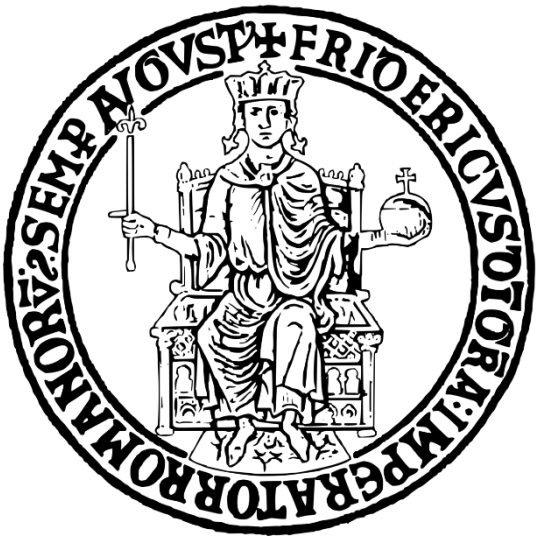
University of Naples Federico II
Department of Agricultural Sciences



PhD in Food Sciences

XXXIII Cycle (2018-2021)

Interactions between Aroma Compounds and Polyphenols in Red Wine: Study of their Effects on Sensory Properties through Combined Chemical and Sensory Analytical Approaches

PhD Coordinator

Professor Amalia Barone

PhD Candidate

Elisabetta Pittari

PhD Supervisor

Professor Paola Piombino

Contents

Abbrevations

AHC: Agglomerative Hierarchical Clustering

BCPs: Base Chemical Parameters

DP: Polymerization Degree

GC-FID: Gas Chromatography-Flame Ionized Detector

GC-MS: Gas Chromatography-Mass Spectrometer

GC-O: Gas Chromatography-Olfactometer

HS-SPME: Headspace-Solid Phase Micro Extraction

MDS: MultiDimensional Scaling

MF: Modified Frequency

MUC1: Mucin1

NMR: Nuclear Magnetic Resonance

OAV: Odour Active Value

PCA: Principal Component Analysis

PPhs: Polyphenols

PRPs: Proline-Rich Proteins

PTR-ToF-MS: Proton Transfer Reaction – Time of Flight – Mass Spectrometer

RAS: Retronasal Aroma Simulator

RATD: Retronasal Aroma Trapping Device

QDA: Quadratic Discriminant Analysis

SPME: Solid Phase Micro Extraction

TA: Titratable Acidity

TPC: Total Polyphenol Content

TPI: Total Polyphenol Index

TDS: Temporal Dominance of Sensations

VA: Volatile Acidity

VOCs: Volatile Organic Compounds

Preface

Polyphenols and volatile organic compounds are responsible for two of the main characteristics in defining complexity and quality of wines and for the two main intrinsic drivers of wines consumers purchasing decisions: astringency (mainly for red wines) and aroma perception. It is nowadays known that to fully understand wine chemical and sensory characteristics (i.e., odours, astringency, tastes, flavour, aromas), besides identification and quantification of wine volatile and non-volatile components, it is necessary to study their interactions, that impact wine sensory perception and quality. In literature, these interactions, and those specifically referred to polyphenols and volatiles and between the sensory stimuli elicited by these wine constituents, have been not deeply studied and enough comprehended even if it is a subject of interest for wine researchers and producers to understand consumers' perceptions and choices as well as for precision oenology purposes. Moreover, the subject is of transversal concern in the food field since polyphenols are largely present in other food matrices and because of their antioxidant and healthy properties. Indeed, nowadays the population is turning toward healthier and more sustainable plant-based food products, often discarded by the consumer due to their bitterness/astringency characteristics. Consequently, all scientific knowledge helping in understanding how to smoothen/mask these sensations are welcomed by scientists and food technologists/engineers working in the food field.

Therefore, for their importance and because of the current concern in food research and oenology, the interactions between wine volatile compounds and polyphenols and their physicochemical and sensory effects, represent the main subject of interest of the present PhD thesis. Finally, since polyphenols (e.g., tannins), exhibit antioxidant properties, an overview about the actual knowledge of their modulating role in the protection toward the oxidation of wine aromas, will be also illustrated.

Prefazione

I polifenoli e i composti organici volatili sono responsabili di due caratteristiche fondamentali per la qualità dei vini e di due principali drivers intrinseci del consumo di vino: l'astringenza (soprattutto per i vini rossi) e la percezione dell'aroma. È ormai noto che per comprendere appieno le caratteristiche chimiche e sensoriali del vino (es. odori, astringenza, gusti, flavour, aromi), oltre all'identificazione e quantificazione delle componenti volatili e non volatili del vino, è necessario studiare le loro interazioni, che influiscono sulla percezione sensoriale e sulla qualità del vino. In letteratura queste interazioni, e quelle specificamente riferite ai polifenoli e ai volatili e tra gli stimoli sensoriali originati da questi composti, non sono state studiate a fondo e sufficientemente comprese anche se è argomento di interesse per ricercatori e produttori di vino al fine di capire la percezione e le scelte dei consumatori, oltre che per obiettivi di enologia di precisione. Inoltre, l'argomento è di interesse trasversale in campo alimentare poiché i polifenoli sono largamente presenti in altre matrici alimentari e per le loro proprietà antiossidanti e salutari. Oggi, infatti, la popolazione si sta orientando verso prodotti alimentari a base vegetale più sani e sostenibili, spesso scartati dal consumatore a causa delle loro caratteristiche amare e astringenti. Di conseguenza, tutte le conoscenze scientifiche che aiutano a capire come attenuare/mascherare queste sensazioni sono benvenute da scienziati e tecnologi/ingegneri alimentari che lavorano nel campo alimentare.

Pertanto, per la loro importanza e per l'attuale interesse nella ricerca alimentare ed enologica, le interazioni tra composti volatili e polifenoli del vino ed i loro effetti fisico-chimici e sensoriali, rappresentano il principale argomento di interesse della presente tesi di dottorato. Infine, poiché i polifenoli (ad es. i tannini), presentano proprietà antiossidanti, verrà anche illustrata una panoramica sull'effettiva conoscenza del loro ruolo modulatore nella protezione verso l'ossidazione degli aromi del vino.

Background

Part of the content of the present Section is included in:

Interactions between polyphenols and volatile compounds in wine:

a literature review on physicochemical and sensory insights

Pittari, E.[1], Moio, L.[1] & Piombino, P. [1, *]

[1] Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, 83100 Avellino, Italy

* Corresponding author:

Professor Paola Piombino

Published in ***Applied Sciences, 2021, 11(3), 1157,***
DOI: 10.3390/app11031157

1. General Introduction

Wine has always been considered a protagonist in the culture and history of the Mediterranean civilization. Writers, poets, scientists have written and continue to write about what is still called "The Nectar of the Gods". There are those who, like Pablo Neruda, writes: "Day-coloured wine, night-coloured wine, wine with purple feet or wine with topaz blood, wine, starry child of earth"; or who like Ernest Hemingway, pays homage to wine, defining it as: “One of the most civilized things in the world and one of the most natural things of the world that has been brought to the greatest perfection, and it offers a greater range for enjoyment and appreciation than, possibly, any other purely sensory thing.”

It is nowadays known that wine consumption has a fundamental importance in our tradition, importance attributable, amongst other characteristics, to its strong communicative power that comes from its sensory properties. Indeed, in the last decades, wine demand has constantly moved from an everyday and nutritional purpose to the pleasure of drinking and, therefore, to the consumption of quality products (Corduas et al., 2013 and references therein).

Wine is a complex food product, consisting of a wide and diverse range of chemical compounds, whose composition and quality depend on several factors, among which type and quality of grapes, *terroir*, applied viticultural practices and processing techniques employed during winemaking, from harvesting to commercialization, play a fundamental role (Jackson & Lombard, 1993).

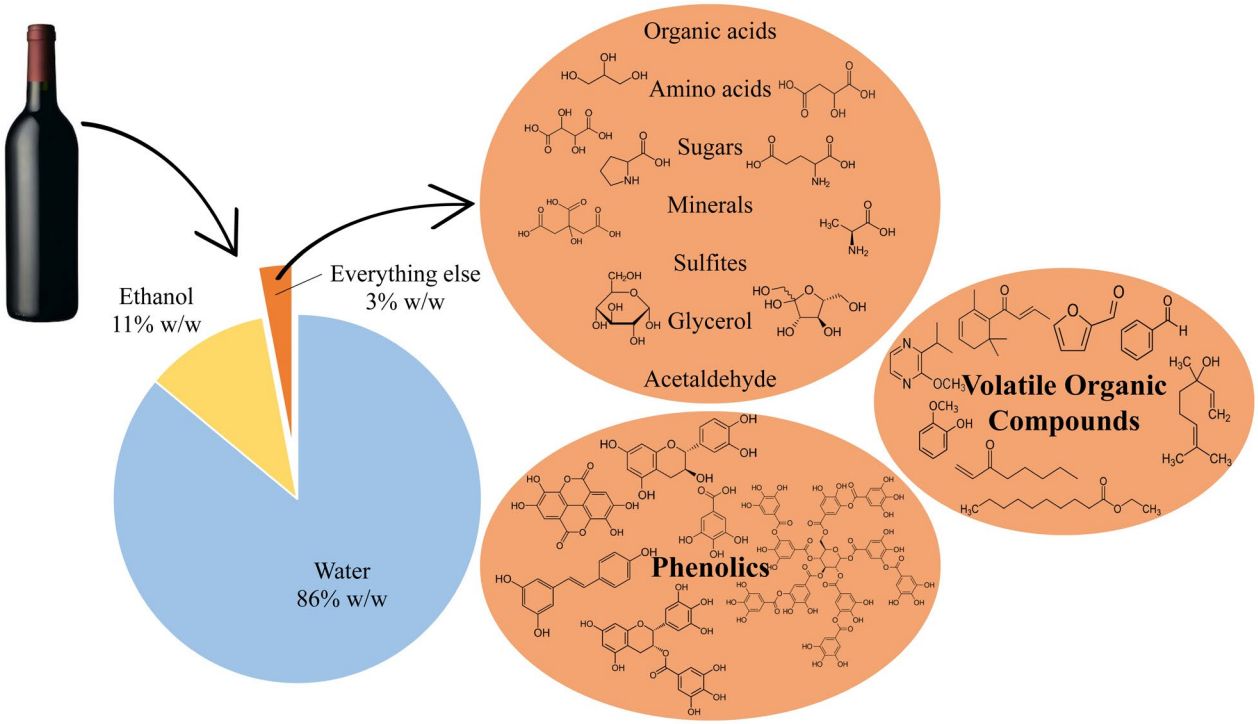
Figure 1 shows the composition of a representative dry red table wine, expressed in % weight-for-weight (w/w) basis. Water and ethanol, which constitute about 97% (w/w) of its composition, are the two main components of this alcoholic beverage, with water occupying its 86% (w/w). The remaining 3% (w/w) is characterised by many volatile and non-volatile components belonging to several chemical classes, (Waterhouse et al., 2016 and references therein).

These “minor” compounds, even if present at low concentrations (lower than 10 g/L) result extremely important for wine quality, being responsible for most of its oenological and sensory characteristics, such as colour and flavour (odour/aroma, taste, mouthfeel) (Waterhouse et al., 2016 and references therein).

However, among them, polyphenols (PPhs) and volatile organic compounds (VOCs) are responsible for two of the most important wine hedonic properties, and they are liable for two of the main characteristics in defining

complexity and quality of wines and for the two main intrinsic drivers of wines consumers purchasing decisions: astringency (mainly for red wines) and aroma perception (Peynaud, 1987; Green, 1993; Jover et al., 2004; Charters & Pettigrew, 2007; King et al., 2010; Sáenz-Navajas et al., 2016; Soares et al., 2017).

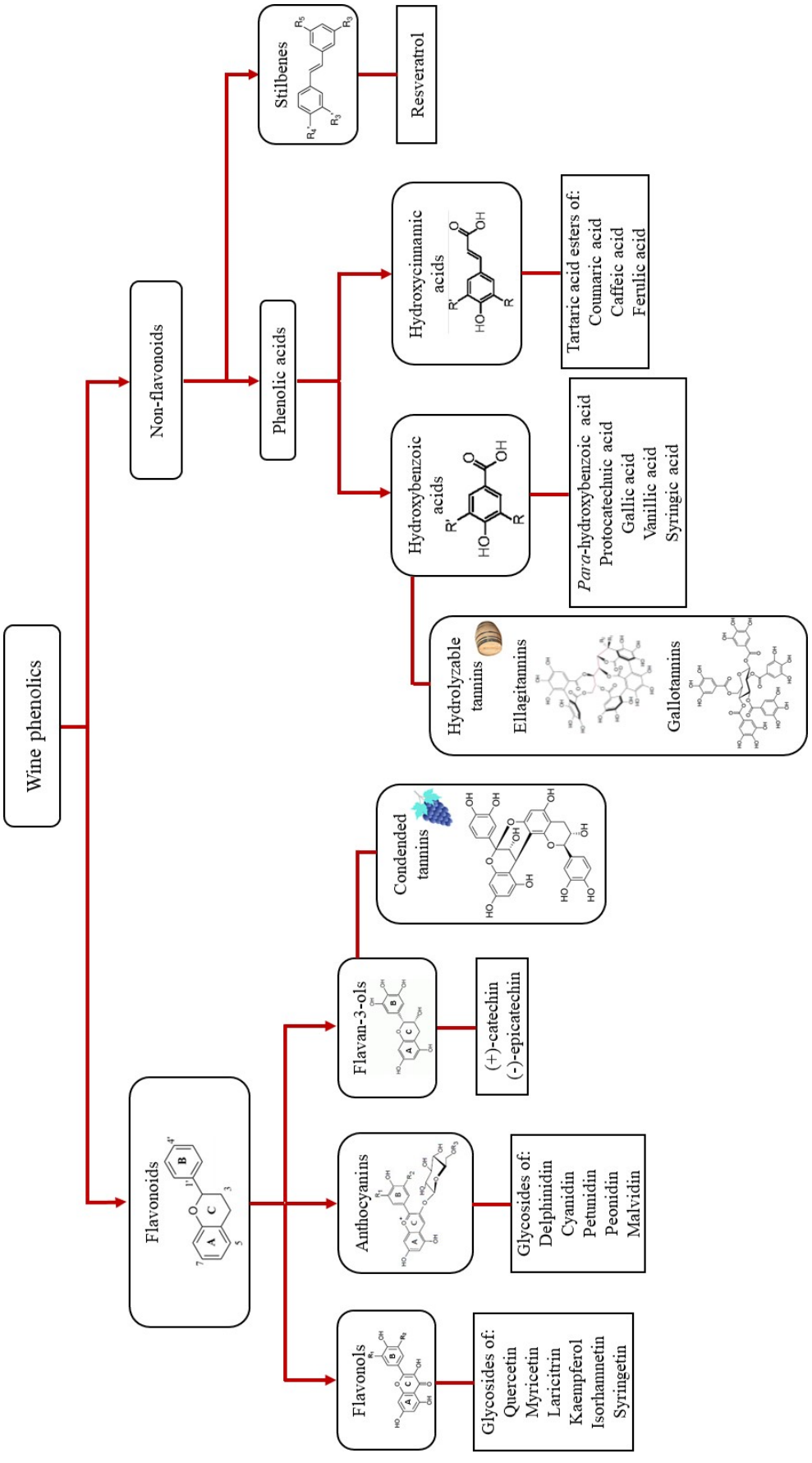
Figure 1. Composition of a representative dry red table wine expressed in % w/w. Adapted from Waterhouse et al. (2016).



1.1. Wine polyphenols and their sensory properties

Characterised by the same hydroxy-substituted benzene ring structure, many phenolic compounds, usually divided in non-flavonoids and flavonoids compounds, have been identified in grapes and wines, as shown in Figure 2. Since their oenological interest is multifactorial – as they are responsible for wine colour and its stability, for wine longevity thanks to their antioxidant activity, and for important wine's oral characteristics, such as astringency and bitterness – polyphenols biosynthesis, chemical, physicochemical, and sensory properties have been widely studied in the literature.

Figure 2. Classification of wine phenolics. Adapted from Gambuti & Moio (2017).



Non-flavonoid compounds include phenolic acids [hydroxybenzoic (C6-C1) and hydroxycinnamic (C6-C3) acids], and stilbenes (Kennedy et al., 2006; Rentzsch et al., 2009; Waterhouse et al., 2016). The most abundant hydroxybenzoic acids are represented by *para*-hydroxybenzoic, protocatechuic, gallic, vanillic, and syringic acids (Baderschneider & Winterhalter, 2001). Gallic acid, mainly present in grape seeds, is considered the most important phenolic acid, since it represents the precursor of all hydrolysable tannins. Hydroxycinnamic acids are normally found in grapes and wines as hydroxycinnamates, which are tartaric acid esters of coumaric, caffeic and ferulic hydroxycinnamic acids. Hydroxycinnamic acids and hydroxycinnamates represent the major phenolic compounds in white wines – responsible for their colour – and in red and white grape juices; moreover, they are the main class of non-flavonoid phenolics in red wines (Kennedy et al., 2006; Rentzsch et al., 2009; Ugliano & Henschke, 2009; Waterhouse et al., 2016). Finally, another group of non-flavonoids is represented by stilbenes. The most important stilbene compound, resveratrol, is a phytoalexin used by vines to defend themselves from *Botrytis cinerea* attacks and other forms of aggression (Langcake & Pryce, 1976; Siemann & Creasy, 1992). It is a compound of great interest since it is an antioxidant and a free radical scavenger, and therefore it plays an important role in human and animal biological and therapeutic activities (Savouret & Quesne, 2002). Apart from hydroxycinnamic acids, non-flavonoid compounds are found as non-coloured compounds and, in most newly fermented wines, they are present at low concentrations (Kennedy et al., 2006; Rentzsch et al., 2009; Waterhouse et al., 2016).

Flavonoids are characterised by a C6-C3-C6 skeleton. In large sense, flavonoids include anthocyanins, flavan-3-ols, flavonols, flavanonols, and flavones (Baderschneider & Winterhalter, 2001). Among them, flavan-3-ols, flavonols and anthocyanins are particularly important to the quality of red wines (Waterhouse et al., 2016 and references therein). Flavan-3-ols are the most abundant class of flavonoids in grapes. They are benzopyrans with a saturated carbon chain between C2 and C3 and a hydroxyl function in C3. In grapes the most abundant flavan-3-ols monomers are (+)-catechin and its isomer (-)-epicatechin (Garrido & Borges, 2013). Flavonols are characterized by the presence of a keto group at position C4, a double bond between atoms C2 and C3, and a hydroxyl group in C3. In grapes they are present mainly in the glycosilated form of the six main aglycones: quercetin, myricetin, laricitrin, kaempferol, isorhamnetin and syringetin. Finally, anthocyanins are flavonoids compounds responsible for the colour of red grapes and wines. They are glycosides of the delphinidin,

cyanidin, petunidin, peonidin and malvidin anthocyanidins, characterised by a flavylium cation-based structure (Waterhouse et al., 2016 and references therein).

Even if the term *tannin* is widely used to describe all polymeric polyphenols (Waterhouse et al., 2016), tannins represent a wide class of phenolic compounds characterised by high molecular weight, and usually divided in non-flavonoids polymers, namely hydrolysable tannins, and oligomers and polymers of flavan-3-ols, namely condensed tannins or proanthocyanidins (Lesschaeve & Noble, 2005). Hydrolysable tannins are hydroxybenzoic acids polymers, usually composed of two subgroups: gallotannins and ellagitannins, that are polyol (generally D-glucopyranose) respectively esterified with either gallic acids or ellagic acids. They are extracted from oak barrels or chips during ageing or added as oenological tannins during winemaking processes (Sarneckis et al., 2006; Versari et al., 2013). Condensed tannins or proanthocyanidins are naturally present in red wines, since they are extracted from grapes seeds and skins during the maceration process and then modified during winemaking and ageing processes (Waterhouse et al., 2016). They are polymers composed of flavan-3-ol units. Proanthocyanidins differ in their constitutive units [(+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin], their sequences, the positions of interflavanic linkages, (C4-C6 or C4-C8 in the B-type series, with additional C2-O-C7 or C2-O-C5 bonds in A-type structures), their lengths and the presence of substituents (e.g., galloyl or glucosyl groups) (Cheynier, 2005; Versari et al., 2013).

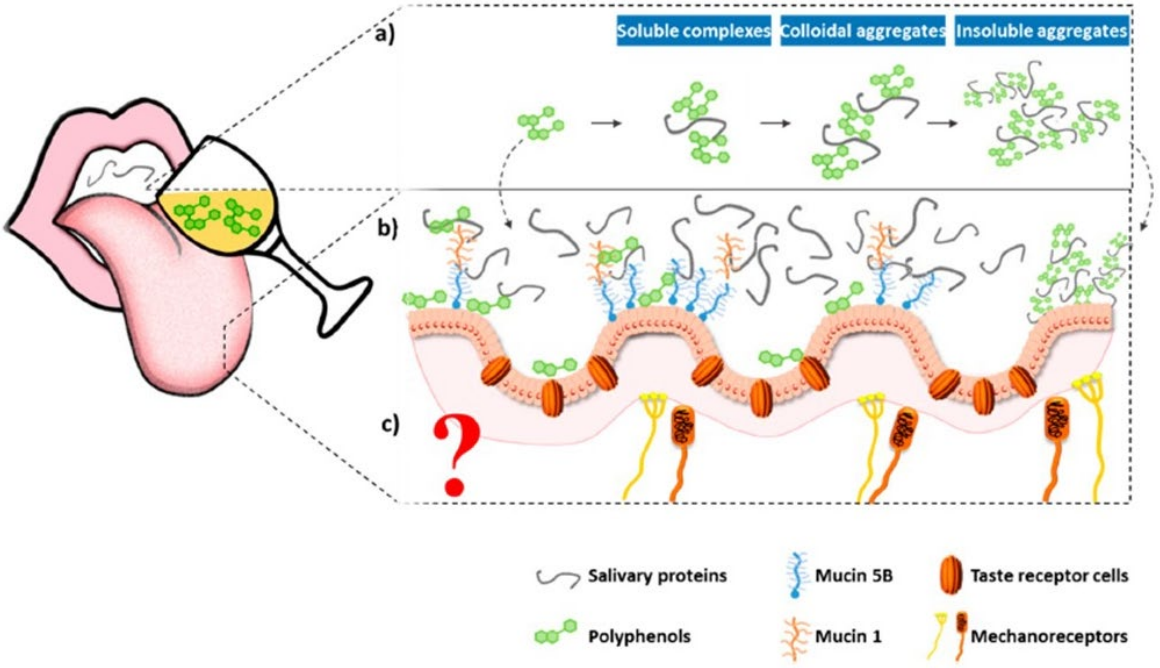
Among all phenolic compounds, flavan-3-ols monomers [(+)-catechin and (-)-epicatechin], their oligomers and polymers (condensed tannins or proanthocyanidins) and hydrolysable tannins are the most abundant phenolic compounds in wine (Lesschaeve & Noble, 2005).

1.1.1. In-mouth sensory properties elicited by polyphenols

From a sensory point of view, tannins are involved in major oral sensations, such as astringency and bitterness, as reviewed recently (Soares et al., 2020). The American Society for Testing of Materials defined 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).

Over the years, several theories have been developed to describe astringency perception mechanisms, as represented in Figure 3.

Figure 3. Proposed mechanisms for astringency onset: (a) interaction and precipitation of salivary proteins; (b) interaction of phenolic compounds (PC) with oral cells and/or mucosal pellicle; (c) activation of oral mechanoreceptors. Cited from Soares et al. (2020).



Being able to interact and precipitate proteins by forming noncovalent complexes mostly correlated to conformationally accessible hydrophobic regions of both molecules (Haslam & Lilley, 1988; Spencer et al., 1988; Hatano & Hemingway, 1996), the interaction between astringent agents (i.e., tannins) and salivary proteins has been proposed as one of the main phenomena in explaining wine astringency perception. Initial steps of astringency perception imply a face-to-face stacking between the aromatic groups of polyphenols and the carbon–hydrogen skeleton of the pyranic rings of condensed tannins with surface exposed amino acid residues of salivary proteins. These complexes, in subsequent aggregation and precipitation steps, cause a drying and grainy sensation in the mouth that decreases salivary lubrication between oral tissues and increases friction in the oral cavity (Bate-Smith, 1954, 1973; Breslin et al., 1993; Kallithraka et al., 1998; Jöbstl et al., 2004; Bajec & Pickering, 2008; McRae & Kennedy, 2011; Soares et al., 2011; Soares et al., 2017). These tannins-proteins interactions depend on several characteristics of both tannins (i.e., size, structure, charge, position of the galloyl group) and proteins (i.e., amino acid composition, spatial structure, size, charge). Even if it is difficult to find a common principle, in general terms greater interactions between proteins and tannins occur when tannins are characterized by a higher polymerization degree, molecular weight, and number of galloyl groups (galloylation degree) apparently because the number of interaction sites increase with size

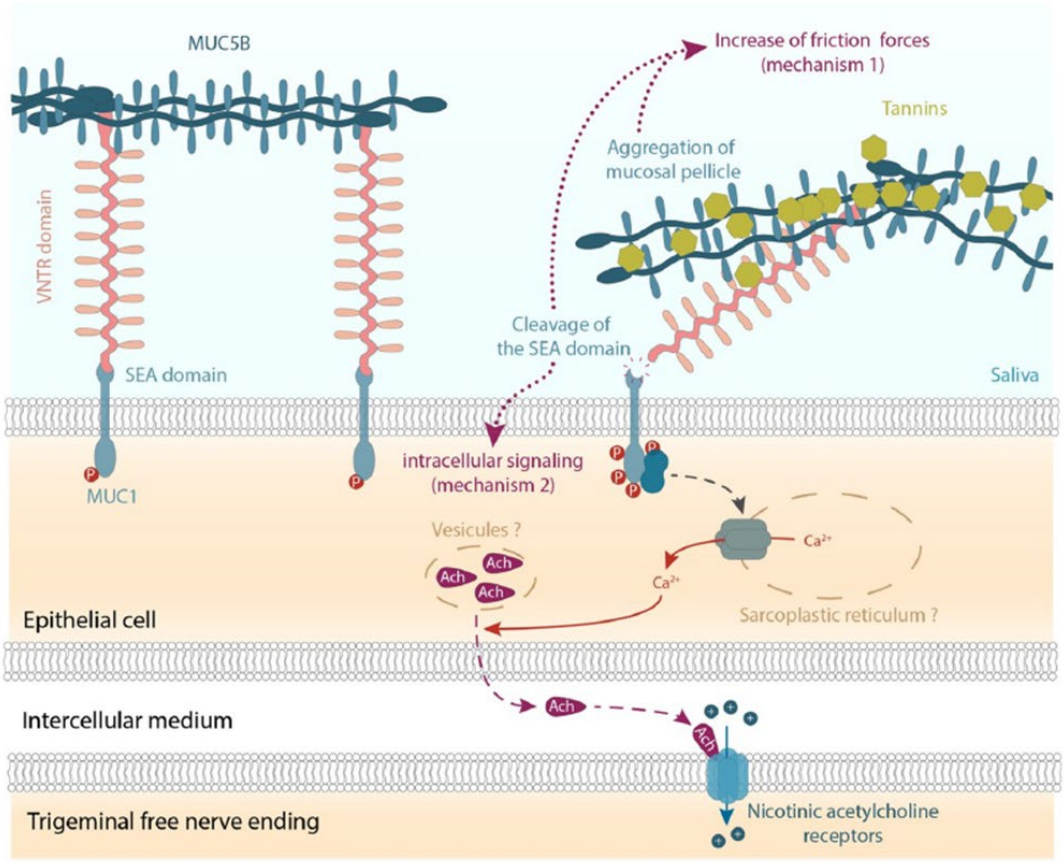
(Ricardo-da-Silva et al., 1991; Cheynier et al., 1992; De Freitas & Mateus, 2012). Regarding proteins, proline-rich proteins (PRPs) normally display a stronger affinity for tannins. This is because this amino acid, not allowing the formation of the alpha helices, gives to the protein an open form more accessible to tannins (Hagerman & Butler, 1981). However, is it important to highlight that the molecular mechanisms by which the aggregates could generate the sensation of astringency remains unknown (Canon et al., 2021).

While this tactile nature of astringency has been investigated since 1954 (Bate-Smith, 1954), astringency has also been defined as a trigeminal sensation. This "lubrication" theory of astringency asserts that after astringent compounds strip the oral cavity of mucosal and epithelial proteins that confer lubrication, the increased friction between the surfaces of the oral cavity activates mechanosensors of somatosensory nerves located in the mouth and trigeminal nerve (Lyman & Green, 1990; Charlton et al., 2002; Bajec & Pickering, 2008; Chen & Engelen, 2012; Jiang et al., 2014; Schöbel et al., 2014). However, specific data about which mechanoreceptors are activated is still unknown.

Very recently, Canon et al. (2021) proposed an alternative hypothesis on the astringency molecular mechanism, represented in Figure 4. This hypothesis involves tethered MUC1 (mucin 1) since this protein is involved in both the lubrication of the epithelial cell surface and the anchoring of the salivary proteins. Authors suggested two sequential mechanisms that could be involved in astringency perception. In the first mechanism, "the dissociation of MUC1 induces the release of one of its subunits (α-subunit) that ensures the lubricating properties of the protein. At the same time, MUC1 dissociation could pull out the mucosal pellicle, increaing the friction forces at the surface of the oral mucosa". In the second step "the SEA module (sea urchin sperm protein domain of the α-subunit) dissociation induces an intracellular signal, leading to the release of neurotransmitters that activate the free ends of the trigeminal nerve located in the oral epithelium".

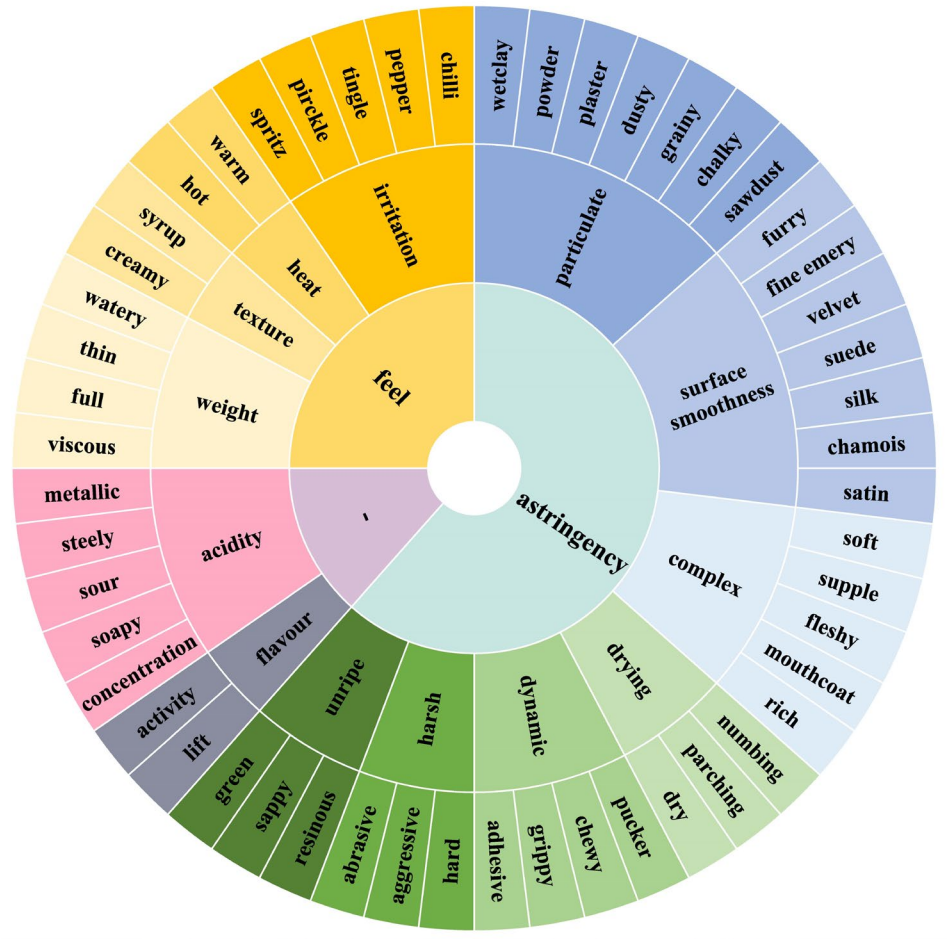
It is important to highlight that elucidating the molecular mechanisms underpinning astringency perception is an always actual research topic in the food industry; indeed, despite decades of research, the exact chemosensory mechanisms at the base of this sensation, as such as the nature of the receptors activated remain unknown.

Figure 4. Alternative hypothesis on the molecular mechanisms underpinning astringency sensation. The aggregation of the mucosal pellicle by tannins will lead to the disruption of the two subunits of MUC1 inducing two different mechanisms: (1) pull out of the mucosal pellicle and (2) intracellular signaling, leading to the release of neurotransmitters. Cited from Canon et al. (2021).



Because of its wide complexity, astringency, together with other red wine mouthfeel properties, has been arranged into a hierarchical vocabulary. In this vocabulary, defined as “The red wine mouth-feel wheel”, astringency sensation is described with 33 different terms, grouped in seven categories, among which two terms are frequently referred to smoother astringency characteristics (complex and surface smoothness), while the other five usually describe stronger stimuli (drying, harsh, unripe, dynamic, and particulate) (Gawel et al., 2000) (Figure 5).

Figure 5. Red wine Mouth-feel Wheel – showing first-, second-, and third-tier terms – used to describe the mouth-feel characteristics of red wine.
Adapted from Gawel et al. (2000); created with XLStat Sensory.



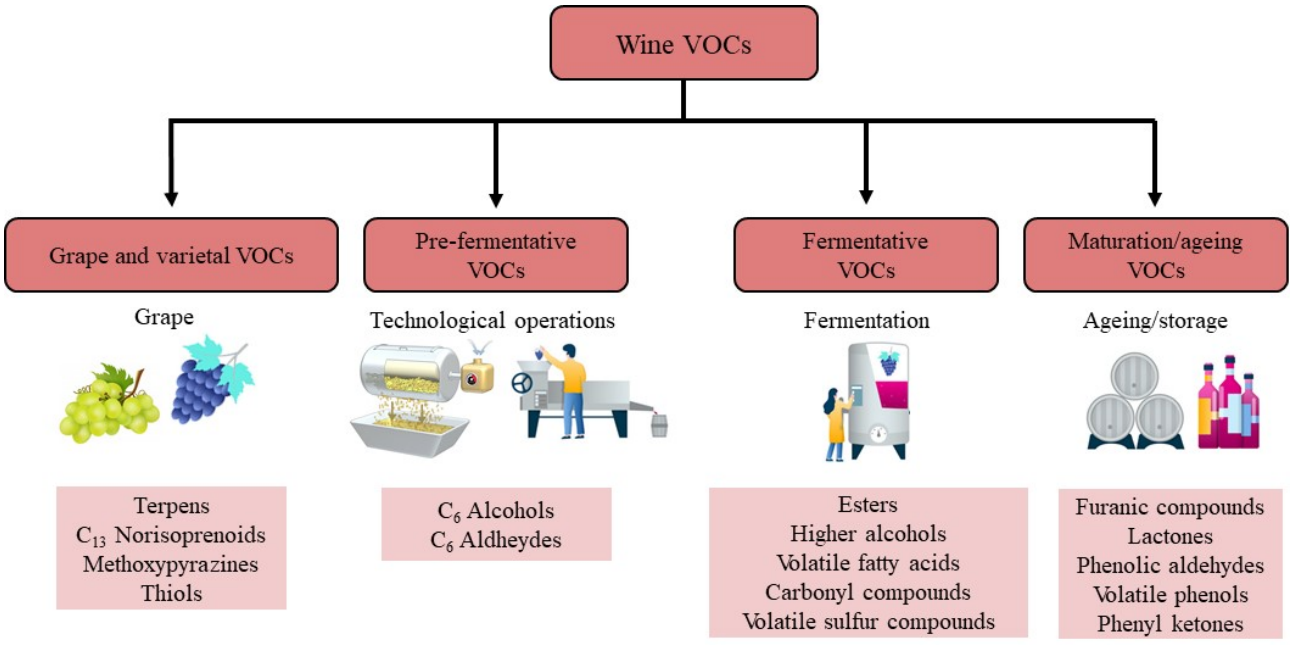
Besides astringency, even if information is scarce (Soares et al., 2020), there is scientific evidence showing that polyphenols can be additionally responsible for the perception of bitterness in wine (Robichaud & Noble, 1990; Peleg et al., 1999; Hufnagel & Hofmann, 2008; Soares et al., 2013; Soares et al., 2017; Soares et al., 2018). Indeed, it has been observed that (epi)catechin monomers are more bitter than dimers (procyanidins B3 more bitter than B4, and B6), and trimers (trimers C1 and C2) (Peleg et al., 1999). Moreover, recent results have suggested that several phenolic compounds, such as pentagalloylglucose hydrolysable tannins, (-)-epicatechin, procyanidin trimer C2, procyanidin B2-3-O-gallate and some ellagitannins activate bitter taste receptors (Soares et al., 2013; Soares et al., 2018).

Astringency and bitterness are key factors for food selection. Aprioristically, when they are perceived in high intensities, they can impair the consumption of a particular food product. However, even if astringency and bitterness represent repulsive sensations for consumers, when well balanced with the other oral sensations, they can add structure/body and persistence to red wines (Varela & Gambaro, 2006) and can be perceived as strongly linked to its quality (Saenz-Navajas et al., 2012 and references therein).

1.2. Wine aroma compounds and olfactory characteristics

More than 800 VOCs have been identified in wines, with a concentration range varying from hundreds of mg/L to µg/L or ng/L levels (Li, 2006). However, only some of them work as odour-active molecules, mainly in reason of concentrations above their sensory perception threshold but also because of synergistic or masking effects at peri/sub-threshold levels (Guth 1997; Culleré et al., 2004; Zhang et al., 2007; Li et al., 2008; Ferreira, 2010; Cameleyre et al., 2018 and references therein). In wine, VOCs are divided in: (i) grape and varietal VOCs; (ii) pre-fermentative VOCs; (iii) fermentative VOCs; (iv) maturation/ageing VOCs (Ruiz et al., 2019), as shown in Figure 6.

Figure 6. Classification of wine aroma according to its origin and chemical families predominant in each type of aroma. Adapted from Perestrelo et al. (2020).



Most of wine aromas, and the most important contributors to the overall wine "bouquet", belong to the third cited group (fermentative VOCs), and are therefore produced or released during wine fermentation due to microbial activity (Bartowsky 2005; Belda et al. 2016). However, each group and family of aroma compounds, as such as the complex matrix in which these compounds are dissolved, varies greatly among different types of wines, depending on several variables (e.g., *terroir*, grape variety, microbial starter, fermentation process, aging, bottling, etc.) with different predominant aromas in each case, conferring a specific olfactory profile to each wine.

Grape and varietal VOCs are constituted by different chemical compounds; their origin is grapes since they are present in the cells of berries. These chemical compounds can be found in two forms. They exist as free volatile molecules – methoxypyrazines, varietal thiols and monoterpenoids – and in the form of flavorless precursors (glycosidic, aminoacidic) – unsaturated fatty acids, phenolic acids, S-cysteine conjugates, dimethylsulfide precursors, carotenoids and glycoconjugates (Baumes et al., 2009; Ruiz et al., 2019). Due to the metabolic activity of yeasts during fermentation, these non-odorant precurosos are transformed to aromas that are of great relevance in the sensory perception of wines. In general, grape and varietal VOCs are more concentrated in grape skins; therefore, increasing the contact between grape skins and must leads to a higher concentration of these compounds in the must (Castro-Vázquez et al., 2002; Tamborra et al., 2004; Sánchez-Palomo et al., 2006). The sensory detection threshold of grape varietal aroma compounds is low (μg/L or ng/L), despite their very low concentrations. This explains why these VOCs have a strong effect on aroma and flavour (Waterhouse et al., 2016 and references therein). Wine alkyl-2-methoxypyrazines (MPs) aroma is generally described as green, bell pepper, vegetal, earthy, leafy, peas, asparagus, musty, raw potatoes (Mozzon et al., 2016 and references therein). Volatile thiols are of great importance for the organoleptic quality of wines because of their contribution to the pleasant 'herbaceous' (e.g., boxwood, broom), 'fruity' (e.g., grapefruit and citrus zest, passion fruit), 'mineral', 'smoky', and 'toasty' aromas associated to highly molecular weights and low volatility molecules (Rauhut, 2017 and references therein). Finally, the most important and odoriferous monoterpenoids in wines are linalool, (E)-hotrienol, citronellol, geraniol, nerol, α-terpineol (monoterpene alcohols), and (−)-cis-rose oxide. Their identification and quantification are of great interest in the oenological research since they are responsible for the typical floral, fruity, and citrus (Strauss et al., 1986) aroma "bouquet" of wines.

Pre-fermentative aromas are VOCs formed during the first processing steps such as crushing, pressing, and skin contact, or by thermal, chemical, and enzymatic reactions in the must. The main pre-fermentative VOCs are represented by six carbon atoms (C_6) aldehydes and alcohols (Crouzet, 1986). Several C_6 aldehydes and alcohols have been identified in grapes (e.g., 1-hexanal, cis-2-hexenal, trans-2-hexenal, cis-3-hexenal, 1-hexanol, 2-hexenol, trans-2-hexenol, cis-2-hexenol, trans-3-hexenol, cis-3-hexenol), all characterised by low olfactory thresholds (in the range of µg/L), and responsible for herbaceous and unripe fruit aromas. However, while at low concentrations (indicatively less than 0.5 mg/L), they may contribute to the overall wine aroma complexity, at high doses (few mg/L) they can be responsible for wine undesirable vegetal notes (Ribereau-Gayon et al., 2006).

Fermentation VOCs derive from (i) the alcoholic fermentation, the anaerobic biochemical process of sugars conversion to ethanol, carbon dioxide and energy, conducted by *Saccharomyces* wine yeasts, and (ii) the malolactic fermentation, the enzymatic decarboxylation of L-malic acid to L-lactic acid and carbon dioxide, conducted by lactic acid bacteria. During these processes, a wide variety of volatile metabolites are generated, which include by-products responsible for the background aroma of any wine that strongly contribute to wine sensory profile and "aromatic bouquet". Their accumulation depends on several fermentation conditions (e.g., yeast strain and species, chemical, physical, and nutrient must composition such as sugar content, temperature, pH, SO_2, oxygen levels, aeration and more). These important compounds include esters, higher alcohols, volatile fatty acids, carbonyls, and volatile sulfur compounds (Styger et al., 2011), all characterized by different olfactory properties. Esters normally contribute to pleasant wine fruity aromas, and, in a lesser extent, to the floral and sweet sensory profile of white and red wines (Lambrechts & Pretorius, 2000). Except for 2-phenylethanol, characterised by a pleasant rose-like aroma, fermentation-derived higher alcohols have been described with solvent, fusel, boiled potato notes. However, suitable concentrations of these aroma compounds (lower than 300 mg/L) have been shown to add sensory complexity to wines (Rapp & Versini, 1996). Volatile fatty acids have been described with unpleasant aromas of rancid, sweat, pungent, vinegar-like, fatty, butter, or cheese-like notes and, in general, their sensory contribution in real wine is a general contribution to the vinous character (Lambrechts & Pretorius 2000). The majority of fermentative sulfur compounds are associated with off-flavours, being described with rotten egg, putrefaction, cabbage, cauliflower, onion, rubber, asparagus, truffle, garlic, potato, cheese, natural gas, sulfurous (Waterhouse et al., 2016 and references

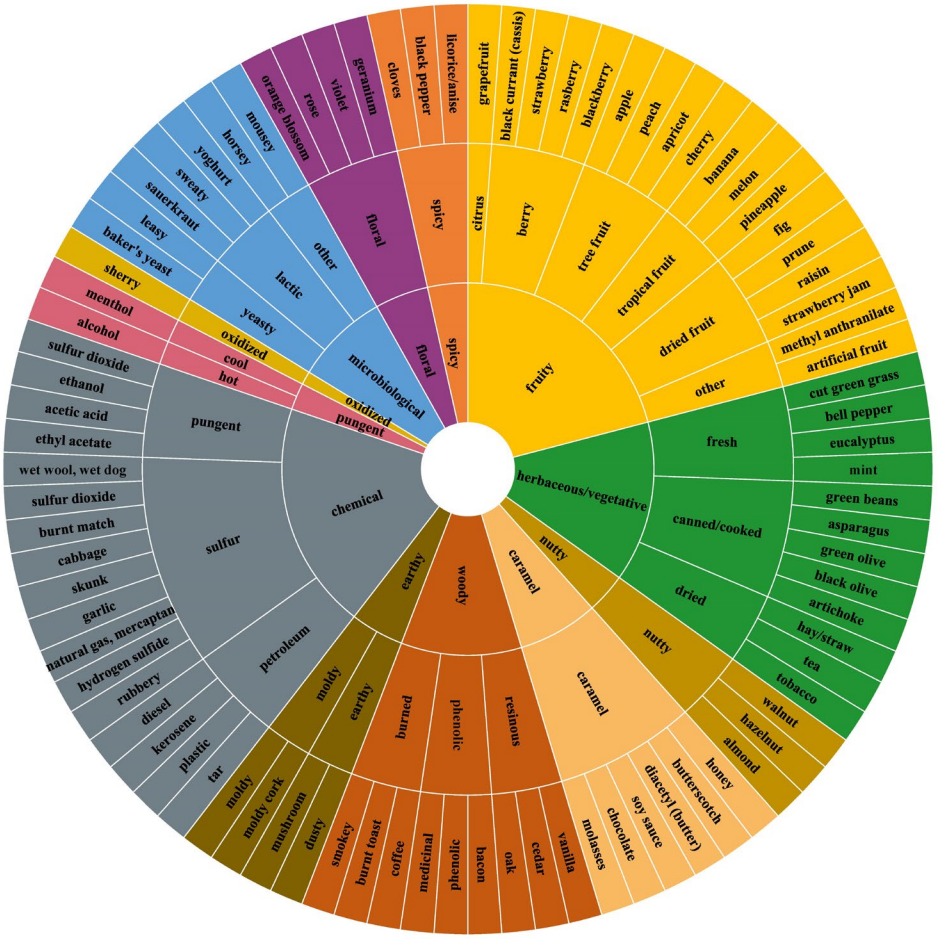
therein). However, as reported by some authors, hydrogen sulfide and dimethyl sulfide might add complexity to wine aromatic "bouquet" when present at concentrations up to around 100 μg/L (Ugliano & Henschke, 2009; Siebert et al., 2010; Rauhut, 2017).

Maturation/ageing VOCs refer to the aromas that develop during wine ageing and/or extracted from wood barrels. Generally, wine ageing leads to a loss of the typical aromas associated to varietal and fermentation VOCs, and to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration (i.e., appearance of oxidative aromas, as presented below, Background Section-4.2.). Oak wood confers numerous specific compounds to wine, many of which are formed during toasting of the wood (Vivas & Glories, 1993). VOCs extraction from oak barrels depends mainly on several factors (e.g., quantity of compounds that are potentially extractable, contact time between wine and oak wood, wine composition, microbiological transformations) (Spillman et al., 1998 a and b). The main maturation/ageing VOCs are represented by furanic compounds, lactones, phenolic aldehydes, volatile phenols, and phenyl ketones (Pérez-Coello & Díaz-Maroto, 2009). They are responsible for the so-called "*boisè*" character, and they are characterised by woody, spicy, liquorice, toasty, smoky, cocoa, coconut, leather, and vanilla notes, among others.

When volatilized from the wine matrix, wine aromas can reach – through the orthonasal (nose) and retronasal (mouth) paths – the olfactory bulbs and trigger receptors stimulating the perception of the corresponding odour whose intensity and quality mostly depend on their nature and concentration. Since olfaction generates diverse and complex perceptions in wine and the assessment of olfactory characteristics is one of the most difficult and discriminative aspects of wine tasting (Jackson, 2009), over time, wine olfactory properties have been classified in an increasingly detailed manner.

As an example, Figure 7, defined "The Wine Aroma Wheel" (Noble et al., 1987), shows the organoleptic complexity of wine with a vocabulary including around 90 different olfactory descriptors.

Figure 7. Wine Aroma Wheel showing first-, second-, and third-tier terms.
Adapted from Noble et al. (1987); created with XLStat Sensory.



1.3. Wine polyphenols-aromas interactions

Even if wine PPhs and VOCs have been widely studied in literature, it has been demonstrated that to fully understand wine chemical and sensory characteristics (i.e., odours, astringency, bitterness, tastes, flavour, aromas perception), besides identification and quantification of wine volatile and non-volatile components, it is necessary to study their interactions (Pozo-Bayón & Reineccius, 2009; Sáenz-Navajas et al., 2010; Villamor & Ross, 2013; Paravisini & Guichard, 2017). Indeed, the concept of flavour (in French *flaveur*), is defined as "the set of sensations perceived by the organ of smell, by the gustatory buttons and by the oral cavity, including thermal, tactile, chemical, kinesthetics sensations, painful, etc." and it represents the interaction between taste/in-mouth properties and olfactory sensations.

In literature, the interactions between non-volatile and volatile wine fractions, those specifically referring to polyphenols and volatiles and between the sensory stimuli elicited by these wine constituents, have been not

deeply studied and enough comprehended, even though the topic is of great interest in oenology. Moreover, the subject is of transversal concern in the food field since polyphenols are largely present in other food matrices and because of their antioxidant and healthy properties. Indeed, nowadays the population is more aware of healthy problems and new concerns about climate change, to the point that an ever-increasing number of consumers are advocating the reduction/elimination of consumption of animal products and turning toward healthier and more sustainable plant-based foods. These products, that can represent a valid food alternative, are often discarded by the consumer due to their bitterness/astringency characteristics. Consequently, all scientific knowledge helping in understanding how to smoothen/mask these sensations are welcomed by scientists and food technologists/engineers working in the food field.

Bilateral sensory effects have been suggested by different studies carried out with different approaches, estimating and/or measuring the sensory impact of the interactions between wine PPhs and VOCs both in orthonasal and retronasal conditions by *in-vitro* or *in-vivo* studies on aroma release. Some authors have explored the influence of aromas on mouthfeel perceptions of Chardonnay wines (Sereni et al., 2016). It was found that it is important to consider both volatile and non-volatile wine fractions when attempting to establish the relationship between chemical composition and mouthfeel as the volatile fractions, in some cases, might influence the mouthfeel sensations.

From a physicochemical perspective, a special attention has been paid, of late, at understanding what happens in retronasal simulated and real conditions, reproducing - by model mouths or by real *in-vivo* settings - the aroma release during wine tasting. These approaches based on the evidence that, together with other in-mouth variables such as wine sip volume (Genovese et al. 2015), salivary components can interact not only with wine polyphenols but also with VOCs, significantly affecting their release and perception (Genovese et al., 2009; Piombino et al., 2014).

Notwithstanding the unclear mechanisms at the base of those interactions, it is known that they impact wine sensory perception and quality (Sereni et al., 2016; Cameleyre et al., 2018). However, depending on the methodological approaches, on the wine matrix or model solutions compositions (e.g., pH, ethanol, and sugar content), on the tested VOCs and tannin types and corresponding concentrations applied, different results are found in literature. Hence, studying PPhs-VOCs chemical and sensory interactions and, moreover, investigating cross-modal interactions of wine odour–mouthfeel stimuli is a subject of interest for wine

researchers and producers to understand consumers' perceptions and choices as well as for precision oenology purposes.

Therefore, for their importance and because of the current concern in food research and oenology, wine VOCs-PPhs interactions and their physicochemical and sensory effects, represent the main subject of interest of the present PhD thesis.

The actual scientific knowledge on the influence of VOCs-PPhs interactions on wine chemical and sensory properties will be presented as follows.

Finally, since PPhs (e.g., tannins), exhibit antioxidant properties, an overview about the actual knowledge of their modulating role in the protection toward the oxidation of wine aromas, will be also illustrated.

2. Aromas influence on in-mouth sensations

Cross-modal interactions are defined as "the functional integration of information transmitted by anatomically distinct senses that relies on a multimodal processing" (Etiévant et al., 2016).

Regarding flavour perception, the integration between different chemosensory characteristics may lead to cross-modal interactions in which the perception of a specific aroma compound can influence the perceived intensity of a gustatory molecule and vice versa (Delwiche, 2004).

Cross-modal sensory interactions and their effects on the sensory perception have been explored in food and beverage matrices, mostly in model systems (Poinot et al., 2013), but also studying real products, such as cheese (Niimi et al., 2014), cider (Symoneaux et al., 2015), cocoa and milk beverages (Labbe et al., 2006), desserts (Tournier et al., 2009), olive oil (Caporale et al., 2004), and yoghurt (Saint-Eve et al., 2004).

Regarding the effects of aromas on tastes perception, it has been shown that specific olfactory characteristics can modulate the perception of sweet, acid, salty, and bitter tastes, either enhancing or decreasing/masking their perception. For example, in food products it has been observed that the perception of vanilla, caramel, strawberry, and other fruity notes could have an enhancing effect on sweetness perception (Labbe et al., 2006; Tournier et al., 2009; Symoneaux et al., 2015), cocoa notes on bitterness (Labbe et al., 2006), lemon notes on perceived acidity (Valentin et al., 2006), and animal, soy sauce and cheese notes on saltiness (Djordjevic et al., 2004; Lawrence et al., 2009; Nasri et al., 2011). At the contrary, some authors have observed a masking effect played by caramel aromas on sourness perception (Stevenson et al., 1999; Boakes & Hemberger, 2012), or by hay, animal, and earthy notes on cider sweet taste perception (Symoneaux et al., 2015).

Therefore, since the observation of the ability of aroma compounds of potentially modifying the perceived tastes of a foods and beverages products, cross-modal interactions in general, and taste-aroma interactions in particular, are nowadays investigated as a valid alternative or as a complementary approach to the modulation of a specific taste. For example, in the case of sweet and salty foods, in the context of worldwide nutritional recommendations to lower salt and sugar consumption, food technologists and engineers are focusing on the utilisation of cross-modal interactions to reach novel ways to reduce the amount of salt or sugar in their products while maintaining original taste and acceptability (Hutchings et al., 2018).

Therefore, it is possible to imagine using cross-modal interactions to limit undesirable tastes in foods and beverages and, at the same time, to enhance pleasant in-mouth properties.

2.1. Wine aromas effects on astringency and tastes perception

Regarding wine matrices, astringency is amongst the least understood in-mouth sensation. This can be due to several different reasons related to its complexity of physiology/mechanisms and elicited sensations including multimodality, among which the interactions with other stimuli such as aromas or tastes can play a role.

Therefore, in late years, the oenological research has focused on the comprehension of the possible sensory effects of aromas-polyphenols interactions in wines, trying to gain knowledge on the possible influence of VOCs sensory properties on wine tastes and particularly on PPhs in-mouth sensory proprieties, mostly astringency and, in a lesser extent, bitterness.

Wine astringency represents one of the most appreciated and desired sensory characteristics in red wines. However, as already reported above, astringency, together with bitterness, when not well managed and balanced, represent repulsive sensations for consumers. Therefore, as for food products, also in the case of wines cross-modal interactions could be used by winemakers to manage astringency and bitterness, besides all the reasons exposed above.

However, up to date, a limited number of works focused on sensory cross-modal interactions in wines, and very few works investigated the influence of olfactory characteristics on in-mouth sensations. Furthermore, most of the studies focused on wine-like solutions, and, only a few, on real wine matrices, showing, moreover, contradictory results.

Some studies have shown a significant effect of the olfactory characteristics on the perception of astringency, tastes, and other in-mouth sensations. For example, in one of the first study Sáenz-Navajas et al. (2010b), applying a construction/deconstruction method, suggested that the addition of a volatile fruity extract obtained from a Chardonnay white wine to the dearomatized non-volatile extract obtained from a red wine decreased astringency and bitterness and increased sweet perception. Vice versa, the substitution of a white wine volatile matrix with a red wine one, caused an increase in astringency perception and a decrease in sweetness. In a subsequent experiment (Sáenz-Navajas et al., 2018), it has been demonstrated that the green mouthfeel character of red wines, associable to the unripe astringency sub-quality, is positively correlated with vegetal aromas and negatively correlated with woody, ripe fruit, and oxidized ones. Moreover, the relations between aromas and astringency have been further underlined by Ferrer-Gallego et al. (2014), studying astringent model solutions with the presence of 2 g/L of catechin or epicatechin. Authors observed that the addition of specific

volatile compounds with fruity, leather, and smoked notes (due to isoamyl acetate, ethyl hexanoate, damascenone, 4-ethylphenol and 4-ethylguaiacol) increased astringency persistence and intensity.

At the contrary, other studies present in literature suggest that olfactory features do not impact in-mouth sensations. Results from a very recent work (Sáenz-Navajas et al., 2020), conducted with and without nose clips, reported that except for the oily mouthfeel attribute (which the authors hypothesised to be masked by earthy aromas and enhanced by alcoholic notes), the perception of aromas did not have an impact on the other palate sensations of red wines, including numerous astringency descriptors (i.e., dry, sticky, dusty, grainy, sandy, coarse, fleshy, mouthcoating, silky and gummy). Those results support the findings of a previous study conducted by de-la-Fuente-Blanco et al. (2017). Using a descriptive analysis technique based on intensity rating performed by three groups of participants (novices, trained and expert consumers), the study concluded that aroma–astringency interactions were quantitatively not relevant in determining the astringency intensity levels of red wines, regardless of consumers' expertise level. By contrast, bitterness increased with animal aromas, however only in the novice group.

Therefore, gaining knowledge on the modulation effects of aromas on astringency and taste perception in wine appears very difficult, since data are still scarce and, sometimes, controversial. Indeed, integrative brain processes, such as cross-modal interactions, could explain why it is difficult to find a direct correlation between specific compounds or chemical structures and diverse astringency sensations that are of great interest for research and production. Hence, wine cross-modal sensory interactions remain an unclear subject of interest that needs to be explored further. The investigation of this aspect is of great interest for oenologists and winemakers to manage and control wine quality and it might be useful to better comprehend consumers' preferences/acceptance, with the goal of satisfying their demand.

For these reasons one of the main objectives of the present PhD thesis is to determine how the perception of wine aroma could modulate the perception of wine astringency, also trying to contribute to the knowledge of the influence of wine aromas on sweetness, acidity, and bitterness perception.

3. Polyphenols effects on volatiles release and perception

Wine volatiles are characterized by different chemical and physicochemical properties affecting their binding and release behaviour. The volatility and solubility of aroma compounds represent the two main physicochemical properties driving the partitioning of the volatile substances between the liquid and the gas phases. This is strongly influenced by other wine constituents present in the medium, such as simple-molecules (ethanol, sugars, and glycerol) and macromolecules (proteins, polysaccharides, and polyphenols) (Goldner et al., 2009; Robinson et al., 2009; Paravisini & Guichard, 2016 and reference therein; Piombino et al. 2019). Winemaking procedures and stabilization treatments (maceration, filtration, fining), as well as ageing processes (polymerization and precipitation), or even the grape variety, impact on macromolecules involved in these interactions, with potential effects on mouthfeel balance and perceivable olfactory profile.

Apart from short sections on polyphenols impact on VOCs (Villamor & Ross, 2013; Paravisini & Guichard, 2016), previous to the work conducted in the frame of this PhD project (Pittari et al., 2021), no published review focused on this topic.

3.1. Molecular insights

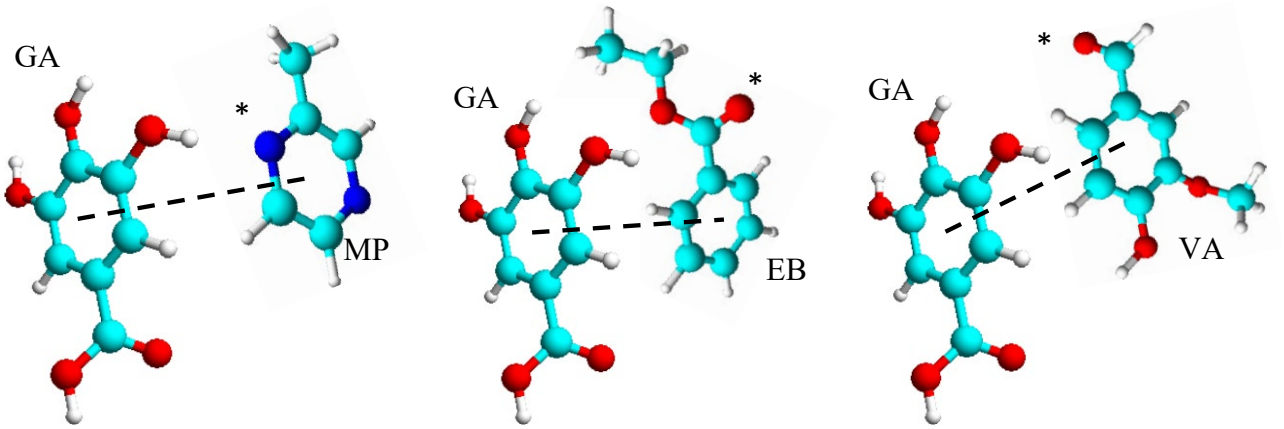
The pioneer research conducted on wine PPhs-VOCs interactions and, consequently, on the effects of polyphenols on aromas release, dates to the late 90s (Dufour & Bayonove, 1999). Authors evaluated the influence of phenolic compounds such as (+)-catechin, epicatechin and a highly condensed tannin fraction (extracted from wine), on some linear or aromatic wine aromas with different hydrophobicity, added in 10% hydroalcoholic or wine model solutions. A dynamic exponential dilution technique, and ^{1}H NMR to probe the interactions at the molecular level, were used. General decreases of volatility for isoamyl acetate, ethyl hexanoate, benzaldehyde and limonene were correlated to increasing concentrations of (+)-catechin (0-12 g/L), with the latter less retained at low catechin concentrations (0-5 g/L). Unlike catechin, the tannin fraction induced a slight decrease of benzaldehyde release and a salting out of limonene with no effect on the two esters, thus suggesting that monomeric or oligomeric/polymeric PPhs can differently impact volatility. At molecular level, the NMR study focused on aromas-monomeric polyphenols interactions. Similar weak bimolecular bindings were reported for intermolecular complexation of isoamyl acetate, ethyl hexanoate, and

benzaldehyde with catechin. Both catechin and epicatechin showed a higher affinity to benzaldehyde than for 3,5-dimethoxyphenol. Monomers had a higher affinity for benzaldehyde than for themselves.

In subsequent work, Jung and collaborators (Jung et al., 2000), applying ^{1}H NMR spectroscopy analyses, have explained the supramolecular assembly at the base of specific VOCs-PPhs interactions by noncovalent bonds. Authors have shown that the addition of gallic acid to model solutions containing 2-methylpyrazine, vanillin or ethyl benzoate has reduced their volatility mostly due to π-π stacking of the galloyl ring of the phenolic compound with the aromatic ring of the odorant molecule, with secondary hydrogen-bonding effects helping in stabilizing the complex and enhancing the specificity, as represented in Figure 8. Furthermore, the supramolecular complexation also depends on the structural nature of the VOC, with 2-methylpyrazine and vanillin interacting more strongly than ethyl benzoate.

These molecular insights have represented the starting point for further research conducted in the last decades, by means of updated approaches/methodologies, and aimed at understanding how volatility and sensory perception of wine aromas could be affected by the presence of polyphenols.

Figure 8. Proposed mechanisms illustrating π-π stacking interactions (black dotted line) and hydrogen bonding (* suggested involved atom) of the galloyl ring of the phenolic compound with the aromatic ring of the odorant molecule: (a) gallic acid (GA) and 2-methylpyrazine (MP); (b) gallic acid and ethyl benzoate (EB); (c) gallic acid and vanillin (VA) (adapted from Jung et al. (2000); created with ACD Labs, Freeware, 2020). Cited from Pittari et al. (2021).



The two studies cited, suggested hydrophobicity of both PPhs and VOCs as a main driving force in explaining bimolecular aroma-phenolic compound interactions, and then significantly involved in the modification of VOCs release. Supporting results were obtained by a different approach (Aronson & Ebeler, 2004). The

authors prepared model solutions of ethyl benzoate (2 to 16 mg/L in 1% ethanol-water mixture) and 2-methylpyrazine (60 to 300 mg/L in water) and investigated their interactions with gallic acid (10 mM) through HS-SPME/GC-MS and sensory analysis. Their results are aligned to molecular evidence: the addition of gallic acid significantly decreased the headspace partitioning of the two VOCs and their perceived aromatic intensity. The variation in VOCs response to polyphenols do not only depend on the concentration or on the chemical characteristics of both VOCs and PPhs, but also depend on other matrix characteristics such as ionic strength and ethanol content. These variables can impact polyphenols' structure, aggregation, solvation, colloidal state (Poncet-Legrand et al., 2003) and involvement in "salting-out" and/or hydrophobic phenomena that are likely to impact their interactions with VOCs. All the mentioned factors may affect how PPhs interact with VOCs and consequently, their release and perception.

3.2. Polyphenols effects on VOCs release

Different studies have investigated the effects of polyphenols on wine odour. In most of the studies, static or dynamic HS-SPME/GC-MS or FID analyses have been applied to investigate aroma release from the matrix. Some of them have combined chemical and sensory experiments to study and compare the two effects while only a few studies performed GC-O analysis.

The different analytical methodologies were applied to model solutions and real wines with significant compositional differences or deodorized/reconstituted wines.

These different approaches, notwithstanding their advantages and drawbacks, tested the behaviour of several VOCs belonging to different chemical classes. Different results, sometimes contradictory and difficult to interpret, have been reported. To compare these results, they were summarized in Table 1, which was organized listing VOCs belonging to the same chemical class accordingly to their increasing hydrophobicity expressed as $\log P_{octanol/water}$.

Considering that ethanol can affect PPhs structure/colloidal state and solubility, as well as VOCs solubility, release, and perception, in Table 1 we have also reported its concentration used in the studies. However, since in all relevant studies ethanol levels ranged between 10 to 12 % (v/v), it is difficult to hypothesize a significant effect on both PPhs and VOCs chemical characteristics. In literature, differences in PPhs particle size and colloidal state (Poncet-Legrand et al., 2003; Zanchi et al., 2007), as well as in VOCs solubility, release, and

sensory perception, have always been observed at different ethanol levels when higher than 2 % (v/v) (Ickes & Cadwallader, 2017 and references there in).

As further information, in Table 1, we also specified the type of matrix as well as the nature and/or the content of PPhs that were tested in the different studies to consider different wine systems. Grape tannins and polyphenols extracted from grape skins or seeds are characterized by different properties. The highest concentration of tannins in grape berry derives from grape skins, which differ from seed tannins in terms of polymerization degree (DP) and amount of gallates (Pinelo et al., 2006). The average DP for skin tannins is higher than the average DP for seed tannins, which tend to be in monomeric form rather than polymerized. Also, real wines (i.e., white, young red, and old red wines) are widely different in terms of polyphenolic characteristics. They differ in terms of total phenolic content, which can vary from around 200 mg/l of gallic acid equivalents (GAEs) in white wines, to 2000 mg/L in young-reds and 3500 mg/L or more in aged-red wines (Waterhouse et al., 2016). Moreover, older red wines are normally characterized by a decrease of several low molecular weight phenolic compounds and anthocyanins and higher concentrations of polymeric pigments, while younger wines have higher concentrations of anthocyanins and other phenolic compounds (Chira et al., 2011; McRae et al., 2012; Li & Sun, 2019).

Considering all the described frameworks, in the following paragraphs, we attempted to link and critically discuss the observed effects of polyphenols on VOCs release (Table 1).

3.2.1. Effects on terpenoids

Terpenoids are varietal compounds essentially coming from grapes as enzymatically produced secondary metabolites of the terpenoid pathway and existing as saturated/unsaturated and cyclic/acyclic hydrocarbons, that can contain alcohol, aldehyde, ketone, ester, ether, and acetal functionalities. These compounds are also present as terpene glycosides that can be hydrolysed to free volatile aglycones in different phases of wine production and life, mainly by yeast glycosidases during fermentation, and by the acidic conditions during wine storage. From a sensory point of view, terpenes are largely responsible for citric, floral, and balsamic aromas (González-Barreiro et al., 2014). The effect of polyphenols on monoterpenoids volatility has been mostly observed in deodorized/reconstituted real wines (Rodríguez-Bencomo et al., 2011), and linalool in model wine solutions (Mintropoulou et al., 2011). According to data reported in the literature and summarized in Table 1, a common trend seems to come out. Indeed, independently from the VOC hydrophobicity within

the tested range (2.67≤logP≤3.47) and from the type of matrix representative of different PPhs compositions, the release of the tested terpenoids, decreases at increased tannin concentrations (Mintropoulou et al., 2011; Rodríguez-Bencomo et al., 2011). Interestingly, considering the real wine matrices, it can be noted that while the aged-red wine has significantly retained terpinen-4-ol, β-citronellol and nerol, in the young-red one a significant retention effect has been observed for all the terpene compounds, including α-terpineol and linalool (Rodríguez-Bencomo et al., 2011). Based on results by Dufour & Bayonove (1999) suggesting that monomeric or oligomeric/polymeric polyphenols can differently impact aromas volatility, this behaviour could be linked to the lower concentration of polymeric polyphenols (Del Álamo et al., 2004) of young-red wines compared to aged ones. Considering the sensory importance of these odorants and that they act in combination with each other (Ferreira, 2010), it could be hypnotized that the PPhs composition of wine could negatively impact the olfactory perception of monoterpenes.

Some results of a recent work (Wang et al., 2021) referred to a possible sensory impact on terpenes perception as an effect of the presence of gallic and *p*-coumaric acids. Independently of phenolic acids composition and concentration, both acids tended to decrease the production and volatilization of free terpenes during fermentation, with *p*-coumaric acid showing a greater restraining effect. Studies of linalool and its terpene glycoside have shown that the main driving forces in their interactions with these phenolic acids are dispersive interactions and hydrogen bonding. Sensory analyses confirmed a decrease in the perception of some aromatic notes related to the presence of free terpenes (e.g., tropical, and sweet fruit aromas), albeit not enough to be statistically significant. The authors concluded that the matrix effect of phenolic acids can effectively control the release and modulate the global feature of wine aromas.

3.2.2. Effects on esters

Esters are mainly produced by yeasts during alcoholic fermentation. Their concentration and relative proportion are strongly influenced by several fermentation parameters (i.e., oxygen level, fermentation temperature, yeast strain characteristics, yeast assimilable nitrogen levels). From a sensory point of view, they are considered as one of the most important families of compounds lending fruity characters in wines. In most GC-O studies present in literature, esters are included in the list of the compounds with the highest OAV (Odour Active Value/unit), an index computed to estimate the olfactory potency of an odorant in terms of the

ratio of the concentration of the volatile compound to its odour detection threshold (Waterhouse et al., 2016 and references therein).

The influence of polyphenols on esters' release and, in some cases, on their perception, has been investigated on several compounds belonging to this family, as reported in Table 1. Some observations can be made despite the absence of a clear and unique trend that could be explained by the wide range of polarity within the group of esters tested ($1.26 \leq \log P \leq 5.71$). Around 2.85 seems to be a cut-off logP value, indeed there is a switch of esters behaviour depending on the PPhs levels in the matrix. Esters characterized by lower logPs tended to a lower release at smaller PPhs levels, while raised at higher ones. This suggests that for poorly hydrophobic esters, there is the prevalence of a retention phenomenon at low PPhs concentrations and a tendency of salting-out effects at higher PPhs concentrations. Alternatively, the release trend of esters characterized by higher logPs, decreased independently from the PPhs level, suggesting that hydrophobicity represents the main driving force of highly hydrophobic esters release. The only exception to this behaviour is the opposite trend observed for ethyl octanoate in oak barrel aged red wine. Both monomers, catechin and gallic acid, tested at 2 g/L, did not affect the release of the more polar esters (ethyl isobutanoate, ethyl butanoate, isoamyl acetate), while the release of the hydrophobic ethyl octanoate decreased in presence of catechin (logP=1.37) but not in presence of gallic acid (logP=0.59), suggesting that hydrophobicity of PPhs could be significant (Lorrain et al., 2013). In the case of ethyl 2-methyl butanoate, unlike ethyl butanoate, the sensory impact of release differences determined by GC-MS was confirmed by GC-O data. In fact, the olfactometric score increased when the volatile matrix of a white wine was replaced by the volatile extract of an aged red wine (Sáenz-Navajas et al., 2010a). Results from sensory assessment, are not completely in line with instrumental ones with ethyl octanoate, ethyl isobutanoate and ethyl butanoate being perceived as less intense in presence of catechin at 2 g/L (Lorrain et al., 2013).

Based on results reported above and on the knowledge that esters act synergistically in imparting fruity notes to wine (Ferreira, 2010), the observed changes of most hydrophobic esters at increased levels of PPhs, could have a significant sensory impact on wine fruity aroma. Also, the observed decreases of isoamyl acetate, a molecule having an important olfactory role in wine, could be significant (Ferreira, 2010).

3.2.3. Effects on alcohols

Alcohols are a group of volatile compounds mainly produced as fermentative by-products of yeasts amino acids metabolism, via the Ehrlich pathway. Their production is strongly influenced by several fermentation parameters (e.g., fermentation temperature, yeast strain characteristics, yeast assimilable nitrogen levels, turbidity) (Waterhouse et al., 2016 and references therein). From a sensory point of view, except for β-phenylethanol, described with floral/rose notes, the other alcohols are described with fusel, oily, alcoholic, ethereal terms. Some authors have suggested that alcohols may contribute not only to the vinous aroma but also to its aromatic complexity of wines. However, at high concentrations they can mask certain aromas (Etievant, 1991; Ferreira, 2010).

Based on results reported in the literature and schematized in Table 1, it seems difficult to draw trends or conclusions. The entire set of compounds show low hydrophobicity ($0.76 \leq \log P \leq 2.03$), which could be a reason for other variables driving their interactions with PPhs. Looking at β-phenylethanol, a "salting-out" effect at high tannins concentrations and independently from their nature, was observed (Mintropoulou et al., 2011). Considering its relatively low hydrophobicity ($\log P = 1.36$) and the presence of an aromatic ring on its structure, the formation of π-π interactions of the galloyl ring of the phenolic compound with the aromatic ring of the odorant molecule might explain its reduction in volatility at low tannins concentration (Dufour & Bayonove, 1999; Jung et al., 2000). At high tannins concentrations, it could be possible that the decrease in the potential binding sites for odorants has occurred because of the low ethanol concentration (10% v/v) contained in the model solutions. Indeed, it has been shown that at relatively low ethanol concentrations (8–10% v/v), more tannins self-aggregation occurs, making them less available to interact with aroma compounds (Poncet-Legrand et al., 2003; Zanchi et al., 2007; Villamor et al., 2013). This could explain the two aromatic alcohols showing opposite trends at the corresponding lowest PPhs levels: benzyl alcohol raised over the headspace of a real matrix (oak barrel aged red wine) containing 12% v/v ethanol and TPC=230 (Rodríguez-Bencomo et al., 2011); β-phenylethanol lowered in a model wine solution with 10% v/v ethanol and 0.5-1 g/L of skin tannins extract (Mintropoulou et al., 2011).

From a sensory perspective, it is not possible to speculate on the impact of the observed variations.

3.2.4. Effects on volatile phenols

Volatile phenols are a family of volatiles, that comprise (i) volatile phenols formed during the fermentation process and released from grape-derived glycosides and (ii) volatile phenols formed during the fermentation process by the metabolism of hydroxycinnamic acids, precisely by yeasts of the genus *Brettanomyces/Dekkera*, through decarboxylation of trans ferulic and *trans* ferulic and *trans para*-coumaric acid, and (iii) volatile phenols extracted when storing wine in contact with toasted oak wood (Chatonnet et al., 1992; Pérez-Coello et al., 2009; Ristic et al., 2015). While some of them contribute positively to wines aroma complexity (i.e., guaiacol and eugenol), others (i.e., 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol) are involved in the appearance of unpleasant notes. Consequently, it is important for winemakers to manage the increase and/or the production of these VOCs, and to understand which conditions favour their perception or otherwise. For this reason, the influence of polyphenols on the release of different volatile phenols ($1.32 \leq \log P \leq 2.61$) and in some cases on their sensory perception, has been evaluated both in model solutions and real wines (Table 1). Except for guaiacol and eugenol, characterized by the lowest logP values and better volatilized in the presence of grape tannins at 0.5-1.5 g/L (Villamor et al., 2013), the release of volatile phenols was essentially reduced by PPhs.

Important results regarding the effects of polyphenols on the two volatile phenols 4-ethylphenol and 4-ethylguaiacol in model solutions have been understood from a recent study (Petrozziello et al., 2014). The authors showed that at increasing polyphenols concentration, a significant and linear decrease in the volatility of these two VOCs has been observed due to π-π interactions. Additionally, performing sensory tests, they showed that the unpleasant and characteristic "phenolic'' taint, due to the presence of 4-ethylphenols (Chatonnet et al., 1992; Chatonnet et al., 2006; Silva et al., 2004), has been significantly higher in the trials with lower polyphenol content, highlighting a consistent and significant masking effect of polyphenols on the perception of the Brett-character, due to the presence of those two VOCs. This result may be of great interest in winemaking since controlling the concentration and the sensory impact of these compounds in wine is an always current topic in oenology.

3.2.5. Effects on acids

Volatile aliphatic organic acids are compounds produced during alcoholic fermentation as by-products of fatty acids. As all the other fermentative aromas, acids production strongly depends on fermentation parameters.

Fatty acids, such as butyric acid, isobutyric acid, hexanoic acid, octanoic acid, nonanoic acid, and decanoic acid, possess unpleasant aromas, are normally described with rancid, pungent, fatty, or cheese-like notes and, their sensory contribution in real wine is a general contribution to the vinous character (Lambrechts & Pretorius, 2000). However, volatile aliphatic organic acids concentration is usually correlated with their corresponding ethyl esters, with the latter being characterised by a more powerful odour. Indeed, as an example, acetic acid itself is described with pungent, vinegar-like descriptors; however, the off-odour associated with volatile acidity appears to be primarily due to the more powerful ethyl acetate, formed by esterification of acetic acid (Waterhouse et al., 2016).

In literature, the effect of polyphenols has been reported on butyric, hexanoic and octanoic acids (Table 1). When analysed in a reconstituted sample made of the volatile extract of an aged-red wine and a non-volatile extract of a Chardonnay white wine, the release of the three compounds decreased (Sáenz-Navajas et al., 2010a). The intensity of butyric acid, tested by GC-O analyses, was negatively affected. Octanoic acid, having a higher logP=3.05 compared to the other two, showed different behaviours depending both on the concentration and the nature of PPhs.

No conclusions can be drawn on this class of VOCs, due to the scarcity of results.

3.2.6. Effects on ketones

Ketones are mainly derived from lipid oxidation, as well as from the citrate and glucose metabolism. This group of VOCs is characterized by a wide array of odours varying from baked/dehydrated fruits to earthy and floral, among others. Norisoprenoidic ketones such as β-damascenone and α-ionone provide fruity/baked fruit or floral notes. Acetoin and diacetyl mostly result in a buttery flavour while other compounds such as 1-octen-3-one has herbaceous, mushroom, and earthy aromas.

The two VOCs α-ionone and β-damascenone are characterized by similar logP values (3.99 and 4.04, respectively), higher compared to that of 1-octen-3-one (logP=2.18). Both the norisoprenoidic ketones showed similar trends: at high tannins concentrations, and in presence of the high polymerized ones, their release decreased (Rodríguez-Bencomo et al., 2011). Conversely, the release of 1-octen-3-one, at high grape tannins concentrations, increased in model wine solutions. However, results from HS-SPME-GC–O techniques carried out by trained panellists show lower GC-O scores for 1-octen-3-one in presence of tannins (Sáenz-Navajas et

al., 2010a). Further at high ethanol (14% v/v), fructose (2 g/L) and tannins concentrations (1.5 g/L), odour thresholds have been seen to be higher for β-damascenone and 1-octen-3-one (Villamor et al., 2013).

The observed results on β-damascenone and 1-octen-3-one could be interesting from a sensory point of view. β-damascenone is reported as a compound able to enhance the fruity character due to ethyl esters in red wine. Thus, considering the observed negative impact at increasing levels and polymerization of PPhs on the release of esters and β-damascenone (Rodriguez-Bencomo et al., 2011) it can be argued that this could correspond to a significant diminution of the fruity character of red wines, especially in aged and/or woody ones. Concerning 1-octen-3-one, a compound involved in the cork taint (Pons et al., 2011; Cravero, 2020), it could be interesting to test if the concentration and the nature of PPhs, could be useful in managing the sensory impact of this off-flavour.

3.2.7. Effects on oxygen heterocycles (furans/lactones)

Furans and lactones are VOCs normally related to wine ageing. Furans in wines are generated by the thermal degradation of sugars due to acid-catalysed reactions, or even through Maillard reaction. Lactones are essentially formed by yeasts during alcoholic fermentation, though significant odorant lactones are usually accumulated during wine ageing. They can impart powerful nuances to wines, especially in oxidative conditions (Oliveira and Silva et al., 2008). Oxygenated heterocycles reported in Table 1 have different functionalities such as ketonic, aldehydic or alcoholic and range from very polar compounds like sotolon (4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one) with logP= -0.29, to the more hydrophobic cis-whiskey lactone with logP=2.63. Data suggest that at increasing tannins concentrations, VOCs with logP values higher than 1, show a decrease in volatility. More specifically, the retention effect of the real wine matrices was higher for the oak-barrel aged one compared to the young-red one for γ-nonalactone (logP=1.94), trans-whiskey lactone (logP=1.97) and cis-whiskey lactone (logP=2.63). On the contrary, VOCs with lower logP values (i.e., 5-methyl furfural with logP=0.67), independently from the matrix type, have shown a "salting-out" effect (Rodríguez-Bencomo et al., 2011). Furthermore, GC-O data have shown that the most hydrophilic and polar VOCs, sotolon (logP= -0.29), furaneol (logP= -0.08) and ethyl furaneol (logP= 0.43), even if not instrumentally detected by GC-MS, were characterized by higher GC-O scores in presence of a red wine non-volatile extract compared to a white one (Sáenz-Navajas et al., 2010). These results could be linked to the very low detection thresholds characterizing these furans, all in the order of µg/L, with sotolon having the lowest (1-6 µg/L).

A sensory implication of these observations could be that the perception of these molecules involved in oxidative notes of wines, could be favoured in the presence of PPhs. On the contrary, coconut/woody-spicy/sweety odours due to lactones could be less perceivable at increasing concentrations of PPhs. However, there are no scientific data supporting this hypothesis since no works have been conducted on the hypothetical sensory effects of polyphenols on these VOCs, that could be an interesting aspect to consider for future research.

Table 1. Aroma compounds affected by the presence of polyphenols in wine matrix with different characteristics: release and orthonasal sensory perception trends. Cited from Pittari et al. (2021).

Aromas characteristics				Matrix characteristics				Effects		Ref.
Compound	Descriptors*	Concentration	logP (o/w) *	Type of matrix	%Ethanol (v/v)	Added tannins	Tannin content	Effects on release	Effects on orthonasal perception	
MONOTERPENOIDS										
α-Terpineol	Pine, terpenic, lilac, citrus, woody, floral	0-0.433 mg/L	2.67	White wine	12		TPC=230	↓ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓ (ns)		
Linalool	Citrus, floral, sweet, bois de rose, woody, green blueberry	1 mg/L	2.97	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↓		
		0-0.498 mg/L		White wine	12		TPC=230	↑ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓ (ns)		
Terpinen-4-ol	Peppery, woody, earthy, musty, sweet	0-0.665 mg/L	3.26	White wine	12		TPC=230	↑ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
β-Citronellol	Floral, leathery, waxy, rose, citrus	0-1.563 mg/L	3.3	White wine	12		TPC=230	↓ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
Nerol	Sweet, natural, citrus, magnolia	0-7.838 mg/L	3.47	White wine	12		TPC=230	↓ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
ESTERS										
Diethyl succinate	Fruity, apple, cooked apple, ylang	20 mg/L	1.26	Model wine solution	10	Skin tannins extract	0.5-3 g/L	↓		Mintropoulou et al., 2011
							3-10 g/L	↑		
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↑		
Ethyl isobutanoate	Sweet, ethereal, fruity, alcoholic, fusel, rummy	200 µg/L	1.66	Model wine solution	12	Catechin	2 g/L	NA	↓	Lorrain et al., 2013
						Gallic acid	2 g/L	NA	NA	
Isobutyl acetate	Sweet, fruity, ethereal, banana, tropical	0-0.675 mg/L	1.78	White wine	12		TPC=230	↓		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		
Butyl acetate	Ethereal, solvent, fruity, banana	0-0.713 mg/L	1.78	White wine	12		TPC=230	↓ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		
Ethyl butanoate	Fruity, fruit juice, pineapple, cognac	200 µg/L	1.8	Model wine solution	12	Catechin	2 g/L	NA	↓	Lorrain et al., 2013
						Gallic acid	2 g/L	NA	NA	
		-		Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
		0-1.456 mg/L		White wine	12		TPC=230	↓ (ns)		Rodriguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		

* The Good Scents Company; TPC = Total Polyphenol Content (it is expressed in mg/L gallic acid); TPI = Total Polyphenol Index; ↑ = increase; ↓ = decrease; (ns) = not significant; NA = Not Affected; ND = Not Detected

Table 1. Continued.

Aromas characteristics				Matrix characteristics				Effects		Ref.
Compound	Descriptors*	Concentration	logP (o/w) *	Type of matrix	%Ethanol (v/v)	Added tannins	Tannin content	Effects on release	Effects on orthonasal perception	
Ethyl 2-methyl butanoate	Sharp, sweet, green apple, fruity	-	2.16	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓ (ns)		Sáenz-Navajas et al., 2011
		0-0.803 mg/L		White wine	12		TPC=230	↓ (ns)		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑		
				Oak barrel aged-red wine			TPC=2142	↑		
Isoamyl acetate	Sweet, fruity, banana, solvent	4 mg/L	2.25	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↑		Mintropoulou et al., 2011
		200 µg/L		Model wine solution	12	Catechin	2 g/L	NA	NA	Lorrain et al., 2013
						Gallic acid	2 g/L	NA	NA	
		-		Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
		0-1.619 mg/L		White wine	12		TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓		
Ethyl hexanoate	Sweet, fruity, pineapple, waxy, green banana	-	2.85	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al. 2010
		0-2.356 mg/L		White wine	12		TPC=230	↓		Rodríguez-Bencomo et al. 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓ (ns)		
Ethyl cinnamate	Sweet, balsamic, fruity, spicy, powdery, berry plum	0-0.825 mg/L	2.99	White wine	12		TPC=230	↓		Rodríguez-Bencomo et al. 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓		
Ethyl octanoate	Fruity, winey, waxy, sweet, apricot, banana, brandy, pear	1 mg/L	3.84	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↓		
		600 µg/L		Model wine solution	12	Catechin	2 g/L	↓	↓	Lorrain et al., 2013
						Gallic acid	2 g/L	NA	NA	
		-		Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
		0-2.124 mg/L		White wine	12		TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		
Ethyl decanoate	Sweet, waxy, fruity, apple, grape, oily, brandy	1.5 mg/L	4.86	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↓		
		0-0.931 mg/L		White wine	12		TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓		
Ethyl dodecanoate	Sweet, waxy, floral, soapy, clean	2 mg/L	5.71	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
						Seed tannins extract	0.5-5 g/L	↑		
							5-10 g/L	↓		
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↓		

* The Good Scents Company; TPC = Total Polyphenol Content (it is expressed in mg/L gallic acid); TPI = Total Polyphenol Index; ↑ = increase; ↓= decrease; (ns) = not significant; NA = Not Affected; ND = Not Detected

Table 1. Continued.

Aromas characteristics				Matrix characteristics				Effects		Ref.
Compound	Descriptors*	Concentration	logP (o/w) *	Type of matrix	%Ethanol (v/v)	Added tannins	Tannin content	Effects on release	Effects on orthonasal perception	
ALCOHOLS										
Isobutanol	Ethereal, winey	80 mg/L	0.76	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↓		
Benzyl alcohol	Floral, rose, phenolic, balsamic	0-1.563 mg/L	1.1	White wine	12		TPC=230	↑		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		
3-methyl-1-butanol	Fusel, alcoholic, whiskey, fruity, banana	50 mg/L	1.16	Model wine solution	10	Grape tannins	0.5-1.5 g/L	↓		Villamor et al. 2013
2-methyl-1-butanol	Roasted, winey, onion, fruity, fusel, alcoholic, whiskey	150 mg/L	1.29	Model wine solution	10	Skin tannins extract	0.5-10 g/L	↑		Mintropoulou et al., 2011
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	slight ↑		
β-phenylethanol	Floral, rose, dried rose	50 mg/L	1.36	Model wine solution	10	Skin tannins extract	0.5-1 g/L	↓		Mintropoulou et al., 2011
							1-10 g/L	↑		
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↑		
trans-3-hexen-1-ol	Green, cortex, privet, leafy, floral, petal, oily, earthy	0-0.875 mg/L	1.61	White wine	12		TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑ (ns)		
Hexanol	Ethereal, fusel, oily, fruity, alcoholic, sweet, green	6 mg/L	2.03	Model wine solution	10	Seed tannins extract	0.5-10 g/L	↓		Mintropoulou et al., 2011
				Model wine solution	10	Grape tannins	0.5-1.5 g/L	↓		Villamor et al., 2013
		0-2.200 mg/L		White wine	12		TPC=230	↓ (ns)		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑		
ACIDS										
Butyric acid	Sharp, acetic, cheesy, buttery, fruity	-	0.79	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
Hexanoic acid	Sour, fatty, sweaty, cheesy	-	1.92	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
Octanoic acid	Fatty, waxy, rancid, oily, vegetable, cheesy	200 mg/L	3.05	Model wine solution	10	Skin tannins extract	0.5-1 g/L	↓		Mintropoulou et al., 2011
							1-10 g/L	↑		
						Seed tannins extract	0.5-5 g/L	↑		
							5-10 g/L	↓		
						Seed/Skin tannins mixture (4:1 w/w)	1-10 g/L	↑		
		-		Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	↓		Sáenz-Navajas et al., 2010
		0-4.656 mg/L		White wine	12		TPC=230	↑		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑ (ns)		
				Oak barrel aged-red wine			TPC=2142	↑ (ns)		

* The Good Scents Company; TPC = Total Polyphenol Content (it is expressed in mg/L gallic acid); TPI = Total Polyphenol Index; ↑ = increase; ↓= decrease; (ns) = not significant; NA = Not Affected;

ND = Not Detected

Table 1. Continued.

	Aromas characteristics			Matrix characteristics				Effects		Ref.
Compound	Descriptors*	Concentration	logP (o/w) *	Type of matrix	%Ethanol (v/v)	Added tannins	Tannin content	Effects on release	Effects on orthonasal perception	
VOLATILE PHENOLS										
Guaiacol	Phenolic, smoky, spicy, vanilla, woody	4 mg/L	1.32	Model wine solution	10	Grape tannins	0.5-1.5 g/L	↑		Villamor et al., 2013
Eugenol	Sweet, spicy, clove, woody	0.5 mg/L	2.27	Model wine solution	10	Grape tannins	0.5-1.5 g/L	↑		Villamor et al., 2013
		0-0.400 mg/L		White wine			TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine	12		TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
4-ethylguaiacol	Spicy, smoky, bacon, phenolic, clove	135 µg/L	2.43	Model wine solution	Not specified	Grape polyphenolic extract	0-3 g/L	↓	↓	Petruzziello et al., 2014
4-ethylphenol	Phenolic, castoreum, smoky, guaiacol	440 µg/L	2.58	Model wine solution	Not specified	Grape polyphenolic extract	0-3 g/L	↓	↓	Petruzziello et al., 2014
4-vinylphenol	Chemical, phenolic, medicinal, sweet	0-0.432 mg/L	2.61	White wine			TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine	12		TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
KETONES										
1-octen-3-one	Herbal, mushroom, earthy, musty, dirty	1 mg/L	2.18	Model wine solution	10	Grape tannins	0.5-1.5 g/L	↑		Villamor et al., 2013
		-		Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	ND		Sáenz-Navajas et al., 2011
α-ionone	Sweet, woody, floral, violet, orris, tropical, fruity	0-0.228 mg/L	3.99	White wine			TPC=230	↑ (ns)		Rodríguez-Bencomo et al., 2011
				Young-red wine	12		TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓		
β-damascenone	Natural, sweet, fruity, rose, plum, grape, raspberry, sugar	-	4.04	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	ND		Sáenz-Navajas et al., 2010
		0-0.425 mg/L		White wine	12		TPC=230	↑ (ns)		Rodríguez-Bencomo et al. 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
OXYGEN HETEROCYCLES (FURANS/LACTONES)										
Sotolon	Sweet, caramellic, maple, sugar, burnt sugar, coffee	-	-0.29	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	ND		Sáenz-Navajas et al., 2010
Furaneol	Sweet, cotton candy, caramellic, strawberry, sugar, brown sugar	-	-0.08	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	ND		Sáenz-Navajas et al., 2010
Ethyl furaneol	Sweet, caramellic, candy, butterscotch	-	0.43	Red wine non-volatile extract + white wine VOCs extract	12		TPI=60.1	ND		Sáenz-Navajas et al., 2010
5-methyl furfural	Spicy, caramellic, maple	0-1.475 mg/L	0.67	White wine	12		TPC=230	↑		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↑		
				Oak barrel aged-red wine			TPC=2142	↑		
γ-nonalactone	Coconut, creamy, waxy, sweet, buttery, oily	0-0.413 mg/L	1.94	White wine	12		TPC=230	↓		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		
***trans*-whiskey lactone**	Spicy, coconut, clove, celery, incense	0-0.868 mg/L		White wine			TPC=230	↑		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓ (ns)		
				Oak barrel aged-red wine			TPC=2142	↓		
***cis*-whiskey lactone**	Sweet, spicy, coconut, vanilla	0-0.682 mg/L	2.63	White wine	12		TPC=230	↑ (ns)		Rodríguez-Bencomo et al., 2011
				Young-red wine			TPC=1820	↓		
				Oak barrel aged-red wine			TPC=2142	↓		

* The Good Scents Company; TPC = Total Polyphenol Content (it is expressed in mg/L gallic acid); TPI = Total Polyphenol Index; ↑ = increase; ↓= decrease; (ns) = not significant; NA = Not Affected; ND = Not Detected

To summarise, the release of all the tested terpenoids decreases at increasing tannin concentrations. For esters, around 2.85 seemed to be a cut-off logP value: esters characterized by lower logPs diminished at smaller PPhs levels, while they were raised at higher ones, suggesting that for poorly hydrophobic esters, there is the prevalence of retention phenomena at low PPhs concentrations and the prevalence of salting-out effects at higher PPhs concentrations. Conversely, the release of esters characterized by higher logPs, except for ethyl octanoate, decreased independently from the PPhs level, suggesting that hydrophobicity represents the main driving force of highly hydrophobic esters release. Except for guaiacol and eugenol, the release of volatile phenols (e.g., 4-ethylphenol and 4-ethylguaiacol) was essentially reduced by PPhs. At increasing tannins concentrations, oxygen heterocycles VOCs with logP values higher than 1 (e.g., γ-nonalactone, trans-whiskey lactone, and cis-whiskey lactone), show a decrease in volatility. To the contrary, VOCs with lower logP values (i.e., 5-methyl furfural), independently from the matrix type, have shown a "salting-out" effect. Globally, the observed trends seem to suggest that in orthonasal conditions, for VOCs with a greater hydrophilic character, an increase in PPhs determines a greater release (salting-out), which is probably because this increase reduces the solvating capacity that the water molecules have toward VOCs. On the contrary, VOCs with a greater hydrophobic character are more retained at increasing PPhs concentration, likely in reason of hydrophobic intermolecular interactions occurring between them.

3.3. Polyphenols effects on aromas release in oral conditions: the role of saliva

When considering PPhs-VOCs interactions in oral modality, the effects described above for orthonasal conditions, can change. During wine tasting, and in general during food consumption, aroma compounds are transported to the nasal cavity by following the retronasal route (nasopharynx). Along this path there is a dilution and a change in VOCs repartition between the condensed and the gas phases due to the mixing of wine with saliva, to their interaction with the oral/pharyngeal cavity during the transfer to the olfactory receptors through the breath airflow. Several factors (e.g., anatomical, physicochemical, physiological, mechanical, etc.) can be implicated in VOCs release and perception in retronasal conditions (Salles et al., 2011). Individual oral physiology characteristics, such as salivary flow rate, protein content and composition, antioxidant capacity, temperature, mucosa, swallowing and tongue force, oral volume, respiratory flow, and other oral physiological components could vary amongst individuals and with matrix composition, affecting wine aromas release

(Noble, 1996; Buettner & Beauchamp, 2010; Muñoz-González et al., 2014; Piombino et al. 2014; Muñoz-González et al., 2019; Muñoz-González et al., 2020). However, among all, saliva can be considered as a main factor so that its effects on food and beverages flavour perception have been frequently investigated in the last decades.

Saliva can directly play a modulating role on polyphenols perception (PPhs-saliva interactions) and on aroma release and perception (VOCs-saliva interactions) during wine tasting, so we can argue that saliva could play a further indirect role by affecting PPhs-VOCs interactions (Gawel, 1998; Guichard, 2006; Cheynier & Sarni-Manchado, 2010; McRae & Kennedy, 2011; Piombino et al., 2014; Laguna et al., 2017; Mosca & Chen, 2017; Ployon et al., 2017; Soares et al., 2017). While the first evidence on the molecular mechanisms explaining astringency as the sensation elicited by the interaction and precipitation of salivary proteins by tannins, have been published around 50 years ago (Bate-Smith, 1954), the direct impact of saliva on VOCs release and perception has started to be shown more recently (Roberts & Acree, 1995; van Ruth & Roozen, 2000; Friel & Taylor, 2001; Buettner, 2002 a and b). Numerous phenomena have been proposed to explain the changes in release amount, kinetic, and nature of VOCs in the presence of saliva. Salivary proteins have binding sites available to trap volatiles. In fact, mucin and other salivary proteins can directly bind specific aroma compounds, through covalent and non-covalent interactions (hydrophobic and electrostatic interactions, Van der Waals forces, formation of Schiff bases) inducing a modification in their release (van Ruth & Roozen, 2000; Friel & Taylor, 2001; Pagès-Hélary et al., 2014; Ployon et al., 2017; Ployon et al., 2020). Salivary enzymes present in human saliva can catalyse reactions, able to transform some volatile molecules into other odorants, and can hydrolyse bound volatiles from non-volatile precursors (Svensson, 1988; Bohren et al., 1989; Buettner, 2002 a and b; Pagès-Hélary et al., 2014; Ployon et al., 2017). Moreover, saliva can directly impact VOCs dilution affecting their release to the oral cavity, since the repartition of molecules within the system wine-saliva-air is different compared to the wine-air system (Ployon et al., 2017). The first works hypothesizing a role played by saliva on PPhs-VOCs interactions during white and red wine tasting have been conducted using model mouth systems, in *in-vitro* conditions, with either human either artificial saliva, or comparing both types (Genovese et al., 2009; Mintropoulou et al., 2011). In recent years, the development of procedures and methodologies allowing the quantification of aroma release in real *in-vivo* conditions has improved results useful to understand how saliva-polyphenols interactions could impact the release and the

perception of wine aroma. Different innovative approaches were used, such as the application of retronasal trapping devices that allowed to entrap the exhaled breath of the panellists (Muñoz-González et al., 2014), or intra-oral SPME procedures (Esteban-Fernández et al., 2018; Perez-Jiménez et al., 2019; Muñoz-González et al., 2020), or the monitoring of the nasal cavity exhalations through PTR-ToF-MS during real wine tasting sessions (Muñoz-González et al., 2019).

3.3.1. Impact of saliva on aroma through the modulation of polyphenols-VOCs interactions

The contradictory results available in the current literature could be at list partially due to the different approaches that were used (i.e., model solutions or real wines, artificial or real human saliva, different aromas and polyphenols concentrations, different analytical methods), so that it is difficult to get general conclusions on causes and effects of aromas–saliva–polyphenols interactions. Therefore, as already observed (Esteban-Fernández et al., 2018), the effect of polyphenol-salivary proteins interactions on aroma release is very little known. Some hypotheses have been presented as possible causes of the different VOCs release behaviour in retronasal conditions, compared to orthonasal ones, in presence of different polyphenols at different concentrations. In the PPhs-VOCs-saliva systems, not only dilution, interaction and salting-out effects can occur, but also the balance among the following phenomena should be considered: inhibition by PPhs of salivary enzymes activity in "metabolizing" VOCs; competitions between PPhs and VOCs in interacting with salivary proteins; hydrophobic VOCs inclusion in PPhs-saliva complexes. These phenomena have been argued based on results from *in-vivo* trials and mainly observed on wine volatile esters.

Saliva contains several enzymes (e.g., esterases, aldehyde dehydrogenases, aldose reductases, peroxidases, etc…) originating from salivary glands, oral tissues, and microbiota (Nakamura & Slots, 1983; Ihalin et al., 2006). These enzymes may be able to catalyse biochemical reactions, metabolizing certain classes of aromas (e.g., esters, aldehydes, ketones, alcohols, thiols) by transforming them into different odorants. Oral enzymes can also hydrolyse odourless aroma precursors (glycosidic or aminoacidic) with the consequent production of odorous aglycones (Svensson, 1988; Bohren et al., 1989; Hemingway et al., 1999; Buettner, 2002 a and b; Starkenamann et al. 2008; Pagès-Hélary et al., 2014; Ployon et al., 2017). In presence of phenolic compounds, it has been shown that some enzymatic activities may be inhibited (Juntheikki & Julkunen, 2000; Weng et al., 2018). In the specific case of esters, it has been hypothesized that the activity of carboxyl esterase involved in their metabolism might be inhibited in presence of phenolic compounds, thus leading to a lower hydrolysis of

esters in solutions and, consequently, to a higher concentration of molecules that can be released. This hypothesis is supported by results obtained in both *in-vitro* (Genovese et al., 2009) and *in-vivo* (Muñoz-González et al., 2014) conditions. Genovese and co-workers (2009) investigated the influence of human and artificial saliva on the release of white and red wine VOCs by SPME/GC-MS analyses using a model mouth system called retronasal aroma simulator (RAS). In the experiment with human saliva, containing salivary enzymes, authors observed a significant lower decrease of some VOCs concentrations (i.e., ethyl butanoate, 3-mehtylbutyl acetate, ethyl hexanoate, hexyl acetate and ethyl dodecanoate) in red wine headspace, compared to the white one. Successively, interesting results have been pointed out in a more recent study involving a panellists' group that was classified as lower releaser based on their real-time breathing profile monitored by a tailor-made retronasal aroma trapping device (RATD) that allowed to entrap the exhaled breath of the panellists, and consequent GC-MS analysis (Muñoz-González et al., 2014). Authors have shown a higher release of ethyl hexanoate during the consumption of a young-red wine, characterised by the highest polyphenolic content, in comparison with a white, and aged-red, a sparking and a sweet wine (Muñoz-González et al., 2014). Since the same effect was not observed on the other analysed ester (i.e., isoamyl acetate), the presence of tannins could have played an inhibition activity on certain salivary enzymes implicated in the metabolism of ethyl esters, in the mouth. Similar phenomena have been observed by using a more innovative *in-vivo* PTR-ToF-MS approach monitoring the nasal cavity of nine subjects after they rinsed their mouths with three different samples (a control wine and the same wine with two different commercial oenological tannins added). The presence of tannins (50 mg/L) corresponded to a higher release of ethyl decanoate, that was significant at the first and fourth minute of monitoring after swallowing (Muñoz-González et al., 2019), in the prolonged aroma release condition, responsible for the aroma persistence (Linforth & Taylor, 2000; Buettner et al., 2001; Buettner et al., 2004; Buffo et al., 2005). Authors suggested that "the presence of tannins could have inhibited certain salivary enzymes implicated in the metabolism of aroma compounds, such as ethyl esters, in the mouth" (Muñoz-González et al., 2019).

Moreover, a modification of VOCs release might be due to competitions between PPhs and VOCs in interacting with salivary proteins. Salivary proteins (e.g., mucins, α-amylases, etc…) have demonstrated their ability to interact with aroma compounds through hydrophobic and other kinds of non-covalent interactions (electrostatic interactions, Van der Waals forces) (Lubbers et al., 1998), modifying their release and perception

(Guichard, 2006). However, phenolic compounds have shown to strongly interact with mucin (Asquit et al., 1987; Charlton et al., 2002), likely competing with aromas in their interaction with saliva. For example, in *in-vitro* conditions and investigating the influence of the presence/absence of saliva on the release of red and white wine VOCs, a lower decrease for some alcohols (i.e., 2-methyl-1-propanol, 3+2-ethyl-1-butanol, 3-methyl-1-pentanol and 1-hexanol) was found with human or artificial saliva in red wine compared to the white wine (Genovese et al., 2009). Also, Mintropoulou and co-authors (Mintropoulou et al., 2011) showed a different modulating effect of artificial saliva (with no added enzymes) on some VOCs release in model solutions with added tannins. Following interaction with saliva, authors observed a lower decrease for isoamyl acetate, ethyl hexanoate, octanoate, decanoate, dodecanoate, 2-methyl-1-butanol and linalool, in presence of tannins.

However, in presence of tannins, some VOCs, due to their ability to participate in the formation of large complexes with salivary proteins and wine carbohydrates (Mintropoulou et al., 2011), might be encapsulated, leading to a lower retronasal release. This phenomenon has been observed for some hydrophobic esters (i.e., isoamyl acetate and ethyl hexanoate) in different works (Esteban-Fernández et al., 2018; Perez-Jiménez et al., 2019), such as for guaiacol and, in a lesser extent, for β-ionone (Perez-Jiménez et al., 2019). These authors suggested an interaction of these VOCs with salivary proteins-PPhs complexes, resulting in a lower immediate release in red wines (Esteban-Fernández et al., 2018) or in wines added with different types of phenolic extracts (Perez-Jiménez et al., 2019).

A further aspect possibly affected by the PPhs-VOCs-saliva interactions could be the flavour persistence during wine tasting (Muñoz-González et al., 2019). Polyphenols are responsible for astringency and bitterness perception, two oral sensations characterised by an extended persistence as showed by TDS (temporal dominance of sensations) studies (Etaio et al., 2016). Based on some evidence the long-time of development and the dynamic persistence might be at least partially linked to the multistage mechanism underpinning PPhs-saliva interactions (Jöbstl et al., 2004). The results presented by Jöbstl and co-workers show that polyphenol-protein binding produces a more cross-linked and hydrophobic protein, that could enhance hydrophobic trapping/inclusion of small molecules such as VOCs. Moreover, specific aroma compounds-mucosa interactions occurring after swallowing could contribute to the formation of a coating on the throat and pharynx, that could increase the liquid/air free surface, thus modulating some VOCs release over time (Ployon

et al., 2017), under the expiration flows in specific *in-vivo* conditions. This phenomenon, together with the inhibition by PPhs of salivary enzymes activity in "metabolizing" VOCs, could explain the higher ethyl decanoate release over time once the wine was expectorated (Muñoz-González et al., 2019). Also, Perez-Jiménez and co-workers (Perez-Jiménez et al., 2019) have suggested a modification in VOCs behaviour over time. After the fourth minute from the expectoration, in prolonged aroma release conditions, the behaviour of some VOCs release changed for some individuals. For three of the six subjects participating to the study, a higher release of ethyl hexanoate and isoamyl acetate has been observed when tasting the wide added with the red wine phenolic extract mainly composed of anthocyanins. For two individuals, a higher release of guaiacol has been show for all the investigated phenolic extracts. However, some of these cited results are in contradiction with data from a very recent study (Muñoz-González et al., 2020), that showed, through an intra-oral SPME procedure and consequent GC-MS analysis, a lower immediate and prolonged esters' retronasal release (i.e. ethyl butanoate, isoamyl acetate, ethyl pentanoate, hexanoate, octanoate and, decanoate) in the wine added with a moderate total polyphenol content (TPC) compared to the wine with a low TPC, 661±33 and 402±10 mg gallic acid/L, respectively.

All of this highlights the high complexity and scarce knowledge of PPhs-VOCs-saliva interactions and their effects on VOCs release and perception in retronasal settings. Current research is paying attention to individual salivary characteristics and composition (e.g., flow, protein content, antioxidant capacity) and interindividual differences, trying to understand if the interindividual diversity could modulate the in-mouth aroma release by affecting PPhs-VOCs interactions (Perez-Jiménez et al., 2019; Muñoz-González et al., 2019).

To summarise, it has been observed that in the presence of phenolic compounds, some salivary enzymatic activities may be inhibited (e.g., carboxyl esterase), thus leading to a lower hydrolysis of some VOCs (e.g., esters) in solutions and, consequently, to a higher release. Moreover, phenolic compounds have shown to strongly interact with mucin, "competing" with aromas in their interaction with saliva and resulting in some VOCs being characterized by a lower decrease in volatility. Finally, in the presence of tannins, some VOCs, due to their ability to participate in the formation of large complexes with salivary proteins and wine carbohydrates, might be encapsulated, leading to a lower retronasal release. Despite these observations, the impact of PPhs–VOCs–saliva interactions on aroma release during wine consumption deserves further investigation and, in this context, additional research is needed to clarify the contribution of interindividual

differences in terms of saliva composition (i.e., studies with a higher number of subjects will be necessary), since it seems that in these systems, salivary characteristics and interindividual differences may play a crucial role on VOCs release and perception.

3.4. Polyphenols effects on aromas sensory perception

Few experiments have been conducted to measure the sensory impact of polyphenols on aromas perception in orthonasal and retronasal conditions.

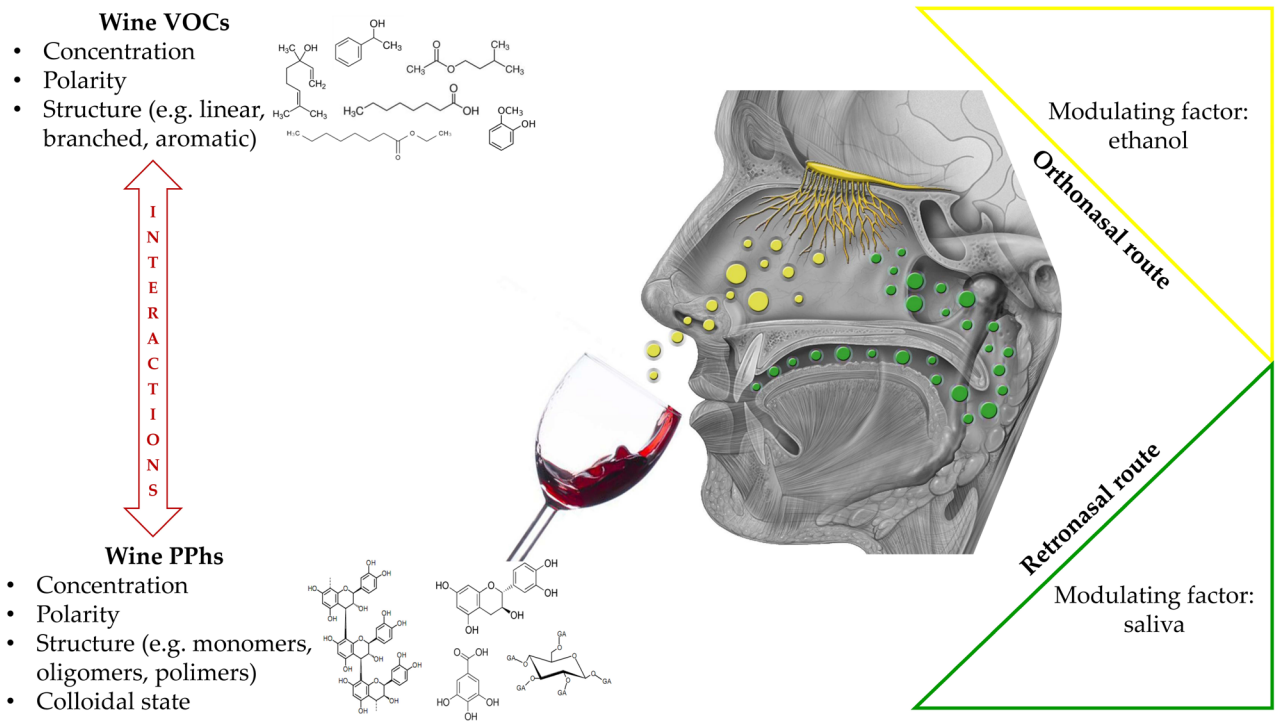
In orthonasal conditions, Lund and co-workers (Lund et al., 2009), investigated the effects of three polyphenols naturally present in white wines on the perception of four key aroma compounds from a Sauvignon Blanc wine. Their results showed that the perception of isobutyl methylpyrazine and ethyl decanoate was suppressed by catechin, caffeic acid, and somehow by quercetin or its degradation products. Regarding the two former phenolic compounds, non-covalent bonds (π-π interaction and hydrogen bonding) between their large-OH groups and the aroma compound might have reduced its perception (Dufour & Bayonove, 1999; Jung et al., 2000). The perception of 3-mercaptohexanol (described as passionfruit skin/stalk), was suppressed when catechin and quercetin were added, while it was enhanced by caffeic acid. 3-Mercaptohexyl acetate was the least affected volatile, suggesting that the acetate group was less suitable to interact with phenolics, compared to the indoxyl. Considering red wine aroma perception in orthonasal conditions, some authors have found that in wines characterized by high polyphenols concentrations (5.4-7.2 g/L), the intensity of perceived fruity, citrus, strawberry, cooked fruit, and floral odours was significantly lower compared to wines with low polyphenols concentrations (1.4-3.2 g/L). A tendency, even if not significant, to the accentuation of spicy, herbaceous, and sweet pepper notes was also observed. However, neither changes in headspace (HS-SPME-GC-MS analyses) nor in matrix concentration (physicochemical composition) resulted significantly related to the relative changes in sensory intensity (Goldner et al., 2011).

In retronasal conditions and focusing on PPhs effects on the prolonged aroma release, a sensory study conducted on a Syrah wine adjusted to two concentrations of ethanol and tannins, has pointed out that the duration (length in mouth) of bell pepper flavour (due to presence of 3-isobutyl-2-methoxypyrazine) was longer at higher tannin concentrations. A possible explanation could be found in a change in release kinetic: the formation of 3-isobutyl-2-methoxypyrazine-tannins complexes (Jung et al., 2000; Aronson & Ebeler,

2004), that could have resulted in this aroma compound being retained in solution, yielding to a more gradual release of its bell pepper flavour over time (Baker and Ross, 2014). Finally, in the same study cited above (Muñoz-González et al., 2020), a sensory descriptive analysis was performed to compare intra-oral SPME data with sensory assessments. Results showed that wines added with phenolic extracts exhibited lower retronasal intensity for the attributes "banana" and "apple", aromatic notes associated with isoamyl acetate and ethyl hexanoate. At the contrary, the attribute "honey" correlated to β-phenylethanol, was scored slightly higher in the wines with phenolic extracts, as such as the attribute "chemical", correlated to guaiacol, however without a relationship to a higher oral release of this latter VOC. The authors suggested that the phenolic extracts that were tested might exert an effect on the prolonged aroma release. This seems an interesting research perspective to approach by means of dynamic sensory methods coupled with real-time instrumental techniques to test over time if polyphenols might induce a modification on the long-lasting aroma perception of aroma attributes.

To simply schematise the main variables potentially involved in the PPhs–VOCs interactions that may occur in wine, a representation is pictured in Figure 9.

Figure 9. Simplified schematic representation of the main variables potentially involved in the PPhs-VOCs interactions that may occur in wine. Cited from Pittari et al. (2021).



Taking all of this in mind, one of the main objectives of the present PhD thesis is to determine how different polyphenols composition could influence wine volatiles. In particular, the potential modulation of polyphenols on the release and the perception of wine aromas will be studied.

4. PPhs modulating role toward wine aromas oxidation

Wine sensory quality strongly depends on the complex phenomena taking place during the whole winemaking process, in which barrel and bottle ageing play an important role. High quality red wines normally require long ageing periods to reach its *optimum* fullness in terms of colour, olfactory, taste and aromatic characteristics. However, during ageing, unfavourable processes (i.e., unsuitable temperature, humidity, and storage or transport conditions), could favour the so-called early wine ageing, influencing wine sensory quality and leading to a reduction of its shelf-life. Among these unfavourable processes, oxygen exposure (prior and after bottling) represents one of the main critical factors able to modify wine quality, either improve or damage its sensory properties (Ugliano, 2013). Since the pioneering work of Pasteur, numerous studies have been dedicated at characterizing the impact of oxidation on wine quality. In the case of high-quality red wines, it is currently accepted that a slow and constant aeration through the different steps of winemaking and ageing has a positive effect on wine sensory quality, while a fast and excessive oxidation can significantly alter this quality, negatively impacting colour, odour, aroma, flavour, and mouthfeel properties (Ugliano, 2013; Ferreira et al., 2014). Indeed, early oxidative ageing is one of the main widespread worldwide defects in oenology (Ugliano, 2013; Franco-Luesma, et al., 2019) and it corresponds to an early sensory deterioration and a short wine shelf-life impacting the commercial value of the wine.

Being most of the high-quality red wines characterised by moderate-long bottle ageing periods, preventing early oxidative ageing processes, and avoiding the loss or the deterioration of some important olfactory and aromatic characteristics, enhancing the wine aroma longevity and shelf-life is of fundamental importance for Italian winemakers and oenologists. Moreover, thinking that almost 40% of world wine production is now exported, it is important to control the chemical changes occurring during unfavourable storage and freight conditions. Indeed, the storage and freight conditions of wine prior to consumption may lead to a reduction in wine quality because of unintended physical and chemical changes in the wine.

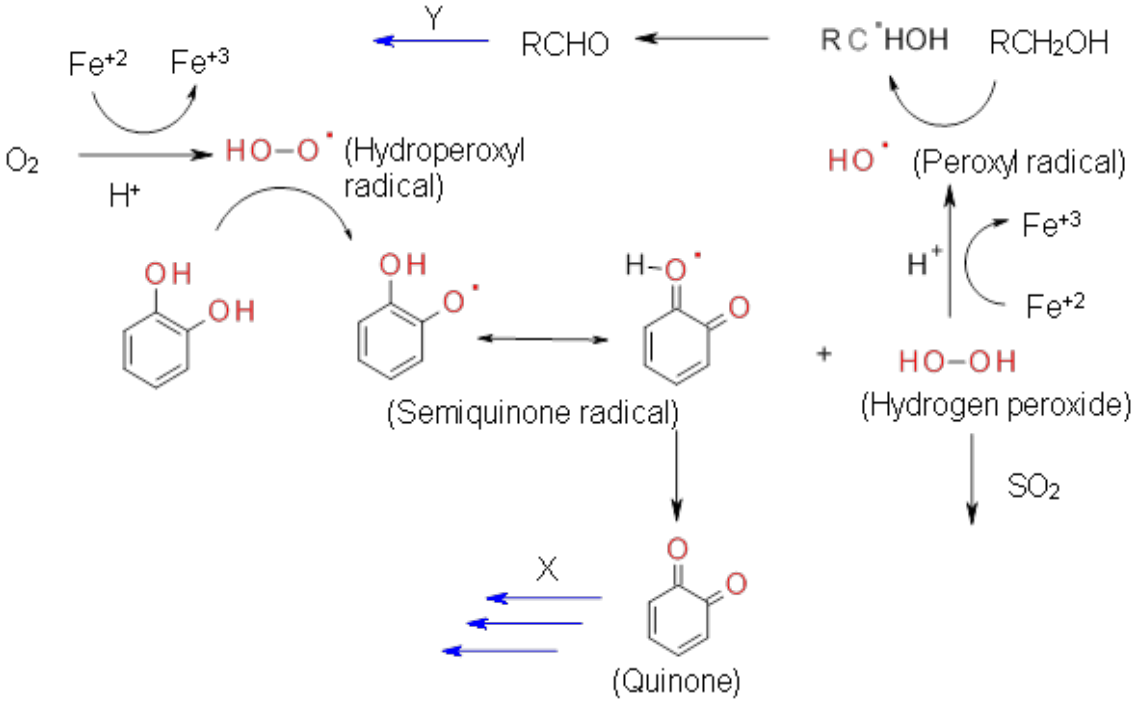
4.1. Mechanisms of oxidation

Two types of oxidation mechanisms can occur during the winemaking process, usually defined as enzymatic and non-enzymatic oxidation. Enzymatic oxidation, or enzymatic browning, almost entirely occurs in grape must (Oliveira et al., 2011). Non-enzymatic oxidation, or wine chemical oxidation, prevails in fermented wine

(Oliveira et al., 2011). However, during red wine processing the impact of enzymatic oxidation is limited (Cheynier et al., 2000) compared to chemical oxidation that represents a very common and difficult problem to manage, against which oenologists and winemakers must constantly interface.

During chemical oxidation of wine, polyphenols characterised by *ortho*-dihydroxybenzene (a catechol ring) or 1,2,3-trihydroxybenzene (a galloyl group) moieties, such as (+)-catechin/(−)-epicatechin, gallocatechin, gallic acid and its esters, and caffeic acid, are the most readily oxidized wine constituents (Singleton, 1987, 2000; Kilmartin et al., 2001; Danilewicz, 2003; Li et al., 2008). These substrates are sequentially oxidized to semiquinone radicals and benzoquinones, while oxygen is reduced to hydrogen peroxide. The whole process, schematised in Figure 10, is mediated by the redox cycle of Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu^{+} (Danilewicz et al., 2008).

Figure 10. Reductive oxidation ladder and primary oxidation products.
Cited from Waterhouse & Laurie (2006).



Once formed, *ortho*-quinones, that are highly unstable and reactive compounds, can be involved in different chemical reactions with other wine components (Quideau et al., 2011), including in nucleophilic conjugate addition reactions with some phenols, thiols, and amines. At the same time, hydrogen peroxide can react with Fe^{2+} ions via the Fenton reaction to produce a hydroxyl radical which is extremely reactive and can react with

various wine constituents, causing the formation of aldehydes and ketones. In this case, ethanol will be primarily oxidised to produce acetaldehyde (Waterhouse & Laurie, 2006).

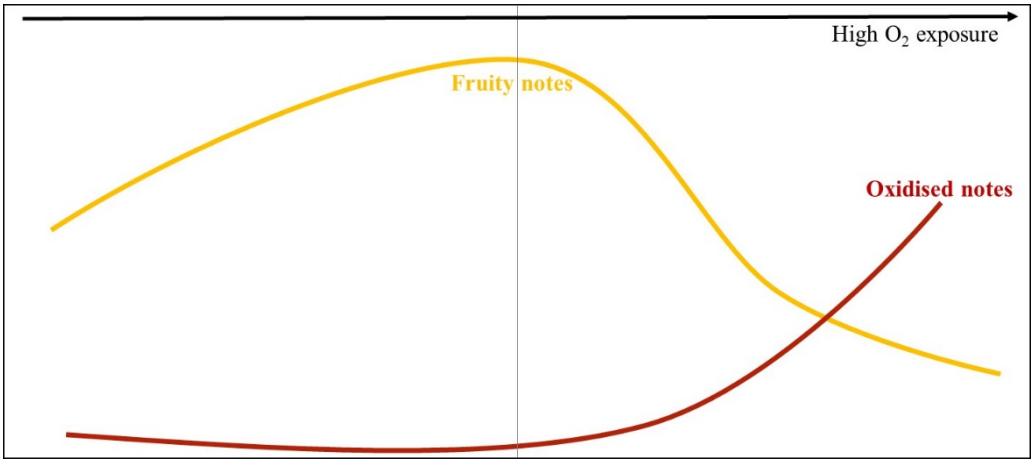
4.2. Chemical and sensory effects of oxidation

Oxidative transformation of wine compounds can modify the structure and the properties of molecules belonging to different chemical families, affecting compounds involved in wine colour and flavour (i.e., olfaction, gustation, and oral somatosensory inputs).

During the last two decades, numerous studies aimed at gaining a deeper understanding of the molecular origin of aroma evolution through wine ageing and oxidation. They characterized the evolution of wine VOCs in terms of quantity and quality during the oxidation processes, in some cases trying to observe their perception pattern during wine tasting. It is nowadays commonly accepted that the easily sensory recognizable symptoms of oxidation are i) browning (mainly in white wines), ii) loss of freshness and fruity aromas, and iii) appearance of oxidised aromas (i.e., raisin, overripe character, rancid, dried fruit, caramel, farm-feed, cooked vegetables, boiled potato, hay, sweet and Madeira/Porto notes) (Escudero et al., 2000; Escudero et al., 2002; Silva Ferreira et al., 2003; Culleré et al., 2007; Ugliano, 2013).

In Figure 11, the effect of high oxygen exposure on the aroma evolution of a bottled wine is represented.

Figure 11. The effect of high oxygen exposure on the aroma of bottled wine. Adapted from Ugliano et al. (2010).



These aromatic changes related to excessive oxygen exposure are due to the oxidation of VOCs, which leads to the formation of new active aroma compounds and to the decrease/disappearance of several VOCs (Escudero et al., 2000).

Under oxidative conditions, it can be observed the generation/increase of several volatiles, involved in the appearance of the above-mentioned oxidised notes. The major contributors identified as responsible for the oxidative notes include various aldehydes, lactones, and acetals (Escudero et al., 2000; Escudero et al., 2002; Silva Ferreira et al., 2003; Culleré et al., 2007; Ugliano, 2013). The most important aldehyde identified in wine is acetaldehyde. It represents up to 90% of the total amount of aldehydes found in wine (Nykänen, 1986). During oxidation, it is formed by the reaction of the hydroxyl radical and ethanol (Waterhouse & Laurie, 2006). In the free form, acetaldehyde is responsible of green apple, overripe/bruised apple, grassy, pungent, nutty, and sherry notes. Besides acetaldehyde, other volatiles can be highly responsible for the occurrence of oxidative aromatic notes, such as methional, phenylacetaldehyde, aliphatic aldehydes (i.e., trans-2-nonenal) and sotolon. Methional and phenylacetaldehyde, which have a great impact on the aroma of both red and white wines, are supposed to be formed via the Strecker reaction of dicarbonyl compounds with methionine, and phenylalanine amino acids, respectively (Escudero et al., 2000; Rizzi, 2006).

Another aspect to consider and manage during wine oxidation processes, is the potential decrease/loss of several volatiles. For example, during oxidation polyfunctional thiols decrease significantly, especially in white wines, leading to loss of freshness (Ugliano, 2013). Moreover, studying the effects of oxidation on young Chardonnay white wines, Patrianakou & Roussis (2013) observed that under semi-oxidative and forced oxidative conditions, the concentration of five important wine esters (i.e., ethyl acetate, hexanoate, octanoate, decanoate, and isoamyl acetate) decreased. Picariello et al. (2020) observed that ethyl esters and acetates decrease during oxidation also in red wines produced by Corvina grapes. However, the nature of esters decrease during oxidation processed and the reactions involved is not clear, as esters can be also easily hydrolysed (Patrianakou & Roussis, 2013; Carrascon et al., 2015).

More recently, Carrascon and co-workers (Carrascon et al., 2015) observed that at low levels of SO_2, β-damascenone, E-whiskylactone, and methyl vanillate are the preferred targets of free radical species. Carrascon et al. (2015) reported that the concentration of isoeugenol, vanillin and ethyl vanillate increases after exposing wine to oxygen, while their increase was not correlated to O_2 consumption.

4.3. Polyphenols' antioxidant activity

Different classes of wine polyphenols exhibit antioxidant properties. Two mechanisms could be involved in the antioxidant capacity of polyphenols: i) scavenging of reactive oxygen species (ROS) and reactive nitrogen species (Takahama et al., 2002; Takahama et al., 2007) and ii) ion chelation (Melidou et al., 2005). The chelation of Fe^{2+} ions by polyphenols increases their oxidation to Fe^{3+} ions in the presence of oxygen. This effect depends on the polyphenol structure and it is increased when Fe^{2+} ions are bound to a galloyl group (Perron et al., 2010). The chelation of Fe^{2+} ions with their oxidation to Fe^{3+} ions decreases the quantity of Fe^{2+} that could participate in the Fenton reaction that is at the origin of the production of hydroxyl radicals (Perron et al., 2010).

Some works have investigated the possible antioxidant activity of white and red wines polyphenols (i.e., phenolic acids), in some cases in combination of SO_2 and/or glutathione, on wine VOCs during ageing, storage, and oxidation processes (Roussis et al., 2005; Lambropoulos & Roussis, 2007; Roussis et al., 2007; Roussis & Serganitis, 2008). Data show that phenolic compounds can impart an antioxidant activity to wine and acting as natural preservatives, delaying the decrease of some esters and terpenes (i.e., isoamyl acetate, ethyl caproate, ethyl caprylate and linalool) due to the oxidation processes, in this way reducing the SO_2 application as antioxidant compound.

A more recent study (Ferreira et al., 2014) investigated the effects of the relationships between the wine chemical composition and the formation of Strecker aldehydes and among other correlations, a negative one was found with some polyphenols (i.e., pyranoanthocyanin pigments, and total flavonols). Moreover, it has been observed that the utilization of SO_2 in combination with catechin can slow down the loss of polyfunctional thiols (i.e., 3-MH) (Carrascon et al., 2015).

In red wines, the antioxidant capacity has been mainly attributed to tannins (Kennedy et al., 2006). As already reported above, tannins are usually divided into two groups: i) oligomers and polymers of flavan-3-ols, namely condensed tannins or proanthocyanidins (from grapes), and ii) non-flavonoids polymers, namely hydrolysable tannins (from wood) (Lesschaeve & Noble, 2005).

Besides their extraction during winemaking and wine ageing in wooden barrels, both proanthocyanidins and hydrolysable tannins can be added in wine as oenological tannins. Their use in winemaking is a long-used and common technological practice. Up to date, they are only authorized by the International Organization of Vine

and Wine (OIV) to facilitate the clarification of wines and musts (OIV, 2015). However, they are also used by winemakers for other properties, such as their impact on wine flavour and antioxidant capacity. The antioxidant property is nowadays one of the main researched properties to protect wines against oxidation (González-Centeno et al., 2012; Magalhães et al., 2014; Versari et al. 2013). Oenological tannins can be very useful in protecting musts and white wines against browning and oxidation (Versari et al., 2013).

However, polyphenols antioxidant properties are controversial, since phenolic compounds in general, and tannins in particular, can show very different antioxidant properties depending on their composition (Magalhães, et al., 2014; Vignault et al., 2018). Therefore, the actual ability of tannins to protect wine aromas from the early oxidative ageing it is still an unclear topic in wine research (Ferreira et al., 2014), that need to be further explored.

For this reason, in the frame of our project, we want to explore the role of polyphenols in modulating the protection of wine aroma toward the oxidation.

References

Aronson, J.; Ebeler, S.E. Effect of polyphenol compounds on the headspace volatility of flavors. *Am. J. Enol. Vitic.* **2004**, 55, 13–21.

Asquit, T.N.; Uhlig, J.; Mehansho, H.; Putman, L.; Carlson, D.M.; Butler, L. Binding of condensed tannins to salivary proline-rich glycoproteins: The role of carbohydrate. *J. Agric. Food. Chem.* **1987**, 35, 331–334.

ASTM. 2004. Standard Definitions of Terms Relating to Sensory Evaluation of Materials and Products. Annual Book of ASTM Standards, American Society for Testing and Materials, Philadelphia.

Baderschneider, B.; Winterhalter, P. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *J. Agric. Food. Chem.* **2001**, 49(6), 2788–2798.

Bajec, M.R.; Pickering, G.J. Astringency: Mechanisms and perception. *Crit. Rev. Food Sci. Nutr.* **2008**, 48(9), 858-875.

Baker, A.K.; Ross, C.F. Sensory evaluation of impact of wine matrix on red wine finish: A preliminary study. *J. Sens. Stud.* **2014**, 29, 139-148.

Bartowsky, E.J. Oenococcus oeni and malolactic fermentationmoving into the molecular arena. *Aust. J. Grape Wine Res.* **2005**, 11, 174–187.

Bate-Smith, E.C. Astringency in foods. *Food Processing and Packaging*, **1954**, 23, 124-127.

Bate-Smith, E.C. Haemanalysis of tannins: The concept of relative astringency. *Phytochemistry*, **1973**, 12, 907–912.

Baumes, R. Wine aroma precursors. In: Wine chemistry and biochemistry. Moreno-Arribas, M.V., Polo, M.C. (Eds.). Springer, New York, 2009.

Belda, I.; Ruiz, J.; Alastruey-Izquierdo, A.; Navascués, E.; Marquina, D.; Santos, A. Unraveling the enzymatic basis of wine "flavorome": a phylo-functional study of wine related yeast species. *Front Microbiol.* **2016**, 7, 12.

Boakes, R.A.; Hemberger, H. Odour-modulation of taste ratings by chefs. *Food Qual. Prefer.* **2012**, 25, 81–86.

Bohren, K.M.; Bullock, B.; Wermuth, B.; Gabbay, K.H. The aldo-keto reductase superfamily – cDNA and deduced amino-acid sequences of human aldehyde and aldose reductases. *J. Biol. Chem.* **1989**, 264(16), 9547–9551.

Breslin, P.A.S.; Gilmore, M.M.; Beauchamp, G.K.; Green, B.G. Psychophysical evidence that oral astringency is a tactile sensation. *Chem. Senses,* **1993**, 18, 4, 405–417.

Buettner, A. Influence of human saliva on odorant concentrations. 2. Aldehydes, alcohols, 3-alkyl-2-methoxypyrazines, methoxyphenols, and 3-hydroxy-4, 5-dimethyl-2(5H)-furanone. *J. Agric. Food. Chem.* **2002a**, 50(24), 7105-7110.

Buettner, A. Influence of human salivary enzymes on odorant concentration changes occurring in vivo. 1. Ester and thiols. *J. Agric. Food. Chem.* **2002b**, 50(11), 3283-3289.

Buettner, A. Investigation of potent odorants and afterodor development in two Chardonnay wines using the buccal odor screening system (BOSS). *J. Agric. Food Chem.* **2004**, 52, 2339–2346.

Buettner, A.; Beauchamp, J. Chemical input–sensory output: Diverse modes of physiology–flavour interaction. *Food Qual. Prefer.* **2010**, 21, 915–924.

Buettner, A.; Beer, A.; Hannig, C.; Settles, M. Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging-consequences for retronasal aroma stimulation. *Chem. Senses*, **2001**, 26, 1211–1219.

Buffo, R.; Rapp, J.; Krick, T.; Reineccius, G. Persistence of aroma compounds in human breath after consuming an aqueous model aroma mixture. *Food Chem.* **2005**, 89, 103–108.

Cameleyre, M.; Lytra, G.; Barbe, J.C. Static headspace analysis using low-pressure gas chromatography and mass spectrometry, application to determining multiple partition coefficients: A practical tool for understanding red wine fruity volatile perception and the sensory impact of higher alcohols. *Anal. Chem.* **2018**, 90, 10812− 10818.

Canon, F.; Belloir, C.; Bourillot, E.; Brignot, H.; Briand, L.; Feron, G.; Lesniewska, E.; Nivet, C.; Septier, C.; Schwartz, M.; Tournier, C.; Vargiolu, R.; Wang, M.; Zahouani, H.; Neiers, F. Perspectives on Astringency Sensation: An Alternative Hypothesis on the Molecular Origin of Astringency. *J. Agric. Food Chem.* **2021**, 69 (13), 3822-3826.

Canon, F.; Paté, F.; Cheynier, V.; Sarni-Manchado, P.; Giuliani, A.; Pérez, J.; Durand, D.; Li, J.; Cabane, B. Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. *LANGMUIR*, **2013**, 29(6), 1926-1937.

Canon, F.; Ployon, S.; Mazauric, J.P.; Sarni-Manchado, P.; Réfrégiers, M.; Giuliani, A.; Cheynier, V. Binding

site of different tannins on a human salivary proline-rich protein evidenced by dissociative photoionization tandem mass spectrometry. *Tetrahedron*, **2015**, 71(20), 3039-3044.

Caporale, G.; Policastro, S.; Monteleone, E. Bitterness enhancement induced by cut grass odorant (cis-3-hexen-l-ol) in a model olive oil. *Food Qual. Prefer.* **2004**, 15, 219–227.

Carrascon, V.; Fernandez-Zurbano, P.; Bueno, M.; Ferreira, V. Oxygen consumption by red wines. Part II: differential effects on color and chemical composition caused by oxygen taken in different sulfur dioxide-related oxidation contexts. *J. Agric. Food Chem.* **2015**, 63, 10938-10947.

Castro-Vázquez, L.; Pérez-Coello, M.S.; Cabezudo, M.D. Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes. *Anal. Chim. Acta,* **2002**, 458, 39-44.

Cejudo-Bastante, M.J.; Castro-Vázquez, L.; Hermosín-Gutiérrez, I.; Pérez-Coello, M.S. Combined effects of prefermentative skin maceration and oxygen addition of must on color-related phenolics, volatile composition, and sensory characteristics of Airén white wine. *J. Agric. Food Chem.* **2011a**, 59, 12171–12182.

Cejudo-Bastante, M.J.; Pérez-Coello, M.S.; Hermosín-Gutiérrez, I. Effect of wine micro-oxygenation treatment and storage period on colourrelated phenolics, volatile composition and sensory characteristics. *LWT-Food Sci. Technol.* **2011b**, 44, 866–874.

Charlton, A.J.; Baxter, N.J.; Khan, M.L.; Moir, A.J.G.; Haslam, E.; Davies, A.P.; Williamson, M.P. Polyphenol/peptide binding and precipitation. *J. Agric. Food Chem.* **2002**, 50, 1593−1601.

Charlton, A.J.; Baxter, N.J.; Lilley, T.H.; Haslam, E.; McDonald, C.J.; Williamson, M.P. Tannin interactions with a full-length human salivary proline-rich protein display a stronger affinity than with single proline-rich repeats, *FEBS Letters*, **1996**, 382, 289-292.

Charters, S.; Pettigrew, S. The dimensions of wine quality. *Food Qual. Prefer.* **2007**, 18, 997-1007.

Chatonnet, P.; Dubourdieu, D.; Boidron, J.; Lavigne, V. Synthesis of volatile phenols by Saccharomyces cerevisiae in wines. *J. Sci.* **2006**, 62, 191–202.

Chatonnet, P.; Dubourdieu, D.; Boidron, J.; Pons, M. The origin of ethylphenols in wines. *J. Sci.* **1992**, 60, 165–178.

Chen, J.; Engelen, L. Food oral processing: Fundamentals of eating and sensory perception. Chen, J.; Engelen, L. (Eds); Wiley-Blackwell, John Wiley & Sons Ltd Chichester, UK, 2012.

Cheynier, V. Polyphenols in foods are more complex than often thought. *American Journal of Clinical Nutrition*, **2005**, supp 80:1S, 223S-229S.

Cheynier, V.; Fulcrand, H.; Moutounet, M. Ossidazione dei polifenoli nei mosti d'uva (Oxidation of polyphenols in wine musts). *Vignevini*, **2000**, 27(11), 52–56.

Cheynier, V.; Rigaud, J.; Ricardo-da-Silva, J.M. Structure of procyanidins oligomers isolated from grape seeds in relation to some of their chemical properties. M.H. Hemingway, P.E. Laks (Eds.), Plant polyphenols, chemical properties and their significance, Plenum Press, New York, 1992; pp. 281-294.

Cheynier, V.; Sarni-Manchado, P. Wine taste and mouthfeel. In Managing wine quality: Viticulture and wine quality, 1st ed.; Reynolds, A.G. (Ed.); Woodhead Publishing: Cambridge, United Kingdom, 2010; pp. 29-72.

Chira, K.; Pacella, N.; Jourdes, M.; Teissedre, P.L. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chem*. **2011**, 126, 1971–1977.

Corduas, M.; Cinquanta, L.; Ievoli, C. The importance of wine attributes for purchase decisions: A study of Italian consumers' perception. *Food Qual. Pref.* **2013**, 28, 407-418.

Cravero, M.C. Musty and moldy taint in wines: A review. *Beverages*, **2020**, 6, 41.

Crouzet, J. Les enzymes et l'arôme des vins. *Rev. Fran. OEnol.* **1986**, 102, 42–49.

Culleré, L.; Cacho, J.; Ferreira, V. An assessment of the role played by some oxidation-related aldehydes in wine aroma. *J. Agric. Food Chem.* **2007**, 55, 876-881.

Culleré, L.; Cacho, J.; Ferreira, V. Analysis for wine C5-C8 aldehydes through the determination of their O-(2,3,4,5,6-pentafluorobenzyl) oximes formed directly in the solid phase extraction cartridge. *Anal. Chim. Acta*, **2004**, 524, 201-206.

Danilewicz, J.C. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *Am. J. Enol. Vitic.* **2003**, 54, 73–85.

Danilewicz, J.C.; Seccombe, J.T.; Whelan, J. Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *Am. J. Enol. Vitic.* **2008**, 59, 128–136.

Delwiche, J. The impact of perceptual interactions on perceived flavor. *Food Qual. Prefer.* **2004**, 15(2), 137–146.

Djordjevic, J.; Zatorre, R.J.; Jones-Gotman, M. Odor-induced changes in taste perception. *Exp. Brain Res.* **2004**, 159(3), 405–408.

De Freitas, V.; Mateus, N. Protein/Polyphenol Interactions: Past and Present Contributions. Mechanisms of Astringency Perception. *Curr. Org. Chem.* **2012**, 16, 724–746.

de la Fuente-Blanco, A.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V.; Sáenz-Navajas, M.P. Cross-modal interactions and effects of the level of expertise on the perception of bitterness and astringency of red wine. *Food Qual. Prefer.* **2017**, 62, 155–161.

Del Álamo, S.M.; Fernandez Escudero, J.A.; De Castro Torio, R. Changes in phenolic compounds and colour parameters of red wine aged with oak chips and in oak barrels. *Food Sci Technol Int.* **2004**, 10, 233–241.

Dufour, C.; Bayonove, C.L. Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *J. Agric. Food Chem.* **1999**, 47, 678–684.

Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **2002**, 77, 325-331.

Escudero, A.; Cacho, J.; Ferreira, V. Isolation and identification of odorants generated in wine during its oxidation: a gas chromatography−olfactometric study. *Eur. Food Res. Technol.* **2000**, 211, 105–110.

Esteban-Fernández, A.; Muñoz-González, C.; Jiménez-Girón, A.; Perez-Jiménez, M.; Pozo-Bayón, M.Á. Aroma release in the oral cavity after wine intake is influenced by wine matrix composition. *Food Chem.* **2018**, 243, 125–133.

Etaio I.; Meillon, S.; Pérez-Elortondo, F.J.; Schlich P. Dynamic sensory description of Rioja Alavesa red wines made by different winemaking practices by using Temporal Dominance of Sensations. *J. Sci. Food Agric.* **2016**, 96(10), 3492-3499.

Etievant, P.X. Wine. In Volatile Compounds in Foods and Beverages; Maarse, H. (Ed.); CRC Press: New York, 1991; pp 483−546.

Etievant, P.; Guichard, E.; Salles, C.; & Voilley, A., Flavor From Food to Behaviors, Wellbeing and Health Woodhead Publishing, Cambridge, United Kingdom, 2016.

Ferrer-Gallego, R.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. *Food Res. Int.* **2014**, 62, 1100–1107.

Ferreira, V. Volatile aroma compounds and wine sensory attributes. In Managing Wine Quality, 2nd ed.; Reynolds, A.G., Ed.; Woodhead Publishing: Sawston, Cambridge, UK; Volume Viticulture and Wine Quality, 2010, pp. 3-28.

Ferreira, V.; Bueno, M.; Franco-Luesma, E.; Culleré, L.; Fernández-Zurbano, P. Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *J. Agric. Food Chem.* **2014**, 62, 10015-10027.

Franco-Luesma, E.; Honoré-Chedozeau, C.; Ballester, J.; Valentin, D. Oxidation in wine: Does expertise influence the perception?. LWT – *Food Science and Technology,* **2019**, 116, Article 108511.

Friel, E.N.; Taylor, A.J. Effect of salivary components on volatile partitioning from solution. *J. Agric. Food. Chem.* **2001**, 49(8), 3898-3905.

Gambuti, A.; Moio, L. Phenolic compounds in wines. In Phenolic Compounds in Food: Characterization and Analysis 1st ed. Nollet, L.M.L., Gutierrez-Uribe, J.A., Eds.; CRC Press, New York, US, 2017.

Garrido, J.; Borges, F. Wine and grape polyphenols — A chemical perspective. *Food Res. Int.* **2013**, 54(2), 1844-1858.

Gawel, R. Red wine astringency: A review. *Aust. J. Grape Wine Res.* **1998**, 4, 73–95.

Gawel, R.; Oberholster, A.; Francis, I.L. A "Mouth-feel Wheel": Terminology for communicating the mouth feel characteristics of red wine. *Aust. J. Grape Wine Res.* **2000**, 6, 203–207.

Genovese, A.; Moio, L.; Sacchi, R.; Piombino, P. Sip volume affects oral release of wine volatiles. *Food Res. Int.* **2015**, 77, 426-431.

Genovese, A.; Piombino, P.; Gambuti, A.; Moio, L. Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations. *Food Chem.* **2009**, 114, 100-107.

Godden, P.; Leigh, F.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; HoJ, P.; Robinson, E., Wine bottle closures: physical characteristics and effect on composition and sensory properties of a Semillon wine. 1. Performance up to 20 months post-bottling. *Austr. J. Grape Wine R.* **2001**, 7, 64-105.

Goldner, M.C.; di Leo, L.P.; van Baren, C; Bandoni A. Influence of polyphenol levels on the perception of aroma in Vitis vinifera cv. Malbec wines. *S. Afr. J. Enol. Vitic.* **2011**, 32, 21–27.

Goldner, M.C.; Zamora, M.C.; Di Leo Lira, P.; Gianninoto, H.; Bandoni, A. Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens. Stud.* **2009**, 24, 243-257.

González-Barreiro, C.; Rial-Otero, R.; Cancho-Grande, B.; Simal-Gándara, J. Wine Aroma Compounds in Grapes: A Critical Review. *Crit. Rev. Food Sci. Nutr.* **2015**, 55, 202-218.

González-Centeno, M.R.; Jourdes, M.; Femenia, A.; Simal, S.; Rosselló, C.; Teissedre, P.L. Proanthocyanidin composition and antioxidant potential of the stem Winemaking byproducts from 10 different grape varieties (Vitis vinifera L.). *J. Agric. Food Chem.* **2012**, 60, 11850–11858.

Green, B.G. Oral astringency: A tactile component of flavor. *Acta Psychol*. **1993**, 84, 119–125.

Guichard, E. Flavour retention and release from protein solutions. *Biotechnol. Adv.* **2006**, 24, 226-229.

Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, 45, 3027-3032.

Hagerman, A.E.; Butler, L.G. Protein precipitation method for the quantitative determination of tannins. *J. Agric. Food Chem.* **1978**, 26, 809-812.

Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical Biology and Medicine,* **1994**, 16(6), 845-850.

Haslam, E.; Lilley, T.H. Natural astringency in foodstuffs a molecular interpretation. *Crit. Rev. Food Sci. Nutr.* **1988**, 27, 1-40.

Hatano, T.; Hemingway, R.W. Association of (+)-catechin and catechin-(4Rf8)-catechin with oligopeptides. *Chem. Commun.* **1996**, 2537-2538.

Hemingway, K.M.; Alston, M.J.; Chappell, C.G.; Taylor, A.J. Carbohydrate-flavour conjugates in wine. *Carbohydrate Polymers*, **1999**, 38(3), 283–286.

Hufnagel, J.C.; Hofmann, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008**, 56, 9190–9199.

Hutchings, S.C.; Low, J.Y.Q.; Keast R.S.J. Sugar reduction without compromising sensory perception. An impossible dream? *Critical Reviews in Food Science and Nutrition*, **2018**, 8398, 1-21.

Ickes, C.M.; Cadwallader, K.R. Effects of Ethanol on Flavor Perception in Alcoholic Beverages. *Chem. Percept.* **2017**, 10, 119–134.

Ihalin, R.; Loimaranta, L.; Tenovuo, J. Origin, structure, and biological activities of peroxidases in human saliva. *Arch. Biochem. Biophys.* **2006**, 445(2), 261–268.

Jackson, D.I.; Lombard, P.B. Environmental and management practices affecting grape composition and wine quality – a review. *Am. J. Enol. Vitic.* **1993**, 44, 409-430.

Jackson, R.S. Wine Tasting: A Professional Handbook, 2nd ed; Elsevier-Academic Press: Oxford, UK, 2009.

Jiang, Y.; Gong, N.N; Matsunami, H. Astringency: A more stringent definition. *Chem. Senses*, **2014**, 39, 467–469.

Jöbstl, E.; O'Connell, J.; Fairclough, P.A.; Williamson, M.P. Astringency – a molecular model for polyphenol/protein binding. *Fibre Diffraction Review*, **2004**, 12, 66-69.

Jover, A.J.V.; Montes, F.J.L.; Fuentes, M.d.M.F. Measuring perceptions of quality in food products: The case of red wine. *Food Qual. Prefer.* **2004**, 15, 453–469.

Jung, D.M.; de Ropp, J.S.; Ebeler, S.E. Study of interactions between food phenolics and aromatic flavors using one- and two-dimensional 1 H NMR spectroscopy. *J. Agric. Food Chem.* **2000**, 48, 407–412.

Juntheikki, M.R.; Julkunen-Tiitto, R. Inhibition of b-glucosidase and esterase by tannins from Betula, Salix, and Pinus species. Journal of Chemical Ecology, 2000, 26(5), 1151–1165.

Kallithraka, S.; Bakker, J.; Clifford, M.N. Evidence that salivary proteins are involved in astringency. *J. Sens. Stud.* **1998**, 13, 29-43.

Kennedy, J.A.; Saucier, C.; Glories, Y. Grape and wine phenolics: history and perspective. *Am. J. Enol. Vitic.* **2006**, 57, 239-248.

Kilmartin, P.A.; Zou, H.; Waterhouse, A.L. A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *J. Agric. Food Chem.* **2001**, 49, 1957–1965.

King, E.S.; Kievit, R.L.; Curtin, C.; Swiegers, J.H.; Pretorius I.S.; Bastian, S.E.P.; Francis, I.L. The effect of multiple yeasts coinoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference. *Food Chem*. **2010**, 122, 618–26.

Kwiatkowski, M.; Skouroumounis, G.K.; Lattey, K.A.; Waters, E.J. The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a

Cabernet Sauvignon wine during two years' storage. *Austr. J. Grape Wine R.* **2007**, 13, 81-94.

Labbe, D.; Damevin, L.; Vaccher, C.; Morgenegg, C.; Martin, N. Modulation of perceived taste by olfaction in familiar and unfamiliar beverages. *Food Qual. Prefer.* **2006**, 17, 582–589.

Laguna, L.; Bartolomé, B.; Moreno-Arribas, M.V. Mouthfeel perception of wine: Oral physiology, components and instrumental characterization. *Trends Food Sci. Tech.* **2017**, 59, 49–59.

Lambrechts, M.G.; Pretorius, I.S. Yeast and its importance to wine aroma-a review. *S. Afr. J. Enol. Vitic.* **2000**, 21, 97–129.

Lambropoulos, I.; Roussis, I.G. Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by caffeic acid and gallic acid. *Food Res. Int.* **2007**, 40(1), 176-181.

Langcake, P.; Pryce, R.J. The production of resveratrol by Vitis Vinifera and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, 9, 77-86.

Lawrence, G.; Salles, C.; Septier, C.; Busch, J.; Thomas-Danguin, T. Odour–taste interactions: A way to enhance saltiness in low-salt content solutions. *Food Qual. Prefer.* **2009**, 20, 241-248.

Lesschaeve, I.; Noble, AC. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *Am. J. Clin. Nutr.* **2005**, 81, 330-335.

Li, H. Wine Tasting; China Science Press: Beijing, China, 2006.

Li, H.; Tao, Y.S.; Wang, H. Impact odorants of Chardonnay dry white wine. *Eur. Food Res. Technol.* **2008**, 227, 287–92.

Li, L.; Sun, B. Grape and wine polymeric polyphenols: Their importance in enology. *Crit. Rev. Food Sci Nutr.* **2019**, 59, 563-579.

Linforth, R.; Taylor, A.J. Persistence of volatile compounds in the breath after their consumption in aqueous solutions. *J. Agric. Food Chem.* **2000**, 48, 5419–5423.

Lopes, P.; Silva, M.A.; Pons, A.; Tominaga, T.; Lavigne, V.; Saucier, C.; Darriet, P.; Teissedre, P.L.; Dubourdieu, D., Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. *J. Agric. Food Chem.* **2009**, 57, 10261-10270.

Lorrain, B.; Tempere, S.; Iturmendi, N.; Moine, V.; de Revel, G.; Teissedre, P.L. Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chem.* **2013**, 140, 76–82.

Lubbers, S.; Landy, P.; Voilley, A. Retention and release of aroma compounds in foods containing proteins. *Food Technol.* **1998**, 52(5), 68.

Lund, C.M.; Nicolau, L.; Gardenr, R.C.; Kilmartin, P.A. Effect of polyphenols on the perception of key aroma compounds from Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2009**, 15(1), 18-26.

Lyman, B.J.; Green, B.G. Oral astringency: effects of repeated exposure and interactions with sweeteners. *Chem. Senses.* **1990**, 15, 151–164.

Magalhães, L.M.; Ramos, I.I.; Reis, S.; Segundo, M.A. Antioxidant profile of commercial oenological tannins determined by multiple chemical assays. *Aust. J. Grape Wine Res.* **2014**, 20, 72-79.

McRae, J.M.; Dambergs, R.G.; Kassara, S.; Parker, M.; Jeffery, D.W.; Herderich, M.J.; Smith, P.A. Phenolic compositions of 50 and 30 year sequences of Australian red wines: The impact of wine age. *J. Agric. Food Chem.* **2012**, 60, 10093–10102.

McRae, J.M.; Kennedy, J.A. Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. *Molecules*, **2011**, 16, 2348–2364.

Melidou, M.; Riganakos, K.; Galaris. D. Protection against nuclear DNA damage offered by flavonoids in cells exposed to hydrogen peroxide: The role of iron chelation. *Free Radical Biology and Medicine,* **2005**, 39 (12), 1591-1600.

Mitropoulou, A.; Hatzidimitriou, E.; Paraskevopoulou, A. Aroma release of a model wine solution as influenced by the presence of non-volatile components. Effect of commercial tannin extracts, polysaccharides, and artificial saliva. *Food Res. Int.* **2011**, 44, 1561−1570.

Moio, L.; Ugliano, M.; Genovese, A.; Gambuti, A.; Pessina, R.; Piombino, P. Effect of antioxidant protection of must on volatile compounds and aroma shelf life of Falanghina (Vitis vinifera L.) wine. *J. Agric. Food Chem.* **2004**, 52, 891-897.

Mosca, A.C.; Chen, J. Food-saliva interactions: Mechanisms and implications. *Trends in Food Sci. Technol.* **2017**, 66, 125–134.

Mozzon, M.; Savini, S.; Boselli, E.; Thorngate, J.H. The herbaceous character of wines. *Italian Journal of Food Science*, **2016**, 28(2), 190–207.

Muñoz-González, C.; Brulé, M.; Feron, G.; Canon, F. Does interindividual variability of saliva affect the release and metabolization of aroma compounds ex vivo? The particular case of elderly suffering or not from hyposalivation. *J. Texture Stud.* **2019**, 50, 36-44.

Muñoz-González, C.; Canon, F.; Feron, G.; Guichard, E.; Pozo-Bayón, M.Á. Assessment wine aroma persistence by using an in vivo PTR-ToF-MS approach and its relationship with salivary parameters. *Molecules*, **2019**, 24, 1277.

Muñoz-González, C.; Feron, G.; Canon, F. Physiological and oral parameters contribute prediction of retronasal aroma release in an elderly cohort. *Food Chem.* **2020**, 128355.

Muñoz-González, C.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.Á. Impact of the nonvolatile wine matrix composition on the in vivo aroma release from wines. *J. Agric. Food Chem.* **2014**, 62, 66−73.

Muñoz-González, C.; Perez-Jiménez, M.; Pozo-Bayón, M.Á. Oral persistence of esters is affected by wine matrix composition. *Food Res. Int.* **2020**, 135, 109286.

Nakamura, M.; Slots, J. Salivary enzymes. Origin and relationship to periodontal disease. *J. Periodontal Res.* **1983**, 18, 559–569.

Nasri, N.; Beno, N.; Septier, C.; Salles, C.; Thomas-Danguin, T. Cross–modal interactions between taste and smell: Odour-induced saltiness enhancement depends on salt level. *Food Qual. Prefer.* **2011**, 22, 678-682.

Niimi, J.; Eddy, A.I.; Overington, A.R.; Heenan, S.P.; Silcock, P.; Bremer, P.J.; Delahunty, C.M. Aroma–taste interactions between a model cheese aroma and five basic tastes in solution. *Food Qual. Prefer.* **2014**, 31, 1–9.

Noble, A.C. Taste–aroma interactions. *Trends Food Sci. Technol.* **1996**, 7, 439–443.

Noble, A.C.; Arnold, R.A.; Buechsenstein, J.; Leach, E.J.; Schmidt, J.O.; Stern, P.M. Modification of a standardized system of wine aroma terminology. *Am. J. Enol. Vitic*. **1987**, 38, 143-146.

Nykänen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, 37, 84–96.

OIV. International Oenological Codex. COEI-1-TANINS:2015. http://www.oiv.int/public/medias/4093/e-coei-1-tanins.pdf

Oliveira, C.M.; Ferreira, A.C.S.; De Freitas, V.; Silva, A.M.S. Oxidation mechanisms occurring in wines. *Food Res. Int.* **2011**, 44(5), 1115-1126.

Oliveira e Silva, H.; Guedes de Pinho, P.; Machado, B.P.; Hogg, T.; Marques, J.C.; Câmara, J.S.; Albuquerque, F.; Silva Ferreira, A.C. Impact of forced-aging process on Madeira wine flavor. *J. Agric. Food Chem.* **2008**, 56, 11989-11996.

Pagès-Hélary, S.; Andriot, I.; Guichard, E; Canon, F. Retention effect of human saliva on aroma release and respective contribution of salivary mucin and a-amylase. *Food Res. Int.* **2014**, 424–431.

Paravisini, L.; Guichard, E. Interactions between aroma compounds and food matrix. In Flavour: From Food to Perception, 1st edition; Guichard, E., Salles, C., Morzel, M., Le Bon, A.M., Eds; John Wiley & Sons: Hoboken, New Jersey, US, 2017; pp. 208-234.

Pasteur, M.L. Etudes sur le vin (2nd ed). ; Librairie F. Savy: Paris, France, 1875.

Patrianakou, M.; Roussis, I.G. Decrease of Wine Volatile Aroma Esters by Oxidation. *S. Afr. J. Enol. Vitic.* **2013**, 34, 241-245.

Peleg, H.; Gacon, K.; Schlich, P.; Noble, A. C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **1999**, 79, 1123–1128.

Perestrelo, R.; Silva, C.; Gonçalves, C.; Castillo, M.; Câmara, J.S. An Approach of the Madeira Wine Chemistry. *Beverages*, **2020**, 6, 12.

Pérez-Coello, M.S.; Diaz-Maroto, M.C. Volatile compounds and wine aging. In Wine chemistry and biochemistry; Moreno-Arribas, M.V., Polo, M.C. Eds; Springer: New York, 2009, pp. 295–311.

Perez-Jiménez, M.; Chaya, C.; Pozo-Bayón, M.Á. Individual differences and effect of phenolic compounds in the immediate and prolonged in-mouth aroma release and retronasal aroma intensity during wine tasting. *Food Chem.* **2019**, 285, 147-155.

Perron, N.R; Wang, H.C.; DeGuire, S.N.; Jenkins, M.; Lawson, M.; Brumaghim. J.L. Kinetics of iron oxidation upon polyphenol binding. *Dalton Transactions*, **2010**, 39(41), 9982-9987.

Petit, E.; Jacquet, R.; Pouységu, L.; Deffieux, D.; Quideau, S. Reactivity of wine polyphenols under oxidation conditions: Hemisynthesis of adducts between grape catechins or oak ellagitannins and odoriferous thiols. *Tetrahedron,* **2019**, 75(5), 551-560.

Petrozziello, M.; Asproudi, A.; Guaita, M.; Borsa, D.; Motta, S.; Panero, L.; Bosso, A. Influence of the matrix

composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. *Food Chem.* **2014**, 149, 197-202.

Peynaud, E. The taste of wine: The art and science of wine appreciation. 1st ed.; Macdonald Orbis: London, United Kingdom, 1987.

Picariello, L.; Slaghenaufi, D.; Ugliano, M. Fermentative and post-fermentative oxygenation of Corvina red wine: influence on phenolic and volatile composition, colour and wine oxidative response. *J. Sci. Food Agric.* **2020**, 100, 2522-2533.

Pinelo, M.; Arnous, A.; Meyer, A.S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.* **2006**, 17, 579–590.

Piombino, P.; Genovese, A.; Esposito, S.; Moio, L.; Cutolo, P.P.; Chambery, A.; Severino, V.; Moneta, E.; Smih, D.P.; Owens, S.M.; Gilbert, J.A.; Ercolini, D. Saliva from obese individuals suppresses the release of aroma compounds from wine. *PLoS ONE*, **2014**, 9, e85611.

Piombino, P.; Moio, L.; Genovese, A. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res Int.* **2019**, 116, 548-558.

Pittari, E.; Moio, L.; Arapitsas, P.; Curioni, A.; Gerbi, V.; Parpinello, G.P.; Ugliano, M.; Piombino, P. Exploring Olfactory–Oral Cross-Modal Interactions through Sensory and Chemical Characteristics of Italian Red Wines. *Foods*, **2020**, 9, 1530.

Pittari, E.; Moio, L.; Piombino, P. Interactions between Polyphenols and Volatile Compounds in Wine: A Literature Review on Physicochemical and Sensory Insights. *Appl. Sci.* **2021**, 11, 1157.

Ployon, S.; Belloir, C.; Bonnotte, A.; Lherminier, J.; Canon, F.; Morzel, M. The membrane-associated MUC1 improves adhesion of salivary MUC5B on buccal cells. Application to development of an in vitro cellular model of oral epithelium. *Arch. Oral Biol.* **2016**, 61, 149−155.

Ployon, S.; Brulé, M.; Andriot, I.; Morzel, M.; Canon, F. Understanding retention and metabolization of aroma compounds using an in vitro model of oral mucosa. *Food Chem.* **2020**, 318, 126468.

Ployon, S.; Morzel, M.; Belloir, C.; Bonnotte, A.; Bourillot, E.; Briand, L.; Lesniewska, E.; Lherminier, J.; Aybeke, E.; Canon, F. Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs. *Food Chem.* **2018**, 253, 79-87.

Ployon, S.; Morzel, M.; Canon, F. The role of saliva in odour release and perception. *Food Chem.* **2017**, 226, 212-220.

Poinot, P.; Arvisenet, G.; Ledauphin, J.; Gaillard, J.L.; Prost, C. How can aroma–related cross–modal interactions be analysed? A review of current methodologies. *Food Qual. Prefer.* **2013**, 28, 304–316.

Poncet-Legrand, C.; Cartalade, D.; Putaux, J.L; Cheynier, V.; Vernhet, A. Flavan-3-ol aggregation in model ethanolic solutions: incidence of polyphenol structure, concentration, ethanol content, and ionic strength. *Langmuir*, **2003**, 19, 10563–10572.

Pons, M.; Dauphin, B.; La Guerche, S.; Pons, A.; Lavigne-Cruege, V.; Shinkaruk, S.; Bunner, D.; Richard, T.; Monti, J.-P.; Darriet, P. Identification of Impact Odorants Contributing to Fresh Mushroom Off-Flavor in Wines: Incidence of Their Reactivity with Nitrogen Compounds on the Decrease of the Olfactory Defect. *J. Agric. Food Chem*. **2011**, 59, 3264–3272.

Pozo-Bayón M.Á., Reineccius G. Interactions Between Wine Matrix Macro-Components and Aroma Compounds. In: Wine chemistry and biochemistry. Moreno-Arribas, M.V., Polo, M.C. (Eds.). Springer, New York, 2009.

Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouysegu, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angewandte Chemie-International Edition*, **2011**, 50(3), 586-621.

Rapp, A.; Versini, G. Influence of nitrogen on compounds in grapes on aroma compounds in wines. *J. Int. Sci. Vigne et Vin*, **1996**, 51, 193–203.

Rauhut, D. Usage and Formation of Sulphur Compounds. In: Biology of Microorganisms on Grapes, in Must and in Wine, 1st ed. König, H.; Unden, G.; Fröhlich, J. Eds. Springer-Verlag, Berlin, 2017.

Rentzsch, M.; Schwarz, M.; Winterhalter, P.; Hermosin-Gutierrez, I. Formation of hydroxyphenyl-pyranoanthocyanins in Grenache wines: precursor levels and evolution during aging. *J. Agric. Food Chem.* **2007**, 55 (12), 4883–4888.

Rentzsch, M.; Wilkens, A.; Winterhalter P. Non-flavonoid Phenolic Compounds. In: Moreno-Arribas M.V., Polo M.C. (eds) Wine Chemistry and Biochemistry. Springer, New York, NY, 2009.

Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. Handobbok of Oenology, Vol I. John Wiley & Sons Ltd, Chichester, England, 2006.

Ricardo-da-Silva, J.M.; Cheynier, V.; Souquet, J.M.; Moutounet, M.; Cabanis, J.C.; Bourzeix, M. Interaction of grape seed procyanidins with various proteins in

relation to wine fining. *J. Sci. Food Agric.* **1991**, 57, 111– 125.

Ristic, R.; Boss, P.; Wilkinson, K. Influence of fruit maturity at harvest on the intensity of smoke taint in wine. *Molecules*, **2015**, 20, 8913.

Roberts, D.D.; Acree, T.E. Simulation of retronasal aroma using a modified headspace technique: Investigating the effect of saliva, temperature, shearing, and oil on flavour release *J. Agric. Food. Chem.* **1995**, 43(8), 2179-2186.

Robichaud, J.L.; Noble, A.C. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **1990**, 52, 343–353.

Robinson, A.L.; Ebeler, S.E.; Heymann, H.; Boss, P.K.; Solomon, P.S.; Trengove, R.D. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, 57, 10313–10322.

Rodríguez-Bencomo, J.J.; Muñoz-González, C.; Andújar-Ortiz, I.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.Á. Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. *J. Sci. Food Agric.* **2011**, 91, 2484–2494.

Roussis, I.G.; Lambropoulos, I.; Papadopoulou, D. Inhibition of the decline of volatile esters and terpenols during oxidative storage of Muscat-white and Xinomavro-red wine by caffeic acid and N-acetyl-cysteine, *Food Chem.* **2005**, 93(3), 485-492.

Roussis, I.G.; Lambropoulos, I.; Tzimas, P.; Protection of volatiles in a wine with low sulphur dioxide by caffeic acid and glutathione. *Am. J. Enol. Vitic*, **2007**, 58, 274 – 278.

Roussis, I.G.; Sergianitis, S. Protection of some aroma volatiles in a model wine medium by sulphur dioxide and mixtures of glutathione with caffeic acid or gallic acid. *Flavor Fragr. J.* **2008**, 23, 35–39.

Ruiz, J.; Kiene, F.; Belda, I.; Fracassetti, D.; Marquina, D.; Navascués, E.; Calderón, F.; Benito, A.; Rauhut, D.; Santos, A.; Benito, S. Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine. *Applied Microbiol. Biotechnol.* **2019**, 103:7425–7450.

Sáenz-Navajas, M.P.; Arias, I.; Ferrero-del-Teso, S.; Fernández-Zurbano, P.; Escudero, A.; Ferreira, V. Chemo-sensory approach for the identification of chemical compounds driving green character in red wines. *Food Res. Int.* **2018**, 109, 138–148.

Sáenz-Navajas, M.P; Avizcuri, J.M; Echávarri, J.F; Ferreira, V.; Fernández-Zurbano, P.; Valentin, D. Understanding quality judgements of red wines by experts: Effect of evaluation condition. *Food Qual. Prefer.* **2016**, 48, 216-227.

Sáenz-Navajas, M.P.; Campo, E.; Avizcuri, J.M.; Valentin, D.; Fernández-Zurbano, P.; Ferreira, V. Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: Wine reconstitution strategies and sensory sorting task. *Anal. Chim. Acta,* **2012**, 732, 64–72.

Sáenz-Navajas, M.P.; Campo, E.; Cullere, L.; Fernandez-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010a**, 58, 5574–5585.

Sáenz-Navajas, M.P.; Campo, E.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chem.* **2010b**, 121, 1139–1149.

Sáenz-Navajas, M.P.; Ferrero-del-Teso, S.; Jeffery, D.W.; Ferreira, V.; Fernández-Zurbano, P. E ect of aroma perception on taste and mouthfeel dimensions of red wines: Correlation of sensory and chemical measurements. *Food Res. Int.* **2020**, 131, 108945.

Saint-Eve, A.; Paci Kora, E.; Martin, N. Impact of the olfactory quality and chemical complexity of the flavouring agent on the texture of low fat stirred yogurts assessed by three different sensory methodologies. *Food Qual. Prefer.* **2004**, 15, 655–668.

Salles, C.; Chagnon, M.C.; Feron, G.; Guichard, E.; Laboure, H.; Morzel, M.; Semon, E.; Tarrega, A.; Yven, C. In-mouth mechanisms leading to flavor release and perception. *Crit. Rev. Food Sci. Nutr.* **2011**, 51, 67–90.

Sanchez-Palomo, E.; Diaz-Maroto, M.; Gonzalez Vinas, M.; Soriano-Perez, A.; Perez-Coello, M. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. *Food Control,* **2007**, 18, 398–403.

Sanz, M.L.; Martínez-Castro, I.; Moreno-Arribas, M.V.. Identification of the origin of commercial enological tannins by the analysis of monosaccharides and polyalcohols. *Food Chem.* **2008**, 111, 778-783.

Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Austr. J. Grape Wine R.* **2006**, 12, 39-49.

Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Aust. J. Grape Wine R.* **2006**, 12, 39–49.

Savouret, J.F.; Quesne, M. Resveratrol and cancer: a review. *Biomed. Pharmacother.* **2002**, 56, 84-87.

Schöbel, N.; Radtke, D.; Kyereme, J.; Wollmann, N.; Cichy, A.; Obst, K.; Kallweit, K.; Kletke, O.; Minovi, A.; Dazert, S.; Wetzel, C.H.; Vogt-Eisele, A.; Gisselmann, G.; Ley, J.P.; Bartoshuk, L.M.; Spehr, J.; Hofmann, T.; Hatt, H. Astringency is a trigeminal sensation that involves the activation of G protein-coupled signaling by phenolic compounds. *Chem. Senses,* **2014**, 39, 471-487.

Sereni, A.; Osborne, J.; Tomasino, E. Exploring retro-nasal aroma's influence on mouthfeel perception of Chardonnay wines. *Beverages*, **2016**, 2, 7.

Siemann, E.H; Creasy, L.L. Concentration of the Phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, 43, 49-52.

Silva Ferreira, A.C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, 51, 1377-1381.

Silva, P.; Cardoso, H.; Gerós, H. Studies on the wine spoilage capacity of Brettanomyces/Dekkera spp. *Am. J. Enol. Viticult.* **2004**, 55, 65–72.

Singleton, V.L. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Viticult.* **1987**, 38, 69–77.

Singleton, V.L.A. Survey of wine ageing reactions, especially with oxygen. Proceedings of the ASEV 50th Anniversary Annual Meeting, Davis, California: American Society for Enology and Viticulture. 2000, pp. 323–336.

Singleton, V.L.; Kramling, T.E. Browning of white wines and an accelerated test for browning capacity. *Am. J. Enol. Vitic.* **1976**, 27, 157–160.

Skouroumounis, G.K.; Kwiatkowski, M.; Francis, I.L.; Oakey, H.; Capone, D.; Duncan, B.; Sefton, M.A.; Waters, E.J. The impact of closure type and storage conditions on the composition, colour and flavour properties of a Riesling and a wooded Chardonnay wine during five years' storage. *Austr. J. Grape Wine R.* **2005**, 11, 369-384.

Soares, S.; Brandão, E.; Guerreiro, C.; Soares, S.; Mateus, N.; de Freitas, V. Tannins in food: Insights into the molecular perception of astringency and bitter taste. *Molecules*, **2020**, 25, 2590.

Soares, S.; Brandão, E.; Mateus, N.; de Freitas, V. Sensorial properties of red wine polyphenols: Astringency and bitterness. *Crit. Rev. Food Sci. Nutr.* **2017**, 57, 937-948.

Soares, S.; Kohl, S.; Thalmann, S.; Mateus, N.; Meyerhof, W.; de Freitas, V. Different phenolic compounds activate distinct human bitter taste receptors. *J. Agric. Food Chem.* **2013**, 61, 1525–1533.

Soares, S.; Silva, M.S.; García-Estévez, I.; Gromann, P.; Brás, N.F.; Brandão, E.; Mateus, N.; De Freitas, V.; Behrens, M.; Meyerhof, W.; et al. Human bitter taste receptors are activated by different classes of polyphenols. *J. Agric. Food Chem.* **2018**, 66, 8814–8823.

Soares, S.; Vitorino, R.; Osório, H.; Fernandes, A.; Venâncio, A.; Mateus, N.; Amado, F; de Freitas, V. Reactivity of human salivary proteins families toward food polyphenols. *J. Agric. Food Chem.* **2011**, 59, 5535–5547.

Spencer, C.M.; Cai, Y.; Martin, R.; Gaffney, R.H.; Goulding, P.N.; Magnolato, D.; Lilley, T.H.; Haslam, E. Polyphenol complexation some thoughts and observations. *Phytochem.* **1988**, 27, 2397–2409.

Spillman, P.J.; Iland, P.G.; Sefton, M.A. Accumulation of volatile oak compounds in a model wine stored in American and Limousin oak barrels. *Aust. J. Grape Wine Res.* **1998a**, 4, 67–73.

Spillman, P.J.; Pollnitz, A.P.; Liacopoulos, D.; Pardon, K.H.; Sefton, M.A. Formation and degradation of furfuryl alcohol, 5-methylfurfuryl alcohol, vanillyl alcohol, and their ethyl ethers in barrel-aged wines. *J. Agric. Food Chem.* **1998b**, 46, 657–663.

Starkenmann, C.; Le Calvé, B.; Niclass, Y.; Cayeux, I.; Beccucci, S.; Troccaz, M. Olfactory perception of cysteine-S-conjugates from fruits and vegetables. *J. Agric. Food Chem.* **2008**, 56(20), 9575-9580.

Stevenson, R.J.; Prescott, J.; Boakes, R.A. Confusing tastes and smells: How Odours can influence the perception of sweet and sour tastes. *Chem. Senses*, **1999**, 24(6), 627–635.

Strauss, C.R.; Wilson, B.; Gooley, P.R.; Williams, P.J. Role of monoterpenes in grape and wine flavor. Parliment, T.H.; Croteau, R. Eds.; Biogeneration of Aromas, ACS Symposium series, Vol. 317, American Chemical Society, USA, 1986, pp. 222-242.

Styger, G.; Prior, B.; Bauer, F.F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, 38, 1145.

Svensson, B. E. Abilities of peroxidases to catalyse peroxidase-oxidase oxidation of thiols. *Biochemistry Journal*, **1988**, 256, 757–762.

Symoneaux, R.; Guichard, H.; Le Quéré, J.M.; Baron, A.; Chollet, S. Could cider aroma modify cider mouthfeel properties? *Food Qual. Prefer.* **2015**, 45, 11–17.

Takahama, U.; Ryu, K.; Hirota, S. Chlorogenic acid in coffee can prevent the formation of dinitrogen trioxide by scavenging nitrogen dioxide generated in the human oral cavity. *J. Agric. Food Chem.* **2007**, 55 (22), 9251-9258.

Takahama, U.; Takayuki, O.; Sachiko, H. Oxidation of Quercetin by Salivary Components. Quercetin-Dependent Reduction of Salivary Nitrite under Acidic Conditions Producing Nitric Oxide. *J. Agric. Food Chem.* **2002**, 50 (15), 4317-4322.

Tamborra, P.; Martino, N.; Esti, M. Laboratory tests on glycosidase preparations in wine. *Anal. Chim. Acta,* **2004***,* 513(1), 299-303.

Tournier, C.; Sulmont-Rosse, C.; Semone, E.; Issanchou, S.; Guichard, E. A study on texture-taste-aroma interactions: Physico-chemical and cognitive mechanisms. *Int. Dairy J.* **2009**, 19, 450–458.

Ugliano M., Henschke P.A. Yeasts and Wine Flavour. In: Moreno-Arribas M.V., Polo M.C. (eds) Wine Chemistry and Biochemistry. Springer, New York, NY, 2009.

Ugliano, M. Oxygen contribution to wine aroma evolution during bottle aging. *J. Agric. Food Chem.* **2013**, 61, 6125-6136.

Ugliano, M.; Kwiatkowski, M.; Travis, B.; Francis, I.L.; Waters, E.J.; Herderich, M.; Pretorius, I.S. Post-bottling management of oxygen to reduce off-flavour formation and optimize wine style. *Austral. New Zealand Wine Industr. J.* **2009**, 24, 24−28.

Ugliano, M., Kwiatkowski, M., Vidal, S., Capone, D., Solomon, M., Dieval, J.B., Aagaard, O. & Waters, E.J. The role of copper and glutathione addition and oxygen exposure in the evaluation of key aroma compounds of Sauvignon Blanc. Poster presented at 14th AWITC, Adelaide, 2010.

Valentin, D.; Chrea, C.; Nguyen, D.H. Taste-odour interactions in sweet taste perception. In Optimising Sweet Taste in Foods; Spillane, W.J., Ed. 2006; pp. 66-84.

van Ruth, S.; Roozen, J. Influence of mastication and saliva on aroma release in a model mouth system. *Food Chem.* **2000**, 71, 339–345.

Varela, P.; Gámbaro, A. Sensory descriptive analysis of Uruguayan Tannat wine: Correlation to quality assessment. *J. Sens. Stud.* **2006**, 21, 203-217.

Versari, A.; du Toit, W.; Parpinello, G.P. Oenological tannins: a review. *Austr. J. Grape Wine R.* **2013**, 19, 1-10.

Vignault, A.; González-Centeno, M.R.; Pascual, O.; Gombau, J.; Jourdes, M.; Moine, V.; Iturmendi, N.; Canals, J.M.; Zamorac, F.; Teissedre, P.L. Chemical characterization, antioxidant properties and oxygen consumption rate of 36 commercial oenological tannins in a model wine solution. *Food Chem.* **2018**, 268, 210-219.

Villamor, R.R.; Evans, M.A.; Mattinsonc, D.; Ross, C.F. Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Res. Int.* **2013**, 50, 38–45.

Villamor, R.R; Ross, C.R. Wine matrix compounds affect perception of wine aromas. *Annu Rev Food Sci Technol.* **2013**, 4, 1-20.

Vivas, N. ; Glories, Y. Étude de la flore fongique du chêne (Quercus sp.) caractéristique du séchage naturel des bois destinés à la tonnellerie, *Cryptogamie–Mycologie*, **1993**, 14 (2), 127-148.

Wang, X.J.; Li, Y.K.; Song, H.C.; Tao, Y.S.; Russo N. Phenolic matrix effect on aroma formation of terpenes during simulated wine fermentation - Part I: Phenolic acids. *Food Chem.* **2021**, 341(2), 128288.

Waterhouse, A.L.; Laurie, V.F. Oxidation of wine phenolics: A critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **2006**, 57, 306–313.

Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. Understanding wine chemistry 1st ed.; Waterhouse, A.L, Sacks, G.L., Jeffery D.W., Eds.; John Wiley & Sons Ltd.: Chichester, UK, 2016.

Weng, Z.M.; Ge, G.B.; Dou, T.Y.; Wang, P.; Liu, P.K.; Tian, X.H.; Qiao, N.; Yü, Y.; Zou, L.W.; Zhou, Q.; Zhang, W.D.; Hou, J. Characterization and structure-activity relationship studies of flavonoids as inhibitors against human carboxylesterase 2. *Bioorganic Chem.* **2018**, 77, 320–329.

Zanchi, D.; Vernhet, A.; Poncet-Legrand, C.; Cartalade, D.; Tribet, C.; Schweins, R.; Cabane, B. Colloidal dispersions of tannins in water–ethanol solutions. *Langmuir*, **2007**, 23, 9949–9959.

Zhang, M.; Xu, Q.; Duan, C.; Qu, W.; Wu, Y. Comparative study of aromatic compounds in young red wines from Cabernet Sauvignon, Cabernet Franc, and

Cabernet Gernischet varieties in China. *J. Food Sci.* **2007**, 72, 248–52.

Project objectives

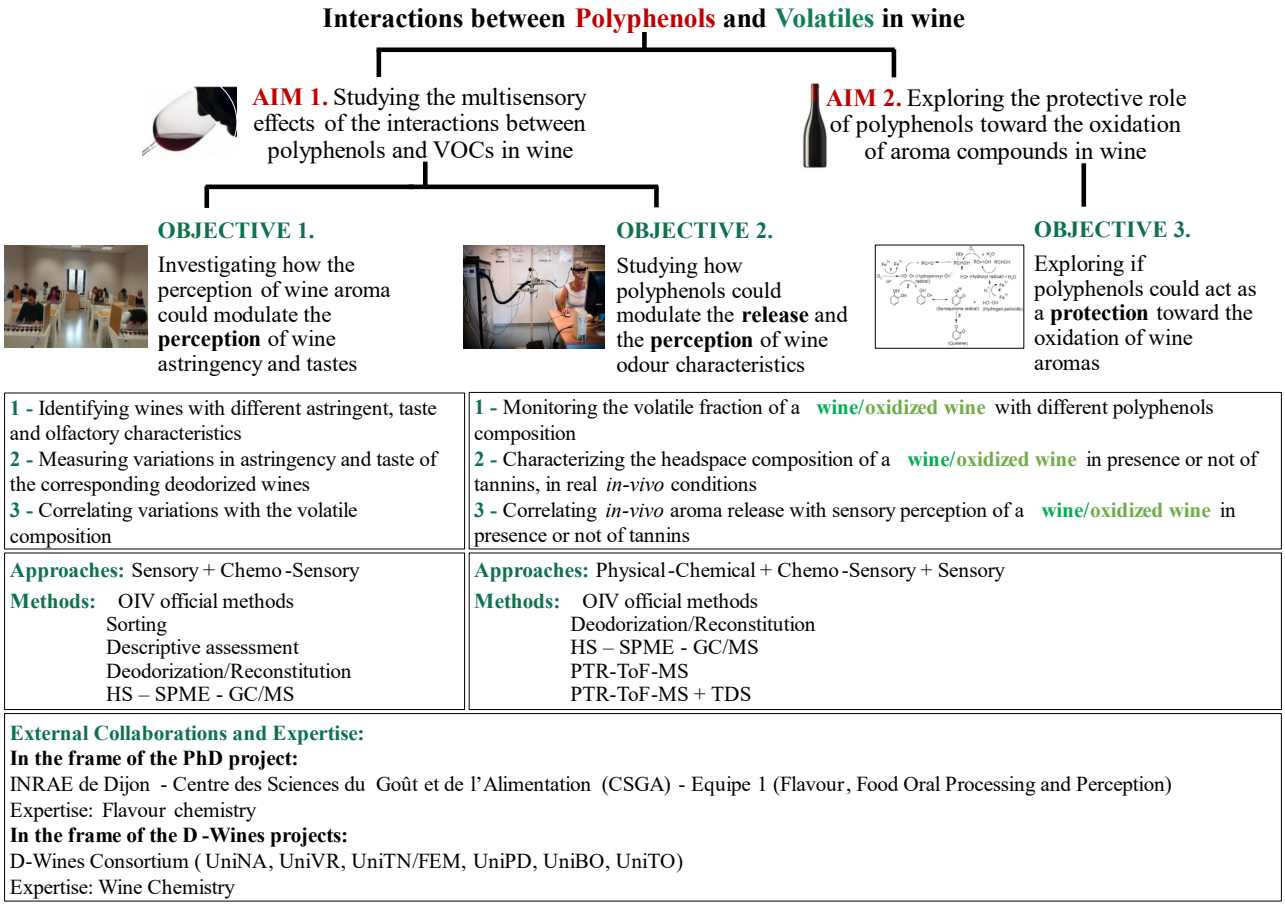
In the whole previously described framework, the main aim of this PhD thesis is to study the interactions between polyphenols and volatile compounds in wine to gain knowledge on the effects of these interactions on wine sensory perception and chemical stability.

The specific research questions of this thesis are referred to the following objectives schematised in Figure 1:

- investigating how the perception of wine aroma could modulate the perception of wine astringency and tastes;
- studying how polyphenols could modulate the release and the perception of wine odour characteristics;
- exploring if polyphenols could act as a protection toward the oxidation of wine aromas.

Each objective and research question will be presented as different chapter.

Figure 1. Scheme of this PhD thesis project. Aims, objectives, methodologies, and external collaborations.



Chapter I – Impact of red wine aroma on astringency and taste perception

The present chapter aims at studying and developing the first research question: investigating how the perception of wine aroma could modulate the perception of wine astringency and tastes.

We wanted to reach this goal by testing olfactory–oral cross-modal interactions in real wines, considering the great complexity typical of the wine matrix. Therefore, we needed a large set of commercial wines characterised by a wide diversity in terms of general sensory characteristics and, in particular in terms of astringency properties.

For this reason, in a preliminary study, we first studied the astringency diversity of single cultivar red wines produced from many Italian autochthonous grape varieties. Following multivariate statistical analyses, such as agglomerative hierarchical clustering (AHC) following multidimensional scaling (MDS), ANOVA, principal component analysis (PCA) and quadratic discriminant analysis (QDA), we characterized the astringency profiles of the single cultivar wines by developing the so-called 'astringency spectra'.

Once characterized these wines in terms of astringency profiles, and tested the correlations between sensory and chemical parameters – including total phenols and proanthocyanidins – we investigated odour–astringency and odour–taste cross-modal sensory interactions in a reduced set of samples, exploiting the sensory diversity of 10 single-cultivar Italian red wines and we tested and compared the correlations between sensory (odour descriptors, astringency sub-qualities, and tastes) and chemical compositional parameters (total phenols, proanthocyanidins, ethanol, reducing sugars, pH, titratable acidity, volatile acidity) both in the presence and in the absence of VOCs.

This first chapter has been divided into two sections. Part I is an edited version of the published paper **Piombino et al. (2020)** that aims at characterising, from a sensory point of view, the diverse astringency of single cultivar Italian red wines and correlating astringency sub-qualities with chemical composition.

Part II is an edited version of the published paper **Pittari et al. (2020)** that aims at exploring olfactory–oral cross-modal interactions through sensory and chemical characteristics of Italian red wines.

Part I

This part is an edited version of:

Preliminary sensory characterisation of the diverse astringency of single cultivar Italian red wines and correlation of sub-qualities with chemical composition

Piombino, P.[1,*], **Pittari, E.[1]**, Gambuti, A.[1], Curioni, A.[2], Giacosa, S.[3], Mattivi, F.[4,5], Parpinello, G.P.[6], Rolle, L.[3], Ugliano, M.[7] & Moio, L.[1]

[1] Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, 83100 Avellino, Italy

[2] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, 35020 Padova, Italy

[3] Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, 10095 Torino, Italy

[4] Center Agriculture Food Environment, University of Trento, 38123Trento, Italy

[5] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all' Adige, 38010 Trento, Italy

[6] Department of Agricultural and Food Sciences, University of Bologna, 40126 Bologna, Italy

[7] Department of Biotechnology, University of Verona, 37029 Cariano (VR), Italy

* Corresponding author:

Professor Paola Piombino

Published in ***Australian Journal of Grape and Wine Research, 2020, 26(3), 233-246***
DOI: 10.1111/ajgw.12431

1. Introduction

According to the Organisation Internationale de la Vigne et du Vin (OIV) (2017), Italy is the grape-producing country with the greatest number of cultivars. This results from centuries of human selection, which has led to a tight cultivar–environment relationship. This rich ampelographic heritage, composed nowadays of around 500 cultivars – considering those listed in the Italian National Catalogue of Grapevine Varieties (Lacombe et al., 2011) – includes red grapes with different composition of phenolic substances (Mattivi et al., 2002, 2009). The corresponding wines present a wide spectrum of sensory features, including diverse astringency.

This means diversified mouthfeel characteristics, as reported in the different Disciplinary Regulations of Italian Wines (https://www.politicheagricole.it/). Some of these grapes are used to produce well-known wines, such as Chianti or Barolo, which, despite their richness in tannin and intense mouthfeel, are appreciated by consumers and represent some of the best examples of Italian red wines (Piacenza et al., 2009; de Luca et al., 2019). At the end of the last century, there was a renaissance of Italian wines and, at the beginning of the 21st century, a rising trend of propagation (a parameter evaluating the market interest in cultivars) was observed (Mannini, 2004). The annual nursery production of grafting grew from 300 000 to 1 700 000 for Nebbiolo, from 200 000 to 1 000 000 for Aglianico and from 100 000 to 1 000 000 for Primitivo. Nowadays, there is international interest in Italian cultivars, for example some red cultivars, such as Sangiovese, Montepulciano, Barbera, Lambrusco, and Nero d'Avola, are now grown in several Australian regions, such as the Riverina, Barossa Valley, McLaren Vale, Riverland, and King Valley (Wine Australia, 2019).

In view of a such wide biodiversity, of the increase in high quality products and of the economic potential, it is quite surprising that the astringency of Italian red wines has never been systematically investigated and compared from a sensory point of view. Several Italian wines have been studied in terms of the composition of their PPhss. Data about their astringency as sensory parameters can only be recovered for some of them in a fragmentary way as a result of the impact of viticultural/oenological practices on the sensory profile (Boselli et al., 2004; Gerbi et al., 2006; Gambuti et al., 2009; Torchio et al., 2010; Pagliarini et al., 2013; Patrignani et al., 2017). Moreover, data on different cultivars are not comparable because of the methodological/terminology differences (oenology, sensory techniques, phenolic analysis, and vocabulary). This lack is one of the reasons

why today it is not possible to identify specific astringency characters as one typical feature of any Italian wine.

Without this knowledge, winemakers are not supported either by the knowledge of the strengths and weaknesses of a specific grape cultivar, or by a shared sensory model. In the current market, the ability to associate a certain product to specific sensory attributes and territories is often a vehicle to commercial success. As a result, a more comprehensive characterisation of the astringency of Italian red wines would provide an opportunity to support/consolidate their international image, with positive commercial outcomes. Indeed, the commercial value of a wine is related to its intrinsic (e.g., sensory features) and extrinsic (e.g., geographical origin) characteristics and both influence wine purchase and repurchase (Charters & Pettigrew, 2007; Mueller et al., 2010; Sáenz-Navajas et al., 2016).

Among the many sensory characteristics of red wine, astringency is of great oenological interest because of its strong link with the perceived quality (Sáenz-Navajas et al., 2011 and references therein). Due to its wide complexity exposed above (Background Section), some authors (Vidal et al., 2017) spoke about of a 'polarisation of astringency' related to terms: those related to soft textures opposite to those related to rough textures and aggressiveness. Our consideration is that the less pleasant astringency sensations could positively impact the perceived quality when present in a well-balanced wine. This appears to be supported by the fact that they are often present in premium wines suitable for long ageing. In contrast, those astringency sensations considered as pleasant could lead to less appreciated wines if not combined with other descriptors. Vidal et al. (2017) expected that both low and extremely high overall astringency intensity could be perceived as indicators of low quality in Tannat wines, being the typicity of this product linked to its astringency. We hypothesise that red wines can differ according to the balance between 'strong' and 'smooth' sensations defining their astringency. These two terms were already adopted to differentiate wines according to their astringency. Based on the characterisation of the intensity and sub-qualities of astringency, several groups of Tannat wines were identified: those characterised by intermediate astringency (described as dry, rough and mouth-coating); those eliciting smooth astringency characteristics (described as velvety, silky and suede); and those characterised by their strong astringency (described as hard, harsh, and aggressive) (Vidal et al., 2017). Overall sensory intensity and persistence of red wines are positively correlated with astringency (Peynaud, 1987), and therefore to tannin

concentration (Gonzalo-Diago et al., 2013). A relationship between tannin concentration and wine allocation grade, that is related to market value, has also been described (Mercurio et al., 2010). Several authors studied the astringency of red wines through their sub-qualities (Green, 1993; Gawel et al., 2000; Francis et al., 2002; Vidal et al., 2004; Ferrer-Gallego et al., 2014; Vidal et al., 2018), showing that astringency is not only complex but also a time-dependent sensation. Recent studies investigated the development of astringency sub-qualities over time by approaching this subject through temporal measurements (Guinard et al., 1986; Cadena et al., 2014; Vidal et al., 2016; Kang et al., 2019). They highlighted the importance of assessing astringency through a holistic chemosensory approach. It includes complementary information derived from static and/or temporal sensory assessments and chemical analysis. Some of these papers characterised the astringency features of a specific wine. The authors investigated astringency sub-qualities and the correlation between these sensory variables and chemical composition (Vidal et al., 2016).

In a similar manner, but for the first time on a large set of Italian red wines made 100% from native grape cultivars, in this experiment we mainly studied the astringency diversity of red wines from 11 cultivars representative of Italy: Teroldego, Corvina, Raboso Piave, Nebbiolo, Sangiovese, Sagrantino, Montepulciano, Cannonau, Aglianico, Primitivo and Nerello Mascalese. These cultivars are used to produce different wines labelled with Denomination of Origin Controlled (DOC) and Guaranteed Origin (DOCG).

To reach our goal the astringency sub-qualities of an initial set of 111 commercial wines were investigated by sensory analysis adopting a two-step analytical strategy composed of a sorting task and a sensory assessment through a numerical category scale. Multivariate statistical analyses, such as agglomerative hierarchical clustering (AHC) following multidimensional scaling (MDS), ANOVA, principal component analysis (PCA) and quadratic discriminant analysis (QDA) allowed a step-by-step definition of a reduced set of representative samples used to develop astringency profiles of single cultivars called ‘astringency spectra’.

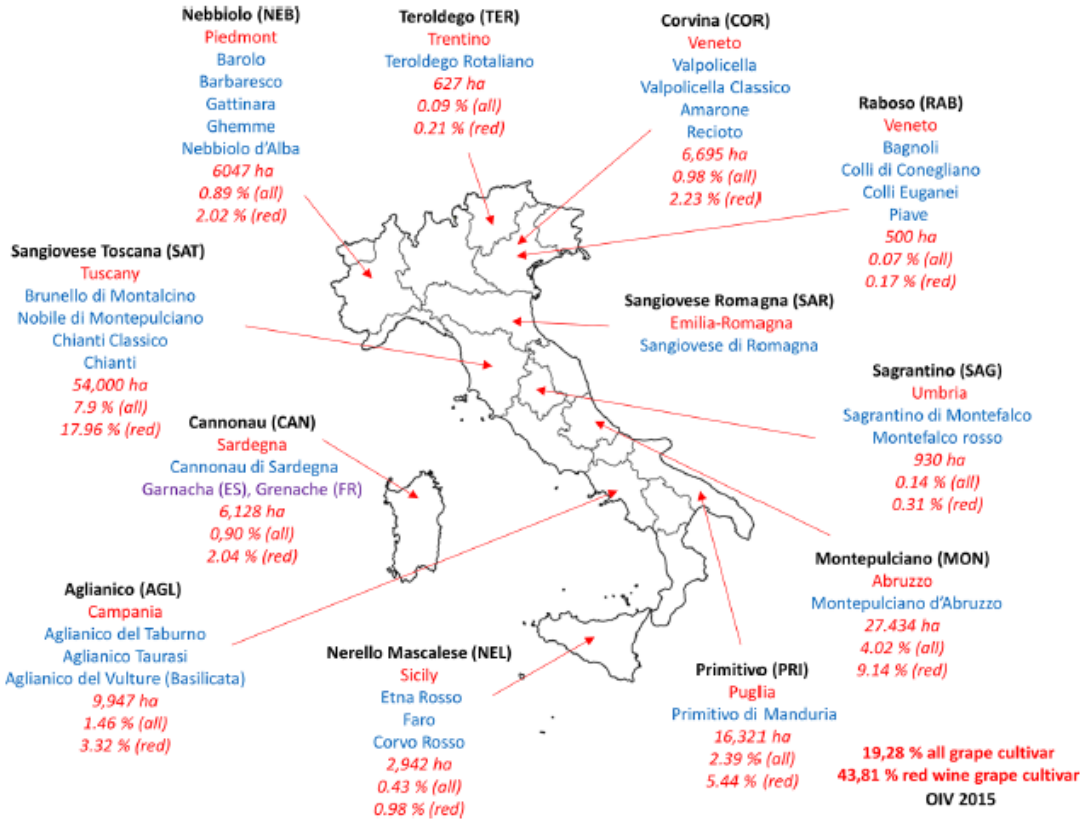
Furthermore, the wide diversity in PPhss and astringency features of Italian red wines was exploited as an opportunity to investigate the correlation between specific in-mouth sensory variables (single astringency sub-qualities and tastes) and some aspects of chemical composition, particularly PPhss measured with different methods, macromolecules, and basic chemical analyses.

2. Materials and Methods

2.1. Wine samples

One hundred and eleven Italian red wines, 100% single cultivar, vinified in 2016 from 11 Italian grape cultivars, harvested in the corresponding main geographical areas of production (12 regions), were sampled from the commercial wineries where they were produced. For that reason, oenological parameters varied. The set of wines was composed of 11 Teroldego Rotaliano (from Trentino TER), seven Corvina (from Veneto: COR), nine Raboso Piave (from Veneto: RAB), 13 Nebbiolo (from Piemonte: NEB), 19 Sangiovese (12 from Romagna: SAR; seven from Toscana: SAT), 10 Sagrantino di Montefalco (from Umbria: SAG), 9 Montepulciano (from Abruzzo: MON), 9 Cannonau (from Sardegna: CAN), 10 Aglianico (from Campania: AGL), 11 Primitivo (from Puglia: PRI) and 3 Nerello Mascalese (from Sicilia: NER). As reported by Arapitsas et al. (2020), "in 2015, the above-mentioned cultivars accounted for 44% of the red grape vine-cultivated area of Italy and, therefore, constitute a representative portion of Italian oenological biodiversity (Figure 1)".

Figure 1. Distribution of the wine sample set according to their cultivar (black) and region (red). The principal denomination of origin of each cultivar/region is also shown (light blue). The cultivation area refers to the whole of Italy for each cultivar for the year 2015 (OIV, 2018). Cited from Arapitsas et al. (2020).



Wines were fermented in stainless steel vats, at commercial scale, at wineries among the most representative in each area of production and sampled before malolactic fermentation and before wood ageing. All samples were protected with 50 mg/L of free SO2 before bottling; bottles were closed with a Select Green 500 cork type (Nomacorc, Rivesaltes, France) prior to storage at constant cellar temperature (12±2°C) until analysis.

2.2. Experiment 1: Selection of wines

This step was carried out to select the most representative wines belonging to each grape cultivar and to have first indication of the astringency features of the wines.

2.2.1 Sorting task

Panel: the jury was composed of 14 people (seven males, seven females; 22–49 years) recruited from students and staff members of the Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II. They were selected based on their interest, availability, and ability in recognising oral stimuli. They all were expert wine tasters and had previous experience with sensory tests on wine. The study protocol has been approved by the Ethics Committee of University of Naples Federico II. All participants were volunteers and before participating in the study they signed an informed consent form defining the type of research, voluntary participation, and agreement to sip and spit reference solutions and wines. All data were collected anonymously.

Panel training (phase 1: familiarisation with in-mouth sensations): to familiarise with the astringency vocabulary, judges were provided with a list of seven terms defining the diverse astringency categories (designated hereinafter as 'sub-qualities') of red wine as described at the first level of the mouthfeel wheel (Gawel et al., 2000): drying, harsh, unripe, dynamic, particulate, complex and surface smoothness. Assessors were provided with a sheet with the Italian translation of the definitions reported by Gawel et al. (2000). After the theoretical introduction, nine taste/mouthfeel references were presented to the jury to develop a consensual list of terms describing the oral sensations elicited by each standard (Tables 1, 2). The same references were employed to exercise the jury to recognise and discriminate the different oral sensations and to help in the application of terms consistently to the corresponding definitions. The references (20 mL in covered disposable plastic cups) were presented in water and in red table wine. A 5-year-old Pinot Noir was used as reference for

the surface smoothness (Cliff et al., 2007). Tannic acid and four commercial tannin-based products were used as sensory references for astringency and its sub-qualities (Table 1). The appropriate concentration was chosen through preliminary intra-laboratory tests. The association of the terms to these references was obtained by asking the assessors to take a sip (15 mL), to move the sample (15 s) while wetting the whole mouth and then record the most intense sensations. Only descriptors cited at least by 85% of the jury were matched to the terms as reported in Table 1 and considered as consensually associated to the corresponding sensory reference.

At the end of each tasting session the perceived sensations were discussed to agree on a common definition (Table 2). Relationships and redundancies among the terms were discussed. At the end of the training, it was consensually decided that the terms 'surface smoothness' and 'particulate' were to be labelled as 'velvet' and 'powdery' astringent sensations, respectively. To help in memorisation and in consistent use of terms, as well as to prevent overlapping, a consensus was found on simplified descriptions for the terms. They were schematised (Table 2) and a sheet with the simplified descriptions was attached to the wall of each booth during all the subsequent sessions. The first session was considered introductory, so that data collected only from the second and third training sessions were employed to calculate the frequency of citations for matching standards with descriptor/s and to test panellist performance.

Table 1. References and corresponding consensual descriptors used to train the assessors in recognising and distinguishing among the different in-mouth sensations (tastes and astringency sub-qualities).

References	Concentration (g/L)[1]	Descriptors[2,3]	Producers
Fructose	2	Sweet	J.T. Baker (Avantor; Radnor, PA, USA)
Tartaric acid	4	Sour	Chem-Lab (Eernegem, West-Vlaanderen, Belgium)
Caffeine	2	Bitter	ACEF (Piacenza, Italy)
Tannic acid	2	Astringent	J.T. Baker (Avantor; Radnor, PA, USA)
Tannin VR color (catechin and ellagic tannins formulation)	4	Drying and harsh	Laffort (Bordeaux, France)
Tannin VR grape (proanthocyanidic tannins extracted from grape skin and seeds)	2	Particulate/powder and Unripe	Laffort (Bordeaux, France)
Tannin plus (tannins formulation)	4	Complex and drying	Laffort (Bordeaux, France)
Tannins galalcool (gallic tannins from gallnuts in granulated form)	2	Unripe	Laffort (Bordeaux, France)
Red wine (Pinot Noir 5 years old)	-	Surface smoothness/velvet	St. Michael Eppan (Trentino-Alto Adige, Italy)

[1]Both in distilled water and in table red wine (pH, 3.2; ethanol, 12.5% v/v; TA, 7.7 g tartaric acid/L; residual sugars, 1.5 g/L; total anthocyanins, 36 mg/L; BSA reactive tannins, 112 mg/L); [2]Agreed definitions are reported in Table 2; [3]Consensaul association frequency ≥85%.

Table 2. Definitions of the terms considered to assess astringency.

Terms	Agreed definitions	Simplified definitions
Astringency[1]	Oral tactile sensation mainly characterised by dryness and roughness	-
Drying[2]	Lack of lubrication and dehydration feeling in the mouth	No lubrication + dehydration
Harsh[2]	Unbalanced in-mouth sensation of dryness, roughness (irregularities and lack of smoothness) and bitterness	Astringency + roughness + bitterness (combined and aggressive/excessive)
Dynamic[2]	Sensations impacting on fluidity of oral movement	Lack of fluidity
Particulate (as Powdery)[2]	Oral sensation associated with the touch of powdery matter	Powdery at touch
Unripe	Unbalanced in-mouth sensation of astringency, sourness and green aroma	Astringency + acid + herbaceous (combined and aggressive/excessive)
Surface smoothness (as Velvet)[2]	Oral texture sensation associated with the touch of velvet	Velvet at touch
Complex[2]	Balanced in-mouth sensation of smooth astringency, acidity and retronasal stimulation	Astringent + acid + flavoured (combined and not aggressive/excessive)

[1]As defined by **Vidal et al. (2016)**. [2]Agreed definitions elaborated by starting from those reported by **Gawel et al. (2000)**.

Panel training (phase 2: familiarisation with sorting): assessors were introduced to the sorting procedure. For this purpose, eight red wines (30 mL in covered ISO wine glasses) from different cultivars were presented. Judges were asked to introduce the sample into their mouth, focus on the perception of astringency and sort samples according to their similarity in astringency sub-qualities on which they were trained. Panellists were asked to label each group with the dominant sub-quality/s perceived among the seven on which they were trained. Judges could make as many groups of similar samples as possible, and groups of single samples were permitted. Between two samples, assessors were asked to rinse the mouth by drinking bottled still water (Evian), to eat some apple slices, then drink a second time and finally wait at least 30 s before the subsequent evaluation. At the end, it was checked if the definitions of terms needed to be refined in this context of wines representative of the sample set under investigation. After discussion, no changes were made, and the consensus was confirmed on all the definitions reported in Table 2. During the discussion judges were also asked about the roughness/aggressiveness of the different sensations: drying, harsh, dynamic, unripe, and particulate were mostly perceived as strong/aggressive while complex and velvet as smooth/not aggressive.

Samples analysis: wines were evaluated by sorting according to an intra-cultivar experimental design meaning that all the wines from a given cultivar were sorted in the same session. In this way, an intra-cultivar sorting

was performed to investigate similarities and dissimilarities among wines belonging to the same cultivar (from seven Corvina to 13 Nebbiolo). Due to the limited number of samples (only three), Nerello Mascalese was not included in this first intra-cultivar experimental step so that a total of 108 samples were analysed by sorting. Judges attended 11 sessions corresponding to the number of single cultivar wines (Sangiovese wines were divided into two sessions according to geographical origin). The evaluation procedure was the same of the training. Assessors were asked to group samples according to the similarity in their astringency sub-qualities and label the groups. Thirteen samples, corresponding to the maximum number of wines sampled within a single cultivar, were evaluated during each session. When less than 13 wines were available, 'fake' samples were obtained by blending available wines of the same cultivar; data about these samples were not considered. Samples (30 mL) were presented according to a randomised arrangement in covered ISO approved wine glasses labelled with three-digit random codes. All wines were served at room temperature (21±1°C) and were evaluated in individual booths.

2.3. Experiment 2: Sensory assessment of wines

The aim of this step was to obtain a sensory descriptive assessment of in-mouth features (tastes and astringency sub-qualities) of a reduced number of wine samples selected as the most representative within each single cultivar wine.

2.3.1. Samples

A set of 77 wines was analysed: 74 (five SAT and five SAR; eight TER; seven NEB, RAB, CAN, SAG, MON, COR, PRI and AGL) were selected according to the results of the sorting and three were the Nerello Mascalese (NER) wines.

2.3.2. Descriptive analysis

Panel training: The nine taste/mouthfeel references listed in Table 1 were presented to the jury to train them to score the intensity of different in-mouth sensations on the following numerical category scale: 1 = very low, 2 = low, 3 = medium, 4 = high, and 5 = very high, with half values allowed. Materials and serving conditions were the same as above.

To familiarise the jury with the evaluation procedure, nine samples (three each in duplicate of RAB, SAG and TER) were tested prior to the analytical sessions, as a run-through. The procedure and the conditions were the same as described previously. Data were employed to test the performance of panellists.

Sample analysis: The 77 wines were analysed in terms of astringency and taste by using the terms listed in Table 2, and by scoring the intensity of the perceived descriptors on the scale applied during the training. The sensory assessment was performed according to an inter-cultivar experimental design meaning that 11 wines corresponding to the 11 single cultivar wines were evaluated during each of the seven sessions. Each sample (25 mL) was served as previously described. Panellists were asked to taste each sample by focusing on astringency by paying attention not only to the most intense sensation but also to that/those catching their attention the most during the tasting time, describing and scoring the diverse sensations by using the seven terms corresponding to the seven sub-qualities, and finally by scoring taste sensations (sweet, acid, bitter). Judges were informed that, based on data from training sessions, at least three of the astringency descriptors were expected to be higher than the minimum value on the scale, but no limitations were imposed. Judges were asked to rinse their mouth between two samples.

2.4. Chemical analysis of wines

Ethanol, reducing sugars, volatile acidity (VA) and titratable acidity (TA) were measured according to Organisation Internationale de la Vigne et du Vin (OIV) methods (Organisation Internationale de la Vigne et du Vin 2015). pH was determined by potentiometry (InoLab 730 pH meter, WTW, Weilheim in Oberbayern, Germany). Total phenols were measured by Folin–Ciocalteu assay (Singleton et al., 1999). The concentration of proanthocyanidins was determined after acid hydrolysis with warming (Bate-Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst (Di Stefano et al., 1989; Torchio et al., 2010). Analyses were in triplicate.

2.5. Data analysis

To visualise groupings of wine samples due to astringency similarities analysed by sorting, MDS analysis followed by AHC analysis were performed and the cooccurrence similarity matrices were considered. As previously reported (Sáenz-Navajas et al., 2012, and references therein), for each assessor, results were

organised under an individual similarity matrix (wines x wines): 1 corresponded to two wines put into the same group while 0 was for two wines put in different groups. The sum of the individual matrices across judges was merged into a co-occurrence matrix representing the global similarity matrix where the higher the number the higher the similarity between samples. This method assumes that samples frequently grouped together were perceived as more similar compared to those sorted into different groups. The proximity matrix (Euclidean distances between the products) was the base for the MDS analysis (SMACOF algorithm). The quality of fit was measured by the stress value (from 0 = perfect fit to 1 = worst fit). As previously reported and applied, a value below 0.2 can be considered as a good agreement between the initial and final configurations, so that this stress value was adopted as a criterion to select the number of dimensions for the MDS spaces. Coordinates of samples in the retained MDS configurations were submitted to a AHC with the Ward criterion. We applied the automatic truncation option, which is based on the entropy and tries to create homogeneous groups. Agglomerative hierarchical clustering was helpful for the interpretation of MDS maps allowing the identification of wines belonging to each cluster. We arbitrary decided to select at least seven samples of each single cultivar wine. In this way at least 50% of each single cultivar sample set was selected, indeed the most numerous set of wines was composed of 13 NEB.

Data from the descriptive sensory assessment were analysed by one-way ANOVA (wine was the factor and judges were considered as random factor), and the mean intensity for each astringency sub-quality was compared (intra- and inter-cultivar) by a Tukey post-hoc test ($P<0.05$).

A PCA was applied to the original in-mouth variables (astringency sub-qualities and tastes) constituted by the sensory scores. Sensory data referring to astringency subqualities were also computed as the geometric mean of frequency and mean intensity [mean sensory modified frequency (MF)] as described by Dravnieks (1982): $MF = (F * I)1/2$, where F is the frequency of citation expressed as a proportion of the maximum frequency of citation (i.e., total number of judges) and I is the mean intensity expressed as a proportion of the maximum rate.

Quadratic discriminant analysis was used to classify the wines assuming the cultivar as a qualitative dependent variable and MF of the astringency sub-qualities as quantitative explanatory variables (inequality of covariance matrices tested by Box test; Jarque-Bera normality test; $\alpha=0.05$). The classes weight correction was applied

because the number of observations for the various classes for the dependent variables was not uniform. The classification functions were used to determine which class (cultivar) an observation (wine) is to be assigned to using values taken for the various explanatory variables. An observation was then assigned to the class with the highest classification function. Only wines that, after cross-validation, were well-classified to the corresponding grape cultivar, were further considered to develop single cultivar astringency patterns. To satisfy the assumption that the number of explanatory variables (six) was lower than each sample size, NER samples (only three) were not included in the discriminant analysis.

Pearson correlation analysis ($P<0.05$) was applied across the whole set of wines (sample size = 77) for the computation of correlations between the intensity of astringency sub-qualities and in-mouth sensory variables or chemical parameters.

Performance of the trained judges was tested by threeway ANOVA (Tukey, $P < 0.05$) with interactions of assessor*session, assessor*sample, sample*session (Vidal et al., 2016).

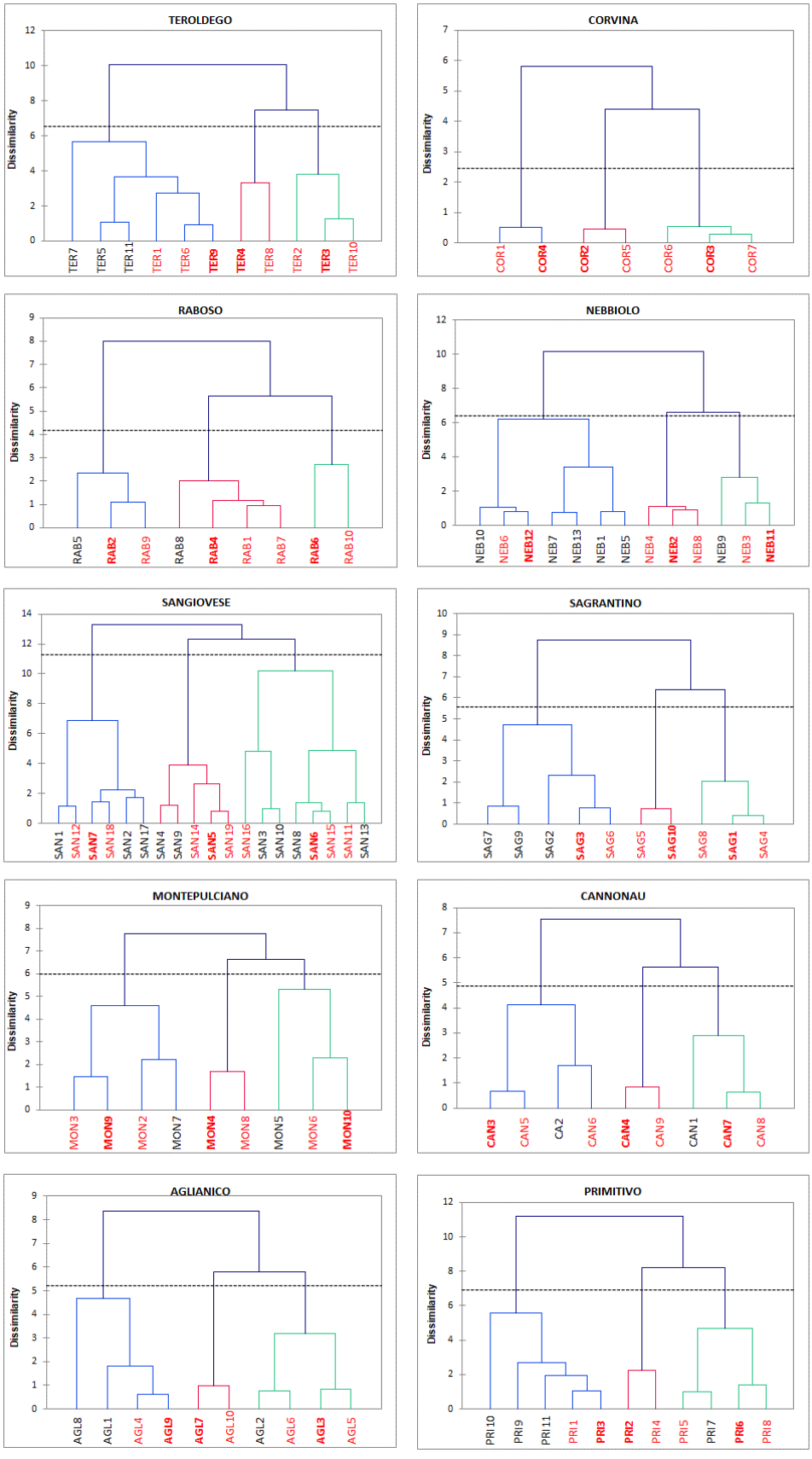
Data were processed with XLStat (version 2018.7), an add-in software package for Microsoft EXCEL (Addinsoft, Paris, France).

3. Results

3.1. Selection of wines

Basic compositional data of the wine samples are shown in Table 3. The ranges of these parameters were large; thus, astringency differences were expected in the set of sampled wines. Data from the wines sorted according to astringency similarities were analysed by AHC after MDS. According to the dendrograms (Figure 2), within each single cultivar wine, samples resulted clustered into three groups represented on three (Sangiovese, Sagrantino, Raboso, Primitivo, Nebbiolo, Corvina) or four (Aglianico, Montepulciano, Cannonau, Teroldego) dimensions on the MDS spaces (not shown).

Figure 2. Dendrograms obtained by agglomerative hierarchical clustering (AHC) performed on data from the sorting test and used for wine selection (red: selected samples; bold: central objects of each cluster).



From these results, we selected samples from each wine type according to the following criteria: the most similar couple of wines, couples including the central object of each cluster, at least three wines from the most homogeneous cluster (lowest within-class variable) when larger than two objects, at least one sample (central object) belonging to each cluster (excluding clusters composed of one sample). When necessary, distances from the MDS output were adopted as additional criteria to select at least 50% of samples from each cultivar. In this manner we reduced the number of samples belonging to each mono-cultivar wine by preserving the representativeness in terms of intracultivar similarities and diversities. The final set of 77 selected wines was then composed of: 10 Sangiovese (five each from Romagna and from Toscana), 8 Teroldego, 7 Nebbiolo, Aglianico, Primitivo, Montepulciano, Cannonau, Raboso Piave, Corvina and Sagrantino, plus 3 Nerello Mascalese.

Table 3. Oenological parameters determined in the 111 single cultivar Italian red wines.

Parameter	Mean	Minimum	Maximum
Ethanol (% v/v)	13.9	11.4	16.6
Reducing sugars (g/L)	2.6	1	20.1
Titratable acidity (g tartaric acid/L)	5.7	4	10
pH	3.6	3.1	4.1
Total phenols (Folin-Ciocalteu) (mg (+)-catechin/L)	2341	704	5449
Proanthocyanidins (mg cyanidin chloride/L)	3373	628	6312

3.2. Description and discrimination of wines

Each astringency sub-quality of the 11 single cultivar wines was compared (Figure 3) and several differences emerged for six out of the seven sub-qualities. According to the significance ($P<0.05$) reported on the top of each box, only some of these differences were significant.

Three main levels of drying intensity were identified: Nebbiolo and Sagrantino showed the highest mean intensity, followed by Raboso, Primitivo and Nerello Mascalese and then by Corvina. Two further intermediate levels corresponded to the drying intensity of the other wines. Sagrantino and Corvina wines represented the highest and lowest values, respectively, of the harsh intensity. Some significant differences were detected among the other wines, except for Sangiovese and Nerello.

Figure 3. Box-plots describing inter-cultivar diversity of each astringency sub-quality in the 11 single cultivar Italian red wines investigated [means (+); central horizontal bars: medians; lower/upper limit of the box: first/third quartile; points above/below the whiskers' up per/lower bounds: outliers; box-plot's horizontal width: no statistical meaning]. Letters reported on the top of each box-plot refer to significant differences tested by ANOVA ($p<0.05$); ns: not significant.

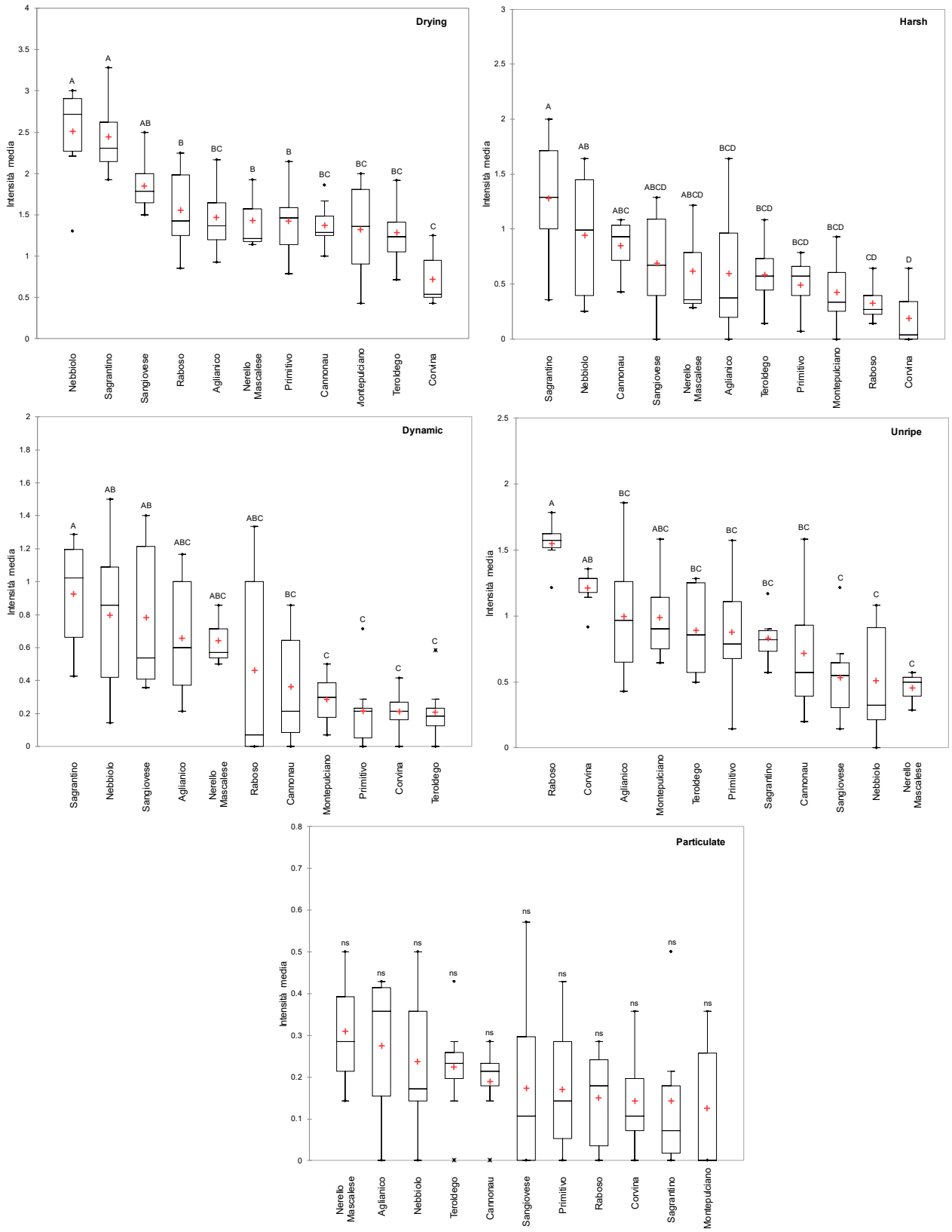
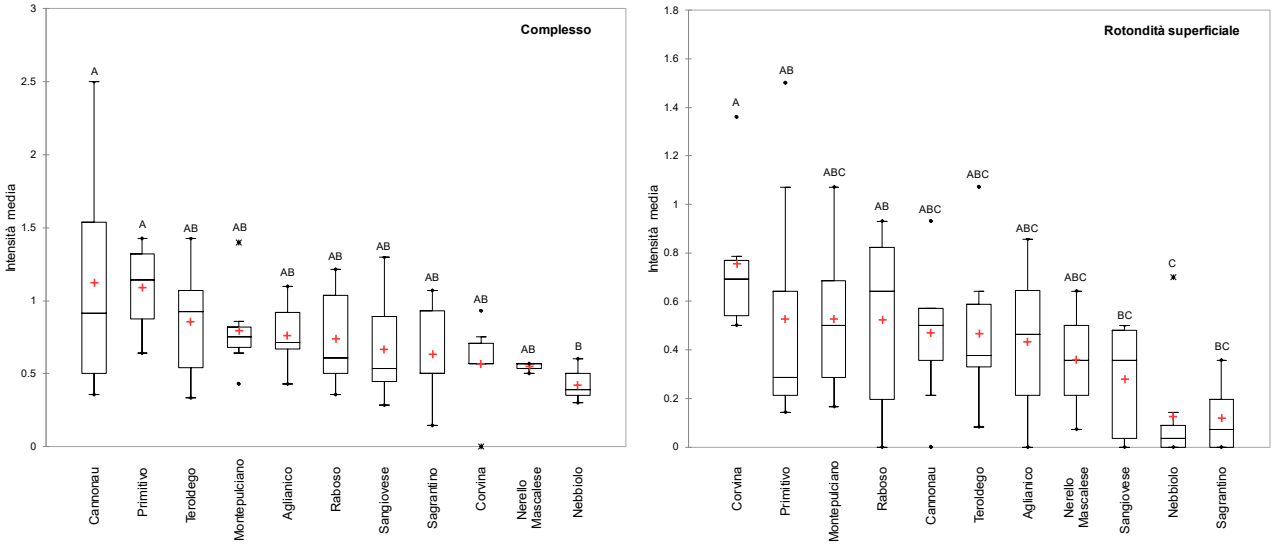


Figure 3. Continued.



For unripe, the highest mean intensity was associated with Raboso, in contrast to Sangiovese, Nebbiolo and Nerello which were less unripe and significantly different from that of Corvina, Montepulciano was not different according to its unripe character. Astringency of Sagrantino was perceived as the most dynamic while Teroldego, Primitivo, Montepulciano and Corvina was less so. For dynamic no differences emerged for all the other wines. Cannonau and Primitivo were different from Nebbiolo which was the less complex.

Corvina was opposite to Nebbiolo with the highest and the lowest values for surface smoothness, respectively. Raboso and Primitivo were more velvet than Nebbiolo, while Sangiovese less than Corvina. Finally, the sub-quality particulate for the 11 single cultivar wines was not significantly different, and therefore, this sub-quality was not considered for the subsequent analyses.

Figure 4 shows the PCA where all in-mouth sensory variables (a) and observations (b) were plotted on the first two components representing 58.81% of the variance. The astringency sub-qualities and the bitter taste are mostly represented on PC1, while the contrast between acid and sweet tastes is represented on PC2. The variables that positively correlated ($P<0.0001$) to each other are dynamic with drying ($R^2=0.565$), harsh with bitter ($R^2=0.771$), acid with unripe ($R^2=0.593$), surface smoothness with complex and sweet ($R^2=0.283$ and $R^2=0.256$, respectively). Drying and dynamic were negatively correlated ($P<0.0001$) to surface smoothness ($R^2 = -0.642$ and $R^2 = -0.463$, respectively). Compared to unripe, harsh showed an opposite correlation to acid taste ($R^2 = -0.577$). Most of the Sangiovese, Nebbiolo and Sagrantino wines showed the largest squared

cosines to positive values of the first factor, where the variables drying and dynamic, harsh, and bitter are well projected. On the other side of the first factor, in the space where the best represented variables are acid, surface smoothness and unripe, different wines showed the largest squared cosines, mainly Corvina and Raboso. Along the second factor, some Raboso, Aglianico and Montepulciano wines were linked to the acid taste, opposite to Cannonau, Primitivo and Teroldego which were linked to the sweet taste. A wide intra-cultivar diversity results for Aglianico wines, which occupy the most diversified positions in the PCA space.

Figure 4. Principal component analysis (PCA) plots, (a) variables and (b) observations calculated on intensity scores (AGL, Aglianico; CAN, Cannonau; COR, Corvina; MON, Montepulciano; NEB, Nebbiolo; PRI, Primitivo; RAB, Raboso Piave; SAG, Sagrantino; SAN, Sangiovese; TER, Teroldego).

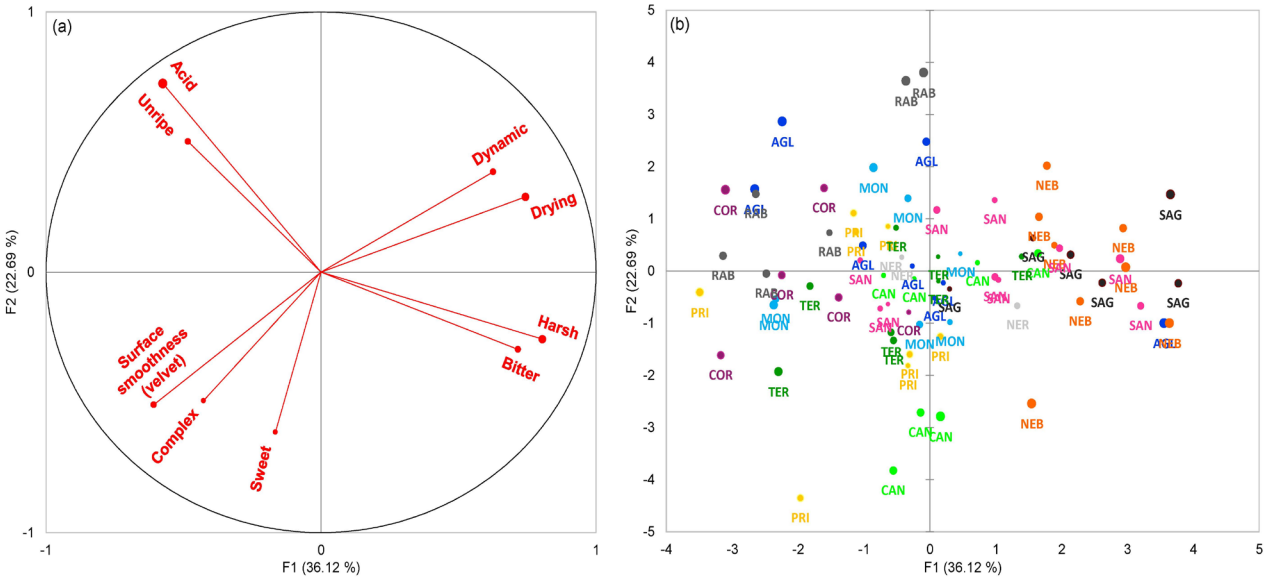
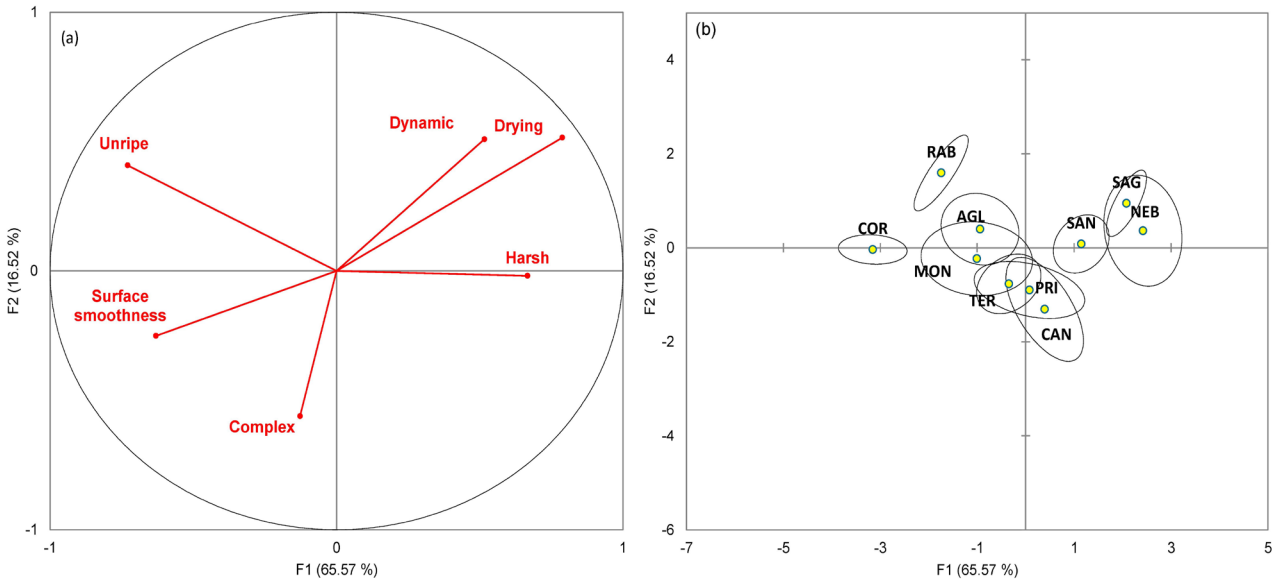


Figure 5 shows the output of the QDA. The goal was to test if the single cultivar wines could be discriminated and clustered only according to their astringency sub-qualities (MF values). As previously applied on olfactory and in-mouth descriptors (Lelièvre et al., 2008), the MF method was applied because it considers both types of values produced by assessors: the frequency of citation of a sensory term and the intensity assigned to it. In this way we properly considered cases in which a term has been used frequently but with low scores, and cases in which the same descriptor has been poorly cited but with high scores.

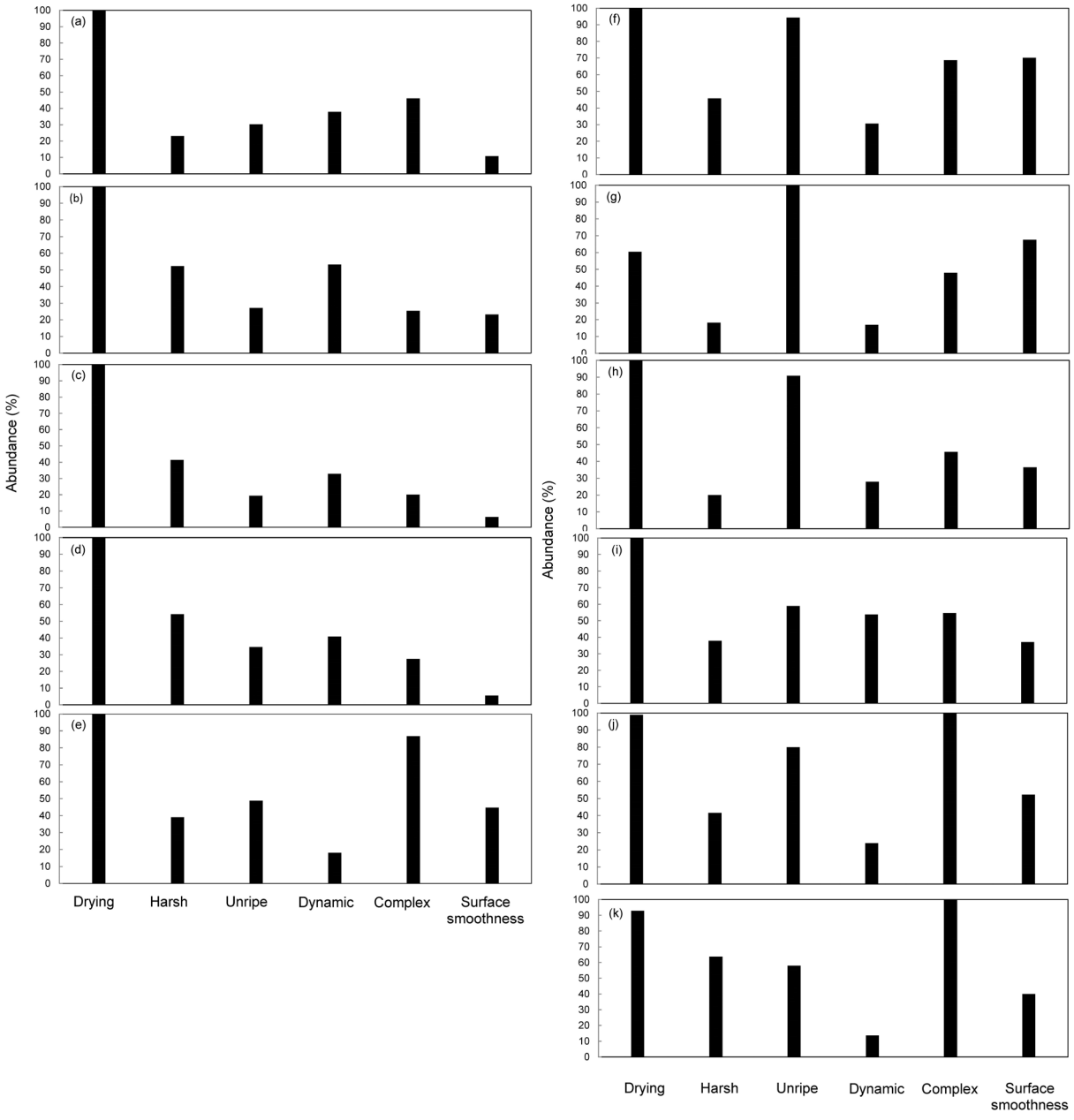
Figure 5. Quadratic discriminant analysis computed using mean sensory modified frequency of astringency sub-qualities (drying, harsh, unripe, dynamic, complex and surface smoothness) as quantitative explanatory variables. (a) Vectors show astringency sub-qualities contributing to the overall variance between single cultivar wines. (b) Ellipses show 95% confidence intervals for each single cultivar wine around the corresponding centroids (AGL, Aglianico; CAN, Cannonau; COR, Corvina; MON, Montepulciano; NEB, Nebbiolo; PRI, Primitivo; RAB, Raboso Piave; SAG, Sagrantino; SAN, Sangiovese; TER, Teroldego).



For each observation (wine sample), the probability of belonging to each group (single cultivar wine) was computed, and each wine was reclassified into the group for which the probability of belonging was the greatest. According to the confusion matrix, 88% of the wines were correctly reclassified: Corvina, Raboso, Nebbiolo, Sagrantino and Sangiovese samples were 100% correctly matched to the corresponding cultivar, followed by Cannonau and Primitivo (85.71%), Teroldego (75.00%), Aglianico (71.43%) and Montepulciano (57.14%).

Only the wines correctly reclassified were considered to develop, for each of the corresponding ten single cultivar wines, a graphical representation of their astringency features. For each single cultivar wine, the astringency sub-quality with the highest MF (mean value over the wines retained in the analysis) was considered as 100 and the MFs of the five remaining sub-qualities were normalised with respect to it. In this manner, as for a typical mass spectrum, we obtained a histogram corresponding to the 'astringency spectrum' of a given single cultivar wine where, the six sub-qualities were conceived as 'fragments' of the whole astringency of that wine (Figure 6).

Figure 6. ‘Astringency spectra’ developed for the single cultivar wines. (a) Sangiovese Toscana (SAT); (b) Sangiovese Romagna (SAR); (c) Nebbiolo (NEB); (d) Sagrantino (SAG); (e) Primitivo (PRI); (f) Montepulciano (MON); (g) Corvina (COR); (h) Raboso (RAB); (i) Aglianico (AGL); (j) Teroldego (TER); (k) Cannonau (CAN).

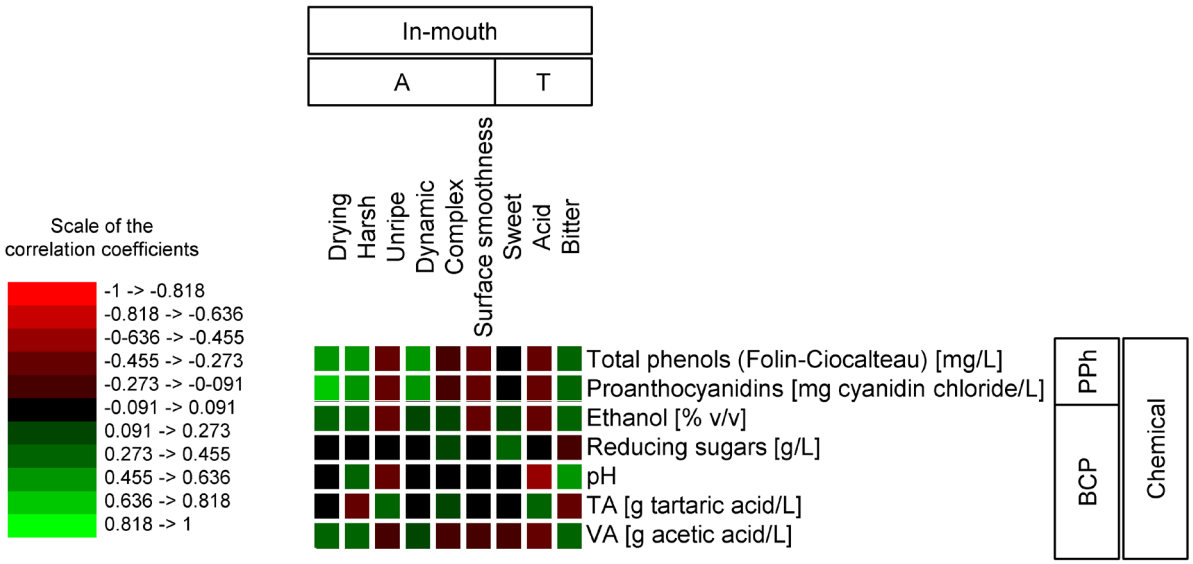


The abundance of each astringency sub-quality was plotted by computing its occurrence relative to the most important subquality detected in that single cultivar wine. In this way we obtained normalised profiles that allowed a comparison of the average relative contribution of each sub-quality to the astringency, within each of the diverse single cultivar wines. The patterns were different from each other, eight wines were dominated by the drying astringency (Figure 6a–f,h,i), two by the complex (Figure 6j,k) and one by the unripe (Figure 6g).

3.3.Correlations

Pearson correlations (P<0.05) were computed to test, across the different single cultivar wines, the association between the variables describing in-mouth sensations (astringency sub-quality: A; taste sensation: T) and a set of chemical variables concerning PPhs, and wine base chemical parameters (BCPs) (mean of triplicate analyses). Figure 7 represents the map of the correlations, whose coefficients are detailed in Table 4). At least one significant correlation was found for each variable and in most cases with a P-value <0.0001.

Figure 7. Map of the correlations (Pearson) between in-mouth and chemical variables (A: astringency sub-qualities; T: tastes; PPhs: phenolic substances; BCP: basic chemical parameters).
Corresponding P-values are reported in Table 4.



The PPhs variables, total phenols and proanthocyanidins, were: (i) highly (P<0.0001) positively correlated to drying (R^2=0.558 and 0.708, respectively), harsh (R^2=0.479 and 0.475) and dynamic (R^2=0.468 and 0.583); (ii) weakly negatively correlated to unripe (R^2 = 0.304 and 0.365) and surface smoothness (R^2 = −0.408 and −0.433); and (iii) not correlated to complex. Among sweet, acid, and bitter tastes, only the two latter showed weak correlation with PPhs parameters. Also, some correlations between BCPs and in mouth variables emerged but only those between pH and acidity (R^2 = −0.562) or bitterness (R^2=0.497) were the strongest (P<0.0001). The VA positively correlated with harsh (R^2=0.444), bitter (R^2=0.405) and drying (R^2=0.311), and negatively to acid (R^2=−0.290) and complex (R^2=−0.265).

Table 4. Correlation coefficients (Pearson) between in-mouth and chemical variables represented in Figure 6.

		Variables	In-Mouth								
			Astringency						Taste		
			Drying	Harsh	Unripe	Dynamic	Complex	Surface smoothness	Sweet	Acid	Bitter
Chemical	PPh	Total phenols (Folin-Ciocalteu) [mg/L]	**0.558**	**0.479**	**-0.304**	**0.468**	-0.159	**-0.408**	-0.079	**-0.347**	**0.425**
		Total proanthocyanidins ([mg cyanidin chloride/L]	**0.708**	**0.475**	**-0.365**	**0.583**	-0.225	**-0.433**	-0.052	**-0.296**	**0.409**
	BCP	Ethanol [% v/v]	**0.363**	**0.396**	**-0.416**	0.179	0.202	**-0.275**	0.171	**-0.421**	**0.278**
		Reducing sugars [g/L]	0.036	-0.010	-0.052	0.013	0.229	0.040	**0.387**	-0.089	-0.093
		pH	0.074	**0.434**	**-0.368**	-0.019	0.056	-0.082	0.031	**-0.562**	**0.497**
		Titratable acidity [g tartaric acid/L]	0.011	**-0.284**	**0.276**	0.020	0.150	0.049	-0.033	**0.451**	**-0.363**
		Volatile acidity [g acetic acid/L]	**0.311**	**0.444**	-0.195	0.172	**-0.265**	-0.134	-0.103	**-0.290**	**0.405**

Values in bold are different from 0 with a significance level $p < 0.05$ (in gray $p<0.0001$)
PPh: PolyPhenols; BCP: Base Chemical Parameters

4. Discussion

4.1. Description and discrimination of wines

From this study we obtained sensory profiles describing the balance among astringent sensations elicited by an extensive sample set of single cultivar Italian red wines representing different styles of astringency. Several studies focusing on molecules known to be responsible for astringency have been conducted on Italian red wines/grapes (Mattivi et al., 2002, 2009) but, for the first time, the astringency diversity of Italian red wines has been systematically investigated and compared from a sensory perspective. As in previous studies on red wine astringency (Ferrer-Gallego et al., 2016; Vidal et al., 2016), this study was carried out in full perceptual conditions (all senses). This allowed the assessment of wine astringency under conditions similar to that occurring during wine consumption, when cross-modal sensory interactions can occur. By merging the results reported through this study it appears possible to state that even if an intracultivar diversity was detected, it was possible to identify a pattern of astringency features common to wines from a given grape cultivar. Indeed, referring to the box-plots (Figure 3), we could gather that the shorter the box, the lower the variability of that sub-quality in that wine type. This suggests a wine feature that has been perceived in a similar manner in all samples by all judges, and therefore likely to be linkable to the grape cultivar (e.g., strong unripe in Raboso and Corvina; very low dynamic in Teroldego, Corvina and Primitivo; absence of velvet character in Nebbiolo and Sagrantino). Such a result suggests that these astringency features could be linked to the grape cultivar.

The detection of single wines or groups with different levels of intensity for the various astringency sub-qualities testifies the inter-varietal astringency diversity. The 11 single cultivar wines were differentiated at least for three different levels of intensity for drying, two for harsh, unripe, dynamic, complex and velvet,

while none for particulate. This indicates that judges showed a good understanding of what the different sub-qualities are, and that the 11 wines were distinguishable mostly according to the strong astringency sensations. The lack of a significant difference among wines for the term particulate (here intended as powdery) agrees with latest results obtained by applying the modified progressive profiling, a dynamic sensory method (Kang et al., 2019). The study reports that, differently from the other sub-qualities, the graininess, which was defined as a sensation of particulate matter on the mouth surface, resulted in a variable not useful to discriminate the astringency of 13 red wines.

The PCA performed on sensory intensities highlighted correlations between the six astringency sub-qualities and tastes (Figure 4). Some of these correlations (e.g., harsh and bitter, unripe and acid) suggest that judges correctly used the sub-qualities descriptors according to their definitions (Table 2). Taste variables occupied three distinct parts on the map. Also, the six astringency sub-qualities were well projected on three distinct areas of the chart, each of them close to a taste variable. The unripe astringency was not correlated to any of the other sub-qualities, suggesting a different 'nature' of this sub-quality compared to the others. The PCA found that in-mouth sensations of Sagrantino, Nebbiolo and Sangiovese were perceived as similar, and mainly associated to strong astringency sub-qualities and bitter taste. The other wines were spread on the opposite side of the chart sharing some common characteristics. The outputs of the QDA (Figure 5) showed that only some of the 11 single cultivar wines were discriminable from others due to their astringency features. Corvina and Raboso were discriminable from the other single cultivar wines and similar to each other, mostly for their unripe character. The discriminability of Nebbiolo, Sagrantino and Sangiovese was highlighted. All the other wines were not well discriminable according to their astringency features. This could be due to a higher degree of intra-varietal variability or to a more balanced contribution of the diverse astringency sensations. Each single cultivar wine showed a unique pattern among the six astringency sub-qualities. The astringency spectrum (Figure 6) of the single cultivar wines that were 100% correctly reclassified (Corvina, Raboso, Nebbiolo, Sagrantino and Sangiovese) can be considered as more reliable than the others. The future assessment of a larger and new distinct representative set of the same single cultivar wines could be useful to validate the astringency profiles that were developed in this study. According to the dominant sub-quality, three groups of wines can be distinguished: those dominated by the drying character, two dominated by the complex sub-

quality and the one dominated by an unripe astringency, namely Corvina. The astringency spectrum of Sagrantino (Figure 6d) and Sangiovese from Romagna (Figure 6b) was similar for the relative contribution of drying, harsh and complex while different mainly for that of surface smoothness and dynamic: the first was rather important in Sangiovese from Romagna and the second almost absent in Sagrantino. This lack of surface smoothness was also detected in Nebbiolo wines (Figure 6c). The scientific literature has no sensory data on Sagrantino wines; however, our results appear in line with previous chemical composition data. A study that measured the amount, the localisation, and the extractability of flavan-3-ols and anthocyanins in 25 high-quality red grapes, classified Sagrantino grapes as the richest in extractable PPhs and proanthocyanidins (Mattivi et al., 2002). Nebbiolo produces wines with high acidity and tannins when young, so that they require long ageing to reach a balance between acidity, astringency, full body, and aroma complexity (Asproudi et al., 2015). Barbaresco wines (100% made with Nebbiolo grapes) are often characterised by light colour and high roughness (Gerbi et al., 2006). Nebbiolo grapes are known to be poor in anthocyanins and rich in proanthocyanidins (Mattivi et al., 2002; Locatelli et al., 2016). Astringency is reported as an important sensory descriptor of Sangiovese from Romagna wines (Pagliarini et al., 2013; Laureati et al., 2014; Patrignani et al., 2017), which show the lowest level of co-pigmentation compared to that of the other wines (Versari et al., 2007). This could correspond to a higher astringency as a consequence of poor inclusion of some astringent monomeric components into the copigmentation stacks (Boulton, 2001; Alvarez et al., 2009; Escribano-Bailón & Santos-Buelga, 2012). Moreover, in recent years, unbalanced Sangiovese wines with excessive alcohol and astringency have been related to climate change (Filippetti et al., 2015). The rising temperature during ripening can negatively affect the acidity and the synthesis of PPhs provoking the rise of sugar accumulation leading to excessive alcohol. Due to the importance of Sangiovese grapes and wines (the principal Italian red cultivar), this issue is of impact also considering the enhancing role of increased ethanol on astringency (Noble, 1999), and the high maximal values observed both for the proanthocyanidins as well as for ethanol (Table 3). For the first time, our results compared Sangiovese wines from the two main areas of production showing different astringency features. Compared to Romagna (Figure 6b), the astringency spectrum of Toscana (Figure 6a) was different for a higher relative contribution of the complex sub-quality and an importantly lower impact of the harsh and dynamic components (mean intensities were significantly different; Tukey: $P<0.05$). Unripe

characterised the profile of Raboso wines (Figure 6h). Raboso Piave grapes are known to have high acidity and unbalanced PPhs with predominant low molecular flavanols (catechin), leading to astringent wines not easy to drink if grape maturity, winemaking, and ageing are not well managed (Mattivi et al., 2006; Corso et al., 2013). For Aglianico (Figure 6i), the pattern showed a balanced contribution of the different sub-qualities other than drying. High release and astringency of seeds tannin compared to other grapes were detected in Aglianico. Studies on winemaking and ageing optimisation to smooth the astringency and balance the sourness, two sensations characterising young Aglianico wines, were carried out (Mattivi et al., 2002; Gambuti et al., 2009). In Montepulciano (Figure 6f) the important contributors, harsh and unripe, were counterbalanced by surface smoothness and complex. Only 57% of our Montepulciano samples were correctly reclassified to the corresponding single cultivar wine and for this reason the resulting astringency spectrum was the least reliable compared to that of the other cultivars. Cannonau (genetically the same cultivar as Grenache) was one of the two wines in which complex dominated (Figure 6k); following an important relative contribution of strong sub-qualities (drying, harsh, unripe) and a good occurrence of surface smoothness is observed. In a comparison with many Italian cultivars (Mattivi et al., 2002), Cannonau exhibited a medium or low-medium level of PPhs having less than 40% of the catechins and proanthocyanidins reactive to vanillin located in the seeds, and extractable proanthocyanidins in the seeds were not exceeding 35% of PPhs. In Primitivo wines the most important astringency sub-qualities were drying and complex, with a good relative contribution of surface smoothness (Figure 6e). Primitivo wines, colour intense but low in tannin concentration, commonly reach a high alcohol concentration and have a ruby-purple colour, with a sensory profile showing a good balance between astringency, body, and pleasantness (Suriano et al., 2016; Trani et al., 2016). The astringency spectrum of Corvina wines (Figure 6g) was the only cultivar dominated by an unripe astringency and, at the same time, by the highest relative contribution of surface smoothness compared to that of other wines. This astringency profile fits in with previous knowledge about Corvina grapes; indeed, it is reported as characterised by a low tannin concentration and a green flavour (herbaceous/balsamic) that has been correlated to a high concentration of hexanols (Paronetto & Dellaglio, 2011) and cyclic terpenes (Slaghenaufi & Ugliano, 2018). Moreover, even if blended with other grapes, it gives the wine a powerful structure but surprising smoothness (Paronetto & Dellaglio, 2011). Finally, Teroldego is generally characterised by an intense ruby colour and by

smoothness in the mouth. Compared to other grapes, Teroldego had the highest extractable anthocyanin, showing an average concentration of extractable proanthocyanidins, with a low proportion from the seeds (Mattivi et al., 2002). Similar to Cannonau, its astringency spectrum (Figure 6j) was dominated by the complex sub-quality. This, together with a good surface smoothness, contrasts with the important contribution of drying and unripe with a net result, in terms of astringency, that suggest a soft mouthfeel.

4.2. Correlations

The significant correlations highlighted between sensory and chemical variables (Figure 7, Table 4) were tested across the 11 different single cultivar wines. Total phenols and proanthocyanidins were positively correlated to drying, harsh and dynamic while only negative correlation coefficients emerged between surface smoothness, unripe and complex; a weak significance was detected only for the first two. This result suggests that none of the two PPhs variables tested can predict/measure the perception of astringency in all its possible nuances. The fact that at least some aspects of astringency could be connected to aroma compounds could partially impact on this result. Indeed, being unripe and complex, two astringency sub-qualities including a retronasal olfactory sensation (Gawel et al., 2000), the volatile composition of the wine could play a significant role on their perception. The absence of a correlation between unripe and PPhs parameters supports the idea of a multidimensional nature of this sensory variable and appears consistent with previous findings. Indeed, in a chemosensory study aimed to characterise the fractions driving different mouthfeel properties in red wines, only the category unripe was not included in the final list of terms generated to describe the in-mouth sensations elicited during the tasting of the different odourless fractions (Sáenz-Navajas et al., 2017). The same authors tried to understand the involvement of volatile organic compounds modulating the perception of the green character of red wine astringency (Sáenz-Navajas et al., 2018). No specific aroma compounds were identified but a high concentration of fusel alcohols was observed and the involvement of interactions between isoamyl alcohol and anthocyanin-derivative fractions and/or tannin was suggested. Among the sensory and chemical parameters considered in this study, proanthocyanidins had the highest correlation coefficient. This is in accord with several studies that linked tannin concentration not only to the overall astringency but also to some sub-qualities describing ‘aggressive’ sensations (dry, pucker, chalk) and,

in accord with our results, to the decrease of smooth sensations (surface smoothness, silky, velvet) (Vidal et al., 2004; Preys et al., 2006; Vidal et al., 2018). A positive correlation was also found between the intensity of dry measured by modified progressive profiling and total tannin concentration (Kang et al., 2019). Among BCP parameters, ethanol had a negative correlation with acid and positive with bitter and this is coherent with the literature; indeed, ethanol tends to increase bitterness perception (Fischer & Noble 1994; Vidal et al., 2004; Sokolowsky & Fischer, 2012) and suppress sourness (Williams, 1972; Gonzalo-Diago et al., 2014). Ethanol was positively correlated with drying and harsh while negatively correlated with unripe and surface smoothness. It has been reported that ethanol decreases protein–tannin interactions and this has been linked to a decrease of the overall intensity of astringency (Waterhouse et al., 2016, and references therein), while our result refers to drying that is a specific sub-quality. This result appears in line with a recent study (Sáenz-Navajas et al., 2020), where a positive correlation was found (even if not significant) between ethanol and dry. According to its definition (Gawel et al., 2000), the drying sub-quality corresponds to a lack of lubrication with dehydration, and ethanol is a dehydrating agent. It is reported that ethanol is astringent at high concentration, due to denaturation and precipitation of salivary proteins (Waterhouse et al., 2016 and references therein). In our experiment, we tested the correlations across the whole set of wines that, according to data reported in Table 3, includes samples with high alcohol concentration. A negative correlation between pH and acid taste was observed, and the pH was also weakly positively correlated to harsh and bitter, in line with the definition of harsh. Some studies have reported on the influence of pH and ethanol on the different astringency sub-qualities (Gawel et al., 2014; Kang et al., 2019). The trends that we observed for unripe appear in line with previous findings. It has been reported (De Miglio et al., 2002) that unripe was rated more intensely as ethanol concentration decreased and as the pH value lowered. It was suggested that the driving force of these effects could be the impact of ethanol and pH on the perceived acidity, and this appears coherent with the definition of unripe.

The TA confirmed the same correlations detected for pH but with an opposite trend. The weak correlation between VA and in-mouth variables could be linked to the maceration conditions during winemaking. Indeed, conditions enhancing the extraction of PPhs, if combined with the ethanol developed and the limited nutrient status, can stress yeast and even bacteria and may lead to a rise in VA. A recent paper identified VA among

the top five predictive variables for drying and mouth-coating astringency subqualities in Tannat wines (Vidal et al., 2018).

According to our results, harsh and unripe were the subqualities that can be affected the most by BCPs, while drying and even more dynamic (no correlations with BCPs) appear to be influenced by the composition of PPhs. Also, complex and surface smoothness, the two sub-qualities describing smooth astringency, were poorly correlated to BCPs. The lack of correlations between complex and PPhs supports the hypothesis that other factors, likely olfactory cues, could play an important role on its perception but specific investigations are necessary.

5. Conclusions

Overall, this experiment gives a first picture of the diverse astringency of red wines from Italian native grapes, including some single cultivar products that have never been investigated before on their astringency. Furthermore, a contribution to the knowledge about the influence of chemical composition on the perception of astringency sub-qualities is given.

The 11 single cultivar wines were differentiated at least for three different levels of intensity for drying, two for harsh, unripe, dynamic, complex and velvet, while none for particulate. Despite the detected intra-cultivar variability, which was expected due to viticultural and oenological differences in commercial wine production, recurrent astringency features were found within wines from a given cultivar: intense unripe in Corvina and Raboso; low dynamic in Teroldego, Primitivo, Corvina and Montepulciano; and no velvet in Sagrantino and Nebbiolo. All samples were produced in the same vintage and had no contact with wood; therefore, it appears reasonable that these recurrent features can essentially be referred to as the astringency of the grape cultivars. The astringency spectrum, a sensory pattern describing the relative balance among six astringency sub-qualities of the single cultivar wines, was different from each other. Further experiments are necessary to validate these profiles on other wines produced from the same cultivars, and in limited perceptual conditions to evaluate the impact of cross-modal sensory interactions.

The correlation study conducted over a set of different wines confirmed the positive correlation between proanthocyanidins and astringency, highlighted that neither total phenols nor proanthocyanidins were able to

measure/predict the perception of astringency in all its nuances, and suggested that the diverse astringency sub-qualities could be affected differently by the chemical parameters, such as ethanol or pH.

References

Alvarez, I.; Aleixandre, J.L.; Garcia, M.J.; Lizama, V.; Aleixandre-Tudo, J.L. Effect of the prefermentative addition of copigments on the polyphenolic composition of Tempranillo wines after malolactic fermentation. *Eur. Food Res. Technol.* **2009**, 228, 501–510.

Arapitsas, P.; Ugliano, M.; Marangon, M.; Piombino, P.; Rolle, L.; Gerbi, V.; Versari, A.; Mattivi, F. Use of untargeted Liquid Chromatography–Mass Spectrometry metabolome to discriminate Italian monovarietal red wines, produced in their different terroirs. *J. Agric. Food Chem.* **2020**, 68, 13353−13366.

Asproudi, A.; Piano, F.; Anselmi, G.; Di Stefano, R.; Bertolone, E.; Borsa, D. Proanthocyanidin composition and evolution during grape ripening as affected by variety: Nebbiolo and Barbera cv. *Journal International des Sciences de la Vigne et du Vin*, **2015**, 49, 59–69.

Boselli, E.; Boulton, R.B.; Thorngate, J.H.; Frega, N.G. Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **2004**, 52, 3843–3854.

Boulton, R.B. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, 52, 67–87.

Cadena, R.S.; Vidal, L.; Ares, G.; Varela, P. Dynamic sensory descriptive methodologies. Time-intensity and temporal dominance of sensations. Varela, P.; Ares, G., eds. Novel techniques for sensory characterization and consumer profiling; CRC Press: Boca Raton, FL, USA; 2014, pp. 333–364.

Charters, S.; Pettigrew, S. The dimensions of wine quality. *Food Qual. Pref.* **2007**, 18, 997–1007.

Cliff, M.A.; King, M.C.; Schlosser, J. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Res. Int.* **2007**, 40, 92–100.

Corso, M.; Ziliotto, F.; Rizzini, F.M.; Teo, G.; Cargnello, G.; Bonghi, C. Sensorial, biochemical and molecular changes in Raboso Piave grape berries applying 'Double Maturation Raisonnée' and late harvest techniques. *Plant Science*, **2013**, 208, 50–57.

De Luca, P.; Pegan, G.; Gonzalo-Penela, C. Insights from a Google keywords analysis about Italian wine in the US market. *Micro & Macro Marketing*, **2019**, 1, 93–116.

De Miglio, P.; Pickering, G.J.; Reynolds, A.G. Astringent sub-qualities elicited by red wine: the role of ethanol and pH. Cullen, C.W., Pickering, G.J. and Phillips, R., eds. Proceedings of the international Bacchus to the future conference; 23 April–15 May 2002; St Catharines, ON, Canada; Brock University Press: St Catherines, ON, Canada; 2002, pp. 31–52.

Di Stefano, R.; Cravero, M.C.; Gentilini, N. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, **1989**, 25, 83–89.

Dravnieks, A. Odor quality: semantically generated multidimensional profiles are stable. *Sciences*, **1982**, 218, 799–801.

Escribano-Bailón, M.T.; Santos-Buelga, C. Anthocyanin copigmentation-evaluation, mechanisms and implications for the colour of red wines. *Curr. Org. Chem.* **2012**, 16, 715–723.

Ferrer-Gallego, R.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. *Food Res. Int.* **2014**, 62, 1100–1107.

Ferrer-Gallego, R.; Brás, N.F.; García-Estévez, I.; Mateus, N.; Rivas-Gonzalo, J.C.; De Freitas, V.; Escribano-Bailón, M.T. Effect of flavonols on wine astringency and their interaction with human saliva. *Food Chem.* **2016**, 209, 358–364.

Filippetti, I.; Movahed, N.; Allegro, G.; Valentini, G.; Pastore, C.; Colucci, E.; Intrieri, C. Effect of post-veraison source limitation on the accumulation of sugar, anthocyanins and seed tannins in Vitis vinifera cv. Sangiovese berries. *Austr. J. Grape Wine R.* **2015**, 21, 90–100.

Fischer, U.; Noble, A.C. The effect of ethanol, catechin concentration, and pH on sourness bitterness of wine. *Am. J. Enol. Vitic.* **1994**, 45, 6–10.

Francis, I.L.; Gawel, R.; Iland, P.G.; Vidal, S.; Cheynier, V.; Guyot, S.; Kwiatkowski, M.J.; Waters, E.J. Characterising mouth-feel properties of red wines. *Aust. N.Z. Wine Ind.* **2002**, 17(3), 18, 21–22, 24–25.

Gambuti, A.; Capuano, R.; Lecce, L.; Fragasso, M.G.; Moio, L. Extraction of phenolic compounds from 'Aglianico' and 'Uva di Troia' grape skin and seeds in model solutions: influence of ethanol and maceration time. *Vitis*, **2009**, 48, 193–200.

Gawel, R.; Oberholster, A.; Francis, I.L. A 'Mouth-feel Wheel': terminology for communicating the mouth-feel characteristics of red wine. *Austr. J. Grape Wine R.* **2000**, 6, 203–207.

Gawel, R.; Day, M.; Van Sluyter, S.C.; Holt, H.; Waters, E.J.; Smith, P.A. White wine taste and mouthfeel as affected by juice extraction and processing. *J. Agric. Food Chem.* **2014**, 62, 10008–10014.

Gerbi, V.; Caudana, A.; Rolle, L.G.; Cagnasso, E.; Zeppa, G. La vinificazione del Nebbiolo. *Quaderni di Viticoltura ed Enologia dell'università di Torino*, **2006**, 28, 167–184.

Gonzalo-Diago, A.; Dizy, M.; Fernández-Zurbano, P. Taste and mouthfeel properties of red wines proanthocyanidins and their relation to the chemical composition. *J. Agric. Food Chem.* **2013**, 61, 8861–8870.

Gonzalo-Diago, A.; Dizy, M.; Fernández-Zurbano, P. Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. *Food Chem.* **2014**, 154, 187–198.

Green, B.G. Oral astringency: a tactile component of flavor. *Acta Psychologica*, **1993**, 84, 119–125.

Guinard, J.X.; Pangborn, R.M.; Lewis, M.J. The timecourse of astringency in wine upon repeated ingestion. *Am. J. Enol. Vitic.* **1986**, 37, 184–189.

Kang,W.; Niimi, J.; Muhlack, R.A.; Smith, P.A.; Bastian, S.E.P. Dynamic characterization of wine astringency profiles using modified progressive profiling. *Food Res. Int.*, **2019**, 120, 244–254.

Lacombe, T.; Audeguin, L.; Boselli, M.; Bucchetti, B.; Cabello, F.; Chatelet, P.; Crespan, M.; D'Onofrio, C.; Eiras Dias, J.; Ercisli, S.; Gardiman, M.; Grando, M.S.; Jandurova, O.; Jung, A.; Kiss, E.; Kozma, P.; Maul, E.; Maghradze, D.; Martinez, M.C.; Muñoz, G.; Pátková, J.K.; Pejic, I.; Peterlunger, E.; Pitsoli, D.; Preiner, D.; Raimondi, S.; Regner, F.; Savin, G.; Savvides, S.; Schneider, A.; Spring, J.L.; Szoke, A.; Veres, A.; Boursiquot, J.M.; Bacilieri, R.; This, P. Grapevine European catalogue: towards a comprehensive list. *Vitis*, **2011**, 50, 65–68.

Laureati, M.; Gaeta, D.; Pagliarini, E. Qualitative and sensory evaluation of Sangiovese red wine obtained from organically and conventionally grown grapes. *Italian Journal of Food Science*, **2014**, 26, 355–362.

Lelièvre, M.; Chollet, S.; Abdi, H.; Valentin, D. What is the validity of the sorting task for describing beers? A study using trained and untrained assessors. *Food Qual. Pref.* **2008**, 19, 697–703.

Locatelli, M.; Travaglia, F.; Coïsson, J.D.; Bordiga, M.; Arlorio, M. Phenolic composition of Nebbiolo grape (Vitis vinifera L.) from Piedmont: characterization during ripening of grapes selected in different geographic areas and comparison with Uva Rara and Vespolina cv. *Eur. Food Res. Technol.* **2016**, 242, 1057–1068.

Mannini, F. Italian indigenous grapevine cultivars: guarantee of genetic biodiversity and economic resource. *Acta Horticulturae*, **2004**, 652, 87–95.

Mattivi, F.; Vrhovsek, U.; Masuero, D.; Trainotti, D. Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Austr. J. Grape Wine R.* **2009**, 15, 27–35.

Mattivi, F.; Zulian, C.; Nicolini, G.; Valenti, L. Wine, biodiversity, technology, and antioxidants. *Annals of the New York Academy of Sciences*, **2002**, 957, 37–56.

Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. Metabolite profiling of grape: flavonols and anthocyanins. *J. Agric. Food Chem.* **2006**, 54, 7692–7702.

Mercurio, M.D.; Dambergs, R.G.; Cozzolino, D.; Herderich, M.J.; Smith, P.A. Relationship between red wine grades and phenolics. 1. Tannin and total phenolics concentrations. *J. Agric. Food Chem.* **2010**, 58, 12313–12319.

Mueller, S.; Osidacz, P.; Francis, I.L.; Lockshin, L. Combining discrete choice and informed sensory testing in a two-stage process: can it predict wine market share? *Food Qual. Prefer.* **2010**, 21, 741–754.

Noble, A.C. Why do wines taste bitter and feel astringent. Chemistry of wine flavour. Volume 714. ACS Symposium Series (American Chemical Society: Washington, DC, USA), 1999, pp. 156–165.

Organisation Internationale de la Vigne et du Vin (2015) Compendium of international methods of must and wine analysis (Organisation Internationale de la Vigne et du Vin : Paris, France).

Organisation Internationale de la Vigne et du Vin (2017) Vine varieties distribution in the world (Organisation Internationale de la Vigne et du Vin : Paris, France). http://www.oiv.int/public/medias/5336/infographie-focus-oiv-2017-new.pdf

Organisation Internationale de la Vigne et du Vin (2018). OIV Focus for 2018; OIV: Paris, France, 2018.

Pagliarini, E.; Laureati, M.; Gaeta, D. Sensory descriptors, hedonic perception and consumer's attitudes to Sangiovese red wine deriving from organically and conventionally grown grapes. *Front. Psychol.* **2013**, 4, 896.

Paronetto, L.; Dellaglio, F. Amarone: a modern wine coming from an ancient production technology. *Adv. Food Nutr. Res.* **2011**, 63, 285–306.

Patrignani, F.; Montanari, C.; Serrazanetti, D.I.; Braschi, G.; Vernocchi, P.; Tabanelli, G.; Parpinello, G.P.; Versari, A.; Gardini, F.; Lanciotti, R. Characterisation of yeast microbiota, chemical and sensory properties of organic and biodynamic Sangiovese red wines. *Ann. Microbiol.* **2017**, 67, 99–109.

Peynaud, E. The taste of wine: the art and science of wine appreciation; Macdonald Orbis: London, England; 1987.

Piacenza, M.; Benfratello, L.; Sacchetto, S. Taste or reputation? What drives market prices in the wine industry? Estimation of a hedonic model for Italian premium wines. *Appl. Econ.* **2009**, 41, 2197–2209.

Preys, S.; Mazerolles, G.; Courcoux, P.; Samson, A.; Fischer, U.; Hanafi, M.; Bertrand, D.; Cheynier, V. Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Anal. Chim. Acta*, **2006**, 563, 126–136.

Sáenz-Navajas, M.P.; Martín-López, C.; Ferreira, V.; Fernández-Zurbano, P. Sensory properties of premium Spanish red wines and their implication in wine quality perception. *Austr. J. Grape Wine R.* **2011**, 17, 9–12.

Sáenz-Navajas, M.P.; Ferrero-del-Teso, S.; Jeffery, D.W.; Ferreira, V.; Fernández-Zurbano, P. Effect of aroma perception on taste and mouthfeel dimensions of red wines: correlation of sensory and chemical measurements. *Food Res. Int.* **2020**, 131, 108945.

Sáenz-Navajas, M.P.; Arias, I.; Ferrero-del-Teso, S.; Fernández-Zurbano, P.; Escudero, A.; Ferreira, V. Chemo-sensory approach for the identification of chemical compounds driving green character in red wines. *Food Res. Int.* **2018**, 109, 138–148.

Sáenz-Navajas, M.P.; Avizcuri, J.M.; Echávarri, J.F.; Ferreira, V.; Fernández-Zurbano, P.; Valentin, D. Understanding quality judgements of red wines by experts: effect of evaluation condition. *Food Qual. Prefer.* **2016**, 48, 216–227.

Sáenz-Navajas, M.P.; Avizcuri, J.M.; Ferrero-del-Teso, S.; Valentin, D.; Ferreira, V.; Fernández-Zurbano, P. Chemo-sensory characterization of fractions driving different mouthfeel properties in red wines. *Food Res. Int.* **2017**, 94, 54–64.

Sáenz-Navajas, M.P.; Campo, E.; Avizcuri, J.M.; Valentin, D.; Fernández-Zurbano, P.; Ferreira, V. Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: wine reconstitution strategies and sensory sorting task. *Anal. Chim. Acta*, **2012**, 732, 64–72.

Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **1999**, 299, 152–178.

Slaghenaufi, D.; Ugliano, M. Norisoprenoids, sesquiterpenes and terpenoids content of Valpolicella wines during aging: investigating aroma potential in relationship to evolution of tobacco and balsamic aroma in aged wine. *Front. Chem.* **2018**, 6, 66.

Sokolowsky, M.; Fischer, U. Evaluation of bitterness in white wine applying descriptive analysis, time-intensity analysis, and temporal dominance of sensations analysis. *Anal. Chim. Acta*, **2012**, 732, 46–52.

Suriano, S.; Alba, V.; Di Gennaro, D.; Basile, T.; Tamborra, M.; Tarricone, L. Major phenolic and volatile compounds and their influence on sensorial aspects in stem-contact fermentation winemaking of Primitivo red wines. *J. Food Sci. Technol.* **2016**, 53, 3329–3339.

Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L. Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Anal. Chim. Acta*, **2010**, 660, 183–189.

Trani, A.; Verrastro, V.; Punzi, R.; Faccia, M.; Gambacorta, G. Phenols, volatiles and sensory properties of primitivo wines from the 'Gioia Del Colle' PDO area. *S. Afr. J. Enol. Vitic.* **2016**, 37, 139–148.

Versari, A.; Parpinello, G.P.; Mattioli, A.U. Characterisation of colour components and polymeric pigments of commercial red wines by using selected UV-Vis spectrophotometric methods. *S. Afr. J. Enol. Vitic.* **2007**, 28, 6–10.

Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Dynamic characterization of red wine astringency: case study with Uruguayan Tannat wines. *Food Res. Int.* **2016**, 82, 128–135.

Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Sensory characterization of the astringency of commercial Uruguayan Tannat wines. *Food Res. Int.* **2017**, 102, 425–434.

Vidal, L.; Antúnez, L.; Rodríguez-Haralambides, A.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Relationship between astringency and phenolic composition of commercial Uruguayan Tannat wines: application of boosted regression trees. *Food Res. Int.* **2018**, 112, 25–37.

Vidal, S.; Courcoux, P.; Francis, I.L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.J.; Cheynier, V. Use of an experimental design approach for evaluation of

key wine components on mouth-feel perception. *Food Qual. Prefer.* **2004**, 15, 209–217.

Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. Understanding wine chemistry; John Wiley: Hoboken, NJ, USA, 2016.

Williams, A.A. Flavour effects of ethanol in alcoholic beverages. *The Flavour Industry*, **1972**, 3, 604–606.

Wine Australia (2019) National vintage report (Wine Australia: Adelaide, SA, Australia). https://www.wineaustralia.com/getmedia/807bf053-3692-448a-9ed5-c0084a47e1bb/Vintage-report-2019_fullversion.pdf

Part II

This part is an edited version of:

Exploring Olfactory–Oral Cross-Modal Interactions through Sensory and Chemical Characteristics of Italian Red Wines

Pittari, E.[1], Moio, L.[1], Arapitsas, P.[2], Curioni, A.[3], Gerbi, V.[4], Parpinello, G.P.[5], Ugliano, M.[6], & Piombino, P[1, *]

[1] Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, 83100 Avellino, Italy

[2] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all' Adige, 38010 Trento, Italy

[3] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, 35020 Padova, Italy

[4] Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, 10095 Torino, Italy

[5] Department of Agricultural and Food Sciences, University of Bologna, 40126 Bologna, Italy

[6] Department of Biotechnology, University of Verona, 37029 Cariano (VR), Italy

* Corresponding author:

Professor Paola Piombino

Published in ***Foods, 2020, 9(11), 1530***
DOI:10.3390/foods9111530

1. Introduction

Wine consumption and preference are sensitive to quality, flavour, and sensory characteristics (Chironi et al., 2013; Vecchio et al., 2019). Flavour results from the integration of all sensations perceived in the mouth and in the nose cavities, including olfactory (orthonasal and retronasal), tastes, and other oral sensations involving tactile and trigeminal perceptions (Prescott, 2012; Small & Prescott, 2005). During tasting, flavour perception is significantly affected by the interactions among sensory stimuli (Noble, 1996), among which odour and astringency play a fundamental role (Peynaud, 1987; Charters & Pettigrew, 2007; Sáenz-Navajas et al., 2016). Some works addressed the study of multimodal interactions (i.e., aroma–aroma, aroma–taste, taste–astringency and aroma–astringency) and their sensory impact (Sáenz-Navajas et al., 2012; de-la-Fuente-Blanco et al., 2016). However, the mechanisms at the base of these interactions remain unclear and need to be further explored, ideally in real wines with different sensory characteristics and matrix composition. The 11 wine types tested and described above (Piombino et al., 2020) showed diverse astringency patterns characterized by a different balance among six astringency sub-qualities (drying, harsh, unripe, dynamic, complex, and surface smoothness). The correlations with compositional parameters were not tested considering the overall astringency as usually done in previous studies but by looking at its six sub-qualities and some chemical parameters, including total phenols and proanthocyanidins. Results partially support the hypothesis that olfactory cues related to wine VOCs might play a role in modulating the perception of some astringency sub-qualities. The exploration of this aspect is of interest as the research outputs are useful for oenologists to manage and control wine quality and to better comprehend consumer preferences/acceptance. Indeed, integrative brain processes, such as cross-modal interactions, could explain why it is difficult to find a direct correlation between specific compounds or chemical structures and astringency sensations that are of great interest for research and production.

For these reasons, the main aims of this experiment were: (i) to investigate both odour–astringency (single sub-qualities) and odour–taste cross-modal sensory interactions in a wide set of real wine matrices, exploiting the sensory diversity of 10 single-cultivar Italian red wines; (ii) to test and compare the correlations between sensory (odour descriptors, astringency sub-qualities, and tastes) and chemical compositional parameters (total phenols, proanthocyanidins, ethanol, reducing sugars, pH, titratable acidity, volatile acidity) both in the

presence and in the absence of VOCs. To do this, a sample set of 74 wines was assessed under two types of evaluation conditions: whole wines (WWs) and corresponding deodorized wines (DWs), meaning wines with or without odorants, respectively. To exclude olfactory perceptions, instead of using nose-clips as in most of the previous studies (Sáenz-Navajas et al., 2012; Sáenz-Navajas et al., 2020), a deodorization procedure was applied to make subjects comfortable with the sensory test and to simulate, as much as possible, the same breathing conditions experienced during a 'normal' wine tasting. Unlike previous methods applied for wine deodorization to study astringency or aroma (Sáenz-Navajas et al., 2010a; Sáenz-Navajas et al., 2010b; Lytra et al., 2012; Muñoz-González et al., 2014), a new deodorization procedure was optimized to avoid the use of solvents and obtain representative deodorized wines that could be safely tasted by judges.

2. Material and Methods

2.1. Chemicals

Fructose (99%) and tannic acid (95%) were purchased from J.T. Baker (Avantor; Radnor, PA, USA). Tartaric acid (99.7%) was provided by Chem-Lab (Eernegem, West-Vlaanderen, Belgium), and caffeine (99.2%) by ACEF (Piacenza, Italy). 2-Phenylethanol (≥99%), citral (95%), linalool (97%), 1-octen-3-one (96%), cis-3-hexen-1-ol (≥98%), ethyl butyrate (≥99%), damascenone (1.1–1.4 wt.%), benzaldehyde (≥99.5%), isoamyl acetate (≥95%), gamma-dodecalactone (≥97%), sotolone (≥97%), 4-ethylguaiacol (≥98%), 4-ethylphenol (99%), eucalyptol (99%), furaneol (≥98%), ethyl caproate (≥99%), eugenol (≥98%), citronellol (95%), phenylacetaldehyde (≥95%), furfuryl acetate (≥98%), 2,4,6-trichloroanisole (99%), 2-methyl-1-propanol (99.5%), methanethiol (≥98%) were all provided by Sigma-Aldrich (St. Louis, MO, USA). Ethanol (food grade, 70%) was supplied by ITW Reagents (Milano, Italy). Tanin VR colour, Tanin VR grape, Tanin plus, Tanin galalcool were all purchased from Laffort (Bordeaux, France).

2.2. Wine Samples

Seventy-four Italian red wines, 100% mono-varietal, vinified in 2016 from 10 Italian grape varieties harvested in 11 regions corresponding to the main geographical areas of production were sampled from the producers. The set of wines included: 10 Sangiovese (Romagna and Toscana), 8 Teroldego Rotaliano (Trentino-Alto

Adige), 7 Corvina (Veneto), Raboso Piave (Veneto), Nebbiolo (Piemonte), Sagrantino (Umbria), Montepulciano (Abruzzo), Cannonau (Sardegna), Aglianico (Campania), and Primitivo (Puglia). Wines were selected from the most representative cellars of each production area, fermented in stainless steel vats at commercial scale, and sampled before malolactic fermentation and oak barrels ageing. Before bottling, all samples were protected with 50 mg/L of free SO_2, and bottles were closed with a Select Green 500 cork type (Nomacorc, Revisaltes, France) and stored at controlled cellar temperature (12±2°C) until analyses.

2.3. Sensory Analysis

The aim was to investigate the impact of the olfactory stimuli on astringency and taste perceptions during a red wine tasting. For this purpose, the 74 wine samples described above (whole wines: WWs) and corresponding 74 deodorized wines (DWs) were characterized for odour, astringency and taste features using a descriptive sensory assessment on a numerical category scale.

2.3.1. Panel

The jury was composed of 14 selected individuals (7 males and 7 females aged between 22 and 49 years) recruited among students and researchers (Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II). They were selected based on their interest, availability, and ability to recognize olfactory and oral stimuli. They were all expert wine tasters with previous experience in performing sensory tests on wine. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Participation was on a voluntary basis and, prior to the experiments, tasters were required to sign an informed consent form disclosing the type of research, voluntary participation, and agreement to taste/smell reference solutions and wines. All data were collected anonymously.

2.3.2. Procedure

Panel training: judges' selection and familiarization with 10 in-mouth sensations (seven astringency sub-qualities: drying, harsh, unripe, dynamic, particulate/powder, complex, and surface smoothness/velvet; and three tastes: sweetness, sourness, and bitterness) were performed according to the procedures and standard

materials previously reported (Piombino et al., 2020). Likewise, panellists were selected and trained on olfactory stimuli by providing them a list of 11 odour families (fruity; dehydrated fruits; dried fruits: nuts; floral; vegetal; spicy; toasted; woody; earthy; alcoholic; off-odours: phenolic, sulphurous, cork taint, maderised/oxidised) selected from the literature (Noble et al., 1987) and 24 odour standards representative of different odour families and wine volatiles (Table 4). Panellists were asked to smell each standard (20 mL of water solution in covered disposable plastic cups served according to a randomized order) to recognize the corresponding odour descriptor/s or family/ies and to score the intensity on the following numerical category scale: 1=very low, 2=low, 3=medium, 4=high, and 5=very high, with half values allowed. One introductory session (no data collected) and two real sessions were carried out. Data collected in the 2nd and 3rd sessions were used to calculate the frequency of citations for standard correctly matched with descriptor/s. Only the terms with an association frequency (percentage of judges that consensually matched the correct descriptor to a given standard solution) ≥85% were considered as consensually associated to the corresponding standards (Table 4). At the end of each training session, the perceived sensations. Only the terms with an association frequency (percentage of judges that consensually matched the correct descriptor to a given standard solution) ≥85% were considered as consensually associated to the corresponding standards (Table 4). At the end of each training session, the perceived sensations were discussed with the participants to prevent overlapping and redundancies among terms and to help their memorization.

Finally, to familiarize the jury with the application of the procedure to real wines as well as to test panellists' performances, 10 commercial wines (selected among samples under investigation) were assessed (two replicates) using the same evaluation procedure as run-through prior to the real analytical sessions. Subjects were provided with water and required to wait at least 15 s between each sample.

Sensory assessment: WWs and DWs were analysed by descriptive sensory assessment using the same vocabulary and the five-point numerical category scale employed during the training. A total of 296 samples, meaning (74 WWs + 74 DWs) x two replicates, were assessed during 15 sessions (10 wines/session; the two missing wines were obtained by blending some available wines but data on these "fake" samples were not considered in the analyses). Each session was split into 2 sub-sessions with an imposed break of 15 min, and the evaluations of WWs or corresponding DWs were performed in each sub-session. All participants evaluated

the 74 WWs by first smelling and scoring odours intensities, and then by tasting for astringency sub-qualities and tastes according to the procedure previously described (Piombino et al., 2020). The same tasting procedure was repeated on DWs in a separated sub-session. Subjects were not informed about the nature of the samples. For each sample, 25 mL were served in covered glasses (ISO, 1997) coded with three-digits and presented in a randomized order. Wines were served at room temperature (21±1°C) and evaluated in individual booths (ISO, 2007).

Table 4. Reference compounds and corresponding consensual descriptors used to train the assessors in recognizing and distinguishing among the different olfactory stimuli.

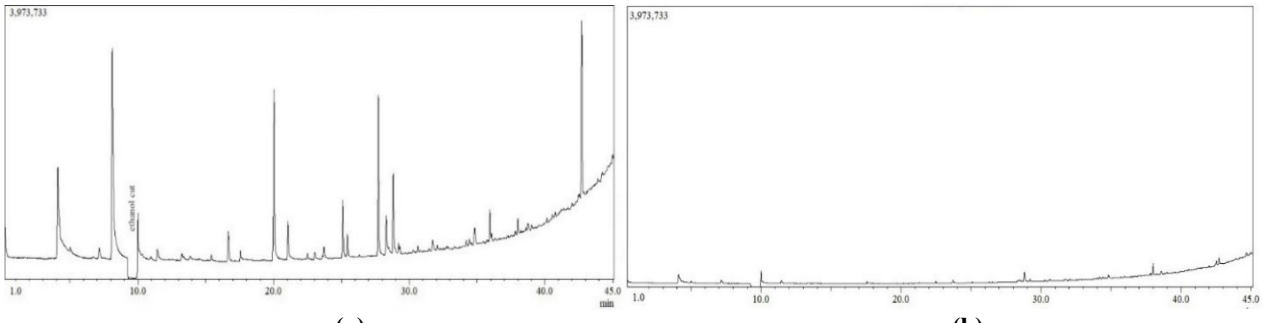
Reference compounds	Concentration (µg/L)[1]	Consensual descriptors[2]	Desriptors[3]
2-phenylethanol	159	Floral, rose	Floral, rose, dried rose
citral	76.8	Terpenic, citric, fruity	Sharp, lemon, sweet
linalool	14.3	Terpenic, floral	Citrus, floral, sweet, bois de rose, woody, green, blueberry
1-octen-3-one	1.7	Mushroom, earth, musk, vegetal	Herbal, mushroom, earthy, musty, dirty
cis-3-hexen-1-ol	157.5	Herbaceous, green, vegetal	Fresh, green, grassy, foliage, vegetable, herbal, oily
ethyl butyrate	27.7	Fruity	Fruity, juicy, fruity, pineapple, cognac
damascenone	14.4	Apple pie, backed apple	Natural, sweet, fruity, rose, plum, grape, raspberry, sugar
benzaldehyde	696.6	Bitter almond	Sharp, sweet, bitter almond, cherry
isoamyl acetate	10.4	Fruity, banana	Sweet, fruity, banana, solvent
gamma-dodecalactone	20.8	Fruity, peach, dehydrated apricot	Fatty, peach, sweet, metallic, fruity
sotolone	2	Fenugreek, fennel, liquorice, nut, raisins	Sweet, caramellic, maple, sugar, burnt sugar, coffee
4-ethylguaiacol	118.2	Phenolic, smoked, woody	Spicy, smoky, bacon, phenolic, clove
4-ethylphenol	21	Phenolic, horse sweat	Phenolic, castoreum, smoky, guaiacol
eucalyptol	30.1	Eucalyptol, balsamic, vegetal	Eucalyptus, camphoreous, medicinal, herbal
2,5-dimethyl-4-hydroxy-3(2H)-furanone	7	Cotton candy, caramel, backed, toasted	Sweet, cotton candy, caramellic, strawberry, sugar, brown sugar
ethyl caproate	35.4	Fruity, pineapple	Sweet, fruity, pineapple, green banana, waxy
eugenol	30.9	Cloves, spicy	Sweet, spicy, clove, woody
citronellol	48	Terpenic, floral	Floral, leathery, waxy, rose, citrus
phenylacetaldehyde	11.1	Honey, beeswax, fruity	Green, sweet, floral, hyacinth, clover, honey, cocoa
furfuryl acetate	79.7	Fruity, banana, sweet	Sweet, fruity, banana, horseradish
2,4,6-trichloroanisole	11.7	Cork taint	-
2-methyl-1-propanol	668	Amylic, chemical, grappa	Ethereal, winey
3-(methylthio)-1-propanol	46.8	Garlic, sulfurous, cooked vegetable	Sulfurous, onion, sweet, soup, vegetable
ethanol	4.0 g/L	Alcoholic, ethereal, sharp	Strong, alcoholic, ethereal, medical

[1]In distilled water; [2]Association frequency (percentage of judges that consensually associated the correct descriptor to a given standard solution) ≥85%; [3]The Good Scents Company

2.4. Deodorization and Reconstitution of Wines

Drawing from the methods previously reported (Rodríguez-Bencomo et al., 2011; Lytra et al., 2012), a new rapid (~2h) deodorization procedure was optimized to obtain representative and safe deodorized wines (DWs). The whole wines (WWs) were deodorized during the two days preceding the date fixed for the corresponding session of assessment. Wines were deodorized one by one (two replicates) as follows: 360 mL of wine were weighed and treated in an ultrasound bath (Transsonic 460 H, Elma, Germany) with water as processing liquid, working at a fixed frequency of 35 KHz, to the minimum intensity (1) in a range between 1–15 (set through a turning knob), and maintained at a controlled low temperature of 20°C for 30 min. The samples were then evaporated at 30°C under reduced pressure (Rotavapor R-210, Büchi, Switzerland). The process was stopped when the samples reached a weight loss of ~95% (~90min). As the deodorization procedure stopped, the samples were weighed and reconstituted, one by one, at the initial weight by adding distilled water and food-grade ethanol at a proper concentration to reach the initial alcohol degree (%v/v) of the wine. DWs were then stored at (12±2°C) till the analysis. Any visual differences between reconstituted wine and real wine were ascertained on a subset of samples randomly chosen within each grape variety, by means of discriminant analysis [triangle test, (ISO, 2004)]: differences resulted not significant (α=0.01). This test, along with an informal check to verify the absence of off-odours and off-tastes, was conducted internally at the laboratory. The efficacy of the deodorization was confirmed by Gas-Cromatography/Mass Spectrometry (GC-MS) analysis (Genovese et al., 2005) of the volatile fraction of the wines prior and after deodorization–reconstitution. Different methods for VOCs isolation were applied for the check: pre-concentration by SPME (Figure 6) and liquid–liquid extraction as previously reported (Piombino et al., 2010; Piombino et al., 2019).

Figure 6. SPME/GC-MS chromatograms (TIC) of a WW sample (a) compared to the corresponding DW (b).



(a) **(b)**

2.5. Chemical Analysis

Ethanol, reducing sugars, volatile acidity (VA), and titratable acidity (TA) were measured according to the Organisation Internationale de la Vigne et du Vin (OIV) methods (OIV, 2015). pH was determined by potentiometry (InoLab 730 pH meter, WTW, Weilheim in Oberbayern, Germany). Total phenols were measured by Folin–Ciocalteu assay (Singleton et al., 1999). The concentration of proanthocyanidins was determined after acid hydrolysis with warming (Bate-Smith reaction) using a ferrous salt (FeSO4) as catalyst (Di Stefano et al., 1989; Torchio et al., 2010). Analyses were carried out in triplicate.

2.6. Data Analysis

For the sensory characterization of WWs, two Principal Component Analyses (PCA) were carried out on the correlation matrices (Pearson, $p<0.05$) of the mean intensities over wines of each grape variety rated by the 14 judges for significant in-mouth sensations and odours and tested using multi-way ANOVAs.

A three-way ANOVA (judges as random factor, grape variety, and perception modality as fixed factors; Tukey, $p<0.05$) with interactions (grape variety*perception modality) was computed to test the discrimination effect of in-mouth descriptors and to evaluate the impact of the perception modality (with and without VOCs, WWs and DWs respectively) on astringency sub-qualities and tastes perception across the 74 red wines under investigation. A two-way ANOVA (judges as random factor and wine variety as fixed factor; Tukey, $p<0.05$) was also computed to test the discrimination effect of olfactory descriptors across the 74 wine samples.

To test the impact of olfactory cues on the perception of the astringency sub-qualities in the 10 mono-varietal wines, other two-way ANOVAs (judges as random factor and wine as fixed factor; Tukey, $p<0.05$ and 0.1) were performed on the intensity scores of astringency sub-qualities in WWs and corresponding DWs of each wine type.

Pearson correlation analyses ($p< 0.05$) were applied to the whole set of wines (sample size: 74) for the computation of correlations between specific odour descriptors and in-mouth sensory variables, and between these latter for WWs or DWs and chemical parameters.

Performance of the trained judges was tested by a three-way ANOVA (Tukey, $p<0.05$) with three interactions: assessor*session, assessor*sample, sample*session (Vidal et al., 2016).

Data was processed with XLStat (version 2019.6), an add-in software package for Microsoft EXCEL (Addinsoft, Paris, France).

3. Results and Discussion

3.1. Olfactory/in-Mouth Cross-Modal Interactions

The main aim of this study was to investigate the impact of olfactory cues on tastes and astringency sub-qualities during red wine tasting. To account for the wide sensory diversity that different red wines can show, the experiments were carried out on 74 wines selected among the 111 Italian red wines (11 grape varieties), whose astringency has been recently studied (Piombino et al., 2020).

As a first step, we tested the sensory diversity of the 74 wines produced with 10 grape varieties.

As a result, the discrimination effect of oral and olfactory descriptors among the 74 wines was tested by ANOVA and results are reported in Tables 5 and 6, respectively.

Table 5. Three-way ANOVA (judges as random factor, grape variety, and perception modality as fixed factors) computed to test the discrimination effect of in-mouth descriptors and to evaluate the impact of the perception modality (with and without odours, WWs and DWs, respectively) on oral sensory perception of the 74 red wines investigated.

Oral descriptor	Model		Grape variety		Perception modality		Perception modality* Grape variety	
	F	*p*	F	*p*	F	*p*	F	*p*
Drying	15.488	**<0.0001**	14.557	**<0.0001**	0.191	0.662	1.438	0.157
Harsh	10.697	**<0.0001**	11.253	**<0.0001**	6.534	**0.011**	0.575	0.836
Unripe	11.541	**<0.0001**	6.744	**<0.0001**	11.293	**0.001**	2.046	**0.026**
Dynamic	10.241	**<0.0001**	16.396	**<0.0001**	11.001	**0.001**	1.976	**0.032**
Particulate/powder	5.858	**<0.0001**	1.064	0.387	2.567	0.109	0.891	0.541
Complex	12.593	**<0.0001**	3.658	**<0.0001**	54.233	**<0.0001**	1.368	0.189
Surface smoothness/velvet	7.881	**<0.0001**	10.517	**<0.0001**	4.313	**0.038**	0.807	0.622
Sweet	6.277	**<0.0001**	5.112	**<0.0001**	8.710	**0.003**	0.397	0.948
Sour	6.913	**<0.0001**	16.876	**<0.0001**	0.002	0.963	0.911	0.522
Bitter	7.915	**<0.0001**	10.126	**<0.0001**	13.342	**0**	1.149	0.321

In bold significant differences (Tukey, $p < 0.05$).

Table 6. Two-way ANOVA (judges as random factor and grape variety as fixed factor) computed to test the discrimination effect of olfactory descriptors among the 74 red wines investigated.

Olfactory descriptor	Model		Grape variety	
	F	*p*	F	*p*
Fruity	11.779	**<0.0001**	2.663	**0.003**
Dehydrated fruits	5.621	**<0.0001**	3.674	**<0.0001**
Dried fruits (nuts)	2.836	**<0.0001**	1.824	0.052
Floral	13.841	**<0.0001**	3.787	**<0.0001**
Vegetal	4.757	**<0.0001**	6.862	**<0.0001**
Spicy	6.549	**<0.0001**	2.478	**0.006**
Toasted	4.975	**<0.0001**	2.450	**0.007**
Woody	6.406	**<0.0001**	1.166	0.31
Earthy	1.903	0.006	1.679	0.081
Alcoholic	2.680	**<0.0001**	1.883	**0.044**
Off-odours	5.766	**<0.0001**	4.508	**<0.0001**

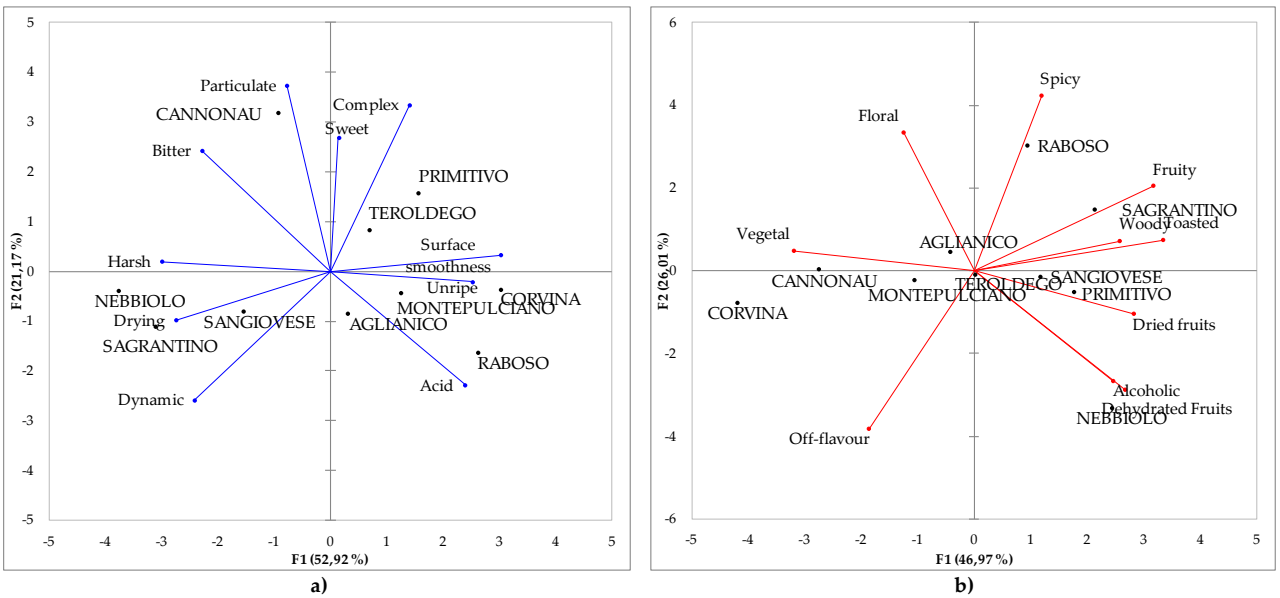
In bold significant differences (Tukey, $p < 0.05$).

The sensory features of the 10 single-varietal wines are shown in two separated PCAs computed on the mean intensities of oral (astringency and taste) characteristics (Figure 6a) and olfactory attributes (Figure 6b), respectively. The first biplot (Figure 6a) accounts for more than 74% of the variance, while the second (Figure 6b) for around 73%. The charts show the sensory diversity of the 10 wine types.

In Figure 6a, Corvina and Raboso show the largest squared cosines to positive values of F1, where the variables surface smoothness, unripe and sour taste are well projected. Montepulciano and Aglianico occupy the same area but show lower squared cosines. On the opposite side of F1, Nebbiolo, Sagrantino and Sangiovese are all well correlated to harsh, drying, and dynamic astringency. Particulate, complex, and sweet sensations are well represented on positive F2 and correlated with Cannonau, while Primitivo and Teroldego are mostly correlated with complex and smooth astringency.

Figure 6b shows that the sample set was representative of wines with different olfactory characteristics. F1 represents the contrast between wines with dominant vegetal odours, mainly Corvina and Cannonau, and those presenting different notes: fruity, toasted, and woody odours such as Sagrantino; dried fruits like Primitivo; dehydrated fruits and alcoholic notes like Nebbiolo. On F2, opposite to off-flavours, there are wines with spicy and floral odours, such as Raboso and Aglianico, respectively. This latter wine along with Montepulciano, Sangiovese and Teroldego, has low squared cosines suggesting a lower and/or more balanced contribution of different odours.

Figure 6. Principal component analysis (PCA) plots carried out on the correlation matrices (Pearson, $p < 0.05$) of the mean intensities over the 10 single-varietal wines rated by the 14 judges for significant: (a) oral (astringency and tastes) characteristics and (b) olfactory attributes.



Except for particulate/powder astringency, all the other nine in-mouth descriptors showed significant effects for the fixed factor grape variety (Table 5). Eight out of 11 olfactory descriptors resulted significantly different depending on the grape variety (Table 6): dried fruits (nuts) and woody were not significant and so it was the earthy descriptor, which was not considered for further analyses – including the PCA reported in Figure 6b – due to the lack of significance of its model. These first results confirm an inter-varietal sensory diversity of the 10 monovarietal wines. This diversity represents the assumption for the investigation of cross-modal sensory interactions between olfactory cues and in-mouth sensations during red wine tasting.

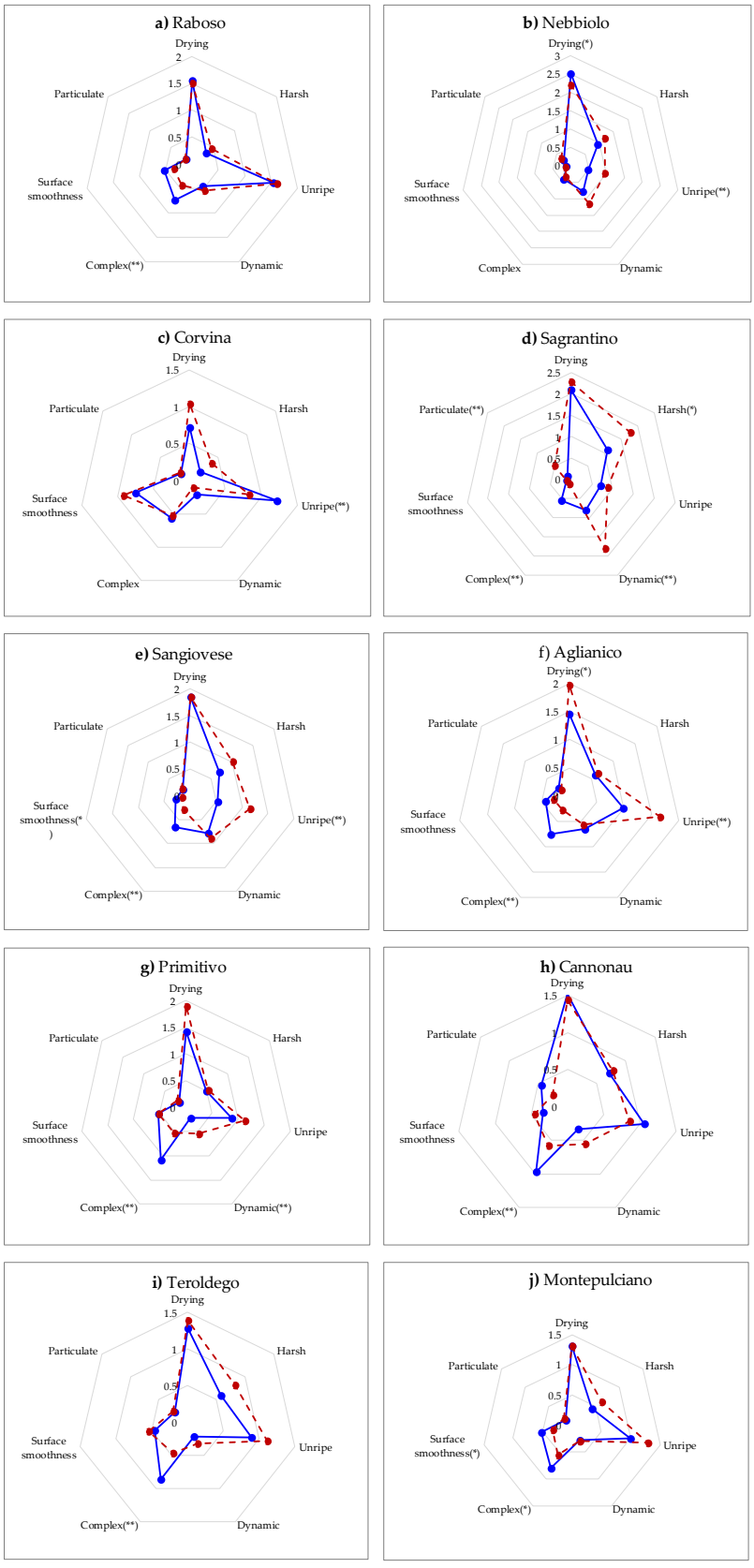
The spider-plots in Figure 7 illustrate how the astringency sensory profile of each of the 10 single-varietal wines changed after deodorization. We worked under the assumption of representative deodorized samples not only because our procedure has been developed from previous ones (Rodríguez-Bencomo et al., 2011; Lytra et al., 2012). We also considered that rotary evaporation at low temperature (30°C) represents a method largely applied during the preparative steps for polyphenols analysis in several food matrices (including wine) by several methods (Stalikas, 2007). Based on the actual literature (Luo et al., 2010; García Martín & Sun, 2013; Liu et al., 2016; Zhang et al., 2016; Bonaldo et al., 2020; Celotti et al., 2020), possible chemical, physical chemical, and rheological changes of polyphenols due to ultrasound and/or evaporation treatments are not favoured under the working conditions applied and, moreover, there is no clear evidence of their significant

and/or irreversible impact on the sensory characteristics of the wine. As an example, the reported maximum effects of ultrasound treatment on the chromatic characteristics of wine is $\Delta E^*=2.8$ (Zhang et al., 2016). Considering that the theoretical limit of perception reported for the human eye is $\Delta E^*\geq 3$ (García Martín & Sun, 2013), this means that differences cannot be detected from a sensory point of view. This is coherent with our results from triangle test showing that the colour of WWs and corresponding DWs were not perceived as different. Moreover, a recent study on the application of ultrasounds to accelerate the autolytic process in wine yeast (Liu et al., 2016), did not detect any effect on sensory parameters, including colour intensity, tonality, body, astringency, acidity, global quality, and bitterness.

The ANOVA highlights several significant differences ($p<0.05$, $p<0.1$) in mean intensities of perceived sub-qualities assessed in WWs and corresponding DWs. At least one significant variation resulted for each wine type. Sagrantino's astringency was impacted the most after deodorization, with four astringency sub-qualities (harsh, dynamic, complex, and particulate) whose mean intensity significantly varied in the absence of olfactory cues. Three significant variations were detected for both Sangiovese (unripe, complex, and surface smoothness) and Aglianico (unripe, complex, and drying) and two for Nebbiolo (unripe and drying) and Primitivo (dynamic and complex). The astringency of the remaining wines was less affected by the absence of VOCs, where significant variations were detected only for one sub-quality, namely complex for Raboso, Cannonau and Teroldego, and unripe for Corvina.

Two sub-qualities were the most frequently impacted by the deodorization: complex was perceived as significantly less intense in 8 out of 10 wine types (Raboso, Sangrantino, Sangiovese, Aglianico, Primitivo, Cannonau, Teroldego and Montepulciano) and unripe in four (Nebbiolo, Corvina, Sangiovese and Aglianico). This is not surprising because both these astringency sub-qualities correspond to sensations including not only oral but also retronasal olfactory perceptions. Indeed, based on the original definitions (Gawel et al., 2000), our jury developed and used consensual definitions as previously reported: complex was intended as a balanced in-mouth sensation of smooth astringency, acidity and retronasal stimulation; unripe corresponded to an unbalanced in-mouth sensation of astringency, acidity, and green aroma.

Figure 7. Spider-plots illustrating the impact of the deodorization on astringency sub-quality profile of each of the 10 single-varietal wines: (a) Raboso, (b) Nebbiolo, (c) Corvina, (d) Sagrantino, (e) Sangiovese, (f) Aglianico, (g) Primitivo, (h) Cannonau, (i) Teroldego and (j) Montepulciano. Significant differences assessed in WWs (blue line) and corresponding DWs (broken red line) are marked with asterisks (*p<0.1, **p<0.05).



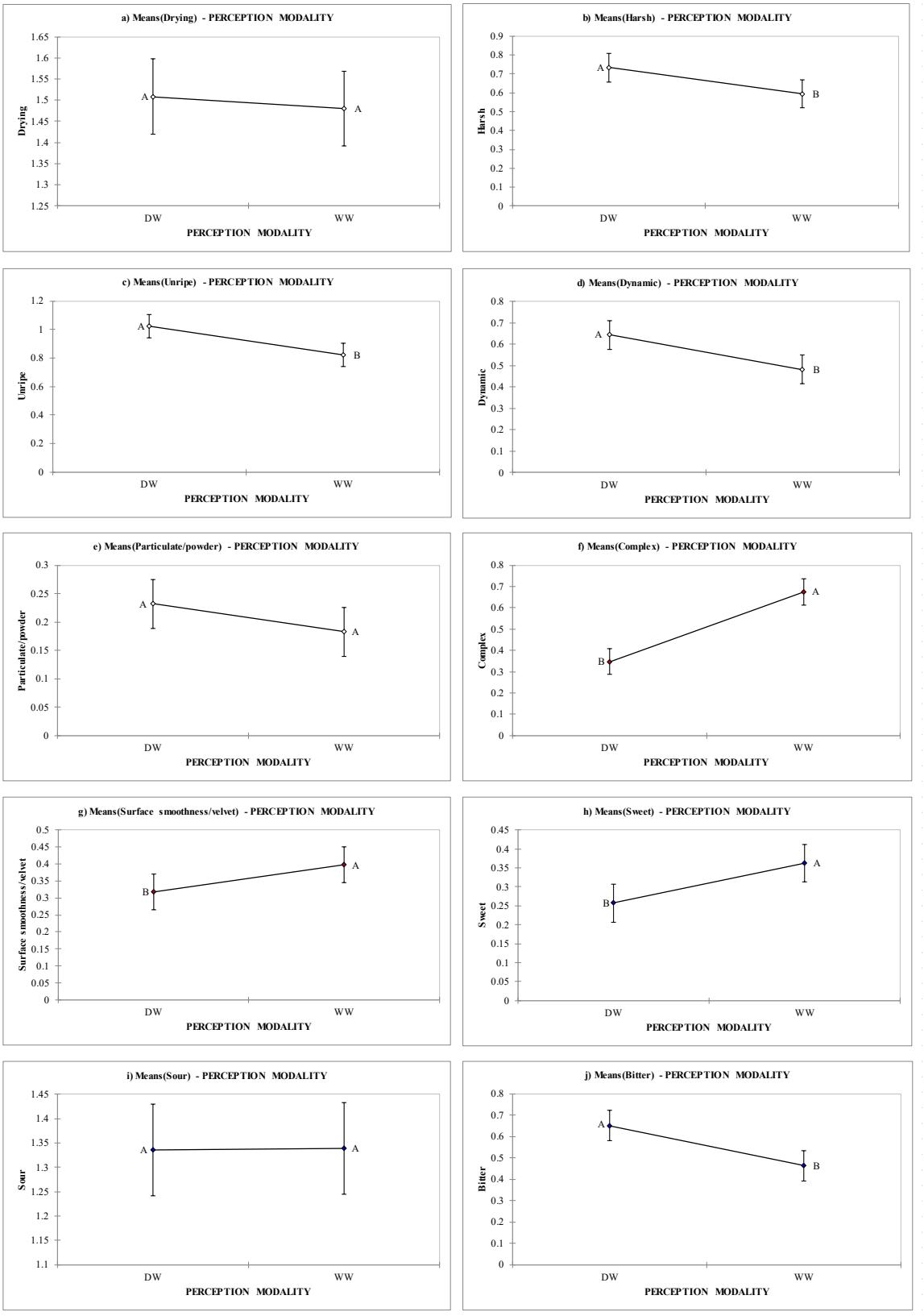
The direction of the variation was always the same for all sub-qualities across all monovarietal wines, except for two terms: drying that slightly varied (p<0.1) in Nebbiolo and Aglianico but in opposite direction; and unripe that increased (p<0.05) for deodorized Nebbiolo, Sangiovese and Aglianico, while decreased (p<0.05) for deodorized Corvina. This result could be linked to the strong vegetal odours detected in these wines (Figure 6b), in line with previously reported high concentration of VOCs, such as cyclic terpenes and hexanols, characteristic of Corvina wines and responsible for its vegetal/herbaceous/balsamic character (Slaghenaufi & Ugliano, 2018; Paronetto et al., 2011).

A recent study (Sáenz-Navajas et al., 2018), aimed to identify chemical compounds driving green character in red wines, concluded that it is a multivariate character associated to both aroma and mouthfeel descriptors such as vegetal, astringency, green and dry tannins. Based on this knowledge, our hypothesis is that the strong vegetal odours of Corvina can enhance the perception of unripe astringency. This synergic/additive effect could be the reason why, unlike Nebbiolo, Sangiovese and Aglianico (Figure 7b,e,f) that were not characterized by vegetal odours (Figure 6b), in Corvina the unripe astringency was perceived more intense in WWs (Figure 7c). This hypothesis seems to be supported by a similar trend detected in Cannonau (Figure 7h) which, like Corvina, was strongly characterized by vegetal odours (Figure 7b).

To get a more general result, an ANOVA (p<0.05) was applied across the whole set of 74 wines belonging to the 10 different grape varieties, to evaluate the impact of the perception modality (presence or absence of VOCs) and of the interaction “perception modality*grape variety” on in-mouth sensations assessed in WWs and DWs.

Results reported in Table 5 show that the perception of 7 (harsh, unripe, dynamic, complex, surface smoothness, sweet and bitter) out of 10 in-mouth sensations was significantly affected by odours. Complex astringency is the most impacted by olfactory cues (p<0.0001) while both the unripe and dynamic sub-qualities were significantly affected by the interaction “perception modality*grape variety”. The variation of mean intensities (over 74 wines) of each astringency sub-quality and taste sensation during DWs tasting compared to corresponding WWs is represented in Figure 8.

Figure 8. Variation of mean intensities (over 74 wines) of each astringency sub-quality and taste sensation during DWs tasting compared to corresponding WW: a) Drying, b) Harsh, c) Unripe, d) Dynamic, e) Particulate/powder, f) Complex, g) Surface smoothness/Velvet, h) Sweet, i) Sour and j) Bitter. Significant differences are marked with different letters (p<0.05). Whole Wines (WWs), Deodorized Wines (DWs).



Except for drying and particulate (Figure 8a,e), the other astringency sub-qualities (harsh, unripe, dynamic; Figure 8b–d) were perceived stronger in DWs. This suggests that olfactory perception can smooth these mouthfeel sensations previously described as "strong astringency sensations" (Vidal et al., 2017; Piombino et al., 2020). On the contrary, complex and surface smoothness/velvet (Figure 8f,g) decreased in DWs, suggesting that olfactory cues can enhance smoother aspects of astringency. The lack of impact on particulate astringency could be because wines were not discriminable according to this astringency feature. The perception of drying astringency that, based on results from consumer studies (Vidal et al., 2015), is assumed to be the basic astringent sensation because the most easily associated to the global term astringency, was not significantly impacted by olfactory cues. A similar result has been already reported (Sáenz-Navajas et al., 2020).

Moving to taste sensations (Figure 8h–j), it can be observed that the perception of olfactory stimuli significantly impacted bitterness and sweetness. Bitterness increased in the absence of VOCs, in accordance with previous data (Sáenz-Navajas et al., 2020), whereas perceived sweetness decreased. Those results seem to confirm the ones of an earlier study (Sáenz-Navajas et al., 2012), in which wine sweetness and bitterness perceptions were found to be significantly impacted by aromas. Moreover, previous findings on the effect of aromas on cider tastes showed that, overall, aromas significantly modulated sweetness perception for ciders with a sugar content of around 35–40 g/L (Symoneaux et al., 2015). Although the residual sugar content of our samples was 1 to 20 g/L (Table 7), our results are in line with the mentioned work. Sourness sensation did not show significant differences between WWs and DWs, meaning that the perception of olfactory stimuli did not influence this taste attribute.

According to Figure 6b, the large set of wines showed a wide array of sensory characteristics matching the large range of basic compositional data reported in Table 7.

Table 7. Oenological parameters determined in the 74 single cultivar Italian red wines.

Parameter	Mean	Minimum	Maximum
Ethanol (% v/v)	13.89	11.42	16.62
Reducing sugars (g/L)	2.64	1.1	20.1
Titratable acidity (g tartaric acid/L)	5.75	3.99	9.99
pH	3.55	3.07	4.1
Total phenols (Folin-Ciocalteu) (mg (+)-catechin/L)	2354.46	703.59	5448.55
Proanthocyanidins (mg cyanidin chloride/L)	3364.8	627.75	6312.37

Thanks to this diversity, we tried to go deeper into our investigation on cross-modal interactions in red wine tasting, by performing a Pearson correlation analysis to statistically test the relationships between specific olfactory notes and single astringency sub-qualities and tastes. Results report a total of 21 significant (p<0.05) correlations, 17 significant correlations between odours and astringency sub-qualities and 4 between odours and tastes. However, in most cases the computed r value is very low and likely linked to a casual effect. For each astringency sub-quality, one to four significant correlations to olfactory descriptors were found. Fruity was slightly correlated with the complex astringency (r=0.308). In a previous study (Sáenz-Navajas et al., 2010a), it was observed that the addition of a fruity aroma extract coming from a Chardonnay white wine caused a significant decrease in the perception of the global astringency in different red wine matrices. Lately, this output was not confirmed (Sáenz-Navajas et al., 2020). The descriptor dehydrated fruit positively correlated with drying (r=0.459; p<0.0001) and harsh astringency (r=0.286), while negatively correlated with surface smoothness (r= −0.341). This could suggest that these three sub-qualities are parts of one unique sensation, where smoothness complements strong sensations such as drying and harsh. A similar consideration was recently reported for silky and dry mouthfeel descriptors (Sáenz-Navajas et al., 2020). Dried fruit was the only odour descriptor never correlated with in-mouth sensory variables. Floral aromas showed very weak relationships: positive with the complex sensation (r=0.275) and negative with harsh (r= −0.284). Vegetal odours were the only ones related to four sub-qualities. The correlation with unripe (r=0.385; p<0.0001) and surface smoothness (r=0.237) astringency was positive while the correlation with drying (r = −0.340) and dynamic (r= −0.291) was negative. Spicy only correlated with complex (r=0.462; p<0.0001) but it had the largest coefficient both within the whole dataset and, compared to the other odours correlated to this sub-quality: fruity and floral positively and off-flavour (r= −0.30) negatively.

These relationships are based only on a statistical approach and as already stated, the low r values, suggest a casual effect. However, the three largest and significant correlations (p<0.0001) that were found – spicy and complex, dehydrated fruits and drying, vegetal and unripe – seems to be confirmed from a cognitive point of view. Indeed, according to Figure 6b, Raboso were the spiciest wines and after deodorization their astringency was perceived as significantly (p<0.05) less complex (Figure 7a), confirming the significant and positive correlation previously reported (r=0.462). Nebbiolo was characterized by dehydrated fruits odours (Figure 6b)

and the average astringency of deodorized Nebbiolo was perceived as less drying (p<0.1), in line with the computed positive correlation (r=0.459). Finally, in accordance with the positive significant correlation (r=0.385) between vegetal odours and unripe astringency, in Corvina wines, which were strongly characterized by vegetal notes (Figure 6b), the unripe astringency was perceived significantly (p<0.05) less intense in DWs compared to WWs (Figure 7c). A similar finding (even if not significant) was observed for Cannonau, which was the only other monovarietal wine associated with vegetal odours (Figure 6b). The green character has been negatively correlated to consumers' preference of red wines, resulting in intensified vegetal notes and masked by woody aromas (Sáenz-Navajas et al., 2018). Our results support both these conclusions: woody odours were significantly (p<0.05) correlated with unripe astringency, even if with a small negative correlation coefficient (r= −0.257). Moreover, alcoholic notes were negatively correlated (r= −0.340) with unripe astringency. These results are interesting and need to be verified by further experiments.

Few significant correlations were detected between olfactory characteristics and taste sensations and, also in this case, the r values were very low. Sweet taste did not correlate to any odour, while sourness was positively correlated to floral, and bitterness showed a low negative correlation with floral and a positive correlation with the dehydrated fruits and off-flavour. This latter descriptor was intended as inclusive of different kinds of wine off-odours (phenolic, sulphurous, cork taint, maderised/oxidised); however, the most cited off-odour was the phenolic/stable/animal taint (data not shown). For this reason, the positive correlation highlighted between bitterness and off-flavour seems to support previous results (de-la-Fuente-Blanco et al., 2017), according to which bitterness was enhanced by animal aromas.

Overall, our findings suggest that during red wine tasting, odour–oral cross-modal interactions could modulate the perception of specific astringency sub-qualities and tastes. Specific olfactory characteristics such as spicy, dehydrated fruits and vegetal odours, could drive this modulation effect for complex, drying, and unripe sub-qualities, and this should be further explored by specific experiments.

3.2. Olfactory Cues and Correlations between Sensory and Chemical Variables

In red wine astringency research, one of the biggest challenges is to find analytical methods able to predict the perceived astringency. Several studies investigated the correlation between astringency as a sensory parameter

and measurements essentially based on compositional/metabolomic (Hufnagel & Hofmann, 2008), spectrophotometric (e.g., 280 and 230 nm) (Boulet et al., 2016), and precipitation techniques (Ferrer-Gallego et al., 2012). Thanks to these studies and to those investigating how other wine components (e.g., ethanol, pH, etc.) can influence astringency perception, our knowledge about this sensory stimulus has greatly expanded. However, most of these studies tested the correlation between chemicals and the overall astringency but did not pay attention to the different sub-qualities of this attribute. According to our recent results (Piombino et al., 2020), and a few further studies addressing this subject (Vidal et al., 2018; Sáenz-Navajas et al., 2020), the current analytical methods are not able to predict astringency in all its sensory nuances, and their predictive power varies depending on the parameter/method applied.

We argue that odour–oral cross-modal interactions can affect the correlations between chemical and sensory parameters, thus interfering with the estimation of their predictive power. To test this hypothesis, we computed Pearson correlations between sensory (odour descriptors, astringency sub-qualities, and tastes) and chemical compositional parameters (total phenols, total proanthocyanidins, ethanol, reducing sugars, pH, titratable acidity, volatile acidity) across the 74 whole wines (WWs) and the corresponding deodorized wines (DWs). In this way, we were able to compare the correlations under two different tasting conditions: in the presence and in the absence of VOCs. This comparison is reported in Tables 8 and 9, where several significant correlations ($p<0.05$, $p<0.0001$) were found. In most cases, the number of significant correlations and the magnitude of the correlation coefficients were higher for DWs than for WWs. In only a few cases, the magnitude of the correlation coefficients decreased, the direction of the sign switched, or the relationship got or lost its statistical significance. Among correlations between sub-qualities (Table 8), the unripe astringency was the only one showing a lower number of significant correlations and lower correlation coefficients in DWs compared to WWs. In the absence of olfactory cues, the unripe astringency is slightly negatively correlated with harsh and complex, while in the presence of odours, a weak negative relationship was detected also for drying, dynamic and particulate. The unripe mouthfeel was also significantly related to tastes. A good positive correlation with sourness was confirmed in the absence of VOCs, which seems coherent with the significant correlation with pH and titratable acidity. Moreover, in the absence of odours, the weak correlation with total proanthocyanidins and ethanol was lost and, among the considered sub-qualities, unripe became the only one not correlated with

chemical parameters linked to polyphenols. These results support the idea that the unripe astringency is a multisensory feeling greatly impacted by VOCs perceptions.

As for unripe, also the complex sub-quality is defined as a mouthfeel including aroma sensations. However, unlike unripe, the magnitude of correlation coefficients with other sub-qualities increases in the absence of VOCs and the correlation with total phenols and proanthocyanidins became significant even if with low r values. Another result from this comparison refers to the particulate sub-quality. A higher number of significant correlations (from 3 to 8) with other sub-qualities, tastes and polyphenol parameters emerged in the absence of VOCs. This could suggest that odours can have a role in modulating the perception of this sensation; however, only low r values were computed.

Particulate and dynamic were never correlated to tastes in WWs, while in DWs they were both significantly correlated to bitter and, dynamic was also negatively correlated to sour. Unlike for WWs, for DWs all the seven astringency sub-qualities were significantly correlated to the bitter taste, with the largest correlation coefficients (WWs=0.754; DWs=0.785) confirmed between bitterness and harsh astringency.

Moving onto the correlations between chemical and sensory parameters, the magnitude of the correlation coefficients increased with wine deodorization. On the one hand, the absence of VOCs led to greater positive correlations between drying, harsh, dynamic sub-qualities and total polyphenols, total proanthocyanidins, ethanol and volatile acidity. On the other hand, the negative correlations of complex and surface smoothness with total phenols and proanthocyanidins were stronger for DWs. As an example, for DWs, total proanthocyanidins showed the greatest positive correlation coefficients with drying and dynamic, which increased from 0.571 to 0.703, and from 0.304 to 0.737, respectively, when compared to WWs. The correlations between volatile acidity and drying, harsh and dynamic became significant for DWs but not for WWs. All these results confirm previous findings on correlations between sensory and chemical parameters (Vidal et al., 2018; Piombino et al., 2020; Sáenz-Navajas et al., 2020) and show the impact of cross-modal oral/olfactory sensory interactions on red wine perception.

Table 8. Correlation coefficients (Pearson) between astringency and chemical variables. Comparison between WW and DW.

Variables	Drying		Harsh		Unripe		Dynamic		Complex		Surface smoothness		Particulate	
	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
Drying	**1**	**1**	**0.391**	**0.440**	**-0.245**	0.001	**0.623**	**0.642**	**-0.278**	**-0.470**	**-0.651**	**-0.629**	0.060	**0.290**
Harsh	**0.391**	**0.440**	**1**	**1**	**-0.350**	**-0.230**	**0.285**	**0.590**	**-0.261**	**-0.344**	**-0.341**	**-0.406**	**0.253**	**0.260**
Unripe	**-0.245**	0.001	**-0.350**	**-0.230**	**1**	**1**	**-0.289**	-0.130	0.001	**-0.232**	0.044	-0.204	**-0.259**	-0.073
Dynamic	**0.623**	**0.642**	**0.285**	**0.590**	**-0.289**	-0.130	**1**	**1**	**-0.372**	**-0.501**	**-0.473**	**-0.465**	0.140	**0.238**
Complex	**-0.278**	**-0.470**	**-0.261**	**-0.344**	0.001	**-0.232**	**-0.372**	**-0.501**	**1**	**1**	**0.268**	**0.590**	-0.182	**-0.232**
Surface smoothness	**-0.651**	**-0.629**	**-0.341**	**-0.406**	0.044	-0.204	**-0.473**	**-0.465**	**0.268**	**0.590**	**1**	**1**	-0.155	**-0.376**
Particulate	0.060	**0.290**	**0.253**	**0.260**	**-0.259**	-0.073	0.140	**0.238**	-0.182	**-0.232**	-0.155	**-0.376**	**1**	**1**
Sweet	-0.056	-0.137	-0.077	-0.181	**-0.313**	**-0.353**	-0.114	-0.013	**0.355**	**0.252**	**0.289**	**0.293**	0.110	0.071
Sour	-0.197	-0.137	**-0.597**	**-0.526**	**0.538**	**0.597**	-0.043	**-0.284**	-0.095	-0.001	-0.009	-0.005	-0.173	-0.195
Bitter	**0.306**	**0.366**	**0.754**	**0.785**	**-0.237**	**-0.295**	0.130	**0.451**	-0.197	**-0.259**	-0.187	**-0.288**	0.080	**0.304**
Total phenols (Folin-Ciocalteau) [mg/L]	**0.469**	**0.622**	**0.284**	**0.506**	-0.189	0.166	**0.240**	**0.599**	-0.170	**-0.375**	**-0.292**	**-0.414**	**0.238**	**0.318**
Total proanthocyanidins [mg/L]	**0.561**	**0.703**	**0.297**	**0.577**	**-0.279**	0.110	**0.304**	**0.737**	-0.207	**-0.427**	**-0.304**	**-0.569**	0.163	**0.295**
Ethanol [% v/v]	**0.394**	**0.476**	**0.262**	**0.396**	**-0.264**	-0.137	0.094	**0.461**	0.016	-0.051	-0.178	-0.171	0.069	0.129
Reducing sugars [g/L]	-0.013	-0.014	-0.015	-0.165	0.059	0.055	-0.057	-0.017	0.206	0.125	0.043	0.196	0.109	-0.055
pH	-0.010	-0.010	**0.335**	**0.466**	**-0.274**	**-0.376**	-0.023	0.166	0.024	0.165	-0.071	0.106	0.134	0.055
TA [g tartaric acid/L]	0.084	0.163	**-0.248**	**-0.313**	**0.258**	**0.493**	0.080	-0.066	-0.041	-0.186	0.033	-0.197	-0.032	0.025
VA [g acetic acid/L]	0.193	**0.361**	0.201	**0.447**	-0.067	-0.158	0.215	**0.413**	-0.156	-0.198	-0.056	-0.165	0.051	0.086

In bold significant differences (Tukey, $p < 0.05$) (grey: $p < 0.0001$).

The correlations that were detected in WWs between tastes and all the other sensory and chemical parameters (Table 9) were confirmed and reinforced in DWs. The only correlation that was not significant in WWs and became slightly significant in DWs was the one between reducing sugars and sweetness (from 0.099 to 0.595). This suggests that the overall aroma might modulate the perception of sweetness in red wine, but further investigation is necessary. The significant positive correlation between pH and bitterness was stronger in DWs. Among all the mentioned significant correlations, only a few can be considered good correlations ($r > \pm 0.7$). According to these, we can conclude that: bitterness and harsh astringency perceptions are strongly related independently from odour in-mouth multi-modal interactions; total proanthocyanidins is the better predictive chemical parameter for both drying and dynamic astringency, but the estimation of its predictive power is strongly affected by olfactory–oral cross-modal interactions.

Table 9. Correlation coefficients (Pearson) between taste and chemical variables. Comparison between WW and DW.

Variables	Sweet		Sour		Bitter	
	WW	DW	WW	DW	WW	DW
Drying	-0.056	-0.137	-0.197	-0.137	**0.306**	**0.366**
Harsh	-0.077	-0.181	**-0.597**	**-0.526**	**0.754**	**0.785**
Unripe	**-0.313**	**-0.353**	**0.538**	**0.597**	**-0.237**	**-0.295**
Dynamic	-0.114	-0.013	-0.043	**-0.284**	0.13	**0.451**
Complex	**0.355**	**0.252**	-0.095	-0.001	-0.197	**-0.259**
Surface smoothness	**0.289**	**0.293**	-0.009	-0.005	-0.187	**-0.288**
Particulate	0.11	0.071	-0.173	-0.195	0.08	**0.304**
Sweet	**1**	**1**	**-0.277**	**-0.398**	**-0.243**	-0.131
Sour	**-0.277**	**-0.398**	**1**	**1**	**-0.668**	**-0.716**
Bitter	**-0.243**	-0.131	**-0.668**	**-0.716**	**1**	**1**
Total phenols (Folin-Ciocalteu) [mg/L]	-0.043	-0.118	-0.089	-0.179	0.168	**0.471**
Total proanthocyanidins [mg/L]	-0.067	-0.163	-0.102	-0.189	0.198	**0.498**
Ethanol [% v/v]	0.036	0.173	-0.21	**-0.331**	0.167	**0.327**
Reducing sugars [g/L]	0.099	**0.595**	-0.016	-0.079	0.019	-0.161
pH	-0.022	0.135	**-0.508**	**-0.656**	**0.371**	**0.529**
TA [g tartaric acid/L]	-0.058	-0.115	**0.459**	**0.621**	**-0.276**	**-0.424**
VA [g acetic acid/L]	-0.089	0.032	0	**-0.359**	0.145	**0.435**

In bold significant differences (Tukey, $p < 0.05$) (grey: $p < 0.0001$).

To the best of our knowledge, this is the first time that this kind of comparison has been done. In our opinion, this approach, if applied to a wider variety of chemical parameters, could be helpful to research aimed at

understanding which compounds and structures are related to different mouthfeel sensations. Results confirm the importance of cross-modal interactions on red wine perception and can help to optimize the current predictive analytical parameters/methods. Even if wine deodorization is time consuming, it offered interesting results and its further comparison with other approaches (e.g., nose clips) could represent an interesting future perspective. Only a few and recent reports focus on the impact of the odour stimuli on the perception of single sub-qualities rather than overall astringency, and no experiment was ever carried out on very diverse Italian red wines (Parpinello et al., 2019; Arapitsas et al., 2020; Piombino et al., 2020).

References

Arapitsas, P.; Ugliano, M.; Marangon, M.; Piombino, P.; Rolle, L.; Gerbi, V.; Versari, A.; Mattivi, F. Use of untargeted Liquid Chromatography–Mass Spectrometry metabolome to discriminate Italian monovarietal red wines, produced in their different terroirs. *J. Agric. Food Chem.* **2020**, 68, 13353−13366.

Bonaldo, F.; Guella, G.; Mattivi, F; Catorci, D.; Arapitsas, P. Kinetic investigations of sulfite addition to flavanols. *Sci. Rep.* **2020**, 10, 12792.

Boulet, J.C.; Trarieux, C.; Souquet, J.M.; Ducasse, M.A.; Caillé, S.; Samson, A.; Williams, P.; Doco, T.; Cheynier, V. Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chem.* **2016**, 190, 357–363.

Celotti, E.; Stante, S.; Ferraretto, P.; Román, T.; Nicolini, G.; Natolino, A. High Power Ultrasound Treatments of Red Young Wines: Effect on Anthocyanins and Phenolic Stability Indices. *Foods*, **2020**, 9, 1344.

Charters, S.; Pettigrew, S. The dimensions of wine quality. *Food Qual. Pref.* **2007**, 18, 997-1007.

Chironi, S.; Ingrassia, M. Wine label design as a strategic tool to attract consumers: A marketing study on Sicilian wine positioning work. *Rivista di Economia Agraria*, **2013**, 1, 7–21.

de-la-Fuente-Blanco, A.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V.; Sáenz-Navajas, M.P. Cross-modal interactions and effects of the level of expertise on the perception of bitterness and astringency of red wine. *Food Qual. Pref.* **2017**, 62, 155-161.

de-la-Fuente-Blanco, A.; Sáenz-Navajas, M.P.; Ferreira V. Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions. *Aust. J. Grape Wine R.* **2016**, 23, 162-169.

Di Stefano, R.; Cravero, M.C.; Gentilini, N. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, **1989**, 25, 83–89.

Ferrer-Gallego, R.; Rui, G.; Rivas-Gonzalo, J.C; Escribano-Bailóna, M.T. Interaction of phenolic compounds with bovine serum albumin (BSA) and α-amylase and their relationship to astringency perception. *Food Chem.* **2012**, 135, 651-658.

García Martín J.F.; Sun D.W. Ultrasound and electric fields as novel techniques for assisting the wine ageing process: The state-of-the-art research. *Trends Food Sci. Tech.* **2013**, 33, 40–53.

Gawel, R.; Oberholster, A.; Francis, I.L. A "Mouth-feel Wheel": Terminology for communicating the mouth feel characteristics of red wine. *Austr J. Grape Wine R.* **2000**, 6, 203-207.

Genovese, A.; Dimaggio, R.; Lisanti, M.T.; Piombino, P.; Moio, L. Aroma composition of red wines by different extraction methods and gas chromatography SIM/mass spectrometry analysis. *Ann. Chim.* **2005**, 95, 383-394.

Hufnagel, J.C.; Hofmann, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008**, 56, 9190–9199.

ISO. (1997). 3951. Sensory analysis – Apparatus – Wine-tasting glass. International Organization for Standardization.

ISO. (2004). 4120. Sensory analysis – Methodology – Triangle test. International Organization for Standardization.

ISO. (2007). 8589. Sensory analysis – General guidance for the design of test rooms. International Organization for Standardization.

Liu, L.; Loira, I.; Morata, A.; Suárez-Lepe, J.A.; González, M.C.; Rauhut, D. Shortening the ageing on lees process in wines by using ultrasound and microwave treatments both combined with stirring and abrasion techniques. *Eur. Food Res. Technol.* **2016**, 242, 559–569.

Luo, H.; Schmid, F.; Grbin, P.R.; Jiranek, V. Viability of common wine spoilage organisms after exposure to high power ultrasonics. *Ultrason. Sonochem.* **2010**, 19, 415–420.

Lytra, G.; Tempere, S.; de Revel, G.; Barbe, J.C. Impact of Perceptive Interactions on Red Wine Fruity Aroma. *J. Agric. Food Chem.* **2012**, 60, 12260–12269.

Muñoz-González, C.; Feron, G.; Guichard, E.; Rodríguez-Bencomo, J.J.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.A. Understanding the Role of Saliva in Odour Release from Wine by Using Static and Dynamic Headspace Conditions. *J. Agric. Food Chem.* **2014**, 62, 8274-8288.

Noble, A.C. Taste-aroma interactions. *Trends Food Sci. Tech.* **1996**, 7, 439–444.

Noble, A.C.; Arnold, R.A.; Buechsenstein, J.; Leach, E.J.; Schmidt, J.O.; Stern, P.M. Modification of a standardised system of wine aroma terminology *Am. J. Enol. Vitic.* **1987**, 38, 143-146.

Organisation Internationale de la Vigne et du Vin (2015). Compendium of international methods of must and wine analysis (Organisation Internationale de la Vigne et du Vin: Paris, France).

Paronetto, L.; Dellaglio, F. Amarone: a modern wine coming from an ancient production technology. *Adv. Food Nutr. Res.* **2011**, 63, 285–306.

Parpinello, G.P.; Ricci, A.; Arapitsas, P.; Curioni, A.; Moio, L.; Segade, S.R.; Ugliano, M. Versari, A. Multivariate characterisation of Italian monovarietal red wines using MIR spectroscopy. *OENO One*, **2019**, 4, 741-751.

Peynaud, E. (1987). The taste of wine: The art and science of wine appreciation. London: Macdonald Orbis.

Piombino, P.; Genovese, A.; Gambuti, A.; Lamorte, S.A.; Lisanti, M.T.; Moio, L. Effects of off-vine bunches shading and cryomaceration on free and glycosilated flavours of Malvasia delle Lipari wine. *Int. J. Food Sci. Tech.* **2010**, 45, 234–244.

Piombino, P.; Moio, L.; Genovese, A. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res. Int.* **2019**, 116, 548-558.

Piombino, P.; Pittari, E.; Gambuti, A.; Curioni, A.; Giacosa, S.; Mattivi, F.; Parpinello, G.P.; Rolle, L.; Ugliano, M.; Moio, L. Preliminary sensory characterisation of the diverse astringency of single cultivar Italian red wines and correlation of sub-qualities with chemical composition. *Aust. J. Grape Wine R.* **2020**, 26, 233-246. and references therein.

Prescott, J. Chemosensory learning and flavour: Perception, preference and intake. *Physiol. Behav.* **2012**, 107, 553–559.

Rodríguez-Bencomo, J.J.; Muñoz-González, C.; Andújar-Ortiz, I.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.A. Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. *J. Agric. Food Chem.* **2011**, 91, 2484–2494.

Sáenz-Navajas, M.P.; Arias, I.; Ferrero-del-Teso, S.; Fernández-Zurbano, P.; Escudero, A.; Ferreira, V. Chemo-sensory approach for the identification of chemical compounds driving green character in red wines. *Food Res. Int.* **2018**, 109, 138-148.

Sáenz-Navajas, M.P.; Campo, E.; Avizcuri, J.M.; Valentin, D.; Fernández-Zurbano, P.; Ferreira, V. Contribution of non-volatile and aroma fractions to in-mouth sensory properties of red wines: Wine reconstitution strategies and sensory sorting task. *Anal. Chim.* **2012**, 732, 64–72.

Sáenz-Navajas, M.P.; Campo, E.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chem.* **2010a**, 121, 1139-1149.

Sáenz -Navajas, M.P.; Campo, E.; Cullere, L.; Fernandez-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010b**, 58, 5574−5585.

Sáenz-Navajas, M.P.; Ferrero-del-Teso, S.; Jeffery, D.W.; Ferreira, V.; Fernández-Zurbano, P. Effect of aroma perception on taste and mouthfeel dimensions of red wines: Correlation of sensory and chemical measurements. *Food Res. Int.* **2020**, 131, 108945.

Sáenz-Navajas, M.P; Avizcuri, J.M; Echávarri, J.F; Ferreira, V.; Fernández-Zurbano, P.; Valentin, D. Understanding quality judgements of red wines by experts: Effect of evaluation condition. *Food Qual. Pref.* **2016**, 48, 216-227.

Singleton, V.L.; Orthofer, R; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method. Enzymol.* **1999**, 299, 152–178.

Slaghenaufi, D; Ugliano, M. Norisoprenoids, sesquiterpenes and terpenoids content of Valpolicella wines during aging: investigating aroma potential in relationship to evolution of tobacco and balsamic aroma in aged wine. *Front. Chem.* **2018**, 6, 66.

Small, D.M.; Prescott, J. Odor/taste integration and the perception of flavor. *Exp. Brain Res.* **2005**, 166, 345-357.

Stalikas, C.D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.* **2007**, 30, 3268-3295.

Symoneaux, R.; Guichard, H.; Le Quéré, J. M.; Baron, A.; Chollet, S. Could cider aroma modify cider mouthfeel properties? *Food Qual. Pref.* **2015**, 45, 11–17.

Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L. Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Anal. Chim. Acta*, **2010**, 660, 183–189.

Vecchio, R.; Lisanti, M.T.; Caracciolo, F.; Cembalo, L.; Gambuti, A.; Moio, L.; Siani, T.; Marotta, G.; Nazzaro, C.; Piombino, P. The role of production process and information on quality expectations and perceptions of sparkling wines. *J. Sci. Food Agric.* **2019**, 99, 124–135.

Vidal, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. How do consumers describe wine astringency? *Food Res. Int.* **2015**, 78, 321-326.

Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Sensory characterization of the astringency of commercial Uruguayan Tannat wines. *Food Res. Int.* **2017**, 102, 425–434.

Vidal, L.; Antúnez, L.; Giménez, A.; Medina, K.; Boido, E; Ares, G. Dynamic characterization of red wine astringency: case study with Uruguayan Tannat wines. *Food Res. Int.* **2016**, 82, 128–135.

Vidal, L.; Antúnez, L.; Rodríguez-Haralambides, A.; Giménez, A.; Medina, K.; Boido, E.; Ares, G. Relationship between astringency and phenolic composition of commercial Uruguayan Tannat wines: Application of boosted regression trees. *Food Res. Int.* **2018**, 112, 25–37.

Zhang, Q.A.; Shen, Y.; Fan, X.H.; García-Martín, J.F. Preliminary study of the effect of ultrasound on physicochemical properties of red wine. *CyTA J. Food*, **2016**, 14, 55–64.

Chapter II – Impact of red wine phenolics on red wine aromas release and perception

The present chapter aims at studying and developing the second research question: studying how polyphenols could modulate the release and the perception of wine odour and contributing, through a physicochemical and sensory approach, to the knowledge of the role played by the whole wine non-volatile matrix on the release and perception of wine VOCs.

For this purpose, four different wine matrices, obtained by enriching a basic bleed (*saigner*) wine (S) - a real wine matrix very poor in polyphenols -with increasing quantities of a deodorized dry extract obtained from the same pressed wine very reach in polyphenols, were analysed. In this way we obtained four matrices with identical chemical composition of the volatile fraction and increasing concentrations of polyphenols, by using only endogenous compounds for the matrix enrichment. For this purpose, we treated the pressed wine to the deodorization procedure previously described, thus obtaining the corresponding dry extract to progressively add for the matrix enrichment. This allowed us to perform the analyses in conditions that were as representative as possible of the real ones, both in terms of the compositional characteristics of the wine matrices, and in terms of isolation conditions of the volatile fraction with respect to the tasting conditions. Indeed, VOCs release was measured directly from the wine glass, miming the real tasting conditions.

With a physicochemical approach, the VOCs release in the dynamic headspace of the four obtained wine matrices were isolated by a SPME preconcentration. A correlation study between data on volatiles release and that on the chemical composition of the fixed fraction of the different matrices, gave information on the impact of the various constituents of the red wine matrix on the release of VOCs. The results obtained were also correlated with the descriptive data of the olfactory characteristics of the four matrices, obtained by sensory analysis. This made it possible to evaluate the sensory impact of the differences in aromas release in the four matrices.

1. Introduction

According to all that reported in the above sections, it is now common knowledge that identification and quantification of wine VOCs does not allow to fully understand wine flavour and aromas perception, as the interactions between volatile and non-volatile wine matrices influence chemical and sensory properties of wines (Pozo-Bayón & Reineccius, 2009; Sáenz-Navajas et al., 2010; Villamor & Ross, 2013; Paravisini & Guichard, 2017).

The nature of these interactions may differ according to the physicochemical properties of the aroma compound (e.g., molecular size, functional group, solubility, and volatility) and the binding that may occur among the wine components via chemical binding of covalent, hydrophobic, or hydrogen bonds, or via formation of inclusion complexes (Solms et al., 1973; Voilley et al., 1991; Villamor & Ross, 2013).

Besides polyphenols-aroma compounds interactions and PPhss influence on VOCs release and perception that have been extensively reviewed above (Background Section), VOCs partitioning is strongly influenced by other wine matrix characteristics, such as ethanol, and residual sugars content (Goldner et al., 2009; Robinson et al., 2009; Paravisini & Guichard, 2017, and reference therein; Piombino et al., 2019).

Ethanol influence on VOCs release and perception of wine and other alcoholic beverages has been widely studied and recently reviewed (Ickes & Cadwllader, 2017). Data present in literature suggest that ethanol changes wine polarity, modifying VOCs distribution between gas and liquid phases according to their physicochemical characteristics (i.e., logP value), and modulating the overall aroma perception in wines. The effect of increasing amounts of ethanol in decreasing the volatility of numerous important wine VOCs, has been very well documented (Ickes & Cadwllader, 2017 and references therein). For example, some authors have observed that increasing ethanol levels in wine were linearly and negatively correlated with the volatility of several VOCs, mostly esters (i.e., ethyl 2 and 3-methyl butyrate, isoamyl acetate, ethyl hexanoate, octanoate and decanoate), but also terpenes (i.e., linalool, nerol, limonene), ketones (i.e., α and β-ionone, β-damascenone), methoxypyrazines (i.e., isobutyl and isopropyl pyrazine) and volatile phenols (i.e., eugenol, 4-ethylphenol, and 4-ethylguaiacol) (Voilley et al., 1991; Fischer et al., 1996; Hartmann et al., 2002; Robinson et al., 2009; Petrozziello et al., 2014). From a sensory perspective, increasing ethanol concentrations have been

negatively correlated to fruity (Guth 1998; Grosch, 2001; Escudero et al. 2007; Goldner et al., 2009; Villamor et al., 2013a), floral (Grosch, 2001) and herbaceous aromas (Goldner et al., 2009).

Regarding the effects of residual sugars content on the release and perception of food products and beverages aroma compounds, results present in literature show that they are highly dependent on the intrinsic properties of VOCs, such as steric hindrance, polarity, and volatility, and on sugar characteristics. However, referring to wines, a limited number of works focused on the investigation of this topic and, only a few worked on real wine matrices (Paravisini & Guichard, 2017). For example, Robinson et al. (2009) observed that the response of 20 wine VOCs to increasing glucose concentrations was an increase in their volatility. On the contrary, increasing fructose concentrations in model wine solutions led to a reduction on the headspace concentration of 8 detected odorants (Villamor et al., 2013b). Assessing the effects of 5 different non-volatile matrices (white, young-red, aged-red, sparkling, and sweet wines) on the volatility of typical wine aroma compounds, Rodríguez-Bencomo et al. (2011) did not observe a linear and homogeneous trend. Indeed, working with real wine matrices, these authors observed that VOCs response to different residual sugars content might result either in a retention or a salting-out effect, depending on the interactions amongst all wine components.

However, VOCs response to the presence of a specific wine component is strongly influenced by the interactions among all the wine components present in the matrix. As an example, in the same study above cited, Villamor et al. (2013b) observed that the retention of hydrophobic aroma compounds in the wine matrix was higher in formulations with high ethanol, tannins, and fructose concentration (14% ethanol, 1.5 g/L tannins and 2 g/L fructose, respectively).

Thus, the main aim of the present experimental plan was to investigate the impact of the non-volatile matrix composition (i.e., ethanol, pH, titratable acidity, and reducing sugars), and in particular of the phenolic fraction, on the release and perception of wine VOCs and, additionally, to contribute to the knowledge on VOCs behaviour, through a physicochemical and sensory approach.

2. Materials and methods

2.1. Wine samples

We wanted to obtain four wine samples characterised by the same aromatic base and an increasing non-volatile matrix content. To reach this goal, a bleed wine (BW) was used as aromatic base and it was added with the non-volatile matrix of the pressed wine (PW), obtained within the same vinification. The experimental wines (BW and PW) were produced from Aglianico grape variety in a winery located in Campania region (Italy). The BW was obtained by subtracting an aliquot of the fermenting wine from the tank and completing the alcoholic fermentation in absence of the skins. In this way the extraction of polyphenolic compounds was reduced. The PW was obtained from the pressing of the drained pomace at the end of alcoholic fermentation. The PW was used to obtain the non-volatile matrix to add to BW, by the deodorization process described below. The volumes of PW to deodorize and add to BW were calculated respecting the following proportions of addition (the volumes of PW are referred to the wine before the deodorization procedure):

sample B: 1 L of BW + 0 L of PW

sample B1:P0.5: 1 L of BW + 0.5 L of PW

sample B1:P1.5: 1 L of BW + 1.5 L of PW

sample B1:P2: 1 L of BW + 2 L of PW.

2.2. Deodorization and Reconstitution of wines

PW was deodorized following the procedure recently published (Pittari et al., 2020) and according to the volumes reported in Table 1.

Briefly, as a first step, PW wine was treated in an ultrasound bath (Transsonic 460 H, Elma, Germany) with water as processing liquid, working at a fixed frequency of 35 KHz, to the minimum intensity (1) in a range between 1–15 (set through a turning knob), and at a controlled temperature of 20°C for 30 min. Successively, the samples were evaporated at 30°C under reduced pressure (Rotavapor R-210, Büchi, Switzerland), until a weight loss of ~95% was reached (~90min). As the end of the deodorization procedure, the deodorized PW was added to BW, according to the volumes reported in Table 1.

Table 1. Volumes of BW and PW wines used in the preparation of the experimental samples.

Sample	Volume of PW subjected to deodorization (mL)	Volume of BW used for the reconstitution (mL)
B	0	3690
B1:P0.5	1845	3690
B1:P1.5	5535	3690
B1:P2	7380	3690

2.3. Chemical analysis

Alcoholic strength by volume, reducing sugars, pH, volatile acidity (VA) and titratable acidity (TA) were measured according the OIV Compendium of International Methods of Wine and Must Analysis (2020). Total phenols were measured by Folin–Ciocalteu assay (Singleton et al., 1999). The concentration of proanthocyanidins was determined after acid hydrolysis with warming (Bate-Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst (Di Stefano et al., 1989; Torchio et al., 2010). The analyses were performed in triplicate.

2.4. VOCs extraction: SPME procedure

VOCs extraction was carried out by Headspace-Solid Phase Micro-Extraction (HS-SPME). To carry out the analyses under conditions that were as representative as possible of the olfactory perception during wine tasting, the extraction of wine VOCs was carried out in INAO tasting glasses, sealed with a special silicone lid, which allowed the hermetic closure of the glass, as represented in Figure 1.

30 mL of each sample, spiked with 2-octanol as internal standard (IS) at a concentration of 357 µg/L (Sigma Aldrich, St. Louis, USA) were analysed. Before extraction, the samples were equilibrated in a thermostatic bath at 30° C for 10 minutes. A DVB/CAR/PDMS fiber (divinylbenzene/carboxen/polydimethysiloxane; 50/30 µm thickness, coating phase; 2 cm length) was exposed to the headspace of the samples for 30 minutes at 30 °C by An SPME manual fiber holder (Supelco, Bellefonte, PA, USA). Each sample was analysed in triplicate.

Figure 1. Representation of the VOCs extraction phase carried out carried out by Headspace-Solid Phase Micro-Extraction (HS-SPME) in INAO tasting glass.

2.5. GC-MS analysis

The identification of VOCs and their quantification was carried out by GC-MS analyses. The analyses were carried out using a GC/MS-QP2010 quadrupole mass spectrometer (Shimadzu, Shimadzu corp., Kyoto, Japan) equipped with a DB-WAX column (60 m, 0.25 mm i.d., 0.25 µm film thickness) (J&W Scientific Inc., Folsom, CA, 95360, USA). The injector and the electronic source were kept at a temperature of 250 and 230 °C, respectively. The SPME fiber was desorbed in the injector for 10 minutes, in splitless mode. The oven temperature was maintained at 40 °C for 5 min, and then increased by 2 °C/min, up to 220 °C and held for 20 min. Helium was used as a carrier gas with a flow of 1.3 mL/min. Electron impact mass spectra were recorded with an ion source energy of 70 eV.

The identification of the compounds was performed by comparison of their retention times and their mass spectra with those of pure reference standards under the same chromatographic conditions. They were further confirmed by comparison of the mass spectra obtained for each compound with those stored in the database of

the National Institute of Standards and Technology (NIST). The relative concentrations of isolated compounds were expressed as a ratio of the response of each compound against the response of the internal standard.

2.6. Sensory Analysis

The aim was to investigate the sensory impact of the differences in matrix composition on the sensory characteristics of the four obtained wine matrices. For this purpose, the four wine samples were characterized in terms of olfactory, astringency sub-qualities and tastes characteristics using a descriptive sensory assessment on a numerical category scale.

2.6.1. Panel

The jury was composed of 19 selected subjects (9 males and 10 females aged between 21 and 49 years) recruited among students and researchers (Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II). They were all expert wine tasters with previous experience in performing sensory tests on wine. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Participation was on a voluntary basis and, prior to the experiments, tasters were required to sign an informed consent form disclosing the type of research, voluntary participation and agreement to taste/smell reference solutions and wines.

They were recruited based on their interest, and availability. Judges who in the preliminary tests carried out at least 80% of correct identifications, were then subjected to the training phase, aimed at memorizing, and recognizing perceptible odour stimuli in wine. All data were collected anonymously.

2.6.2. Procedure

Panel training: judges' selection and familiarization with oral and olfactory stimuli were performed according to training procedures previously applied. Four training sessions were held on a weekly basis. During this phase, the employed chemical standards were dissolved in aqueous solution and in wine.

Sensory assessment: the four experimental samples were analysed by descriptive sensory assessment using the same vocabulary and the five-point numerical category scale employed during the training (1=very weak, 2=weak, 3=medium, 4=strong, 5=very strong).

Analyses were carried out on a weekly basis and performed in duplicate, in two separated sessions. In each of the two sessions, all judges analysed all the wine samples. Each session was split into 3 sub-sessions with an imposed break of 15 min between 2 successive phases.

The first sub-session was aimed at analysing wines olfactory characteristics.

Panellists were asked to smell each wine samples, to recognize the corresponding odour descriptor/s or family/ies and to score the intensity on the five-point numerical category scale, with half values allowed. Once analysed the four samples, judges were then asked to rank the wine samples by increasing overall odour intensity.

Following the first-time break, the in-mouth profile of the wine samples was evaluated. In this phase, panellists were asked to taste each sample by focusing on the intensity of perceived tastes (sweet, acid, and bitter) and astringency sub-qualities, by paying attention not only to the most intense sensation but also to that/those catching their attention the most during the tasting time.

Finally, after a further time break, the same in-mouth profile analysis (astringency sub-qualities and tastes) was repeated with judges employing nose-clips.

During the sensory assessment, for each sample, 30 mL volumes were served in INAO tasting glasses coded with three-digits and presented in a randomized order. Wines were served at room temperature (21±1°C) and evaluated in individual booths (ISO, 2007). Between the three sub-sessions the sample codes were changed, in order to avoid any memory effect.

2.7. Data Analysis

Chemical and sensory data were subjected to Analysis of Variance (One and Two-way ANOVA with interactions), and the significance of the differences was then tested by post-hoc Tukey test with α=0.05.

The correlations between volatile and non-volatile chemical composition were tested by Pearson correlation analyses ($p< 0.05$).

Moreover, sensory data were also analysed by a Friedman's test followed by a Namenyi's multiple comparison with the aim of determining eventual significant differences between them.

Data was processed with XLStat (version 2019.6), an add-in software package for Microsoft EXCEL (Addinsoft, Paris, France).

3. Results and Discussion

The main aim of the present experimental plan was to investigate the impact of the non-volatile matrix composition (i.e., ethanol, pH, titratable acidity, and reducing sugars), and in particular of the phenolic fraction, on the release and perception of wine VOCs, through a physicochemical and sensory approach.

To account for different non-volatile matrix compositions, and particularly for a wide and significant polyphenolic diversity, the experiments were carried out on 4 wine matrices obtained by adding to a bleed wine (BW) increasing concentrations of the non-volatile matrix of the same pressed wine (PW), notably rich in polyphenols.

3.1. Chemical matrix composition

Basic compositional data (titratable acidity, pH, residual sugars, ethanol) and polyphenolic characteristics (i.e., tannins (BSA reactive), phenols (Fe reactive), free and total anthocyanins) of the 4 reconstituted wines are represented in Figure 2 and 3, respectively. As it can be observed, the 4 matrices show significant different chemical compositions.

Except for the dilution effect of the alcoholic degree that decreased from B to B1:P2, titratable acidity, pH, residual sugars and the four considered phenolic parameters significantly increased from B to B1:P2, as expected. These outcomes are in accordance with data reported in literature regarding press wines composition. As reported by Ribéreau-Gayon et al. (2006), press wines are normally characterised by higher values of all elements - reducing sugars, acidity, and phenolic compounds (anthocyanins and tannins) - except for alcohol, that normally decreases when comparing press and free run wines. Interestingly, also titratable acidity and pH

are normally both characterised by higher values in press wines, because of their higher mineral concentration that leads to an increase in the pH (Ribéreau-Gayon et al., 2006).

Figure 2. Basic chemical composition of wine matrices. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).

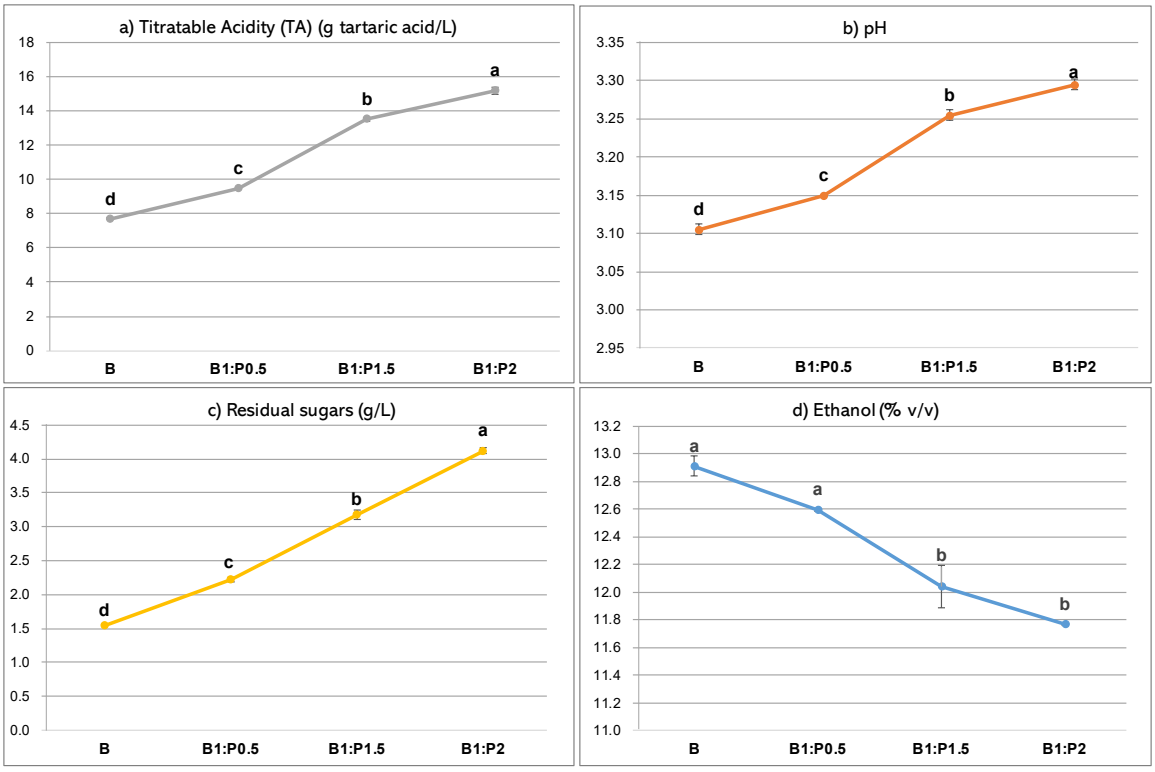
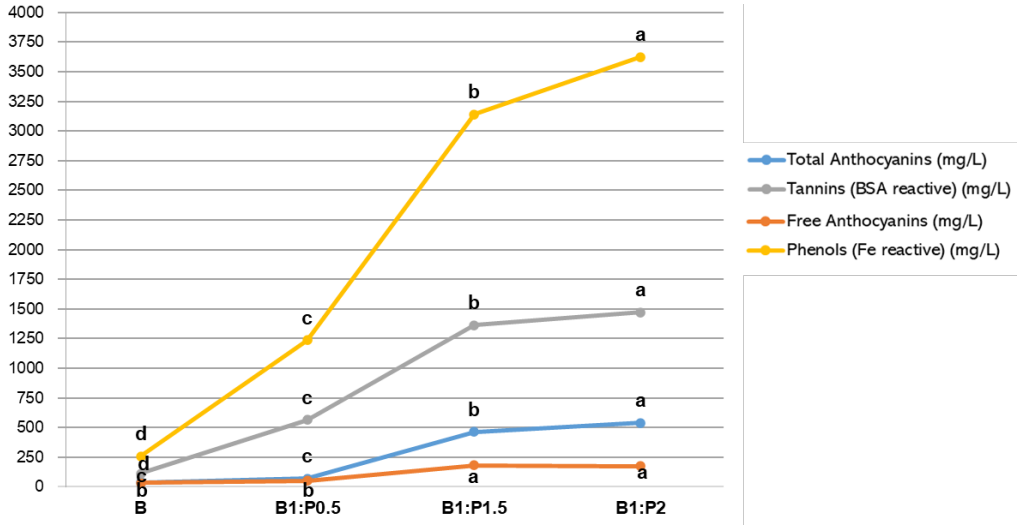


Figure 3. Polyphenolic composition of wine matrices. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).



Therefore, as we expected to obtain four matrices characterised by increasing concentrations of the phenolic fractions without adding exogenous compounds, these results suggest that the objective was achieved. Hence, these compositional differences represented the starting point to further analyse the response of the volatile compounds to the different non-volatile matrix composition and to study the correlations between specific chemical parameters and VOCs release.

3.2. VOCs release response to different matrix compositions

VOCs extraction was carried out by Headspace-Solid Phase Micro-Extraction (HS-SPME), in INAO tasting glasses, to simulate as much as possible the real wine tasting conditions. In total, 34 VOCs were identified in the headspace of the 4 wine samples: 10 esters, among which 2 acetates, 9 alcohols, 5 acids, 5 terpenoids, 1 lactone, 2 volatile phenols, and 2 sulfur molecules. The identified volatile compounds were listed according to the retention time (RT) in Table 2, together with their chemical class and mean concentration in each matrix (expressed in μg/L). VOCs analysis was performed adding an internal standard (IS) (2-octanol) to the wines before extraction. This technique, that presents the advantages to be simple, versatile, not time-consuming (Pati et al., 2021), and to compensate for sample preparation variability (Merkle et al., 2015), has however some limitations that need to be underlined. Indeed, as recently reviewed by Pati et al. (2021), this kind of analysis implies that the response factor of all analytes is supposed to be equal to the one of the IS, meaning that VOCs and IS are equally influenced by matrix effects (Pati et al., 2021).As it is not likely that the IS behaves at the same way as all the analytes, this type of quantification is considered a semiquantitative method and it does not allow to obtain information on the real concentration of volatile compounds in the matrix and to accurately compare the results with the ones obtained with other extraction techniques. However, as the main aim of this experiment was to monitor and compare the behaviour of several VOCs in different matrices, and as previous works with the same purpose applied this kind of analytical approach (Sáenz-Navajas et al., 2010; Rodríguez-Bencomo et al., 2011), this type of analysis has been considered appropriate to detect significant differences. The obtained data can be used as starting point for further targeted analyses applying quantitative methods. Headspace analyses revealed significant differences of VOCs behaviour in the 4

different wine samples, suggesting that the headspace of the different matrices has different composition. Indeed, except for alpha-terpineol and nonanoic acid, that did not show significant differences among the 4 wine reconstituted wines, for all the other 32 identified VOCs significant differences in concentration were found, indicating a relevant impact of the non-volatile fraction on their release. In the following paragraphs, we attempted to investigate the VOCs behaviour according to their chemical class.

Table 2. VOCs identified and quantified by HS-SPME /GC-MS in the four wine matrices. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).

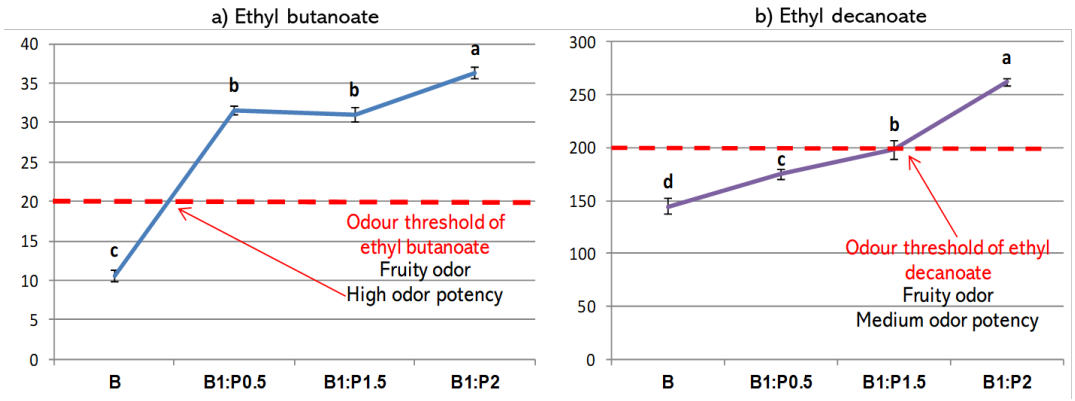
N°	RT	Compound	Chemical class	Mean concentration (µg/L)			
				B	B1:P0.5	B1:P1.5	B1:P2
1	14.976	Ethyl butanoate	Esters	10.611 c	31.518 b	30.948 b	36.184 a
2	18.456	2-methyl-1-propanol (Isobutanol)	Alcohols	185.432 c	201.276 b	197.343 bc	220.761 a
3	20.237	Isoamyl acetate	Esters	63.750 b	75.033 ab	71.579 ab	79.743 a
4	21.829	1-Butanol	Alcohols	2.388 b	3.699 a	3.521 a	2.539 b
5	26.218	3-methyl-1-butanol	Alcohols	4004.876 c	4269.162 b	4100.605 bc	4554.827 a
6	27.736	Ethyl hexanoate	Esters	413.346 b	423.693 b	429.484 b	500.495 a
7	35.160	Ethyl lactate	Esters	162.225 d	230.456 c	255.249 b	348.163 a
8	36.001	1-Hexanol	Alcohols	152.510 d	165.569 b	160.393 c	179.454 a
9	36.701	(E)-3-Hexen-1-ol	Alcohols	1.156 c	1.955 a	1.514 b	2.092 a
10	41.030	Ethyl octanoate	Esters	1112.566 d	1222.916 b	1168.154 c	1479.012 a
11	42.073	Acetic acid	Acids	101.708 d	129.503 c	148.821 b	208.260 a
12	42.705	1-Heptanol	Alcohols	16.000 c	17.765 b	16.799 c	19.794 a
13	44.879	2-ethyl-1-hexanol	Alcohols	5.169 b	6.866 a	7.183 a	7.643 a
14	48.387	Linalool	Terpenoids	16.913 c	19.947 c	24.715 b	28.309 a
15	49.115	1-Octanol	Alcohols	10.782 a	10.824 a	10.037 b	11.216 a
16	49.747	Isoamyl lactate	Esters	22.375 d	28.577 c	29.912 b	38.849 a
17	50.709	Isobornyl acetate	Terpenoids	40.659 a	29.247 b	12.303 c	11.841 c
18	53.005	gamma-Butyrolactone	Lactones	7.435 d	15.379 c	30.008 b	40.569 a
19	53.819	Ethyl decanoate	Esters	144.135 d	174.558 c	197.883 b	261.662 a
20	56.031	Diethyl succinate	Esters	92.859 d	147.864 c	248.901 b	353.632 a
21	57.315	alpha-Terpineol	Terpenoids	5.551 a	5.442 a	5.098 a	5.607 a
22	58.416	3-(methylthio)-1-propanol (Methionol)	S-Compounds	6.459 d	9.276 c	13.940 b	19.366 a
23	61.184	beta-Citronellol	Terpenoids	4.356 ab	4.619 ab	4.047 b	4.690 a
24	62.145	Ethyl phenylacetate	Esters	5.069 d	8.956 c	16.166 b	22.299 a
25	63.791	Phenethyl acetate	Esters	6.557 b	6.907 b	7.105 b	10.329 a
26	65.347	Hexanoic acid	Acids	82.962 ab	93.438 a	76.748 b	89.067 ab
27	65.872	Geranylacetone	Terpenoids	12.539 d	15.712 c	20.026 b	24.575 a
28	68.924	beta-Phenethyl alcohol	Alcohols	729.938 d	944.422 c	1231.477 b	1686.321 a
29	71.113	Benzothiazole	S-Compounds	10.049 a	8.002 b	2.962 c	0.000 d
30	74.769	4-Ethylguaiacol	Phenols	9.809 c	12.738 a	11.715 b	10.086 c
31	76.246	Octanoic Acid	Acids	120.288 a	119.703 a	95.392 b	112.066 ab
32	81.304	Nonanoic acid	Acids	20.451 a	21.356 a	25.873 a	17.370 a
33	81.658	4-ethyl-phenol	Phenols	14.640 a	14.401 a	9.574 b	6.913 c
34	86.138	Decanoic acid	Acids	10.258 a	10.156 a	7.662 b	9.497 ab

3.2.1. Esters

10 Esters, among which 2 acetates, have been identified: ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl lactate, ethyl octanoate, isoamyl lactate, ethyl decanoate, diethyl succinate, ethyl phenylacetate, phenethyl acetate. In all reconstituted wines, esters all showed increasing concentrations (Table 2), indicating a possible salting out effect as the non-volatile composition increased.

Of particular interest results the increased release of ethyl butanoate in B1:P0.5, B1:P1, and B1:P2 (Figure 4a) and ethyl decanoate in B1:P2 (Figure 4b), above their perception thresholds - 20 µg/L (Guth, 1997) and 200 µg/L (Waterhouse et al., 2016 and references therein), respectively. However, it is important to highlight that our results are semiquantitative.

Figure 4. Graphic showing the release of a) ethyl butanoate, and b) ethyl decanoate in the four matrices. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).



3.2.2. Alcohols

The 9 identified alcohols are represented by 2-methyl-1-propanol (isobutanol), 1-butanol, 3-methyl-1-butanol, 1-hexanol, (E)-3-hexen-1-ol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, and beta-phenethyl alcohol. Except for beta-phenethyl alcohol, described with floral/rose notes, the other identified alcohols are described with fusel, oily, alcoholic, ethereal terms, and it has been reported that at high concentrations they can mask certain relevant wine aromas. Based on results reported in Table 2, most of the identified alcohols showed a significant increase at higher concentrations of the non-volatile matrix. Indeed, except for 1-butanol, the release of all the other alcohols increased in B1:P2 sample, suggesting a salting-out effect played by the higher non-volatile matrix composition.

3.2.3. Acids

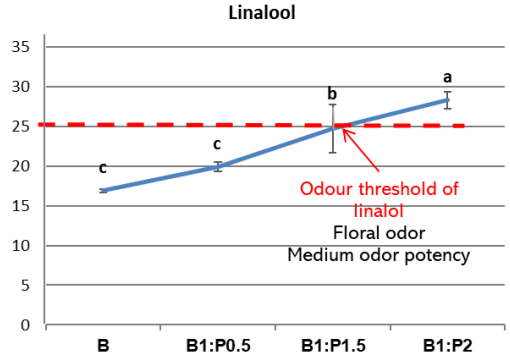
Acetic, hexanoic, octanoic, nonanoic, and decanoic acids have been detected by HS-SPME analyses. Among them, only acetic acid showed a clear and significant trend: it constantly and significantly increased at higher non-volatile matrix concentrations, from B to B1:P2 (Table 2). Acetic acid is the main volatile acid of wine, it is described with pungent, vinegar-like descriptors and when at high concentrations it gives off sensation in the mouth (Zamora, 2009).

3.2.4. Terpenoids

5 Terpenoids have been identified in the four wine matrices: alpha-terpineol, beta-citronellol, isobornyl acetate, geranylacetone, and linalool. Regarding the release of this group of VOCs, different behaviours were observed: isobornyl acetate significantly decreased, while geranylacetone and linalool significantly increased; alpha-terpineol did not significantly vary, and beta-citronellol did not show a clear trend (Table 2).

Interestingly, linalool showed an increased release in B1:P2 above its perception threshold (25 µg/L) (Ferreira et al., 2000 and references therein), as represented in Figure 5. However, it is important to highlight that our results are semiquantitative.

Figure 5. Graphic showing the release of linalool in the four matrices. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).



3.2.5. Lactones

gamma-Butyrolactone is the only identified lactone compound and it is characterised by sweet odorous, like creamy, oily, fatty, and caramel notes. As it can be observed in Table 2, this VOC shows a constant significant increase from B to B1:P2, suggesting that in highly concentrated red wines, the perception of this volatile molecule can be favoured.

3.2.6. Volatile phenols and sulfur compounds

2 Volatile phenols (4-ethylguaiacol, 4-ethylphenol) and 2 sulfur molecules (3-(methylthio)-1-propanol, benzothiazole) were detected.

Regarding volatile phenols, data reported in Table 2 show that 4-ethylguaiacol did not significantly vary when comparing B with B1:P2, while 4-ethylphenol showed a significant decrease at increasing concentration of the non-volatile matrix (Table 2). This latter result could be of great interest for oenologists and winemakers as 4-ethylphenol is involved in the appearance of unpleasant notes, responsible for the "Brett character", one of the main worldwide spread defects, and because controlling its concentration in wine is an always current topic in oenology.

The two sulfur molecules show opposite trends: while 3-(methylthio)-1-propanol (methionol) increased, benzothiazole significantly decreased from B to B1:P2 (Table 2). These sulfur molecules are normally involved in wine olfactory defects such as appearance of sulfurous, onion, rubbery, vegetable, and meaty notes, and they are characterised by high odour potencies (Moio et al., 1994). Moreover, in the case of benzothiazole, its perception threshold is very low (50-200 µg/L) (Chatonnet et al., 1992). As already observed for 4-ethylphenol, the result concerning benzothiazole may be of great interest in winemaking to control its concentration and sensory impact in wine.

3.3. Study of the influence of the non-volatile matrix components on aromas release

Once established how the different non-volatile matrices induced a significant effect on VOCs release, a Pearson correlation test and a linear regression analysis were performed between some of the matrix compositional parameters (Base Compositional Parameters: BCP; Polyphenols: PPhss) and the aroma compounds to better understand the observed behaviours.

Table 3 shows the results of the Person correlation analysis, where significant negative or positive correlations ($p<0.10$, $p<0.05$) are indicated in bold. As it can be observed, for 18 out of the 34 identified VOCs, one to five significant correlations with the chemical parameters were found.

Table 3. Correlation matrix coefficients (Pearson) between
Base Compositional Parameters (BCPs), Polyphenols (PPhss), and Volatile Organic Compounds (VOCs).
In each chemical class of compounds, VOCs have been displayed from the highest to the lowest $logP_{octanol/water}$ value.

	BCPs		PPhs			
VOCs	**Ethanol**	**Residual sugars**	**Total anthocyanins**	**Free anthocyanins**	**Tannins (BSA reactive)**	**Phenols (Fe reactive)**
ESTERS						
Ethyl decanoate	**-0.945**	-0.295	0.884	0.808	0.886	**0.911**
Ethyl octanoate	-0.763	-0.593	0.660	0.540	0.662	0.701
Ethyl hexanoate	-0.822	-0.549	0.763	0.648	0.725	0.766
Isoamyl acetate	-0.801	-0.579	0.744	0.624	0.699	0.742
Ethyl phenylacetate	**-0.993**	-0.094	**0.964**	**0.920**	**0.963**	**0.978**
Phenethyl acetate	-0.783	-0.243	0.626	0.567	0.740	0.749
Ethyl butanoate	-0.825	0.084	0.689	0.683	0.831	0.821
Isoamyl lactate	**-0.923**	-0.298	0.834	0.759	0.865	0.887
Diethyl succinate	**-0.986**	-0.142	**0.955**	**0.903**	**0.948**	**0.967**
Ethyl lactate	**-0.943**	-0.251	0.862	0.794	0.892	**0.912**
TERPENOIDS						
Geranylacetone	**-0.989**	-0.117	**0.949**	**0.902**	**0.956**	**0.972**
Isobornyl acetate	**0.976**	-0.235	**-0.953**	**-0.965**	**-0.997**	**-0.990**
beta-Citronellol	-0.063	-0.845	-0.106	-0.246	-0.062	-0.021
Linalol	**-0.997**	-0.052	**0.964**	**0.927**	**0.974**	**0.986**
alpha-Terpineol	0.197	**-0.984**	-0.262	-0.421	-0.344	-0.286
ALCOHOLS						
1-Octanol	-0.024	**-0.976**	-0.085	-0.248	-0.123	-0.068
2-Ethyl-1-hexanol	**-0.902**	0.099	0.793	0.787	0.907	0.900
1-Heptanol	-0.741	-0.539	0.607	0.498	0.651	0.682
1-Hexanol	-0.788	-0.464	0.656	0.559	0.709	0.736
(E)-3-hexen-1-ol	-0.624	-0.324	0.437	0.369	0.573	0.582
beta-Phenethyl alcohol	**-0.969**	-0.221	**0.926**	0.861	**0.920**	**0.942**
3-methyl-1-butanol	-0.708	-0.567	0.568	0.456	0.615	0.647
1-Butanol	-0.058	0.723	-0.045	0.080	0.175	0.116
2-methyl-1-propanol (Isobutanol)	-0.833	-0.423	0.713	0.621	0.759	0.785
ACIDS						
Decanoic acid	0.612	-0.760	-0.701	-0.809	-0.716	-0.681
Nonanoic acid	0.076	**0.993**	0.002	0.170	0.077	0.016
Octanoic acid	0.643	-0.729	-0.732	-0.834	-0.741	-0.709
Hexanoic acid	0.102	-0.639	-0.303	-0.402	-0.190	-0.169
Acetic acid	**-0.939**	-0.312	0.876	0.797	0.877	**0.903**
LACTONES						
gamma-Butyrolactone	**-0.997**	-0.056	**0.969**	**0.932**	**0.972**	**0.985**
PHENOLS						
4-Ethylphenol	**0.973**	0.082	**-0.985**	**-0.943**	**-0.943**	**-0.961**
4-Ethylguaiacol	0.037	0.591	-0.170	-0.064	0.063	0.008
S-COMPOUNDS						
Benzothiazole	**0.997**	0.032	**-0.980**	**-0.946**	**-0.976**	**-0.988**
3-(methylthio)-1-propanol (Methionol)	**-0.982**	-0.162	**0.947**	0.892	**0.942**	**0.961**

Values in bold are different from 0 with a significance level alpha=0.10 (in grey alpha=0.05)

BCPs: Base Chemical Parameters; PPhs: Polyphenols

Ethanol showed a general negative impact on the release of most of the volatile molecules belonging to the different chemical classes, as previously reviewed (Villamor & Ross, 2013; Paravisini & Guichard, 2016; Ickes & Cadwallader, 2017). More specifically, data obtained (Whiton & Zoecklein, 2000; Goldner et al., 2009; Robinson et al., 2009; Villamor et al., 2013) by headspace solid-phase microextraction analyses (HS-SPME) on wine VOCs release, have shown that higher ethanol levels led to a reduction in the headspace concentration of many volatiles (e.g., ethyl decanoate and hexanoate, ethyl phenylacetate, beta-phenylethanol, acetic acid). Here we have observed that ethanol is significantly correlated with 15 VOCs, among which only 3 showing positive correlations, namely isobornyl acetate, 4-ethylphenol and, benzothiazole. Ethyl phenylacetate, diethyl succinate, geranylacetone, linalool, beta-phenethyl alcohol, gamma-butyrolactone, and 3-(methylthio)-1-propanol showed the stronger negative correlations ($p<0.05$), suggesting a retention effect played by ethanol on most the VOCs considered in the study.

Residual sugars showed few significant correlations with the volatile compounds (Table 3). They have been found to be significantly correlated ($p<0.05$) only with alpha-terpineol ($r= -0.984$), 1-octanol ($r= -0.976$), and nonanoic acid ($r= +0.993$). However, even if data are not significant, a general trend can be observed, suggesting a negative impact of residual sugars on the release of many VOCs belonging to different chemical classes (e.g., esters, terpenoids, alcohols, acids).

Observing VOCs-PPhs correlation (Table 3), homogeneous trends were observed for esters and alcohols, with general positive correlations with the phenolic parameters, which could suggest an enhancing effect of polyphenols on the release of these classes of VOCs. For the other groups of volatile molecules (i.e., terpenoids, acids, and sulfur compounds), a general trend has not been found.

Esters have been widely studied in terms of response to polyphenols, however, as recently reviewed, without observing a clear and unique trend (Pittari et al., 2021). Results in Table 3 show that among the 10 identified esters, only ethyl phenylacetate, diethyl succinate, and ethyl lactate showed significant correlation values with the phenolic parameters ($p<0.10$, $p<0.05$). These results partially support previous findings reported in Table 1 (Background Section), which observed that increasing concentrations of tannins (skin tannins extract and a seed/skin tannins mixture), caused a higher release of diethyl succinate in wine model solutions (Mintropoulou

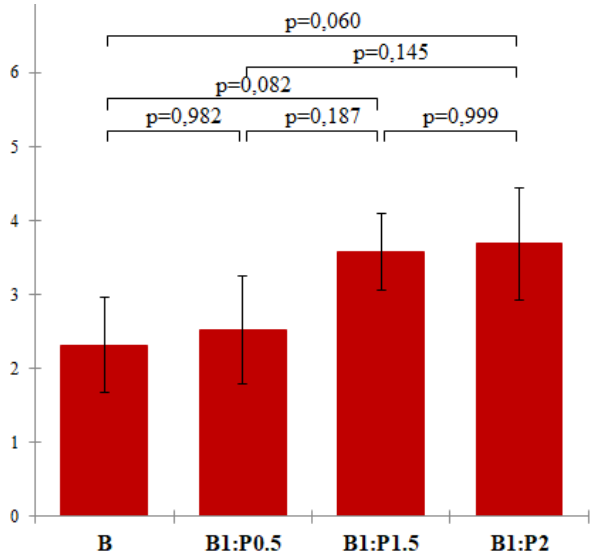
et al., 2011). Moreover, even if not significant, the positive correlations observed for other esters (i.e., isobutyl acetate, butyl acetate, ethyl butanoate and octanoate) with phenolic parameters, agree with data reported in the literature (Mintropoulou et al., 2011; Rodríguez-Bencomo et al., 2011) and schematised in Table 1 (Background Section). Among the 5 identified terpenoids, while linalool and geranylacetone have been found to be significantly positively correlated with the four phenolics ($p<0.10$, $p<0.05$), isobornyl acetate showed strong negative correlations ($p<0.05$) with all the considered PPhs. Finally, beta-citronellol and alpha-terpineol did not significantly correlate to any of the phenolics parameters; however, they both showed negative values of correlations with the PPhs. Those results seem to partially confirm the ones of earlier studies (Mintropoulou et al., 2011; Rodríguez-Bencomo et al., 2011), in which the release of some terpenoids (i.e., alpha-terpineol, beta-citronellol) decreased at increased tannin concentrations, as reported in Table 1 (Background Section). Alcohols and acids were not correlated as much as other chemical groups by the PPhs composition. Indeed, among them, only beta-phenylethanol showed a significant positive correlation ($p<0.10$) with phenolic parameters, namely with total anthocyanins, tannin (BSA reactive) and phenols (Fe reactive) (Table 3). This result agrees with previous findings, reported in Table 1 (Background Section) that showed a "salting-out" effect at high tannins concentrations independently from their nature (Mintropoulou et al., 2011). Considering the relatively low hydrophobicity of beta-phenylethanol (logP=1.36) and the presence of an aromatic ring on its structure, the formation of π-π interactions of the galloyl ring of the phenolic compound with the aromatic ring could have occurred, reducing its volatility (Dufour & Bayonove, 1999; Jung et al., 2000). However, at high tannins concentrations, it could be possible that the decrease in the potential binding sites for odorants has occurred because of the lower ethanol concentration observed in B1:P2 compared to B, that led more tannins self-aggregation, making them less available to interact with some aroma compounds (Poncet-Legrand et al., 2003; Zanchi et al., 2007; Villamor et al., 2013b). gamma-Butyrolactone shows significant positive correlations with all the PPhss, showing an enhancing effect played by these chemical compounds on its release. To the best of our knowledge, this is the first time that the release of this compound has been studied in response to different polyphenolic concentrations, therefore no data are available in the literature. Among the two identified volatile phenols, important results regarding the effects of polyphenols on 4-ethylphenol

have been highlighted (Table 3): it has been found to be significantly and negatively correlated with all the considered parameters. This result is in line with data reported in Table 1 (Background Section) of a recent study (Petrozziello et al., 2014), where the authors showed that at increasing polyphenols concentration, a significant and linear decrease in the volatility of this VOC was observed due to π-π interactions. Interestingly, phenolic parameters have been also negatively correlated with benzothiazole ($p<0.10$, $p<0.05$). This result may be of great interest in winemaking since controlling the concentration and the sensory impact of these compounds in wine is an always current topic in oenology.

3.4. Matrix effect on olfactory perception

Figure 6 shows the ranks of the overall odour intensity assessed in the four matrices. As it can be observed, the trend suggests an increase in the perception of the global aroma intensity passing from B to B1:P2, in line with the observed changes in volatiles release, previously shown. However, these changes of the global aroma intensity are not significant ($p=0.060$).

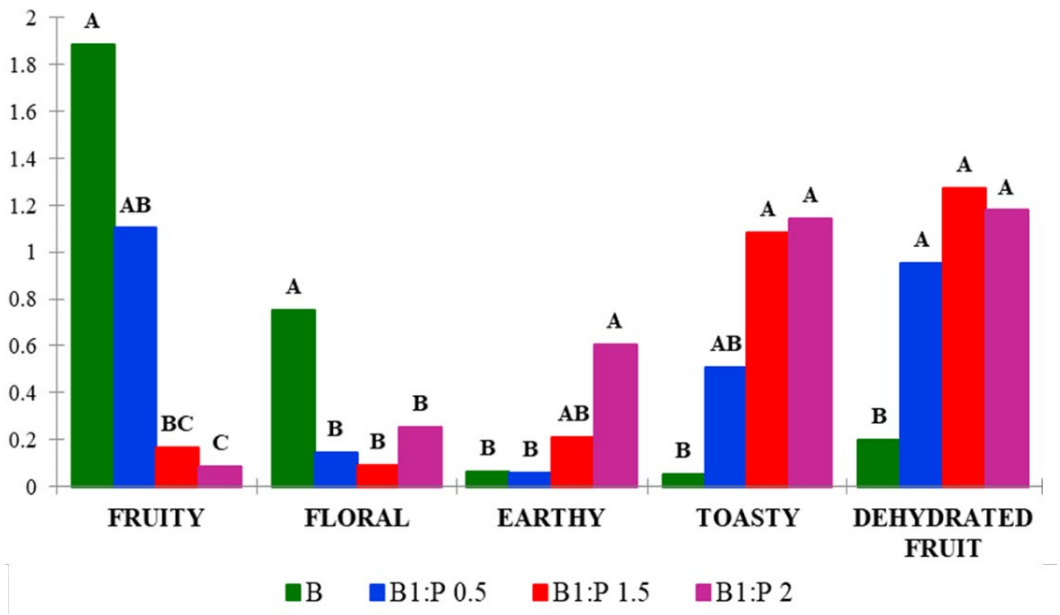
Figure 6. Ranking of the overall odour intensity in the four matrices. Differences were analysed by a Friedman's test followed by a Namenyi's multiple comparison.



Differently from the global odour intensity, significant variations of the relative intensity of specific olfactory notes have been highlighted (Figure 7). Such results show a significant modulating effect of the different non-

volatile matrix composition on the olfactory profile of the wine. In Figure 7 are represented the olfactory descriptors that have shown significant differences (ANOVA, $\alpha>0.05$) between the four wine matrices, namely fruity, floral, earthy, toasty, and dehydrated fruit. Looking at the figure, several observations can be made. Fruity and floral notes decreased from B to B1:P2, while earthy, toasty, and dehydrated fruit significantly increased from B to B1:P2. Moreover, while fruity, earthy, and toasty notes gradually varied from B to B1:P2, floral and dehydrated fruit notes significantly changed as the base wine (B) was enriched with the non-volatile matrix (from B1:P0.5 to B1:P2), then following an almost constant behaviour. The decrease of isobornyl acetate until the 72% could be linked to the decrease of floral odours, while the increase in gamma-butyrolactone (+400%) could be involved in the higher perception of dehydrated fruit.

Figure 7. Wine matrices olfactory profile. Only descriptors with significant differences between the four wine matrices are shown. Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p<0.05$).



It is interesting to notice that even if molecules normally responsible for fruity notes (i.e., esters, some terpenoids) increased from B to B1:P2 (Table 2), these olfactory characteristics significantly decreased. We must consider that, together with some esters and terpenoids, also other VOCs responsible for different type

of olfactory characteristics increased in the enriched matrices (i.e., alcohols, acetic acid, gamma-butyrolactone) (Table 2).

Differences in concentrations and proportions of the same odorants in mixture generate various qualitative and quantitative sensory perceptions that may be responsible for wide aromatic bouquet differences between wines. Indeed, in recent years, many works have investigated the effects of perceptual interactions between odorants, observing the capacity of some volatiles to enhance or mask the perception of other odorants present in the matrix (Atasanova et al., 2005; Lytra et al., 2012; Cameleyre et al., 2015; Ferreira et al., 2016). For example, Atasanova et al. (2005), studying three binary mixtures of fruity (ethyl butyrate, and isoamyl acetate) and woody (beta-methyl-gamma-octalactone, and methoxy-2-phenol) odorants, confirmed the influence on the olfactory perception of sub- and peri-threshold components in odour mixtures. More recently, some authors (Cameleyre et al., 2015; Ferreira et al., 2016) adding higher alcohols (i.e., 3-methyl-1-butanol, 1-butanol) to a model wine solution containing fruity esters, observed a decrease of the perception of fruity notes. Further results came from a work of Lytra et al. (2012), carried out supplementing a fruity fraction with several compounds, among which also acetic acid, and gamma-butyrolactone. Authors showed that these volatiles indirectly contributed to the decrease in the fresh-fruit aroma intensity and indicated that odorants with caramel and lactic aroma had a “masking” effect on the fresh-fruit notes. All these previous evidences suggest that a possible cause for the enhancement of earthy, toasty, and dehydrated fruit odours, and for the decrease of fruity and floral notes that we observed in our experiment, should be found in perceptual interactions. Indeed, investigating the response of a single molecule to different matrix compositions is not enough to totally comprehend its sensory impact, and that studying perceptual interactions is necessary to explain some behaviours. Thus, further studies are needed to better investigate the obtained results, trying to estimate the contribution of the identified compounds, ideally in model solutions containing odour mixtures, to the olfactory profile of wines.

4. Conclusions

Overall, this study has shown that the non-volatile composition of wines strongly influences the volatility of wine aroma compounds, with almost all the volatile compounds having significant different behaviours in the four analysed matrices. Pearson analysis highlighted strong positive and negative correlations between the non-volatile wine components and aroma compounds. Among them, polyphenols (anthocyanins and tannins) showed strong positive correlations with ethyl phenylacetate, diethyl succinate, geranylacetone, linalool, beta-phenylethanol, gamma-butyrolactone, and methionol and strong negative correlations with isobornyl acetate. Negative correlations were also found for 4-ethylphenol, and benzothiazole, two molecules responsible for important wine taint, that therefore could be less perceivable in wine with high polyphenol content.

From a sensory point of view, while the rating of the global odour intensity did not significantly differ among the four matrices, their odour profiles changed. Fruity and floral notes decreased, while earthy, toasty, and dehydrated fruit significantly increased as the non-volatile matrix concentration increased. According to the literature, the decrease of the fruity notes could be explained by perceptual interactions among odorants: the increase of 1-butanol, 3-methyl-1-butanol, acetic acid, and gamma-butyrolactone could have masked the fruity character expected as a consequence of the important rise of esters release. The decrease of isobornyl acetate until the 72% could be linked to the decrease of floral odours, while the increase in gamma-butyrolactone (+400%) could be involved in the higher perception of dehydrated fruit.

However, it is important to highlight that since VOCs analysis was performed applying a semiquantitative method that does not allow to obtain information on the real concentration of volatile compounds in the matrix and to accurately compare the results with the ones obtained with other extraction techniques, further investigations, applying quantitative methods, are necessary to better comprehend these first results.

References

Atanasova, B.; Thomas-Danguin, T.; Chabanet, C.; Langlois, D.; Nicklaus, S.; Etiévant, P. Perceptual Interactions in Odour Mixtures: Odour Quality in Binary Mixtures of Woody and Fruity Wine Odorants. *Chem. Senses*, **2005**, 30(3), 209–217.

Cameleyre, M.; Lytra, G.; Tempere, S.; Barbe, J.C. Olfactory Impact of Higher Alcohols on Red Wine Fruity Ester Aroma Expression in Model Solution. *J. Agric. Food. Chem.* **2015**, 63, 9777-9788.

Chatonnet, P.; Lavigne, V.;, Boidron, J.N.; Dubourdieu, D.; Pons, M. Identification et dosage de sulfures volatils lourds dans les vins par charomatographie enphase gazeuse et phototmétrie de flamme. Sci Ailements, 1992, 12:ì, 513–532.

Di Stefano, R.; Cravero, M.C.; Gentilini, N. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, **1989**, 25, 83–89.

Dufour, C.; Bayonove, C.L. Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *J. Agric. Food Chem.* **1999**, 47, 678–684.

Escudero, A.; Campo, E.; Fariña, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem*. **2007**, 55(11), 4501–4510.

Etievant, P.X. Wine. In Volatile Compounds in Foods and Beverages; Maarse, H. (Ed.); CRC Press: New York, 1991; pp 483−546.

Ferreira, V. Volatile aroma compounds and wine sensory attributes. In Managing Wine Quality, 2nd ed.; Reynolds, A.G. (Ed.); Woodhead Publishing: Sawston, Cambridge, UK; Volume Viticulture and Wine Quality, 2010, pp. 3-28.

Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture,* **2000**, 80(11), 1659–1667.

Ferreira, V.; Sáenz-Navajas, M.P.; Campo, E.; Herrero, P.; de la Fuente, A.; Fernández-Zurbano, P. Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. *Food Chem.* **2016**, 199, 447-456.

Fischer, C.; Fischer, U.; Jakob, L. Impact of matrix variables ethanol, sugar, glycerol, pH and temperature on the partition coefficient of aroma compounds in wine and their kinetics of volatilization. In Proceedings for the Fourth Internatinal Symposium on Cool Climate Enology and Viticulture. T. Henick-Kling et al., Eds. New York State Agricultural Experiment Station, Geneva, 1997, pp. 42-46.

Goldner, M.C.; Zamora, M.C.; Di Leo Lira, P.; Gianninoto, H.; Bandoni, A. Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens. Stud.* **2009**, 24, 243–257.

Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission *Chemical Senses*, **2001**, 26, 533-545.

Guth, H. Comparison of different white wine varieties in odor profiles by instrumental analysis and sensory studies. In: Chemistry of wine flavor. Waterhouse, A.L.; Ebeler, S.E., Eds; American Chemical Society, Washington, DC. 1998, pp. 39–52.

Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food. Chem.* **1997**, 45, 3027–3032.

Hartmann, P.J.; McNair, H.M.; Zoecklein, B.W. Measurement of 3-alkyl-2-methoxypyrazine by headspace solid-phase microextraction in spiked model wines. *American Journal of Enology and Viticulture,* **2002**, 53(4), 285-288.

Ickes, C.M.; Cadwallader, K.R. Effects of Ethanol on Flavor Perception in Alcoholic Beverages. *Chem. Percept.* **2017**, 10, 119–134.

ISO. (2007). 8589. Sensory analysis – General guidance for the design of test rooms. International Organization for Standardization.

Jung, D.M.; de Ropp, J.S.; Ebeler, S.E. Study of interactions between food phenolics and aromatic flavors using one- and two-dimensional 1 H NMR spectroscopy. *J. Agric. Food Chem.* **2000**, 48, 407–412.

Lytra, G.; Tempere, S.; de Revel, G.; Barbe, J.C. Impact of Perceptive Interactions on Red Wine Fruity Aroma *J. Agric. Food Chem*. **2012**, 60 (50), 12260-12269.

Merkle, S.; Kleeberg, K.K.; Fritsche, J. Recent Developments and Applications of Solid Phase

Microextraction (SPME) in Food and Environmental Analysis—A Review. *Chromatography,* **2015**, 2, 293-381.

Mitropoulou, A.; Hatzidimitriou, E.; Paraskevopoulou, A. Aroma release of a model wine solution as influenced by the presence of non-volatile components. Effect of commercial tannin extracts, polysaccharides, and artificial saliva. *Food Res. Int.* **2011**, 44, 1561−1570.

Moio, L.; Etievant, T.P.; Langlois, D.; Dekimpe, J.; Addeo, F. Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis. *J. Dair. Res.* **1994**, 61, 385-394

Organisation Internationale de la Vigne et du Vin (2015). Compendium of international methods of must and wine analysis (Organisation Internationale de la Vigne et du Vin: Paris, France).

Paravisini, L.; Guichard, E. Interactions between aroma compounds and food matrix. In Flavour: From Food to Perception, 1st ed.; Guichard, E., Salles, C., Morzel, M., Le Bon, A.M., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2017; pp. 208–234.

Pati, S.; Tufariello, M.; Crupi, P.; Coletta, A.; Grieco, F.; Losito, I. Quantification of Volatile Compounds inWines by HS-SPME-GC/MS: Critical Issues and Use of Multivariate Statistics in Method Optimization. *Processes,* **2021**, 9, 662.

Petrozziello, M.; Asproudi, A.; Guaita, M.; Borsa, D.; Motta, S.; Panero, L.; Bosso, A. Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. *Food Chem.* **2014**, 149, 197–202.

Piombino, P.; Moio, L.; Genovese, A. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res. Int.* **2019**, 116, 548–558.

Pittari, E.; Moio, L.; Piombino, P. Interactions between Polyphenols and Volatile Compounds in Wine: A Literature Review on Physicochemical and Sensory Insights. *Appl. Sci.* **2021**, 11, 1157.

Poncet-Legrand, C.; Cartalade, D.; Putaux, J.L; Cheynier, V.; Vernhet, A. Flavan-3-ol aggregation in model ethanolic solutions: incidence of polyphenol structure, concentration, ethanol content, and ionic strength. *Langmuir*, **2003**, 19, 10563–10572.

Pozo-Bayón M.Á., Reineccius G. Interactions Between Wine Matrix Macro-Components and Aroma Compounds. In: Wine chemistry and biochemistry. Moreno-Arribas, M.V., Polo, M.C. (Eds.). Springer, New York, 2009.

Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. Handobbok of Oenology, Vol I. John Wiley & Sons Ltd, Chichester, England, 2006.

Robinson, A. L.; Ebeler, S. E.; Heymann, H.; Boss, P. K.; Solomon, P. S.; Trengove, R. D. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, 57, 10313−10322.

Rodríguez-Bencomo, J.J.; Muñoz-González, C.; Andújar-Ortiz, I.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.Á. Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. *J. Sci. Food Agric.* **2011**, 91, 2484–2494.

Sáenz-Navajas, M.P.; Campo, E.; Cullere, L.; Fernandez-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010**, 58, 5574–5585.

Singleton, V.L.; Orthofer, R; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method. Enzymol.* **1999**, 299, 152–178.

Solms, J.; Osman-Ismail, F.; Beyeler, M. The interactions of volatiles with food components *Can. Inst. Food Sci. Technol.* **1973**, 6, 10-16.

Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L. Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Anal. Chim. Acta*, **2010**, 660, 183–189.

Villamor, R.R; Ross, C.R. Wine matrix compounds affect perception of wine aromas. *Annual Review of Food Science and Technology*, **2013**, 4, 1-20.

Villamor, R.R.; Evans, M.A.; Ross, C.F. Effects of Ethanol, Tannin, and Fructose Concentrations on Sensory Properties of Model Red Wines. *Am. J. Enol. Vitic.* **2013a**, 64(3), 342-348.

Villamor, R.R.; Evans, M.A.; Mattinsonc, D.; Ross, C.F. Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of

odorants in a model wine. *Food Res. Int.* **2013b**, 50, 38–45.

Voilley, A.; Beghin, V.; Charpentier, C.; Peyron, D. Interactions between aroma substances and macromolecules in a model wine. *Lebensmittel-Wissenschaft & Technologie,* **1991**, 24(5), 469-472.

Whiton, R.S.; Zoecklein, B.W. Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. *Am J Enol Vitic.* **2000**, 51, 379–382.

Zamora F. Biochemistry of Alcoholic Fermentation. In: Wine Chemistry and Biochemistry, Moreno-Arribas M.V.; Polo M.C. (Eds.); Springer, New York, NY, 2009.

Zanchi, D.; Vernhet, A.; Poncet-Legrand, C.; Cartalade, D.; Tribet, C.; Schweins, R.; Cabane, B. Colloidal dispersions of tannins in water–ethanol solutions. *Langmuir*, **2007**, 23, 9949–9959.

Chapter III – Impact of tannins on red wine aroma oxidation

The present chapter aims at studying and developing the third research question: exploring if polyphenols could act as a protection toward the oxidation of wine aromas. At the same time, within this experimental part, we added further knowledge to the role played by the polyphenols on the release and perception of wine VOCs. However, differently from the experiment exposed in Chapter II, in the present chapter we worked with exogenous oenological tannins.

Moreover, while results exposed in Chapter II refer to static analytical sensory and chemical methods and *in-vitro* conditions, here are reported results obtained through dynamic methods of both sensory and chemical analyses applied under *in-vivo* conditions.

For this purpose, we investigated the impact of two different commercial oenological tannins (i.e., proanthocyanidins and ellagitannins) – belonging to grapes and wood barrels, respectively - on wine flavour (mainly aroma) and volatiles release of a red wine before and after air exposition. Oenological tannins addition in winemaking is a long-used and common technological practice. Up to date, they are authorized by the International Organization of Vine and Wine (OIV) to facilitate the clarification/stabilization of wines and musts, to promote the expression, stabilisation, and preservation of colour in red wines, and to contribute to the antioxidant and antioxidasic protection of compounds of the wine (OIV-OENO 613-2019).

In order to link analytical measurements with sensory evaluation, we performed a real-time *in-vivo* study, coupling a dynamic sensory evaluation technique (TDS – Temporal Dominance of Sensations) with a dynamic chemical nosespace analysis using a Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS), during the consumption of the same red wine in six different conditions: the base wine, the base wine with ellagitannins, the base wine with proanthocyanidins, the oxidized base wine, the oxidized base wine with ellagitannins and the oxidized base wine with proanthocyanidins.

This chapter is an edited version of:

Effects of oenological tannins on aroma release and perception of oxidized and non-oxidized red wine: a dynamic real-time *in-vivo* study by coupling TDS to PTR-ToF-MS

Pittari, E.[1], Piombino, P.[1], Andriot, I.[2,3], Cordelle, S.[2,3], Feron, G.[2], Gourrat, K.[2], Le Quéré, J.L.[2], Neiers, F.[2], Moio, L.[1], Schlich, P.[2,3], Canon, F.[2,*].

[1] Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, 83100 Avellino, Italy

[2] Centre Des Sciences du Goût et de l'Alimentation (CSGA), AgroSup Dijon, CNRS, INRAE, Université de Bourgogne Franche Comté, F-21000 Dijon, France

[3] ChemoSens Platform, CSGA, F-21000 Dijon, France

* Corresponding author:

Francis Canon

Submitted (February 2021) to **Food Chemistry**, revised version under re-submission

1. Introduction

We have already reviewed (Background Section) that oxidation occurs as one of the main chemical phenomena affecting the organoleptic properties of wine during its evolution/ageing (Ugliano, 2013; Ferreira et al., 2014) and that early oxidative ageing is one of the main widespread worldwide defects in oenology (Ugliano, 2013; Franco-Luesma et al., 2019), corresponding to a short wine shelf-life. Oxidative transformation of wine compounds modifies the structure and the properties of molecules belonging to different chemical families, affecting compounds involved in wine colour and flavour. For example, oxidation tends to decrease the level of wine astringency, but also to modify its fruity and floral notes. Moreover, oxidized wines are characterized by the increase or the appearance of the following olfactory descriptors: raisin, overripe character, rancid, dried fruit, caramel, farm-feed, cooked vegetables, boiled potato, hay, sweet and Madeira/Porto (Escudero et al., 2000; Escudero et al., 2002; Silva Ferreira et al., 2003; Culleré et al., 2007; Ugliano, 2013).

It has been observed that wine oxidative notes could be more perceivable during tasting (retronasal) rather than sniffing (orthonasal) from the glass (Piombino et al., 2019) and that some VOCs involved in oxidative notes perception were better released simulating wine tasting in small sips (Genovese et al., 2015). This suggests that the perception of oxidative molecular markers can be impacted by factors affecting their portioning and release, such as the non-volatile matrix composition and saliva. Wine contains different classes of polyphenols (e.g., tannins), which exhibit antioxidant properties, thanks to their scavenging of reactive oxygen and nitrogen species and ion chelation (Waterhouse et al., 2016).

Besides their extraction during winemaking and oak-barrels ageing, both proanthocyanidins and hydrolysable tannins can be added to wine as oenological tannins. Their use in winemaking is a long-used and common technological practice. Up to date, they are only authorized by the International Organization of Vine and Wine (OIV) to facilitate the clarification/stabilization of wines and musts (OIV, 2015). Indeed, due to their hydroxyl groups on aromatic rings, tannins have also the properties to interact with different compounds and especially with proteins present in the wine, which are responsible for instability, or saliva of the consumer (Canon et al., 2013). These interactions can lead to aggregation and precipitation of the interactants (Canon et al., 2013). Moreover, during wine tasting, the aggregation of the mucosal pellicle by tannins is thought to be at the origin of astringency perception (Ployon et al., 2018), and it can also modify the ability of the mucosal pellicle to

interact with aroma compounds and change aroma persistence (Ployon et al., 2020). Indeed, tannins are also used by winemakers for other properties, such as increase of aroma persistence or antioxidant activity (Versari et al., 2013). As a result, a wide range of oenological tannins are present in the market. Their antioxidant capacity is one of the main researched properties to protect wines against oxidation (Versari et al. 2013; Magalhães et al., 2014). Oenological tannins can be very useful in protecting musts and white wines against browning and oxidation (Versari et al., 2013). However, their antioxidant capabilities are controversial, since tannins with different compositions can show very different antioxidant properties (Magalhães, et al., 2014; Vignault et al., 2018), and because tannins oxidation leads to the formation of reactive species such as *ortho*-quinones (Petit et al., 2019) that can modify wine VOCs patterns, as reviewed above (Background Section). These reactions can be at the origin of a decrease of volatile polyfunctional thiols concentration, responsible for varietal fruity notes of many young wines produced from different varieties (Darriet et al., 1995).

Ortho-quinones are also involved in the formation of odour active Strecker aldehydes (Keim et al., 2002). Thus, it appears that the addition of oenological tannins in wine can influence wine aroma perception through different mechanisms, which impact nature, concentration, and release kinetics of aroma compounds.

With this experiment, we wanted to bring to light the impact of this practice on wine flavour. Differently form the experiment in Chapter II, in this study we aimed at deciphering the effect of the addition of exogenous oenological tannins (i.e., proanthocyanidins and ellagitannins) on the sensory perception and aroma release of a red wine before and after air exposition.

In order to link analytical chemical measurements with sensory evaluation, we performed an in-vivo study, coupling Temporal Dominance of Sensations (TDS) (Pineau et al., 2009), a dynamic sensory method, with a dynamic approach of analytical chemistry consisting in the nosespace analysis by Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS). Measurements were carried out during the consumption of the same red wine in six different conditions (3x2 factorial design): the base wine, the base wine with ellagitannins, the base wine with proanthocyanidins, the oxidized base wine, the oxidized base wine with ellagitannins and the oxidized base wine with proanthocyanidins.

2. Materials and methods

2.1. Wine

A commercial Pinot Noir wine, obtained with a standard industrial process from a winery located in Burgundy wine region (France), vintage 2016, with no oak-barrels ageing, was selected as base wine for both *in-vivo* and *in-vitro* experiments. This wine was considered the base wine of the study (BW). The base analytical parameters of the wines (Table 1) (alcohol, residual reducing sugars, glycerol, titratable acidity, volatile acidity, pH, and total dry extract) were determined using a commercial WineScan™ analyser (FOSS A/S, Hillerød, Denmark) composed by an FTIR interferometer (Fourier Transform Infrared Spectroscopy technique) and the integration software (Foss Integrator) provided with built-in calibration curves. This technique is now widespread as a rapid tool for routine wine analysis, and its technical specifications and performances are described by OIV (2010). Before analysis, the samples were centrifuged (2700g ×5 min) to remove turbidity and CO2, if present. From the obtained results, net dry extract was calculated by subtracting the residual reducing sugars content from the total dry extract value (Giacosa et al., 2021).

Table 1. Base parameters of the base wine (BW).

Base wine parameters	Mean	Std. Dev.
Ethanol content (%v/V)	12.99 ±	0.01
Residual sugars (g/L)	0.18 ±	0.00
Glicerol (g/L)	6.58 ±	0.16
Titratable acidity (g tartaric acid/L)	5.21 ±	0.01
Volatile acidity (g acetic acid/L)	0.61 ±	0.00
pH	3.54 ±	0.06
Total dry extract (g/L)	26.07 ±	0.11

All the data are expressed as mean of two analytical replicates

2.2. Oxidation procedure

The oxidation procedure was conducted by saturating the wine samples with air, as previously described by Ferreira et al. (2015) with few modifications. In the specific, air saturation was performed by gentle shaking 250 mL of wine in a closed 500 mL flask for 10 s, successively opening the cup for 10 s to allow fresh air to enter and repeating the same operation two more times.

For the in-vivo experiments, the 250 mL of air-saturated wine were then transferred in dark amber glass bottles of 500 mL with a screwed cap, resulting in headspace volume to liquid volume (V_{HS}/V_L) ratio of 1, and directly stored in an incubator (XB112, France Etuves, Chelles, France) in the dark at +25 °C for seven days, when the first saturation cycle was considered complete (Ferreira et al., 2015). At that time, the samples were considered ready for *in-vivo* experiments.

For the *in-vitro* experiments, following the air-saturation, 5.5 mL volumes of each sample were aliquoted and distributed in screw capped vials of 11 mL, resulting in the same V_{HS}/V_L ratio equal to 1 as for the *in-vivo* part. Finally, the samples were stored in the incubator in the dark at +25°C for seven days. After seven days (t=1week), the saturation cycle was considered complete, the five vials representing oxidized samples were taken out from the incubator and analysed.

2.3. In-vivo experiments

2.3.1. Wine samples preparation

The BW was treated with two different commercial tannin extracts: i) a commercial extract of oak ellagitannins (Laffort, Bordeaux, France) at 5 g/hL that led to a wine coded as Base Wine Ellagitannins (BWE), and ii) a commercial grape seed extract rich in proanthocyanidins (Laffort, Bordeaux, France) at 20 g/hL, that led to the Base Wine Proanthocyanindins (BWP). These three samples (BW, BWE, BWP) were then submitted to the oxidation procedure described above to obtain the oxidized base wine (OW) and the oxidized base wine spiked with ellagitannins at 5 g/hL (OWE) and proanthocyanidins at 20 g/hL (OWP), resulting in six wine samples: BW, BWE, BWP, OW, OWE, OWP. These two concentrations have been chosen based on the recommendation provided by Laffort and through preliminary intra-laboratory sensory tests. For both tannins, we used the highest recommended concentration.

Tannins were added to 50 mL of BW 45 min before the experiment giving BWE and BWP. After 15 minutes of incubation at room temperature, 10 mL of samples, which correspond to 1 sip, were put into the glasses for sensory evaluation. Bottles were closed with a vacuum wine stopper and stored at 10 °C up to the next session. 10 mL of oxidized samples were taken from the bottles stored into the incubator and put into the glasses.

The samples were served in tulip shape 100 mL (±10) volume black glasses covered with a lid to avoid sample evaporation before sensory evaluation. Each sample was prepared in triplicate (3 glasses of 10 mL, each corresponding to 1 sip). Products were presented in an anonymous manner with random three-digit codes (using the same three-digit code for the replicate of each sample).

2.3.2. Subjects

The jury was composed of 17 subjects aged between 22 and 59 years (10 females – mean age= 39±13; 7 males – mean age= 42±13) recruited from the Centre des Sciences du Goût et de l'Alimentation (INRAE, Dijon, France) and selected based on their interest, motivation, and availability. They all have been informed and have signed a consent form. They all were wine consumers and had previous experiences in performing sensory tests on wine and TDS sensory measurements. They were asked not to drink any coffee or tea, not to smoke and not to eat any food (chewing-gum included) 1 h before the sessions.

2.3.3. Sensory analysis

2.3.3.1. Panel training

Considering that TDS sensory tests do not require lengthy training (Pineau et al., 2012), and that all participants had experience in TDS evaluation, only 2 training sessions were organized. During each session, subjects were asked to rinse their mouth firstly with a solution of apple pectin (0.1%) (Sigma-Aldrich, Steinheim, Germany), secondly with a solution of sodium bicarbonate (1%) provided by a pharmacy in Dijon (Burgundy, France) and, thirdly with mineral water (Evian, Évian-les-Bains, France) (Esteban-Fernández et al., 2016) and to wait 60 s between each sample.

Session 1. This session aimed at generating a list of aroma descriptors. Judges were asked to assess and describe 7 wine samples in terms of aroma characteristics. The 7 wine samples were: 1) a Santenay 1er Cru 2016 (BW2), obtained from the same winery located in Burgundy (France) than BW; 2) BW2E; 3) BW2P; 4) OW2; 5) OW2E; 6) OW2P; 7) 11 days oxidized BW2.

Session 2. This session aimed at familiarizing the judges with the list of descriptors previously generated, in order to reach a consensus on the definition of each attribute. Judges were asked to assess the aroma characteristics of 9 wine samples, using the list of attributes previously generated, and to score their intensity on the following numerical category scale: 1=very low, 2=low, 3=medium, 4=high, 5=very high. The sample

set was composed as following: 1) BW2; 2) BW2P; 3) OW2; 4) OW2E; 5) OW2P; 6) 10 days oxidized BW2; 7) BW; 8) BWE (ellagitannins at 5 g/hL); 9) BW2 + ellagitannins at 10 g/hL.

During the two training sessions, the panellists were asked to score astringency and tastes (sweet, acid, and bitter) intensities of the samples using the 5-point intensity scale described above. At the end of each training session, the perceived sensations were discussed with the participants to prevent overlapping and redundancies among terms and to help their memorization.

2.3.3.2. Evaluation

General Procedure

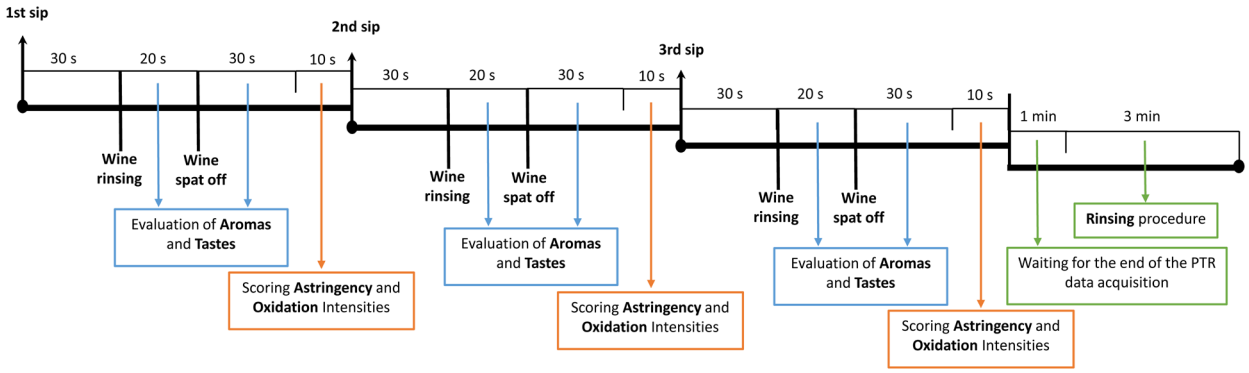
TDS and Nosespace analysis (NS) were performed simultaneously and required individual sessions that were conducted in an air-conditioned room at 23 °C (±0.5). Each session lasted approximately 1 h. During each evaluation session, subjects were connected to a Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS). They were asked to evaluate a single-sip warm-up sample that preceded the six products (3 glasses of one sip per sample): BW, BWE, BWP, OW, OWE and OWP. The six products were analysed in duplicate by each judge; therefore, for each panellist, two individual sessions were performed in two different days. The complete design for the experiment was carried out in 9 days. The presentation orders were set up following a Williams Latin square experimental design balancing order and position effects.

The protocol of the sensory evaluation of one sample is represented in Figure 1. Briefly, the sensory evaluation consisted in evaluating three consecutive repetitions of the same sample. Thus, for each sample, three glasses containing one sip of 10 mL were presented to the subjects. The consumption of the three glasses had to respect a strict protocol, which has been programmed using TimeSens 1.0. software (INRAE, Dijon, France). TimeSens controlled the sequence of events. For each event, instructions and timing were displayed on a screen in front of the subject.

The protocol of consumption consisted of waiting 30 s before putting the first sample in the mouth, allowing to record the blank of the composition of the air from the nasal cavity by the PTR-ToF-MS. Dual-TDS evaluation started just after the panellists took the first sip in their mouth and click on "*Put in the mouth*" button displayed on the screen. Then, they had to keep the wine in mouth during 20 s, while selecting the perceived dominant attributes as a function of time. Inspiration of air by the mouth was allowed. After 20 s, a message

indicated to the subjects that they had to spit off the wine. This step was validated once the subjects clicked on the appropriate button. The evaluation of the dominant sensations continued during 30 s. If the panellists did no longer perceive any aroma and/or taste, they were asked to click on "*No/No more aromas*" and/or "*No/No more tastes*" buttons. After these 30 s, the panellists had 10 s to evaluate astringency and oxidation intensities using two continuous intensity scales (from very low to very high). Then, they had to repeat this sequence two additional times: waiting 30 s, putting the sample in the mouth, and keeping it in mouth during 20 s while evaluating, spitting out the sample, continuing to evaluate the sample for additional 30 s and evaluating astringency and oxidation (10 s). At the end of the 3rd repetition, panellists were asked to wait 1 minute before the end of the PTR-ToF-MS acquisition. The whole TDS evaluation for one product lasted around 5 minutes in total. Between two successive samples, the judges had 3 minutes to clean their mouth as above exposed: firstly rinsing with a solution of apple pectin (0.1%), secondly with a solution of sodium bicarbonate (1%) and, thirdly with mineral water (Evian, Évian-les-Bains, France).

Figure 1. Dual-TDS-Multi Sips protocol followed by the panellists for products 'evaluation.



Sensory analysis

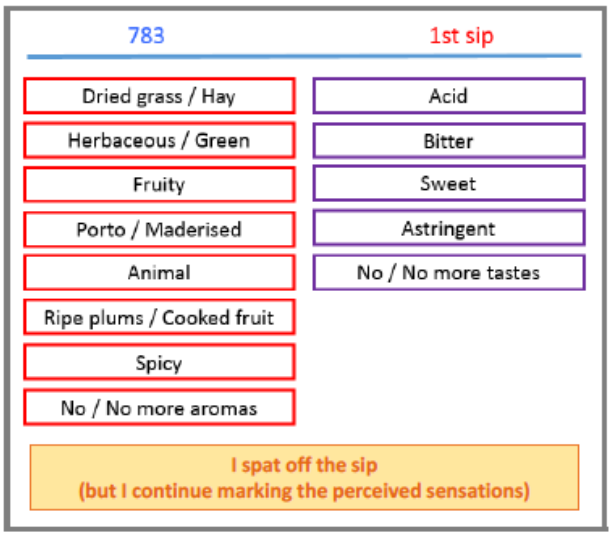
Data were recorded by TimeSens 1.0 (INRAE, Dijon, France). The Dual-TDS screens were designed in French and translated to English for foreign judges.

Dual - Temporal Dominance of Sensations - Multi sips: Dual-TDS consists of an arrangement on the computer screen of attributes belonging to two different sensory modalities in two different columns (Figure 2). Using this type of sensory analysis method, the judges are instructed that they can have only one dominant attribute at the same time in each column at any time. In other words, the selection of a dominant attribute switches off

only the dominant attribute from the same column and not the other one (Schlich, 2017), defining as dominant a sensation that triggers the most attention at any given moment. The subjects had the information that an attribute could be dominant several times during the evaluation and that it was not necessary that all the attributes were selected as dominant during the evaluation of each product.

The following seven aroma attributes were presented simultaneously with the taste attributes on the computer screen, as represented in Figure 2: Dried grass/Hay, Herbaceous/Green, Fruity, Porto/Maderised, Animal, Ripe plums/Cooked fruits and Spicy. For each judge, the attributes were displayed in the same order during the whole sensory evaluation. However, their orders were randomised over the subjects to avoid the risk that they choose preferentially the attributes from the top of the list (Pineau et al., 2012).

Figure 2. Measurement screenshot example of the Multi-TDS procedure (English version).



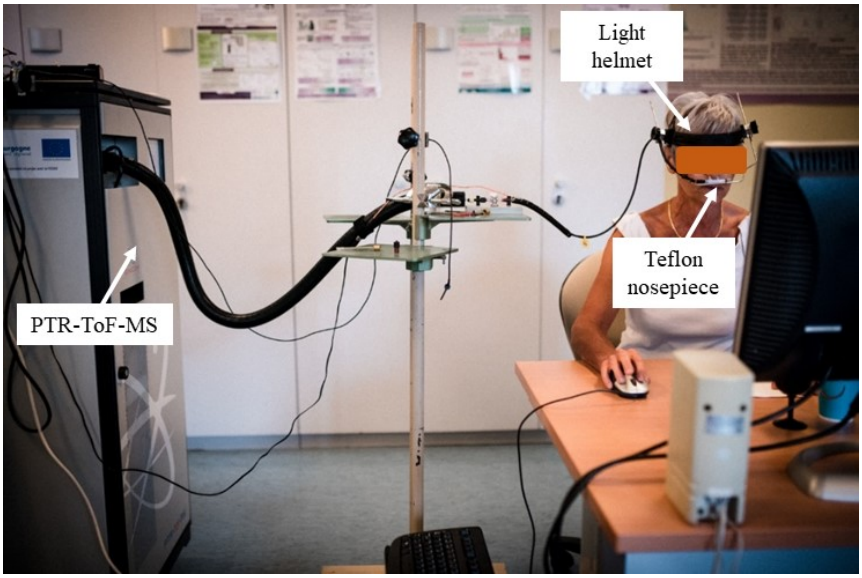
2.3.4. Data treatments

Dual-TDS is equivalent to two TDS run simultaneously. Thus, flavour and taste TDS data were each one analysed separately by the usual TDS curves (Pineau et al., 2009). To compare two products, some TDS curves of differences (Schlich & Pineau, 2016) were produced. TDS curves of differences are obtained as the evolution along time of the difference between dominance rates of two products. Only points corresponding to differences significantly (binomial test, p=0.10) higher or lower than 0 were produced.

2.3.5. Instrumental conditions

The monitoring of the individual's nosespace was done through a Teflon nosepiece, that connected both nostrils of the subjects via a light helmet to a Proton Transfer Reaction-Mass Spectrometer (PTR-MS) instrument equipped with a Time-of-Flight (ToF) analyser (PTR-ToF 8000, Ionicon Analytik, Innsbruck, Austria), as represented in Figure 3.

Figure 3. Representation of the monitoring of the individuals' nosespace through Proton Transfer Reaction-Mass Spectrometer (PTR-MS), while they were performing the sensory evaluation.



Sampling was performed at a total flow rate of 400 mL/min with the transfer line maintained at 110 °C. The helmet allowed subjects to move freely their head during the experiment. Nosespace analysis (NS) was recorded at the same time than the evaluation of Temporal Dominance of Sensations evaluation (TDS). $[H_2O+H]^+$ was used as reagent ion. Parameters of the PTR-ToF-MS instrument were as following: drift pressure of 2.31 mbar, drift temperature of 80 °C, and drift voltage of 390 V, resulting in electric field strength to number density ratio (E/N ratio) of 90 Townsend (Td, $1Td=10^{-17}$ $V.cm^2$). Data were collected using the TofDAQ software provided by the manufacturer of the PTR- ToF- MS. Data acquisition was performed at 1 mass spectrum ranging from *m/z* 0 to 226 per 0.100 s.

2.4. In-vitro experiments

2.4.1. Wine samples preparation

To avoid any bottle effect, three bottles of BW (750 mL) were mixed (final volume: 2250 mL). Successively, 6x350 mL of BW were transferred in 500 mL volume flasks. Four BW samples were mixed with: i) ellagitannins at 5 g/hL and 20 g/hL that led to wines coded as BWE1 and BWE2, respectively, and ii) proanthocyanidins at 20 g/hL and 40 g/hL, that led to the BWP1 and BWP2 wines, respectively. Tannins were added directly to the 350 mL volume wines and left in contact with them for 15 minutes. The two other samples were used as an oxidized reference without tannins and a reference conserved under nitrogen atmosphere. BW, BWE1, BWE2, BWP1 and BWP2 represented the five starting points of the oxidation period (t=0). A volume of 1 mL of each condition was sampled for the analyses and stored in the fridge at +2 °C and took out at the analysis time.

The oxidized wine samples were prepared by submitting the remaining volume of the five wine samples to one week oxidation, as reported above (Section 2.2). The following samples represented the first-week oxidations conditions (t= 1 week): i) oxidized base wine (OW), ii) oxidized base wine spiked with ellagitannins at 5 g/hL (OWE) and at 20 g/hL (OWE2), iii) oxidized base wine spiked with proanthocyanidins at 20 g/hL (OWP) and 40 g/hL (OWP2) and iv) based wine under nitrogen atmosphere (OWN). OWN was stored in the fridge (+2 °C) and took out at the analysis time (t= 1 week).

2.4.2. Aromas solution preparation

An aroma solution was prepared for checking the instrumental repeatability throughout the analyses. Four ketones were chosen: 2-pentanone, 2-hexanone, 2-heptanone and 2-nonanone. They were all purchased from Sigma-Aldrich (Steinheim, Germany). Four independent stock solutions were prepared in absolute ethanol. From those solutions, 2 mL vials were prepared adding each aroma compound to a 13% ethanol solution to obtain a mixture of ketones at a final concentration of 0.1 µmol/L for each aroma compounds, strictly avoiding any headspace. They were stored in the fridge at -80 °C until the analysis sampling.

2.4.3. Volatiles measurement

2.4.3.1. PTR-ToF-MS parameters

Samples volatile compounds were analysed by direct injection – HS analysis. All the measurements were performed using a commercial PTR-ToF-MS instrument (PTR- ToF 8000, Ionicon Analytik GmbH, Innsbruck, Austria) with $[H_2O+H]^+$ as reagent ion (O_2^+ signal intensity was ca. 0.5% of the $[H_2O+H]^+$ one). Succeeding several preliminary tests, parameters of the PTR-MS instrument were chosen and set up as following: drift pressure of 2.31 mbar, drift temperature of 80 °C, transfer line temperature 110 °C and drift voltage of 390 V, resulting in electric field strength to number density ratio (E/N ratio) of 90 Townsend (Td, $1Td=10^{-17}$ V.cm^2). Data were collected using the TofDAQ software provided by the manufacturer of the PTR-ToF-MS. Data acquisition was performed at 1 mass spectrum ranging from *m/z* 0 to 226 per 0.100 s.

For each wine samples, 300 µL were transferred into a 20 mL glass vial for the analyses. For aroma solutions, 1 mL was sampled and transferred into a 20 mL glass vial for the analyses. A new vial was opened for each analysis.

The vials were closed by a 3-way cap with silicon septum. A first way was connected to a Tedlar® bag containing wet air. A second way was connected to the PTR-ToF-MS. Aroma injection was performed through the third way. Two 3-way automatic valves were used to direct the airflow way through two parallel circuits. The circuit connected to the glass vial with the sample is called “indirect”, while the second circuit, directly connected to the Tedlar® bag, is called “direct”. The experiment started with the circuit in direct position. Then, the circuit was turned to the indirect position and the air flow from the Tedlar® bag swept the glass vial headspace to the PTR for 2 min. The composition of the gas was analysed by PTR-ToF-MS analysis.

The measurement order followed a Williams Latin square experimental design, and all the samples, including the aromas solution, were analysed in triplicates.

2.4.4. Data analysis

Mass spectra analysis was performed using IgorPro 6.36 (WaveMetrics, Inc. Portland, USA) with a homemade procedure (Analyse_PTRMS_1.06.02.ipf). To guarantee high mass accuracy throughout the analysis, the mass scale was calibrated following the peaks of known ions ($[H_2{}^{18}O+H]^{1+}$, *m/z*=21.022; $[NO]^{1+}$, *m/z*=29.997; $[C_5H_8+H]^{1+}$, *m/z*=69.069). Area through the time of 194 ions have extracted giving the corresponding curve of

release. For all curves of release the average background signal during the 30 s before introduction of the sample was subtracted for both in-vivo and in-vitro experiments. The curves have been divided in three depending on the time of the respective repetitions. The area under the release curve has been extracted for the 0-50 s period and every 5 s between 0 and 80 s for all repetitions of all experiments. Background subtraction led to negative areas, suggesting that the signal was not coming from the samples. Thus, we eliminated all ions having more than 5 negative areas over all the recorded release curves giving a list of 101 ions. In order to avoid effect due to changes in the ionization condition, we also removed all experiments exhibiting large variations of the amount of $[H_2O+H]^+$ reactant ions. After, this removing there was not anymore significant differences of the amount of $[H_2O+H]^+$ for all selected files.

2.4.5. Statistical analyses

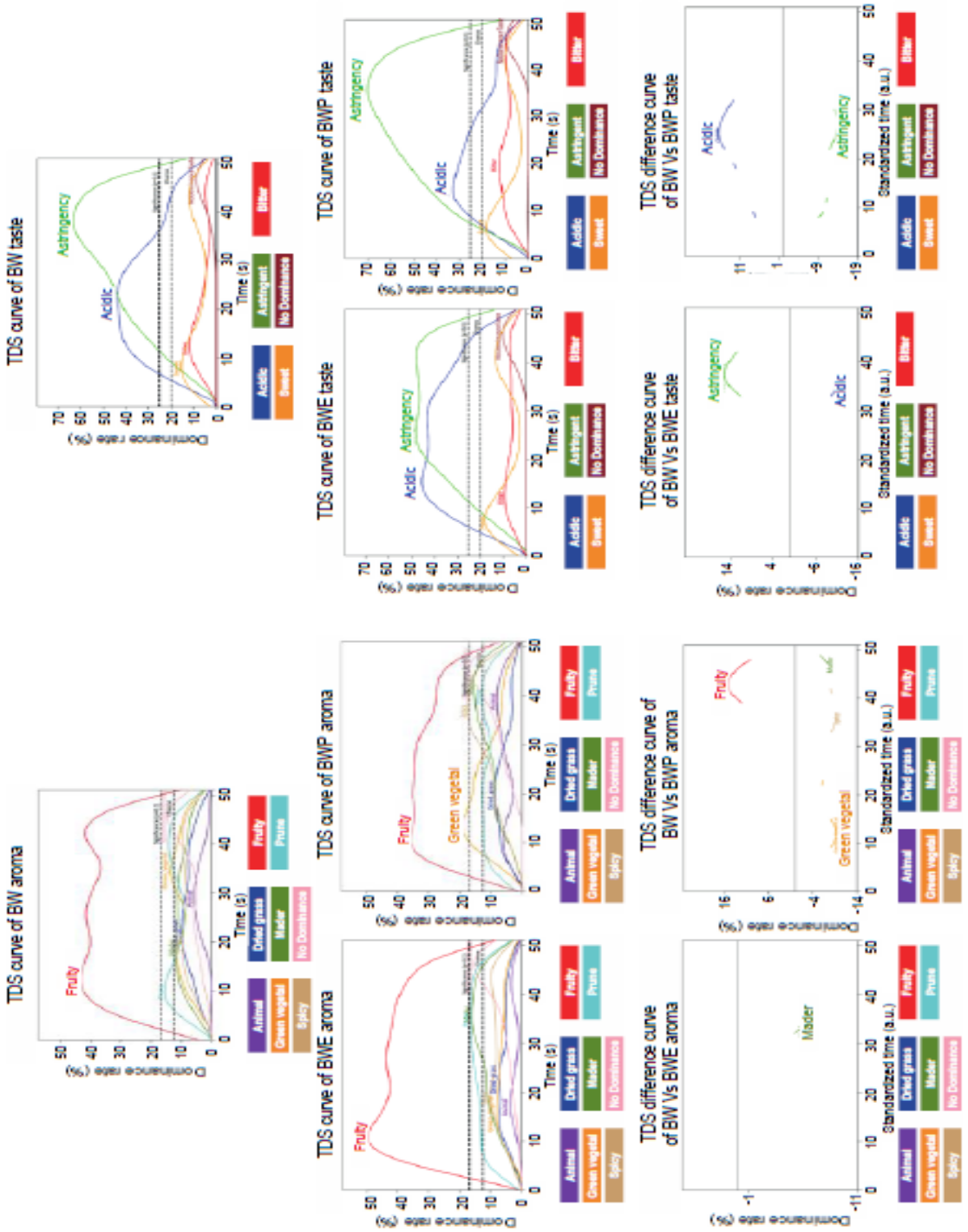
For each studied ion, its 0-50 s area under the curve was analysed with a repeated mixed model of ANOVA using the procedure MIXED from the SAS software. The model featured wines (6 levels) and sips (3 levels) as fixed effects, while panellist and its interaction with wine and sip were random effects with an instructed covariance matrix between them. The sip factor was declared as repeated within panellist by wine and replication with an unstructured covariance matrix. Estimation of the model was done by restricted maximum likelihood (REML). Sip effect was significant for most ions denoting evolutions over time. However, sip by wine interactions were never significant denoting that these evolutions were the same across wine for every ion. Therefore, sip effects will not be reported here, but contributed to a better estimation of the model. Wine effect was significant at p=0.05 for 8 ions and at p=0.15 for 11 others. However, contrast effects comparing each of the 3 wines to its oxidized version were also investigated, as well as contrast effects comparing each pair composed of two of those 3 oxidation effects. Finally, a list of 23 ions featuring either product or contrast oxidation effects was obtained. To compare TDS to PTR-MS results, the 5 s areas under the curve for the 0-50 s period of the 23 affected ions were submitted to a Student t-test (alpha=0.05) as a function of the condition compared.

3. Results and Discussion

3.1. Effect of oenological tannins on base wine flavour perception

TDS curves of the non-oxidized wines with (BWP, BWE) or without (BW) oenological tannins are presented in Figure 4.

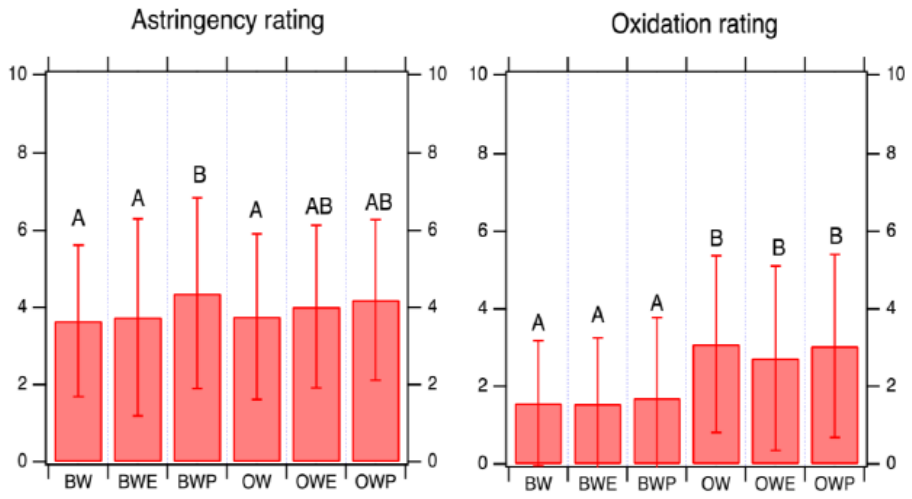
Figure 4. Dominance evolution of the sensory perceptions
of aroma and taste/astringency sensations for BW, BWE and BWP.



Dual TDS-analysis of non-oxidized wines reveals that the three samples have a very similar pattern of dominant sensations through time. Regarding aromas characteristics, fruity is the dominant attribute for the three wines while the dominant attributes for the in-mouth sensations are astringency and acidity. The fruity attributes (i.e., red berries) correspond to the attribute generally reported for non-oaked Pinot Noir wines from Burgundy.

The main difference is observed for BWP that presents a higher dominance of astringency particularly from 20 s, which is typically the time required to reach the maximum of astringency intensity. As a result, BWP sample appears slightly less acid and fruity than BW. Astringency ratings by the subjects at the end of the TDS evaluation confirmed that BWP has a significant higher level of astringency than BW and BWE, which are rated with similar intensities (Figure 5). This result might be explained by the fact that BWP contains the highest tannins concentration (20 g/hL of proanthocyanindins addition against 5 g/hL of ellagitannin for BWE). The decrease of acidity intensity can be at the origin of the slight decrease in the fruity aroma perception, as acidity can impact the perception of fruity sensation (Bonnans & Noble, 1993). Nevertheless, this result indicates that the addition of the two oenological tannins has almost no effect on the perception of BW flavour through the period 0-50 s, which is a prerequisite for our subsequent analyses.

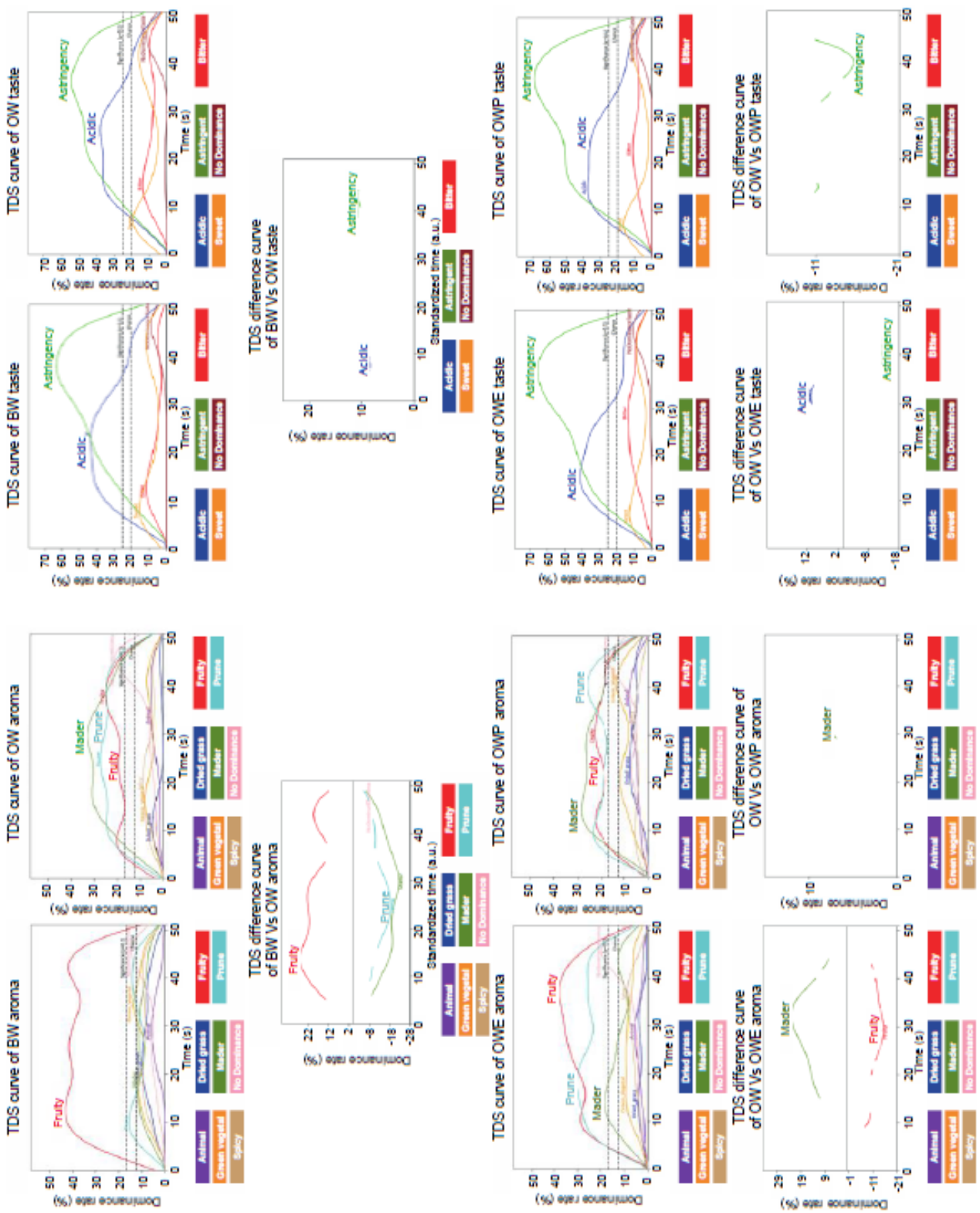
Figure 5. Astringency and oxidation ratings of the different wines using two continuous intensity scales (from very low to very high). Different letters refer to significant differences tested by ANOVA followed by multiple comparison Tukey HSD post-hoc test ($p>0.05$).



3.2. Effect of oxidation on wine flavour perception

TDS curves of the base wine prior (BW) and after oxidized (OW) in the presence of the two kind of tannins (OWP, OWE) are presented in Figure 6. Dual TDS-analysis of the oxidized wines reveals that oxidation has almost no effect on the pattern of dominance of taste and astringency sensations. These observations are confirmed by the astringency ratings (Figure 5). The perception of astringency has been rated significantly more intense in BWP than in BW and BWE, after oxidation only a trend is observed, with no significant differences among the three oxidized samples. This suggest that the observed changes in aroma perception could slightly modulate astringency perception. Indeed, regarding aroma characteristics, oxidation significantly impacts the pattern of dominant sensations of aroma through time. Non-oxidized wine BW is dominated by the fruity attribute, while OW and OWP are dominated by maderised, prune and fruity attributes. OWE is dominated by only two attributes: prune and fruity. The comparison between BW and OW reveals that in BW the fruity attribute is significantly more important during almost all the sensory evaluation while prune and maderised attributes are significantly dominant in OW. In OW the maderised attribute is slightly more dominant than the prune note. This result agrees with the previously reported effects of oxidation, which leads to a decrease of fruity notes and the appearance of oxidative attributes such as maderised/Porto or prune (Culleré et al., 2007; Escudero et al., 2000; Escudero et al., 2002; Silva Ferreira et al., 2003; Ugliano, 2013). Oxidation ratings by the subjects at the end of the TDS evaluation confirmed that OW samples have a significant higher level of perceived oxidation than BW (Figure 5). Concerning the effects of oenological tannins addition prior to oxidation, proanthocyanidins show no effect on the dominance of aroma sensory attributes. At the opposite, compared to OW, ellagitannins induce a decrease of maderised dominance while increasing the fruity one; they show no effect on prune attribute. The different effect observed for proanthocyanidins and ellagitannins could result from different aspects. The base wine (BW) was not aged in oak-barrels meaning that ellagitannins were not present, with their addition being therefore more impacting compared to the addition of proanthocyanidins, already present in BW due to their origin from grape berries. Another reason could be linked to a different impact of the two classes of tannins on the release of wine aromas, as tested in the following paragraphs.

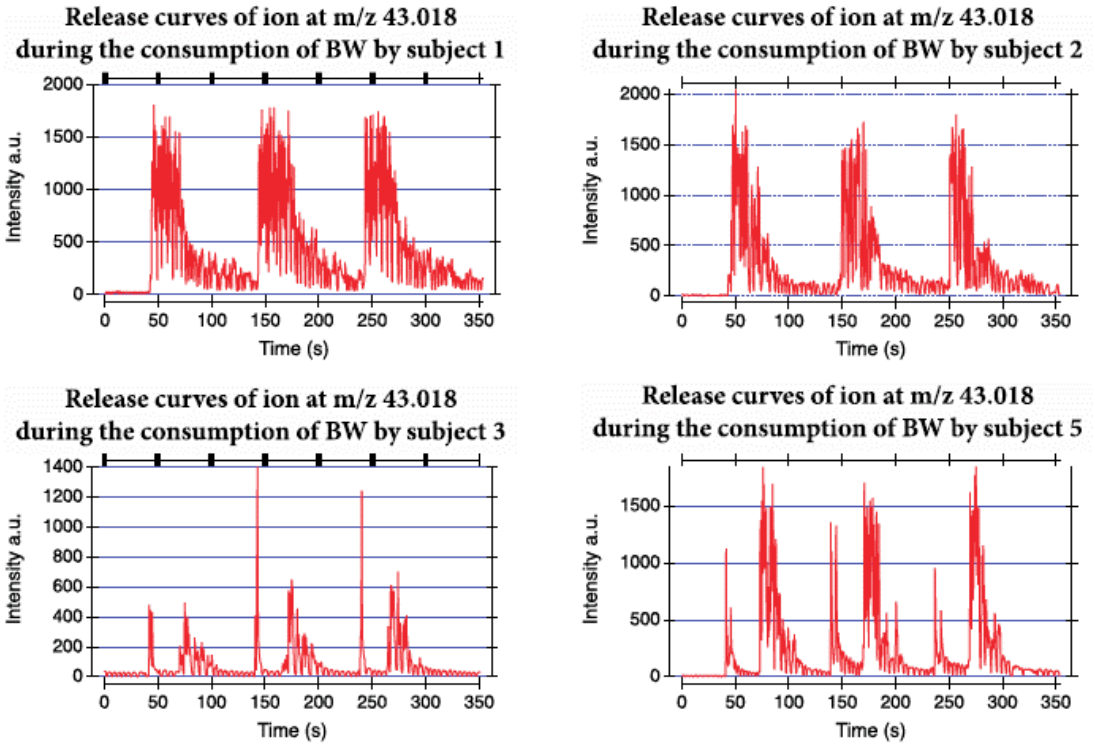
Figure 6. Dominance evolution of the sensory perceptions of aroma and taste/astringency sensations for BW, OW, OWE and OWP.



3.3. Effect of oenological tannins on in-mouth release

In-mouth aroma release is a dynamic process that impacts the variation of the temporal dominance of sensations. In this study, we wanted to investigate if aroma release can be linked to TDS evaluation. All through the dual-TDS experiments, the nasal cavity of the subjects was connected to a PTR-ToF-MS allowing a real-time recording of the release of aroma compounds during the dynamic sensory evaluation of the different wines. Typical release curves are presented in the Figure 7 for ion at *m/z* 43.02. The figure suggests that the release curves of the ion are similar for the same subject while showing interindividual variability. This appears as an interesting research topic that should require further analysis in the future.

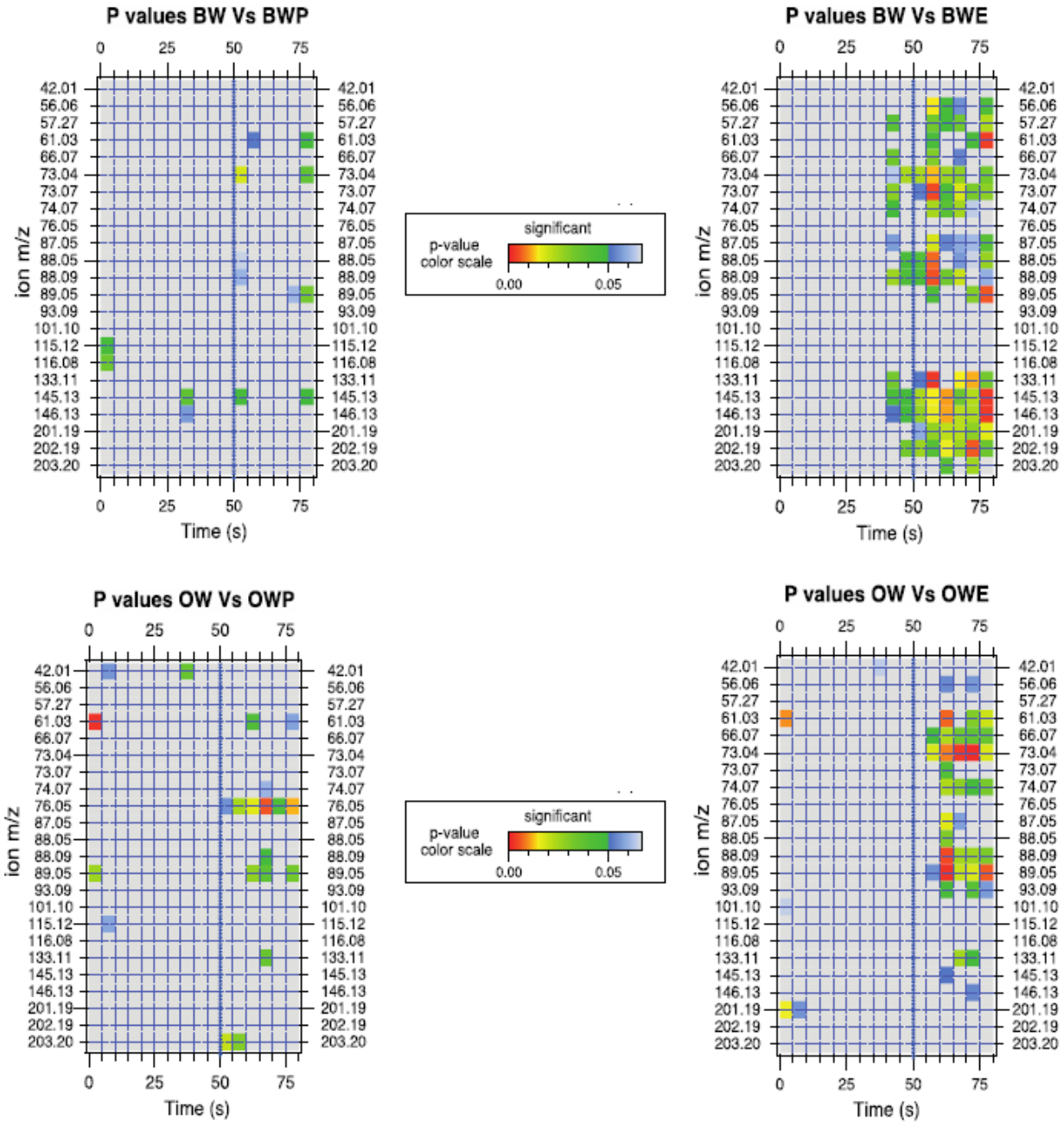
Figure 7. Release curve of ion at m/z 43.018 during the consumption of BW by four different subjects.



The 0-50 s areas under the curve were submitted to a mixed model of ANOVA as described in Section 2.4.5, giving a list of 23 ions significantly affected by the type of wine. To compare TDS and PTR-ToF-MS data, the areas under the curve of the 23 ions were extracted every 5 s from 0 to 80 s and then submitted to a student t-test comparing two different conditions. On the top of Figure 8 is presented the comparison of BW Vs BWE

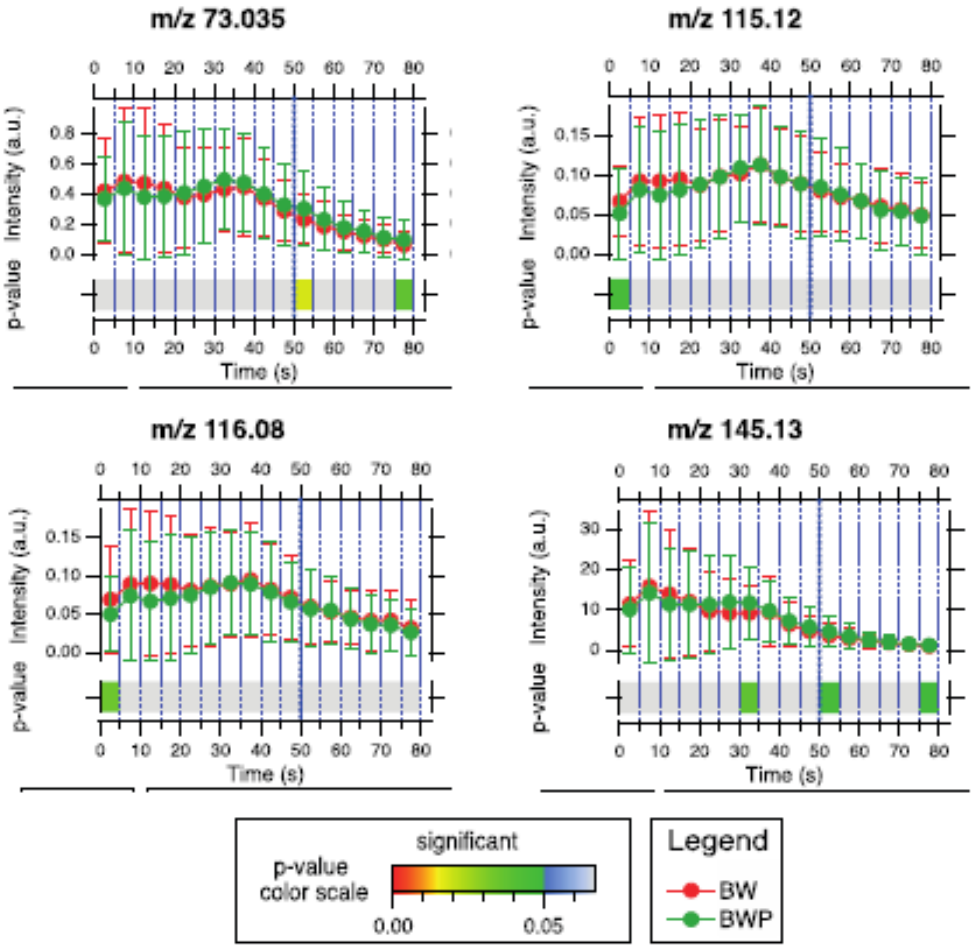
and BW Vs BWP. Over the 0-50 s period, very few differences are observed, indicating that tannins addition did not affect the release of aroma compounds in that specific analysis timing.

Figure 8. Comparison of aroma release of BW Vs BWP, BW vs BWE, OW vs OWP and OW vs OWE. Matrix of the t-test of BW Vs BWP, BW vs BWE, OW vs OWP and OW vs OWE of the areas under the curve every 5 s from 0 to 80 s of the 23 significantly affected ions.



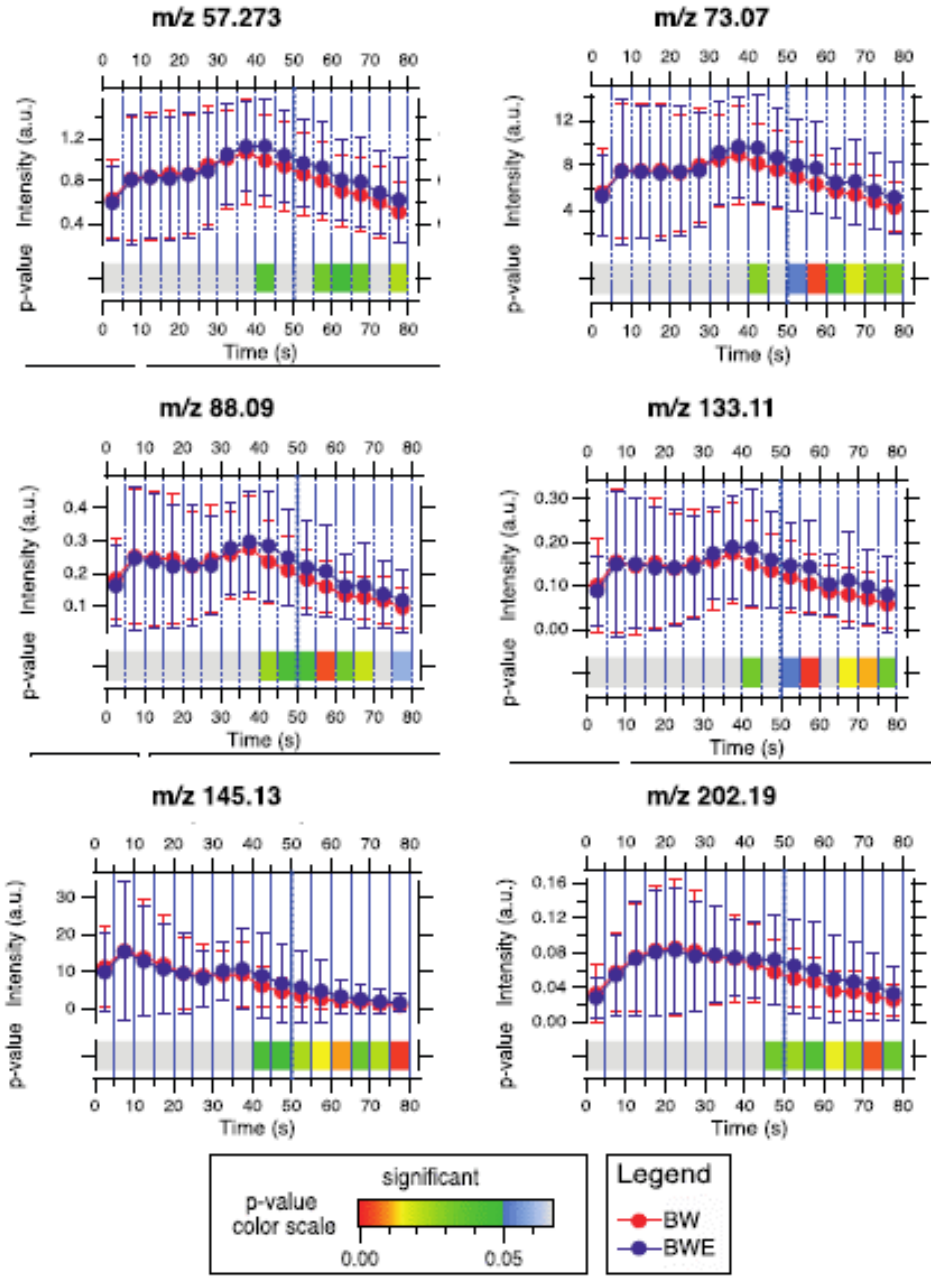
The most impacted ions are represented in Figures 9 and 10. This result agrees with the TDS results, which showed almost no impact on the dominance of sensations over this period (Figure 4).

Figure 9. Comparison of aroma release of BW Vs BWP. Average areas under the curve every 5s from 0 to 80 s for the main significantly affected ions with the respective standard deviations.



However, regarding the 50-80 s, numerous significant differences are observed, particularly concerning the comparison of BW Vs BWE (Figure 10). It is observed that ellagitannins addition increases the release of aroma compounds through this analysis timing, suggesting an enhancing effect of ellagitannins on aroma persistence. This could be explained by the fact that tannins with different nature can differently interact with aroma compounds, affecting their release, as recently reviewed (Pittari et al., 2021). Moreover, aroma compounds can also interact with the oral mucosa (Ployon et al., 2020), and these interactions could be affected by cross-molecular interactions of tannins with the mucosal pellicle, leading to the aggregation of the mucosal pellicle (Ployon et al., 2018). However, as aroma persistence (i.e., 50-80 s) was not evaluated by TDS sensory analysis, further trials to confirm this interesting outcome are necessary.

Figure 10. Comparison of aroma release of BW Vs BWE. Average areas under the curve every 5 s from 0 to 80 s for the main significantly affected ions with the respective standard deviations.



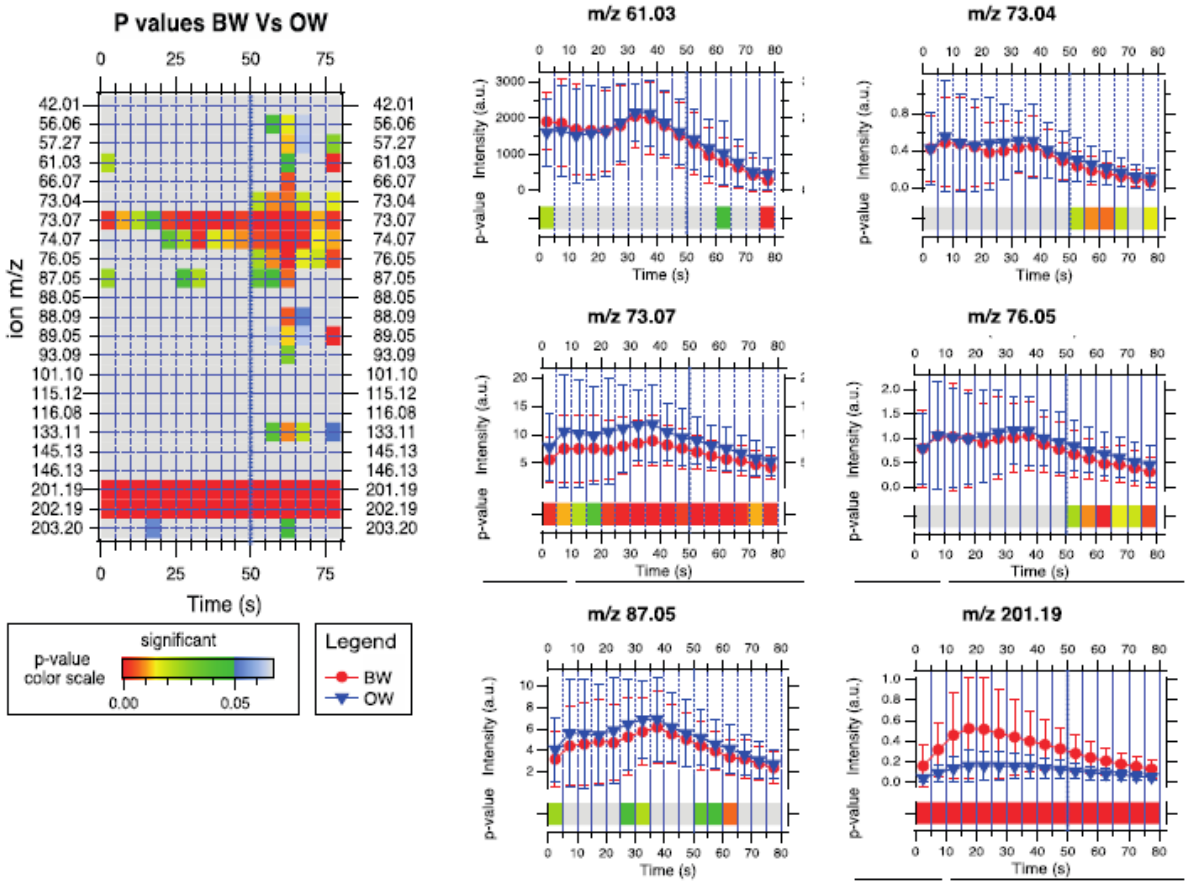
3.4. Effect of wine oxidation on in-mouth aroma release

Figure 11 presents the p-values resulting from the t-test comparing the base wine prior (BW) and after oxidation (OW) as a function of time (every 5 s) for the 23 ions, which are significantly affected by the type of wine during the TDS evaluation period (0-50 s). It reveals that for the 0-50 s period only 4 ions (61.03, 73.07, 87.05, and 201.19) + 2 isotopes [74.07 (^{13}C isotope of 73.07) and 202.19 (^{13}C isotope of 201.19)] are

significantly affected by the oxidation of the base wine when considering areas under the curve for periods of 5 s. The mean 5s-areas of the 4 affected ions are also presented as a function of time with the ones of two other ions (*m/z* 73.04 and 76.05), which show significant differences during the 50-80 s period. These curves show that among the 4 ions with significant differences during the TDS evaluation (0-50 s), the release of the ion 201.19 and of its isotope 202.19, is lower during the consumption of OW. The release of the 432 ion 61.03 is lower during the first 20 s of OW tasting, then increasing until 80 s. These two ions (201.19 and 61.03) can be tentatively attributed to the protonated species of ethyl decanoate ($[C_{12}H_{24}O_2+H]^{1+}$) and acetic acid ($[C_2H_4O_2+H]^{1+}$) (Deuscher et al., 2019), respectively. At the opposite, ions with *m/z* 73.07 and 87.05, which can be tentatively attributed to isobutyraldehyde ($[C_4H_8O^+H]^{1+}$) (Campbell-Sills et al., 2016) and butane-2,3-dione or isovaleraldehyde ($[C_4H_6O_2+H]^{1+}$ or $[C_5H_{10}O+H]^+$) (Deuscher et al., 2019), are more released in OW. Ethyl decanoate is an important wine ester contributing to wine aroma. Its organoleptic profile can be described as fruity, apple, grape (Waterhouse et al., 2016). Together with ethyl hexanoate and ethyl octanoate, ethyl decanoate is considered as being a highly positive aroma compound of young wine "bouquet", introducing fruity flavour notes (Waterhouse et al., 2016). Thus, the decrease of the fruity attribute in OW compared to BW in TDS experiment could be linked to the decrease of ethyl decanoate during wine oxidation. During the parallel *in-vitro* experiment (no saliva) conducted by Headspace - Solid Phase Microextraction – Gas Chromatography - Mass Spectrometry (HS-SPME – GC-MS) analyses (data not shown), we observed a similar result, confirming a significant lower concentration of ethyl decanoate, together with other important wine esters (e.g., ethyl butanoate, 2- and 3-methylbutyrate, hexanoate, octanoate, isoamyl and hexyl acetate), in OW compared to BW. At the opposite, the higher perception of maderised attribute is probably linked to the increase of aldehydes such as isobutyraldehyde or isovaleraldehyde during wine oxidation (Figure 11) as previously observed (Bueno et al., 2016). Indeed, aldehydes are the main cause of the development of oxidation-related off-odours and wine aroma deterioration (Bueno et al., 2016; Ugliano, 2013). Isobutyraldehyde (2-methylpropanal) and isovaleraldehyde (3-methylbutanal) are Strecker aldehydes. Strecker aldehydes can be formed i) from the corresponding precursor alcohols by peroxidation (San Juan et al., 2012) and ii) via Strecker degradation of the corresponding precursor amino acid as secondary reactions of the *ortho*-quinones derivatives formed through the oxidation of wine polyphenols by polyphenoloxidases and/or

molecular oxygen (Rizzi, 2006). The Strecker degradation of amino acids is described as a result of the Maillard reaction and involves the interaction of sugar-derived α-dicarbonyl compounds with free amino acids. In presence of α-dicarbonyl compounds, the amino acid is decarboxylated and deaminated, forming an aldehyde with one carbon atom less than the amino acid and known as "Strecker aldehyde" (Keim et al., 2002). Carbonyl compounds exist in all types of wines, particularly in red wines and in wines that undergo malolactic fermentation. Glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione are the principal α-dicarbonyl compounds found in wine but only α-diketones are relatively abundant in wine. Typically, α-dicarbonyls with n=0 are reported as Strecker degradation reagents but, in principle, any dicarbonyl compound with extended conjugation (n>0) can be used (Rizzi, 2006). The latter structural category can be extended to include *ortho*-quinones, particularly abundant during oxidation processes (Rizzi, 2006).

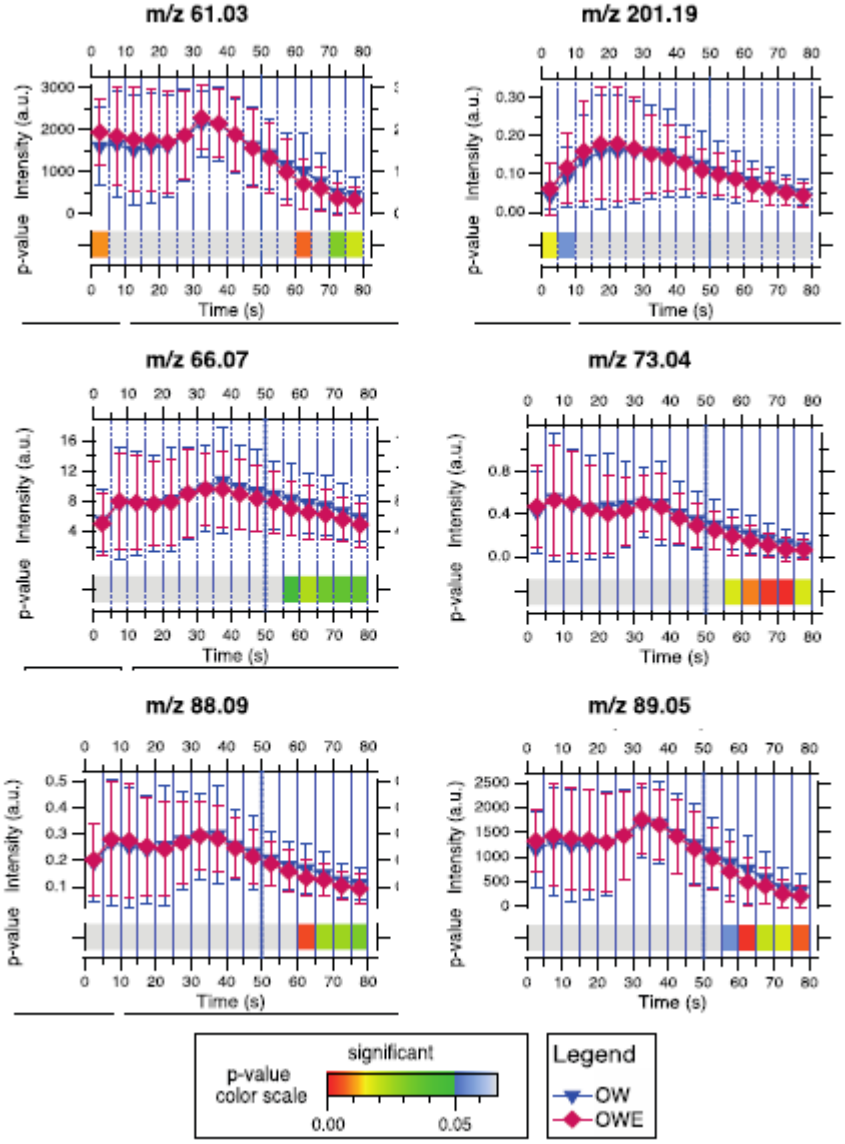
Figure 11. Comparison of aroma release of BW Vs OW. Matrix of the t-test of BW Vs OW of the areas under the curve every 5 s from 0 to 80 s of the 23 significantly affected ions. Average areas under the curve every 5 s for the main significantly affected ions with the respective standard deviations.



3.5. Effect of oenological tannins on in-mouth aroma release of oxidized wine

On the bottom of Figure 8 are also presented the p-values resulting from the t-test comparing the oxidised wine prior (OW) and after the addition of the types of tannins (OWP or OWE) as a function of time (every 5 s) for the 23 ions that are significantly affected by the type of wine during the TDS evaluation period (0-50 s). Comparing the 4 patterns, it is interesting to notice that while the addition of proanthocyanidins to both BW and OW has almost no effect on aroma release, the addition of ellagitannins, at the contrary, influences aroma release in the 50-80 s time in both BW and OW. It is interesting to observe that while ellagitannins increase aroma persistence in the non-oxidized wine, they have a lower effect after oxidation (Figures 8 and 12).

Figure 12. Comparison of aroma release of OW Vs OWE. Average areas under the curve every 5 s from 0 to 80 s for the main significantly affected ions with the respective standard deviations.

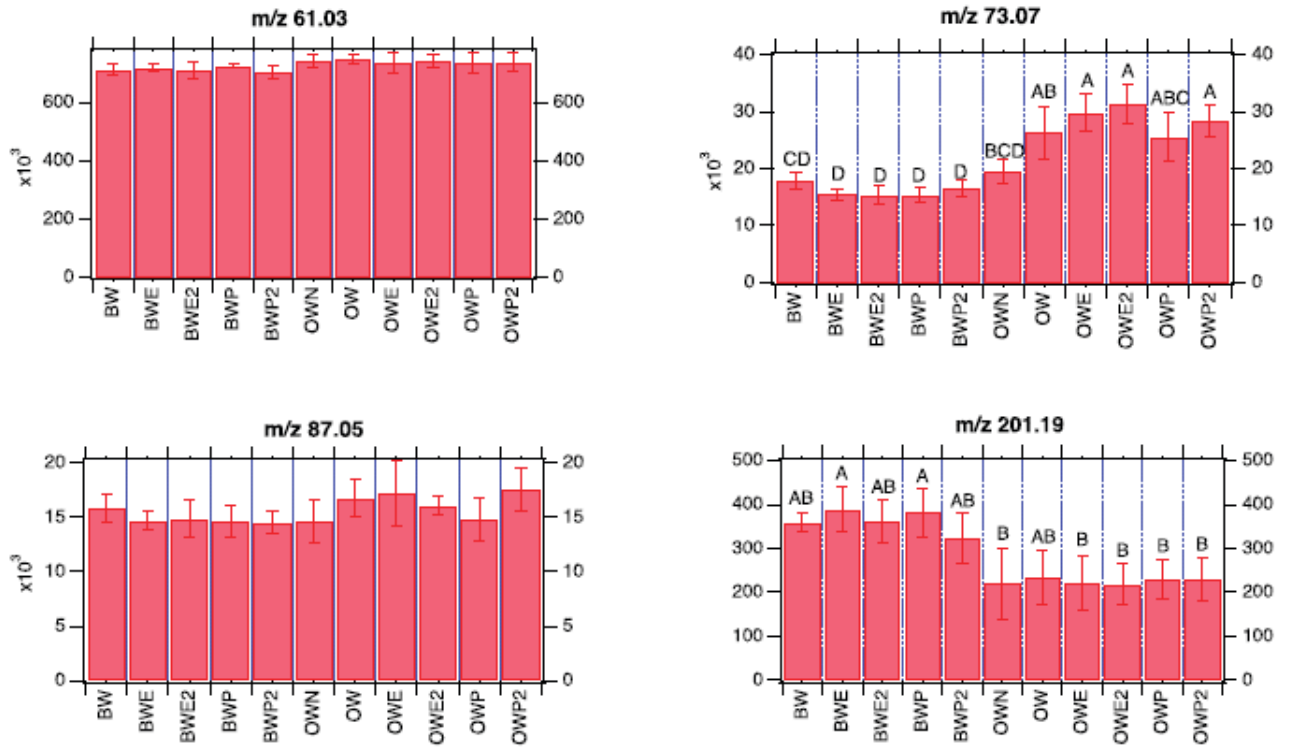


A hypothesis is that the oxidized structures of ellagitannins interact differently with the oral mucosa and aroma compounds decreasing the adsorption/desorption of aroma compounds at the surface of the oral mucosa. These results suggest that ellagitannins could differently impact aroma persistence during red wine tasting, which represents an interesting outcome from an oenological point of view, therefore deserving further investigations.

3.6. Effect of oenological tannins on wine aroma

Ions showing the most significant differences in in-vivo experiments, were also monitored through *in-vitro* analysis. Figure 13 represents the behaviour of the ions with m/z 61.03, 73.07, 87.05 and 201.19, detected by PTR-ToF-MS (no saliva) in all the analysed wine matrices, including the base wine (BW) and the corresponding oxidized wine (OW) spiked with two concentrations of ellagitannins (BWE and BWE2, OWE and OWE2) and proanthocyanidins (BWP and BWP2, OWP and OWP2), as well the base wine oxidized under nitrogen (OWN). The four ions, as already exposed above, are tentatively attributed to acetic acid, isobutyraldehyde, isovaleraldehyde and ethyl decanoate, respectively. The first three compounds are volatile markers of wine oxidation (Ugliano, 2013). While significant trends are not observed for the ions with *m/z* 61.03 and 87.05, significant increase and decrease are observed after oxidation for ions at *m/z* 73.07 and 201.19 respectively (t-test; p-value=0.05). However, whatever the added tannin, no significant difference is observed for both the oxidized and the non-oxidized conditions. The difference of significance observed between *in-vitro* and *in-vivo* data, can be explained by the lower number of observations by condition for the *in-vitro* experiments. Ion at *m/z* 73.07 is significantly higher in OW compared to BW, suggesting that it is formed during wine air exposition and its formation seems to be contrasted by nitrogen (OWN). The formation of this ion seems not to be prevented by the addition of tannins, independently from their nature and concentration. Ion at *m/z* 201.19 is significantly affected by oxidation, but according to T-test OW is not significantly different from BW. This ion is also not significantly affected by the presence of tannins, whatever the condition.

Figure 13. Release of m/z 61.03, 73.07, 87.05 and 201.19 detected by PTR-ToF-MS (no saliva) in all the analysed wine matrices, including the base wine (BW) and the corresponding oxidised wine (OW) spiked with two concentrations of ellagitannins (BWE and BWE2, OWE and OWE2) and proanthocyanidins (BWP and BWP2, OWP and OWP2), as well the base wine oxidised under nitrogen (OWN). Significant differences are marked with different letters ($p<0.05$).



4. Conclusions

The present study allowed deciphering the effect of the addition of oenological tannins on wine perception before and after oxidation, by correlating dynamic sensory evaluation with analytical measurement of aroma release in the nasal cavity. Firstly, the addition of the two tannins had almost no impact on the temporal dominance of sensations of the non-oxidized wine used in this study during the first 50 s. After this period, the study has revealed an increase of the release of numerous ions when ellagitannins were added to the wine, suggesting that these tannins increase aroma persistence. This effect was mainly observed for non-oxidized wines and in a low extent after oxidation. Sensory evaluation has also revealed that the addition of proanthocyanidins increases the astringency perception, while ellagitannins do not. However, it is important to highlight that the added quantity of the latter was smaller. Secondly, wine oxidation induces a decrease of the fruity attribute while increasing the dominance of maderised and prune notes perception. This effect could be related to the decrease of ethyl decanoate release and the increase of Strecker aldehydes release, which can

be responsible for the appearance of oxidative notes. Thirdly, the addition of ellagitannins before oxidation leads to the preservation of the fruity attribute dominance and to the decrease of the maderised one. The only chemical evidence that could be linked to this effect is a significant increase of ethyl decanoate release during the first second of the consumption of the wine containing ellagitannins.

These results provide important information for the use of oenological tannins in winemaking and their potential impact on wine perception. More specifically, it evidences that the presence of ellagitannins can have a positive impact on wine perception, and both on the aroma persistence in young wine and on the preservation of the fruity aroma perception after oxidation. Therefore, they can be useful for winemakers to better understand and manage red wines' oak-barrel ageing. Indeed, according to our results, wood-barrel ageing of young fruity red wines, which corresponds to a storage in the presence of ellagitannins (extracted from the wood to wine) and oxygen (permeated through the wood into the wine), could be a way to preserve fruitiness and smooth astringency. This preservation of fruity aromas could potentially help to counterbalance the contribution of aromas extracted from wood and in masking the appearance of oxidative notes with a positive impact on the sensory shelf-life.

References

Bonnans, S.; Noble, A.C. Effects of sweetener type and of sweetener and acid levels on temporal perception of sweetness, sourness and fruitiness. *Chem. Senses*, **1993**, 18 (3), 273-283.

Bueno, M.; Carrascón, V.; Ferreira, V. Release and Formation of Oxidation-Related Aldehydes during Wine Oxidation. *J. Agric. Food Chem.* **2016**, 64(3), 608-617.

Campbell-Sills, H.; Capozzi, V.; Romano, A.; Cappellin, L.; Spano, G.; Breniaux, M.; Lucas, P.; Biasoli, F. Advances in wine analysis by PTR-ToF-MS: Optimization of the method and discrimination of wines from different geographical origins and fermented with different malolactic starters. *Int. J. Mass Spectrom.* **2016**, 579 397-398, 42-51.

Canon, F.; Paté, F.; Cheynier, V.; Sarni-Manchado, P.; Giuliani, A.; Pérez, J.; Durand, D.; Li, J.; Cabane, B. Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. *LANGMUIR*, **2013**, 29(6), 1926-1937.

Culleré, L.; Cacho, J.; Ferreira, V. An assessment of the role played by some oxidation-related aldehydes in wine aroma. Journal of Agriculture and Food Che *J. Agric. Food Chem*. **2007**, 55, 876-881.

Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.N.; Dubourdieu, D. Identification of a powerful aromatic component of Vitis vinifera L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour and Fragrance Journal*, **1995**, 10, 385–392.

Deuscher, Z.; Andriot, I.; Semon, E.; Repoux, M.; Preys, S.; Roger, J.M.; Boulanger, R.; Labouré, H.; Le Quéré, J.L. Volatile compounds profiling by using proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS). The case study of dark chocolates organoleptic differences. *J. Mass Spectrom.* **2019**, 54(1), 92-119.

Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **2002**, 77, 325-331.

Escudero, A.; Cacho, J.; Ferreira, V. Isolation and identification of odorants generated in wine during its oxidation: a gas chromatography−olfactometric study. *Eur. Food Res. Technol.* **2000**, 211, 105−110.

Esteban-Fernández, A.; Rocha-Alcubilla, N.; Muñoz-González, C.; Moreno-Arribas, M.V.; Pozo-Bayón, M.Á. Intra-oral adsorption and release of aroma compounds following in-mouth wine exposure. *Food Chem.* **2016**, 205, 280-288.

Ferreira, V.; Bueno, M.; Franco-Luesma, E.; Culleré, L.; Fernández-Zurbano, P. Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *J. Agric. Food Chem.* **2014**, 62, 10015-10027.

Ferreira, V.; Carrascon, V.; Bueno, M.; Ugliano, M.; Fernandez-Zurbano, P. Oxygen consumption by red wines. Part I: consumption rates, relationship with chemical composition, and role of SO_2. *J. Agric. Food Chem.* **2015**, 63, 10928-10937.

Franco-Luesma, E.; Honoré-Chedozeau, C.; Ballester, J.; Valentin, D. Oxidation in wine: Does expertise influence the perception?. LWT – *Food Sci. Technol.* **2019**, 116, Article 108511.

Genovese, A.; Moio, L.; Sacchi, R.; Piombino, P. Sip volume affects oral release of wine volatiles. *Food Res. Int.* **2015**, 77 (3), 426-431.

Giacosa, S.; Parpinello, G.P.; Río Segade, S.; Ricci, A.; Paissoni, M.A.; Curioni, A.; Marangon, M.; Mattivi, F.; Arapitsas, P.; Moio, L.; Piombino, P.; Ugliano, M.; Slaghenaufi, D.; Gerbi, V.; Rolle, L.; Versari, A. Diversity of Italian red wines: A study by enological parameters, color, and phenolic indices. *Food Res. Int.* **2021**, 143, Article 110277.

Keim, H.; de Revel, G.; Marchand, S.; Bertrand, A. Method for Determining Nitrogenous Heterocycle Compounds in Wine. *J. Agric. Food Chem.* **2002**, 50(21), 5803-5807.

Magalhães, L.M.; Ramos, I.I.; Reis, S.; Segundo, M.A. Antioxidant profile of commercial oenological tannins determined by multiple chemical assays. *Austr. J. Grape Wine R.* **2014**, 20, 72-79.

OIV. International Oenological Codex. COEI-1-TANINS: 2015. http://www.oiv.int/public/medias/4093/e-coei-1-tanins.pdf.

Petit, E.; Jacquet, R.; Pouységu, L.; Deffieux, D.; Quideau, S. Reactivity of wine polyphenols under oxidation conditions: Hemisynthesis of adducts between grape catechins or oak ellagitannins and odoriferous thiols. *Tetrahedron*, **2019**, 75(5), 551-560.

Pineau, N.; de Bouille, A.G.; Lepage, M.; Lenfant, F.; Schlich, P.; Martin, N.; Rytz, A. Temporal dominance

of sensations: what is a good attribute list?. *Food Qual. Prefer.* **2012**, 26, 159-165.

Pineau, N.; Schlich, P.; Cordelle, S.; Mathonnière, C.; Issanchou, S.; Imbert, A; Rogeaux, M.; Etiévant, P.; Köster, E. Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Qual. Prefer.* **2009**, 20(6), 450-455.

Piombino, P.; Moio, L.; Genovese, A. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res. Int.* **2019**, 116, 548-558.

Pittari, E.; Moio, L.; Piombino, P. Interactions between Polyphenols and Volatile Compounds in Wine: A Literature Review on Physicochemical and Sensory Insights. *Appl. Sci.* **2021**, 11(3), 1157.

Ployon, S.; Brulé, M.; Andriot, I.; Morzel, M.; Canon, F. Understanding retention and metabolization of aroma compounds using an in vitro model of oral mucosa. *Food Chem.* **2020**, 318, 126468.

Ployon, S.; Morzel, M.; Belloir, C.; Bonnotte, A.; Bourillot, E.; Briand, L.; Lesniewska, E.; Lherminier, J.; Aybeke, E.; Canon, F. Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs. *Food Chem.* **2018**, 253, 79-87.

Rizzi, G.P. Formation of strecker aldehydes from polyphenol-derived quinones and alpha-amino acids in a nonenzymic model system. *J. Agric. Food Chem.* **2006**, 54(5), 1893-1897.

San Juan, F.; Cacho, J.; Ferreira, V.; Escudero, A. Aroma Chemical Composition of Red Wines from Different Price Categories and Its Relationship to Quality. *J. Agric. Food Chem.* **2012**, 60(20), 5045-5056.

Schlich, P. Temporal Dominance of Sensations (TDS): a new deal for temporal sensory analysis. *Current Opinion in Food Science*, **2017**, 15, 38-42.

Schlich, P.; Pineau, N. Temporal dominance of sensations, in Time- Dependent Measures of Perception in Sensory Evaluation, ed. by Hort, J.S.E, Kemp, S.E., Hollowood, T., eds, John Wiley & Sons Ltd.: Chichester, UK, 2017, pp. 283– 320.

Silva Ferreira, A.C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, 51, 1377-1381.

Ugliano, M. Oxygen contribution to wine aroma evolution during bottle aging. *J. Agric. Food Chem.* **2013**, 61, 6125-6136.

Versari, A.; du Toit, W.; Parpinello, G.P. Oenological tannins: a review. *Austr. J. Grape Wine R.* **2013**, 19, 1-10.

Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. Understanding wine chemistry; John Wiley: Hoboken, NJ, USA, 2016.

Summarizing results, General conclusions, and Future perspectives

Wine sensory characteristics, and in particular the olfactory ones, are the result of different interactions: those between volatile molecules, the perceptive ones between the different senses, and those between aromas and other non-volatile components of the wine matrix, which being able to modulate the volatility of the volatile molecules, could influence its intensity and perceived sensory quality.

Polyphenols and volatile organic compounds are responsible for two of the most important wine hedonic properties, and they are liable for two of the main characteristics in defining complexity and quality of wines and for the two main intrinsic drivers of wines consumers purchasing decisions: astringency (mainly for red wines) and aroma perception.

Therefore, studying the interactions between polyphenols and volatile compounds is a subject of interest for wine researchers and producers to understand consumers' perceptions and choices as well as for precision oenology purposes.

Investigating the interactions between polyphenols and volatile compounds in such a complex matrix as red wine, is not an easy task. With this thesis project we aimed at adding knowledge on the effects of these interactions on wine sensory perception by considering cross-modal sensory interactions, physical-chemical interactions and, moreover, starting to address chemical aspects.

First objective

In the frame of the first objective, we aimed at investigating how the perception of wine aroma could modulate the perception of wine astringency and tastes.

General results:

Our findings suggest that the perception of the basic astringent sensation (drying astringency) was not significantly impacted by olfactory cues. However, the outcomes highlight that odour characteristics could modulate the perception of the other astringency sub-qualities, suggesting that cross-modal interactions between odours and oral characteristics occur during red wine tasting, and are able to significantly modulate the perception of specific sub-qualities of astringency and taste.

Highlights:

- olfactory perception could smooth the intensity of harsh, unripe, and dynamic sub-qualities, previously described as “strong astringency sensations”, while enhancing the intensity of complex and velvet sub-qualities, previously described as “smooth astringency sensations”;
- unripe, and in a lesser extent complex astringency sub-quality, are complex sensations that would involve the simultaneous intervention of molecules active at both oral and olfactory levels;
- significant positive correlations were found between unripe astringency and vegetal notes, complex astringency and spicy notes and drying astringency and dehydrated fruit notes; significant negative correlations between unripe sub-quality and alcoholic notes were highlighted;
- olfactory perception could smooth the intensity of the bitter taste, while enhancing sweetness intensity;
- odour–oral cross-modal interactions could affect the correlations between chemical and sensory parameters, thus interfering with the estimation of their predictive power.

Practical outcomes:

These obtained results are of practical interest because astringency is a sensation that when perceived in high intensities, can impair the consumption of a particular food product. Therefore, in a perspective of precision oenology, these results could be helpful in the management of wine astringency during winemaking with an alternative approach to the more common technological ones. Moreover, in the food field, these results are useful since nowadays an ever-increasing number of consumers are turning toward healthier and more sustainable plant-based foods, rich in polyphenols. These products, that can represent a valid food alternative, are often discarded by the consumer due to their bitterness/astringency characteristics. Consequently, all scientific knowledge helping in understanding how to smoothen/mask these sensations are welcomed by scientists and food technologists/engineers working in the food field. Finally, since odour–oral cross-modal interactions could affect the correlations between chemical and sensory parameters, thus interfering with the estimation of their predictive power, these results could be used as starting point to create new models and methods to predict astringency.

Second objective

In the frame of the second objective, we aimed at studying how polyphenols, wine compounds involved in the perception of astringency, could modulate the release and the perception of wine odour characteristics.

General results:

We have observed that polyphenols showed a significant impact on the release of the main wine volatile compounds (i.e., esters, alcohol, terpenoids, volatile phenols, sulfur compounds). From a sensory point of view, while the rating of the global odour intensity did not significantly differ among the four matrices with different non-volatile compositions, their odour profiles changed, suggesting a relevant sensory impact.

Highlights:

- polyphenols (anthocyanins and tannins) showed strong positive correlations with ethyl phenylacetate, diethyl succinate, geranylacetone, linalool, beta-phenylethanol, gamma-butyrolactone, and methionol and strong negative correlations with isobornyl acetate;
- a negative correlation between polyphenols (anthocyanins and tannins) and some volatile compounds responsible for wine off-odours, 4-ethylphenols and benzothiazole, was highlighted;
- the increase in the alcohol content would reduce wine volatile composition;
- fruity and floral notes perception decreased, while earthy, toasty, and dehydrated fruit significantly increased as the non-volatile matrix concentration increased.

Practical outcomes:

We have observed that managing the olfactory perception it is possible to control astringency sensation. In the same way, to manage the aromatic quality of a wine, it seems possible to act not only on all the processes involved in the production of volatile molecules, but also on the non-volatile matrix, particularly on the polyphenolic fraction. Polyphenolic fractions seem to have an impact on the release of the main wine volatiles and, therefore, hypothetically they can also influence the perceptibility of odours. In particular, of great oenological interest is the reduction of the release of 4-ethylphenol and benzothiazole, since they are responsible for important wine off-odours, indicating that polyphenols could be used in the management of some wine taints. Finally, our results suggest that the alcohol content of wines should be controlled, as it tends to deplete its volatile fraction. This approach on real matrices has proved to be useful to obtain knowledge on

the role of polyphenols on the sensory perception of wine odours in conditions much more similar to real ones (compared to the use of model solutions).

Third objective

In the frame of the third objective, we aimed at exploring if polyphenols could act as protection toward the oxidation of wine aromas.

General results:

Different tannins (ellagitannins and proanthocyanidins) modulated wine aromas release and perception over time in different ways, both before and after oxidation.

Highlights:

- ellagitannins showed a positive effect on the aromatic persistence of the retronasal aroma of a young red wine;
- wine oxidation led to dominance of Madeira/prune odours and to the disappearance of fruity notes;
- in-mouth aroma release results showed a decrease of ethyl decanoate and an increase of isobutyraldehyde and isovaleraldehyde in the oxidised wine;
- ellagitannins preserved the fruity attribute dominance after oxidation, preventing the increase of the maderised one, typical oxidative note;
- no clear evidences of aroma protection were found regarding in-mouth aroma release; only ellagitannins showed a significant increase of the *in-vivo* release of ethyl decanoate in the first seconds of the consumption of the wine containing ellagitannins, that could probably explain the preservation of the fruity note in that wine.

Practical outcomes:

These results provide important information for the use of oenological tannins in winemaking and their potential impact on wine perception. They show that the presence of ellagitannins can have a positive impact on wine perception, and both on the aroma persistence in young wine and on the preservation of the fruity aroma perception after oxidation. Therefore, they can be useful for winemakers to better understand and manage red wines' oak-barrel ageing. Wood-barrel ageing of young fruity red wines, which corresponds to a

storage in the presence of ellagitannins (extracted from the wood to wine) and oxygen (permeated through the wood into the wine), could be a way to preserve fruitiness and smooth astringency. This preservation of fruity aromas could potentially help to counterbalance the contribution of aromas extracted from wood and in masking the appearance of oxidative notes with a positive impact on the sensory shelf-life.

APPENDIX 1

LIST OF PUBLICATIONS

INTERNATIONAL JOURNAL PUBLICATIONS

1. Piombino, P.; **Pittari, E.**; Gambuti, A.; Curioni, A.; Giacosa, S.; Mattivi, F.; Parpinello, G.P.; Rolle, L.; Ugliano, M.; Moio, L. Preliminary sensory characterisation of the diverse astringency of single cultivar Italian red wines and correlation of sub-qualities with chemical composition. *Australian Journal of Grape and Wine Research,* **2020**, 26, 233–246. DOI: 10.1111/ajgw.12431

2. **Pittari, E.**; Moio, L.; Arapitsas, P.; Curioni, A.; Gerbi, V.; Parpinello, G.P.; Ugliano, M.; Piombino, P. Exploring Olfactory–Oral Cross-Modal Interactions through Sensory and Chemical Characteristics of Italian Red Wines. *Foods*, **2020**, 9, 1530. DOI:10.3390/foods9111530

3. **Pittari, E.**; Moio, L.; Piombino, P. Interactions between Polyphenols and Volatile Compounds in Wine: A Literature Review on Physicochemical and Sensory Insights. *Applied Sciences*, **2021**, 11, 1157. DOI: 10.3390/app11031157

4. **Pittari, E.**; Piombino, P.; Andriot, I.; Cordelle, S.; Feron, G.; Gourrat, K.; Le Quéré, J.L.; Neiers, F.; Moio, L.; Schlich, P.; Canon, F. Effects of oenological tannins on aroma release and perception of oxidized and non-oxidized red wine: a dynamic real-time in-vivo study by coupling TDS to PTR-ToF-MS. Submitted (February 2021) to *Food Chemistry*. Revised version under re-submission.

IN EDITING

1. Piombino, P.; Lisanti, M.T.; **Pittari, E.**; Picariello, L.; Moio, L. Impact of non-volatile matrix components on red wine aroma release and perception of olfactory and oral sensations. For Food Chem.

NATIONAL JOURNAL PUBLICATIONS

1. Piombino, P.; **Pittari, E.**; Gambuti, A.; Curioni, A.; Giacosa, S.; Mattivi, F.; Parpinello, G.P.; Rolle, L.; Ugliano, M.; Moio, L. Verso una descrizione oggettiva della diversa astringenza dei vini rossi italiani. *OICCE TIMES*, **2020**, 83, 27–33.

2. Piombino, P., E. Pittari, P. Arapitsas, A. Curioni, V. Gerbi, G.P. Parpinello, M. Ugliano, & L. Moio, 2021. Study of Cross-Modal Interactions through Sensory and Chemical Characteristics of Italian Red Wines. Submitted (February 2021) to *Infowine*.

POSTERS

1. Piombino, P.; **Pittari, E.**; Gambuti, A.; Rinaldi, A.; Moio, L.; Slaghenaufi, D.; Versari, A.; Rolle, L.; Mattivi, F.; Curioni, A.; Ugliano, M. Astringency diversity of Italian red wines. In Proceedings of the International Conference MACROWINE 2018, 28th-31st May, Saragoza, Spain.

2. Piombino, P.; **Pittari, E.**; Moio, L.; Curioni; A.; Mattivi, F.; Parpiniello, G.P.; Rolle, L.; Ugliano, M. Impact of olfactory cues on the perception of astringency sub-qualities in Italian red wines. In Proceedings of the Eighth European Conference on Sensory and Consumer Research EUROSENSE 2018, 2nd–5th September, Verona, Italy.

3. **Pittari, E.** Interactions between polyphenols and volatile compounds in wine: study of the effects on the chemical stability and on the sensory perception. In Proceedings of the XXIII WORKSHOP ON THE DEVELOPMENTS IN THE ITALIAN PhD RESEARCH ON FOOD SCIENCE, TECHNOLOGY AND BIOTECHNOLOGY 2018, 19th–21st September, Oristano, Italy.

4. Gambuti, A.; Piombino, P.; Rinaldi, A.; **Pittari, E.**; Curioni, A.; Giacosa, S.; Mattivi, F.; Parpinello, G.P.; Perenzoni, D.; Slaghenaufi, D.; Moio, L. Phenolic parameters explaining different astringency properties in red wines. International conference OENO/IVAS 2019, 25th-28th June, Bordeaux, France.

5. Piombino, P.; Lisanti, M.T.; **Pittari, E.**; Picariello, L.; Moio, L. Impact of non-volatile matrix components on red wine aroma release and perception of olfactory and oral sensations. International conference 16th WEURMAN FLAVOUR RESEARCH SYMPOSIUM 2021, 4th-6th May 2021, online. **Winner of the Second Price of the Session "Flavour Perception", assigned by the Scientific Committee.**

6. Piombino, P.; Lisanti, M.T.; **Pittari, E.**; Picariello, L.; Moio, L. Impact of the non-volatile matrix composition on red wine aroma release and perception of olfactory and oral cues. International conference MACROWINE 2021, 23rd-30th June 2021, online.

ORAL COMMUNICATIONS

1. Mattivi, F.; Arapitsas, P.; Perenzoni, D.; Piombino, P.; **Pittari, E.**; Moio, L. Applicazioni della metabolomica in enologia in Trentino. Conference Incontri Rotaliani: Teroldego e Vini di Borgogna, 12th-13th May 2019, Trento, Italy.

2. Piombino, P.; **Pittari, E.**; Moio. L. La diversa astringenza dei vini rossi Italiani. International conference ENOFORUM 2019, 21st-23rd May, Vicenza, Italy.

3. Mattivi, F.; Perenzoni, D.; Piombino, P.; **Pittari, E**. I tannini nei principali vini rossi italiani: Diversità delle concentrazioni e delle strutture molecolari. International conference ENOFORUM 2019, 21st-23rd May, Vicenza, Italy.

4. Piombino, P.; **Pittari, E.**; Ugliano, M.; Curioni, A.; Gerbi, V.; Mattivi, F.; Parpinello, G.P.; Moio, L. Exploring multisensory interactions through the study of astringency diversity of mono-varietal Italian red wines. International conference OENO/IVAS 2019, 25th-28th June, Bordeaux, France.

5. **Pittari, E.**; Andriot, I.; Moio, L.; Le Quéré, J.L.; Schlich, P.; Piombino, P.; Canon, F. A real-time *in-vivo* approach to explore tannins effect on aroma release and perception of red wine after air exposition. International conference 16th WEURMAN FLAVOUR RESEARCH SYMPOSIUM 2021, 4th-6th May 2021, online.

6. **Pittari, E.**; Andriot, I.; Moio, L.; Le Quéré, J.L.; Schlich, P.; Piombino, P.; Canon, F. Effect of oenological tannins on wine aroma before and after oxidation: a real-time study by coupling sensory (TDS) and chemical (PTR-ToF-MS) analyses. International conference MACROWINE 2021, 23rd-30th June 2021, online.