with an increased perceived astringency (Charlton, 2002).

The larger molecular size and structural flexibility of tannins, as well as a higher ratio of catechin subunits and epicatechin or epigallocatechin subunits as well as the presence of more C4-C8 than C4-C6 linkages, were associated with a higher binding of tannins with proteins, namely to a greater specificity for the salivary PRPs (de Freitas and Mateus, 2001; Le Bourvellec *et al*., 2004; Cheynier *et al*., 2006; Dangles *et al*., 2006). Since there are more binding sites for interaction with proline or histidine residues on larger tannins with more structural flexibility, such as gallate groups and free-rotating interflavan bonds, they are more likely to bind to proteins (Deaville, 2007). The larger size of tannins also allows for greater self-association, which favors the aggregation of complexes.

The fact that some aged wines have a tannin concentration similar to that of young wines (Mercurio *et al*., 2008) and yet mature wines are often considered less astringent (Herderich *et al*., 2006) is therefore not surprising. According to the investigation by McRae *et al*., (2010), tannins in aged wines are bigger than tannins in young wines, which is often associated to greater astringency (Vidal *et al*., 2003), as previously mentioned. Thus, the increase in the average size and polymerization of tannins in aged wines, resulting from intramolecular binding due to oxidative processes, associated with a decrease in chain flexibility, may be the most important factor responsible for the lower astringency associated with aged wines.

One important aspect to be considered in the context of the present work is the possible migration of phenolic compounds, including tannins, from the cork stoppers into the wine, as previously discussed in section 1.2.2. In a recent work carried out by Azevedo *et al*., (2022) they reported the study of the interaction of human salivary proteins (SP) with phenolic compounds that migrate from cork stoppers to wine. Among the different tested phenolic compounds, castalagin and vescalagin showed a higher ability to precipitate SP (mainly aPRPs, statherin and P-B peptide). In addition, there also seems to be a matrix effect (presence of other compounds) that could affect these interactions.

This study yields valuable data to understand the influence that these compounds may have on the astringency sensory perception of wine.

Another work showed that polyphenols are susceptible to migrate from cork stoppers to wine (Varea., *et al.,* 2001) and concluded that higher amounts of polyphenols are found in natural cork stoppers than in agglomerated cork stoppers, however, no significant differences were found among the different types of cork stoppers.

The cork surface covered with wider lenticels, crevices and fibrous tissue is of lower quality since the roughness of cork increases the surface of the stoppers.

The release of cork phenols from cork granules, disks, and stoppers of different quality classes A (best quality) to D (worst quality) in synthetic wine was investigated (Gabrielli *et al.,* 2016) aiming to evaluate the release of phenolic compounds from cork stoppers and their effect on protein-haze. In this study authors found that the protein haze increased as the release of cork phenolics concentration increased, mostly if a high ratio of tannin vs protein concentration is achieved. They conclude that protein-haze can increase when low quality and/or uncoated cork stoppers are used.

Gancel. *et al.,* 2023 compare the polyphenols, mainly hydrolysable tannins, transferred from natural and microagglomerated corks treated with supercritical CO2 into hydroalcoholic solutions. In this study, despite the high intra-“natural cork stopper” variability, significant differences were found between both types of stoppers for all polyphenols, the agglomerated corks releasing significantly less polyphenols; i.e., 25 times less. In contrast, suberic acid was extracted from both types of corks in similar concentrations; therefore, its extractability was not impacted by the type of stopper. They carried out a sensory profile of macerates of natural cork stoppers that were perceived with notes of “cardboard, dust, plank, wood” and “cork taint” significantly higher than supercritical CO2 treated microagglomerated cork stopper macerates. The natural cork macerate with the highest content in polyphenol was perceived as being bitter and more astringent than microagglomerated cork stoppers.

Cordes *et al*., 2021 studied suberic acid as a potentially flavour-active contaminant released by some agglomerated corks. Liquid chromatography-mass spectrometry revealed that agglomerated corks from one manufacturer released more than 40 odorless compounds into the wine, which were not released by natural corks or, when release, only in substantially lower amounts. One of these compounds has been identified as suberic acid, a key component of the cork suberin polymer. It has also been detected in agglomerated corks from a second manufacturer. This contamination generates sensory changes on the palate described as the tactile perception of lingering astringency. Additional gustative changes were caused by suberic acid and agglomerated corks. This research combined the trained wine tasters with the unerring accuracy of an electronic tongue (Cordes *et al*., 2021).

Even though the structure and content of tannins influence astringency and salivary protein composition (de Freitas and Mateus, 2001; Cheynier *et al.,* 2006 b; Rinaldi and Moio L, 2021), consumer physiology and psychological factors will also have an impact on how wine astringency is judged and perceived (Mateus, 1999).

The exact composition of saliva varies from individual to individual; the group of proteins is mainly composed by α-amylase, lactoferrin, other enzymes, peptides (agglutinins, immunoglobulins), mucinglycoproteins, and proline-rich proteins (PRPs) as well as histidine-rich proteins (histatins or HRPs), these last being the main tannin-binding proteins (Bajec and Pickering, 2008; de Freitas and Mateus 2001).

Individuals differ not only in terms of saliva protein content, but also in salivary flow rate and saliva viscosity, and the latter has been proven to have a high impact on how astringent is recognized (Demiglio, 2008; Dinnella *et al.,* 2009). It has been proven that saliva flow and certain concentrations of salivary proteins generally decrease the sensation of astringency. Although saliva viscosity and oral lubrication are not directly related, a reduction in saliva viscosity can result in the impression of astringency due to the friction increased (de Wijk and Prinz, 2005).

### *2.3.2 – Factors affecting oral astringency perception.*

As already mentioned, several factors influence the perception of wine astringency, namely phenolic compounds and proteins present in wine and in the oral cavity, as well as several physiological factors, such as saliva composition, oral pH, temperature, oral cavity surface properties and oral fluid composition.

Despite the concentrations and type of phenolic compounds present in wine, other characteristics related to the wine matrix also influence perception of astringency, such as pH, organic acid composition, and ethanol. Other factors that may affect the perception of astringency include the presence of other ions and salts, the sugar concentration, the amount of acetaldehyde and the presence of other compounds that interact with tannins, such as yeast proteins and grape polysaccharides (Mc Rae., *et al.,* 2011).

Below is presented a short discussion regarding this subject.

#### 2.3.2.1 Effect of carbohydrates on astringency and tannin-protein interactions

Carbohydrates are frequently used in food industry as food colloids (gums) and are also naturally present in several food products, thereby affecting their astringent sensation. Therefore, this interaction between carbohydrates and protein/tannin has a great impact in the perception and choice of foodstuffs.

Different polysaccharides reduce the astringency of tannins through different mechanisms of action (Figure 2.5). Arabic gum and β-cyclodextrin bind preferentially to polyphenols, inhibiting protein-tannin interactions, while the polyelectrolytic properties of pectins allow them to bind directly to protein/polyphenol complexes, thus increasing the water solubility of these complexes and preventing their precipitation (McManus *et al.,* 1985; Poncet *et al.,* 2007; Soares *et al*., 2017; Fernandes *et al*., 2014). It is expected that polysaccharides, naturally occurring in wine, like arabinogalactan and rhamnogalacturonan II, the pectic polysaccharides from grape cell walls and mannoproteins from yeast during fermentation, have an important role on wine astringency. All polysaccharides have been shown to reduce perceived astringency to some extent, but acidic polysaccharides have the greatest impact on reducing astringency (Vidal *et al.,* 2004b; Carvalho *et al.,* 2006; Riou *et al*., 2002; Taira *et al.,* 1997).

Sugars have also demonstrated to influence the perception of astringency in wines. Higher concentrations of sucrose in wine have been associated with lower astringency and reduction of the unpleasant "puckering" sensation of young wines (Gawel *et al.,* 2007; Boselli *et al.,* 2006; Saenz *et al.,* 2010)

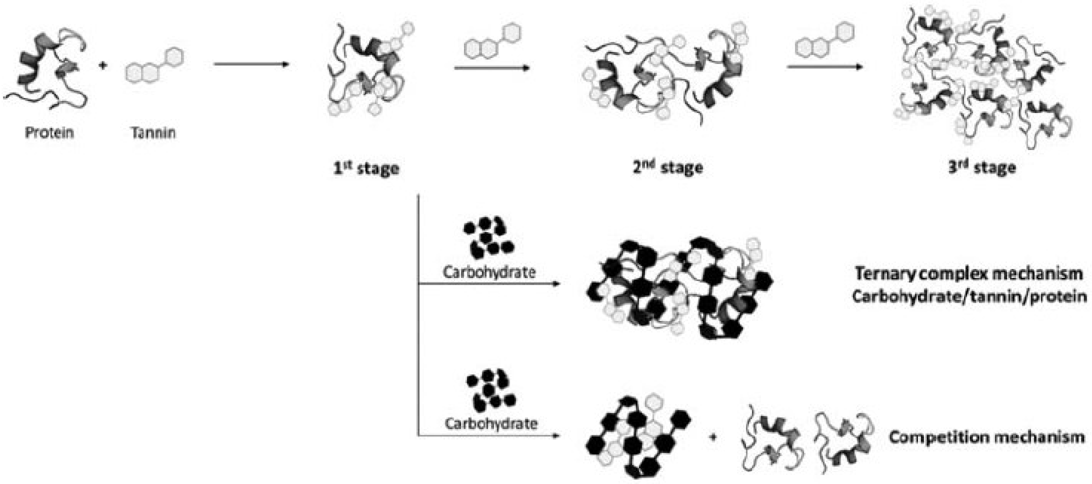


**Figure 2.5.** Schematic representation of the third stages of tannin/protein aggregation and of the two proposed mechanisms by which carbohydrates inhibit tannin/protein interaction (Soares *et al.,* 2017).

### 2.3.2.2 Effect of pH

The pH of wine normally varies between 3.2 and 3.8, and this difference is sufficient to cause variations in astringency. Although the role of acidity in modulating the mouthfeel qualities of red wine has not been explored in depth, it has been proposed that acidity may have an indirect effect on astringency by increasing the effectiveness of binding between polyphenols and salivary proteins. By changing the ionization state of proteins and tannins, the pH of the solution can have an impact on tannin-protein interactions. In fact, reducing the pH of wine and model wine solutions has been shown to increase both the level of astringency and the interaction of tannins with proteins (Fontoin *et al.,* 2008).

Shiraz red wine phenolic content, acidity and mouth perceived textural qualities were examined by Gawel *et al*., (2007a). They stated that "chalk-like" perceptions were negatively correlated with acidity and alcohol content, while "suede-like" sensations were linked to a lack of polyphenols. Roughing astringent characteristics were mostly correlated with the presence of polyphenols, while "puckery" sensations were characterized by high acidity as well as high amounts of pigmented polymers and tannins. However, the results were somewhat ambiguous.

#### 2.3.2.3 Effect of Organic Acids

The interaction between proteins in saliva and vicinal hydroxyl groups of tannins is the accepted mechanism evoking astringency. Many organic acids such as tartaric or suberic acid contain vicinal hydroxyl groups and thus can also bind to proteins. Thus, variable astringency intensities and sub-qualities have been shown for the acids contained in wine, as well as their dependence on pH suggesting that the acidic properties of these acids are an additional cause of their astringency (Lawless *et al*., 1996). Suberic acid was shown to increase wine astringency, but also causes additional changes in the mouthfeel (Cordes *et al.,* 2021). However, increasing the concentration of specific organic acids, such as malic, lactic and tartaric acid, has a less impact on increasing astringency than lowering pH using other acids. However, higher concentrations of organic acids combined with higher acidity have been shown to contribute to the chalky characteristics of red wine (McRae and Kennedy 2011).

Another example of the effect on astringency perception of different acids was reported for malic and tartaric acids. Wines rich in malic acid showed stronger saliva protein reactivity and higher potential astringency than wines rich in tartaric acid, when compared at the same pH, most likely as a result of its superior buffering capacities (Picariello, *et al.*, 2019).

#### 2.3.2.4 Effect of Anthocyanins

Studies on the influence of anthocyanins on astringency are controversial. An anthocyanin fraction added to a model wine solution was felt to be "coarse and chalky" and contributed slightly to the overall astringency (Vidal *et al* 2004a). Other studies have shown that anthocyanins were able to interact with human salivary proteins forming soluble aggregates (Ferrer-Galego *et al.*, 2015), and even precipitated, the cinnamoylated fraction being the most reactive (precipitation between 6.5 and 17.5%), also influencing the perception of astringency (Paissoni *et al.*, 2018).

According to Jackson (2008), free anthocyanins are thought to have no direct impact on red wine astringency. Vidal *et al*., (2004a) demonstrated that although highly purified grape anthocyanins were evaluated similarly to model wine, they did not add astringency or bitterness to the wine. However, several investigations have shown that grape anthocyanin fractions can enhance the astringency of grape skin or seed proanthocyanidins when added to a white wine (Brossaud *et al*., 2001), leading also to an increase in perceived astringency in model wines (Vidal *et al*., 2004a)

#### 2.3.2.5 Effect of Ethanol

Alcohol is another inherent factor in wine that can influence the perception of mouthfeel and of astringency in particular. Ethanol content in red wine is about 11% to 15%, and studies have shown that higher concentrations decrease the perception of astringency in model wines and alter the lack of astringency in wines (Peleg and Noble, 1999). Structural changes in tannins in wines with a high concentration of ethanol may be at least partially responsible for the decrease in astringency with increasing ethanol concentration. This may inhibit the development of protein aggregates by reducing the binding of tannins to proteins as well as the self-association of bound tannins (McRae and Kennedy

2011). Furthermore, higher concentrations of ethanol may increase lubrication of the oral cavity, which decreases the sensation of roughness.

PRP-tannins aggregates were found to cause variations in ethanol solubility in mixtures of grape seed tannins, probably related to variations in tannin structure (Zanchi *et al*., 2009). The resulting astringency can also be affected by changes in tannin solubility in wine. Additionally, increasing the amount of ethanol to increase the viscosity of the solution can reduce the perception of astringency and interactions between proteins and tannins (Demiglio *et al.,* 2008).

### 2.3.2.6 Effect of Temperature

The mouth is a highly vascularized region whose temperature quickly returns to typical values after consumption of hot or cold foods or beverages. Therefore, to study the actual influence of temperature on food perception can be a complex process. Temperature can affect hydrogen bonds and trigger the formation of hydrophobic bonds and, consequently, it is an essential parameter to be consider in protein–phenolic interactions. Moreover, polyphenols bind strongly to proteins at a higher temperature according to a model protein system (Ozdal *et al.,* 2013).

According to Laaksonen *et al*., studies aqueous systems of tannic acid or catechin did not show significant differences in astringency when tested at 7 ºC or 18 ºC (Laaksonen *et al*., 2011).

## 2.4 Sensory perception of astringency and other mouthfeel attributes of wines

A variety of studies explored the sensory mechanisms and properties of foods that determine the perception of astringency and other mouthfeel attributes (Bajec and Pickering 2008). However, sensory evaluation of mouthfeel attributes of wine is not easy due to the complexity of the wine chemical composition and the variety of oral sensations involved (Paissoni *et al*., 2023). In addition, many of these are interrelated influencing the perception of each other.

The sensory properties "in the mouth" of red wines encompass multiple and interactive sensations of acidity, sweetness, bitterness, retronasal perception of aroma (flavour), viscosity, heat and astringency. These sensory properties are often described by experienced wine tasters The importance of wine tasters achieving a common understanding of terms describing the mouthfeel of wine has been demonstrated (Gawel 1997); mouthfeel terms that were not properly defined substantially reduced the communicative value of these descriptions.

Using a focus group, Lee and Lawless (1991) developed terminology to describe the astringent impressions of alum, gallic acid and tartaric acid solutions: 'drying', 'wrinkling', 'sour', 'astringent', 'bitter' and 'hard'.

A hierarchically structured vocabulary of mouthfeels elicited by red wines has been produced by Gawel *et al.* represented as a wheel, this structured vocabulary should help tasters in the interpretation and use of terminology related to the sensations 'in the mouth' produced by red wines (Gawel *et al.,* 2000).

In 2001, Gawel *et al*., used a group discussion involving experienced wine tasters and red wine producers to develop a vocabulary for describing the astringent subqualities of red wines. This led to the development of the 'mouthfeel wheel', in which they organized and presented their findings (Gawel *et al*., 2000). Panel leaders generated definitions for these terms, which were refined after additional wine tasting and further agreement among panel members.

Commercial samples were used as physical standards to represent astringent "surface smoothness" impressions. In this study, Gawel *et al*., (2001) demonstrated that wine tasters can be trained to successfully distinguish astringent subqualities among single varietal Australian red wines, although this is a difficult task.

Side tastes such as bitterness, acidity, and sweetness can highly modulate overall astringency (Fleming 2016). The sensitivity of MRs to astringents as well as basic flavours may elucidate the complexity of red wine astringency, which has been described by 33 different subqualities (Gawell 2001). Among these “hard”, “green” and “rich” were associated with bitterness, acidity and high flavour concentration, respectively (King 2003), “harsh”, “abrasive” and “drying” were found to define astringency as a negative sensation, while the sub-qualities “complex” and “mouth” have been associated with a positive impact during tasting (Gawell 2001).

These sub qualities have also been associated with touch patterns when used to describe the astringent tactile sensations in the mouth elicited by red wines (De Miglio, 2008; Rinaldi 2018). Qualitative astringency traits such as “smooth”, “mouth-coat” and “rich” represented taste drivers for Sangiovese wine (Rinaldi *et al.,* 2020)

Likewise, for Tannat (Vidal *et al.,* 2020) and wines from the Cotes du Rhone and Rioja appellations (Saenz *et al* 2013), the “mouth-coat” attribute contributed to the quality of the wine.

Several reviews have been focused in this area during the last few years (Gawel R, 1998; de Freitas V, Mateus N, 2012; Bajec M. Pickering, 2008; Mc Rae M, Kennedy JA, 2011; Ma W *et al*.,2014; Soares *et al*., 2017; Lisy, Duan CQ, 2019).

The evaluation of the impact of the closures on wine can be performed using a twostep approach: a) investigate if a wine presents sensory perceptible differences when bottled using different closures; b) compare the sensory profile of such wines and eventually characterize the perceptible differences found using sensory descriptive analysis.

Different overall discrimination tests can be used to determine whether a perceptible difference exists between two samples. The duo-trio test developed by Peryam and Swartz (1950) presents a lower carryover effect and lower potential fatigue of assessors than other frequently used tests, namely the triangular test.

Classical or generic sensory descriptive analysis is extensively used tool in sensory science, being able to provide a complete or a partial description of the sensory characteristics of a food product or wine (Heymann *et al*., 2014, Lawless and Heymann, 2010). In this methodology, assessors (usually between 8 and 12) are trained to attribute recognition and scaling, they use a common and agreed sensory language, and products are scored on repeated trials to obtain a quantitative description. The high specialization of descriptive panels allows obtaining detailed, reliable and consistent results (Moussaoui & Varela, 2010).

The panel leader is responsible for selecting, training, and monitoring the assessors (ISO 8586:2012). He also selects the sensory tests to be performed, the statistical calculation to be implemented and advises on the procedure for sensory sessions. Candidate assessors must have basic theoretical training on fundamental concepts related to tasting. After the initial theoretical training, different tests must be carried out to make the most suitable selection possible: tests to detect disabilities, tests to determine sensory awareness, tests to discriminate between stimulus intensity levels. To develop the ability to detect, recognize, describe and discriminate sensory stimuli the select panellists follow a training period. When studying mouthfeel attributes of wines, it is important that evaluators undergo training on aspects related to alcohol content and astringency. It is possible to observe differences in alcohol content and astringency between grape and wood tannins found in wines. Consequently, tests of solutions prepared with different types of tannins (grapes and wood) should be proposed.

It is recommended to prepare solutions of the same concentration for anthocyanidic tannins (grape), gallic tannins and ellagic tannins (oak). Astringency perception is basically a dynamic process that changes and evolves continuously, takes many seconds (15s) to fully develop. Sensory studies TDS (Temporal Dominance of Sensations) on wine state that after sweet, sour and bitter flavours, about 35 seconds after expectoration or ingestion, astringency occurs (Pessina *et al*., 2004).

In order to evaluate some matrix effects on astringency, tests should be carried out with mixtures to evaluate the matrix effect, from previously mentioned studies citing as examples a decrease in astringency with an increase in the amount of ethanol, impact on protein-tannin interactions, viscosity, greater viscosity and bitterness with an increase in ethanol , greater perception of astringency with high acidity or high ionic strength, acids and salts can reduce saliva viscosity, among others. therefore, it is important in training to adapt the tasters to the variations of the matrix in the perception of astringency in order to become familiar with these effects.

# *Chapter 3 - MATERIALS AND METHODS*

## 3.1- Sensory Analysis

Sensory evaluation took place at the sensory analysis facilities of the Escola Superior de Biotecnologia (ESB). These comprised a laboratory and a preparation area complying with the requirements of ISO 8589:2007 standard.

The panel leader was responsible for selecting, training and monitoring the assessors (ISO 8586:2012). He also selected the sensory tests to be performed, the statistical calculation to be implemented and advised on the procedure for sensory evaluation.

It is very important to select candidates in relation to the objective of the sensory tests. In the case of sensory analysis of wines, in some cases it is necessary to have “experts” interested in products of wine origin, and who demonstrate experience in sensory evaluation activities. Candidates must have basic theoretical training on fundamental concepts related to tasting. After the initial theoretical training, different tests must be carried out to make the most suitable selection possible. Final selection can only be made after training and completion of planned tasks.

To carry out this study, fourteen tasters were selected, nine from Amorim Cork S.A. and five from ESB-UCP, comprising eight women and six men, all adults under 65 years old. Panellists from ESB had more than 15 years of experience on wine sensory evaluation, therefore, they were familiar with the evaluation of wine sensory attributes and with the sensory techniques and methodologies applied. Panellist from Amorim Cork had different levels of experience and expertise on wine sensory evaluation, they were selected based on their availability and willingness to participate in the study. Twelve sensory evaluation sessions were held, with an approximate duration of 1 ½ hours each, starting at 9 a.m. The first four sessions focused on homogenization and training of all panellists. In the following eight sessions wines bottled with different stoppers were evaluated. All sessions took place as blind tastings. Panellists were instructed on the sensory evaluation protocol for the appropriate evaluation at the beginning of each session. Sandwiches of sliced white bread and cooked ham as well as milk chocolate were provided in between tastings for palate cleaning.

The sessions took place roughly twice a month, according to the availability of participants from both institutions, from March to December 2022 (with interruption between the months of July to October).

### *3.1.1 Sensory Analysis PHASE 1*

Participants’ ability to perform wine sensory evaluation tests was evaluated/confirmed. Screening tests were performed to rule out any candidate with colour vision deficiency, anosmia, or ageusia, and other issues of concern which may skew assessor response (ISO 3972; ISO 5496). These encompassed: colour vision was checked using an Ishihara test, triangular tests were performed to determine sensory awareness and discrimination ability, and rating tests were used discriminate between stimulus intensity of basic tastes and astringent compounds.

Panel homogenization and training exercises were performed, mainly on gustatory attributes, particularly those related to the astringency of red wine.

The familiarization and training began with a theoretical description of human taste, explanation of in-mouth perception and on good practices in sensory evaluation.

Practical exercises on basic in-mouth attributes (sweet, sour, bitter, astringent, and burning sensation) were held. The following practical exercises focused on refining and differentiating proposed mouthfeel descriptors until an appropriate mouthfeel vocabulary was developed.

Participants evaluated flavoured solutions and wines by rating the intensities of the derived descriptors using anchored scales. Panellists were asked to consult the 'mouthfeel wheel' (Figure 3.1) and a list of descriptor definitions as needed during the evaluation. After the assessments, judges openly discussed the meaningfulness of the terms for mouthfeel characterization of red wine, and irrelevant and/or redundant terms were omitted from the sensory evaluation form.

A descriptive sensory evaluation form and a sensory lexicon were developed and approved. Sensory exercises on the use of sensory scales to evaluate the intensity of the selected descriptive attributes were performed.

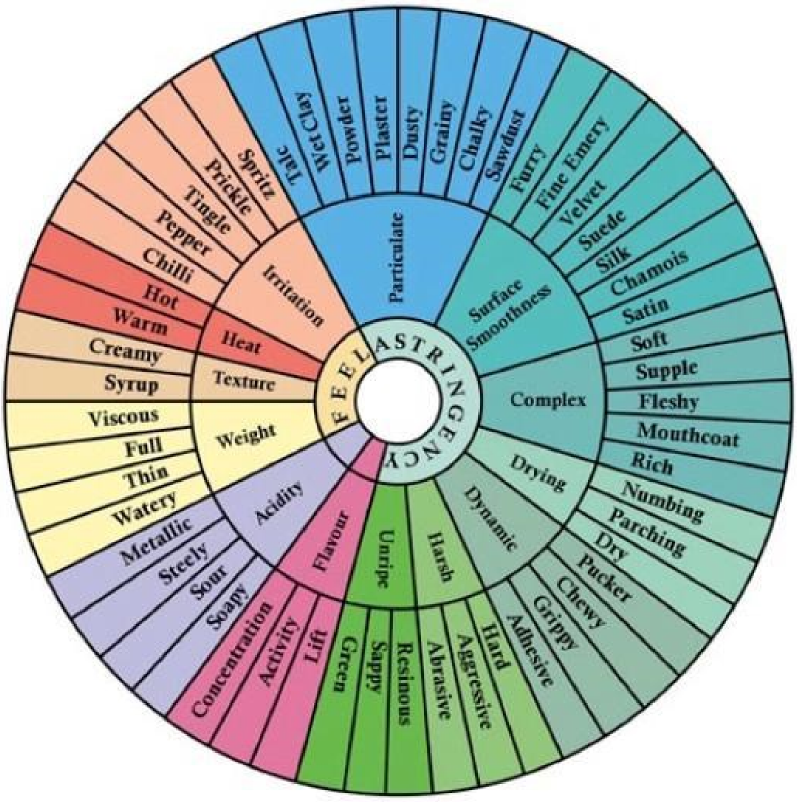


**Figure 3.1** 'Mouthfeel wheel' showing terminology used to describe mouthfeel characteristics of red wine (taken from Gawel *et al*., 2000).

Details regarding the training of panellist (Ishiara tests; Basic tastes; Scales use; Perception tests; Intensity evaluation tests; Sensory perception in the mouth-consensus assessment and Discrimination tests) are shown in ***Appendice I***

### *3.1.2 Sensory analysis PHASE 2*

**Comparative analysis of sensory characteristics of wines (8 Sessions)**.

Wines submitted to sensory analysis were selected by Amorim Cork S.A. and provided before tasting sessions.
For this study eight wines from different countries and regions were used. In every session each wine used was sealed with three different stoppers and the bottling time varied between six and thirty months as indicated in (Table 3.1)

**Table 3.**1 List of wines used, origin and closures used in each session.

| Wines | Country | Region | Bottling time (Months) | Stoppers |
|---|---|---|---|---|
| *Wine 1* | Spain | Ribera del Duero | 30 | **NT**-G1<br>**TD1+1**-G2<br>**MA_D**-G3 |
| *Wine 2* | Portugal | Douro | 28 | **MA_N**-G1<br>**MA_D**-G2<br>**NT**-G3 |
| *Wine 3* | Italy | Trentino | 16 | **MA_D**-G1<br>**MA_N**-G2<br>**VL**-G3 |
| *Wine 4* | Portugal | Douro | 15 | **TD1+1**-G1<br>**MA_N**-G2<br>**MA_D**-G3 |
| *Wine 5* | Italy | Piedmont | 30 | **MA_D**-G1<br>**NT**-G2<br>**MA _N**-G3 |
| *Wine 6* | Italy | Piedmont | 6 | **NT_BW**-G1<br>**NT**-G2<br>**MA_D**-G3 |
| *Wine 7* | France | Bordeaux | 6 | **TD1+1**-G1<br>**MA_D**-G2<br>**TD2+2**-G3 |
| *Wine 8* | Portugal | Península de Setúbal | 20 | **MA_D**-G1<br>**MA_X**-G2<br>**NT**-G3 |

**NT**-Natural Cork **MA**-Micro agglomerated cork **VL**-Vinilok **TD**-Tecnichal Discs

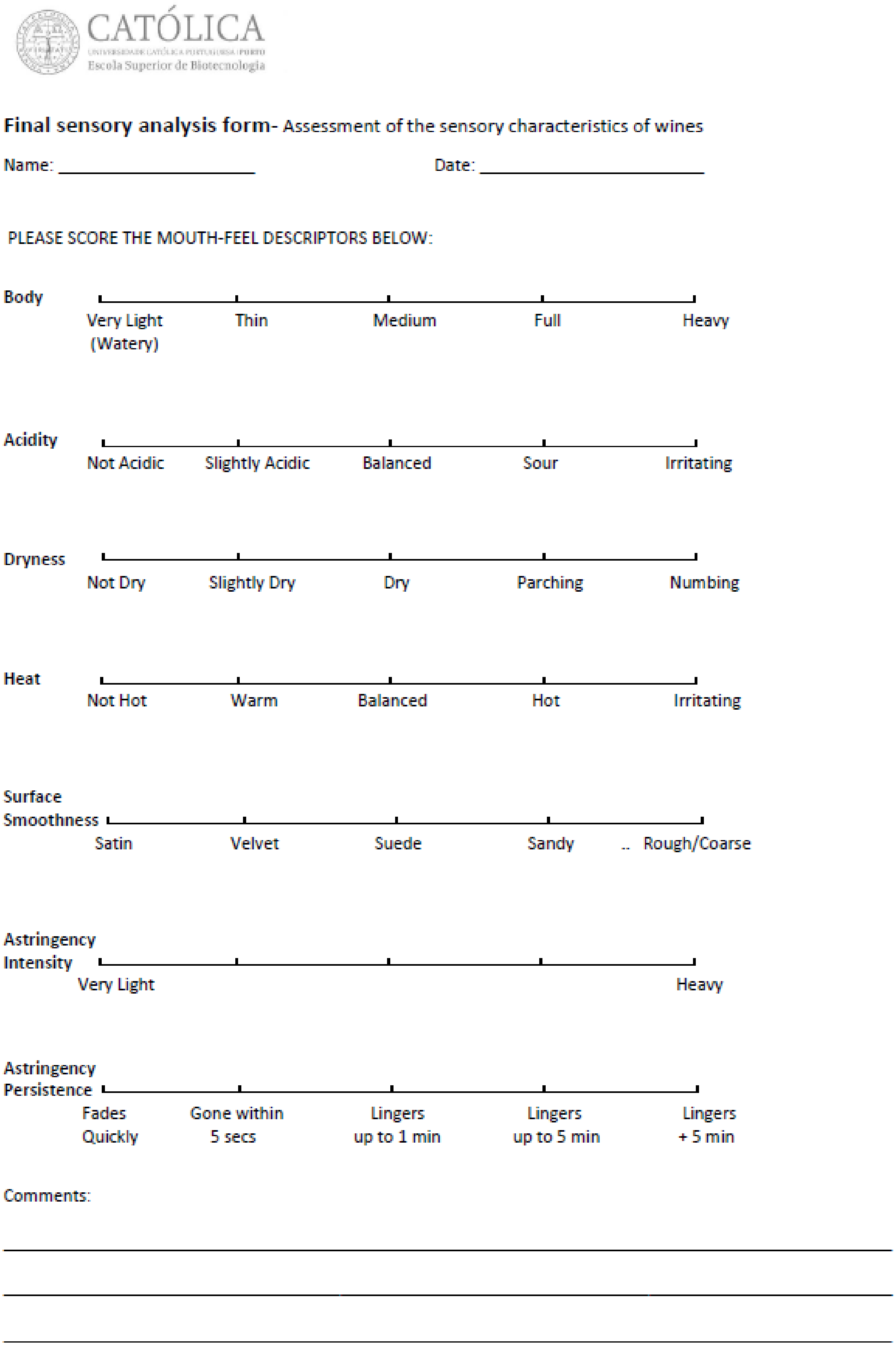
The eight sessions, following the training sessions wine evaluation tests were carried out using Duo-Trio tests (ISO 10399:2017).

The wines were bottled in appropriate bottles (0,75L) and were opened approximately 30 minutes prior to each session. Panellists were served approximately 35 ml of wine per sample using wine tasting glasses.

Sensorial tests were carried out in blind tasting mode (without information on the type of wine and closure used) and the samples coded using three-digit numbers. Assessors were required to taste the wines in the order that they were presented, and they were directed to briefly swirl the sample in the mouth prior to expectoration. Tasters were instructed to clean the palate between tasting sessions using odor/flavour-free water and ham white bread sandwiches. Between tests, the tasters were able to eat milk chocolate to minimize the stress caused by the test.

| **Six duo-trio tests (ISO 10399:2017) were performed sequentially** | |
|---|---|
| Wine stopper A vs. Wine stopper B | Wine stopper B vs. Wine stopper A |
| Wine stopper A vs. Wine stopper C | Wine stopper C vs. Wine stopper A |
| Wine stopper B vs. Wine stopper C | Wine stopper C vs. Wine stopper B |

In the second part of the session, a descriptive sensory evaluation of each wine was carried out using the tasting sheet developed and adapted in the initial training sessions (Figure 3.1). Definitions for the terms used are listed in (Table 3.2).



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**Final sensory analysis form**- Assessment of the sensory characteristics of wines

Name: ____________________ Date: ______________________

PLEASE SCORE THE MOUTH-FEEL DESCRIPTORS BELOW:

| | | | | | |
|---|---|---|---|---|---|
| **Body** | Very Light (Watery) | Thin | Medium | Full | Heavy |
| **Acidity** | Not Acidic | Slightly Acidic | Balanced | Sour | Irritating |
| **Dryness** | Not Dry | Slightly Dry | Dry | Parching | Numbing |
| **Heat** | Not Hot | Warm | Balanced | Hot | Irritating |
| **Surface Smoothness** | Satin | Velvet | Suede | Sandy | .. Rough/Coarse |
| **Astringency Intensity** | Very Light | | | | Heavy |
| **Astringency Persistence** | Fades Quickly | Gone within 5 secs | Lingers up to 1 min | Lingers up to 5 min | Lingers + 5 min |

Comments:

_______________________________________________

_______________________________________________

_______________________________________________

**Figure 3.1** Final sensory evaluation score sheet- Assessment of the sensory characteristics of wines. Adapted (King *et al.*, 2003 and Rankine, 1990).

**Table 3.2** Definitions of mouthfeel descriptors used in sensory evaluation of red wine (adapted from Gawel *et al*., 2000)

| ***Mouthfeel Descriptor*** | ***Definition*** |
|---|---|
| Body | Feelings of weight or density describing how a fluid moves around in the mouth, where low-bodied or watery wines spread faster in the mouth and full-bodied wines flow with more resistance |
| Acidity | Metallic or irritating sensation felt on the sides of the tongue |
| Dryness | Feeling of lack of lubrication in the mouth; pulling away or tightening from internal mouth surfaces |
| Heat | Warming sensation around mouth surfaces |
| Surface Smoothness | Textural sensations felt in the mouth, where a less smooth wine imparts a sensation of coarseness or graininess that is reminiscent of particulates, and a very smooth wine is characterised by a fine texture and a lack of particulates. |

**Table 3.3.** Definitions for terms describing astringent impressions (Adapted from Lawless *et al.,* 1994).

| ***Astringency Term*** | ***Definition*** |
|---|---|
| Drying | Lack of lubrication or moistness resulting in friction between oral surfaces |
| Puckery | Drawing or tightening sensation felt in the mouth, lips and/or cheeks |
| Roughing | Un-smooth texture in the oral cavity marked by inequalities, ridges and/or projections felt when oral surfaces come in contact with one another |
| Astringent | Being the combination of drying, puckery and roughing |

The mouthfeel descriptors were rated on 10-cm continuous scales labelled at five evenly spaced points meant to describe the intensity of the sensation. Panellists were asked to mark on the scale the intensity of each mouthfeel parameter for each sample. Distances from the origin for the attribute scale labels are shown in (Table 3.4).

**Table 3.4** Distance from the origin for attribute scale labels

| | ***Attribute Scale Labels*** | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Distance from the Origin(cm)*** | *Body* | *Acidity* | *Dryness* | *Heat* | *Surface Smoothehness* | *Astrigency Intensity* | *Astrigency Persistence* |
| *0* | Very Ligth | Not acidic | Not dry | Not hot | Satin | Very light | Fades quickly |
| *2.5* | Thin | Slightly acidic | Slightly dry | Warm | Velvet | - | Gone within 5 secs |
| *5* | Medium | Balanced | Dry | Balanced | Suede | - | Lingers up to 1 min |
| *7.5* | Full | Sour | Parching | Hot | Sandy | - | Lingers up to 1 min |
| *10* | Heavy | Irritating | Numbing | Irritating | Rought/Coarse | Heavy | Lingers + 5 min |

After sensorial test sessions the results were compiled and treated statistically.

For the duo-trio test the number of correct identifications were computed, and the number of correct responses obtained compared with the minimum number of correct responses needed to conclude that a perceptible difference exists based a duo-trio test according to the ISO 10399:2017 standard for a probability $\alpha$=0.05 (Figure 3.2).

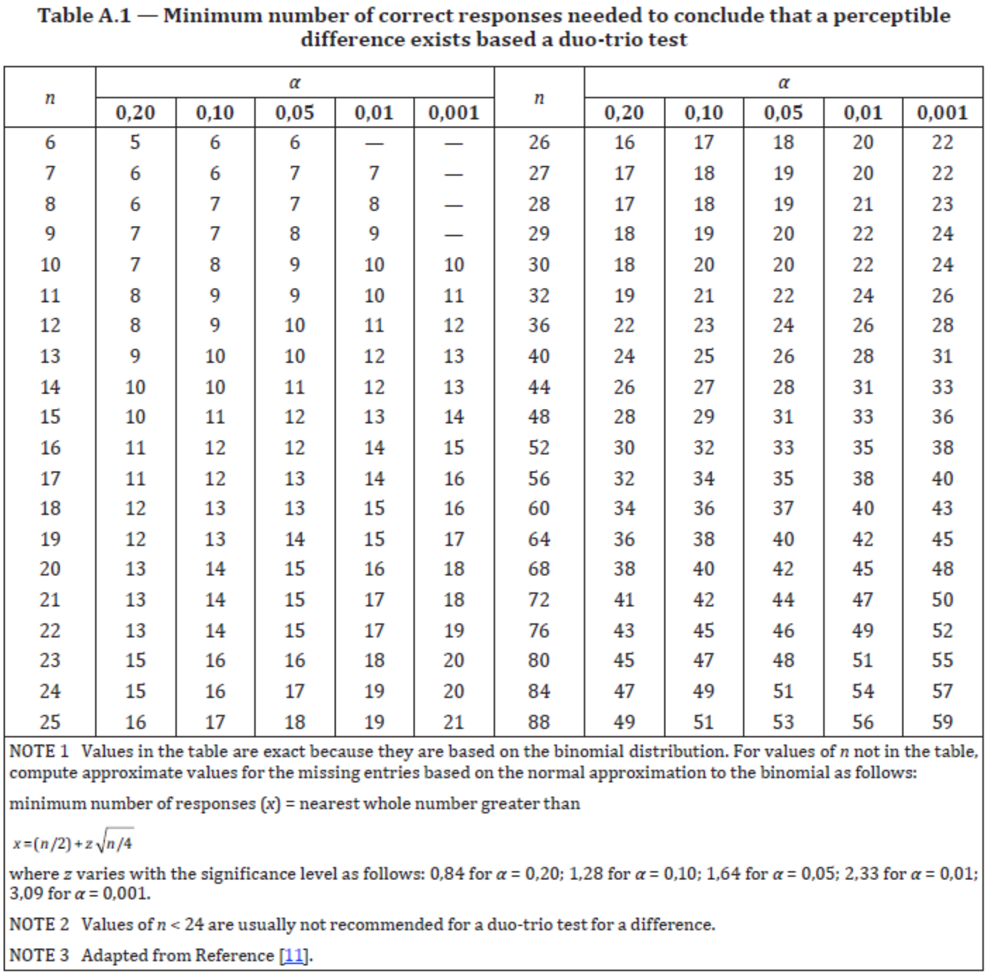


**Table A.1 — Minimum number of correct responses needed to conclude that a perceptible difference exists based a duo-trio test**

| $n$ | $\alpha$ | | | | | $n$ | $\alpha$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0,20 | 0,10 | 0,05 | 0,01 | 0,001 | | 0,20 | 0,10 | 0,05 | 0,01 | 0,001 |
| 6 | 5 | 6 | 6 | — | — | 26 | 16 | 17 | 18 | 20 | 22 |
| 7 | 6 | 6 | 7 | 7 | — | 27 | 17 | 18 | 19 | 20 | 22 |
| 8 | 6 | 7 | 7 | 8 | — | 28 | 17 | 18 | 19 | 21 | 23 |
| 9 | 7 | 7 | 8 | 9 | — | 29 | 18 | 19 | 20 | 22 | 24 |
| 10 | 7 | 8 | 9 | 10 | 10 | 30 | 18 | 20 | 20 | 22 | 24 |
| 11 | 8 | 9 | 9 | 10 | 11 | 32 | 19 | 21 | 22 | 24 | 26 |
| 12 | 8 | 9 | 10 | 11 | 12 | 36 | 22 | 23 | 24 | 26 | 28 |
| 13 | 9 | 10 | 10 | 12 | 13 | 40 | 24 | 25 | 26 | 28 | 31 |
| 14 | 10 | 10 | 11 | 12 | 13 | 44 | 26 | 27 | 28 | 31 | 33 |
| 15 | 10 | 11 | 12 | 13 | 14 | 48 | 28 | 29 | 31 | 33 | 36 |
| 16 | 11 | 12 | 12 | 14 | 15 | 52 | 30 | 32 | 33 | 35 | 38 |
| 17 | 11 | 12 | 13 | 14 | 16 | 56 | 32 | 34 | 35 | 38 | 40 |
| 18 | 12 | 13 | 13 | 15 | 16 | 60 | 34 | 36 | 37 | 40 | 43 |
| 19 | 12 | 13 | 14 | 15 | 17 | 64 | 36 | 38 | 40 | 42 | 45 |
| 20 | 13 | 14 | 15 | 16 | 18 | 68 | 38 | 40 | 42 | 45 | 48 |
| 21 | 13 | 14 | 15 | 17 | 18 | 72 | 41 | 42 | 44 | 47 | 50 |
| 22 | 13 | 14 | 15 | 17 | 19 | 76 | 43 | 45 | 46 | 49 | 52 |
| 23 | 15 | 16 | 16 | 18 | 20 | 80 | 45 | 47 | 48 | 51 | 55 |
| 24 | 15 | 16 | 17 | 19 | 20 | 84 | 47 | 49 | 51 | 54 | 57 |
| 25 | 16 | 17 | 18 | 19 | 21 | 88 | 49 | 51 | 53 | 56 | 59 |

NOTE 1 Values in the table are exact because they are based on the binomial distribution. For values of $n$ not in the table, compute approximate values for the missing entries based on the normal approximation to the binomial as follows:

minimum number of responses ($x$) = nearest whole number greater than

$x = (n/2) + z\sqrt{n/4}$

where $z$ varies with the significance level as follows: 0,84 for $\alpha = 0,20$; 1,28 for $\alpha = 0,10$; 1,64 for $\alpha = 0,05$; 2,33 for $\alpha = 0,01$; 3,09 for $\alpha = 0,001$.

NOTE 2 Values of $n < 24$ are usually not recommended for a duo-trio test for a difference.

NOTE 3 Adapted from Reference [11].

**Figure 3.2**-Table A1 from ISO 10399:2017

The results of the descriptive sensory evaluation of each wine were computed by calculating the numerical classifications corresponding to the attribute intensities for each taster and sample marked on the score sheet.

Analysis of outliers was performed using the Grubbs test ($p<0.05$) and the Z score test ($Z>2$). Outliers were replaced by the mean of the ratings for the sample and parameter. Mean, standard deviation, median, first and third quartiles were calculated for each attribute. Data normality was verified and the results for each sealed wine and for each attribute compared using a two-way ANOVA (data normality accepted) or non-parametric Kruskal-Wallis test (data normality rejected). For the attributes of the wines in which significant differences were found ($p<0.05$), post-hoc tests by Tukey, Fisher and Bonferroni (ANOVA) and Steel-Dwass-Critchlow-Fligner (Kruskal-Wallis) were performed.

Additionally, for sealed wines in which sensory perceptible differences were found using duo-trio test, but no significant differences in intensities of attributes were identified, a multidimensional analysis of results was carried out using a Generalized Procrustes Analysis (GPA). GPA analysis is used to obtain a consensual representation of the wines sensory profile while minimizes the effects of differences in the use of scales by tasters.

## 3.2 Physical-chemical analysis

### 3.2.1 Sulfur dioxide ($SO_2$) determination

Free sulfur dioxide (F $SO_2$) and total sulfur dioxide (T $SO_2$) were analysed at the Comissão de Viticultura da Região dos Vinhos Verdes (CVRVV) using Potentiometric Titration-MI 241, ed.04 (without deduction of interferents). This analyzes were provided by Amorim Cork SA.

### 3.2.2 Chromatic characteristics of wine

The "chromatic characteristics" of a wine are its luminosity and chromaticity. Luminosity depends on transmittance and varies inversely with the intensity of colour of the wine. Chromaticity depends on dominant wavelength (distinguishing the shade) and purity. Conventionally, the chromatic characteristics of red and rosé wines are described by the intensity of colour and shade, in keeping with the procedure adopted as the working method.

Principle of the method OIV-AS2-07B:2009

A spectrophotometric method whereby chromatic characteristics is expressed conventionally, as Intensity of colour (CI) and the shade or tone of colour (CT).

- (CI) is given by the sum of absorbances (or optical densities) using a 1 cm optical path and radiations of wavelengths 420, 520 and 620 nm.

- (TC) is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm.

The optical density at 280 nm is a measure of the absorbance of light by a sample at that wavelength.

This measurement is commonly used in winemaking to determine the total polyphenol index (TPI) of a sample. The TPI is a measure of the total amount of phenolic compounds in a sample, including flavonoids, phenolic acids, and tannins and can be used as an indicator of the antioxidant capacity of a sample. Analyzes provided by Amorim Cork SA carried out by CVRVV.

### 3.2.3 Volatile Organic Compounds (VOCs) analysis

The bottle closures can impact the volatile composition of wines due to different permeability to oxygen and the absorption and/or migration of (VOCs) from and into the wine.

Acquisition Method to characterize the VOCs: Headspace solid-phase microextraction with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). Analyzes provided by Amorim Cork SA carried out by Faculty of Pharmacy, Porto University (FFPU).

**Table3.5** – Quantified volatile compounds, descriptor, and olfactory perception threshold (OPT)

| **COMPOUND** | ***DESCRIPTOR*** | ***OPT*** |
|---|---|---|
| ***Aldehydes*** | | |
| Benzaldehyde | Almond, bitter | 1-2 mg/L |
| Octanal | Citrus | 2.5 µg/L |
| Phenylacetaldehyde | Bitter, green, honey | 1 µg/L |
| Nonanal | Green, pungent | 1 µg/L |
| Decanal | Grassy, orange skin | 1 mg/L |
| β-cyclocitral | Floral, sweet | 5 ng/L |
| ***Esters*** | | |
| Diethyl succinate | Fruity | 200 mg/L |
| ***Ketones*** | | |
| *2-heptanone* | Sweet,fruity,woody | - |
| *2-nonanone* | Sweet, herbal | - |
| *2-undecanone* | Green, floral | - |
| *β-damascenone* | Apple, honey, smoky | 0.05 µg/L |
| ***Furans*** | | |
| *Furfural* | Almond, bread | 14.1 mg/L |
| *5-methyl-2-furfural* | Almond, burnt sugar | 16 mg/L |

The data processing of physical and chemical results was carried out using Kruskal-Walli’s test (multiple comparisons by comparing the mean rank of each column with the mean rank of every other column; correction for multiple comparison with the Dunn's test) for * p-value < 0.05.

GraphPad Prism (version 10.0.0, GraphPad Software, LLC) was used.

# *Chapter 4 - RESULTS AND DISCUSSION*

This chapter presents the results obtained for each of the eight wines analyzed, including the results of sensory analysis, physical-chemical parameters, and analysis of volatile compounds.

## 4.1 Session 1 Wine 1

Stoppers: NT (G1), TD1+1 (G2), MA _D (G3)
14 Panellists

### *4.1.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis*

**Table 4.1** Duo-trio test results

| **Comparison** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Correct answers** | 8 | 11 | 7 | 9 | 7 | 6 |
| **Conclusion (α =0.05)** | Perceptible differences | | Absence of significant differences | | Absence of significant differences | |

NT (G1), TD1+1 (G2), MA _D (G3)

**Table 4.2** Results of the descriptive analysis test

| **Attribute** | | **Mean** | **Standard deviation** | **1st Quartile** | **Median** | **3rd Quartile** |
|---|---|---|---|---|---|---|
| ***Body*** | G3 | 5.4 | 1.4 | 4.5 | 5.2 | 6.5 |
| | G2 | 6.0 | 1.6 | 5.3 | 6.3 | 6.9 |
| | G1 | 5.9 | 1.0 | 5.2 | 5.9 | 6.5 |
| ***Acidity*** | G3 | 4.8 | 1.4 | 3.2 | 5.2 | 6.0 |
| | G2 | 4.8 | 1.2 | 4.3 | 4.8 | 5.9 |
| | G1 | 5.7 | 1.3 | 4.7 | 5.8 | 6.0 |
| ***Dryness*** | G3 | 5.8 | 1.4 | 5.0 | 5.9 | 6.8 |
| | G2 | 6.8 | 2.1 | 5.7 | 6.9 | 8.1 |
| | G1 | 5.4 | 1.8 | 4.5 | 5.6 | 6.5 |
| ***Heat*** | G3 | 6.0 | 1.5 | 5.0 | 6.0 | 6.8 |
| | G2 | 5.9 | 1.7 | 5.0 | 6.1 | 6.6 |
| | G1 | 5.0 | 1.6 | 4.0 | 5.2 | 5.9 |
| ***Surface smoothness*** | G3 | 5.5 | 1.3 | 4.6 | 5.3 | 6.5 |
| | G2 | 6.1 | 1.2 | 5.8 | 6.1 | 6.5 |
| | G1 | 5.5 | 1.5 | 4.4 | 5.8 | 6.9 |
| ***Astringency*** | G3 | 6.2 | 1.6 | 5.1 | 5.6 | 7.4 |
| | G2 | 6.7 | 1.3 | 6.0 | 6.7 | 7.7 |
| | G1 | 6.1 | 1.2 | 5.3 | 6.2 | 6.8 |

NT (G1). TD1+1 (G2). MA _D (G3)

**Table 4.3** -Statistical comparison of the intensities of wine attributes

| Attribute | | Statistical comparison | Attribute | | Statistical comparison |
|---|---|---|---|---|---|
| ***Body*** | G3 | ANOVA p=0.65<br>absence of significant differences | ***Heat*** | G3 | ANOVA p=0.03<br>* G3 a. G2 ab. G1 b |
| | G2 | | | G2 | |
| | G1 | | | G1 | |
| ***Acidity*** | G3 | Kruskal-Wallis p=0.26<br>absence of significant differences | ***Surface smoothness*** | G3 | ANOVA p=0.18<br>absence of significant differences |
| | G2 | | | G2 | |
| | G1 | | | G1 | |
| ***Dryness*** | G3 | ANOVA p=0.04<br>* G3 ab. G2 a. G1 b | ***Astringency*** | G3 | ANOVA p=0.39<br>absence of significant differences |
| | G2 | | | G2 | |
| | G1 | | | G1 | |

*different letters (a.b) indicate statistically significant differences ($p \leq 0.05$)

NT (G1). TD1+1 (G2). MA _D (G3)

Sensory perceptible differences were found between wine 1 sealed with stoppers **NT** and **TD1+1**. The evaluation of the sensory profile indicated the presence of perceptible differences in the intensity of attributes dryness between **TD1+1** and **NT** wines and heat between **MA_D** wines and **NT**.

### *4.1.2 Results from physical-chemical analysis*

**Table 4.4**- Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **NT** | 24 | 62 | 57.0 | 3.6 | 4.8 | 1.4 | 9.8 | 0.7 |
| **TD1+1** | 23 | 62 | 58.0 | 3.6 | 4.8 | 1.4 | 9.8 | 0.7 |
| **MA_D** | 26 | 66 | 57.7 | 3.4 | 4.7 | 1.4 | 9.5 | 0.7 |

No significant differences were found among the wine 1 sealed with **NT**. **TD1+1**. and **MA_D** stoppers.

### *4.1.3 Results from volatile compounds analysis*

**Table 4.5**- Compounds showing tendencies between wine1. sealed with different stoppers.

| Compound | Comparison | % Variation (*p*-value) |
|---|---|---|
| Decanal | This compound showed a tendency (not significant) for higher levels in wines sealed with MA_D compared with NT and TD1+1 | |

Statistical significance assessed using Kruskal-Wallis ANOVA.

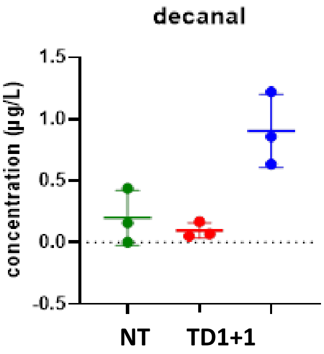


**Figure 4.1**- Boxplots of compounds showing tendencies between (wines1). Statistical significance assessed using Kruskal-Wallis ANOVA.

No significant differences were found among (wines1) sealed with **NT**. **TD1+1**. and **MA_D** stoppers. However. decanal showed a tendency for higher levels in wines sealed with **MA_D** compared with wines sealed with natural cork stopper **NT** and **TD 1+1.**

## 4.2 Session 2 Wine2

Stoppers: MA _N (G1). MA_ D (G2). NT (G3)

10 Wine Tasters

### 4.2.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.6** - Duo-trio test results

| **Comparison** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Correct answers** | 5 | 4 | 7 | 8 | 4 | 6 |
| **Conclusion (α =0.05)** | Absence of significant differences | | Perceptible sensory differences | | Absence of significant differences | |

MA _N (G1). MA_ D (G2). NT (G3)

**Table 4.7** Results of the descriptive analysis test

| **Attribute** | | **Mean** | **Standard deviation** | **1st Quartile** | **Median** | **3rd Quartile** |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 6.3 | 1.2 | 5.4 | 6.5 | 7.3 |
| | G2 | 6.3 | 1.0 | 5.5 | 6.3 | 7.1 |
| | G3 | 6.4 | 0.8 | 5.9 | 6.3 | 7.0 |
| ***Acidity*** | G1 | 5.3 | 1.9 | 3.5 | 5.8 | 6.6 |
| | G2 | 5.3 | 1.3 | 4.9 | 5.5 | 5.9 |
| | G3 | 5.7 | 1.4 | 5.1 | 5.7 | 6.3 |
| ***Dryness*** | G1 | 5.3 | 1.1 | 5.1 | 5.2 | 5.8 |
| | G2 | 6.0 | 0.8 | 5.4 | 6.0 | 6.6 |
| | G3 | 6.2 | 1.0 | 5.9 | 6.2 | 6.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Heat*** | G1 | 5.8 | 1.3 | 5.3 | 5.9 | 6.4 |
| | G2 | 5.8 | 1.0 | 5.3 | 6.0 | 6.6 |
| | G3 | 5.9 | 1.1 | 5.2 | 6.0 | 6.4 |
| ***Surface smoothness*** | G1 | 5.4 | 0.8 | 5.1 | 5.4 | 6.0 |
| | G2 | 6.2 | 0.2 | 6.0 | 6.2 | 6.2 |
| | G3 | 5.8 | 0.7 | 5.4 | 5.8 | 6.4 |
| ***Astringency - Intensity*** | G1 | 5.9 | 1.3 | 5.0 | 5.9 | 6.4 |
| | G2 | 6.1 | 0.8 | 5.4 | 6.3 | 6.7 |
| | G3 | 6.0 | 1.0 | 5.3 | 6.2 | 6.8 |
| ***Astringency - Persistance*** | G1 | 5.8 | 0.9 | 5.3 | 5.8 | 6.4 |
| | G2 | 6.4 | 0.4 | 6.2 | 6.4 | 6.8 |
| | G3 | 6.2 | 1.3 | 5.2 | 6.5 | 7.0 |

**Table 4.8** -Statistical comparison of the intensities of wine attributes

| Attribute | | Statistical comparison | Attribute | | Statistical comparison |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.78<br>absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.04<br>* G3 ab. G2 a. G1 b |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | Kruskal-Wallis p=0.82<br>absence of significant differences | ***Astringency Intensity*** | G1 | ANOVA p=0.87<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.04<br>* G3 a. G2 ab. G1 b | ***Astringency Persistance*** | G1 | ANOVA p=0.23<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Heat*** | G1 | ANOVA p=0.92<br>absence of significant differences | | | |
| | G2 | | | | |
| | G3 | | | | |

*different letters (a.b) indicate statistically significant differences (p≤0.05)
MA _N (G1). MA_ D (G2). NT (G3)

Sensory perceptible differences were found between wines sealed with **MA_D** stoppers and **NT** stoppers. The evaluation of the sensorial profile of these wines indicated the presence of significant differences in the intensities of the attributes **Dryness** for wines **NT** and **MA_N** and **Roughness** between wines **MA_D** stopper and **MA_N**.

### 4.2.2 Results from physical-chemical analysis

**Table 4.9**-Quantification of sulfur dioxide and colour parameters. Mean values.

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **MA _N** | 19.7 | 56.0 | | 3.1 | | | | |
| **MA_ D** | 18.7 | 51.7 | | 3.1 | | | | |
| **NT** | 19.7 | 61.3 | | 3.2 | | | | |

**Table 4.10**-Parameters showing significant differences between wines.

| Parameter | Comparison | % Variation (*p*-value) |
|---|---|---|
| $TSO_2$ | NT *vs.* MA_D | 18.7 ± 2.5 (*) |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

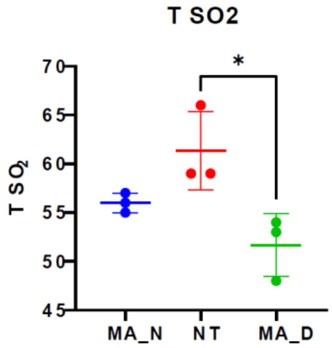


**Figure 4.2**- Boxplots of compounds showing significant differences between wines1. Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

Total sulfur dioxide was found in significant higher levels in wines sealed with **NT** compared with **MA_D**. No other significant differences were found among the wines sealed with the different stoppers.

### 4.2.3 Results from volatile compounds analysis

No significant differences were found among the wines sealed with natural cork **NT**. Microagglomerated **MA_N** and **MA_D**.

## 4.3 Session 3 Wine 3

Stoppers: MA_D (G1). MA_N (G2). VL (G3)

11 Wine Tasters

### 4.3.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.11**- Duo-trio test results

| **Comparison** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Correct answers** | 8 | 5 | 10 | 6 | 5 | 6 |
| **Conclusion ($\alpha$ =0.05)** | Perceptible sensory differences | | Perceptible sensory differences | | Absence of significant differences | |

MA_D (G1). MA_N (G2). VL (G3)

**Table 4.12**- Results of the descriptive analysis test

| Attribute | | Mean | Standard deviation | 1st Quartile | Median | 3rd Quartile |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 6.2 | 1.0 | 5.6 | 6.4 | 6.8 |
| | G2 | 6.0 | 0.8 | 5.5 | 6.2 | 6.5 |
| | G3 | 5.8 | 1.1 | 4.8 | 6.0 | 6.7 |
| ***Acidity*** | G1 | 5.1 | 1.6 | 3.8 | 5.4 | 6.5 |
| | G2 | 4.8 | 1.6 | 3.9 | 5.3 | 5.9 |
| | G3 | 5.5 | 1.8 | 5.4 | 6.3 | 6.6 |
| ***Dryness*** | G1 | 5.3 | 1.1 | 4.4 | 5.2 | 6.2 |
| | G2 | 5.3 | 1.3 | 4.2 | 5.3 | 6.2 |
| | G3 | 5.2 | 1.7 | 3.8 | 6.1 | 6.4 |
| ***Heat*** | G1 | 5.7 | 1.2 | 5.1 | 5.9 | 6.5 |
| | G2 | 5.8 | 1.0 | 5.1 | 5.9 | 6.4 |
| | G3 | 5.7 | 1.2 | 4.6 | 6.1 | 6.4 |
| ***Surface smoothness*** | G1 | 5.5 | 0.9 | 4.8 | 5.5 | 6.3 |
| | G2 | 5.4 | 0.9 | 4.7 | 5.4 | 5.9 |
| | G3 | 5.2 | 1.4 | 4.2 | 5.1 | 6.2 |
| ***Astringency - Intensity*** | G1 | 5.3 | 1.7 | 4.5 | 5.0 | 6.2 |
| | G2 | 5.7 | 1.9 | 4.7 | 5.7 | 7.0 |
| | G3 | 6.3 | 1.6 | 5.2 | 6.4 | 7.5 |
| ***Astringency - Persistance*** | G1 | 6.2 | 1.1 | 5.6 | 6.2 | 7.1 |
| | G2 | 6.2 | 0.7 | 6.0 | 6.0 | 6.4 |
| | G3 | 5.9 | 1.3 | 5.1 | 6.0 | 7.0 |

MA_D (G1). MA_N (G2). VL (G3)

**Table 4.13** -Statistical comparison of the intensities of wine attributes

| Attribute | | Statistical comparison | Attribute | | Statistical comparison |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.62 absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.61 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | ANOVA p=0.19 absence of significant differences | ***Astringency Intensity*** | G1 | ANOVA p=0.30 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.99 absence of significant differences | ***Astringency Persistance*** | G1 | Kruskal-Wallis p=0.87 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Heat*** | G1 | ANOVA p=0.96 absence of significant differences | | | |
| | G2 | | | | |
| | G3 | | | | |

*different letters (a.b) indicate statistically significant differences ($p \leq 0.05$)

MA_D (G1). MA_N (G2). VL (G3)

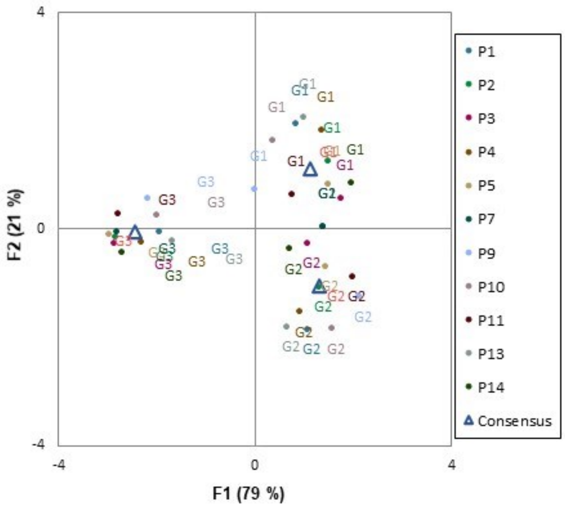


**Figure 4.3** - Representation of wine3 evaluations by different tasters by GPA

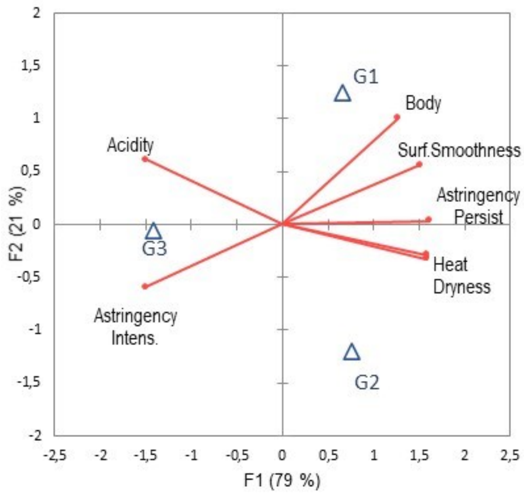


**Figure 4.4** - Representation of evaluated wines3 and attributes evaluated by GPA
MA_D (G1). MA_N (G2). VL (G3)

Sensory perceptible differences were found between the wines sealed with the **MA_N** stopper and the other two wines. This difference was not reflected in significant differences in the intensity of the evaluated sensory attributes; however. the representation of the tasters' results using the GPA method showed that wine sealed with **VL** glass stoppers tended to be more acidic and astringent than wines sealed with microagglomerate stoppers.

### 4.3.2 Results from physical-chemical analysis

**Table 4.14**-Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **MA_D** | 18 | 119 | 58.2 | 3.6 | 3.5 | 0.9 | 8.0 | 1.0 |
| **MA_N** | 17 | 123 | 57.5 | 3.7 | 3.6 | 0.9 | 8.2 | 1.0 |
| **VL** | 16 | 128 | 59.8 | 3.8 | 3.7 | 0.9 | 8.4 | 1.0 |

**Table 4.15**- FQ Parameters showing significant differences between wines sealed with different stoppers

| Parameter | Comparison | % Variation (*p*-value) |
|---|---|---|
| FSO2 | VL *vs.* **MA_D** | -11.1 ± 0.0 (*) |
| OD 420 | **VL** *vs.* MA_D | 4.6 ± 4.3 (*) |
| OD 620 | **VL** *vs.* MA_D | 5.1 ± 9.1 (*) |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - p < 0.05)

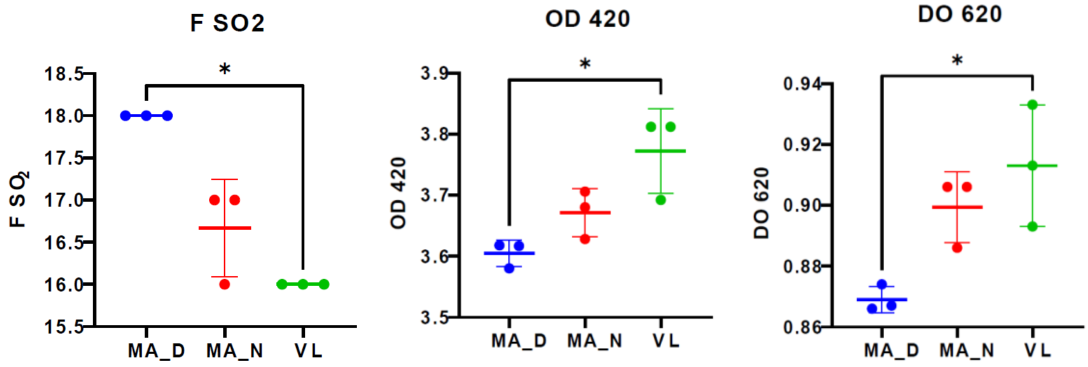


**Figure 4.5** Boxplots of compounds showing significant differences between wines3 Statistical significance assessed using Kruskal-Wallis ANOVA. (* - p < 0.05)

Overall. the red wines3 sealed with **MA_D** showed a significant higher level of free sulfur dioxide compared with **VL**. OD 420 and OD 620 are significant higher in wines sealed with **VL** compared with **MA_D**.

### 4.3.3 Results from volatile compounds analysis

No significant differences were found among the wines sealed with **MA_N**. **MA_D** and **VL** stoppers.

## 4.4 Session 4 Wine 4

### 4.4.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

Stoppers: TD 1+1 (G1). MA_N(G2). MA_D (G3)

11 Wine Tasters

**Table 4.16** Duo-trio test results

| **Comparison** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Hits** | 5 | 6 | 5 | 6 | 6 | 5 |
| **Conclusion ($\alpha$ =0.05)** | Absence of significant differences | | Absence of significant differences | | Absence of significant differences | |

**Table 4.17** Descriptive analysis results

| **Attribute** | | **Mean** | **Standard deviation** | **$1^{st}$ Quartile** | **Median** | **$3^{rd}$ Quartile** |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 6.7 | 1.0 | 5.9 | 6.4 | 6.7 |
| | G2 | 6.6 | 1.4 | 5.7 | 6.2 | 7.5 |
| | G3 | 6.6 | 0.9 | 5.9 | 6.3 | 7.2 |
| ***Acidity*** | G1 | 6.4 | 0.6 | 6.0 | 6.4 | 6.5 |
| | G2 | 6.2 | 0.9 | 5.9 | 6.0 | 7.1 |
| | G3 | 6.3 | 0.9 | 5.9 | 6.1 | 7.1 |
| ***Dryness*** | G1 | 6.5 | 1.0 | 5.8 | 6.6 | 7.3 |
| | G2 | 5.9 | 1.3 | 5.0 | 5.4 | 6.5 |
| | G3 | 5.7 | 1.3 | 5.0 | 5.6 | 6.7 |
| ***Heat*** | G1 | 6.4 | 1.3 | 5.9 | 6.1 | 7.2 |
| | G2 | 6.0 | 0.9 | 5.2 | 6.0 | 6.8 |
| | G3 | 5.5 | 1.4 | 5.1 | 5.5 | 6.3 |
| ***Surface smoothness*** | G1 | 6.7 | 0.8 | 6.3 | 6.6 | 7.2 |
| | G2 | 6.2 | 0.9 | 5.6 | 6.2 | 6.6 |
| | G3 | 6.1 | 1.8 | 5.7 | 6.4 | 6.8 |
| ***Astringency - Intensity*** | G1 | 7.4 | 0.7 | 6.9 | 7.4 | 7.9 |
| | G2 | 7.1 | 1.7 | 5.9 | 7.1 | 8.6 |
| | G3 | 6.9 | 0.8 | 6.8 | 7.0 | 7.3 |
| ***Astringency - Persistance*** | G1 | 7.1 | 1.3 | 6.1 | 7.2 | 8.2 |
| | G2 | 6.3 | 1.2 | 6.0 | 6.1 | 7.0 |
| | G3 | 6.3 | 1.2 | 5.5 | 6.6 | 6.9 |

TD 1+1 (G1). MA_N(G2). MA_D (G3)

**Table 4.18**-Statistical comparison of the intensities of wine attributes.

| **Attribute** | | **Statistical comparison** | **Attribute** | | **Statistical comparison** |
|---|---|---|---|---|---|
| ***Body*** | G1 | Kruskal-Wallis p=0.90 absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.57 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | ANOVA p=0.80 absence of significant differences | ***Astringency Intensity*** | G1 | ANOVA p=0.61 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.28 absence of significant differences | ***Astringency Persistance*** | G1 | ANOVA p=0.22 absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Heat*** | G1 | ANOVA p=0.09 absence of significant differences | | | |
| | G2 | | | | |
| | G3 | | | | |

*different letters (a.b) indicate statistically significant differences (p≤0.05)
TD 1+1 (G1). MA_N(G2). MA_D (G3)

No sensorially perceptible differences were found between the evaluated wines sealed with **MA_N**. **MA_D** and **TD1+1** stoppers.

### 4.4.2 Results from physical-chemical analysis

**Table 4.19**- Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **TD 1+1** | 17 | 88 | 70.7 | 4.3 | 5.2 | 1.4 | 10.8 | 0.8 |
| **MA_N** | 18 | 82 | 70.0 | 4.2 | 5.1 | 1.4 | 10.7 | 0.8 |
| **MA_D** | 16 | 89 | 71.5 | 4.3 | 5.2 | 1.4 | 10.9 | 0.8 |

**Table 4.20**- Parameters showing significant differences between wines. sealed with different stoppers.

| Parameter | Comparison | % Variation (*p*-value) |
|---|---|---|
| OD 420 | MA_D *vs.* MA_N | 2.1 ± 2.1 |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

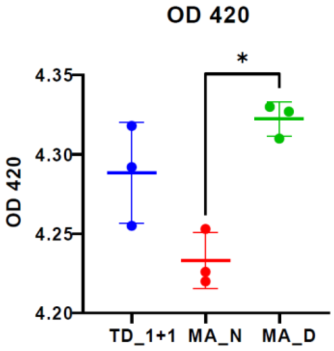


**Figure 4.6**- Boxplots of compounds showing significant differences between wines4 Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

**OD 420** was found in significant higher levels in wines sealed with **MA_D** compared with **MA_N**. No other significant differences were found among the wines sealed with the different stoppers.

### 4.4.3 Results from volatile compounds analysis

**Table 4.21**- Compounds showing significant differences and tendencies between wines. sealed with different stoppers.

| Compound | Comparison | % Variation (*p*-value) |
|---|---|---|
| hexyl acetate | This compound showed a tendency for lower levels in wines sealed with TD1+1 compared with MA_N and MA_D | |
| furfural | MA_N *vs.* MA_D | 7.02 ± 0.89 (*) |

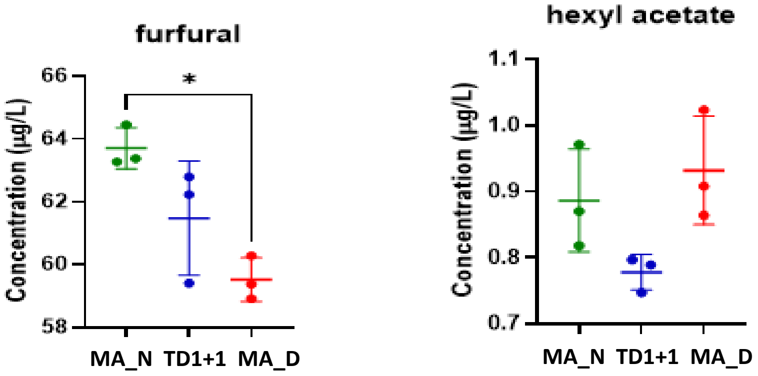


**Figure 4.7**- Boxplots of compounds showing significant differences and tendencies between wines4. Statistical significance assessed using Kruskal-Wallis ANOVA. (* - p < 0.05)

Overall. the red wines sealed with **TD1+1** showed a tendency for lower levels of hexyl acetate compared with **MA_N** and **MA_D**. Furfural was found in significant higher levels in wines sealed with **MA_N** compared with **MA_D**. No other significant differences were found among the wines sealed with the different stoppers.

## 4.5 Session 5 Wine 5

Stoppers: MA_D (G1). NT (G2). MA_N (G3)
8 Wine Tasters

### 4.5.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.22** Duo-trio test results

| **Comparasion** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Correct answers** | 6 | 9 | 4 | 6 | 7 | 4 |
| **Conclusion (α =0.05)** | Perceptible sensory differences | | Absence of significant differences | | Absence of significant differences | |

MA_D (G1). NT (G2). MA_N (G3)

**Table 4.23**–Descriptive analysis results

| Attribute | | Mean | Standard deviation | 1st Quartile | Median | 3rd Quartile |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 5.8 | 0.7 | 5.3 | 5.8 | 6.3 |
| | G2 | 5.4 | 0.7 | 5.2 | 5.4 | 5.8 |
| | G3 | 5.3 | 0.5 | 4.8 | 5.4 | 5.8 |
| ***Acidity*** | G1 | 6.4 | 1.1 | 6.2 | 6.5 | 6.9 |
| | G2 | 6.0 | 2.0 | 4.8 | 6.1 | 7.7 |
| | G3 | 5.6 | 1.2 | 4.7 | 5.7 | 6.1 |
| ***Dryness*** | G1 | 5.9 | 0.9 | 5.4 | 5.7 | 6.1 |
| | G2 | 5.6 | 0.7 | 4.9 | 5.9 | 6.1 |
| | G3 | 6.2 | 0.7 | 5.9 | 6.1 | 6.4 |
| ***Heat*** | G1 | 6.8 | 0.8 | 6.2 | 6.5 | 7.5 |
| | G2 | 6.3 | 0.9 | 5.6 | 6.6 | 6.9 |
| | G3 | 7.1 | 0.9 | 6.5 | 7.4 | 7.7 |
| ***Surface smoothness*** | G1 | 6.2 | 1.6 | 5.6 | 6.2 | 6.9 |
| | G2 | 5.8 | 1.4 | 5.2 | 5.9 | 6.9 |
| | G3 | 6.0 | 1.3 | 4.8 | 6.4 | 6.8 |
| ***Astringency - Intensity*** | G1 | 6.3 | 1.5 | 5.3 | 6.1 | 6.7 |
| | G2 | 6.1 | 1.3 | 5.2 | 6.3 | 6.8 |
| | G3 | 5.8 | 0.9 | 5.5 | 6.1 | 6.4 |
| ***Astringency - Persistance*** | G1 | 6.6 | 1.9 | 5.1 | 6.3 | 7.9 |
| | G2 | 5.9 | 1.0 | 5.4 | 5.8 | 6.2 |
| | G3 | 6.2 | 1.8 | 5.2 | 6.1 | 6.9 |

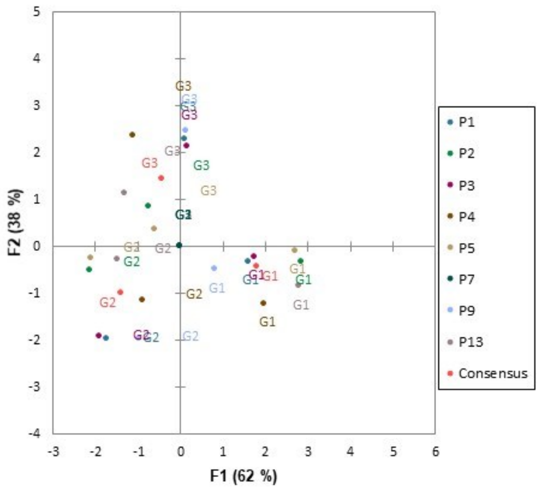
MA_D (G1). NT (G2). MA_N (G3)

**Table 4.24** -Statistical comparison of the intensities of wine attributes

| **Attribute** | | **Statistical comparison** | **Attribute** | | **Statistical comparison** |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.25<br>Absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.77<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | ANOVA p=0.14<br>Absence of significant differences | ***Astringency Intensity*** | G1 | ANOVA p=0.70<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.46<br>Absence of significant differences | ***Astringency Persistance*** | G1 | Kruskal-Wallis p=0.42<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |

| | | | |
|---|---|---|---|
| ***Heat*** | G1<br>G2<br>G3 | ANOVA p = 0.13 absence of significant differences | |

*different letters (a.b) indicate statistically significant differences (p≤0.05)
MA_D (G1). NT (G2). MA_N (G3)



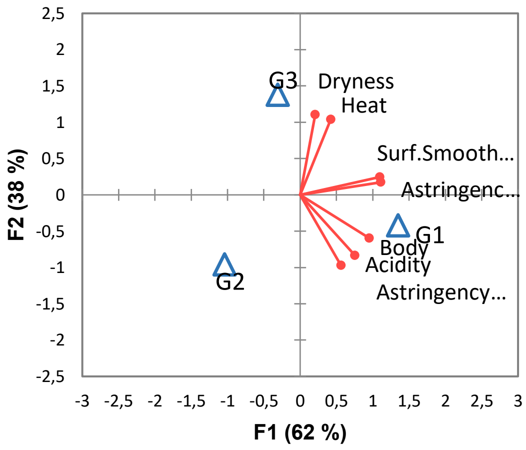


**Figure 4 . 8** - Representation of wine5 evaluations by different tasters by GPA

**Figure 4.9** - Representation of evaluated wines5 and attributes evaluated by GPA MA_D (G1). NT (G2). MA_N (G3)

Sensory perceptible differences were found between wines sealed with a **MA_D** stopper and **NT** stopper. This difference was not reflected in significant differences in the intensity of the evaluated sensory attributes. however. the representation of the results of the tasters' evaluations using the GPA method showed that the wine sealed with **MA_D** stopper presented greater Body and Acidity . with the **NT** stopper lower Roughness. and the wine sealed with the **MA_N** tended to have higher Dryness and Heat and lower Astringency Intensity

### 4.5.2 Results from physical-chemical analysis

**Table 4.25**: Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **MA_D** | 13 | 57 | 42.3 | 2.7 | 3.0 | 0.6 | 6.3 | 0.9 |
| **NT** | 14 | 52 | 43.6 | 2.8 | 3.1 | 0.7 | 6.6 | 0.9 |
| **MA_N** | 13 | 58 | 42.3 | 2.7 | 3.0 | 0.6 | 6.3 | 0.9 |

No significant differences were found among the wine 5 sealed with **NT**. **MA_D**. and **MA_N** stoppers.

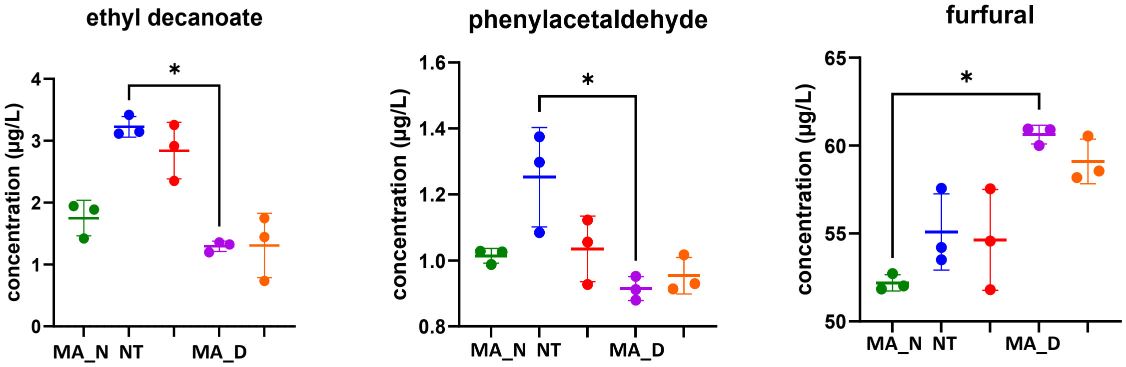
### 4.5.3 Results from volatile compounds analysis

**Table 4.26** Compounds showing significant differences between red wines. sealed with different stoppers.

| Compound | Comparison | % Variation (*p*-value) |
|---|---|---|
| ethyl decanoate | **NT** *vs*. MA_D | 149 ± 4.77 (*) |
| phenylacetaldehyde | **NT** *vs*. MA_D | 36.9 ± 8.26 (*) |
| furfural | MA_N *vs*.**MA_D** | -13.9 ± 0.719 (*) |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - p < 0.05)

**Figure 4.10**- Boxplots of compounds showing significant differences between wines5. Statistical significance assessed using Kruskal-Wallis ANOVA. (* - p < 0.05)



Overall. the wine 5 sealed with **NT** showed significant higher levels of ethyl decanoate and phenylacetaldehyde compared with **MA_D**. Furfural showed significant lower levels in wines sealed with **MA_N** compared with **MA_D**.

## 4.6 Session 6 Wine 6

Stoppers: NT_ BW (G1). NT (G2) MA_D (G3)

11 Wine Tasters

### 4.6.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.27**- Duo-trio test results.

| Comparasion | G1/G2 | G2/G1 | G3/G2 | G2/G3 | G1/G3 | G3/G1 |
|---|---|---|---|---|---|---|
| **Hits** | 7 | 6 | 7 | 8 | 7 | 4 |
| **Conclusion ($\alpha$ =0.05)** | Perceptible sensory differences | | Perceptible sensory differences | | Absence of significant differences | |

NT_ BW (G1). NT (G2) MA_D (G3)

**Table 4.28** Descriptive analysis results

| Attribute | | Mean | Standard deviation | 1st Quartile | Median | 3rd Quartile |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 3.9 | 1.1 | 3.4 | 4.0 | 4.5 |
| | G2 | 3.7 | 1.2 | 2.7 | 4.2 | 4.7 |
| | G3 | 4.1 | 1.4 | 3.0 | 4.0 | 5.0 |
| ***Acidity*** | G1 | 6.5 | 1.8 | 5.5 | 6.0 | 8.4 |
| | G2 | 6.1 | 2.3 | 5.6 | 6.2 | 6.9 |
| | G3 | 6.4 | 2.2 | 5.5 | 5.9 | 6.9 |
| ***Dryness*** | G1 | 6.3 | 2.2 | 4.6 | 6.0 | 8.3 |
| | G2 | 6.3 | 2.4 | 4.7 | 6.0 | 8.4 |
| | G3 | 7.0 | 2.3 | 6.0 | 7.0 | 8.5 |
| ***Heat*** | G1 | 5.6 | 1.8 | 4.3 | 5.5 | 7.4 |
| | G2 | 5.5 | 2.1 | 3.7 | 5.5 | 7.5 |
| | G3 | 5.8 | 1.8 | 5.1 | 6.0 | 6.8 |
| ***Surface smoothness*** | G1 | 5.8 | 2.0 | 4.4 | 5.5 | 7.4 |
| | G2 | 5.8 | 2.1 | 4.4 | 5.9 | 7.0 |
| | G3 | 6.5 | 2.1 | 4.6 | 6.7 | 7.6 |
| ***Astringency - Intensity*** | G1 | 7.5 | 2.1 | 5.7 | 8.3 | 9.3 |
| | G2 | 7.7 | 2.6 | 5.8 | 9.2 | 9.7 |
| | G3 | 8.0 | 2.1 | 6.7 | 9.2 | 9.4 |
| ***Astringency - Persistance*** | G1 | 6.6 | 1.5 | 6.1 | 7.2 | 7.5 |
| | G2 | 7.2 | 1.2 | 6.5 | 7.3 | 8.2 |
| | G3 | 7.3 | 1.4 | 6.4 | 7.3 | 7.9 |

NT_ BW (G1). NT (G2) MA_D (G3)

**Table 4.29** -Statistical comparison of the intensities of wine attributes.

| Attribute | | Statistical comparison | Attribute | | Statistical comparison |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.78<br>absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.69<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | ANOVA p=0.92<br>absence of significant differences | ***Astringency Intensity*** | G1 | Kruskal-Wallis p=0.68<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.87<br>absence of significant differences | ***Astringency Persistance*** | G1 | ANOVA p=0.48<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Heat*** | G1 | ANOVA p=0.94<br>absence of significant differences | | | |
| | G2 | | | | |
| | G3 | | | | |

*different letters (a.b) indicate statistically significant differences (p≤0.05)
NT_ BW (G1). NT (G2) MA_D (G3)

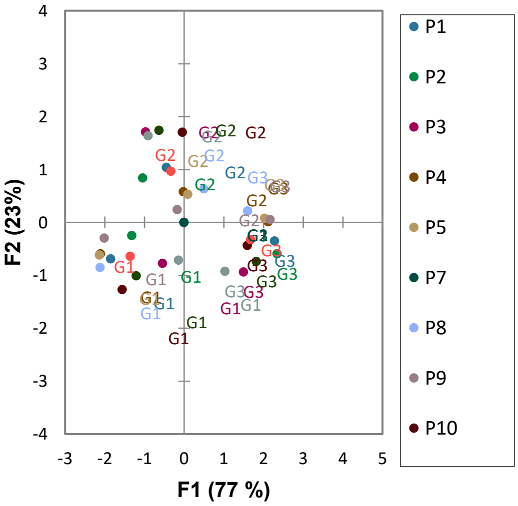


**Figure 4.11** - Representation of wine evaluations by different tasters by GPA

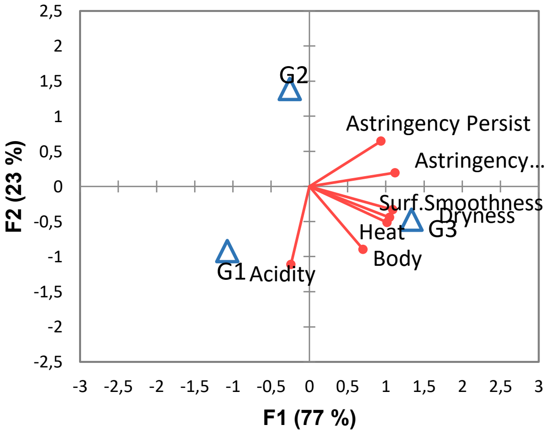


**Figure 4.12** - Representation of evaluated wines and attributes evaluated by GPA NT_ BW (G1). NT (G2) MA_D (G3)

Sensory perceptible differences were found between wines bottled with a **NT_BW** stopper and **NT** stopper and between wines bottled with **NT** stopper and **MA_D** stopper.

This difference was not reflected in significant differences in the intensity of the evaluated sensory attributes; however. the multidimensional representation of the results of the tasters evaluations using the GPA method showed that the wine bottled with **NT_BW** cork stopper tended to have a higher acidity than the wine bottled with **NT** cork stopper and that the wine bottled with **MA_D** cork stopper had a higher Heat. Dryness. Roughness than **NT** wine.

### 4.6.2 Results from the physical-chemical analysis

**Table 4.30** -Quantification of sulfur dioxide and colour parameters

| | **Free $SO_2$** | **Total $SO_2$** | **OD 280 nm** | **OD 420 nm** | **OD 520 nm** | **OD 620 nm** | **C. Intensity (420+520+620)** | **Tone C. (420/520)** |
|---|---|---|---|---|---|---|---|---|
| **NT_ BW** | 13 | 94 | 50.5 | 1.9 | 1.8 | 0.4 | 4.1 | 1.0 |
| **NT** | 15 | 99 | 50.6 | 1.9 | 1.8 | 0.4 | 4.1 | 1.1 |
| **MA_D** | 16 | 93 | 50.8 | 1.9 | 1.8 | 0.4 | 4.0 | 1.1 |

No significant differences were found among the wine 6 sealed with a **NT_BW**. **NT** and **MA_D** stoppers.

### 4.6.3 Results from volatile compounds analysis

No significant differences in the levels of volatile compounds were found among the wine 6 sealed with **NT_BW**. **NT** and **MA_D** stoppers.

## 4.7 Session 7 Wine 7

Stoppers: TD1+1 (G1). MA_D (G2). TD2+2 (G3)
12 Wine Tasters

### 4.7.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.31** Duo-trio test results

| **Comparasion** | **G1/G2** | **G2/G1** | **G3/G2** | **G2/G3** | **G1/G3** | **G3/G1** |
|---|---|---|---|---|---|---|
| **Correct answers** | 10 | 9 | 7 | 5 | 8 | 4 |
| **Conclusion ($\alpha$ =0.05)** | Perceptible sensory differences | | Absence of significant differences | | Absence of significant differences | |

TD1+1 (G1). MA_D (G2). TD2+2 (G3)

**Table 4.32** Descriptive analysis results

| **Attribute** | | **Mean** | **Standard deviation** | **1st Quartile** | **Median** | **3rd Quartile** |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 5.1 | 1.2 | 4.0 | 5.0 | 6.1 |
| | G2 | 5.8 | 1.0 | 5.2 | 5.7 | 6.4 |
| | G3 | 5.4 | 1.2 | 4.8 | 5.1 | 5.7 |
| ***Acidity*** | G1 | 6.7 | 1.5 | 6.0 | 6.2 | 8.0 |
| | G2 | 6.7 | 1.2 | 5.9 | 6.4 | 7.3 |
| | G3 | 6.8 | 1.0 | 6.4 | 6.8 | 7.1 |
| ***Dryness*** | G1 | 6.2 | 1.1 | 5.2 | 6.1 | 7.0 |
| | G2 | 6.5 | 1.2 | 6.0 | 6.3 | 7.6 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
|  | G3 | 6.2 | 1.1 | 5.3 | 6.2 | 7.1 |
| ***Heat*** | G1 | 5.9 | 1.0 | 5.4 | 6.0 | 6.4 |
|  | G2 | 6.1 | 1.0 | 5.9 | 6.2 | 6.7 |
|  | G3 | 6.4 | 0.9 | 6.0 | 6.4 | 6.9 |
| ***Surface smoothness*** | G1 | 5.9 | 0.8 | 5.5 | 6.0 | 6.3 |
|  | G2 | 6.3 | 0.5 | 6.0 | 6.2 | 6.4 |
|  | G3 | 6.5 | 0.7 | 6.2 | 6.5 | 7.1 |
| ***Astringency - Intensity*** | G1 | 6.4 | 1.3 | 5.4 | 6.6 | 7.4 |
|  | G2 | 6.9 | 1.4 | 5.9 | 7.0 | 8.0 |
|  | G3 | 7.1 | 1.3 | 6.5 | 7.2 | 7.7 |
| ***Astringency - Persistance*** | G1 | 6.6 | 1.2 | 6.0 | 6.6 | 7.6 |
|  | G2 | 7.0 | 1.3 | 5.9 | 6.8 | 7.9 |
|  | G3 | 7.3 | 0.9 | 7.1 | 7.4 | 7.8 |

TD1+1 (G1). MA_D (G2). TD2+2 (G3)

**Table 4.33** -Statistical comparison of the intensities of wine attributes.

| **Attribute** |  | **Statistical comparison** | **Attribute** |  | **Statistical comparison** |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.36<br>absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.05<br>* G1 a. G2 ab. G3 b |
|  | G2 |  |  | G2 |  |
|  | G3 |  |  | G3 |  |
| ***Acidity*** | G1 | ANOVA p=0.96<br>absence of significant differences | ***Astringency Intensity*** | G1 | ANOVA p=0.33<br>absence of significant differences |
|  | G2 |  |  | G2 |  |
|  | G3 |  |  | G3 |  |
| ***Dryness*** | G1 | ANOVA p=0.72<br>absence of significant differences | ***Astringency Persistance*** | G1 | ANOVA p=0.31<br>absence of significant differences |
|  | G2 |  |  | G2 |  |
|  | G3 |  |  | G3 |  |
| ***Heat*** | G1 | ANOVA p=0.45<br>absence of significant differences |  |  |  |
|  | G2 |  |  |  |  |
|  | G3 |  |  |  |  |

*different letters (a.b) indicate statistically significant differences (p≤0.05)

TD1+1 (G1). MA_D (G2). TD2+2 (G3)

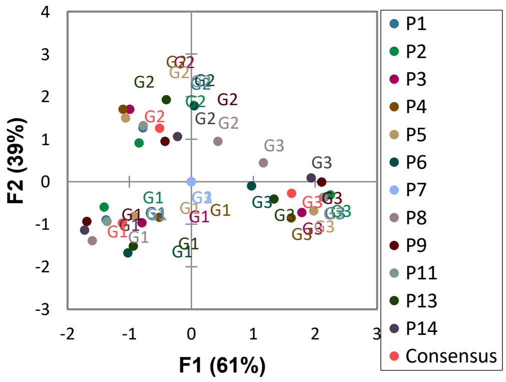


**Figure 4.13** - Representation of wine evaluations by different tasters by GPA

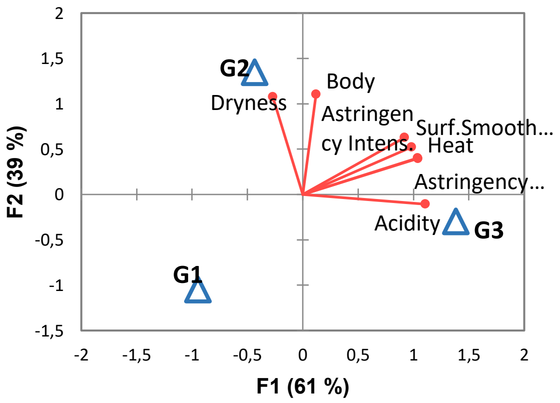


**Figure 4.14**- Representation of evaluated wines and attributes evaluated by GPA TD1+1 (G1). MA_D (G2). TD2+2 (G3)

Sensory perceptible differences were found between wines bottled with **TD1+1** stopper and **MA_D** stopper. There were also sensorially perceptible differences for the **Roughness** attribute between wines bottled with **TD1+1** stopper and **TD 2+2** stopper.

The multidimensional representation of the results of the tasters' evaluations using the GPA method showed the lowest **Roughness** of the wine bottled with **TD1+1** stopper. that the wine bottled with a **MA_D** stopper tended to have a higher **Dryness** and **Body** in particular when compared to the wine bottled with **TD1+** stopper and wine bottled with the **TD2+2** stopper for greater acidity.

### 4.7.2 Results from the physical-chemical analysis

**Table 4.34**- Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **TD1+1** | 12 | 86.7 | 49.6 | 2.7 | 2.5 | 0.6 | 5.8 | 1.1 |
| **MA_D** | 12 | 90.3 | 48.1 | 2.7 | 2.5 | 0.6 | 5.7 | 1.1 |
| **TD2+2** | 12 | 91.3 | 47.7 | 2.6 | 2.5 | 0.6 | 5.7 | 1.1 |

**Table 4.35**- FQ Parameters showing significant differences between these red wines. sealed with different stoppers.

| **Parameter** | **Comparison** | **% Variation (*p*-value)** |
|---|---|---|
| $TSO_2$ | TD2+2 *vs.* **MA_D** | 9.7 ± 1.0 (*) |
| OD 520 | **TD2+2** *vs.* MA_D | 4.7 ± 3.3 (*) |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

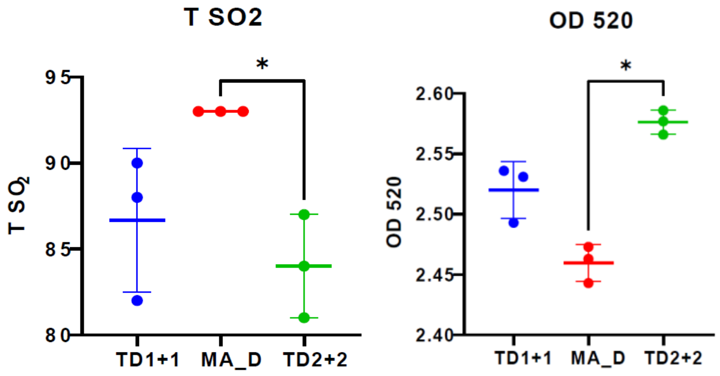


**Figure 4.15** Boxplots of compounds showing significant differences and tendencies between wines7. Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

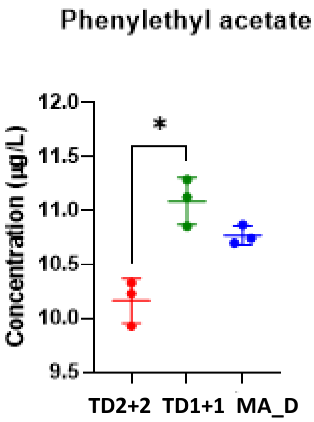
T $SO_2$ was found in significant higher levels in wines sealed with **MA_D** compared with **TD2+2**. **OD 520** is significant higher in wines sealed with with **TD2+2** compared with **MA_D**.  No other significant differences were found among the wines sealed with the different stoppers.

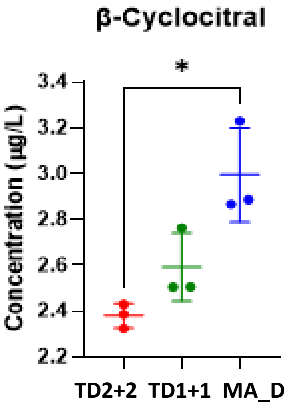
### 4.7.3 Results from the analysis of the volatile compounds

**Table 4.36** Compounds showing significant differences and tendencies between wines sealed with different stoppers.

| Compound | Comparison | % Variation (*p*-value) |
|---|---|---|
| Phenylethyl acetate | TD2+2 *vs.* **TD1+1** | -8.35 ± 1.64 (*) |
| β-Cyclocitral | TD2+2 vs. **MA_D** | -20.5 ± 4.54 (*) |
| 4-*tert*-Butylcyclohexanol | This compound was only detected in wines sealed with MA_D | |
| Decanal | TD2+2 vs. **MA_D** | -94.4 ± 21.4 (***) |
| | TD1+1 *vs.* **MA_D** | -93.2 ± 20.9 (***) |

Statistical significance assessed using Kruskal-Wallis and ordinary one-way ANOVA. (* - p < 0.05; *** - p < 0.001)





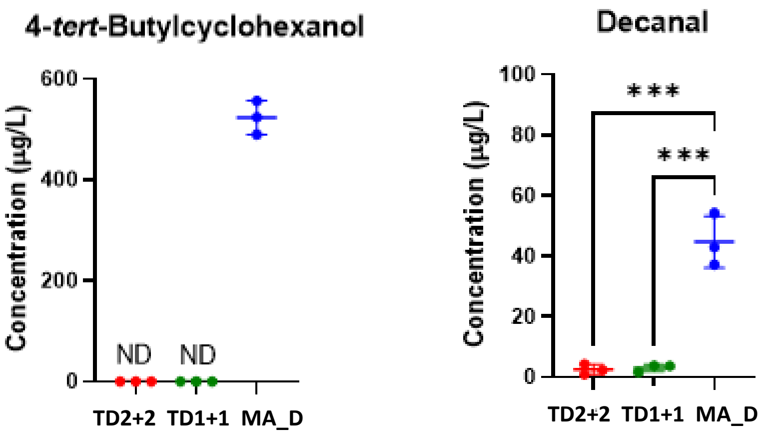


**Figure 4.16** -Boxplots of volatile compounds showing significant differences or tendencies between wines7. Statistical significance assessed using Kruskal-Wallis and ordinary one-way ANOVA. (* - $p < 0.05$; *** - $p < 0.001$). ND – not detected.

Phenylethyl acetate was found in significant lower levels in wines 7 sealed with **TD 1+1** compared with **TD 2+2**. β-cyclocitral and decanal were found in significant lower levels in wines sealed with **TD1+1** compared with **MA_D**. Decanal was also found in significant lower levels in wines sealed with **TD 2+2** compared with **MA_D**. 4-*tert*-buthylcyclohexanol was only detected in wines sealed with **MA_D**.

## 4.8 Session 8 Wine 8

**Stoppers:** MA_D (G1). MA_X (G2). NT (G3).
9 Wine Tasters

### 4.8.1 Sensory evaluation results Duo-Trio test and Descriptive Analysis

**Table 4.37** -Duo-trio test results

| Comparasion | G1/G2 | G2/G1 | G3/G2 | G2/G3 | G1/G3 | G3/G1 |
|---|---|---|---|---|---|---|
| Correct answers | 5 | 4 | 6 | 5 | 3 | 7 |
| Conclusion (α =0.05) | Absence of significant differences | | Absence of significant differences | | Absence of significant differences | |

MA_D (G1). MA_X (G2). NT (G3).

**Table 4.38** Descriptive analysis results MA_D (G1). MA_X (G2). NT (G3).

| Attribute | | Mean | Standard deviation | 1st Quartile | Median | 3rd Quartile |
|---|---|---|---|---|---|---|
| ***Body*** | G1 | 7.2 | 1.6 | 6.0 | 7.2 | 8.3 |
| | G2 | 7.1 | 1.7 | 5.4 | 7.7 | 8.4 |
| | G3 | 7.8 | 0.9 | 6.9 | 7.7 | 8.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Acidity*** | G1 | 5.3 | 1.6 | 4.1 | 6.0 | 6.4 |
| | G2 | 6.0 | 1.6 | 5.0 | 6.0 | 7.3 |
| | G3 | 5.6 | 1.6 | 4.1 | 6.0 | 7.1 |
| ***Dryness*** | G1 | 6.0 | 1.2 | 4.9 | 6.0 | 6.2 |
| | G2 | 6.6 | 1.5 | 5.5 | 6.6 | 7.9 |
| | G3 | 6.5 | 1.6 | 5.9 | 6.2 | 6.7 |
| ***Heat*** | G1 | 6.2 | 0.8 | 6.0 | 6.0 | 6.5 |
| | G2 | 6.5 | 1.4 | 5.7 | 6.0 | 6.7 |
| | G3 | 6.7 | 1.9 | 5.7 | 6.5 | 7.5 |
| ***Surface smoothness*** | G1 | 5.8 | 1.3 | 4.7 | 6.0 | 7.0 |
| | G2 | 6.4 | 1.1 | 5.9 | 6.8 | 7.1 |
| | G3 | 6.0 | 1.5 | 5.5 | 6.0 | 6.5 |
| ***Astringency - Intensity*** | G1 | 5.7 | 1.8 | 4.7 | 6.0 | 7.2 |
| | G2 | 6.8 | 1.9 | 5.9 | 7.2 | 8.0 |
| | G3 | 6.6 | 2.5 | 5.1 | 6.5 | 8.9 |
| ***Astringency - Persistance*** | G1 | 6.6 | 1.7 | 4.6 | 7.1 | 7.8 |
| | G2 | 7.1 | 1.7 | 6.0 | 7.9 | 8.1 |
| | G3 | 7.1 | 2.0 | 6.0 | 7.7 | 8.5 |

MA_D (G1). MA_X (G2). NT (G3)

**Table 4.39** -Statistical comparison of the intensities of wine attributes.

| Attribute | | Statistical comparison | Attribute | | Statistical comparison |
|---|---|---|---|---|---|
| ***Body*** | G1 | ANOVA p=0.39<br>absence of significant differences | ***Surface smoothness*** | G1 | ANOVA p=0.33<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Acidity*** | G1 | ANOVA p=0.03<br>* G1 a. G2 b. G3 ab | ***Astringency Intensity*** | G1 | ANOVA p=0.13<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Dryness*** | G1 | ANOVA p=0.25<br>absence of significant differences | ***Astringency Persistance*** | G1 | ANOVA p=0.57<br>absence of significant differences |
| | G2 | | | G2 | |
| | G3 | | | G3 | |
| ***Heat*** | G1 | ANOVA p=0.44<br>absence of significant differences | | | |
| | G2 | | | | |
| | G3 | | | | |

*different letters (a.b) indicate statistically significant differences (p≤0.05)
MA_D (G1). MA_X (G2). NT (G3)

No sensorially perceptible differences were found between the wines8 evaluated using the duo-trio total discrimination test. however the analysis of the results of the sensorial profile determination test indicated the presence of significant differences for the “Acidity” attribute between the wine bottled with the **MA_D** stopper and **MA_X** stopper.

### 4.8.2 Results of the physical-chemical analysis

**Table 4.40** Quantification of sulfur dioxide and colour parameters

| | Free $SO_2$ | Total $SO_2$ | OD 280 nm | OD 420 nm | OD 520 nm | OD 620 nm | C. Intensity (420+520+620) | Tone C. (420/520) |
|---|---|---|---|---|---|---|---|---|
| **MA_D** | 14 | 67 | 79.8 | 5.9 | 7.8 | 2.1 | 15.8 | 0.8 |
| **MA_X** | 15 | 67 | 80.3 | 5.9 | 7.8 | 2.1 | 15.8 | 0.8 |
| **NT** | 14 | 66 | 80.4 | 5.9 | 7.8 | 2.1 | 15.8 | 0.8 |

**Table 4.41** FQ Parameters showing significant differences between wines. sealed with different stoppers.

| Parameter | Comparison | % Variation (*p*-value) |
|---|---|---|
| $TSO_2$ | NT *vs.* **MA_D** | -7.5 ± 1.6 |
| OD 280 | **NT** *vs.* MA_D | 1.9 ± 0.5 |
| OD 420 | **NT** *vs.* MA_D | 2.9 ± 2.9 |
| OD 620 | **NT** *vs.* MA_D | 4.7 ± 3.8 |
| CI | **NT** *vs.* MA_D | 3.1 ± 1.8 |

Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

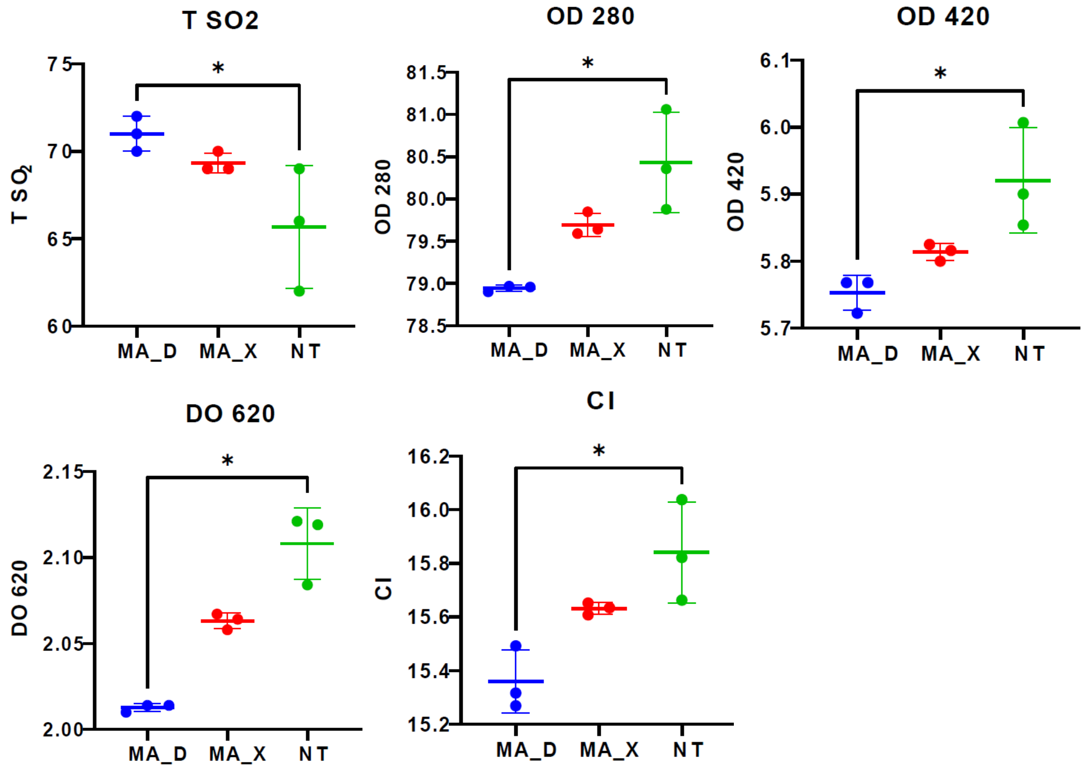


**Figure 4.17** Boxplots of compounds showing significant differences between wines8. Statistical significance assessed using Kruskal-Wallis ANOVA. (* - $p < 0.05$)

T $SO_2$ was found in significant higher levels in wines 8 sealed with **MA_D** compared with **NT**. OD 280; 420 and OD 620 were found in significant higher levels in wines sealed with (**NT**) compared with **MA_D**.

### 4.8.3 Results from volatile compounds analysis

**Table 4.42** Compounds showing tendencies between wines sealed with different stoppers.

| Compound | Comparison | % Variation (*p*-value) |
|---|---|---|
| ethyl decanoate | This compound showed a tendency for higher levels in wines sealed with **NT** compared with MA_D | |
| diethyl succinate | | |
| ethyl octanoate | | |

Statistical significance assessed using Kruskal-Wallis ANOVA.

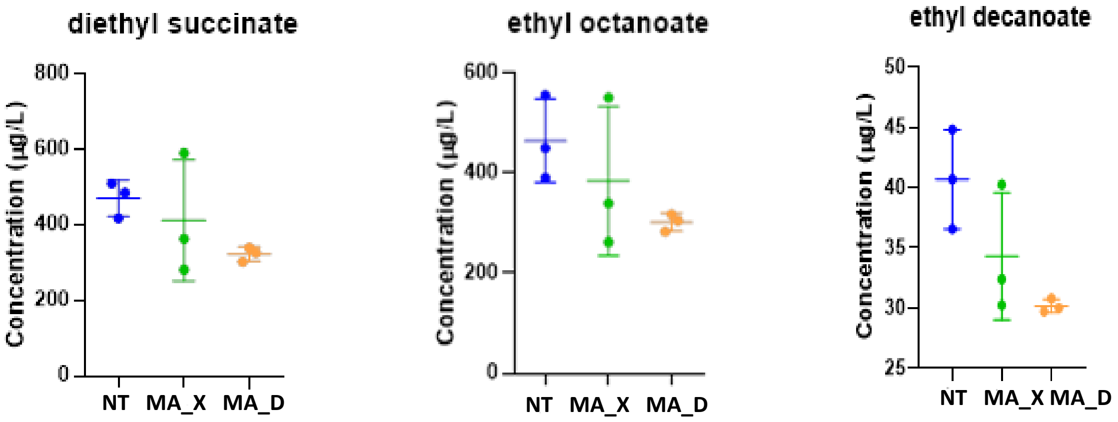


**Figure 4.18** Boxplots of compounds showing tendencies in wines8 sealed with different stoppers. Statistical significance was assessed using Kruskal-Wallis ANOVA.

Overall, the wines sealed with natural cork **NT** showed a tendency for higher levels of diethyl succinate, ethyl octanoate. and ethyl decanoate compared with **MA_D**. Diethyl succinate and ethyl octanoate are the esters present in higher concentrations in this wine. No other significant differences or tendencies were found among the wines.

## 4.9. General discussion

### *4.9.1 Sensory analysis*

The main objective of wine tasting was to perceive significant differences in wines mouthfeel, mainly astringency. The evaluation of astringency is difficult due to the accumulation effect during tasting. In addition, it was also difficult to isolate the mouthfeel from olfactory characteristics. especially because part of the sensory perception in the mouth is due to the retronasal perception associated to volatile compounds that can thus affect the perceived differences. Astringency is known as a perception that increases in intensity and duration with repeated wine tasting. The reason for this is that residual astringency of the previous wine is carried over to the next sample, to whose astringency it adds to the residual astringency is not allowed to decay to zero before the next tasting. This sensory bias is called carry-over effect.

Even considering the mentioned limitations. sensory discrimination tests performed uncovered the existence of perceptible differences between the wines bottled with different stoppers in six of the eight sensory sessions held.

The results of cork stoppers discrimination tests obtained from the sensorial analysis showed a tendency towards sensorially perceptible differences between natural cork stoppers and the technical cork stoppers (microagglomerated or discs). These differences were related to differences in the intensity of sensory mouthfeel attributes of these wines. as shown by the sensory profile results.

When analyzing the results of the descriptive analysis regarding mouthfeel attributes, there was a tendency for the “Dryness” attribute to be more intense in both microagglomerated and disc corks when compared to natural corks. This result may be related to the lower OTR for the microagglomerated and disc corks. thus, causing a reduction in the astringency and wine’s dryness.

This same trend was also verified for the “Heat” attribute. OTR and OIR associated to the stoppers are generally accepted as being among the main factors that affect the sensory attributes of bottled wines.

As discussed before, the synthetic stoppers allow a higher oxygen transmission, whereas the screwcaps allow the lowest, the cork stoppers are associated to intermediate OTR.

Between the different cork stoppers the differences are tenuous, although the natural cork stopper usually shows higher variability in OTR and OIR than other cork stoppers. Natural cork stoppers present closer OTRs and OIRs to technical stoppers. whether microagglomerates or discs, when compared to screwcaps or synthetic stoppers. Therefore. only minor differences in wine sensory attributes were expected in this study.

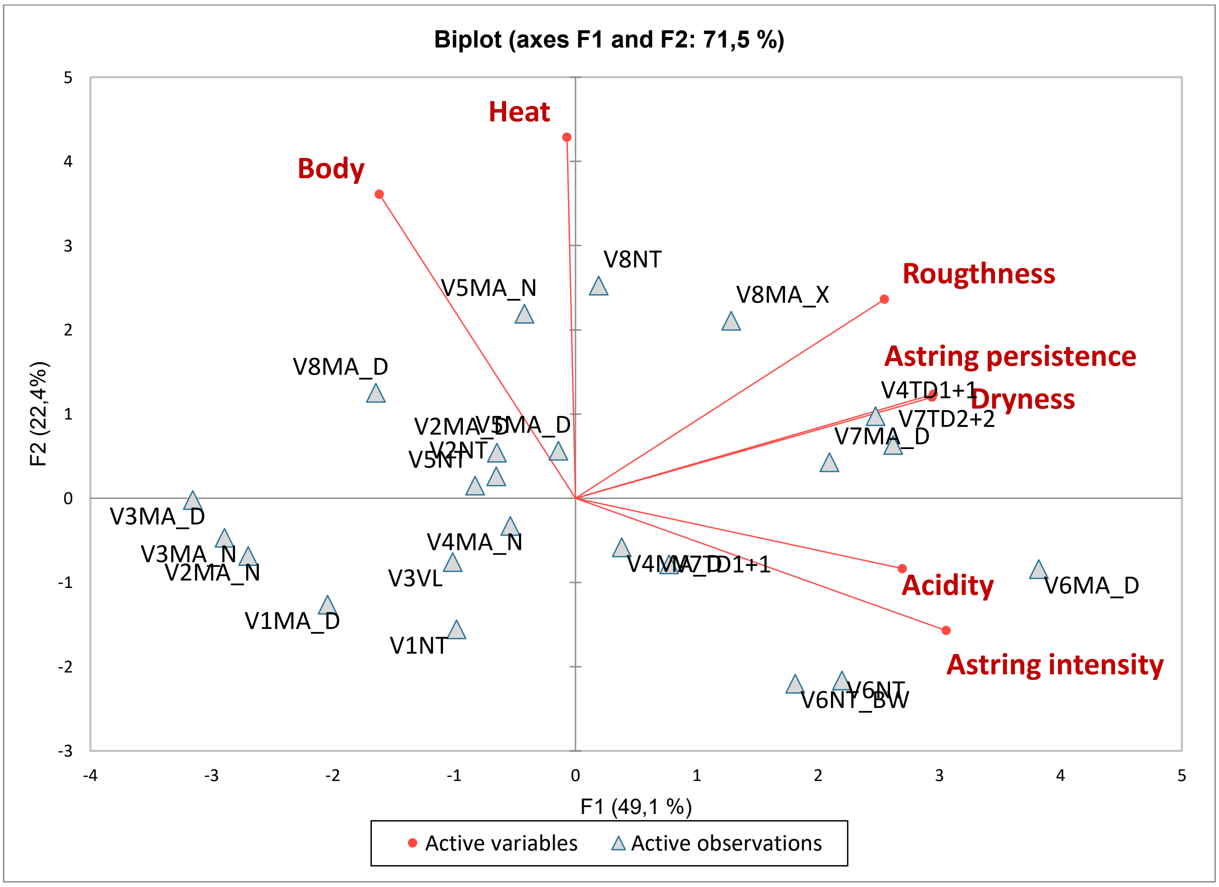


**Figure 4.19** – Bidimensional representation of the sensory profile data obtained by principal components analysis (PCA)

(Figure 4.19) shows the representation of the sensory profile data obtained for all wines and sessions. Although the representation of PCA results only encompasses 71.5% of the overall variability, it shows that persistence of astringency, dryness and roughness were, as expected, well correlated, as it was the acidity and astringency. It also shows that expectedly the variations among wines bottled with difference stoppers was less important than within different wines.

### *4.9.2 Physical-chemical analysis*

Most wines showed no significant differences for free or total sulfur dioxide. The few observed significant differences did not allow to identify any trend dependent on cork type.

Sulfur dioxide measurements showed great variability between replicates. which may influence the failure to find significant differences between wines. This parameter is also very dependent on the dissolved oxygen content during bottling and on pH, variables that were not controlled or known, which may also have contributed to the non-identification of any significant effect of the type of stopper.

Although no significant differences were observed for sulfur dioxide amounts, it is well known that $SO_2$ is the main antioxidant used to protect wine from oxidation and that there is a relationship between the $SO_2$ decrease and the wine oxidation degree as a result of the storage conditions. Stoppers that allow high OTR. such as synthetic closures. usually promote the fastest decrease of $SO_2$ content (Lopes *et al.*, 2009). High OTRs are also responsible for changes in wine components, such as the conversion of anthocyanins into other pigments (de Esteban *et al.*, 2019)

The criteria used to analyse wines' colour was based on that proposed by Glories (1984), who defined colour intensity (CI) as the sum of the absorbances at 420, 520 and 620 nm and tonality (CT) as the quotient of absorbances at 420 and 520 nm.

Regarding these parameters, the significant differences in OD420 worth to mention were observed between wines sealed with **VL** stoppers and those with **MA_D** stoppers. The first showed higher OD420 and significant lower free sulfur dioxide. Considering the representation of the tasters' results using the GPA method. wine sealed with **VL** glass stoppers tended to be more acidic and astringent than wines sealed with microagglomerate stoppers. The observed results are likely associated to a higher oxidation degree in wines sealed with the glass stoppers. In fact, OD420 is a parameter associated to the overall wine's oxidation state (Karbowiak *et al.*, 2010), and the oxidation level is also accepted to be linearly correlated with a decrease of $SO_2$ concentration (Chatonnet el al 2003).

Most wines did not show any significant differences in colour when comparing natural cork and microagglomerated or disc corks. Only wine 8 showed significant OD420 higher levels when sealed with **NT** compared with **MA_D**. This wine sealed with natural cork **NT** also showed a tendency for higher levels of diethyl succinate compared to **MA_D** cork stopper. These two parameters may indicate a tendency for higher oxidation rate when using the natural cork stopper comparing with **MA_D**.

Regarding the total polyphenol Index (TPI), defined as the absorbance measured at 280 nm (OD280). the only significant difference between wines sealed with different stoppers was also observed for wine 8. For this sample, OD280 was significantly higher for the wines sealed with **NT** compared with those sealed with **MA_D,** despite the absence of any perceptible differences among these wines when evaluated using the sensory duo-trio total discrimination test.

As part of a project carried out by INRA and the IFV of Pech Rouge. spectrophotometric measurements were carried out on 21 very different red wines. It was observed that the intensity of astringency was better correlated with the absorbance at 230 nm ($R^2$ = 0.71) than with the Total Polyphenol Index (IPT) determined at an absorbance of 280 nm ($R^2$ = 0.56). In fact. the UV spectra of the main components of red wines show that tannins are the components that most contribute to absorbance at 230 nm, while absorbance at 280 nm involves many absorbing molecules, which do not interfere with astringency, (Boulet *et al* 2016).

### *4.9.3 Volatile compounds analysis*

#### 4.9.3.1 Oxidation markers

Volatile organic compounds (VOCs) are compounds that can be used as potential oxidation markers to establish the quality of stored wines (Ferreira et al 2014). Aldehydes and ketones have been associated with the oxidative degradation of fatty acids present in wax and suberin fractions of cork and are usually associated with unpleasant flavours (Furtado *et al*., 2021).

Significant differences were observed for wine 5. which showed significant higher levels of phenylacetaldehyde when sealed with natural stopper **NT**. comparing with the **MA_D** cork stopper. what can be related to a higher oxidation level due to the higher OTR associated to the **NT** stoppers. In fact, changes in phenylacetaldehyde concentrations can be used as an oxidation marker during ageing (Ferreira *et al.*, 2002).

Wine 7 also showed significant differences in the levels of decanal and β-cyclocitral, both aldehydes. with higher levels of these compounds observed in wines sealed with **MA_D** compared with those sealed with **TD 2+2**. A tendency for higher levels of decanal was also observed for wine 1 when sealed with **MA_D** compared with wines sealed with technical cork discs **TD 1+1**.

#### 4.9.3.2 Esters

Esters such as diethyl succinate are considered as markers of wine aging diethyl succinate is a characteristic volatile compound of the malolactic fermentation in young wines. Typically. diethyl succinate also increases its concentration during wine storage and aging (Rossetti *et al*., 2020).

In the present study, wine 8 sealed with natural cork **NT** showed a tendency for higher levels of diethyl succinate compared to the **MA_D** cork stopper. In the remaining wines there were no significant differences for this parameter. Probably other significant differences would be observed for longer storage times.

Wine 5 when sealed with natural cork **NT** showed a tendency for higher levels of ethyl octanoate and ethyl decanoate. compared with **MA_D**. Wine 8 showed significant higher values of ethyl octanoate when sealed with natural cork than when sealed with **MA_D** cork stopper.

Adsorption of certain VOCs by the stopper materials may also influence the observed results. Natural and technical cork stoppers are known to have the ability to partially adsorb ethyl octanoate and ethyl decanoate. with an increased capacity of adsorption proportional to the increased ester chain length (Capone *et al*., 2003).

The levels of ethyl decanoate. 2-phenylethanol, were found increased in wines bottled with natural cork stoppers compared to those sealed with synthetic closures in different studies, Esters and 2-phenylethanol were described to be responsible for the floral and fruity odour observed in wines sealed with cork stoppers suggesting that cork is the most suitable closure to preserve these aromas (Oliveira *et al.,* 2020).

### *4.9.4 Integration of sensory attributes and physical-chemical analysis*

A multifactorial analysis (MFA) was held to integrate the main sensory profile and physical chemical results for each wine, namely mouthfeel sensory attributes, colour parameters, TPI and VOCs. The bidimensional representation of the wines in the MFA space is presented in (figure 4.20). Similarly, to the representation of the wine’s sensory attributes in (Figure 4.19), it shows that the variations among wines bottled with difference stoppers was much less important than within different wines.

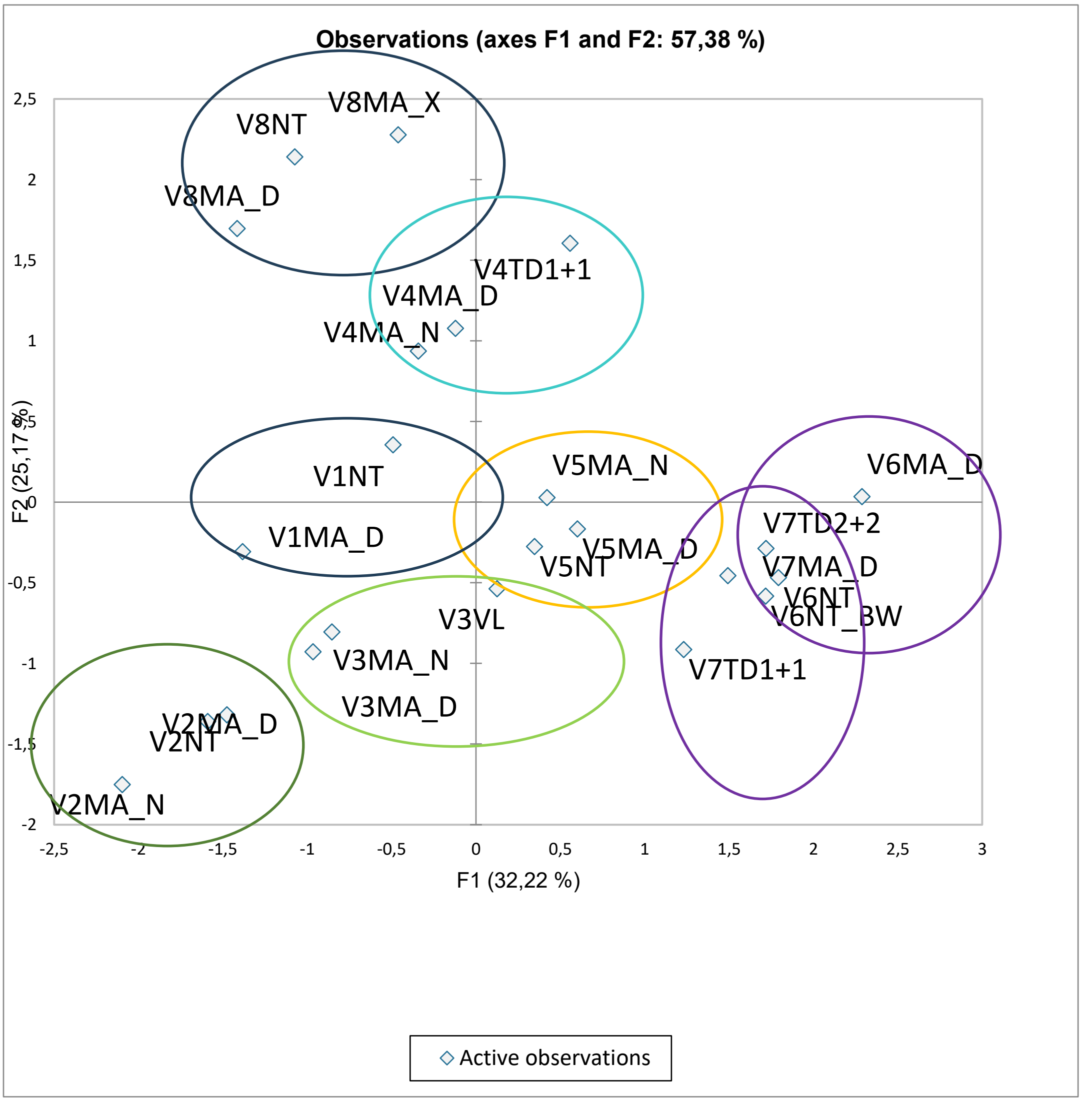


**Figure 4.20** – Bidimensional representation of the wines in the MFA space integrating sensory profile and physical chemical results.

### *4.9.5* Study Limitations

In this study only a small number of commercial wines samples were evaluated, and these were produced in *real* cellar conditions with limited control of variables related to wine production, bottling and aging. Furthermore, important variables were not standardized in this study: bottling time. type of red wine. stopper characteristics (diameter. length. relation between stopper diameter to bottleneck). bottle head space, stopper/wine contact, and cellar storing conditions, like temperature and relative humidity. Therefore, it is expected that these parameters may be even more influential to the wine attributes than the stopper itself (Cardoso *et al.,* 2022), being also known that the influence of the different types of stoppers is highly dependent on wine characteristics (Rossetti *et al.*, 2020).

# *CONCLUSIONS*

In the present study, a sensory procedure was established to detect and characterize differences between wines bottled with different closures. The panellists were familiarized and trained in the evaluation methodologies.

Despite the limitations in controlling parameters that may influence the study, sensory differences were detected among the bottled wines in six of the eight sensory evaluation sessions. The results of cork stoppers discrimination tests obtained from the sensorial analysis showed a tendency towards sensorially perceptible differences between natural cork stoppers and the technical cork stoppers (microagglomerated or discs).

To explain the differences detected. data from sensory analysis was evaluated using uni or multidimensional statistical techniques, allowing to uncover significant differences in the intensity of wine’s attributes. Furthermore, the representation of the panellists' individual results using the GPA method made possible to demonstrate the existence of consensus among the tasters in the gustatory assessment of the wines and to highlight differences in their sensory profile.

The complementary information obtained from the statistical analysis of the relationship between the physical-chemical parameters and the sensory attributes allowed a better understanding of the changes during storage resulting from the use of different closures.

However, only a few wines showed significant differences in physical and chemical parameters analyzed, when comparing natural cork closures to other closures, likely due to a complex set of other factors also influencing the measured parameters.

Most wines showed no significant differences for free or total sulfur dioxide. The few observed significant differences did not allow to identify any trend dependent on cork stopper type.

Also, most wines did not show any significant differences in colour when comparing natural cork and microagglomerated or disc corks. Only one wine showed significant differences in OD 420nm levels.

Similarly, significant differences regarding the total polyphenol Index (TPI) were observed for only one wine sealed with different stoppers.

Considering the results obtained for VOCs. Phenylacetaldehyde, decanal, β-cyclocitral, diethyl succinate, ethyl octanoate and ethyl decanoate, significant differences were also identified in few wines bottled with different stoppers.

Despite the few observed differences, probably related also to other parameters not controlled in the study, wines bottled with natural cork stoppers showed a somewhat lower astringency character when compared to wines sealed with microagglomerated closures or technical discs.

The results also indicate that the determination of mouthfeel profiles for red wines is a complex process that is dependent on several inherent wine conditions/elements. as well as the interactions among them.

Additional research is needed to investigate how specific variables, when individually altered, impact the mouthfeel attributes and to establish clear connections between the physical-chemical composition of red wine and its sensory attributes.

Importantly, this work contributed to improve my knowledge about phenolic compounds, wine oxidation, the types of cork stoppers and their main characteristics. The familiarization with ISO standards and methodologies used for panellists selection and training, for wine characterization, and for statistical sensory data analysis, were also a challenging and valuable knowledge acquired carrying out this research and thesis.

# FUTURE WORK

Future studies can contribute to consolidate and deepen knowledge about the impact of closures on the physical-chemical and sensory characteristics of wines.

Considering the results obtained as well as some difficulties encountered during this study, some suggestions for future work are enumerated:

1. To evaluate a higher number of samples and increase the number of panel tasters.
2. To Standardized: bottling time, type of wine, stopper characteristics (diameter. length. relation between stopper diameter to bottleneck), bottle head space, stopper/wine contact, and cellar storing conditions, like temperature and relative humidity.
3. Use a minimum of 5 replicates for physical-chemical analyses on each wine sealed with every type of cork stopper. The absence of statistical differences with only observed trends may be attributed to the limited number of replicates. which was only 3.
4. Establish a consistent time interval between tastings to facilitate the evaluation of attributes.
5. To determine the physical-chemical characteristics of wines at the point of bottling, to compare them with the corresponding values upon bottle opening.
6. Select stoppers with characteristics as close as possible regarding to diameter, length, relation stopper diameter/ bottleneck.

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.

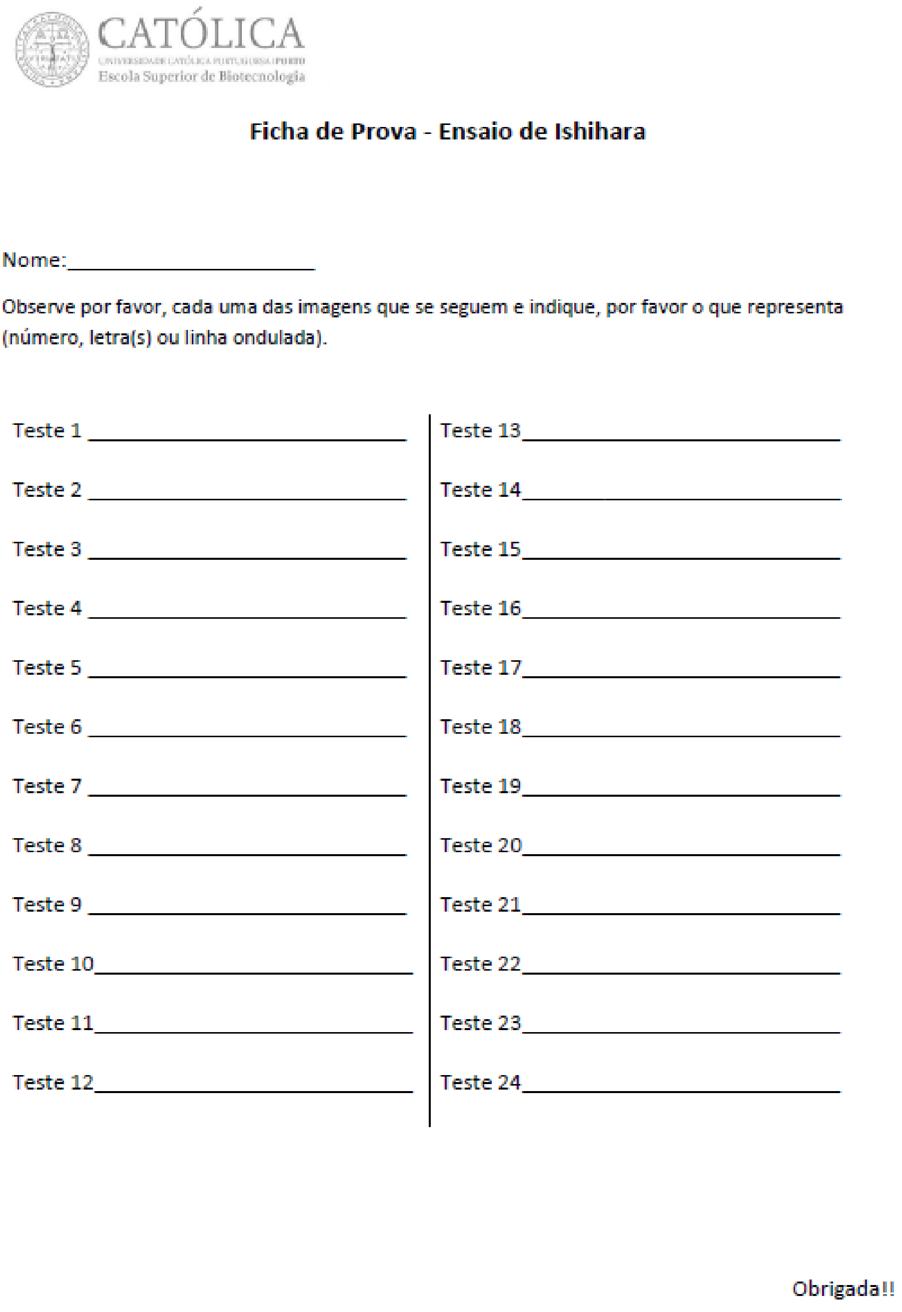
# *APPENDICES*

## Appendice I

### I.1Sensory Analysis-Phase1- (4 Sessions)-Panel Training and familiarisation

#### *I.1.1 Ishihara Test- 24 plates*

Assessors were tested checking colour vision using Ishiara Tests
The test consists of several Ishihara plates. which are a type of pseudoisochromatic plate. Each plate depicts a solid circle of coloured dots appearing randomized in colour and size. Within the pattern are dots which form a number or shape clearly visible to those with normal colour vision. and invisible. or difficult to see. to those with a red-green colour vision defect. Other plates are intentionally designed to reveal numbers only to those with a red-green colour vision deficiency and be invisible to those with normal red-green colour vision. The full test consists of 38 plates. but the existence of a severe deficiency is usually apparent after only a few plates. There are also Ishihara tests consisting of 10. 14 or 24 test plates. We used 24 plates. （Figure I.1)



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**Ficha de Prova - Ensaio de Ishihara**

Nome:______________________

Observe por favor, cada uma das imagens que se seguem e indique, por favor o que representa (número, letra(s) ou linha ondulada).

Teste 1 ____________________________
Teste 2 ____________________________
Teste 3 ____________________________
Teste 4 ____________________________
Teste 5 ____________________________
Teste 6 ____________________________
Teste 7 ____________________________
Teste 8 ____________________________
Teste 9 ____________________________
Teste 10____________________________
Teste 11____________________________
Teste 12____________________________

Teste 13____________________________
Teste 14____________________________
Teste 15____________________________
Teste 16____________________________
Teste 17____________________________
Teste 18____________________________
Teste 19____________________________
Teste 20____________________________
Teste 21____________________________
Teste 22____________________________
Teste 23____________________________
Teste 24____________________________

Obrigada!!

**Figure I.1** Ishihara Test Sheet

### I.1.2 Basic Tastes introducing relating to sensory analysis (ISO 5492:2008)

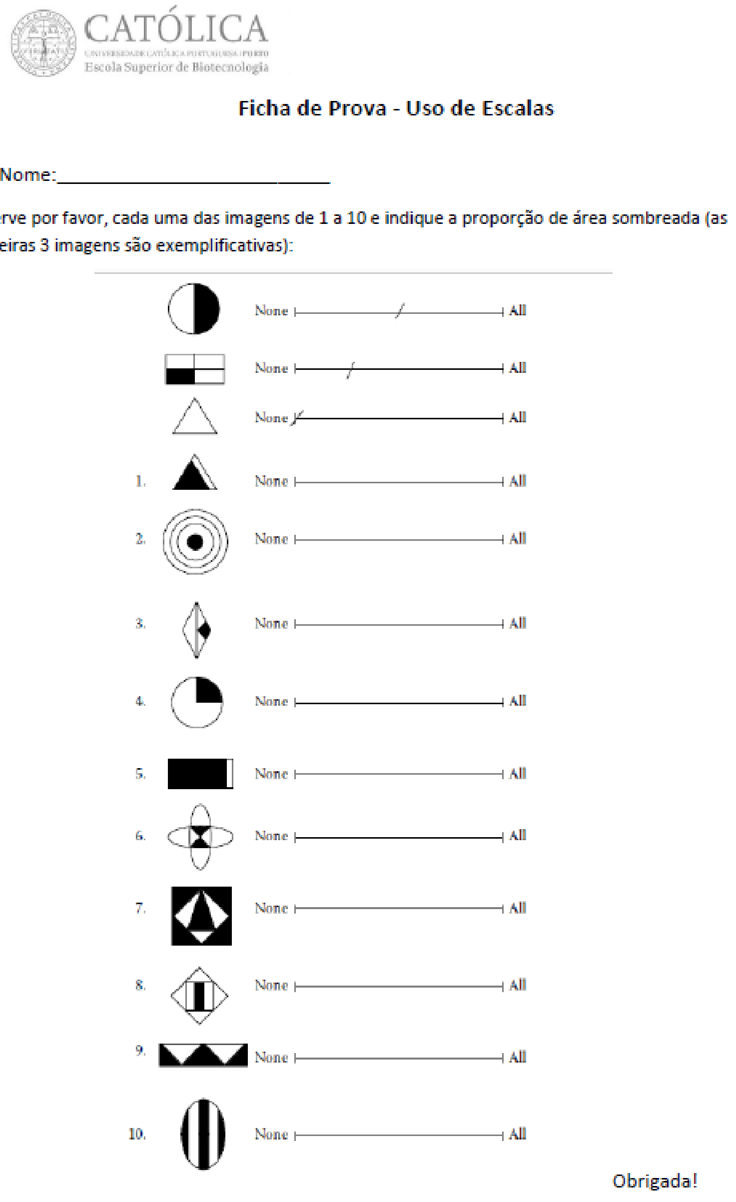
To standardize concepts. the basic flavours were introduced to the panellists as well as the definitions of each one following ISO 5492:2008. (Table I.1)

Table I.1 Basic Tastes relating to sensory analysis (ISO 5492:2008)

| | ***Basic taste***<br>*any one of the distinctive tastes: acid/sour. bitter. salty. sweet. umami*<br>*Other tastes that may be classified as basic are alkaline and metallic.* |
|---|---|
| **Acidity** | acid taste. basic taste produced by dilute aqueous solutions of most acid substances (e.g. citric acid and tartaric acid) |
| **Sourness** | sour taste. gustatory complex sensation. generally due to presence of organic acids<br>In some languages “sour” is not a synonym for “acid”.<br>Sometimes this term has a negative hedonic sense. |
| **Bitterness** | bitter taste. basic taste produced by dilute aqueous solutions of various substances such as quinine or caffeine |
| **Saltiness** | salty taste. basic taste produced by dilute aqueous solutions of various substances such as sodium chloride. |
| **Sweetness** | sweet taste. basic taste produced by dilute aqueous solutions of natural or artificial substances such as sucrose or aspartame. |
| **Astringency** | astringent. complex sensation. accompanied by shrinking. drawing or puckering of the skin or mucosal surface in the mouth. produced by substances such as kaki tannins or sloe tannins. |

### I.1.3 Scale use tests

Panellists were also trained to use scales. They were asked to observe the images presented in the appendix form indicating the proportion of shaded area. (Figure I.2)



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Ficha de Prova - Uso de Escalas

Nome:__________________________

Observe por favor, cada uma das imagens de 1 a 10 e indique a proporção de área sombreada (as primeiras 3 imagens são exemplificativas):

None All

None All

None All

1. None All
2. None All
3. None All
4. None All
5. None All
6. None All
7. None All
8. None All
9. None All
10. None All

Obrigada!

**Figure I.2-** Scales Use Test sheet.

### I.1.4 Perception Tests

Perception Tests were used to train the basic flavours and assess bitterness, sourness and astringency perception using aqueous solutions.

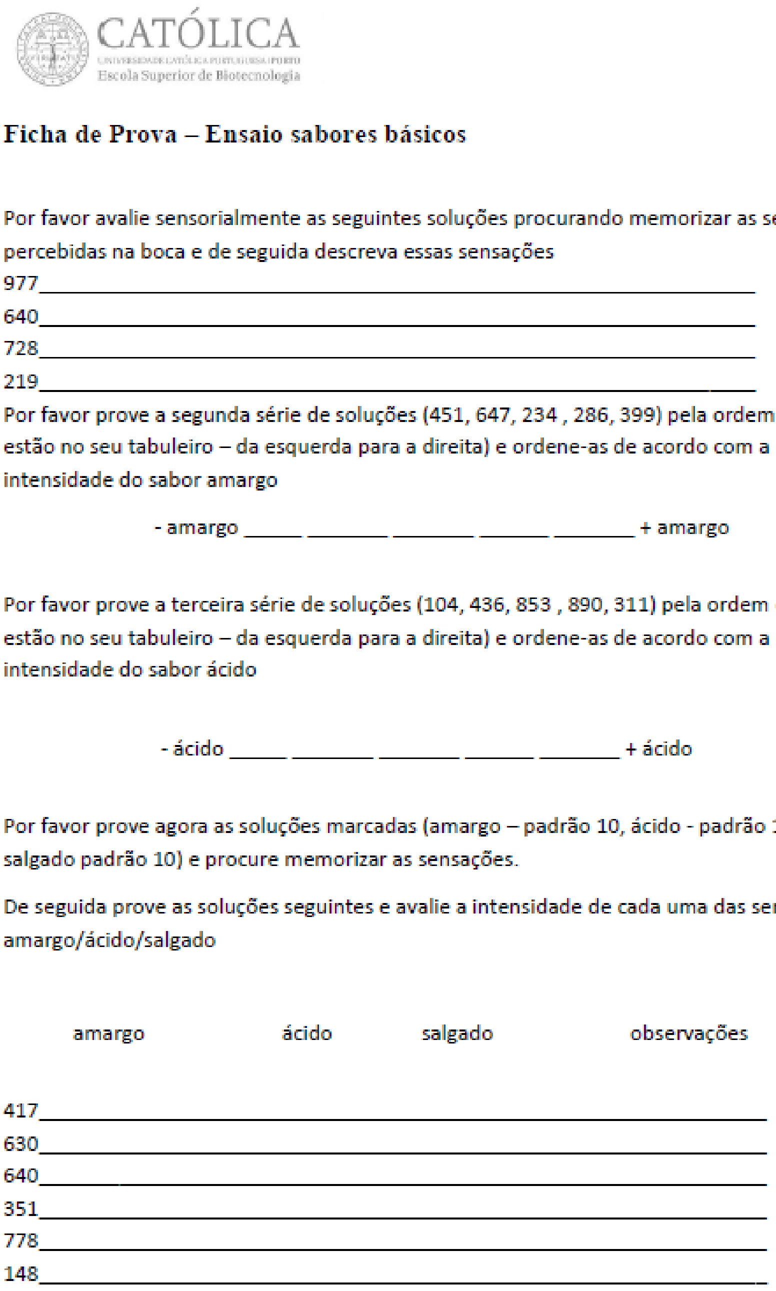
Tasters were introduced first to the theory of astringency and extensively trained to differentiate astringency from bitterness and sourness using tannic acid, caffeine, skin tannin and tartaric acid. Tasters were asked to taste solutions, memorize the sensations perceived in the mouth and describe those sensations.

### *I.1.4.1 Intensity evaluation test – Sorting*

Intensity evaluation tests were applied to order bitterness and acidity intensity using five solutions. Tasters were instructed to taste the series of 5 solutions (Table I.2) in the order they were on their board (left to right) and to order them according to the intensity of bitter and sour taste. Using Test Sheet (Figure I.3)

**Table I.2.** Reagents and concentrations of aqueous solutions Taste/Sensations

| *5 Solutions Sort the Degree of Bitterness* | | |
|---|---|---|
| | **Product Added** | **Concentration-g/L** |
| ***Bitter*** | Cafeine | 0<br>0.15<br>0.22<br>0.34<br>0.51 |
| *5 Solutions Sort the Degree of Sourness* | | |
| | **Product Added** | **Concentration-g/L** |
| ***Sour*** | Tartaric Acid | 0<br>0.1<br>0.2<br>0.4<br>0.6 |



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**Ficha de Prova – Ensaio sabores básicos**

Por favor avalie sensorialmente as seguintes soluções procurando memorizar as sensações percebidas na boca e de seguida descreva essas sensações

977________________________________________________

640________________________________________________

728________________________________________________

219________________________________________________

Por favor prove a segunda série de soluções (451, 647, 234 , 286, 399) pela ordem em que estão no seu tabuleiro – da esquerda para a direita) e ordene-as de acordo com a intensidade do sabor amargo

- amargo _____ _______ _______ ______ _______ + amargo

Por favor prove a terceira série de soluções (104, 436, 853 , 890, 311) pela ordem em que estão no seu tabuleiro – da esquerda para a direita) e ordene-as de acordo com a intensidade do sabor ácido

- ácido _____ _______ _______ ______ _______ + ácido

Por favor prove agora as soluções marcadas (amargo – padrão 10, ácido - padrão 10 e salgado padrão 10) e procure memorizar as sensações.

De seguida prove as soluções seguintes e avalie a intensidade de cada uma das sensações amargo/ácido/salgado

| | amargo | ácido | salgado | observações |
|---|---|---|---|---|
| 417 | | | | |
| 630 | | | | |
| 640 | | | | |
| 351 | | | | |
| 778 | | | | |
| 148 | | | | |

**Figure I.3** Basic Flavours test sheet I

### *I.1.4.2 Intensity evaluation test – Classification*

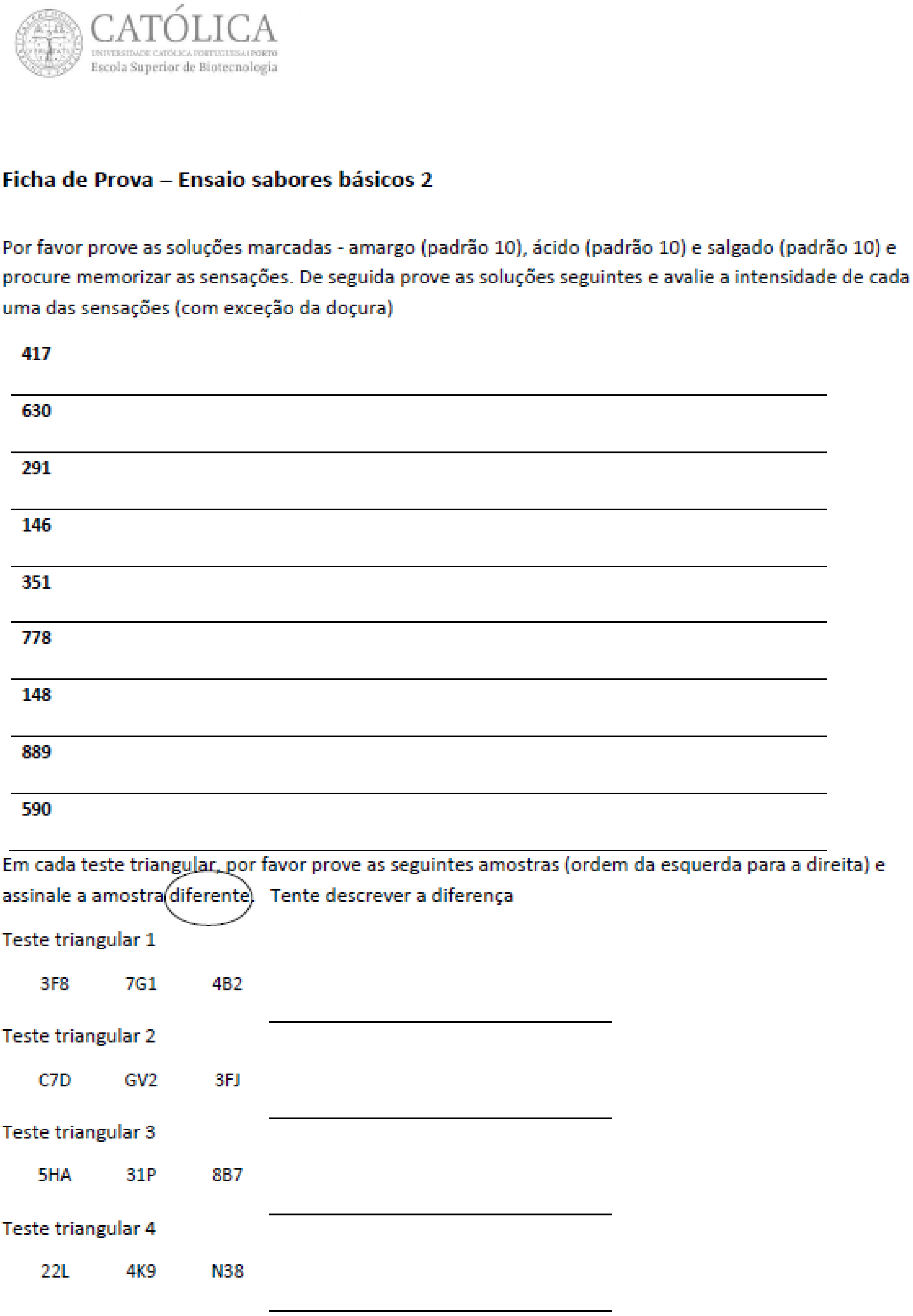
Panellist were asked to taste “n” samples and rate sourness, bitterness, and saltiness comparing with standards (Intensity 10).

Instructions were given to taste the standard solutions (Bitter-Standard (ST)10, Acid ST10 and Salty ST10) and try to memorize the sensations. Then they were instructed to taste the prepared solutions Table I3 and evaluated the intensity of each sensation bitter/sour/salty from 0 to 10 comparing with Standards. Using sensory analysis form (Figure I.4)

**Table I.3**. Reagents and concentrations of aqueous solutions Intensity evaluation tests-Classification.

| *Indicative concentrations of solutions used to detect a stimulus* | | |
|---|---|---|
| | **Product Added** | **Concentration g/L** |
| ***ST 10 Bitter*** | Cafeine | 0.8 |
| ***ST 10 Sour*** | Citric Acid | 0.8 |
| ***ST 10 Salty*** | Sodium Chloride | 3.5 |
| ***Sweet+Sour*** | Sacarose+Citric Acid | 50+1.0 |
| ***Sweet+Salty*** | Sacarose+Sodium Chloride | 50+5.5 |
| ***Sweet+Salty+Sour*** | Sacarose+Sodium Chloride+Citric Acid | 50+3+1 |
| ***Bitter*** | Cafeine | 0.8 |
| ***Sweet+Bitter*** | Sacarose+Cafeine | 25+0.8 |
| ***Sweet+Bitter+Sour*** | Sacarose+Cafeine+Citric Acid | 25+0.6+0.6 |

| *Indicative concentrations of mix solutions mix used to classify intensity* | | |
|---|---|---|
| | **Product Added** | **Concentration g/L** |
| ***ST 10 Bitter*** | Cafeine | 0.8 |
| ***ST 10 Sour*** | Citric Acid | 1.0 |
| ***ST 10 Salty*** | Sodium Chloride | 3.0 |
| ***Sweet+Sour*** | Sasarose+Citric Acid | 25+0.8 |
| ***Sweet+Salty*** | Sacarose+Sodium Chloride | 25+3.0 |
| ***Sweet+Sour*** | Sacarose+Citric Acid | 25+0.3 |
| ***Sweet+Salty+Sour*** | Sacarose+Sodium Chloride+Citric Acid | 25+3.0+0.5 |
| ***Sweet+Bitter*** | Sacarose+Cafein | 25+0.2 |
| ***Sweet+Bitter*** | Sacarose+Cafein | 25+0.6 |
| ***Sweet+Bitter+Sour*** | Sacarose+Cafein+Citric Acid | 25+0.4+0.5 |
| ***Sweet+Bitter+astrigent*** | Sacarose+Cafein+Tanin | 25+0.4+1 gota |
| ***Sweet+Sour+astrigent*** | Sacarose+Citric Acid+Tanin | 25+0.5+1 gota |



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**Ficha de Prova – Ensaio sabores básicos 2**

Por favor prove as soluções marcadas - amargo (padrão 10), ácido (padrão 10) e salgado (padrão 10) e procure memorizar as sensações. De seguida prove as soluções seguintes e avalie a intensidade de cada uma das sensações (com exceção da doçura)

**417** ____________________

**630** ____________________

**291** ____________________

**146** ____________________

**351** ____________________

**778** ____________________

**148** ____________________

**889** ____________________

**590** ____________________

Em cada teste triangular, por favor prove as seguintes amostras (ordem da esquerda para a direita) e assinale a amostra diferente. Tente descrever a diferença

Teste triangular 1

3F8 7G1 4B2 ____________________

Teste triangular 2

C7D GV2 3FJ ____________________

Teste triangular 3

5HA 31P 8B7 ____________________

Teste triangular 4

22L 4K9 N38 ____________________

**Figure I.4** Basic Flavours test Sheet II

To train the perception of astringency in wines. red wines spiked with oenological products were used. to increase the astringency sensation and improve the mouthfeel sensation.

Panellists were asked to use the vocabulary and definitions from the 'mouthfeel wheel' (Gawel *et al.*, 2000) to characterise the intensity of perceived in-mouth sensations.

Panell members discussed perceived mouthfeel qualities of the wines during and following expectoration. Descriptors were individually generated by the judges and the panel leader proposed definitions for these terms with reference to the 'mouthfeel wheel'. These descriptors and definitions were further discussed among the group until the panellists reached agreement regarding the relevance of each descriptor. Judges were encouraged to take the 'mouthfeel wheel' as well as the of terms and definitions for further reflection.

### I.1.5 Sensory perception in the mouth consensus assessment

In addition to astringenc,. it was also intended to evaluate the concept of "body" and "complexity" (Table I.4)

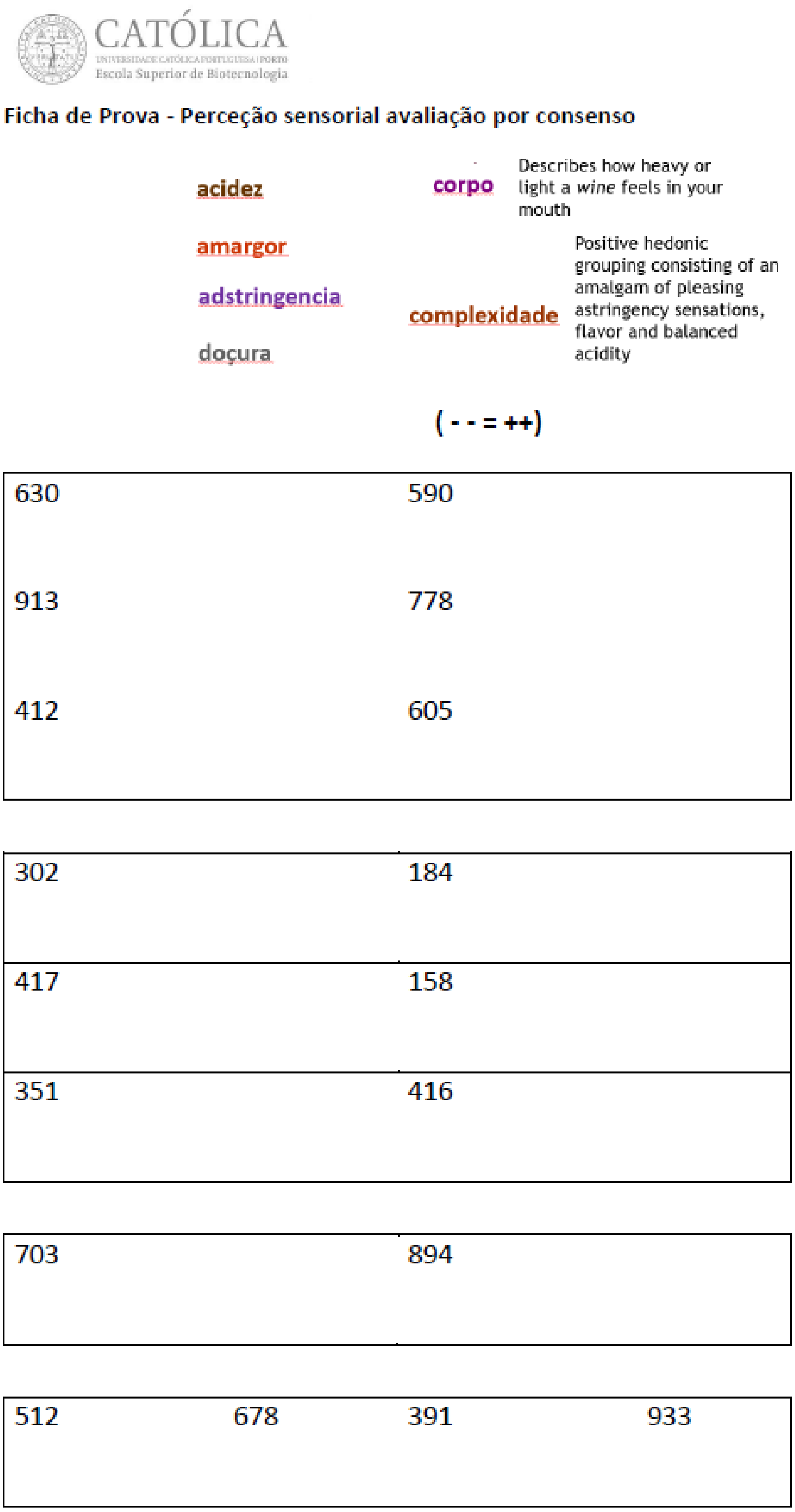
**Table I.4**

| *Mouthfeel Descriptor* | *Definition* |
|---|---|
| **Body** | Describes how heavy or light a wine feel in your mouth (consistency. compactness of texture. fullness. richness. flavour or substance of a product |
| **Complexity** | Positive hedonic grouping consisting of an amalgam of pleasing astringency sensations. flavour. and balanced acidity. |

Panellists were instructed to taste spiked wines (Table I.5) and describe the main differences found ( - - = ++) regarding Acidity. Astringency, Bitternes,. Sweetness, Body and Complexity.

**Table I.5**

| *Wine Samples* | *Product added to commercial wine* | *Concentration* |
|---|---|---|
| *630 and 590* | Solo Dark | 240 mg/L |
| *913 and 778* | Opera Seed | 240 mg/L |
| *412 and 605* | Glycerol | 6 g/L |
| *302 and 184* | Opera Grape | 240 mg/L |
| *417 and 158* | Quinine | 0.8 g/L |
| *351 and 416* | Cafeine | 0.6 g/L |



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**Ficha de Prova - Perceção sensorial avaliação por consenso**

acidez

amargor

adstringencia

doçura

corpo - Describes how heavy or light a *wine* feels in your mouth

complexidade - Positive hedonic grouping consisting of an amalgam of pleasing astringency sensations, flavor and balanced acidity

( - - = ++)

| | |
|---|---|
| 630 | 590 |
| 913 | 778 |
| 412 | 605 |

| | |
|---|---|
| 302 | 184 |
| 417 | 158 |
| 351 | 416 |

| | |
|---|---|
| 703 | 894 |

| | | | |
|---|---|---|---|
| 512 | 678 | 391 | 933 |

**Figure I.5** Sensory perception test consensus evaluation sheet

### I1.6 Discrimination and description training tests

Panellists also evaluated the base wines, spiked with commercial tannins at different levels, to further understand the ranges of astringency perceived in wine.

With the aim of training and leveling the tasters for phase2, discrimination tests (triangular tests) (Table I.6) were applied. In each triangle test instructions were given to taste the samples (left to right order) and mark the “different” sample. It was also asked to describe the difference found.

**Table I.6** Triangular Tests

| | ***Product Added -Proenol*** | ***Concentration*** |
|---|---|---|
| *Test1* | Opera Seed | 80 mg/L |
| *Test2* | Solo Ruby | 160 mg/L |
| *Test3* | Solo Dark | 80 mg/L |
| *Test4* | Crystal Balance | 3 g/L |

**Table I.7** Description of enological products

| | ***Enological Product Description*** |
|---|---|
| **Opera Seed** | Proanthocyanidin condensate extracted from ripe grape pips. Reinforces the balance of wines as it contributes as a complement to the tannic structure. |
| **Solo Ruby** | Extreme quality French oak ellagic Increases the wine's power. volume. structure. intensity of its aromas. as well as its sweetness. |
| **Solo Dark** | High quality American oak ellagic tannin. Provides tannins that become extremely smooth in the mouth. but present and structuring. accompanied by volume and persistence. |
| ***Crystal Balance*** | Cellulose Gum Solution Carboxymethylcellulose (CMC) solution at 5%. For inhibiting the formation and growth of potassium bitartrate crystals in wines. ensuring their stabilization over time. |
| **Opera Grape** | Grape proanthocyanidin. Provides powerful but extremely round and integrated tannins. Contributes to a significant increase in structure and persistence. through ripe. smooth. and very round tannins. |

In the last training session two additional tests were carried out using synthetic wine spiked with tannic acid (TA) and Glycerol (G).

Panellist were instructed to taste the samples in the presented position and to order them from least astringent to most astringent. (TA addition) (Table I.8) Test1 and from least bodied to most bodied (glycerol addition). (Table I.8) Test2

**Table I.8** *TEST 1* *TEST 2*

| **SWL+(TA)** | **TA g/L** | **SWS+(G)** | **G g/L** |
|---|---|---|---|
| 1 | **1.40** | **1** | **17.6** |
| 2 | **1.00** | **2** | **12.6** |
| 3 | **0.40** | **3** | **7.6** |
| 4 | **0.20** | **4** | **3.8** |
| 5 | **0.08** | **5** | **0.0** |
| 6 | **0.00** | | |

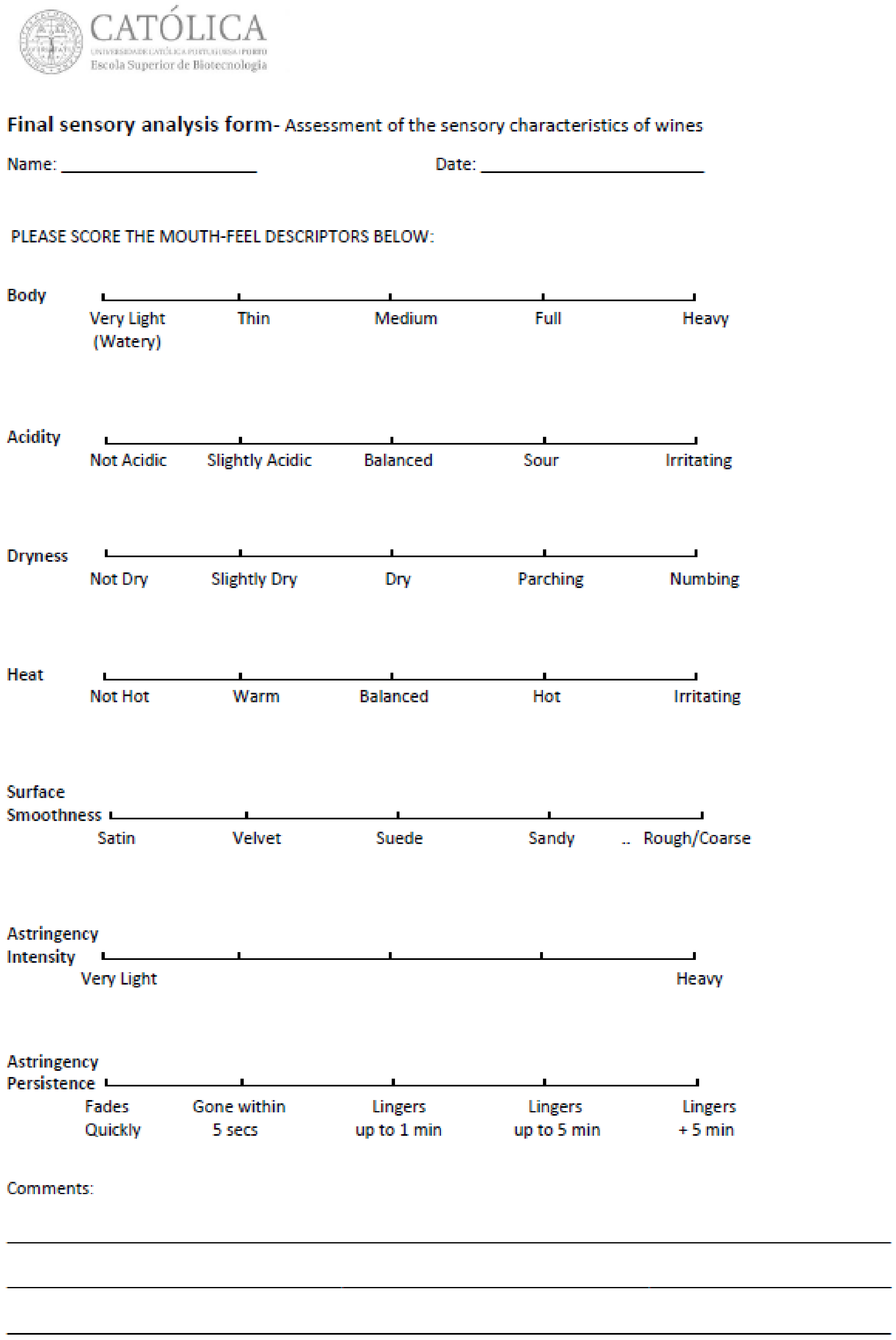
**Synthetic wine solution (SWS)** - 60 g/L Ethanol + 2 g/L Tartaric acid + 10 g/L Sugar

Also. in this session, tree additional triangular tests were applied and the test form that would be used in phase 2 sessions was presented.to panellist (Figure I.6)

The panellist was instructed to taste the wine indicated as reference (REF) and to memorize its characteristics. Then they were instructed to taste the wines with the three-digit numbers and mark which one was the same as the REF The 5 bottles of red wine used were provided by Amorim Cork S.A.

At the end of phase 1 it was ensured that the panellists were familiar with duo-trio test. (format, task and evaluation procedure) the assessors possess the same level of qualification and knowledge of astringency perception and knew the test objectives as required by ISO 8586.

In addition. consensus was achieved during the training sessions through discussions to minimize the carry-over effects between samples. the panellists agreed that the use of break with ingestion of milk chocolate and bread with ham helped them to better discriminate between the samples. especially for the evaluation of astringency.



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**Final sensory analysis form-** Assessment of the sensory characteristics of wines

Name: ____________________ Date: ______________________

PLEASE SCORE THE MOUTH-FEEL DESCRIPTORS BELOW:

| | | | | | |
|---|---|---|---|---|---|
| Body | Very Light (Watery) | Thin | Medium | Full | Heavy |
| Acidity | Not Acidic | Slightly Acidic | Balanced | Sour | Irritating |
| Dryness | Not Dry | Slightly Dry | Dry | Parching | Numbing |
| Heat | Not Hot | Warm | Balanced | Hot | Irritating |
| Surface Smoothness | Satin | Velvet | Suede | Sandy | .. Rough/Coarse |
| Astringency Intensity | Very Light | | | | Heavy |
| Astringency Persistence | Fades Quickly | Gone within 5 secs | Lingers up to 1 min | Lingers up to 5 min | Lingers + 5 min |

Comments:

______________________________________________________________________

______________________________________________________________________

______________________________________________________________________

**Fig I.6** Final sensory analysis form- Assessment of the sensory characteristics of wines. Adapted from (King *et al*., 2003 and Rankine. 1990)