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New approaches for understanding the formation of mouthfeel properties in wines and grapes
Autor/es
Sara Ferrero del Teso
Director/es
Purificación Fernández Zurbano y María Pilar Sáenz Navajas
Facultad
Facultad de Ciencia y Tecnología
Titulación
Departamento
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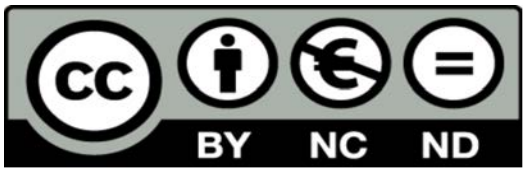

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TESIS DOCTORAL

Nuevas estrategias para comprender la formación de las sensaciones táctiles en boca de vinos y uvas

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New approaches for understanding the formation of mouthfeel properties in wines and grapes

Memoria presentada por:

SARA FERRERO DEL TESO

Para optar al grado de Doctor con Mención de Doctor Internacional

Programa de Doctorado en Enología, Viticultura y Sostenibilidad

Dirigida por las doctoras:

Dra. PURIFICACIÓN FERNÁNDEZ ZURBANO

Dra. MARÍA PILAR SÁENZ NAVAJAS

FEBRERO, 2022

INFORME DIRECTORES

Dña. Purificación FERNÁNDEZ ZURBANO, Profesora Titular del Departamento de Química, Área Química Analítica de la Universidad de La Rioja e Investigadora del Instituto de Ciencias de la Vid y el Vino en el grupo de Investigación Laboratorio de Análisis del Aroma, sabor y Enología (LAAE-Rioja) y Dña. María Pilar SÁENZ NAVAJAS, Científica Titular del Consejo Superior de Investigaciones Científicas (CSIC) en el Instituto de Ciencias de la Vid y el Vino en el grupo de investigación Laboratorio de Análisis del Aroma, sabor y Enología (LAAE-Rioja).

CERTIFICAN

Que la presente memoria, titulada **"New approaches for understanding the formation of mouthfeel properties in wines and grapes"**, presentada por Dña. Sara FERRERO DEL TESO para optar al grado de Doctor por la Universidad de La Rioja, ha sido realizada bajo nuestra dirección autorizando su presentación para proseguir los trámites oportunos y proceder a su calificación por el tribunal correspondiente.

Logroño, febrero 2022

Fdo. Purificación FERNÁNDEZ ZURBANO Fdo. María Pilar SÁENZ NAVAJAS

Este trabajo ha sido realizado gracias a los siguientes contratos concedidos a Dña. Sara Ferrero del Teso:

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Proyecto: Nuevas herramientas y conceptos cuantitativos para la construcción de vinos tecnológicamente mejores, más estables y con menos sulfitos de referencia **(AGL-2014-59840)**. Financiado por el Ministerio de Economía y Competitividad-Gobierno de España.

Proyecto: Verdor, astringencia y dureza en vinos tintos de las variedades Garnacha y Moristel. Caracterización sensorial y molecular y gestión integral en bodega **(RTC 2016-4935-2)**. Financiado por el Ministerio de Economía y Competitividad-Gobierno de España.

The present doctoral thesis has derived in seven published scientific papers. Complete references are listed below:

Sáenz-Navajas, M. P., Avizcuri, J. M., Ferrero-del-Teso, S., Valentin, D., Ferreira, V., & Fernández-Zurbano, P. (2017). Chemo-sensory characterization of fractions driving different mouthfeel properties in red wines. *Food Research International*, *94*. https://doi.org/10.1016/j.foodres.2017.02.002

Sáenz-Navajas, M. P., Arias, I., Ferrero-del-Teso, S., Fernández-Zurbano, P., Escudero, A., & Ferreira, V. (2018). Chemo-sensory approach for the identification of chemical compounds driving green character in red wines. *Food Research International*, *109*. https://doi.org/10.1016/j.foodres.2018.04.037

Sáenz-Navajas, M. P., Ferrero-Del-Teso, S., Romero, M., Pascual, D., Diaz, D., Ferreira, V., & Fernández-Zurbano, P. (2019). Modelling wine astringency from its chemical composition using machine learning algorithms. *Oeno One*, *53*(3). https://doi.org/10.20870/oeno-one.2019.53.3.2380

Ferrero-del-Teso, S., Arias, I., Escudero, A., Ferreira, V., Fernández-Zurbano, P., & Sáenz-Navajas, M. P. (2019). Effect of grape maturity on wine sensory and chemical features: The case of Moristel wines. *LWT- Food Science and Technology, 118*. https://doi.org/10.1016/j.lwt.2019.108848

Sáenz-Navajas, M. P., Ferrero-del-Teso, S., Jeffery, D. W., Ferreira, V., & Fernández-Zurbano, P. (2020). Effect of aroma perception on taste and mouthfeel dimensions of red wines: Correlation of sensory and chemical measurements. *Food Research International*, *131*. https://doi.org/10.1016/j.foodres.2019.108945

Ferrero-del-Teso, S., Suárez, A., Jeffery, D. W., Ferreira, V., Fernández-Zurbano, P., & Sáenz-Navajas, M. P. (2020). Sensory variability associated with anthocyanic and tannic fractions isolated from red wines. *Food Research International*, *136*. https://doi.org/10.1016/j.foodres.2020.109340

Ferrero-del-Teso, S., Suárez, A., Ferreira, C., Perenzoni, D., Arapitsas, P., Mattivi, F., Ferreira, V., Fernández-Zurbano, P., Sáenz-Navajas, M. P. (2021). Modeling grape taste and mouthfeel from chemical composition. *Food Chemistry*, *371*. https://doi.org/10.1016/J.FOODCHEM.2021.131168

In addition, the work in this thesis has contributed to the publication of:

Arias-Pérez, I., Ferrero-Del-Teso, S., Sáenz-Navajas, M. P., Fernández-Zurbano, P., Lacau, B., Astraín, J., … Escudero, A. (2020). Some clues about the changes in wine aroma composition associated to the maturation of “neutral” grapes. *Food Chemistry*, *320*. https://doi.org/10.1016/j.foodchem.2020.126610

Con todo mi cariño,

a mis directoras

"El secreto está en aprender de alguien

que quiera que tu crezcas".

Anónimo.

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A todos, muchas gracias.

PRESENTACIÓN

Presentación

La presente memoria de tesis doctoral se ha desarrollado dentro de los grupos de investigación: “Laboratorio de Análisis Sensorial y Calidad de Alimentos (LASCAL)” de la Universidad de la Rioja y el “Laboratorio de Análisis del Aroma, sabor y Enología (LAAE-Rioja)” del Instituto de Ciencias de la Vid y el Vino junto con la Universidad de Zaragoza, cuya línea de investigación se centra entre otros en la modelización de las notas sensoriales (aroma, gusto, sensaciones táctiles en boca y color) del vino y de su calidad a partir de su composición química.

El sabor resulta de la interacción entre los estímulos aromáticos, del gusto y de las sensaciones mediadas por el nervio trigeminal (sensaciones táctiles en boca). Entre los muchos aspectos que aún deben ser estudiados, este trabajo se ha centrado especialmente en el estudio de las propiedades táctiles en boca, y su correlación con la composición química. A pesar de su importancia en formación del sabor, especialmente en vinos tintos, y por ende en la percepción de la calidad relacionada directamente con el grado de aceptabilidad y satisfacción del consumidor, la formación de las sensaciones táctiles es hasta el momento la menos entendida. Esta Tesis surge para avanzar en la búsqueda de compuestos químicos individuales o grupos de compuestos responsables de tales propiedades. La identificación de los factores implicados en las sensaciones táctiles que incrementan la aceptabilidad del producto por parte del consumidor proporcionará a la industria herramientas útiles para aumentar la aceptación de los vinos y así añadir valor a los mismos.

De acuerdo con la literatura previa, es ampliamente reconocido que los compuestos fenólicos contribuyen de forma activa a la formación del perfil organoléptico del vino, especialmente a la astringencia. Si bien la “astringencia” es el atributo de sensación táctil en boca más ampliamente estudiado en los vinos tintos, no se puede obviar la existencia de otras dimensiones sensoriales táctiles que deben ser consideradas para comprender la formación de estas propiedades en boca

del vino. Así, al comenzar este trabajo de doctorado se sabía que parte de la base del problema en la comprensión de los mecanismos que originan las sensaciones táctiles en boca, reside en la falta de un léxico adecuado que describa de forma objetiva cada sensación. El principal desafío es encontrar el compuesto o grupos de compuestos responsables de las diferentes sensaciones táctiles que se originan en la boca, lo que impide desarrollar referencias que ilustren los atributos y por lo tanto un vocabulario claro y bien definido.

Por ello, la primera sección se ha dedicado al desarrollo de una estrategia química sensorialmente dirigida para la obtención de fracciones inodoras con propiedades de gusto y táctiles consistentes que permitió generar una lista de términos relacionados con las propiedades táctiles y se empleó con éxito en la descripción de vinos y fracciones de uvas y vinos mediante metodologías sensoriales alternativas.

En la segunda sección se combina estudios sensoriales con análisis químicos para comprender conceptos generales como “carácter verde” y “madurez”.

En la tercera sección, diferentes estudios centrados en el vino como matriz principal se presentan y se estudian los impulsores químicos de las propiedades táctiles en boca del vino.

Finalmente, en la sección cuarta, el estudio de las sensaciones táctiles en boca del vino se aborda, acoplando estrategias sensoriales y químicas en fracciones fenólicas más simples con origen tanto en la uva como en el vino.

OBJECTIVES/

OBJETIVOS

Objectives

The general objective of this Doctoral Thesis is to increase knowledge about mouthfeel formation and the chemicals modulating this percept in wines and grapes by applying sensory approaches in combination with chemical strategies.

The specific objectives are:

1. To develop a semi-preparative fractionation method for isolating groups of compounds displaying different sensory properties.
2. To generate a wide vocabulary related to mouthfeel properties able to characterise phenolic fractions and wines
3. To identify the sensory and chemical variables involved in green character of red wines and define their link with taste and mouthfeel properties.
4. To determine the sensory and chemical factors affected by grape maturity and triggering differences in wine taste and astringency.
5. To predict wine sensory astringency from chemical composition using non-linear mathematical models by machine learning algorithms.
6. To define the sensory space of a wide range of red wines in terms of taste and mouthfeel and investigating the potential aroma-taste and aroma-mouthfeel interactions.
7. To investigate the contribution of wine metabolome to wine mouthfeel properties applying a non-targeted instrumental approach.
8. To predict taste and mouthfeel differences elicited by grape phenolic fractions from chemical measurements.
9. To evaluate the sensory variability of phenolic fractions of tannins and anthocyanins and their derivatives.
10. To evaluate the oxygen consumption rates of different phenolic fractions.
11. To evaluate the sensory and chemical impact of oxidative and anoxic ageing of different phenolic fractions.

Objetivos

El objetivo general de esta Tesis Doctoral es aumentar el conocimiento sobre la formación de las sensaciones en boca y los compuestos y parámetros químicos que modulan esta percepción en vinos y uvas mediante la aplicación de diferentes estrategias sensoriales en combinación con químicas.

Los objetivos específicos son:

1. Desarrollar un método semipreparativo de fraccionamiento que permita separar grupos de compuestos con diferentes propiedades sensoriales.
2. Desarrollar un vocabulario amplio relacionado con las propiedades sensoriales en boca, que permita caracterizar las fracciones fenólicas y los vinos.
3. Identificar las variables sensoriales y químicas involucradas en el carácter verde de los vinos tintos y definir su implicación en las propiedades gustativas y en boca.
4. Determinar los factores sensoriales y químicos que se ven afectados por la madurez de la uva y que inducen las diferencias gustativas y de astringencia del vino.
5. Predecir la astringencia sensorial del vino a partir de la composición química utilizando modelos matemáticos no lineales mediante algoritmos de aprendizaje automático (*machine learning*).
6. Definir el espacio sensorial de una amplia gama de vinos tintos en términos del gusto y las sensaciones percibidas en boca e investigar las posibles interacciones aroma-gusto y aroma-sensación en boca.
7. Investigar la contribución del perfil metabolómico del vino en las propiedades sensoriales percibidas en boca aplicando un enfoque instrumental no dirigido.
8. Predecir las diferencias en las propiedades gustativas y las propiedades sensoriales percibidas en la boca causadas por la fracción fenólica de la uva a partir de variables químicas.

9. Evaluar la variabilidad sensorial de fracciones fenólicas de taninos y fracciones de antocianos y sus derivados.
10. Evaluar las tasas de consumo de oxígeno de diferentes fracciones fenólicas.
11. Evaluar el impacto sensorial y químico del envejecimiento oxidativo y anóxico en diferentes fracciones fenólicas.

STRUCTURE

STRUCTURE

This Doctoral Thesis consists of a series of works focused on elucidating the formation of mouthfeel perception evoked by red wines and grapes, by integrating sensory and chemical approaches.

- SUMMARY/ RESUMEN
- INTRODUCTION

It includes a general introduction in which a review is made regarding the concept of flavour, highlighting the relevant role that mouthfeel has in it. It is reviewed the knowledge that exists to date regarding the formation of the mouthfeel. Finally, a short review on the chemical and sensory analysis methods and techniques employed in the study of mouthfeel, focusing on wine as a product.

- SECTIONS

It has been structured in the following 4 sections:

- SECTION I. DEVELOPMENT OF A CHEMOSENSORY STRATEGY FOR WINE AND PHENOLIC FRACTIONS

The study of the first section comprises the development of a chemosensory strategy, which allowed to generate vocabulary related to mouthfeel features in wines and fractions.

- SECTION II. APPLICATION OF CHEMOSENSORY STRATEGIES FOR UNDERSTANDING MOUTHFEEL-RELATED CONCEPTS.

The next section combines sensory studies with chemical analysis to understand global concepts including the multidimensional and ill-defined term of “green character” (applying the strategy developed in Section I) and the concept of grape maturity. It comprises two chapters that are focused on understanding wine flavour formation and how taste and mouthfeel modulate it.

❖ SECTION III. STUDY OF MOUTHFEEL PERCEPTION IN RED WINES

Different studies focused on wine as main matrix are presented in Section III, in which the chemical drivers of wine mouthfeel properties are studied. It comprises three chapters.

❖ SECTION IV. STUDY OF MOUTHFEEL IN GRAPE AND WINE FRACTIONS.

Finally, in section IV, the study of mouthfeel in wine coupling sensory and chemical strategies is focused in simpler phenolic fractions with origin in both grape and wine. It comprises three chapters.

❖ GENERAL CONCLUSION/ CONCLUSIONES GENERALES

It includes the most outstanding general conclusions of the thesis and its importance in the wine industry.

❖ ANNEXES

Includes Annexes with supplementary material or material that complements the results obtained in some of the chapters.

Due to the fact that the doctoral thesis opts for the international mention, the language chosen for its writing has been English, however, complying with what is stipulated in the doctoral program, the summary and the conclusions are written in both Spanish and English.

SUMMARY/

RESUMEN

Summary

It is of great interest for the wine sector to understand the factors that increase the acceptability of the product. The identification of these factors will provide the industry with useful tools to increase the acceptance of our wines and thus add value to them. The degree of acceptability and consumer satisfaction is directly related to the perception of quality. Perceived quality is a multidimensional variable, comprising both intrinsic (organoleptic properties) and extrinsic (label design, packaging...) factors of the product, whose interaction with the consumer and its characteristics (for example: level of expertise with wine or culture) give rise to the formation of perceived quality. Intrinsic properties, especially flavour, play a very important role in quality formation, especially in repurchase situations. Flavour is the result of the integration of aromatic stimuli, taste and sensations mediated by the trigeminal nerve (tactile sensations in the mouth). In the last decade there has been a significant increase in the interest for understanding flavor formation, which implies understanding how this multisensory integration occurs at both the perceptual and cognitive levels.

Among the senses involved in flavour (smell, taste, tactile sensations), the formation of tactile sensations is generally the least understood, which is especially important in the case of wine. The perception of tactile properties occurs mainly in the oral cavity, where mechanical and chemical processes take place. To date, despite advances in instrumental chemical analysis, the taste properties (acid, sweet, bitter, salty and umami) and mouthfeel sensations of food in general and wine in particular, can only be determined by sensory strategies. These techniques make it possible to obtain descriptions of the products and identify the differences that exist among them. This information is of great interest to the industry, as it helps to increase the understanding of the factors that are involved in consumer's preference and the perceived quality of the product. This knowledge can be used in the development of new products, as well as in the development of quality control

strategies, among others. However, it is difficult to obtain a complete sensory profile in terms mouthfeel properties of wine, especially for red wines. This difficulty lies in the fact that the compounds that originate these properties are mostly unknown. This fact generates a lack of an adequate standardised vocabulary that objectively describes the dimensions of wine mouthfeel, which definitely hinders the study and therefore the understanding of the mechanisms that originate these sensations. En este contexto, el objetivo principal de esta Tesis Doctoral ha sido aumentar el conocimiento sobre la formación de las propiedades táctiles en boca y los compuestos y parámetros químicos que conducen y modulan esta percepción en vinos y uvas. Para lograr tal objetivo, se trabajó bajo la premisa de que la comprensión de las sensaciones táctiles en boca inducidas por los vinos debe abordarse combinando estrategias tanto sensoriales como químicas. In this context, the main objective of this Doctoral Thesis has been to increase knowledge about the formation of mouthfeel properties and the chemical compounds and parameters that lead and modulate this perception in wines and grapes. To achieve this goal, we worked under the premise that the understanding of mouthfeel properties induced by wines must be addressed by combining both sensory and chemical strategies.

Thus, the first section was devoted to develop a sensory-directed chemical strategy to obtain odourless fractions with consistent taste and mouthfeel properties. The fractions from three wines were sensory characterised using different techniques: sorting task, repertory grid, triangulation, and Rate-All-That-Apply (RATA) with wine experts. The most surprising result was the sensory properties of the anthocyanic fraction (containing anthocyanins and derivatives), which was especially dry, bitter and persistent as the original wine. In addition, this approximation allowed to generate a list of 18 terms related to mouthfeel properties and was successfully used in the description of wines and fractions by RATA analysis.

Next, this senso-chemical methodology was applied in the study of the "green character" of red wines. At the beginning of this study the "green character" was an ill-defined term. Winemakers declared that it was a default linked to mouthfeel

sensations and taste of certain red wines. They noted that it was a sensation that they were observing recurrently in their wines associated with climate change and that was generating a depreciation of their products. In order to identify the compounds or groups of compounds responsible for this sensation, first of all, the study of the definition of this ill-defined descriptor was addressed. Thus, the "green character" resulted to be a multidimensional term associated with dimensions of aroma, taste and mouthfeel sensations, which was effectively negatively correlated with wine preference. Then, following the strategy of section I, different odourless fractions of wines with high and low green character were obtained. One of the most outstanding results was that the anthocyanic fraction of one of the greenest wines was especially "adherent". This fraction was suggested as responsible for the green character in that wine. These results, in line with those obtained in section I, suggested that anthocyanins and their derivatives could be involved in the formation and modulation of certain mouthfeel properties of some red wines. To deepen in the study of the sensory properties of the anthocyanic fraction, a large-scale study was carried out considering 42 red wines. Two different fractions were obtained for each wine containing tannins or anthocyanins, respectively, and they were chemically and sensory characterized in terms of taste and mouthfeel sensations. The results confirmed the sensory activity of the anthocyanic fractions studied, which varied significantly in the terms: bitterness, dryness, granulosity and body among the fractions derived from the wines studied.

The study of the mouthfeel properties of red wines requires considering tannins (this work focuses mainly on condensed tannins, polymerised flavanols also known as proanthocyanidins). The literature is consistent with respect to the sensory activity of these compounds, stating that they are mainly responsible for wine astringency (i.e., dryness). The modulation of this sensation during the ripening of the grape and the ageing of the wine is widely recognised. However, the correlation between the changes that occur in the chemical composition during grape ripening and wine ageing and the sensory changes remain relatively unknown. In order to

increase knowledge in this field, the effect of grape maturity and oxygen supply on both the taste and mouthfeel properties of grapes and red wines was analysed.

As far as the ripeness of the grape is concerned, it represents an important factor that determines the composition of the grape and consequently the sensory properties of the wines made with these berries. The study consisted of the elaboration of a total of 21 wines (7 wines in triplicate) with grapes of the Moristel variety. These grapes were harvested at different points of maturity (each point separated by one or two weeks) in two plots of very different characteristics. The results of the study showed that the ripeness of the grape generated significant sensory effects on the astringency (i.e., dryness) of the wine and on the fruity aromas of the wines ('black fruit', 'red fruit' and 'raisins'). An important result found in this study is related to the fact that grapes harvested early, even just one week in advance of the optimal point (defined as the one in which maximum values of aromas of red and black fruits and a moderate astringency in wines are obtained) have given rise to wines with higher levels of oxidation aldehydes (acetaldehyde, metional, phenylacetaldehyde and isoaldehydes). These oxidation notes are related to lower levels of certain polyphenols capable of reacting with the aforementioned aldehydes, called in previous works as ARPs (aldehyde-reactive polyphenols). This fact is also supported by negative correlations found between the aldehydes involved and various parameters related to polyphenols (IPT, concentration of tannins or pigments).

Further, the effect of oxidative and reductive ageing (key in the production of long-living wines) on the chemical composition and sensations in the mouth (mouthfeel and taste) has been studied. Therefore, the evolution of a young red wine and its phenolic fractions has been evaluated in the absence and presence of oxygen. Oxygen consumption was measured using a non-invasive method based on luminescence, in order to increase knowledge about the oxygen consumption of the different compounds in wine. In parallel, replicates of the same samples were kept in anoxia (not oxygenated). After exposure to oxygen, all samples were kept inside

an anoxic chamber (in the absence of oxygen). Both chemical and sensory analyses were carried out at two different times, 6 weeks after oxygenation and 24 weeks later. The chemical analysis of the samples showed an important modulating effect of the non-tannic phenolic fractions (flavanols, flavonols, anthocyanins and derived pigments) on the tannic activity (measured as the variation of enthalpy of interaction between proanthocyanidins and a hydrophobic surface) manifested by the tannic fraction of the wine. Thus, the activity of tannins can be reduced by simple addition of non-tannic phenolic compounds. As for the study of oxygen consumption, the results showed that the presence or absence of manganese can make differences in the oxygen consumption rate of fractions with the same phenolic composition. From the study of the effect of oxygen on the chemical parameters analysed, it should be noted that while oxidative ageing induces an increase in the parameter of tannic activity, anoxic ageing, both in oxygenated and non-oxygenated samples, induces changes in the structure of tannins increasing the % of prodelphinidins. Regarding sensory changes induced by oxygen, the most significant changes related to mouthfeel properties were observed in the fractions containing proanthocyanidins, in samples evaluated after 6 weeks of oxygen consumption. These sensory differences between non-oxygenated and oxygenated samples disappear after both samples are aged under anoxic conditions.

The study of the mouthfeel properties of wines was extended to the study of the phenolic fractions of grapes. This study is of great importance since the phenolic compounds present in wines have their origin in the grape. The phenolic compounds of the grape are extracted mainly from the skins and seeds during the maceration and fermentation processes. In this context, a total of 31 grape extracts were obtained and subsequently sensory characterized (through non-verbal and verbal strategies, the latter developed in this thesis) and further submitted to targeted chemical analyses (i.e., quantification of known compounds). The results showed significant sensory differences among the 31 polyphenolic grape extracts. Sensory variables were predicted from chemical parameters by PLS regression. The activity

and concentration of the tannins together with their average degree of polymerisation resulted to be good predictors of the sensation of dryness. The concentration of the polymeric pigments capable of precipitating with ovalbumin seems to be involved in the "adherent" perception and the flavonols in the "bitter" taste. These results increase knowledge about the properties of grape and suggests this methodology to infer grape quality.

Finally, untargeted chromatographic methods were developed and applied with the aim of identifying molecular markers that generating different taste and mouthfeel properties. This methodology overcomes the main limitations of classical directed instrumental techniques since they allow us to consider both unknown metabolites as well as those occurring at low concentrations and that, without a doubt, can play a determining role in the formation of taste and mouthfeel properties. For this study, a total of 42 wines were sensory characterised and their metabolomic profile was obtained by non-directed analysis using UPLC-HRMS-QTOF instrumental technique. The results of this study allowed to obtain very satisfactory PLS regression models predicting sensory variables from chemical parameters. Among the most interesting markers found are those derived from sulfonated flavanols, which according to the PLS models obtained, are involved in reducing the mouthfeel sensation of wine dryness. Similarly, the results suggest that amino acids and peptides are involved in modulating the dryness and oily attributes. In addition, it was possible to confirm the sensory role of anthocyanins and their derivatives in the perception of taste and mouthfeel of red wines. These results establish the basis for formulating different hypotheses related to the sensory activity of different compounds of red wines and whose involvement in taste and mouthfeel will be confirmed with reconstitution studies.

The results of this Doctoral Thesis show how different strategies that combine chemical and sensory techniques have proven to be effective in the study of different oenological concepts ("green character", maturity or oxidative aging), and have allowed to expand the knowledge about the properties of taste and mouthfeel

generated by wines. The development of a broad sensory vocabulary related to wine mouthfeel in section 1 has allowed to deepen the study of the compounds and / or factors causing the different sensations perceived through the development of mathematical models. In addition, the discriminant capacity of mouthfeel terms has been demonstrated in wine and in polyphenolic fractions obtained from grapes and wines. While the strategies developed during this work, which employ chemical-sensory methods along with both directed and undirected instrumental techniques, have shown satisfactory results, they still have limitations and the development of new analytical tools is a key factor for success in understanding properties of taste and mouthfeel sensations. The determination of the molecular structure of the compounds involved in taste and mouthfeel sensations is a great challenge that is fundamental to be able to fully understand their role. In this context, techniques such as voltammetry and spectrofluorometry could increase the variety of sensory attributes satisfactorily modelled.

Resumen

Resulta de gran interés para el sector vitivinícola lograr comprender los factores que incrementan la aceptabilidad del producto por parte del consumidor. La identificación de estos factores proporcionará a la industria herramientas útiles para aumentar la aceptación de nuestros vinos y así añadir valor a los mismos. El grado de aceptabilidad y satisfacción del consumidor está directamente relacionado con la percepción de la calidad. La percepción de la calidad es una variable multidimensional, que comprende tanto factores intrínsecos (propiedades organolépticas) como extrínsecos (diseño de la etiqueta, envase...) del producto, cuya interacción con el consumidor y sus características (por ejemplo: nivel de conocimiento sobre el producto o cultura) dan lugar a la formación de la calidad percibida.

Las propiedades intrínsecas, especialmente el sabor (flavour en inglés), tienen un papel muy importante en la formación de la calidad, especialmente en situaciones de recompra. El sabor es el resultado de la integración de los estímulos aromáticos, del gusto y de las sensaciones mediadas por el nervio trigémino (sensaciones táctiles en la boca). En la última década se ha producido un aumento significativo en el interés por comprender la formación del sabor, lo que implica comprender cómo se produce esa integración multisensorial tanto a nivel perceptivo como cognitivo.

Entre los sentidos involucrados en el sabor (olfato, gusto, sensación táctil), la formación de las sensaciones táctiles es en general la menos entendida, lo que es especialmente importante en el caso del vino. La percepción de las propiedades táctiles ocurre principalmente en la cavidad oral, donde tienen lugar procesos mecánicos y químicos. Hasta la fecha, a pesar de los avances en el análisis químico instrumental, las propiedades gustativas (acido, dulce, amargo, salado y umami) y sensaciones táctiles en boca de los alimentos en general y del vino en particular, solo pueden ser determinadas mediante estrategias sensoriales. Estas técnicas permiten obtener descripciones de los productos e identificar las diferencias que existen entre

ellos. Esta información es de gran interés para la industria, ya que ayuda a aumentar la comprensión sobre los factores que hacen que incremente la preferencia del producto por parte del consumidor y la calidad percibida del mismo, y puede ser empleada en el desarrollo de nuevos productos, así como en el desarrollo de estrategias de control de calidad, entre otros.

Sin embargo, resulta difícil obtener un perfil sensorial completo en términos de las propiedades táctiles en boca del vino. Esta dificultad reside en el hecho de que los compuestos que originan estas propiedades en su mayoría son desconocidos. Lo que a su vez genera una falta de un adecuado vocabulario estandarizado que describa objetivamente las dimensiones de las sensaciones táctiles del vino, lo que definitivamente dificulta el estudio y por ende la comprensión de los mecanismos que originan estas sensaciones.

En este contexto, el objetivo principal de esta Tesis Doctoral ha sido aumentar el conocimiento sobre la formación de las propiedades táctiles en boca y los compuestos y parámetros químicos que conducen y modulan esta percepción en vinos y uvas. Para lograr tal objetivo, se trabajó bajo la premisa de que la comprensión de las sensaciones táctiles en boca inducidas por los vinos debe abordarse combinando estrategias tanto sensoriales como químicas. Así en primer lugar (primera sección), se desarrolló una estrategia química sensorialmente dirigida para la obtención de fracciones inodoras con propiedades de gusto y táctiles consistentes. Las fracciones procedentes de tres vinos fueron analizadas sensorialmente mediante diferentes técnicas sensoriales: tarea de clasificación, técnica de rejilla, triangulación y Rate-All-That-Apply (RATA) con expertos en vino. El resultado más sorprendentemente, fueron las propiedades sensoriales de la fracción antociánica (conteniendo antocianos y derivados), que fue especialmente descrita como seca, amarga y persistente al igual que el vino original. Además, esta aproximación permitió generar una lista de 18 términos relacionados con las propiedades táctiles y se empleó con éxito en la descripción de vinos y fracciones mediante análisis RATA.

A continuación, esta metodología senso-química fue aplicada en el estudio del "carácter verde" de vinos tintos. Al comienzo de este estudio el "carácter verde" era un término mal definido y que a priori los enólogos declaraban que era una sensación ligada a las sensaciones táctiles y al gusto de ciertos vinos tintos. Los enólogos anotaban que se trataba de una sensación que estaban observando recurrentemente en sus vinos asociada al cambio climático y que estaba generando una depreciación de sus productos. Con el objetivo de identificar compuestos o grupos de compuestos responsables de esta sensación, en primer lugar, se abordó el estudio de la definición de este descriptor mal definido. Así, el "carácter verde" resultó ser un término multidimensional asociado a dimensiones tanto de aroma como de gusto y sensaciones táctiles en boca, que efectivamente estaba negativamente correlacionada con la preferencia de los vinos. A continuación, siguiendo con la estrategia de la sección I, se obtuvieron diferentes fracciones inodoras de vinos con alto y bajo carácter verde. Uno de los resultados más destacados, fue que la fracción antociánica (libre de taninos) de uno de los vinos con puntuación especialmente alta para el término "verde", resultó especialmente "adherente". Dicha fracción, fue sugerida como responsable del carácter verde en ese vino. Estos resultados en línea con los obtenidos en la sección I, sugirieron que las antocianos y derivados podían estar implicados en la formación y modulación de ciertas propiedades táctiles de algunos vinos tintos.

Para ahondar en el estudio de las propiedades sensoriales de la fracción antociánica se realizó un estudio a gran escala (considerando 42 vinos tintos). Dos fracciones diferentes obtenidas de vinos sensorialmente diferentes (las mismas fracciones aisladas en la sección I), que contenían taninos o antocianos, respectivamente, fueron analizadas química y sensorialmente en términos de gusto y sensaciones táctiles en boca. Los resultados confirmaron la actividad sensorial de las fracciones antociánicas estudiadas, que variaron significativamente en los términos: amargor, sequedad, granulosidad y cuerpo entre las fracciones derivadas de los vinos estudiados.

El estudio de las propiedades táctiles de los vinos tintos requiere considerar los taninos (este trabajo se centra principalmente en los taninos condensados, flavanoles polimerizados también conocidos como proantocianidinas). La literatura es congruente respecto a la actividad sensorial de estos compuestos, afirmando que son los principales responsables de la astringencia del vino. La modulación de esta sensación durante la maduración de la uva y el envejecimiento del vino es ampliamente reconocida. Sin embargo, la correlación de los cambios que ocurren en la composición química durante la maduración de la uva y el envejecimiento del vino y los cambios sensoriales que se producen, siguen siendo relativamente desconocidos. Con el fin de aumentar el conocimiento en este campo, se analizó el efecto de la madurez de la uva y el aporte de oxígeno en las propiedades tanto del sabor como táctiles de uvas y vinos tintos.

En lo que se refiere a la madurez de la uva, ésta representa un factor importante que determina la composición de la uva y en consecuencia las propiedades sensoriales de los vinos elaborados con dichas bayas. El estudio consistió en la elaboración de un total de 21 vinos (7 vinos en triplicado) con uvas de la variedad Moristel. Estas uvas fueron vendimiadas en diferentes puntos de madurez (cada punto separado por una o dos semanas) en dos parcelas de muy diferentes características. Los resultados del estudio mostraron que la madurez de la uva generó efectos sensoriales significativos sobre la astringencia del vino y en los aromas frutales de los vinos (‘fruta negra’, ‘fruta roja’ y ‘pasas’). Un importante resultado constatado en este estudio está relacionado con el hecho de que uvas vendimiadas de manera temprana, incluso con tan solo una semana de antelación respecto al punto óptimo (definido como aquel en que se obtienen máximos valores de aromas a frutas rojas y negras y una astringencia moderada en los vinos) han dado lugar a vinos con mayores niveles de aldehídos de oxidación (acetaldehído, metional, fenilacetaldehído e isoaldehídos). Estas notas de oxidación están relacionadas con los menores niveles de ciertos polifenoles capaces de reaccionar con los aldehídos citados, denominados en trabajos previos como ARPs (aldehyde-reactive

polyphenols). Una conexión está también apoyada por las correlaciones negativas encontradas entre los aldehídos implicados y varios parámetros relacionados con los polifenoles (IPT, concentración de taninos o pigmentos).

En cuanto al efecto que el envejecimiento oxidativo y reductivo, claves en la elaboración de vinos más longevos, tiene sobre la composición química y las sensaciones en boca (sensaciones táctiles y gusto), este ha sido estudiado empleando un vino tinto joven y sus fracciones fenólicas, en ausencia y presencia de oxígeno. El consumo de oxígeno se midió mediante un método no invasivo basado en luminiscencia, con el fin de aumentar el conocimiento sobre el consumo de oxígeno de los diferentes compuestos del vino. Paralelamente, réplicas de las mismas muestras se mantuvieron en anoxia (no oxigenadas). Después de la exposición al oxígeno, todas las muestras se mantuvieron dentro de la cámara anóxica (en ausencia de oxígeno). Los análisis tanto químicos como sensoriales se llevaron a cabo a dos tiempos distintos, 6 semanas después la oxigenación y 24 semanas después. Mediante el análisis químico de las muestras, se ha puesto de manifiesto un importante efecto modulador de la composición fenólica no tánica (flavanoles, flavonoles, antocianos y pigmentos derivados) sobre la alta actividad tánica (medida como la variación de entalpía de interacción entre las proantocianidinas y una superficie hidrofóbica) manifestada por la fracción tánica del vino, de tal forma que esta puede ser reducida por simple adición de los compuestos fenólicos no tánicos. En cuanto al estudio del consumo de oxígeno, los resultados mostraron que la presencia o ausencia de manganeso puede marcar diferencias en la velocidad de consumo de oxígeno de fracciones con la misma composición fenólica. Del estudio del efecto del oxígeno sobre los parámetros químicos analizados, cabe destacar que mientras el envejecimiento oxidativo induce un incremento en el parámetro de actividad tánica, el envejecimiento anóxico, tanto en muestras oxigenadas como no oxigenadas, induce cambios en la estructura de los taninos aumentando el % de prodelfininidas. En cuanto a los cambios sensoriales en boca debidos al oxígeno, los cambios más significativos relacionados con las

propiedades táctiles evaluadas se observaron en las fracciones que contienen proantocianidinas, en muestras evaluadas después de 6 semanas de consumo de oxígeno. Estas diferencias sensoriales entre muestras no oxigenadas y oxigenadas desaparecen después de que ambas muestras son envejecidas en condiciones anóxicas.

El estudio de las propiedades táctiles en boca del vino y los compuestos implicados en éstas, se amplió al estudio de las fracciones fenólicas de la uva. Este estudio es de gran importancia ya que los compuestos fenólicos presentes en los vinos tienen su origen en la uva. Los compuestos fenólicos de la uva se extraen principalmente de los hollejos y pepitas durante los procesos de maceración y fermentación. En este contexto, se obtuvieron un total de 31 extractos de uva y posteriormente se caracterizaron sensorial (mediante estrategias no verbales y verbales, ésta última desarrollada en esta tesis) y químicamente dirigida (es decir, cuantificación de compuestos conocidos). Los resultados mostraron diferencias sensoriales significativas entre los 31 extractos polifenólicos de uva. Las variables sensoriales se predijeron a partir de los parámetros químicos mediante regresión PLS. La actividad y concentración de los taninos junto con el grado medio de polimerización de los mismos, resultaron ser buenos predictores de la sensación de sequedad, mientras que la concentración de los pigmentos poliméricos capaces de precipitar con ovoalbúmina, parece estar involucrada en la percepción "adherente" y los flavonoles en el sabor "amargo”. Estos resultados aumentan el conocimiento sobre las propiedades de la uva y proponen esta metodología para inferir la calidad de la uva.

Finalmente, se desarrollaron y aplicaron análisis cromatográficos no dirigidos con el objetivo de identificar marcadores moleculares que pueden generar sensaciones táctiles en la boca. Esta metodología supera las principales limitaciones que presentan las técnicas instrumentales dirigidas clásicas ya que permiten considerar tanto metabolitos desconocidos como los que se encuentran en bajas concentraciones y que, sin duda, pueden desempeñar un papel determinante en la

formación de las propiedades táctiles en boca. Para este estudio, se caracterizaron sensorialmente un total de 42 vinos y su perfil metabolómico fue obtenido mediante análisis no dirigido empleando la técnica instrumental UPLC-HRMS-QTOF. Los resultados de este estudio, permitieron obtener modelos de regresión PLS muy satisfactorios prediciendo variables sensoriales a partir de parámetros químicos. Entre los marcadores encontrados están los derivados de flavanoles sulfonados, los cuales, de acuerdo con los PLS obtenidos, están implicados en la reducción de la sensación táctil de sequedad del vino. De manera similar, los resultados sugieren que los aminoácidos y péptidos están involucrados en la modulación de los atributos sequedad y graso. Además, se pudo confirmar el papel sensorial de las antocianos y sus derivados en la percepción del gusto y las sensaciones táctiles en boca de los vinos tintos. Estos resultados establecen las bases para formular diferentes hipótesis relacionadas con la actividad sensorial de diferentes compuestos de los vinos tintos y cuya implicación en el gusto y las sensaciones táctiles en boca se confirmarán con estudios de reconstitución.

Los resultados de esta tesis doctoral muestran como diferentes estrategias que acoplan las técnicas químicas con las sensoriales han demostrado ser efectivas en el estudio de diferentes conceptos enológicos (“carácter verde”, madurez o envejecimiento oxidativo), y han permitido ampliar el conocimiento sobre las propiedades del gusto y táctiles en boca generadas por los vinos. Así, el desarrollo de un amplio y bien definido vocabulario sensorial relacionado con las sensaciones táctiles de los vinos en la sección 1, ha permitido profundizar en el estudio de los compuestos y/o factores causantes de las diferentes sensaciones percibidas mediante el desarrollo de modelos matemáticos. Además, ha sido demostrada su capacidad discriminante al aplicarse tanto en vino, como en fracciones polifenólicas obtenidas de uvas y vinos. Si bien las estrategias desarrolladas durante esta tesis doctoral, que emplean métodos químico-sensoriales junto con técnicas instrumentales tanto dirigidas como no dirigidas, han mostrado resultados satisfactorios, todavía tienen limitaciones y el desarrollo de nuevas herramientas

analíticas es un factor clave para el éxito en la comprensión de propiedades del gusto y las sensaciones táctiles. La determinación de la estructura molecular de los compuestos implicados en el gusto y las sensaciones táctiles se plantea como un gran reto que es fundamental para poder comprender plenamente su papel. En este contexto, técnicas como voltamperometría y espectrofluorometría, podrían aumentar la variedad de atributos sensoriales satisfactoriamente modelados.

Índice

Índice

INTRODUCTION

General introduction, bibliographic review

1. Introduction

The vine is one of the most important crops worldwide, both economically and culturally. In ancient times and currently, the importance of its cultivation for society is based on being the source of products of great economic value, such as wine (Moricca et al., 2021).

Wine has a specific law approved by the General Courts, Law 24/2003, of July 10, on Vine and Wine, reflection of its relevant cultural and socio-economic importance (Miranda Escolar & Fernández Morueco, 2011).

1.1. Cultural and economic importance of wine in Spain

Wine has been consumed for centuries in different regions of the Mediterranean area, being part of the lifestyle, culture and diet since antiquity. Therefore, the study of the origin of grape and wine production, represents an important issue in understanding the evolution of societies. Some works focus on developing analytical methods to detect organic markers related to wine in archaeological ceramics (Blanco-Zubiaguirre et al., 2019), while others focus on grapevine seeds analysis (Moricca et al., 2021).

In the Iberian Peninsula, the origin of winemaking is dated with the arrival of the Phoenicians, around 1.100 BC. in the south of the Peninsula, in the area of Gadir, that is today known as Cádiz (Pérez-Jordà, Peña-Chocarro, & Pardo-Gordó, 2021). It is important to name, an archaeological finding, an exceptionally preserved winepress (*lagar*) from the 3rd century BC (Ruiz-Mata, 2018). This highlights the cultural importance that the wine sector represents in Spain, what, it is also a contribution to the history of viticulture on a world scale.

It is easy to find in history and literature, testimony of what wine represents in our culture. Thus, it is written in "*El libro del Buen amor*" (1330-1343) of the clergyman, Juan Ruiz, the Arcipreste of Hita: "*Es el vino muy bueno en su mesma natura, muchas bondades tienen, si se toma con mesura*". Special mention to "*El*

lazarillo de Tormes" (1554), the quintessential work of the Spanish "picaresca", where wine plays a leading role, and the wine culture is evident once again. In it you can read: *"Yo, como estaba hecho al vino, moría por él…"*.

Wine also has a place in the famous work of Miguel de Cervantes "El *Quijote*" (1615), which says in the chapter XXXV: "…y había dado tantas cuchilladas en los cueros, creyendo que las daba en el gigante, que todo el aposento estaba lleno de vino", referring to leather wine containers, one of the oldest methods of storing wine.

It is also not unusual to find references to wine in art. Artists have captured over the centuries the importance of wine in society through their works. It is the case, of the painting of Diego Velázquez "*El triunfo de Baco*" (1628-1629), known colloquially as "*Los borrachos*" (Figure 1a). The painting is an allegory about wine. "*La Vendimia" o "El otoño*" (1786), of Francisco de Goya y Lucientes, is part of a set about the four seasons (Figure 1b). In this case, the author uses the harvest to ilustrate autumn. Even the world-renowned Pablo Ruiz Picasso, one of the creators of cubism, captured it in various works. It is mentioned here "*La botella de vino*" (1922) (Figure 1c).

a) "*El triunfo de Baco*" (1628-1629).

b) "*La Vendimia"* (1786).

c) "*La botella de vino*" (1922).

Figure 1. References to wine in art: Painting of a) Diego Velázquez; b) Francisco de Goya; c) Pablo Ruiz Picasso.

Concerning the economic importance of wine, the wine sector represents a very important part of the Spanish economy, which is supported by its numbers (Figure 2). Spain is the world leader in vineyard area, with 961.000 hectares, which represents over 13% of the total world surface. It is the third largest producer of wine, and the second in export of wine in terms of volume and the third in terms of value (OIV, 2021).

The gross added value (GVA) is defined as the added value generated by the set of producers in a given sector along the different stages of the production process. Thus, the grape and wine value chain is made up of many actors (Goncharuk, 2017, Skreli & Drini, 2019). The wine sector, which includes viticulture, the winemaking and its commercialisation, contributes to the gross added value (GVA) by 2.2% of the Spanish GVA according to the report "*Economic and Social Importance of the Wine Sector in Spain*" (2020), carried out by International Financial Analysts (AFI) for the Interprofessional Wine Organization of Spain (OIVE).

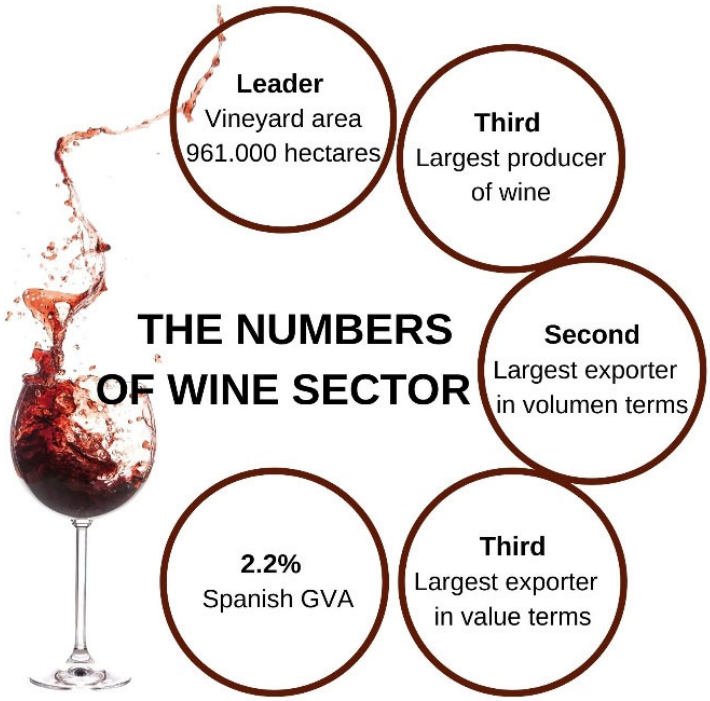

Figure 2. The wine sector in numbers. Data source OIV, 2021. Self-made infographic employing Canva free application.

The stakeholders are involved in growing, processing, and selling the wine that consumers drink. In addition, enotourism, culture and leisure are involved, highlighting wine museums spread throughout different parts of the Iberian Peninsula (Figure 3).

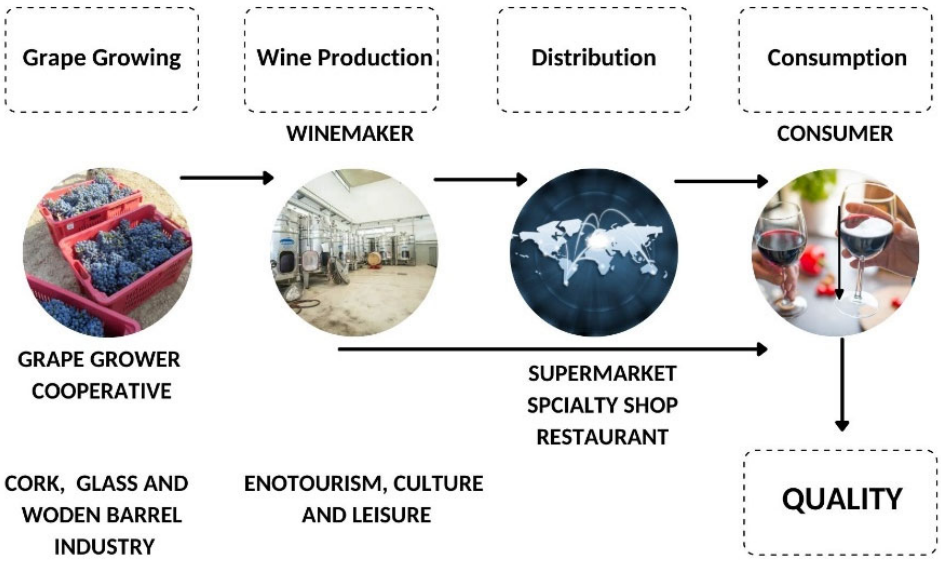

Figure 3. Wine value chain, adapted from Goncharuk (2017). Self-made infographic employing Canva free application.

The last link in the chain of the wine industry is the consumer, which plays a relevant role as the rest of the agents involved depend on their positive response to the product. Satisfaction can be defined as "*a post-selection evaluative judgment, relative to a specific purchase decision*" (Espejel-Blanco & Fandos-Herrera, 2008). It means, if the perceived quality is greater than or equal to that expected, the consumer will be satisfied. Hence, their degree of satisfaction is directly related to their perception of quality. For this reason, one of the biggest challenges of the wine industry is to understand the drivers of perceived quality. Thus, to produce wines appreciated by consumers.

1.2. Wine quality

Currently, in such a globalised market, it is very important to seek for quality in wine, an important consumer requirement. The development that has taken place in the last century in the wine sector has been crucial, and the wine has experienced an increase in quality, due to the improvement in the technology applied in its production.

The understanding of perceived quality of wine is a key clue for providing the wine industry with tools for increasing the acceptance of products and thus add value to the product. There is a wide range of scientific literature aimed to understand the wine quality concept studied from very diverse perspectives (Araujo et al., 2021; Charters & Pettigrew, 2007; Ferreira et al., 2009; Goldstein et al., 2008; Jones, White, Cooper& Storchmann, 2005; Mira de Orduña, 2010; Parr et al., 2020; Reynolds, 2010; Sáenz-Navajas et al., 2015; Sherman, Coe, Grose, Martin, & Greenwood, 2020). All these works agree in that it is necessary to consider both consumer and its interaction with the characteristics of the product when trying to understand perceived quality and thus wine acceptability and satisfaction with the wine. The perception of the product varies according to previous experiences of the consumer, being its culture as well as its involvement with wine and expertise important factors modulating consumer behaviour among others (Joy, Charters, Wang, & Grohmann, 2020; Sáenz-Navajas et al., 2016). For example, concerning the last factor, the perception of consumers is different from that of wine experts, even consumers who are very familiar and knowledgeable with wine (i.e., high-involved consumers) have a different perception in comparison with those who are less involved. A plethora of works has shown that this is an important mediator of wine quality (Aurifeille, Quester, Lockshin, & Spawton, 2002; Bruwer & Huang, 2012; Cox, 2009; Hollebeek et al., 2007; Lockshin, Quester, & Spawton, 2001; Lockshin, Spawton, & Macintosh, 1997; Torri et al., 2013).

At this point, it is important to mention the two processes that consumers follow to form the overall product perception and consequently perceived quality: "bottom up" and "top-down" cognitive processes (Parr, 2008). On the one hand, as a result of the bottom-up process the consumer gathers the information related to the characteristics of the wine itself. In this process the sensory-active compounds activate the sensory receptors present in the organs and generate a signal: sensation. On the other hand, the top-down cognitive process takes place when the information obtained from the sensory organs (i.e., sensation) reaches our brain and is interpreted, based on expectations and thus on previous knowledge (see Figure 4).

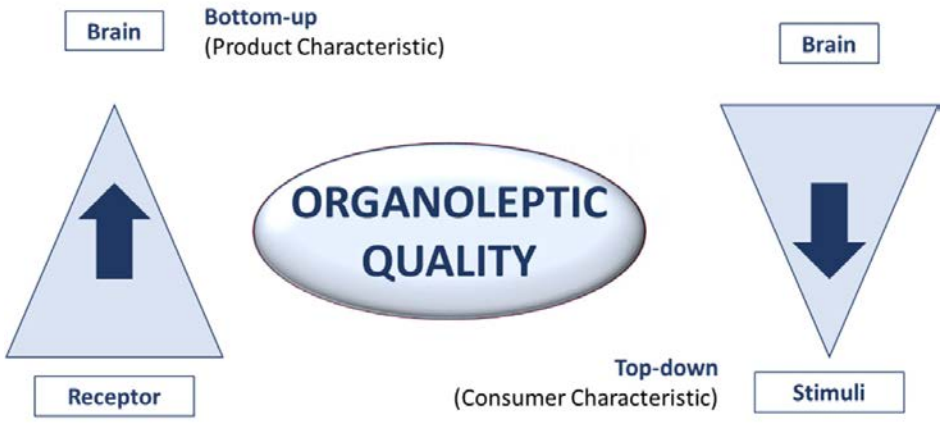

Figure 4. Global cognitive process

Therefore, in order to understand quality perception and thus consumer behaviour, the global cognitive process, which is the result of the integration of both bottom-up and top-down processes, should be considered. That is to say, the perceived quality has to be tackled by understanding the interaction of the product with the consumer.

Product-related factors refer to both intrinsic and extrinsic categories (Verdú-Jover, Lloréns Montes, & Fuentes, 2004; Veale & Quester, 2009). Intrinsic cues are related to a product´s flavour, while extrinsic cues refer to properties which are not physically part of the wine (e.g., bottle weight, place of bottling, type of wine or

appellation…). Extrinsic cues appear to be important in both expected (before being consumed) and experienced (after being consumed) perception of the product especially when less-experienced consumers experiment difficulty in the evaluation of intrinsic quality of the product. The importance of extrinsic cues on wine appreciation also lies in the fact that in most wine purchase situations consumers cannot taste wine and thus evaluate intrinsic factors, what forces them to rely on wine's extrinsic cues. However, the intrinsic characteristics of previously experienced wines play a major role in repurchase situations (D'Alessandro & Pecotich, 2013). While consumers rely on both types of cues when selecting a product, there is a wide range of works focused on understanding the impact of extrinsic cues on wine appreciation, but much less is known about intrinsic cues (i.e., flavour). In terms of intrinsic quality, the absence of aroma defaults is the first requirement for a quality wine. When these annoying molecules are not present, the sensations in the mouth are essential for perceived quality and thus the acceptability of the product. Spence & Piqueras-Fizman (2016) suggest that oral-somatosensory attributes of food and beverages are important drivers for the formation of perception, and therefore consumers´ perceived quality, acceptance and preferences. Similarly, Agorastos (2020), citing Guinard & Mazzucchelli (1996), propose mouthfeel as an important factor, together with gustation and smell, contributing to the overall food perception and acceptance of the consumers. Regarding wine quality, Cheynier & Sarni-Manchado (2010) support the idea that taste and mouthfeel are key factors of wine quality and furthermore, recent work (Araujo et al., 2021) has demonstrated that mouthfeel attributes are related to high quality Pinot Noir Wines, what justifies its study and confirms its importance for the wine industry.

The formation of mouthfeel is overall the least understood, but it is an important factor to consider in the overall wine quality. Thus, considering the importance of taste and mouthfeel in red wine quality and appreciation, the main goal of the present doctoral thesis is to increase the understanding of the molecular basis of red wine palate properties.

In this context, this introduction aims to provide an overview of the concepts of taste and mouthfeel and their linkage to flavour and perceived quality and further examine the present knowledge about the mechanisms and compounds that cause mouthfeel and taste with focus on red wines.

To accomplish such goal, the role of mouthfeel and taste on the concept of flavour will be discussed, and cross-modal interactions will be considered to explain the formation of taste and mouthfeel percepts in both food/beverages in general and in wine in particular. Besides, the confusion surrounding the mouthfeel concept will be highlighted. Then, an exhaustive review of mechanisms involved in the formation of mouthfeel proposed in recent years is presented. The sensory active compounds modulating mouthfeel and taste as well as the factors affecting their perception (such pH, alcoholic grade…) will be described. Finally, the methodology developed up to date, both at sensory and chemical levels, for the determination and evaluation of mouthfeel will be introduced.

2. Definition of flavour

The term flavour has experienced an evolution in its meaning, as suggested by Prescott & Stevenson (2015), who highlighted the confusion around this term.

In the literature, the terms taste and flavour are usually employed indistinguishably, which can lead confusion. According to Spence, Auvray, & Smith, (2014), this makes difficult that researchers provide advances on the topic of flavour. Therefore, it seems pertinent to try to clarify the definition of flavour and the role of taste and mouthfeel on the overall flavour perception.

Scientifically, taste is strictly the sensation experienced by means of the taste buds. It is possible to distinguish five taste classes: sweet, sour, bitter, salty, and umami. The perception of taste takes place through *taste receptor cells* (TRC) located in the taste buds placed in the tongue. While salty and sour tastes are mediated by ion channels, sweet, bitter, and umami are mediated by G-protein coupled receptors (Drayna, 2005; Kim, Breslin, Reed, & Drayna, 2004; Swiegers, Chambers, & Pretorius, 2005).

However, in everyday language, it is common to hear that the loss of taste is associated with blocked nose, due to the consideration of taste as flavour, from a wrong point of view. In this context, the retronasal smell is not considered, nevertheless in the multisensory perception of flavour are involved the three main senses olfactory, gustatory and somatosensory system and odorant molecules can reach the olfactory epithelium via the nose (orthonasal) or the mouth (retronasal olfaction) (Small, Gerber, Mak, & Hummel, 2005). The aforementioned loss of taste can induce to certain confusion because strictly talking it should be referred to as a loss of perceived flavour, because tastes (salty, sweetness, sourness, bitterness and umami) can still be perceived with blocked nose. This confusion has been attributed to the fact that even if taste and aroma are perceived in different physical parts (aroma in the nose and taste in the mouth), both percepts are perceived simultaneously while consuming the wine and are integrated in our brain to form

the overall flavour perception. Thus, there is a repeated co-exposure of both types of stimuli making odours to take on the properties of tastes, from both hedonic and perceptual perspectives through associative learning (Prescott, 2015). One consequence is that the hedonic properties of tastes become attached to the odour. That is, odours that are usually paired with the sweet taste become liked; while odours usually accompanied by bitter taste are most probably disliked (Prescott, 2015). The second consequence is that odours are described in terms of tastes (e.g., the sweet smell of vanilla or the sour smell of lemon). This has been attributed to the integration and simultaneous learning (through repeated exposure) of tastes and aromas, and are thought to be different from the hedonic perception. The multisensory integration of different senses to form the overall flavour perception has developed a growth of interest in the last decades. This concept of flavour as a result of sensory integration was already suggested by Gibson (1966), who presented an ecological perspective of perception (West & Gibson, 1966).

In overall, flavour is a multisensory experience, which integrates the whole sensations perceived in the mouth and nose (Auvray & Spence, 2008; Campo, Reinoso-Carvalho, & Rosato, 2021; Prescott, 2012; Small & Prescott, 2005; Spence, 2016; Spence & Piqueras-Fiszman, 2016).

Another point to consider is that there has been some kind of confusion concerning which senses are directly involved in flavour and which are only modulatory (Spence, 2019). While aroma and taste have been unequivocally considered modalities involved in flavour, trigeminal inputs have been underestimated (Lawless, 1989), which can be attributed to a lack of consensus around this percept as will be further discussed.

3. Definition and role of mouthfeel on flavour

According to Spence (2019, p.227) mouthfeel was not necessary considered as being part of flavour: *"The trigeminal sense, giving rise to burning and cooling sensations (e.g., of chilli and menthol, respectively), is also considered a constitutive, if not always a necessary, component of flavour perception".* Differently, there is a plethora of works that accept that the trigeminal system contributes to flavour perception (Abdi, 2002; Agorastos, 2020; Spence, Auvray, & Smith, 2014; Spence & Piqueras-Fiszman, 2016; Viana, 2011). Accordingly, the present Doctoral thesis will consider oral-somatosensory cues elicited by the trigeminal system being part of flavour perception as has been widely recognised by most recent scientific literature.

Another point that merits to be discussed is the lack of accordance about the use of the terms "trigeminal sensations" and "oral-somatosensory cues". Some authors discern between them (Spence, 2015, 2016; Spence et al., 2014). Thus, it is possible to read in Spence (2015, p.24), "*On top of these two senses (gustatory and olfactory), trigeminal inputs also contribute to flavour perception. As for the other senses, such as vision, audition, and oral somatosensation …".* Similarly, in Spence (2016, p.373): "*Tactile cues concerning the mouthfeel, texture, temperature, and trigeminal cues relating for example to the burning sensation associated with spicy foods are important too"*. The definition proposed by the International Standards Organization (ISO 5492 1992, 2008), in which flavour is defined as a "*Complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting. The flavour may be influenced by tactile, thermal, painful and/or kinaesthetic effects*" also contribute to this confusion as it can be extracted from Spence & Piqueras-Fiszman, (2016, p.60). It is easy to see, that readers might become confused. When authors employ that difference, it must be assumed that trigeminal sensations refer to perceptions that arise from chemical stimuli (chemesthesis), while oral-somatosensory cues refer to physical stimuli (somesthesis). Notwithstanding, this classification does not seem to be accurate from a physiological point of view. The somatosensory system provides and processes

information derived from somatic sensations, as pain, temperature or touch and more specifically the somatosensory information from the face is transmitted via the trigeminal nerve. The trigeminal somatosensory complex includes the main sensory nucleus (lemniscal pathway) that deals with the mechanoreceptive aspects of somesthesis and the spinal trigeminal nucleus (spinal trigeminal pathway), that deals with pain and temperature aspects of chemesthesis (Ten Donkelaar, Broman, & Van Domburg, 2020). In that sense, it can be concluded that general trigeminal inputs include chemesthesis and somesthesis receptors both mediated by the trigeminal nerve (Figure 5).

Thus, the term mouthfeel, describes a set of chemical or physical sensations in the mouth, in concrete tactile perceptions that take place in the oral cavity. The mouthfeel sensations are related to stimuli conveyed to the brain by the trigeminal system (Agorastos, 2020; Hewson, Hollowood, Chandra, & Hort, 2009), which is well in line with Gawel, Smith, Cicerale, & Keast, (2018) that defined the mouthfeel sensations as ‘’*The tactile, irritant and thermal sensations resulting from the activation of chemosensory and somatosensory receptors within the oral cavity by chemical stimuli*’’ and more recently, with Canon, Caillé, Sarni-Manchado, & Cheynier, (2022) referring to mouthfeel as “*Mouthfeel is the mixed experience derived from sensations in the mouth that relate to physical or chemical properties of a stimulus*”.

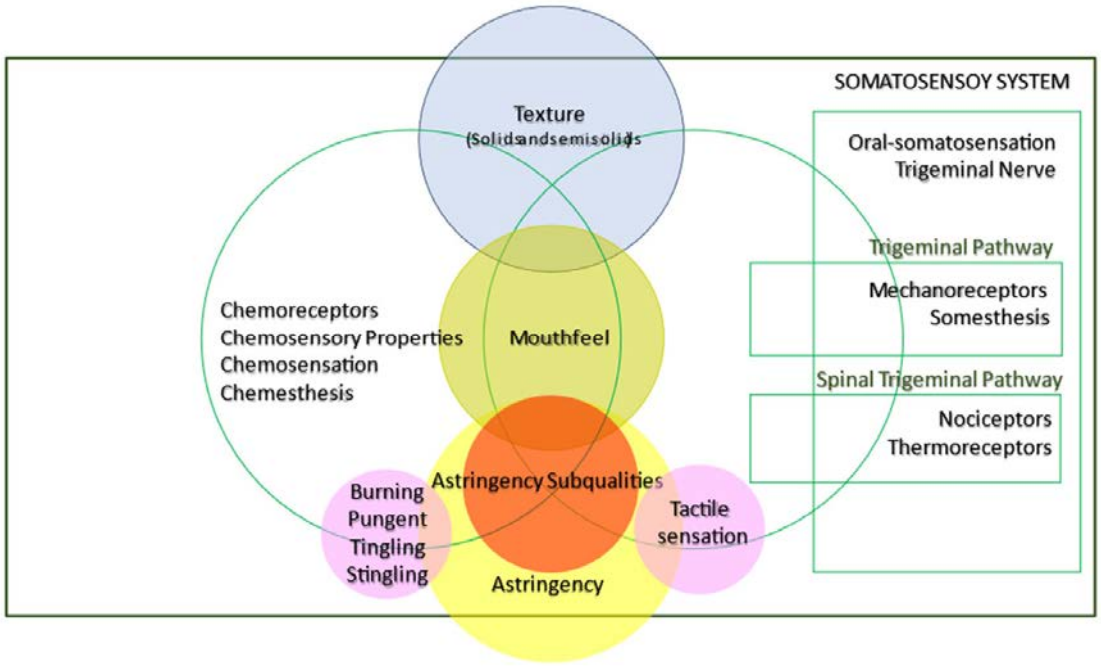

Figure 5. Mouthfeel propose scheme

Accordingly, mouthfeel is the result of the activation of the trigeminal nerve and it is the term employed to describe the sensations perceived in mouth that are neither odours nor tastes, comprising somesthetic and chemesthetic sensations. As stated above, this term as well as its physiology is not free of controversy. Although some works make no differences between mouthfeel and taste (Byrne, 2016), most of the scientific literature unequivocally define and differentiate both percepts (Guinard & Mazzucchelli, 1996; Gawel, 1997; Gawel, 1998; Gawel, Oberholster, & Francis, 2000; Breslin, Gilmore, Beauchamp, & Green, 1993; Green, 1993).

In summary, the literature review supports the idea that mouthfeel is an important component of flavour perception, independent from taste perception, and it is the result of the activation of chemosensory and somatosensory receptors, involving touch, pain and temperature, both mediated by the trigeminal nerve as illustrated in Figure 6.

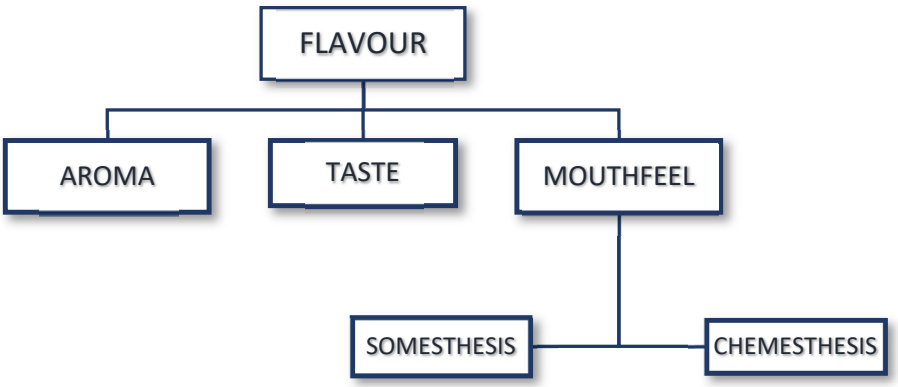

Figure 6. Senses involved on flavour

4. The physiological basis of mouthfeel

Once the wine is in the mouth, a set of sensory inputs get the central nervous system via gustatory, olfactory, and somatosensory peripheral pathways. Therefore, it becomes necessary to provide an overview of the nervous system to understand the mechanisms involved in mouthfeel formation (de Araujo, Geha & Small, 2012).

4.1. Somatosensory system

The somatic sensory system or somatosensory system is the part of the nervous system that detects and allows the perception of pain, temperature, head and body position (proprioception), head and body movement (kinesthesia), and touch (Jacobs, 2011). The main difference with other sensory systems is based on the receptors distribution which are located throughout the body instead of concentrated in specialised locations and respond to different stimuli.

The somatosensory cortex is the region of the brain which is responsible for receiving and processing sensory information and it is found within the parietal lobes (Figure 7).

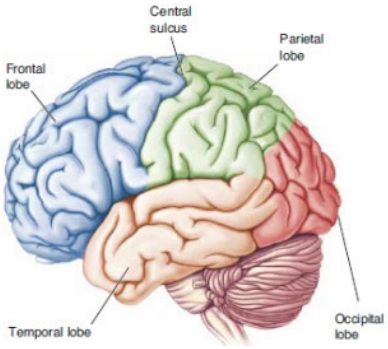

Figure 7. The lobes of the human cerebrum. Reproduced from: Bear, M. F., Connors, B. W., & Paradiso, M. A. (2007). Neuroscience: Exploring the brain (3rd ed.). Lippincott Williams & Wilkins Publishers.

Thus, the somatosensory system conveys information from the periphery to the brain, using peripheral mechanoreceptors, chemoreceptors, thermoreceptors, and nociceptors (classification done according their function, i.e., how the receptor converts the stimuli) which transmit information towards the brain. Therefore,

different types of receptor cells are responsible for the different stimuli. Most of the sensory receptors in the somatic sensory system are mechanoreceptors (Bear, Connors, & Paradiso, 2007).

Receptors are broadly described as encapsulated or unencapsulated according to their structure. The neurons that have non encapsulated endings, like free nerve endings neurons or Merkel´s disks, are responsible for sensing pain and temperature, while neurons with encapsulated nerve endings, like Meissner corpuscles, Pacinian corpuscles and Ruffini corpuscles, answer to pressure and touch stimuli (Drew, Rugiero, & Wood, 2007) (Figure 8).

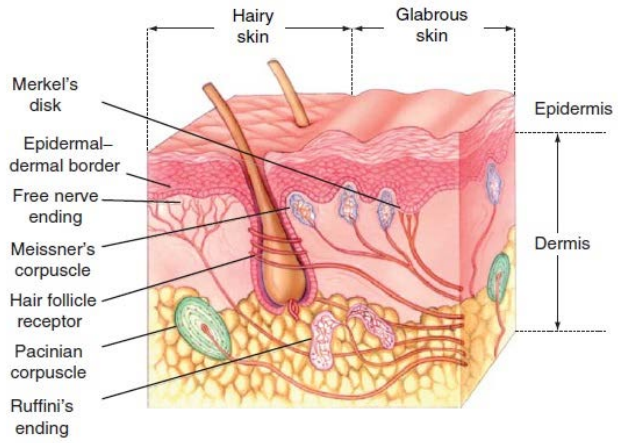

Figure 8. Somatic sensory receptors in the skin. Reproduced from: Drew, L. J., Rugiero, F., & Wood, J. N. (2007). Touch. In *Current Topics in Membranes* (Vol. 59, pp. 425–465). https://doi.org/10.1016/S1063-5823(06)59016-7

Regarding nociceptors and thermoceptors, they exist as free nerve endings in the skin, and are stimulated by nociceptive stimuli that produce the sensation of pain or by changes in skin temperature.

Nociceptors can be activated by mechanical, thermal or chemical stimuli, which can be defined as polymodal receptors. Hereby, different stimuli evoke different nociceptors reactions (i.e., when a thermal stimulus is applied on the skin, in a range of non-painful temperature, non-nociceptive thermoreceptors are activated,

however, whenever the thermal stimulus applied reaches high levels, nociceptive thermoreceptors are activated (Purves, et al., 2001); which is in accordance with Hayes (2016), who explains that TRPV1 receptor is a polymodal nociceptor that responds to heat pain from temperatures above 42.5 °C).

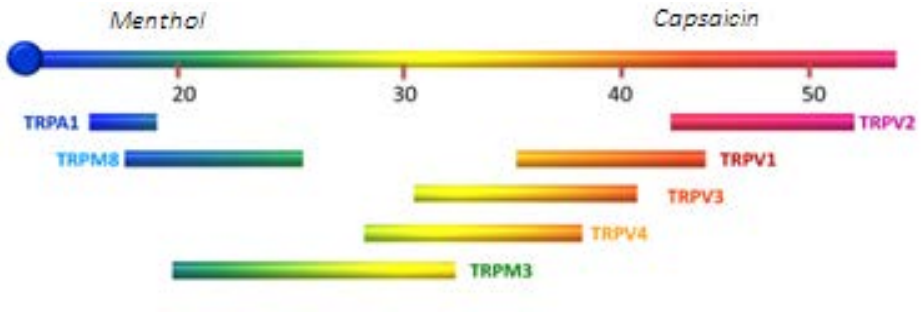

Figure 9. Thermotransient receptor potential (TRP) channels. Adapted from: Ferrandiz-Huertas, C., Mathivanan, S., Wolf, C. J., Devesa, I., & Ferrer-Montiel, A. (2014). Trafficking of ThermoTRP Channels. Membranes, 4(3), 525–564. https://doi.org/10.3390/membranes4030525

Thermoreceptors are neurons that are sensitive to temperature. Transient receptor potential (TRP) ion channels have different physiological functions, one of them thermosensitivity. According with Ferrandiz-Huertas, Mathivanan, Wolf, Devesa, & Ferrer-Montiel (2014) (Figure 9), thermosensory ion channels (thermoTRPs) comprise a set of TRP ion channels that are activated by fluctuations in the temperature. Most of them are non-selective cation channels and humans possess 28 different TRP channels, among these, the vanilloid TRP channels (TRPV), the melastatin (TRPM), and the ankyrin (TRPA) show special importance as thermoreceptors. The vanilloid receptors: TRPV1, TRPV2, TRPV3, and TRPV4 are activated by heat sensation, while cold sensation is reported by melastatin-related TRPM8 and the ankyrin repeat-rich TRPA1 receptors (Drew et al., 2007).

4.2. Face somatosensory system

The somatosensory information of the face is transmitted by trigeminal nerve. Trigeminal nerve (Fifth cranial nerve, CN V) has three branches: the ophthalmic, maxillary, and mandibular nerves, which provide sensory innervation to the face, as well as to the oral and nasal cavities (Figure 10). Furthermore, other cranial nerves: the facial (VII), glossopharyngeal (IX), and vagus (X), provide supplementary information. They surround taste buds, and different classes of receptors and transmit information via the trigeminal system (Walker, 1990; Traurig, 2008).

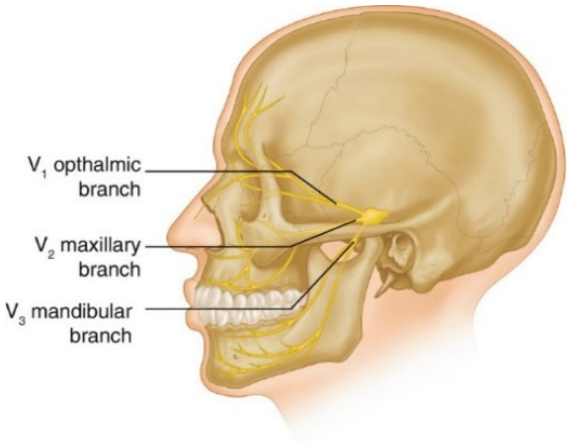

Figure 10. Trigeminal nerve. Reproduced from: White T., Rastogi R., Singh T.S.S. (2019) Trigeminal Neuralgia. In: Pain.. Abd-Elsayed A. (eds) Springer, Cham. https://doi.org/10.1007/978-3-319-99124-5_129

4.3. Somatosensory pathways

The sensory information is carried to the brain by different pathways (Table 1).

Table 1. Somatosensory Pathways. Adapted from: Open-Access Neuroscience Electronic Textbook. Department of Neurobiology and Anatomy at The University of Texas Health Science Center at Houston, McGovern Medical School.

Somatic sensation	Somatosensory Pathway (Body)	Somatosensory Pathway (Face)
Pain	Spinothalamic	Spinal Trigeminal
Temperature		
Touch	Column-Medial Lemniscal	Main Sensory Trigeminal
Proprioception		

While the main sensory trigeminal pathway carries and processes discriminative touch information from the face, the spinal trigeminal pathway carries and processes pain and temperature information from the face (Table 1). The main difference between both is that, although both pathways decussate, they cross at different anatomical levels (Figure 11). In the main sensory trigeminal pathway, the primary axon remains on the same side of the spinal cord until it connects with the second neuron in the chain, the axon of this second neuron crosses the midline immediately, whereas in the spinal trigeminal pathway the afferents synapse first on second order neuron and the axon decussate immediately and ascend to the thalamus.

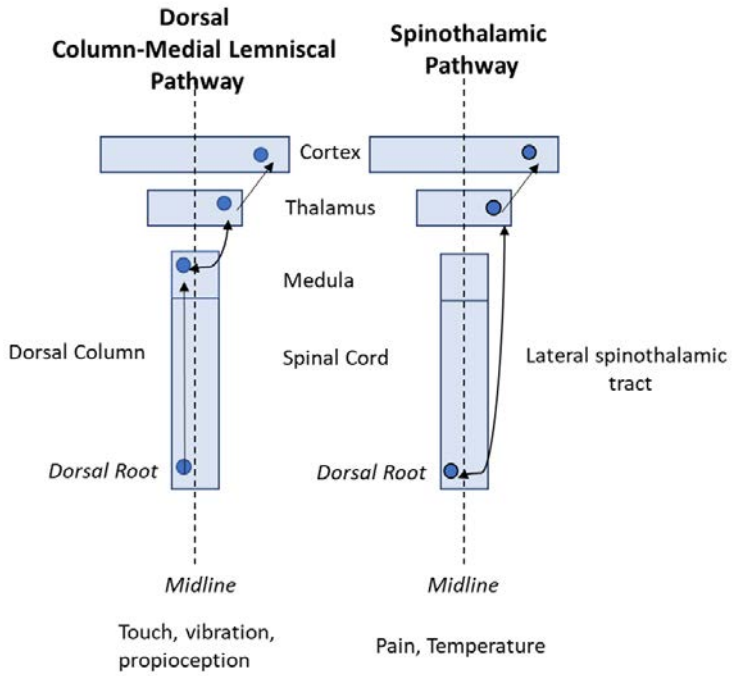

Figure 11. Somatosensory pathways. Adapted from Bear, M. F., Connors, B. W., & Paradiso, M. A. (2007). Neuroscience: Exploring the brain (3rd ed.). Lippincott Williams & Wilkins Publishers.

In this context, the present Doctoral Thesis will consider olfactory, gustatory and mouthfeel (oral-somatosensory cues elicited by the trigeminal system) as being part of flavour perception.

5. Cross-modal interactions in flavour

Considering that wine flavour is a whole, being the result of the integration of aroma, taste and mouthfeel, the understanding of wine taste and mouthfeel should be approached from a holistic perspective, and consequently, the importance of their interaction with aroma cannot be neglected. The perception of each sensation is affected by the presence of other stimuli perceived by other senses (i.e., cross-modal sensory interaction) (Prescott, 2012; Small & Prescott, 2005). In this context aroma-taste, taste-mouthfeel and mouthfeel-aroma interactions will be discussed.

5.1. Aroma-taste interactions

As mentioned previously, odours can acquire taste qualities. According to Spence (2015), it is suggested that the brain combine tastes and smells that commonly appear together (i.e., associative learning). The interactions can be odour-induced taste enhancement (i.e., a particular taste is increased by the presence of particular odours) or taste-induced odour enhancement (i.e., a particular odour is increased by the presence of particular taste) (Prescott, 1999; Prescott, Johnstone, & Francis, 2004; Prescott & Stevenson, 2015). While the interaction between taste and aroma has been widely studied in food systems (Caporale, Policastro, & Monteleone, 2004; Labbe, Damevin, Vaccher, Morgenegg, & Martin, 2006; Lethuaut et al., 2005; Niimi et al., 2014; Saint-Eve, Paçi Kora, & Martin, 2004; Small & Prescott, 2005, 2005; Symoneaux, Guichard, Le Quéré, Baron, & Chollet, 2015; Tournier et al., 2009; Verhagen & Engelen, 2006), only few works deal with such interactions in wine. Thus, Arvisenet el al (2016) studied taste-aroma interactions in model wines and showed that an increase of aroma perception modulated by taste (sweet, sour) depends on panellist expertise (untrained, trained or experts). While no significant differences were found on the orthonasal intensity, scores of perceived retronasal aroma were higher when the sugar concentration was high. More recently in Arvisenet et al. (2019) conclude that it exists an aroma enhancement by sweetness as it was before observed and that acid concentration influences aroma compounds release, although no effect on fruitiness, nor on the

global intensity was perceived. Other authors have studied such interactions in original wined concluding that bitter taste can be masked by fruity aromas as perceived by a trained panel (Sáenz-Navajas et al 2010), while its perception was shown to be enhanced by animal aroma for less-experienced consumers (de-la-Fuente-Blanco, Fernández-Zurbano, Valentin, Ferreira, & Sáenz-Navajas, 2017).

5.2. Taste-mouthfeel and aroma-mouthfeel interactions

There is a wide number of works that focus on aroma and taste interactions in food systems, however, less is known about taste-mouthfeel and aroma-mouthfeel interactions. Concerning taste-mouthfeel, Slocombe, Carmichael, & Simner, (2016) evaluated the influence of tactile features of the food on taste, showing that an experimental foodstuff was rated as significantly sourer if it had a rough (versus smooth) surface. Previously, Christensen (1980) reported that an increase of sweetness enhanced rated viscosity while citric acid reduced viscosity ratings. Capsaicin has been shown to reduce the perceived intensity of tastes thresholds (Verhagen & Engelen, 2006), but this masking effect is probably due to a desensitization induced by compounds triggering trigeminal sensations rather than to perceptual interactions (Labbe, Gilbert, & Martin, 2008).

In the case of aroma-mouthfeel interactions, Kora, Latrille, Souchon, & Martin (2003) showed that the olfactory perception enhanced yogurt astringency. Regarding cold trigeminal perception, the effect of aroma and cooling perception has been widely studied. Thus, Petit et al. (2007) showed significant interactions between aroma and trigeminal perceptions in a congruent (melon odorant, cooling agent, and green colouring) but not in an incongruent mixture (pineapple odorant, cooling agent, and purple coloring), being enhanced the olfactory intensity by coldness due to perceptual interactions. In the opposite case Labbe et al. (2008), reported that olfaction strongly influenced taste and trigeminal perceptions, showing that an increase in mint odorant concentration enhanced coldness when

products were evaluated with and without nose clips, which confirmed its activity in inducing trigeminal sensations.

Aroma-mouthfeel interactions in wine have chiefly been focused on the general concept of astringency. On the one hand, De-la-Fuente-Blanco, Fernández-Zurbano, Valentin, Ferreira, & Sáenz-Navajas (2017) studied the capacity of sourness, bitterness and aroma to modulate astringent perception, however no significant effect for astringency intensity was found in any case. On the other hand, Sáenz-Navajas et al., (2010), measured the effect of different volatile extract compositions on the astringency perception, results showed that reconstituted red wines show lower astringency scores in the presence of volatile fruity extracts from white wines. Accordingly, Sáenz-Navajas et al., (2012) reported that red-black fruit odour nuances are able to modulate the perceived astringency, thus, results derived from the study showed a strong reduction in astringency. Recently, Pittari et al. (2020) evaluated original wines, and their corresponding deodorised wines, in order to evaluate cross-modal interactions. In that case, authors found significant differences between perceived astringency sub-qualities before and after wine deodorization, suggesting that astringency subqualities are modulated by both astringent and odorant compounds.

Considering that most works on aroma-mouthfeel interactions in wine have been focused on aroma-astringency interactions, and that there are few papers dealing with this concept, Section III-Chapter 2 is devoted to study the effect of aroma on mouthfeel properties in the case of red wines.

6. Mouthfeel in wine

6.1. Mouthfeel as concept

As described above, mouthfeel is a relevant sense in the overall food flavour perception. Although this term is widely used in the literature (Gawel, Iland, & Francis, 2001; Gawel, Oberholster, & Francis, 2000; Jowitt, 1974; Langstaff, Guinard, & Lewis, 1991; Langstaff & Lewis, 1993), and despite its sensory relevance, there is a lot of confusion surrounding this term. It is important to highlight that whereas texture is commonly employed to describe solid and semi-solid foods, mouthfeel encompasses all the tactile properties perceived in the mouth (Guinard & Mazzucchelli, 1996).

The mouthfeel evoked by wines involves multiple and interacting sensations. In wine sensory analysis, a wide range of sensory attributes are employed to describe mouthfeel properties including “astringency”, “dry”, “puckery”, “oily”, “silky”, “fleshy”, “velvety”, “dusty”, or “sticky” among others, which constitute different subqualities of mouthfeel. It is worth to highlight, that even though “astringency” is one of the most if not the most studied mouthfeel attributes in red wines, it cannot be neglected that there are other sensory dimensions that should be considered if we want to understand wine mouthfeel. Moreover, it is a multidimensional term that includes various subqualities (e.g., dry, pucker or sticky, among others).

In order to classify the mouthfeel terms employed to describe wines Gawel, Oberholster, & Francis, (2000) developed a “Mouth-feel Wheel” (Figure 12), which included two main categories of terms: astringency and non-astringency terms. Within the non-astringency category, six more specific categories (flavour, acidity, weight, texture, heat and irritation) are included. They are related to several sensory modalities (aroma, taste and mouthfeel), which would induce misunderstanding.

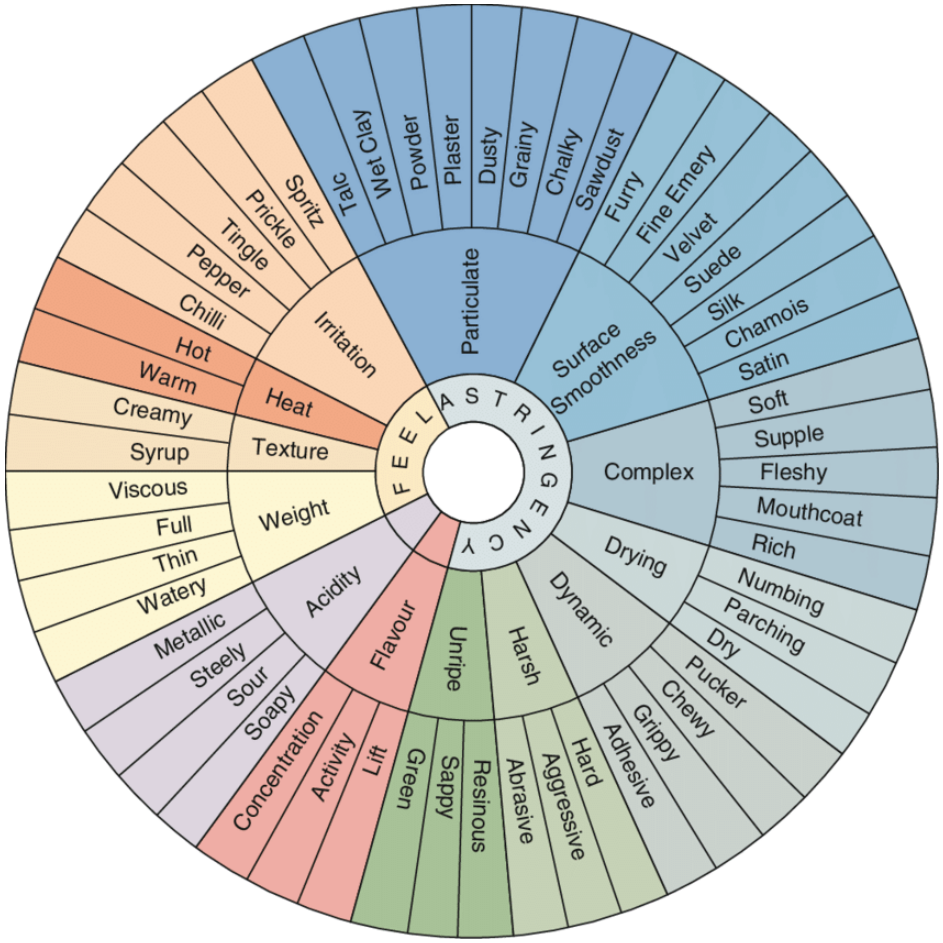

Figure 12. Wine mouth-feel wheel. (Source: Gawel et al. 2000)

Regarding the astringency category, it is formed by seven specific terms: particulate, surface smoothness, complex, drying, dynamic, harsh and unripe. Their definitions are to certain degree confusing as some terms are linked to hedonic properties. For example, the complex category is defined as "a positive hedonic grouping consisting of an amalgam of pleasing astringency sensations, flavour and balanced acidity". Similarly, the terms harsh and unripe are related to negative hedonic properties. Besides, some of the terms included in the astringency category are related to other sensory modalities different from mouthfeel such as aroma for green (vegetal), which leads to multidimensional terms. Moreover, there are terms that are defined based on the level of intensity of a given term, thus they are not related to mouthfeel different dimensions. Among them are aggressive (excessive astringency), abrasive (excessive astringency of a strongly roughing nature), soft

(light and finely textured astringency) or supple (low to moderate astringency). Lawless and Civille (2013) already highlighted that those terms related to mouthfeel should be reviewed and clarified to widen its applications in formal descriptive analysis. Thus, it is important to define a useful and clear vocabulary to describe different mouthfeel dimensions of wines.

As summary, despite the important value that mouthfeel has in consumer's acceptance, chiefly in wines, mouthfeel properties are not well defined and the different chemical structures involved in its formation are not fully understood. This could be attributed to the difficulty in approaching its study (Spence, 2019), which results in a far lower number of works dealing with mouthfeel in comparison with other modalities. Among the few papers dealing with mouthfeel properties, most of them are focused on "astringency" and its mechanisms (as reviewed in next sections).

In order to increase the understanding of mouthfeel dimensions of red wines, Section I-Chapter 1 was developed. Herein, the main challenge is to generate a wide vocabulary related to mouthfeel properties and to find the compound or groups of compounds responsible for different mouthfeel dimensions, which ultimately will allow to develop references illustrating mouthfeel attributes.

6.2. Mechanism of astringency perception: tannin–protein binding

Although astringency is currently accepted as a tactile sensation, not long before, this statement was questioned (Schiffman, Suggs, Sostman, & Simon, 1991). Accepted this assertion, the term astringency refers to an oral sensation, commonly described as drying, that is elicited by some foods and beverages containing polyphenols. The term astringency, comes from latin *adstringere,* which means "to bind", and is associated with polyphenols, mainly tannins, with ability to bind and precipitate salivary proteins (Breslin, Gilmore, Beauchamp, & Green, 1993; Green, 1993).

Phenolic compounds are one of the most important quality variables contributing to the organoleptic properties of red wines. Tannins are polyphenolic compounds with the ability of complexing and precipitating proteins. Tannins can be classified according to their structure into two classes, hydrolysable and condensed tannins (Figure 13). Condensed tannins (or proanthocyanidins) involve the polymerisation of the monomeric flavanols (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechingallate, which are differentiated by the benzenic ring substituted by one or more hydroxyl groups (-OH). Regarding hydrolysable tannins, they are extracted from oak barrels or can be added during winemaking. The hydrolysable tannins are oligomeric forms of gallic acid and can be specified as gallotannins or ellagitannins depending on whether they are constituted of gallic or ellagic acid moieties. (Monagas, Bartolomé, & Gómez-Cordovés, 2005; Nel, 2018; Sarneckis et al., 2006; Schofield, Mbugua, & Pell, 2001; Soares et al., 2020).

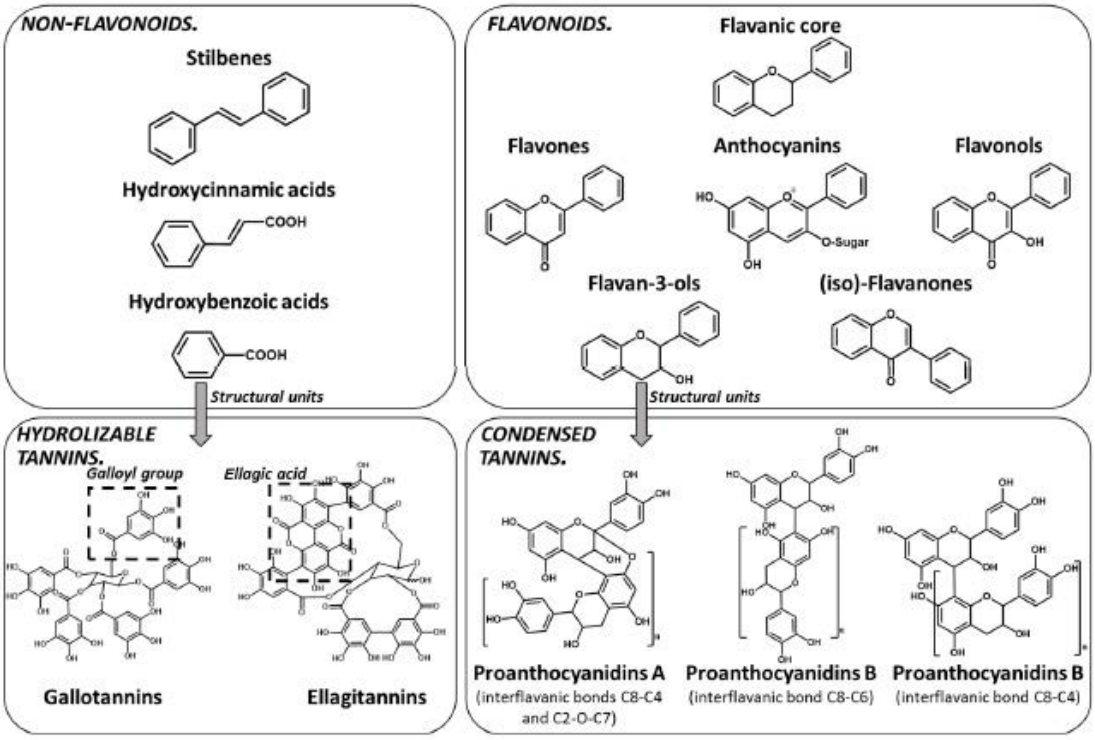

Figure 13. Tannin's classification and phenolic compounds families (chemical structure core and classical division). Reproduced from: Soares, S., Brandão, E., Guerreiro, C., Soares, S., Mateus, N., & De Freitas, V. (2020). Tannins in food: Insights into the molecular perception of astringency and bitter taste. Molecules, 25(11), 1–26. https://doi.org/10.3390/molecules25112590

Despite the lack of fully understanding of the molecular basis of astringency, three main possible mechanisms involving polyphenol–protein binding have been suggested.

The first is related to the central admitted mechanism inducing astringency perception, which involves the precipitation of tannins with proline-rich salivary proteins (insoluble aggregates) in the oral cavity, evoking a tactile sensation, due to a decrease of lubrication and increase of friction between surfaces, via mechanoreceptors (Bate-Smith, 1954, 1973; de Freitas & Mateus, 2012; Upadhyay, Brossard, & Chen, 2016) (Figure 14).

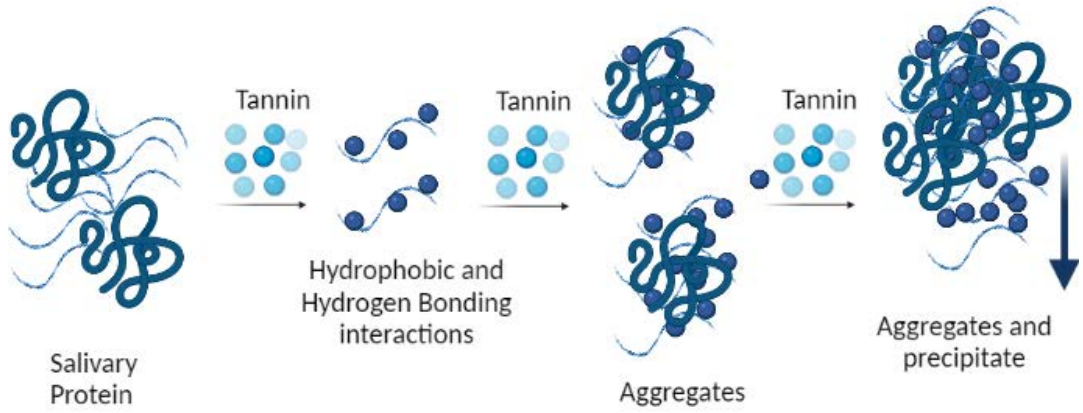

Figure 14. Mechanisms of interaction tannin-protein. Adapted from: McRae, J. M., Falconer, R. J., & Kennedy, J. A. (2010). Created by BioRender.com (2021). Retrieved from https://app.biorender.com

Concerning the second proposed mechanism, it involves low-molecular weight polyphenols that induce astringency by complexing salivary proteins without precipitation to form soluble aggregates. According to the study of Obreque-Slier, Lopez-Solis, Peña-Neira, & Zamora-Marín (2010), astringency correlates better with tannin-gelatin interaction than tannin-gelatin precipitation. Suggesting that, a friction mechanism is enough to trigger astringency sensation (Brossard et al., 2021; Cala et al., 2010; Canon et al., 2013; Kallithraka, Barker, & Clifford, 1998; Pascal, Poncet-Legrand, Cabane, & Vernhet, 2008; Perez-Gregorio, Mateus, & De Freitas, 2014; Schwarz & Hofmann, 2008; Susana Soares et al., 2018, 2011; Yokotsuka & Singleton, 1987). However, the role that soluble complexes play in astringency perception is still unclear.

The third mechanism suggests that oral epithelial cells can bind polyphenols. The oral cavity is lubricated by a salivary film. Its disruption due to the formation of insoluble salivary complexes have shown to decrease saliva lubrication, and consequently cell surfaces and receptors are exposed (Figure 15). This makes that protein-polyphenol complexes are able to binding to oral cells. So, the loss of oral saliva pellicle allows the interaction of both aggregates and free astringent compounds with oral tissue through receptors (Gibbins & Carpenter, 2013; Payne,

Bowyer, Herderich, & Bastian, 2009; Ranc et al., 2006; Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009; Sónia Soares et al., 2019; Susana Soares et al., 2016).

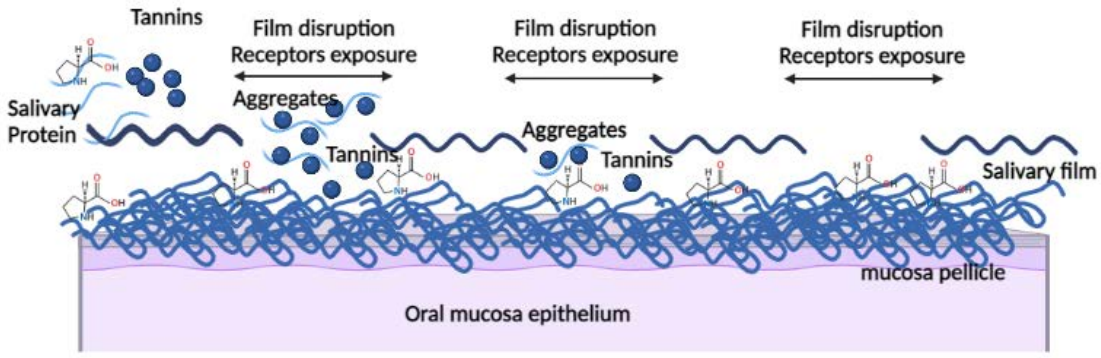

Figure 15. Aggregation of salivary proteins reduces lubrication, inducing film disruption, and possible exposure of cell receptors. Adapted from: Gibbins & Carpenter, (2013). Created by BioRender.com (2021). Retrieved from https://app.biorender.com

Due to the complexity of the process, and considering condensed tannin as principal candidates triggering astringency sensation, lot of works focus on the study of tannin-protein interactions. Different molecular composition of proanthocyanidins (level of galloylation), their mean degree of polymerisation (mDP) or their molecular weight are chemical properties conditioning their binding with proteins, and consequently, affecting sensory properties. McRae, Falconer, & Kennedy, (2010) proposed to measure the thermodynamics of interaction between proline-rich proteins and different types of tannins to explain astringency, concluding that the interactions varied with tannin structure. Vidal et al. (2003), employed purified proanthocyanidin fractions, that differed in mDP, degree of galloylation and epigallocatechin content, concluded that mDP seems to be a significant parameter differentiating among fractions. Furthermore, the different sensory attributes employed by the panel, suggests that different astringent subqualities used to describe fractions, could be associated with these molecular differences, a rougher sensation correlated with an increased degree of galloylation of the fractions and the presence of epigallocatechin units in the proanthocyanidin tended to lower the 'coarse' perception. In the same context, Gonzalo-Diago, Dizy,

& Fernández-Zurbano (2013) obtained proanthocyanidin fractions coming from six different young red wine, which were sensory and chemically analysed. They concluded that concentration of proanthocyanidins was the major determinant of the differences perceived in the astringency. Additionally, the extension flavanol units linked to the proanthocyanidins seem to have a different impact on the evaluated astringent subqualities (velvety and puckering/drying)

The phenolic composition of the wine (both quantitative and qualitative) has its main origin in grapes, therefore grape factors, namely its degree of ripeness should be considered when identifying chemical factors affecting sensory properties. Phenolic concentration reaches a maximum at veraison and then decreases (Bautista-ortín et al., 2012; Ferrer-Gallego, García-Marino, Miguel Hernández-Hierro, Rivas-Gonzalo, & Teresa Escribano-Bailón, 2010; Fournand et al., 2006; Frost et al., 2021; Sherman, Greenwood, Villas-Boâs, Heymann, & Harbertson, 2017). Concerning their qualitative properties (i.e., structure), they have an important impact on protein binding such as the mDP and the percentage of prodelphinidins, which increase with maturity, and the percentage of galloylation, which decreases (Gil et al., 2012). Considering the importance of this issue, in Section II-Chapter 2, the effect of grape maturity on wine flavour in the case of Moristel wines was studied.

Another important factor modulating phenolic composition, especially the polymerisation of tannins, is wine ageing. In accordance with Chira, Pacella, Jourdes, & Teissedre, (2011) and Chira, Jourdes, & Teissedre, (2012), during wine ageing a decrease in the mDP and in astringency perception is observed. Notwithstanding, lot of works are focused on determining and quantifying tannins changes during ageing, bottle storage or even microoxygentanion conditions, but few of them perform a sensory analysis to check whether these observed chemical changes induce mouthfeel changes. Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano (2014) reported a low impact of oxygen dose on mouthfeel properties, only a decrease in global in-mouth intensity was observed. Differently, Gambuti, Rinaldi, Ugliano, &

Moio, (2013) reported differences in astringency after 42 months of ageing in bottle accompanied by a decrease in a saliva precipitation index.

Thus, despite the fact that oxygen has been linked to changes in phenolic composition, a clear correlation between chemical transformation of phenolics and their direct impact on sensory properties (e.g., astringency decrease) is far to be understood. In order to shed light on this issue, Section IV-Chapter 3 has tackled the study of the effect of the oxygen on proanthocyanidins (and other wine phenolic fractions).

6.3. Mechanisms involving protein binding with compounds different from tannins

The phenomenon of astringency seems to be mainly driven by the interaction between polyphenolic compounds and salivary proteins. While tannin-protein interactions are the most widely reported, it is noteworthy to appoint that, different classes of polyphenols (different from tannins) are able to bind oral proteins. Thus, wine phenolic acids interact with salivary proteins, and seem to have a synergy effect on perceived astringency (Ferrer-Gallego et al., 2017). Among the different theories trying to explain the different binding mechanisms between the diverse kind of phenolic compounds and salivary proteins it is possible to find some works focused on anthocyanins (Ferrer-Gallego et al., 2015). According to these authors, anthocyanins are able to interact with saliva proteins forming soluble aggregates, which suggests a potential role of these phenolics on wine astringency. However, during wine storage grape anthocyanins participate in reactions forming new pigments (e.g., pyranoanthocyanins formation). In this context, García-Estévez et al., (2017) studied the interaction between salivary proteins and pyranoanthocyanins concluding that pyranoanthocyanins could contribute to astringency sensation. In 2018, Paissoni et al., in parallel studies to those carried out in this Doctoral Thesis, analysed grape anthocyanins and focused on the interaction between bovine serum albumin (BSA) and salivary proteins, with three different fractions of anthocyanins (glucosylated, acetylated and cinnamoylated). Results showed that, the cinnamoylated anthocyanins were the most reactive to salivary proteins, which was in accordance with sensory results since it was the fraction with the lowest sensory threshold. The attributes more employed by participants to describe all the anthocyanin-related fraction were “astringent” and “bitter”. Although the role of the different classes of anthocyanins on wine astringency is still far to be known, it is clear that the study of astringency mechanisms should be extended to other classes of polyphenols different from polymerised flavanols, including anthocyanins. To this concern, the development of approaches combining both chemical isolation and

sensory confirmation should be considered as a necessary tool for bringing new insights in this field of knowledge. Through this Doctoral Thesis, it is attempted to contribute to this understanding. Therefore, in Section I-Chapter 1, six different fractions of wine were obtained by semipreparative liquid chromatography and Solid-Phase Extraction (SPE). Fractions were sensory and chemically characterised (among them a fraction containing anthocyanin-derivative pigment). In Section IV-Chapter 2 two different fractions of wine, one of them containing principally anthocyanins, were chemically and sensory analysed, seeking for understanding its contribution to taste and mouthfeel properties.

6.4. Other mechanisms of mouthfeel perception: non-bound based

Mouthfeel involves several interactive mechanisms in the mouth. Without exception, once wine is in the mouth, several sensory fibres are activated. These sensations are mediated by the somatosensory system. Non-bound/free astringent compounds evidence that sensory perception of mouthfeel is not only due to an increasing of friction by protein-polyphenol binding mechanism, but also by other processes. In this context, it has been reported that to notice astringency, the attenuation of the saliva proteins film is not always necessary (Rossetti et al., 2009). Different sensations are modulated by the activation of different specialised somatosensory neurons (receptors) surrounding taste buds and located in the oral cavity, that convey the stimuli via trigeminal nerve, evoking sensations of touch, temperature and pain. The trigeminal nerve endings can be stimulated by physical or chemical stimuli, that will be discussed in the following sections.

6.4.1. Physical receptor activation

Mechanoreceptors are receptors trigeminally innervated and sensitive to mechanical deformation (Breslin et al., 1993; Weiffenbach, 1993). They convey information regarding mechanical events, including touch, pressure, vibration and proprioception, as it has been widely explained above. It has been shown that mechanoreceptors are involved in mouthfeel perception, highlighting dynamic processes. Besides them, among the four different types of in-mouth papillae, filiform papillae have been suggested to be involved in mouthfeel perception. They cover most of the tongue and are very innervated by free nerves endings, being the unique papillae that do not contribute to taste. However, there is a lack of knowledge about epithelium interaction with wine components, so the role of filiform papillae remains unknown (Laguna, Bartolomé, & Moreno-Arribas, 2017; Moayedi, Michlig, Park, Koch, & Lumpkin, 2021).

In accordance with the above mentioned literature, the precipitation of salivary proline-rich proteins by polyphenols reducing the lubrication and activation of

mechanoreceptors, is not the unique mechanism involved in the perception of mouthfeel properties.

Mechanisms mediated by receptors involving ion channels are related to several neural signalling processes, mostly in sensory receptor cells. Mechanical forces can gate some ion channels. Among them, transient receptor potential (TRP) channels are a group of ion channels found in a variety of tissues, involved in detection and transduction of sensory stimuli. Those are polymodal, meaning that they can be activated by physical and chemical stimuli through different molecular mechanisms (Figure 16).

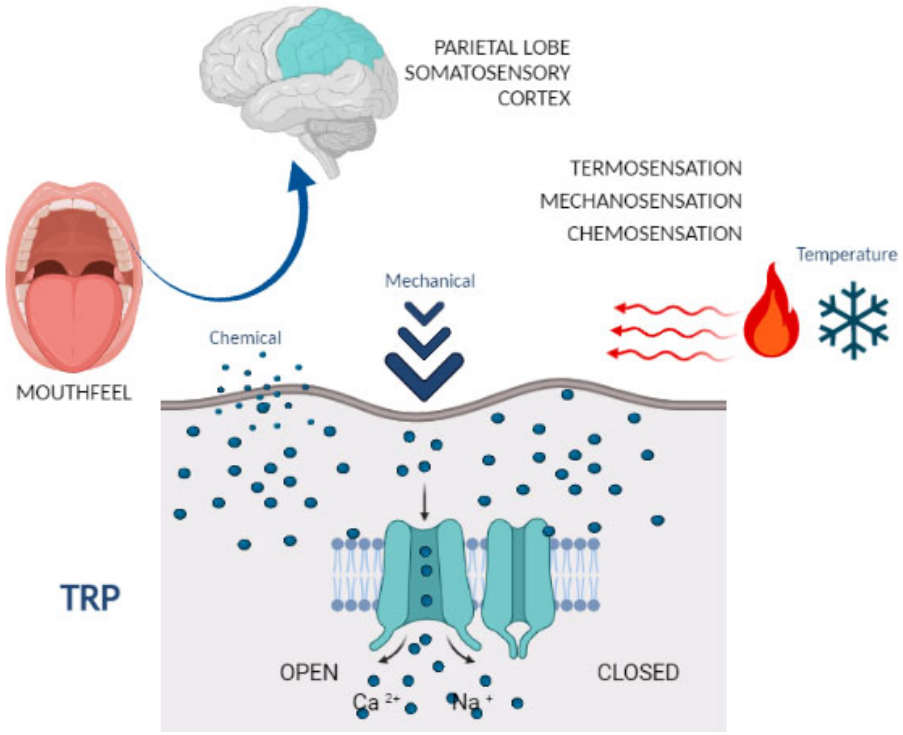

Figure 16. Mechanism of Transient receptor potential (TRP) channels. Created by BioRender.com (2021). Retrieved from: https://app.biorender.com

A mechanical stimulus could act on a TRP channel directly or alternatively, mechanical force could alter the lipid-bilayer, turning in a tension that opens the channel (Christensen & Corey, 2007; De Araujo, Geha, & Small, 2012; Pedersen & Nilius, 2007; Schöbel et al., 2014)

Some TRP channels are activated by changes in the temperature, known as thermoTRPs. Widely studied is the TPRV1, that can be activated by noxious heat (>42 °C), conversely, TRPM8 is a cold-sensitive ion channel and TRPA1 has been reported to be activated by noxious cold. In addition to thermal sensitive receptor channels TRPV1, TRPM8 and TPRA1 have manifested to have chemoreceptive functions in the oral cavity (Simon, De Araujo, Gutierrez, & Nicolelis, 2006).

6.4.2. Chemical receptor activation: Chemesthesis

Chemical activation of receptors is known as chemesthesis, and arises mostly from chemically sensitive receptors of sense of pain and temperature. In other words, chemesthesis comprises somesthetic sensations, rather than olfactory or gustatory, caused by chemical stimuli (Cometto-Muñiz & Simons, 2015). It is important to note, that the chemosensitivity avoid the input of toxins in the body, acting like a protective system, since it conveys irritant sensations that allows its detection and avoid its consumption (Green, 2012; Haley & Mcdonald, 2021). In the case of ophthalmic and maxillary branches of the trigeminal nerve, the chemicals can induce a sneeze or breath holding, avoiding the irritants to enter the body during the breathing (Schiffman, 2007).

Chemesthesis involves sensations such as warm, cool, tingle and burn that occur due to chemical compounds, which stimulate the trigeminal nerve in the oral cavity (Dalton & Byrnes, 2016).

Transient receptor potential (TRP) channels, are activated by changes in the temperature, but also, respond to the presence of some chemicals. Thus, TRPM8, is activated by menthol producing a cooling sensation and TRPV1 receptor, respond to capsaicin (found in chili peppers) with an increase of tongue temperature. According to above, the ion-channels responding to the last two chemicals are also thermoreceptors that respond to warm/hot and cool/cold temperatures, respectively. However, it has been reported that some TRPV1 agonist compounds different of capsaicin produced rapid desensitization of the sensory terminals but

lacking of capsaicin pungency characteristics, which should still be clarified (Viana, 2011).

It has been shown that, taste receptor cells (TRCs) can be modulated by non-taste chemical compounds, sine the insular cortex (responsible of taste), is activated by water and temperature affect the ability to transduce tastant information (De Araujo, Geha, & Small, 2012; Simon et al., 2006).

In the case of carbon dioxide, it stimulates free endings (oral mechanoreceptors) of trigeminal nerve, due to bubbles. Notwithstanding, works suggest that the sensation is not only due to a physical component arising from the mechanical action of bubbles, but also the carbonic “tingling” and “irritant” sensations are due to the chemical activation of oral nociceptors, leading to excitation of trigeminal neurons (Hewson, et al., 2009).

7. Compounds contributing to taste and mouthfeel in wine

Once the mechanisms involving taste and mouthfeel perception have been reviewed, the compounds known to elicit these sensations in wine will be discussed.

The literature is wide on this topic, but above all, there are lot of works focused on astringency (mouthfeel), and bitter taste elicited by flavanols. Broadly, phenolic compounds with lower molecular weight have been described to be more bitter, and proanthocyanidins with higher mDP are reported to be more astringent than their smaller counterparts (Brossaud, Cheynier, & Noble, 2001; Hufnagel & Hofmann, 2008; Peleg, Gacon, Schlich, & Noble, 1999; Robichaud & Noble, 1990).

Important to highlight is the controversy related to the capacity of monomers of flavanols to elicit astringency. It has been suggested that the oral epithelial cells may be directly stimulated by these compounds activating transient receptor protein (TRP) channel. However, this hypothesis is in contradiction with Carpenter (2013), who observed that the TRPV1 channels were not activated by a variety of monomeric black tea flavanols. This suggesting that human oral epithelial cells are not directly stimulated by monomers during normal drinking and thus not involved in the development of astringency. Following this research on monomeric flavanols, Schobel et al. (2014) proposed that galloylation is a necessary condition to trigger the chemosensory response of astringency. However, a general accord in the scientific literature cannot be found, and thus it seems necessary to advance in the development of techniques to measure physiological oral responses to different individual or fractions of known compounds.

Despite these controversies, what it is clear is that flavanol structures and their concentration are important, but are insufficient to totally explain mouthfeel and bitterness in red wines.

7.1. Compounds and factors contributing to mouthfeel in wine

Mouthfeel is a multidimensional oral sensation. In wine, mouthfeel sensations include multiple tactile perceptions such as astringency, mouthcoating, prickling or viscosity among others. During the last decade, there has been an increasing number of works focused on the study of the chemical constituents that are able to elicit these perceptions, however, there is an important lack of understanding to this regard. The absence of reference materials evoking different and well-defined mouthfeel properties and standard lexicon hinders the study of the mechanism eliciting distinct mouthfeel perceptions.

Mouthfeel is affected not only by phenolic composition, but by the entire wine matrix including wine chemical variables such as ethanol, pH, polysaccharides, lipids, their interaction with oral cavity or saliva composition (Brossard et al., 2021; Fontoin, Saucier, Teissedre, & Glories, 2008; Gawel, Smith, & Waters, 2016; Guinard, Pangborn, & Lewis, 1986; Kallithraka, Bakker, & Clifford, 1997; Phan, Hoffman, & Tomasino, 2021; Ramos-Pineda, García-Estévez, Dueñas, & Escribano-Bailón, 2018) or even by physical properties such as temperature (Ross, & Weller, 2007). As it has been mentioned above, volatile composition has been reported to be able to modified mouthfeel perception and thus, it should be considered.

With respect to ethanol content, it has been shown that astringency sensation decreases in the presence of increasing amounts of ethanol (Demiglio & Pickering, 2008). This has been explained in terms of the disruption of hydrophobic interactions by ethanol (Gawel, 1998; McRae, Ziora, Kassara, Cooper, & Smith, 2015), resulting in a decrease of tannin–protein interaction strength and thus of astringency. However, results obtained with red commercial wines have shown that higher ethanol contents are correlated to higher astringency (Watrelot, Byrnes, Heymann, & Kennedy, 2016), which are well in line with results obtained in Section III-Chapter 1.

The phenol-protein interaction is also affected by pH. Lower pH levels are related to higher perceived astringency, which is associated to increases in the binding of

astringent compounds to oral epithelial cells when the salivary film is disrupted in more acidic conditions (Payne et al., 2009).

While the effect on mouthfeel of polysaccharides seems to be smaller than the effect of wine pH and ethanol, the polysaccharides are reported to be involved in increases of viscosity. Furthermore, they have been shown to disrupt the interaction and aggregation between salivary proteins and tannins, which would indirectly result in a decrease of astringency perception (Gawel, Smith, & Waters, 2016; Mateus, Carvalho, Luís, & De Freitas, 2004; Ozawa, Lilley, & Haslam, 1987).

Although lipids are found in wine at low concentrations, these minor components or their interactions with other components have been reported to modulate certain mouthfeel properties in wines. A recent study has shown that phospholipids can alter mouthfeel in model wine solution by increasing the perception of wine viscosity (Phan et al., 2021). These results should be further investigated in real wine contexts.

Carbon dioxide has been reported to enhance astringency in model solutions (Hewson et al., 2009), which is in accordance with results observed in apple cider by Symoneaux, Le Quéré, Baron, Bauduin, & Chollet (2015).

By means of an untargeted approach Skogeron et al. (2009) found that the concentration of amino acids in white wine was a good predictor of wine body, which justifies additional investigation to understand the relationship between aminoacids and mouthfeel sensations.

7.2. Compounds and factors contributing to bitter taste in wine

Bitterness also plays a relevant role in the quality perception of red wine (Cheynier & Sarni-Manchado, 2010). Unlike mouthfeel, bitter taste is elicited by taste receptor cells located in taste buds embedded in the epithelium of papillae on the tongue and soft palate. Bitter taste is perceived by 25 members of the G protein-coupled receptors (GPCR)superfamily, referred to as T2Rs (Aliani & Eskin, 2017).

The role of monomeric phenolics such as flavonols on wine bitterness has been reported by different authors. In 1996, Vaia & McDaniel, in order to clarify the impact that quercetin could have in wines, quercetin was sensory evaluated by free choice profiling by set of different concentration dilution of quercetin in model wine, reporting the elicitation of bitter sensation among others. Later, this time in wine matrix, Preys et al., (2006) revealed a slightly chemical-sensory relationship between myricetin and quercetin aglycones and bitter attribute. It is noteworthy, the approach followed by Sáenz-Navajas et al. (2010), in which taste-active fractions were sensory and chemically characterised. Bitterest fractions were suggested associated to flavonols (myricetin, quercetin and their glycosides). More recently, Ferrer-Gallego et al. (2016) sensory evaluated the effect of the addition of quercetin 3-O-glucoside on wines, and results showed that the addition of this flavonol induce an increase in bitterness.

As for other important class of polyphenols in red wines, anthocyanins in the form of monomers, oligomers and polymers are conventionally thought to be responsible exclusively for the colour of red wines. This understanding is based on studies carried out with grape anthocyanins (containing mainly anthocyanin glucosides along with their monoglucoside acetate and coumarate counterparts) by Vidal et al. (2004) that concluded that coloured tannin-like polyphenolic compounds from wine and pomace do not contribute to bitterness to wine. However, more recently, Soares et al., (2013) showed that the presence of glucose residues in the anthocyanin molecule could be important in bitterness elicitation, because malvidin-3-*O*-glucoside activated TAS2R7, while its aglycon form did not. Well in accordance with this result, Paissoni et al. (2018), assessed anthocyanins differing in the B ring substitution, and in presence of a monoglucoside such as acetyl-monoglucoside, caffeoyl-monoglucosides and *p*-coumaroyl-monoglucoside, all the solutions tasted were described as bitter.

Concerning amino acids and peptides, a wide number of them have been described as bitter. Bitterness of peptides has commonly been related to the L-

configuration and the presence of amino acids with hydrophobic side chains. Desportes, Charpentier, Duteurtre, Maujean, & Duchiron (2001) demonstrated that peptides isolated from wine were able to generate bitter and umami tastes and considered that interactions with other components and synergistic effects certainly occur. More recently, a study integrating non-target metabolomics with sensory data focused on dill has shown that increases in amino acids and organic acids were related to increases in bitter/pungent taste in dill (Castro-Alves et al., 2021). Thus, the role of amino acids on wine bitterness would deserve to be further examined.

8. Methodology to understand the chemical bases of mouthfeel

8.1. Chemical approaches

It has been highlighted, that among the three principal senses involved in flavour, mouthfeel is overall the least understood. In the last decade, most works focused on its understanding have been mainly focused on the chemical characterisation of compounds driving mouthfeel, more specifically on the identification and quantification of astringent phenolic compounds (Derdelinckx & Jerumanis, 1984; Karchesy, Bae, Chalker-Scott, Helm, & Foo, 1989; Kennedy & Taylor, 2003; Somers, 1967).

Conventional oenological analysis, employing spectroscopic techniques like total polyphenol index (TPI) estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970), are widely employed in wineries. Boulet, Ducasse, & Cheynier, (2017) have proposed the measurement of absorbance at 230 nm, which seem to provide better prediction of wine astringency. However, this could not be corroborated, and significant higher correlations employing 230 nm as wavelength were not found during the development of this work.

Chromatographic techniques have been successfully employed to identify relevant wine components causing astringency (targeted methods). Since the concentration of compounds as well as their chemical structure are involved in astringency formation (e.g., galloyl flavanol derivates seem to be more astringent than non-galloylated) some studies are focussed on determine the chemical composition and structure of phenolic compounds (e.g., tannins). With this regard proanthocyanidin acid-catalised cleavage in presence of a nucleophile and subsequent analysis of the breakdown products by liquid chromatography, have been applied in order to further characterised tannins (Arapitsas, Perenzoni, Guella, & Mattivi, 2021; Kennedy & Jones, 2001; Labarbe, Cheynier, Brossaud, Souquet, & Moutounet, 1999; Pinasseau et al., 2016; Vivas et al., 2004).

To this regard, it is worth noting, an interesting different approach developed to the study of tannin activity, proposed by Revelette, Barak, & Kennedy (2014) consisting on measuring the interaction between tannins and a hydrophobic surface (polystyrene divinylbenzene column). The method is based on the thermodynamics of interaction, considering that the retention factor of individual molecules is affected by temperature. The properties of the stationary and the mobile phase determine the retention and selectivity of the separated molecules in chromatographic process. The analytical method is carried out at four different column temperatures and monitored at 280 nm and 520 nm. In addition to tannin activity, tannin concentration, and percentage of pigmented tannins are determined (Peng, Iland, Oberholster, Sefton, & Waters, 2002).

According to the following equation the enthalpy of interaction between tannin and a hydrophobic surface can be calculated plotting the logarithm of the alternative retention factor (K_{alt}) versus the reciprocal of the column temperature in kelvin^{-1} at each of the four temperatures (Figure 17).

$$\ln k_{alt} = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R}$$

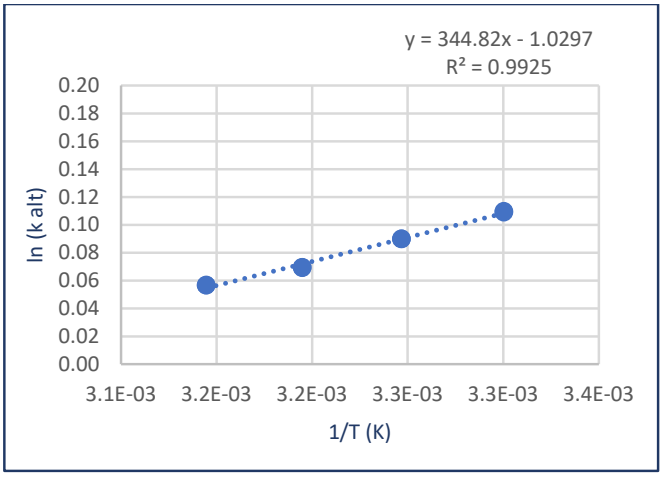

Figure 17. Van't Hoff plot of the alternative retention factor (K_{alt}) versus the reciprocal temperature (Kelvin^{-1}) for the retention of red wine tannins on polystyrene divinylbenzene. Reproduced from a real experiment carried out in Section IV.

The tannin activity is described as adhesiveness or stickiness, and is concentration independent. From all this, it is extracted that tannin activity is an intensive property, describing the impact of the whole tannin system rather than individual compounds.

Other chemical methods are based on the polyphenol-protein interaction. This interaction is supposed to be directly related to the perception of astringency (Pires, Pastrana, Fucinõs, Abreu, & Oliveira, 2020). The classical gelatin index (Glories, 1984), which is not a very reproducible method, is calculated as the difference between absorbance values measured before and after gelatin precipitation. Alternatively, Llaudy, et al., (2004), proposed the use of ovalbumin as precipitation agent and tannic acid solutions as standard. Other methods combine protein precipitation and bisulphite bleaching (Harbertson, Picciotto, & Adams, 2003), which allows to determine both tannin concentration and pigment composition. Polymeric pigments, which are resistant to bleaching, are differentiated from bleachable anthocyanins, and among the firsts, small polymeric pigments (SPP), which do not precipitate with protein, are differentiated from large polymeric pigments (LPP), which do precipitate.

By other hand, Guerreiro et al., (2012) proposed a method, which aimed to simulate a sensory system (i.e., mouth conditions). It consists in a protein immobilised on a sensory gold surface, and its interaction with polyphenols is measured by Surface Plasma Resonance (SPR) (Figure 18). The mechanism consists in the measurement of the angle variation, which depends on the thickness of the metal, that varies when the molecular interaction between protein and polyphenols takes place. The protein α-amylase and the polyphenol pentagalloyl glucose (PGG) were employed in the study.

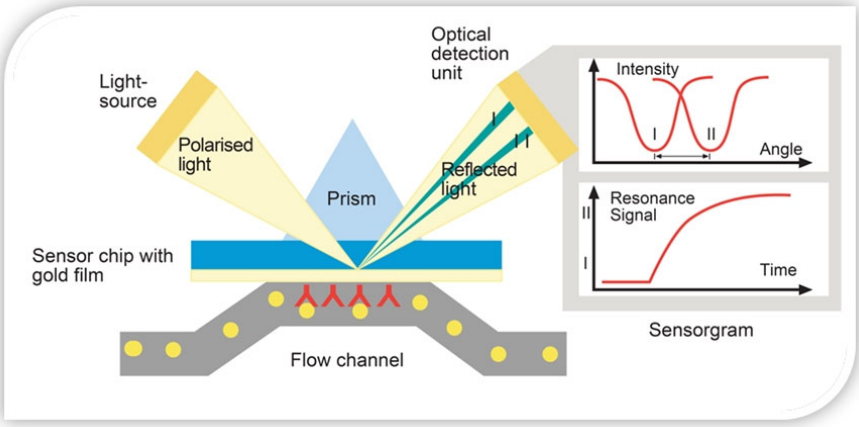

Figure 18. The Biacore optical biosensor detection system based on Surface plasmon resonance (SPR) detects changes in the refractive index. Schematic view of the optical and flow channel setup of a Biacore™ system (reproduced from www. Biobest.com)

Watrelot et al., (2016) immobilised polyphenols (flavan-3-ol monomers, dimers and oligomers) and analysed qualitatively the parameters of interactions of these flavonoids with both proteins and pectins. Guerreiro et al., (2017) went one step further by employing red wine and saliva, combining Localised Surface Plasmon Resonance (LSPR) and Molecular Imprinted Polymers (MIP). Gombau et al., (2019), followed a chemosensory approach, studying the interaction between mucin and oenological tannins by SPR, and correlating the results with the sensory assessment.

Interestingly, the dynamics aspects of astringency have been recently considered in the study of wine astringency by applying rheological and tribology instrumental techniques. Considering astringency as tactile sensation perceptible on oral surfaces, by the contact between the tongue and palate, as well as between the upper lip and the gum (Green, 1993; Breslin et al., 1993), these techniques have focused on the study of the lubrication alteration. They work under the hypothesis that astringency is caused by a decrease in salivary protein present in the tongue surface and thus increase surface friction. Some authors have worked on tribology tools (Prakash, Tan, & Chen, 2013; Laguna and Sarkar, 2017; Wang, et al., 2020, Laguna, Fiszman, & Tarrega, 2021). Tribology is described as the study of friction, wear, and lubrication between two moving surfaces (Jost, 1990). The approach of

Brossard et al., (2016) consisted in a lubrication test employing human saliva and different astringent compounds. The friction coefficient was calculated and correlated with sensory results. These results were in contradiction with previous works that were not able to find any relationship between the sensory features and physical parameters (de Wijk and Prinz 2005; Vardhanabhuti et al., 2011).

Concerning rheological properties, they have been evaluated in a few works. Rheology is the study of deformation (solids) and flow (semiliquids and liquids) of matter. The study of flow behaviour is employed in fluids which cannot be defined by a single value of viscosity, and is described as the resistance against flow. It is assumed that higher viscosity in wine, involves higher resistance in its transportation to the back of the mouth. It is noteworthy, that studies agree in the importance of saliva rheological properties in the overall oral process (de Castilhos, Betiol, de Carvalho, & Telis-Romero, 2017; Laguna, Álvarez, Simone, Moreno-Arribas, & Bartolomé, 2019; Laguna, Bartolomé, & Moreno-Arribas, 2017; Laguna, Fiszman, & Tarrega, 2021; Stokes, Boehm, & Baier, 2013; Trávníček et al., 2016). These different instrumental techniques, not traditionally applied in wine science, could be useful in increasing knowledge related to mouthfeel mechanisms, which are still in its infancy.

8.2. Combination of sensory and chemical approaches

Important to highlight is that although main works are focused on the developing of chemical approaches, its combination with sensory responses is acknowledged to provide a more complete information of the wine (Ross, 2009). To this regard, there is an increasing number of research lines coupling sensory and chemical approaches to identify sensory-active compounds modulating wine taste and mouthfeel (Ferrer-Gallego et al., 2016; Hufnagel & Hofmann, 2008; Piombino et al., 2020; Preys et al., 2006).

In line with these approaches, before this Doctoral Thesis, our research team had adopted a classical strategy derived from flavour chemistry. The methodology involves two main steps (Figure 19): 1) isolation and identification of sensory-active

fractions, and 2) reconstitution experiments to confirm their real sensory role in wine models (Sáenz-Navajas et al., 2012; Sáenz-Navajas, Campo, Fernández-Zurbano, Valentin, & Ferreira, 2010; Sáenz-Navajas, Ferreira, et al., 2010).

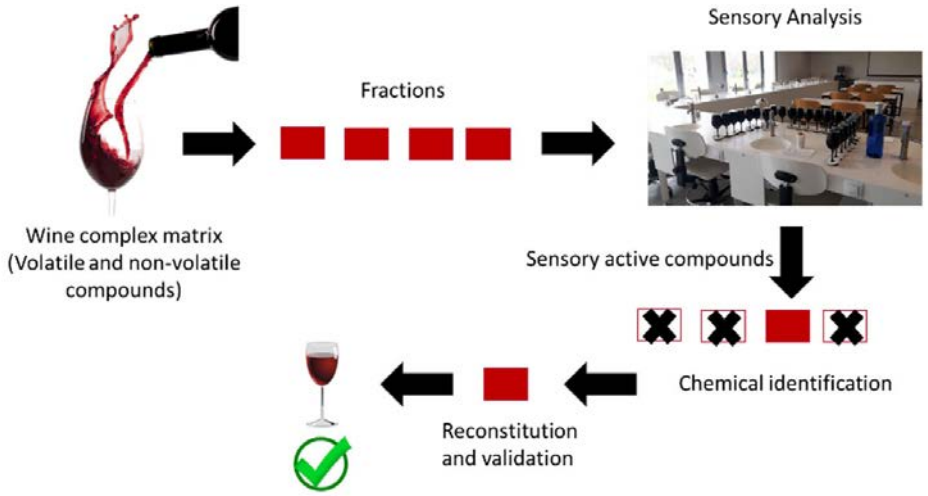

Figure 19. Classical methodology.

Important to remark is that this approach and paradigms derived from classical flavour chemistry have limitations. This strategy only works to identify molecules with explicit sensory effect or agonist molecules (i.e., molecules eliciting specific sensory properties), but they ignore "silent" or antagonist molecules (i.e., molecules able to block the sensory signals of others). The identification of these last molecules is important for understanding wine taste and mouthfeel. For example, caftaric acid and its GRP derivative have been shown to be mutually antagonistic (Gawel, Schulkin, Smith, & Waters, 2014; Gawel, Smith, Cicerale, & Keast, 2018).

In order to overcome the limitations of classical approaches, untargeted chemical analytical methods are proposed to shed light on the unknown world of the sensory activity of polyphenols and their interaction with other wine molecules (Arapitsas & Mattivi, 2018; Vallverdú-Queralt et al., 2017). They seem to have high potential for exploring the relationships between sensory active polyphenols and sensory properties (Yang et al., 2018). In spite of the potential of using metabolomics

to unravel the sensory activity of compounds (sensometabolome as defined by Hufnagel and Hofmann) (Hufnagel & Hofmann, 2008), this sensometabolomic approach has barely been explored before now in wine.

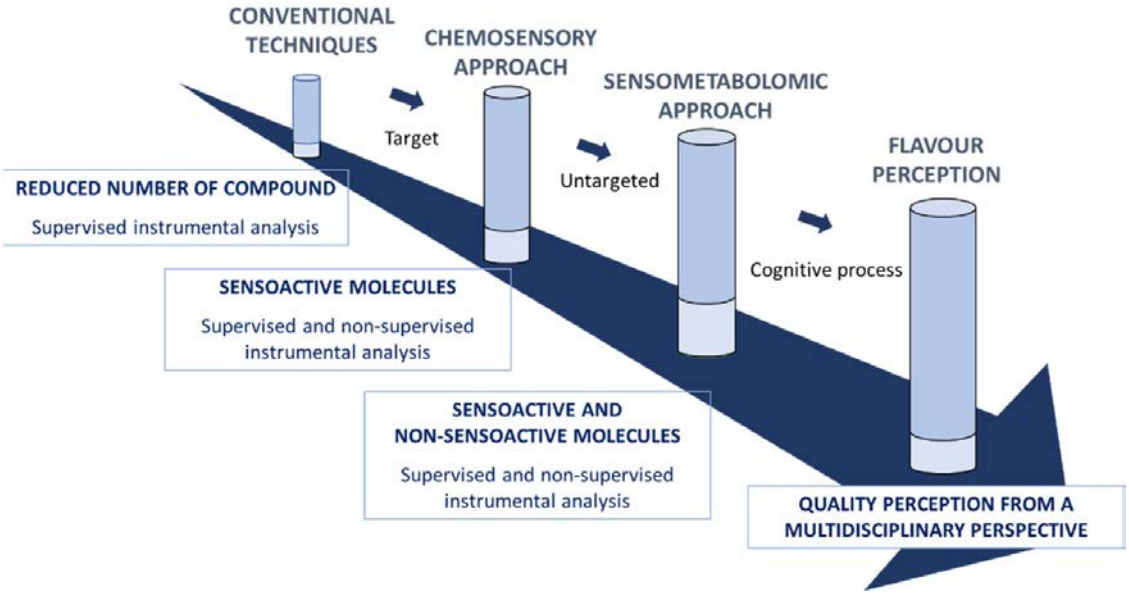

Figure 20. Methodological advance in the knowledge of flavour.

Figure 20 illustrates the evolution of the methods employed to understand the sensory impact of wine components on wine flavour. From the simplest approaches that merely correlate the chemical composition obtained by targeted chemical methods with sensory ratings, to the ones that employ sensory-directed methods coupled with non-targeted chemical approaches.

8.3. Sensory approaches

The last point to be highlighted is the relevance given to the acquisition of sensory data in the present Doctoral Thesis. Therefore, a summary of the methods employed is provided.

Concerning conventional descriptive analysis (DA), it is overall the most widely used in sensory science. It requires an extensive panel training (Lawless & Heymann, 2010), and thus time to get used to the lexicon, normalise the concepts and use the intensity scale correctly (Figure 21). Therefore, references illustrating each sensory

concept are needed, while they are not always available when dealing with mouthfeel properties. Thus, descriptive techniques are limiting when trying to identify and characterise mouthfeel dimensions in wine. Furthermore, descriptive techniques result to be expensive, both in time and human resources.

Figure 21. Panel training with aromatic references to work with conventional descriptive analysis.

To overcome these limitations, the description of mouthfeel properties can be carried out by alternative sensory methods that do not require to achieve consensus among panellists (Valentin et al., 2012). These approaches are specially interesting for the study of mouthfeel dimensions in wine, where there is a lack of standard reference materials illustrating sensory properties. In addition, these alternative methods can be roughly divided into verbal and non-verbal approaches.

Among alternative verbal-based methods, Campo, Ballester, Langlois, Dacremont, & Valentin, (2009) proposed the check-all-that-apply (CATA) method to be more suitable than DA for complex products such as wine. In this approach, panellists are asked to choose from a given list all the attributes they consider that describe the product. In contrast with conventional DA, consensus is not necessary. A variant of CATA is the “pick-K attributes”, which restricts the number of attributes to be selected, and thus highlighting the main sensory features of the product.

Results are expressed as frequency of citation, i.e., ordinal data, which can represent a limitation, mainly because a large number of assessors is required to have enough statistical power. To overcome this limitation, a variant of CATA was developed: Rate-all-that-apply (RATA). This approach consists in selecting the attributes that participants consider that apply to the product and for the attributes selected they also rate their intensity (Ares et al., 2014; Meyners, Jaeger, & Ares, 2016). Since, RATA provides intensities rather than counts it requires a smaller number of panellists than CATA, principally when the panellists are experts or a trained panel (Franco-Luesma et al, 2016).

Regarding alternative non-verbal sensory approaches, similarity-based methods, more specifically free sorting task is among the most widely used to describe and categorise wines (Chollet, Valentin, & Abdi, 2014). It consists in sorting the products of study based on perceived similarities (Figure 22) following a holistic approach rather than analytical as induced by verbal-based approaches. It is possible to add one step more and describe each group aroused in the main task in order to get a raw description of the products, known as labelled sorting task (Faye et al., 2004; Lelièvre, Chollet, Abdi, & Valentin, 2008).

Figure 22. Example of the use of the Sorting Task in one of the experiments carried out in this Doctoral Thesis

The appropriateness of the sensory descriptive method to be applied depends on the aim of the study. Thus, in the present academic work, alternative methods following both verbal and non-verbal approaches have been adopted with wine experts in most of the works in order to identify mouthfeel dimensions, conventional descriptive analysis has been also carried out with a trained panel in Chapter 2, of Section II.

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SECTION I

DEVELOPMENT OF A CHEMOSENSORY STRATEGY FOR WINE AND PHENOLIC FRACTIONS

SECTION I. CHAPTER 1

Chemo-sensory characterization of fractions driving different mouthfeel properties in red wines

1. Introduction

Flavor of food products is driven by color, aroma, taste and mouthfeel. In complex systems, the formation of mouthfeel is overall the least understood. This fact is especially true for the case of complex beverages such as wine. In the scientific literature, the few papers that relate quality perception and mouthfeel properties are limited to a reduced number of terms such as astringency, hot sensation, body or viscosity (Hopfer & Heymann, 2014; Sáenz-Navajas et al., 2016; Varela & Gambaro, 2006). However, based on anecdotal beliefs, it seems that there is awide range of mouthfeel terms driving wine quality perceived by consumerswith different levels of expertise. This is especially important for winemakers as they usually claimto base most of their technical decisions on grape and wine mouthfeel properties. Based on declarations of experts, high quality wines are positively related to in-mouth sensations such as balance, volume/body, round/smooth tannins or soft tannins, while negatively to unbalance, light/short, green sensations, coarse/dry tannins or green tannins among others (Sáenz-Navajas et al., 2016).

Mouthfeel properties seem also to be important quality drivers for wine communicators as they usually mention different subqualities of mouthfeel in their wine descriptions and judgements. For example, they use positive terms such as full, rich, supple, smooth, full texture or donut wine and negative such as thin, limp, watery, angular, harsh, aggressive, rough or angular. Mouthfeel terms (e.g., rough, dry, strong, astringent, harsh, body, hard or tannin) are also included in less experienced consumer vocabulary for describing wines in Spanish language. (Vidal, Giménez, Medina, Boido, & Ares, 2015). However, the valence or hedonic role (positive or negative) of these terms has not been established.

Gawel, Oberholster, and Francis (2000) already developed a mouthfeel wheel based on a hierarchical classification with the main aim of facilitating the communication among wine consumers. However, this wheel has not been generalized and less experienced consumers seem not to understand most of the terms (Vidal et al., 2015). This suggests that there is still a lack of understanding among wine audience when describing wine mouthfeel properties. The main reason could be that compounds present in wine and generating different mouthfeel properties are still unknown, resulting in a lack of reference materials illustrating such mouthfeel properties, and consequently a lack of adequate lexicon, which makes it difficult the interpretation of vocabulary.

There is a wide range of scientific publications aimed at exploring the compounds eliciting different mouthfeel sensation (Ferrer-Gallego, García-Marino, Miguel Hernández-Hierro, Rivas-Gonzalo, & Teresa Escribano-Bailón, 2010; Ferrer-Gallego, Gonçalves, Rivas-Gonzalo, Escribano-Bailón, & De Freitas, 2012; Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Gonzalo-Diago, Dizy, & Fernandez-Zurbano, 2013; Hufnagel & Hofmann, 2008; Saenz-Navajas, Fernandez-Zurbano, Ferreira, & Dizy, 2010; Vidal, Meudec, Cheynier, Skouroumounis, & Hayasaka, 2004). The researches described in these publications are usually carried out by chemists, resulting in impeccable methodologies able to isolate compounds or group of compounds. However, the step involving the sensory characterization of such molecules is usually restricted to the use of a limited and predetermined number of mouthfeel terms such as body/viscosity, astringency, velvety, puckering or tannic intensity.

In this context the main challenge in this field is to find the compound or groups of compounds responsible for mouthfeel properties, which will help to develop references illustrating mouthfeel attributes and further develop a homogeneous and well-defined mouthfeel vocabulary.

Objectives

Our main hypotheses are that 1) in order to build vocabulary, it is of paramount importance to have isolated fractions showing specific mouthfeel-related properties and 2) the subsequent chemical characterization of these fractions will help to identify the chemicals driving such mouthfeel properties. In this context, the specific aims of the present work were 1) to develop a semi-preparative fractionation method for isolating groups of compounds displaying different sensory properties, 2) to use these fractions to generate a wide vocabulary related to mouthfeel properties able to characterize fractions and wines, 4) to chemically characterize fractions and 5) to establish relationships between sensory and chemical composition.

2.Material and Methods

2.1. Chemicals

Bovine serum albumin (BSA, fraction V powder) was purchased from Sigma. Glacial acetic acid, HPLC-grade acetone, HPLC–MS-grade acetonitrile and formic acid, methanol, diethyl ether and absolute ethanol all of them of reagent grade were obtained from Scharlab (Sentmenat, Spain), and potassium metabisulfite from Panreac (Madrid, Spain). Deionized water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use.

Chemicals used for analytical characterization were of analytical reagent grade and were supplied by Sigma-Aldrich (Madrid, Spain) and Extrasynthèse (Genay, France). Purity of chemical standards was over 95% in all cases and most of them over 99%.

2.2. Wines

A set of three wines elaborated in the same winery was selected for the study (codes: W1, W2, W3). Sample information is provided in Table I-1.1. The main criterion for their selection was based on having at least two samples with similar polyphenolic content (measured by absorbance at 280 nm –TPI) but displaying a priori different mouthfeel properties (based on an informal tasting carried out with the winemaker and three researchers of the research team). W1 and W2 had similar TPI but displayed different mouthfeel properties. The third wine, W3, was selected as it had a priori different mouthfeel properties and presented lower TPI than WI and W2, which allowed having wines in a relatively wide TPI range.

Table I-1. 1. Conventional oenological parameters of the studied wines.

	W1	W2	W3
Year	2010	2014	2014
Variety	Tempranillo	Cabernet Sauvignon	Syrah
pH	3.54	3.58	3.58
Volatile acidity[a]	0.37	0.36	0.41
Titratable acidity[b]	6	4.6	6.2
Reducing sugars (g L^{-1})	2.7	3.1	2.5
Lactic acid (g L^{-1})	1	1.4	1.3
Ethanol (%, v/v)	15.3	14.3	14.9
TPI[c]	67.2	68.5	48.7
CI[d]	13.9	17.4	12.2

[a] Expressed as g L^{-1} of acetic acid
[b] Expressed as g L^{-1} of tartaric acid
[c] Total polyphenol index. Calculated as abs at 280nm measured in a 1-cm cuvette and multiplied by 100.
[d] Colour intensity. Calculated as Abs 420 nm + Abs 520 nm + Abs 620 nm measured in a 2-cm quartz cuvette and multiplied by 5.

2.3. Preparation of wine fractions

A total of six fractions per wine were obtained by a two-step methodology.

In the first step, four fractions were collected by a preparative LC method adapted from Remy, Fulcrand, Labarbe, Cheynier, and Moutounet (2000) and Gonzalo-Diago et al. (2013). Therefore, the ethanol of two-hundred milliliters of wine was firstly removed in a rotary evaporator (15 min at 28 ºC). Then, the sample was further freeze-dried in 500 mL-rounded flasks. The extract was redissolved in 20 mL of hydroalcoholic solution (12% ethanol, v/v) and the whole volume was injected into a preparative Millipore LC column (gel: Toyopearl HW-50F; dimensions: 120 mm x 22 mm id; flow rate: 4 mL min-1). A first fraction (F1) was eluted with 240 mL of ethanol/water/formic acid (55:45:1, v/v/v). The second fraction (F2.1) was recovered by elution with 80 mL of acetone (100%). The third (F2.2) and fourth (F2.3) fractions were eluted with 160 and 80 mL of acetone/water at rates of 80:20 and 60:40, respectively. Solvents present in the four fractions were evaporated under vacuum and samples were further freeze-dried.

In the second step, F1 was redissolved in 200 mL of hydroalcoholic solution (12%, v/v) and further submitted to solid-phase extraction (SPE) using an extraction unit (VAC ELUT 20 Station from Varian). SPE cartridges filled with 500 mg of Bond Elut LRC-C18 resins were firstly conditioned by passing 5 mL of methanol and 10 mL of an aqueous solution at pH 2.5 (5 g L^{-1} of tartaric acid, pH adjusted to 2.5 with 0.1 M NaOH). After this, 5 mL of F1 were loaded and sugars and organic acids were washed with 10 mL of aqueous solution at pH 2.5. Then, F1.1 was eluted with 5 mL of diethyl ether, F1.2 with 5 mL of ethyl acetate and F1.3 with 10 mL of methanol. Each cartridge was used a maximum of 5 times. Finally, after F1.3 was eluted, ten-mL of acetonitrile were used as pre-conditioning solvent before conditioning to remove any impurities on the SPE tube. The SPE-procedure was repeated until the 200 mL of F1 were extracted. The extracts of F1.1, F1.2 and F1.3 were gathered in a round flask and further evaporated prior freeze-drying.

The total absence of solvents was assessed by headspace solid phase micro extraction (Carboxen/PDMS 75 µm at 30 ºC x 10 min) and GC with a MS detector (overall system detection limit 1 ng/sample).

The six freeze-dried fractions (F1.1, F1.2, F1.3, F2.1, F2.2, F2.3) were stored at 4 ºC prior sensory and/or chemical analysis. At that time, fractions (coming from 200 mL of original wine) were dissolved in 100 mL (concentrated twice) of hydroalcoholic solution (7% ethanol, v/v; 50 mg L-1 of SO_2; 80 mg L-1 of ascorbic acid) prepared with mineral water (Solan de Cabras®, Spain).

2.4. Chemical characterization of wines and fractions.

2.4.1. Conventional oenological parameters

Ethanol content, pH, reducing sugars, titratable (total) and volatile acidities were determined in original wines by Infrared Spectrometry with Fourier Transformation (IRFT) with a WineScanTM FT 120 (FOSS®), which was calibrated with wines analyzed in accordance with official OIV practices. Malic and Lactic acids were determined by

enzymatic methods using an enzymatic auto analyzer (LISA 200 Wine Analyzer System).

Total polyphenol index (TPI) was estimated as absorbance at 280 nm and color intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Ribéreau-Gayon, 1970). For TPI determination, the abs at 280 nm of samples diluted 1:100 in deionized water was measured in 1-cm-quarz cuvettes. For CI, absorbance of undiluted samples was measured in 2-cm-crystal cuvettes.

2.4.2. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) was carried out as described elsewhere (Harbertson, Picciotto, & Adams, 2003). MPs were the group of compounds bleachable with bisulfite, while SPP and LPP were resistant to bisulfite bleaching. SPP did not precipitate with ovoalbumin, different to LPP. Levels of MP, SPP, and LPP were expressed as absorbance at 520 nm.

2.4.3. Thiolysis assay

Acid-catalyzed degradation in the presence of toluene-α-thiol was performed according to the method described by Gonzalo-Diago et al. (2013).

2.4.4. Quantification of proanthocyanidins

Total proanthocyanidins (TPAs) were measured by vanillin assay in fractions with oligomers (>trimers) and polymers of flavanols (F21, F22, F23) as described elsewhere (Sun, Ricardo-da-Silva, & Spranger, 1998).

Protein-precipitable proantocyanidins (PPAs) were quantified using ovoalbumin as precipitation agent and expressed as tannic acid equivalents as described by Llaudy et al. (2004).

2.4.5. Quantification of low-molecular weight phenolics by UPLC/MS and UPLC/DAD/MS

Compounds of low molecular weight were quantified in F1.1, F1.2 and F1.3. Anthocyanins were identified by UPLC-DAD-MS and quantified by UPLC-DAD and flavonols, flavanols, hydroxycinnamic acids, phenolic acids and aconitic acid by UPLC-MS following the method described by Gonzalez-Hernandez, Avizcuri-Inac, Dizy, and Fernandez-Zurbano (2014).

2.4.6. MALDI-TOF-MS

MALDI-TOF−MS spectra were obtained using a MicroFlex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument was equipped with a pulsed nitrogen laser (337 nm, 3 ns pulse width) and a time-delayed extracted ion source. Spectra were acquired in the positive-ion mode using the reflectron in two mass ranges: 200-800 Da and 800−3500 Da, with a 20 kV accelerating voltage. Freeze-dried fractions coming from 200 mL of original wine were dissolved in 3 mL of acetone/water (60/40), and spectra were run with 2,5-dihydroxybenzoic acid (DHB) as matrix, being the matrix/sample ratio of 1:20, and MicroScout Plate 96 target ground steel (Bruker Daltonics). Matrix solution was prepared at a concentration of 20 mg mL−1 in 30% acetonitrile (v/v) and 0.1% of trifluoroacetic acid. After brief mixing, 1 µL of the mixture was added on the MALDI target and allowed to air-dry. Mass calibration was performed with poly(ethylene glycol) (PEG 2000) as the internal standard. Each recorded spectrum was the sum of 250 laser shots.

2.5. Sensory analysis

In all sensory tasks, participants were instructed to sip the samples via a dark straw (to control the volume they had -2 mL each sip- and to limit carry-over effects) and gently spread out the liquid over the whole mouth cavity. After one minute, they were told to expectorate the sample. The use of a sip (rinsing solutions: water and 1 g L-1 pectin solution) and spit protocol between each sample was imposed as

described elsewhere (Colonna, Adams, & Noble, 2004). Ten-mL samples were served in dark approved wine glasses (ISO 3591, 1977) labelled with 3-digit random codes and covered with plastic Petri dishes according to a random arrangement, different for each participant. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC).

All participants carrying out sensory tests were established winemakers. They were informed that samples were not commercial wines but fractions obtained in the laboratory, in case they accepted to continue in the experiment they signed a consent form. They were not paid for their participation.

2.5.1. Generation of vocabulary and fraction selection

2.5.1.1. Sorting task

Participants. Eighteen wine experts (11 women and 7 men, ranging from 28 to 57 years of age, average = 38) participated.

Samples. Eighteen fractions were evaluated: six fractions (F1.1, F1.2, F1.3, F2.1, F2.2) x 3 wines (W1, W2, W3).

Method. Participants were asked to perform two sorting tasks in two different sessions (each session separated by one week): session 1 for fractions F1.1, F1.2 and F1.3 and session 2 for F2.1, F2.2 and F2.3. In each session, participants received 10 fractions (3 fractions x 3 wines + 1 sample in duplicate to evaluate repeatability) and were asked to sort them on the basis of similarity attending to sensations perceived in mouth (mouthfeel and taste). Even if samples had only a residual aroma, they were instructed not to smell samples orthonasally. Participants could form as many groups as they wished. Upon completion, they recorded the three-digit codes of the samples of each group on a paper sheet and were asked to describe the groups they formed with their own words (maximum of two terms per group). Participants were allowed to taste the samples again, but not to modify the groups they had already established.

Data analysis. For each participant, results were encoded in an individual similarity matrix (samples × samples), in which 1 stands for two samples set in the same group and 0 for different groups. These individual matrices were summed across subjects; the resulting co-occurrence matrix (10 x 10) represents the global similarity matrix where larger numbers indicate higher similarity between samples and the main diagonal accounts for the number of participants. The assumption underlying this method is that samples grouped together are more similar than samples sorted into different groups. The resulting co-occurrence matrix was submitted to a non-parametric MDS analysis (absolute model) in order to obtain a spatial representation of fractions. The quality and the reliability of representations were evaluated by Shepard diagrams and Kruskal´s stress value. Hierarchical cluster analysis with the Ward criterion was performed on the matrix consisting of samples x coordinates of the retained MDS dimensions. All analyses were carried out with XLSTAT (2015 version).

Concerning the terms derived from the description of the groups, an initial list was built with all the terms elicited by participants. This list was firstly reduced by omitting words with hedonic or emotional character (e.g. pleasant, easy, classic, different…) and adverbs going with main words (e.g., very, barely, extremely…). For remaining words, a lemmatization process was performed, i.e. words sharing the same lemma or root (e.g. hard, hardness…) were grouped in the same category. Finally, when a noun and an adjective were grouped to form a category, we selected the adjective to name the category (e.g. bitter and bitterness were grouped under the label bitter).

2.5.1.2. Repertory grid

Participants. Eleven wine experts, different from those participating in the sorting task, (6 women and 5 men, ranging from 31 to 44 years of age, average = 38) participated.

Samples. A total of 12 fractions (3 fractions x 4 triads) were analyzed. The most different samples based on sorting task data were selected.

Method. Based on the repertory grid method proposed by Kelly (1955) in social psychology and further applied by Thomson and McEwan (1988) to food perception, four triads of samples were evaluated in two sessions (held in different days), each composed of two flights. In each flight, participants had to individually taste (following the drinking and rinsing protocol described for sorting task) and select the most different sample in the triad. Then, they were asked to write down the terms (taste and mouthfeel) that differentiate that sample from the other two as well as to cite the terms that made the other two samples to be similar.

Data analysis. The list of collected terms was refined as for sorting task by deleting hedonic and emotional words followed by lemmatization.

2.5.1.3. Triangulation

All terms derived from both sorting task and repertory grid were gathered and grouped in categories according to semantic similarities. This process was performed individually by three experienced researchers, who through a triangulation task (Abric, 2003; Rodrigues, Ballester, Saenz-Navajas, & Valentin, 2015) achieved a final consensual list of terms.

2.5.2. Descriptive analysis (Rate-all-that-apply-RATA)

Participants. Thirty wine experts (20 women and 10 men, ranging from 26 to 58 years of age, average = 37) participated.

Samples. The three original wines (W1-W3) and fourteen fractions (out of the eighteen: 3 wines x 6 fractions) of the three wines were described via a RATA task. These 14 fractions were selected to present important mouthfeel differences based on the sorting task results.

Method. Panelists attended two sessions in two different days. Each session was split into two flights (ca. 30 min each), which were separated by an imposed pause

of 15 min. First session included three wines (1st flight) and five fractions (2nd flight) and second session five (1st flight) and four fractions (2nd flight). Participants were presented with the final list of terms derived from triangulation (total of 23 attributes: four for taste, 18 for mouthfeel and persistence) and they were asked to taste and rate the intensity of exclusively those terms that applied to the sample on a seven-point scale (1 = not intense; 7 = very intense) according to Rate-all-that-Apply (RATA) methodology (Ares et al., 2014; Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014). Terms that did not apply to the sample were allocated a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomized order for each assessor.

Data analysis. First, to find discriminant attributes for the three original wines (W1-W3) a two-way ANOVA (panelists as random and wines as fixed factors) was calculated for each of the 23 terms of the list. Next, two-way ANOVAs (panelists as random and fractions as fixed factors) were performed on the intensity ratings of the 14 fractions. Pair-wise comparison test (SKN) was applied (5% risk) for significant effects. A principal component analysis (PCA) was carried out on the mean intensity scores of the significant terms and the 14 fractions. A hierarchical cluster analysis (HCA) with the Ward criteria was finally applied to all PCs. Clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centers. The terms that best characterized each cluster were identified by using the test-value parameter (Lebart, Morineau, & Piron, 1995). The test-value corresponds to a statistical criterion akin to a standardized variable (zero mean and unit variance). Significance is obtained when the absolute test-value is ≥1.96, which corresponds to an error threshold of 5%.

All statistical analyses were performed using XLSTAT (2015) and SPAD (version 5.5).

3. Results and discussion

3.1 Chemical characterization of fractions

Fractions F1.1 and F1.2 contain in essence phenolic compounds with low molecular weight such as acids and their esters, hydroxycinnamic acids, monomers and dimers of flavanols and flavonols (Table I-1.2).

Fraction F1.3 retain more than 50% of phenolic compounds (measured as absorbance at 280nm: TPI) and 75% of colour intensity (CI) as can be observed in Table I-1.3.

Table I-1.3. Total polyphenol index (TPI), colour intensity (CI), monomeric (MP), small polymeric (SPP) and large polymeric (LPP) pigments measure in the six fractions of the three wines analysed. Marked in bold data higher than the average value calculated with the three wines and six fractions.

		F11	F12	F13	F21	F22	F23
TPI	**W1**	4.2±0.0	4.0±0.0	**32.3±0.3**	3.3±0.0	8.8±0.0	<1
	W2	5.8±0.0	7.3±0.0	**30.9±0.5**	1.5±0.0	5.5±0.0	<1
	W3	4.3±0.0	5.6±0.0	**18.0±0.4**	1.9±0.0	5.1±0.0	<1
CI	**W1**	<0.5	<0.5	**8.4±0.1**	<0.5	1.2±0.0	<0.1
	W2	<0.5	<0.5	**18.5±0.1**	<0.5	<0.5	<0.1
	W3	<0.5	<0.5	**4.9±0.1**	<0.5	0.5±0.1	<0.1
MP	**W1**	<0.1	<0.1	**1.1±0.1**	<0.1	<0.1	<0.1
	W2	<0.1	<0.1	**0.9±0.0**	<0.1	<0.1	<0.1
	W3	<0.1	<0.1	**1.3±0.1**	<0.1	<0.1	<0.1
SPP	**W1**	<0.1	<0.1	**0.2±0.1**	<0.1	**0.1±0.0**	<0.1
	W2	<0.1	<0.1	**0.3±0.0**	<0.1	<0.1	<0.1
	W3	<0.1	<0.1	**0.4±0.0**	<0.1	**0.1±0.0**	<0.1
LPP	**W1**	<0.1	<0.1	**0.9±0.1**	<0.1	**0.2±0.0**	<0.1
	W2	<0.1	<0.1	**2.4±0.0**	<0.1	<0.1	<0.1
	W3	<0.1	<0.1	**0.2±0.1**	<0.1	<0.1	<0.1

The majority of anthocyanin derivatives, including those non-resistant to SO_2 bleaching (MP), resistant to SO_2 bleaching but not precipitating with albumin (SPP), and those resistant to SO_2 and able to precipitate with protein (LPP), are present in F1.3 in the three wines as can be observed in Table I-1.3. Fraction F1.3 of W2 contains the lowest levels of monomeric anthocyanin pigments (MP) and the highest in large polymeric pigments (LPP), followed by W1. Harbertson et al. (2003) suggested that LPP fraction contained anthocyanins linked to polymeric flavanols with more than three catechin or epicatechin subunits. Thus, we had expected to find such anthocyanin-flavanol polymers in F1.3 of W1 and W2. However, the mean degree of polymerization (mDP) (measured by the thiolysis assay) for this fraction was in both cases lower than 1.6 (Table I-1.4), suggesting that F1.3W1 and F1.3W2 would contain at the most anthocyanins linked to two flavanol units, but not polymers itself.

Table I-1. 4. Polymerization range of flavanols (PR), mean degree of polymerization (mDP), total proantocyanidins (TPAs) and precipitable proanthocyanidins (PPAs).

		F11	F12	F13	F21	F22	F23
PR[a]	**W1**				4 - 10	3 - 10	---
	W2				4 - 10	3 - 10	---
	W3				4 - 10	3 - 8	---
mDP[b]	**W1**	1.0	1.2	1.5	2.7	2.2	1.8
	W2	1.0	1.0	1.1	2.5	2.3	1.7
	W3	1.0	1.1	1.2	2.0	1.8	2.0
TPAs[c] (mg L^{-1})	**W1**				283 ± 10	572 ± 5	150 ± 5
	W2				203 ± 5	380 ± 10	208 ± 6
	W3				223 ± 9	412 ± 12	209 ± 4
PPAs[d] (mg L^{-1})	**W1**				107 ± 6	906 ± 12	nd
	W2				51 ± 1	496 ± 6	nd
	W3				107 ± 6	376 ± 12	nd

[a]Polymerization range of flavanols observed in MALDI-TOF spectra.
[b]Mean degree of polymerization of flavanols calculated as (terminal units + extension units)/terminal units calculated by thiolysis method.
[c]Total proanthocyanidins measured by vanillin assay (Sun et al., 1998)
[d]Protein-precipitable proanthocyanidins measured by ovalbumin assay (Llaudy et al., 2004).

Table I-1. 2. Average (±standard deviation) concentration of phenolic compounds quantified by UPLC-MS (acids, flavanols and flavonols) and UPLC-Vis (anthocyanins) in fractions containing low molecular-weight phenolics (F1.1, F1.2, F1.3).

	F1.1			F1.2			F1.3		
	W1	W2	W3	W1	W2	W3	W1	W2	W3
ANTOCYANINS									
∑ Pyranoanthocyanins	nd	nd	nd	nd	nd	nd	4.85 ± 0.03	15.6 ± 0.19	8.98 ± 0.26
∑ Glycosilated anthocyanins	nd	nd	nd	nd	nd	nd	25.4 ± 0.1	160 ± 2	113 ± 1
∑ Acylated anthocyanins	nd	nd	nd	nd	nd	nd	4.71 ± 0.02	37.6 ± 0.1	43.5 ± 0.1
∑ anthocyanin-flavanol dimers	nd	nd	nd	nd	nd	nd	6.96 ± 0.04	24.5 ± 1.5	15.3 ± 0.1
ACIDS									
∑ Hydroxybenzoic acids and derivatives	24.0 ± 0.1	25.7 ± 0.3	18.8 ± 1.3	1.46 ± 0.06	2.74 ± 0.05	0.98 ± 0.00	0.41 ± 0.01	0.95 ± 0.01	0.79 ± 0.00
∑ hydroxycinnamic acids and derivatives	10 8± 2	163 ± 3	53.8 ± 0.0	24.8 ± 0.1	35.5 ± 0.1	14.2 ± 0.01	18.7 ± 0.0	17.8 ± 0.2	18.3 ± 0.1
FLAVANOLS									
∑ (epi)gallocatechins	1.83 ± 0.02	3.58 ± 0.07	0.72 ± 0.03	0.64 ± 0.01	1.01 ± 0.00	0.32 ± 0.02	0.47±0.00	0.60 ± 0.02	nd
∑ (epi)catechins	9.47 ± 0.02	39.7 ± 0.3	37.4 ± 1.1	2.23 ± 0.00	8.35 ± 0.26	9.26 ± 0.10	2.04±0.01	7.62 ± 0.09	nd
∑ gallo(epi)catechin gallates	nd	nd	nd	nd	nd	nd	nd	nd	nd
∑(epi) catechin gallates	nd	nd	nd	nd	nd	nd	nd	nd	nd
∑ dimers	0.32 ± 0.01	1.17 ± 0.06	2.69 ± 0.04	8.24 ± 0.08	28.9 ± 0.05	13.2 ± 0.2	5.36±0.15	9.13 ± 0.14	nd
∑ trimers	nd	nd	nd	3.10 ± 0.06	5.62 ± 0.21	nd	2.63±0.00	2.39 ± 0.03	nd
∑ dimers of (epi) catechin gallates	nd	nd	nd	0.11 ± 0.01	1.90 ± 0.13	0.68 ± 0.08	nd	0.59 ± 0.06	nd
∑ trimers of (epi) catechin gallates	nd	nd	nd	nd	0.58 ± 0.01	0.38 ± 0.04	nd	0.36 ± 0.02	nd
∑ tetramers	nd	nd	nd	nd	nd	0.12 ± 0.01	nd	0.12 ± 0.02	0.38 ± 0.03
FLAVONOLS	4.46 ± 0.07	1.78 ± 0.06	3.05 ± 0.06	26.9 ± 0.16	21.7 ± 0.27	15.2 ± 0.2	19.4±0.00	18.9 ± 0.1	8.85 ± 0.00

This fact was further confirmed by MALDI-TOF MS (aquired in m/z range of 800-3500), where series of oligomeric anthocyanins different from flavanol-derivatives appeared in W1 and W2 but not in W3. Figure I-1.1 shows a first series of signals at m/z 1273.5, 1287.4, 1301.5 and 1315.5, which is consistent with trimers of anthocyanins composed of either two glucosydes (Figure I-1.2a) or one caffeoyl-glucoside moiety (Figure I-1.2b). These trimers would contain at least two malvidins and the third anthocyanin would correspond to cyanidin, delphinidin, petunidin or malvidin, respectively.

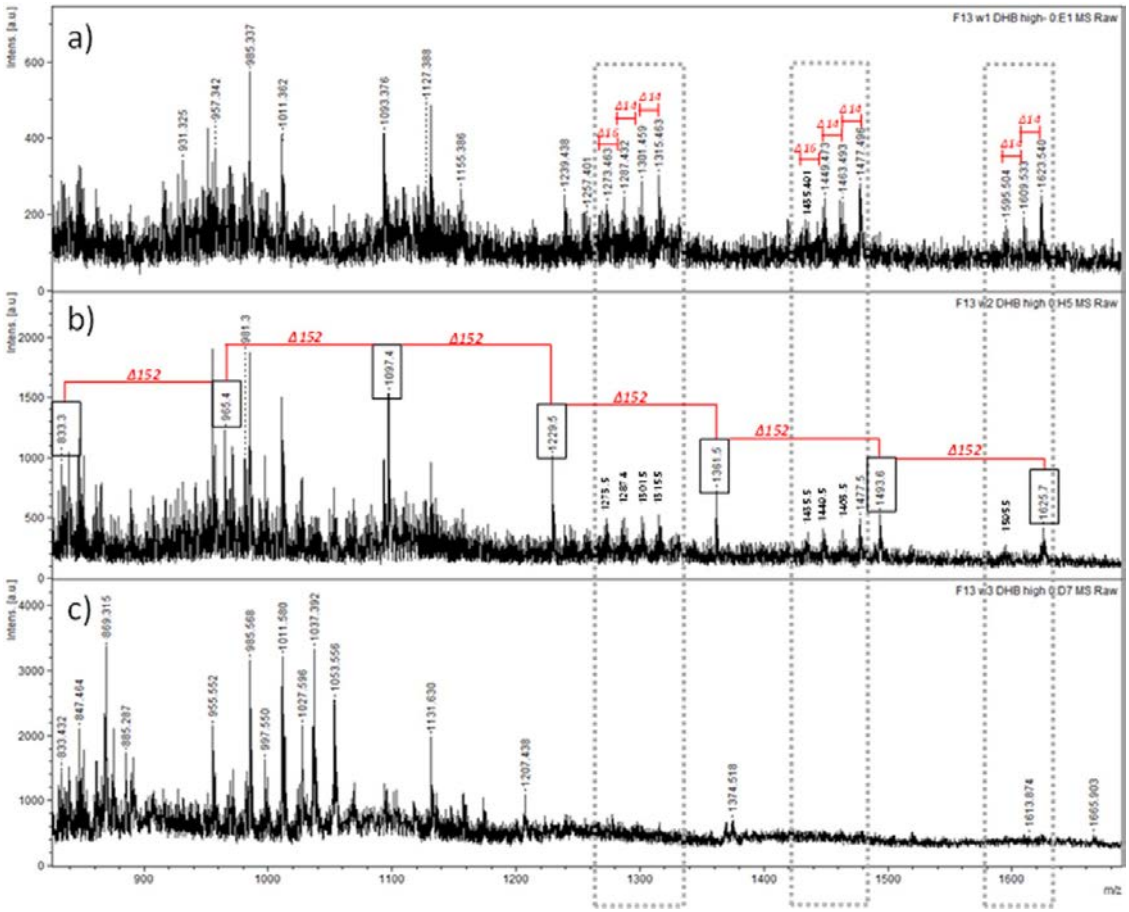

Figure I-1. 1. MALDI-TOF-MS analysis of fraction F1.3 of wines a) W1, b) W2 and c) W3.

Another series of ions at m/z 1449.5, 1463.5 and 1477.5 could be tentatively identified as trimers of anthocyanin-3-O-glucosyde, being two of them malvidin-3-O-glucosyde and the third anthocyanin moiety would be delphinidin-3-O-glucosyde, petunidin-3-O-glucosyde or malvidin-3-O-glucosyde, respectively (Figure I-1.2c). The

presence of such oligomeric anthocyanins in grape skins have been already described by Vidal et al. (2004).

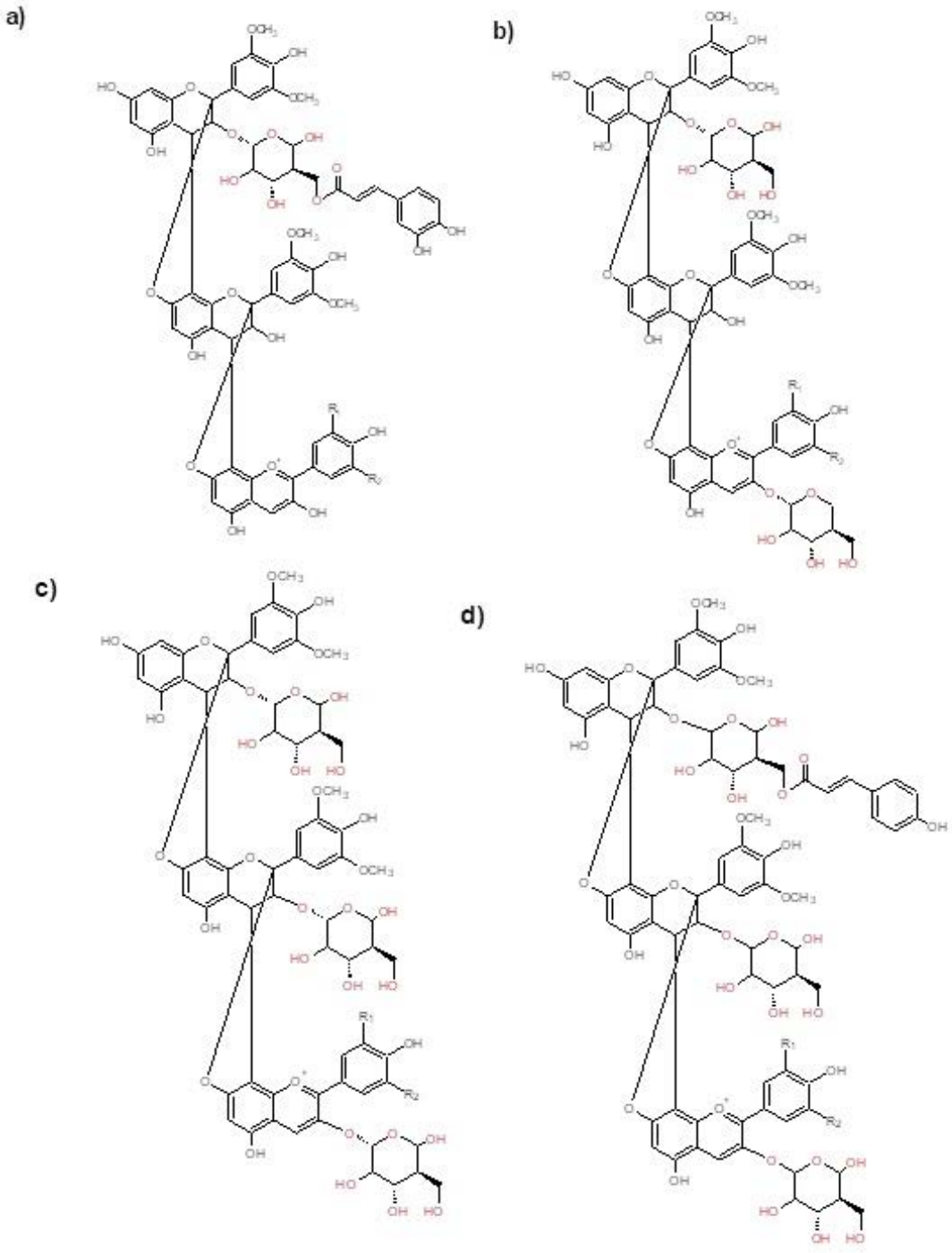

Figure I-1. 2. Tentative identification of molecules found in F13 of W1 and W2.

At higher m/z ratios appear ions at 1595.5, 1609.5 and 1623.5, which could correspond to monocoumaroylated trimers of anthocyanin-3-O-glucosyde with two moieties of malvidin-3-O-glucosyde and the third would be delphinidin-3-O-glucosyde, petunidin-3-O-glucosyde or malvidin-3-O-glucosyde, respectively (Figure I-1.2d). The trimer of malvidin-3-O-glucosyde was already identified in grape pomace by MALDI-TOF MS (Oliveira, Alhinho Da Silva, Teixeira, De Freitas, & Salas, 2015).

The addition of ovoalbumin to fractions F1.3 of wines W1 and W2 confirmed that oligomeric anthocyanins shown in Figure I-1.1 disappeared. This would suggest that these compounds are part of LPP, which are anthocyanin-derivatives precipitating with ovoalbumin (Harbertson et al., 2003). The fact that anthocyanins can precipitate with proteins has been already demonstrated with the monomer of malvidin (Ferrer-Gallego et al., 2015).

Further, it is interesting to note the presence of a series of compounds (m/z 965.4, 1097.4, 1229.5, 1361.5, 1493.6, 1625.7, 1757.7, 1889.7, 2021.8, 2153.9, 2286.0, 2550.0) differing in 132 amu, which corresponds to the molecular weight of pentoses. Thus, these compounds could be tentatively assigned to arabinogalactan-proteins constituted by increasing arabinose moeities, which could be only detected in F1.3W2 (Figure I-1.1).

Fractions F2.1, F2.2 and F2.3 are constituted mainly by flavanols with at least three flavanol moieties based on MALDI-TOF spectra. Figure I-1.3 shows as example the spectrum of F2.1W3. There are ions varying in 16 amu, corresponding to the presence of trihydroxylated flavanols (gallocatechin or epigallocatechins), 152 amu and 288 amu for the incorporation of galloyl and catechin/epicatechin moeities, respectively. In all fractions, galloylated flavanols as well as flavanols composed of (epi)-gallocatechin units are detected.

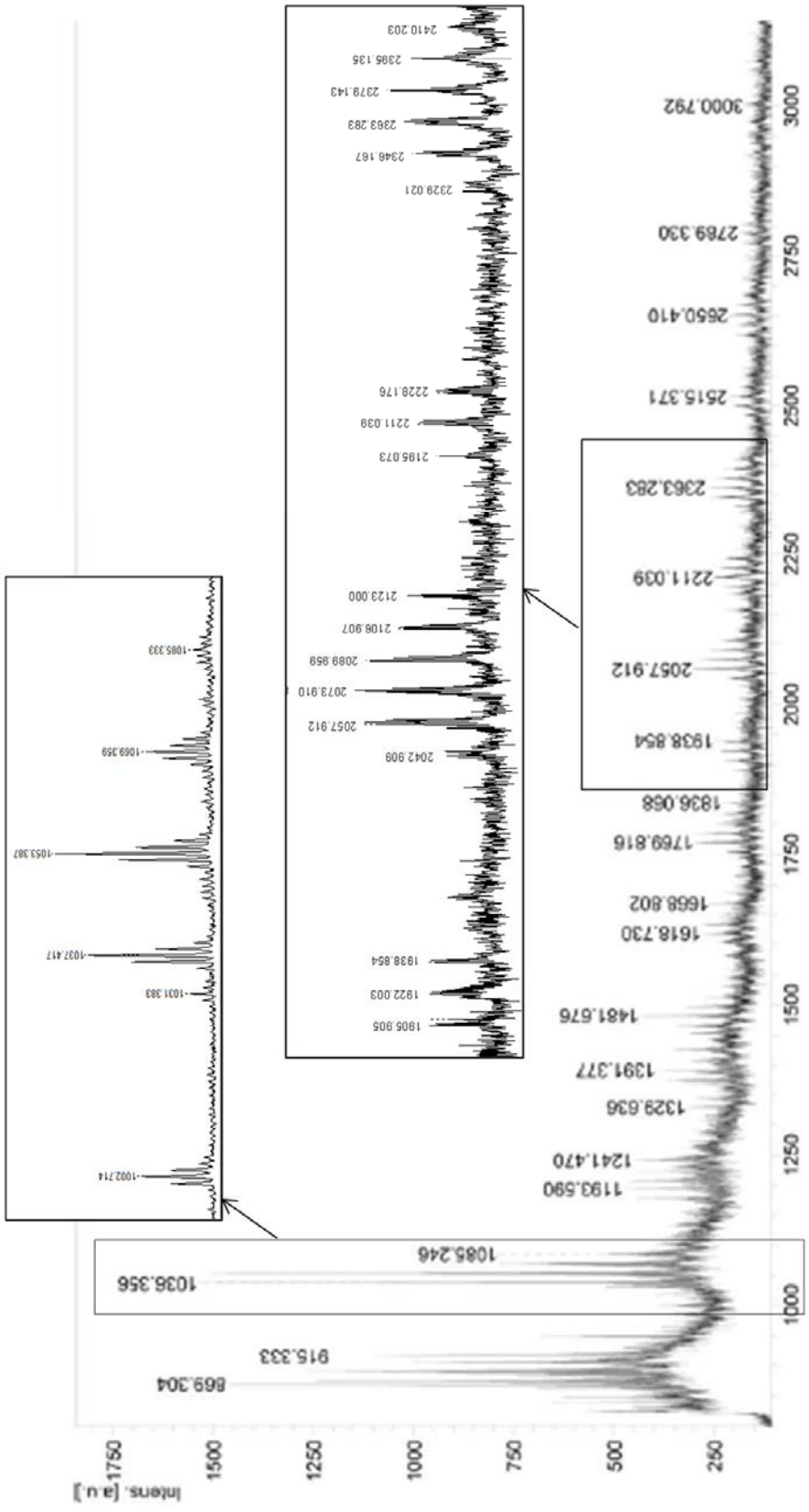

Figure I-1. 3. MALDI-TOF-MS spectra of fraction F2.1 of wine W3.

Interestingly, in fraction F2.3, flavanols higher than dimers are not detected, while in F2.1 and F2.2 from trimers up to decamers are observed (Table I-1.4). This is consistent with the fact that flavanols with lower molecular weight and eluting in F2.3 (60:40 acetone:water) are more polar than those eluting in F2.1 with 100% acetone, which present highest molecular weight, followed by F2.2 (80:20 acetone:water). This is further confirmed by the mDP (Table I-1.4) calculated by the thiolysis assay, which is in the range of 1.7–2.0 for F2.3, 1.8–2.3 for F2.2 and 2.0–2.7 for F2.1. Table I-1.4 shows that F22 of W1 contains the highest levels of total proanthocyanidins (TPAs) as well as those precipitating with ovoalbumin (PPAs), being the PPAs suggested to be involved in astringency perception. Table I-1.5 and Table I-1.6, show the extension and terminal units of flavanol-derivative compounds present in fractions. In general, most terminal units are catechins, while extension units are constituted mainly by epicatechin and epigallocatechin. There is a very small percentage of flavanols constituted by galloylated forms. These data are in accordance with other works characterizing grape skin tannins (Fournand et al., 2006) and wines (Gonzalo-Diago et al., 2013).

Table I-1. 5. Percentage of extension units of polymeric flavanols (Catechin-C, Epicatechin-EC, Catechin gallate-Cg, Epicatechin gallate-ECg) analyzed by thiolysis assay in fractions F2.1, F2.2 and F2.3

% Extension units												
	%C			%EC			%EgC			%ECG		
	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
F21	<1	3	<1	56	59	55	36	29	34	8	8	10
F22	<1	6	1	45	44	38	49	44	53	6	6	9
F23	<1	<1	<1	37	34	35	41	54	49	22	12	16

Table I-1. 6. Percentage of terminal units of polymeric flavanols (Catechin-C, Epicatechin-EC, Catechin gallate-Cg, Epicatechin gallate-ECg) analyzed by thyolisis assay in fractions F2.1, F2.2, F2.3.

%Terminal units												
	%C			%EC			%EgC			%ECG		
	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
F21	30	42	35	28	33	33	19	12	14	12	9	10
F22	59	48	42	20	34	38	10	9	9	5	6	6
F23	<1	50	<1	33	22	36	29	14	28	20	10	19

3.2. Sensory analysis

Odorless fractions (6 fractions x 3 wines), containing different groups of compounds, were further submitted to a series of sensory methodologies with the double aim of firstly generate an ample list of terms related to in-mouth properties (mainly mouthfeel) and 2) make use of this list to characterize fractions.

Two sensory strategies were used to generate vocabulary: sorting task and repertory grid. Sorting task was also used to identify fractions eliciting similar sensory properties. This helped to select samples to be included in each triplet of the repertory grid task and to reduce the number of samples to be submitted to sensory characterization by rate-all-that-apply (RATA) for avoiding carry-over effects.

3.2.1. Selection of fractions and vocabulary generation

Figures I-1.4a and 4b show the clustering of fractions F11-F12-F13 and F21-F22-F23, respectively, derived from sorting tasks. Duplicated fractions (F1.3W3 and F2.2W3) are clustered together, which confirms consistency of the panel when evaluating fractions.

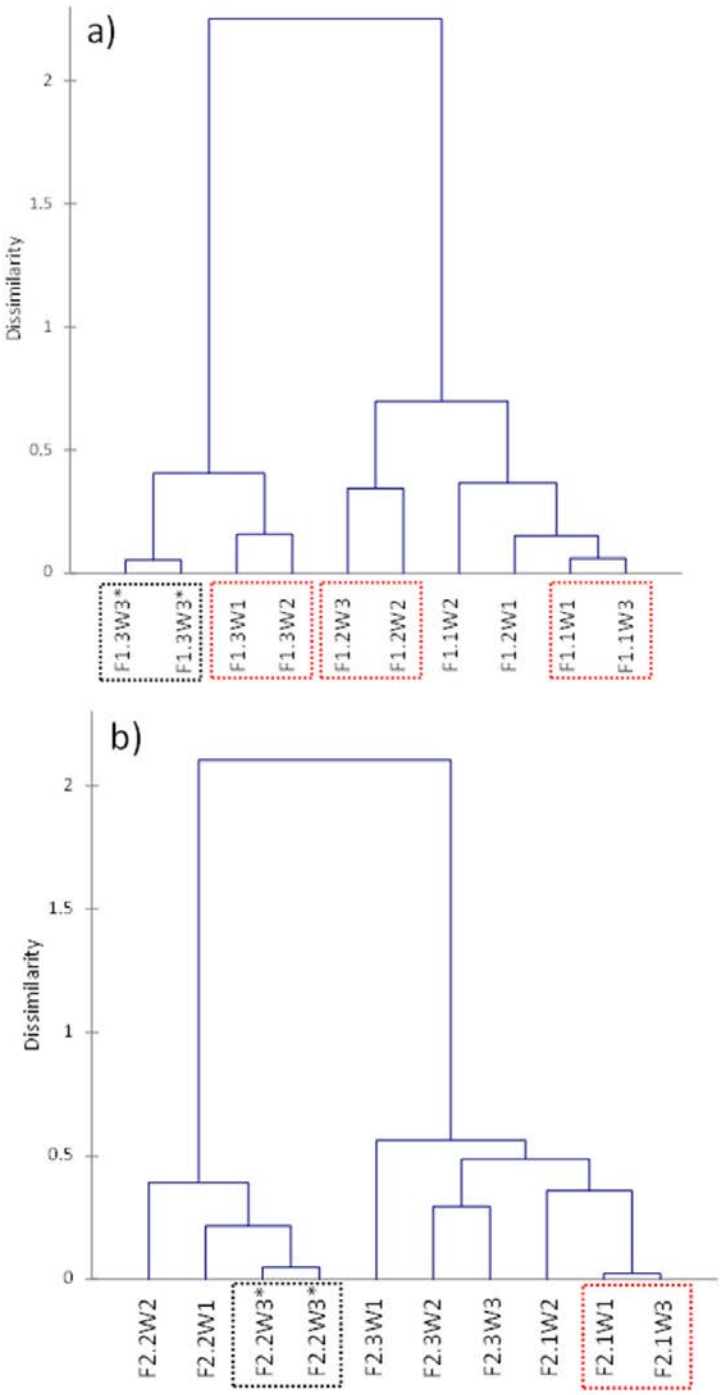

Figure I-1. 4. Hierarchical Cluster Analysis (HCA) calculated with 4 dimensions (Kruskal stress = 0.026) of ordinal multidimensional scaling (MDS) calculated with sorting task data derived from fractions a) F1.1, F1.2 and F1.3 and b) F2.1, F2.2 and F2.3 of the three wines studied (W1, W2 and W3). Fractions F1.3 (F1.3W3*) and F2.2 (F2.2W3) of W3 were presented in duplicate to control repeatability (framed in black).

Sorting task was performed with the first aim of selecting fractions for further analysis and reducing the samples employed in subsequent sensory characterization to avoid carry-over effects. Figure I-1.4a shows that F1.1W3 and F1.1W1 are clustered together as are F1.2W2-F1.2W3 and F1.3W1-F1.3W2. Similarly, F2.1W1 and F2.1W3 are grouped together by most of participants (Figure I-1.4b). Thus, it was decided to discard F1.1W3, F1.2W3, F1.3W1 and F2.1W1 as it is assumed that their sensory characteristics are represented by F1.1W1, F1.2W2, F1.3W2 and F2.1W3, respectively. In contrast, F2.2 and F2.3 of different wines are hardly grouped together (Figure I-1.4b), in all cases less than 35% of participants put them in the same group. Thus, no sample belonging to F2.2 or F2.3 was discarded for subsequent sensory tasks.

The second reason to perform this task was to generate in-mouth (taste and mouthfeel) terms, which also carried out in parallel by a more classical method: repertory grid. Table I-1.7 shows the refined list of attributes derived from both tasks. Twenty-three common attributes are generated, appearing 11 and 8 terms exclusively by sorting task and repertory grid, respectively. Thus, a total of 42 different terms including taste and mouthfeel sensations are generated. Further triangulation process with these 42 terms yielded a final list of 23 terms (Table I-1.8), including four terms related to taste (sour, bitter, sweet and salty), 18 to mouthfeel and one to the length of sensations (persistence).

Table I-1. 7. Common and specific terms generated in sorting task and repertory grid methods.

	COMMON TERMS		
1	Adhesive (*adhesivo*)	2	Alcoholic (*alcohólico*)
3	Astringent (*astringente*)	4	Bitter (*amargo*)
5	Burning (*ardiente*)	6	Coarse (rugoso)
7	Dusty (*polvoriento*)	8	Earthy (*terroso*)
9	Dry (*secante*)	10	Sawdusty (*sensación de serrín*)
11	Grainy (*granuloso*)	12	Hot (*picante*)
13	Oily (*graso*)	14	Salty (*salado*)
15	Silky (*sedoso*)	16	Sticky (*pegajoso*)
17	Sour (*ácido*)	18	Smooth (*suave*)
19	Sweet (*dulce*)	20	Tannic (*tánico*)
21	Unctuous (*untuoso*)	22	Velvety (*aterciopelado*)
23	Watery (*aguado*)		
	SORTING TASK		**REPERTORY GRID**
24	Dense (*denso*)	24	Abrasive (*abrasivo*)
25	Dry on palate (*secante en el paladar*)	25	Chalky (*sensación de tiza*)
26	Dryness on tongue side (*secante en el lateral de la lengua*)	26	Creamy (*cremoso*)
27	Fresh (*fresco*)	27	Fleshy (*carnoso*)
28	Glyceric (*glicérico*)	28	Gummy (*gomoso*)
29	Hard (*duro*)	29	Mouthcoating (*envolvente*)
30	Persistent (*persistente*)	30	Prickly (*punzante*)
31	Rough (*áspero*)	31	Round (redondo)
32	Sandy (*arenoso*)		
33	Viscous (*con volumen*)		
34	Warm (cálido)		

Interestigly, the mouthfeel attributes included in the final list are mostly in accordance with the mouthfeel wheel developed by Gawel et al. (2000) with Australian wine experts. Thus, the majority of the 11 categories (astringency + feel) of Gawel´s wheel are represented: 1. weight (watery), 2. texture (gummy, oily, unctuous), 3. heat and 4. harsh (burning), 5. irritation (hot, prickle), 6. particulate (dusty, grainy, sandy, coarse), 7. surface smoothness (silky), 8. complex (fleshy, mouthcoating), 9. drying (dry, dry on the tongue, dry on the palate) and 10. dynamic (sticky). Only the category unripe (resinous, sappy, green) is not included in our final list, which is not strange given the multidimensionality of attributes belonging to this category.

Table I-1. 8. Final list of terms derived from triangulation and used for Rate-all-that-Apply (RATA) descriptive method.

CATEGORY	INDIVIDUAL TERMS			
Taste	1	Bitter (*amargo*)	3	Salty (*salado*)
	2	Sour (*ácido)*	4	Sweet (*dulce*)
Mouthfeel	5	Burning (*ardiente*)	6	Coarse (rugoso)
	7	Dusty (*polvoriento*)	8	Feeling of dryness (*secante*)
	9	Feeling of dryness on palate (*secante en el paladar*)	10	Feeling of dryness on tongue side (*secante en el lateral de la legua*)
	11	Fleshy (*carnoso*)	12	Grainy (*granuloso*)
	13	Gummy (*gomoso*)	14	Hot (*picante*)
	15	Mouthcoating (*envolvente*)	16	Oily (*graso*)
	17	Prickly (*punzante*)	18	Sandy (*arenoso*)
	19	Silky (*sedoso*)	20	Sticky (*pegajoso*)
	21	Unctuous (*untuoso*)	22	Watery (*aguado*)
Other	23	Persistent (*persistence*)		

3.2.2. Sensory characterization of wines and fractions by RATA

The list of 23 terms generated in previous tasks are employed for characterizing the three wines and their fractions by RATA.

ANOVA results calculated with the intensity ratings of the 23 terms and the three original wines show significant effects for six terms, one belonging to the taste category (salty) and five to mouthfeel (dry, sticky, dusty, coarse and watery). Figure 5 shows the PCA calculated with the six discriminat attributes and the three wines. The first PC, which accounts for more than 70% of the original variance, mainly differenciate between W3, which is described as coarse, watery and dusty, and wines W1 and W2, which are both described as dry, but W1 being sticky and W3 salty.

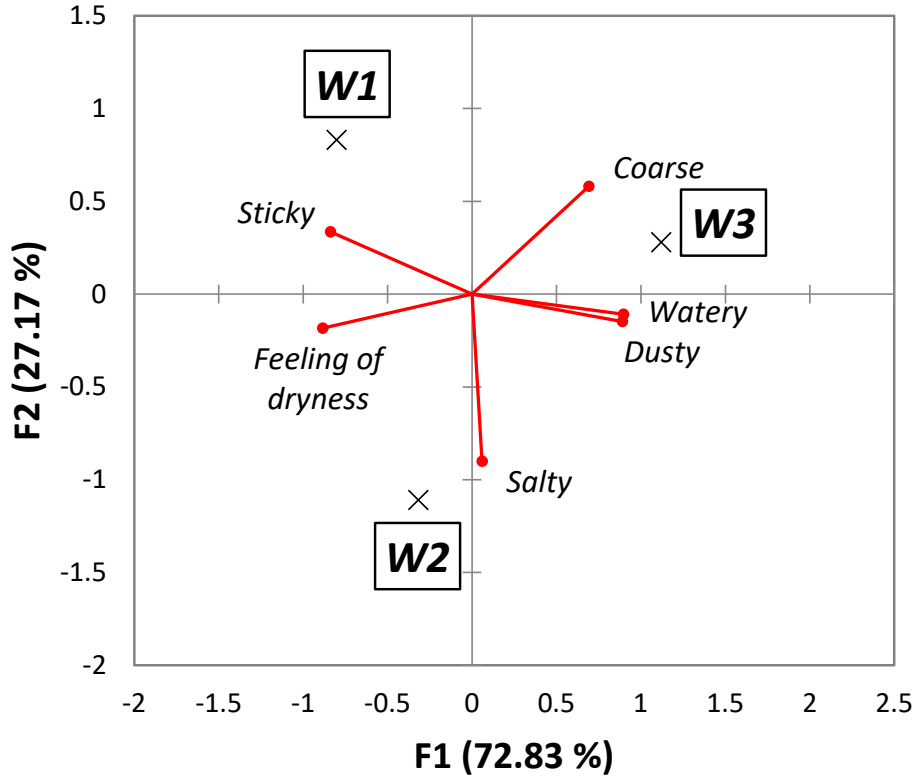

Figure I-1. 5. PCA plot on the principal components 1 and 2 calculated with wines and attributes evaluated by RATA and significantly (P<0.05) different among wines.

ANOVA results also showed that all terms except sticky, salty and gummy discriminate among fractions. This suggests that the fractionation method was effective in isolating groups of compounds able to generate different taste and mouthfeel properties according to expert´s judgements.

PCA (Figure I-1.6) followed by hierarchical cluster analysis (HCA) calculated with the intensity ratings of the 20 discriminant terms and the 14 fractions yielded four clusters of samples (Figure I-1.6a). The first cluster plotted on the left part of the plane is formed by fractions F2.1 and F2.3, being mainly characterized by terms such as: sweet, watery, silky, fleshy, oily and unctuous (Figure I-1.6b). These fractions mainly contain flavanols, dimers and trimers in F2.3 and oligomers from tetramers up to decamers in F2.1. Even if oligomers and polymers of flavanols are thought to be astringent and/or bitter, their concentration is even twice lower than in F2.2. These fractions, which are on the bottom part of the plot, have been described as

coarse, grainy, dry on the tongue and dry on the palate. Oligomers and polymers (from trimers to decamers) present in F2.2 would be responsible for such sensations.

On the top part of the plot are fractions F1.1 and F1.2 (containing phenolic compounds with low molecular weight acids and their esters, hydroxycinnamic acids, monomers and dimers of flavanols and flavonols) as well as F1.3 of W3 (containing anthocyanins not precipitating with proteins: mainly MP and SPP). They are described as bitter, burning and hot. The fourth cluster is formed exclusively by one fraction, F1.3W2, being sandy, dry, dry on the palate, bitter, sour, burning, hot, prickly and persistent. This fraction contains basically anthocyanin-derivative pigments, especially those resistant to SO_2 and precipitating with ovoalbumin (LPP) as can be observed in Table I-1.3.

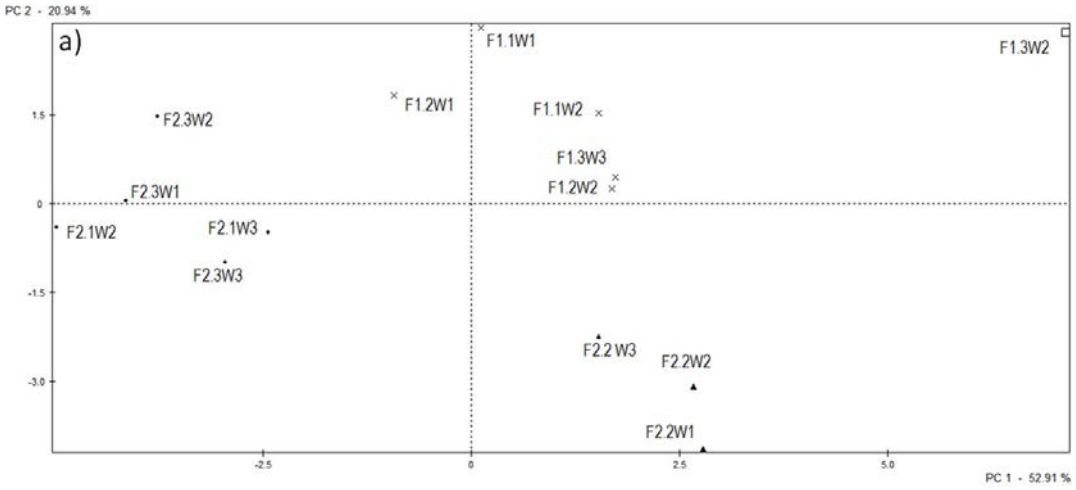

Figure I-1. 6 a. PCA plot on the principal components 1 and 2 (a) projection of fractions

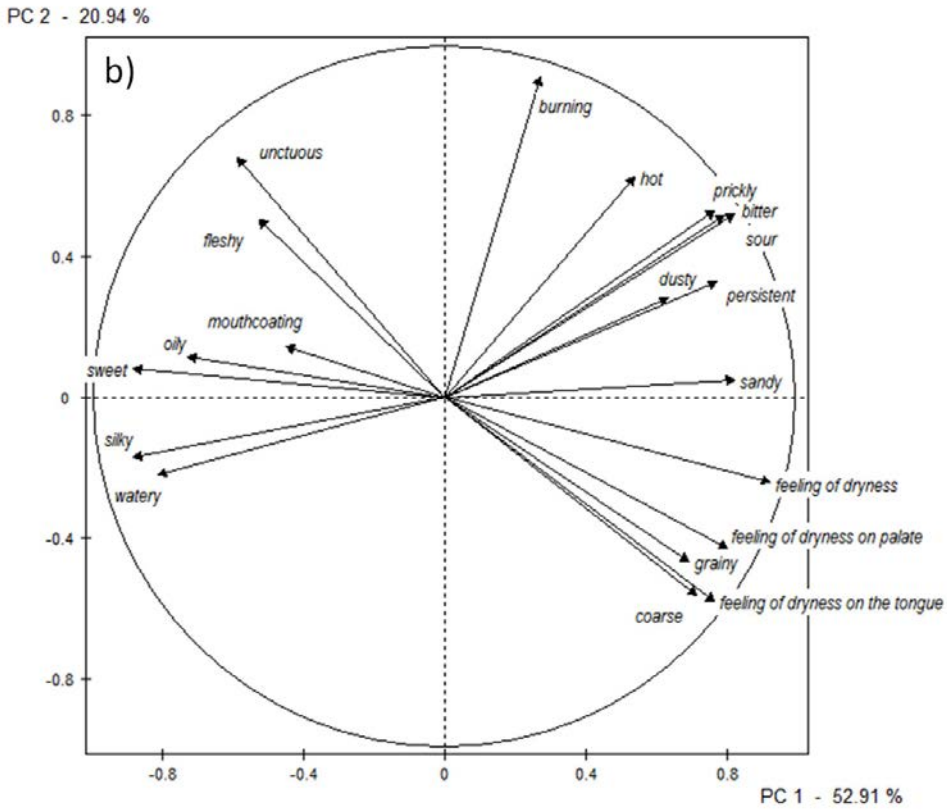

Figure I-1.6 b. PCA plot on the principal components 1 and 2 (b) loadings of the in-mouth attributes.

These results show that for most fractions, the same group of compounds belonging to different wines presents similar in-mouth properties. F1.3 is the sole fraction that generates different sensory properties for the three wines with F1.3W1 and F1.3W2 being similar (based on sorting task results) but different from F1.3W3. According to ANOVA results, these fractions are significantly different ($P<0.05$) for the attributes bitter, dry, silky and persistent, with F1.3W2 being more dry, bitter, and persistent, while F1.3W3 more silky (Figure I-1.7). Taking into account especially that W1 and W2 are scored higher in the feeling of dryness, bitterness and persistency than W3, it seems that fraction F1.3 could contain the group of compounds inducing such sensations in W1 and W2. These compounds seem to be anthocyanin-derivative pigments able to precipitate with ovoalbumin, tentatively attributed to trimers of anthocyanins.

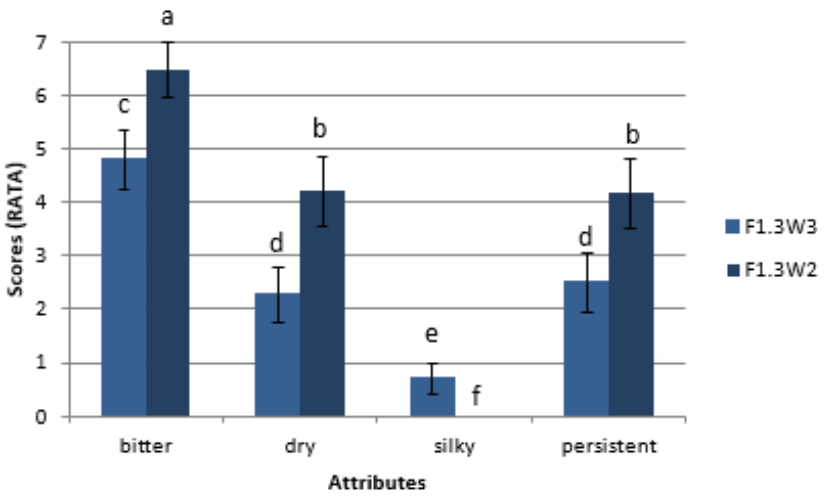

Figure I-1. 7. Mean sensory ratings of the attributes evaluated by RATA and significantly (P<0.05) different for fractions F1.3W2 and F1.3W3. Error bars are calculated as s/(n)1/2; s, standard deviation; n, number of panelists.

It is interesting to note that even if fractions F2.2 for the three wines (containing oligomers and polymers of flavanols) are also rated high in dryness, their intensity is not significantly different among the three wines. Thus, it is not probable that F2.2 would be involved in the difference of dryness perceived in original wines, unlike F1.3.

4. Conclusions

The present manuscript describes a semipreparative method coupled to a sensory method, able to isolate groups of non-volatile compounds eliciting different in-mouth properties. This method has been proved efficient in looking for chemical compounds generating different in-mouth properties in wines. The method has made it possible to develop a wide sensory vocabulary describing relevant in-mouth sensory properties, which has been proved to discriminate between both wines and fractions.

One of the fractions (F1.3) has already shown differences between wines and provided terms close to original wines. A series of oligomeric anthocyanins (trimers glycosilated and/or acylatted) is tentatively suggested to be involved in the dryness, bitterness and persistency perceived in two wines. Notwithstanding, the confirmation of the sensory properties of these olygomeric anthocyanins is needed. Therefore, we are developing analytical strategies aimed at isolating such compounds and further evaluate their real sensory impact in wines by ommission and addition experiments.

It is expected that this method will be essential for making further progresses in the field of the sensory in-mouth properties and also in the elucidation of their chemical base.

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SECCION II

APPLICATION OF CHEMOSENSORY STRATEGIES FOR UNDERSTANDING MOUTHFEEL-RELATED CONCEPTS.

SECTION II. CHAPTER 1

Chemosensory approach for the identification of chemical compounds driving green character in red wines

1. Introduction

Flavour in food and beverages is the result of sensory interactions between sensory active volatile and non-volatile molecules (Prescott, 2012). Understanding the formation of food flavour is of paramount importance for the food industry in general and especially for the industry of complex beverages such as beer or wine. Increasing knowledge about flavour formation allows to have objective tools to manage the production process and optimise the quality of the final product. Traditionally, flavour chemists use two main strategies. The first, involves the complete chemical quantification of known sensory-active molecules and in parallel the sensory description of the product. Both data sets are submitted to statistical analysis with the aim of building models able to predict perceived sensory properties from chemical composition (Regueiro, Negreira, & Simal-Gándara, 2017). Main drawbacks are that a wide range of compounds have to be quantified by a diverse range of chemical methods and the most important is that key unknown sensory active molecules are most probably not being considered. To overcome these drawbacks, flavour chemists use sensory-directed methodologies targeting only compounds eliciting sensory properties in the product object of study and further focusing in the identification and quantification of molecules in sensory-active fractions. For volatile compounds, Gas Chromatography coupled to an olfactory detector (GC-O), which involves the use of GC for separation and the nose of trained judges as detector, has been demonstrated to be a powerful tool to identify sensory-active volatile compounds and is widely used in flavour chemistry (Chin & Marriott, 2015; d'Acampora Zellner, Dugo, Dugo, & Mondello, 2008). Concerning non-volatile compounds, the separation of molecules or group of molecules is usually carried out by preparative liquid chromatography (LC), collected fractions are further sensory

described in terms of taste and mouth-feel properties (Sáenz-Navajas et al., 2017; Scharbert, Holzmann, & Hofmann, 2004). The study of the sensory activity of non-volatile molecules is less explored than volatiles, mainly due to two reasons: it is difficult to describe mouth-feel properties because there is a lack of references and the second reason is that it is more time and resource consuming than GC-O in terms of both fractionation and sensory fatiguing. Once sensory active molecules involved in the formation of flavour are identified by either GC-O or LC followed by sensory evaluation of fractions, reconstitution experiments are carried out to evaluate the real sensory impact of compounds in the matrix object of study. This helps to confirm the complex relationships existing between chemical composition of foods and flavour perception (Regueiro et al., 2017).

This strategy comprising the steps of 1) isolation and identification of sensory-active molecules or groups of molecules and 2) reconstitution experiments to confirm their sensory role has been applied in the present work to identify fractions of both volatile and non-volatile compounds responsible for a recurring undesirable sensory property found in red wines and named by wine experts: “green character”.

Due to climate change, there is a difference in time between technological (related to sugar and acidity content) and phenolic maturity of grapes. Based on declarations of winemakers, the fact that technological maturation is achieved, while phenolics and aroma precursors are still unripe, lead to green wines. During winemaking, the oenologist has to make decisions related to the elaboration of grapes that enter the winery with an acceptable content in sugars or acids but with immature tannins. Such grapes will generate wines with green character, which will induce a decrease of consumers´ acceptance of the product. Given the lack of objective criteria to this concern, such decisions are mainly based on empirical experience. An increase in the chemical and sensory knowledge of such character will allow managing grapes and/or wines with maximum efficiency during winemaking processes.

Objectives

In this context, the main aims of the present work are to: 1) understand the meaning of green character in red wines and establish relationships with simpler sensory descriptors, 2) select wines with different levels of green character and 3) identify volatile and non-volatile compounds involved in the green character in red wines.

2. Material and methods

Figure II-1.1 shows a fluxogram which graphically explains the steps followed in the material and methods section.

2.1. Screening for wines with different levels of green character

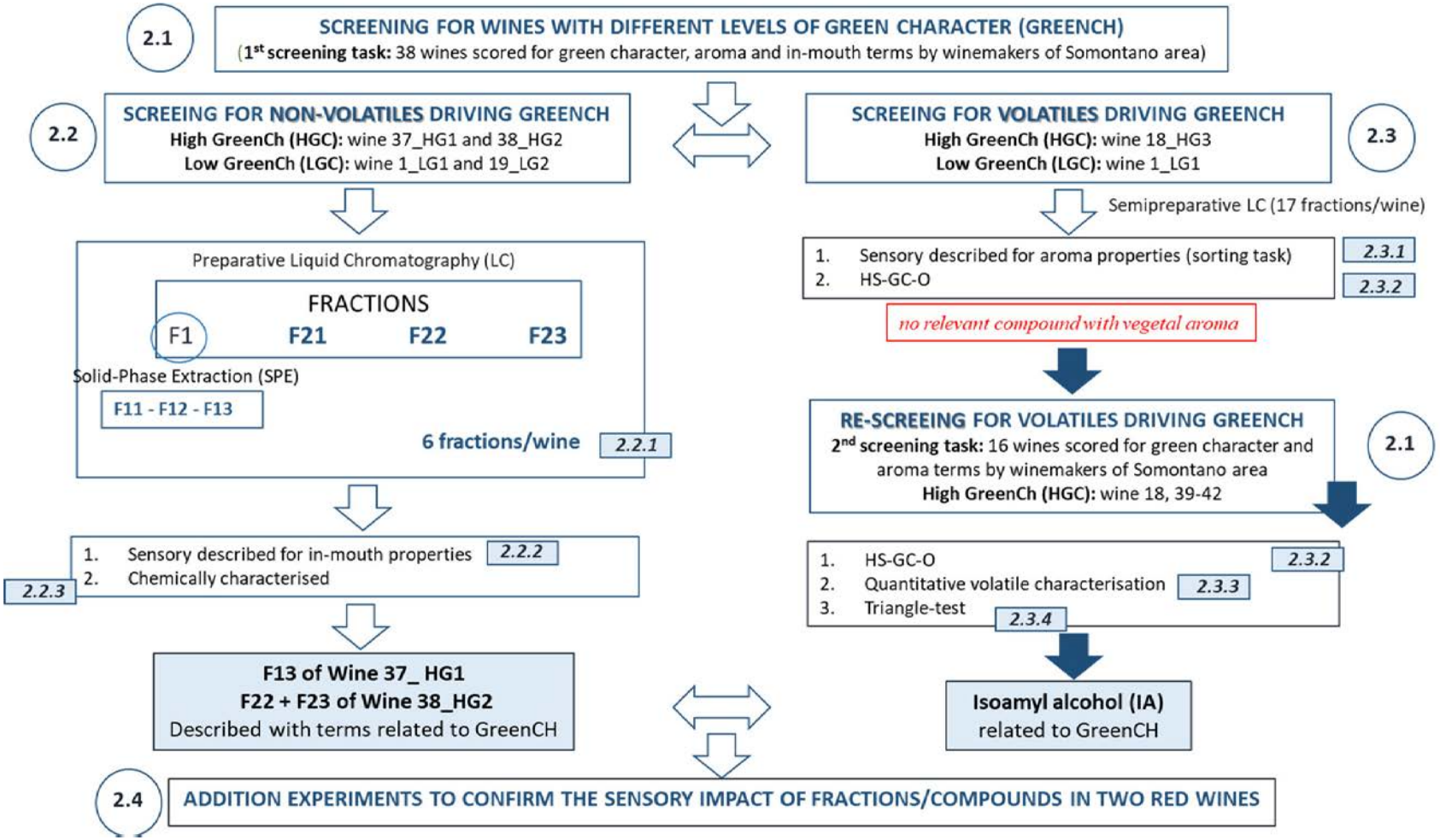
2.1.1. Samples, participants and procedure

A total of 54 red wines were selected in consultation with wine experts. The selection criterion was to have a wide range of samples with different levels of green character according to winemakers from Somontano region. Wines were elaborated mainly in Spain (46), but there were also wines from Italy (3), France (3), Portugal (1) and Chile (1). The alcoholic content ranged from 11.9 to 15.2% (v/v), vintage from 2009 to 2015 and their time in oak barrels from 0 to 24 months. The detailed list of wines is shown in supplementary material 2 and 3.

The panel of experts was composed of fifteen established winemakers of Somontano region (8 women, from 33 to 56 years, median = 45).

Two different tasks were carried out for the screening of wines with green character. The first task (1st screening task) was mainly exploratory. It aimed at globally understanding the term “green character” and linking it to sensory (aroma and/or in-mouth) terms usually employed by wine experts. The second task (2nd screening task) was focused on the screening of aroma compounds driving “green character”.

Figure II-1. 1. Fluxogram which graphically explains the steps followed in the material and methods section.



In the first task, held in year 2015, thirty-eight red wines (Annexe II-1.1) were presented simultaneously. Wine experts were asked to taste each sample from left to right and to score the multidimensional terms: green character and preference. Then, they were presented with the same wines but with different codes and order of presentation. They had to score the intensity of 12 attributes: six aroma (aroma intensity, oxidation, vegetal, fresh fruit, ripe fruit and wood) and six in-mouth (sweet, sour, astringent, oily, green tannins and dry tannins) terms. The intensity was rated on a five-point scale, from 1 (absent) to 5 (very intense). They were free to compare wines before scoring if they wanted. The selection of the intensity scale and attributes was carried out in an independent session in consultation with three experts of the region. These participants were different from those that carried out the description. In this session, they were firstly asked to freely cite terms related to green character. Then, the participants were presented with a global list for all the terms pooled together and a final list was selected by consensus. The scale and terms were those usually employed by the experts in the region and thus more familiar to them.

The second task was held in the year 2016 which involved expanding the space of aroma terms related to green character. It was focused on finding wine exemplars high in green character based exclusively on aroma properties. Therefore, wine experts were presented with 16 red wines (supplementary material 3) simultaneously and were asked to smell each sample from left to right and to score the green character. Then, they were presented with a list of 14 aroma terms (white/yellow fruit, citrus, tropical fruit, red fruit, black fruit, dried fruit, fresh vegetal, cooked vegetables, herbal, reduction, wood, spicy, roasted, animal) compiled from other lists employed in the description of red wines (Noble et al., 1987; Saenz-Navajas, Fernandez-Zurbano, Martin-Lopez, & Ferreira, 2011). Wine experts were asked to rate the intensity of terms that applied exclusively to the particular wine sample on a seven-point scale (1 = not intense; 7= very intense) according to Rate-all-that-Apply (RATA) methodology (Ares et al., 2014; Reinbach,

Giacalone, Ribeiro, Bredie, & Frøst, 2014). Terms that did not apply to the sample were allocated a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomized order for each assessor.

In both tasks (1st and 2nd screening tasks), wines were presented at room temperature, in clear ISO glasses identified only by random three-digit codes. The poured volume per sample was 30 mL. Samples were presented in random order and different for each participant. Mineral water and unsalted crackers were available for palate rinsing. For the first task, participants were asked not to swallow the samples but to expectorate into wine spittoons.

2.1.2. Data analysis

Two-way ANOVAs with assessors as random factor and wine as fixed factor were calculated on the scores of preference, green character, aroma and in-mouth attributes. For significant effects, Fischer post-hoc pairwise comparison (95%) test was performed.

Two principal component analysis (PCA) were calculated with data derived from 1st (38 wines) and 2nd (16 wines) screening tasks and the mean scores (of the 15 winemakers) of the significant in-mouth and/or aroma attributes (active variables) and green character and preference (supplementary variables). All analyses were carried out with XLSTAT (2015 version).

2.2. Screening for non-volatile fractions driving green character

2.2.1. Preparation of wine fractions

Based on the results of the 1st screening task, two wines with high (High Green-HG-: wines 37 and 38) and two with low (Low Green-LG-: wines 1 and 19) green character were selected. These four wines were fractionated. A total of six fractions per wine were obtained by a two-step methodology as described elsewhere (Sáenz-Navajas et al., 2017). Briefly, in the first step, four fractions were collected by a preparative LC method adapted from Remy, Fulcrand, Labarbe, Cheynier, and Moutounet (2000) and Gonzalo-Diago, Dizy, and Fernandez-Zurbano (2013). Therefore, 200 mL of wine were dealcoholized in a rotary evaporator (15 min at 28 ºC). Then, the sample was freeze-dried in 500 mL-rounded flasks. The extract was redissolved in 20 mL of hydroalcoholic solution (12% ethanol, v/v) and the whole volume was injected into a preparative Millipore LC column (gel: Toyopearl HW-50F; dimensions: 120 mm x 22 mm id; flow rate: 4 mL min^{-1}) (Figure II-1.2a). A first fraction (F1) was eluted with 720 mL of ethanol/water/formic acid (55:45:1, v/v/v). The second fraction (F21) was recovered by elution with 80 mL of acetone (100%). The third (F22) and fourth (F23) fractions were eluted with 160 and 80 mL of acetone/water at rates of 80:20 and 60:40, respectively. Solvents present in the four fractions were evaporated under vacuum and samples were further freeze-dried.

In the second step, F1 was redissolved in 200 mL of hydroalcoholic solution (12%, v/v) and further submitted to solid-phase extraction (SPE) using an extraction unit (VAC ELUT 20 Station from Varian) (Figure II-1.2b). SPE cartridges filled with 500 mg of Bond Elut LRC-C18 resins were firstly conditioned by passing 5 mL of methanol followed by 10 mL of an aqueous solution at pH 2.5 (5 g L^{-1} of tartaric acid, pH adjusted to 2.5 with 0.1 M NaOH). This fraction has been described to be especially sour due to the presence of organic acids, which masks other in-mouth attributes such as bitterness or astringency (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014). Thus, sugars and organic acids were removed by loading 5 mL of F1, which was

further washed with 10 mL of aqueous solution at pH 2.5. Then, F11 was eluted with 5 mL of diethyl ether, F12 with 5 mL of ethyl acetate and F13 with 10 mL of methanol. Each cartridge was used a maximum of 5 times. Finally, after F13 was eluted, 10 mL of acetonitrile were used as pre-conditioning solvent before conditioning to remove any impurities on the SPE cartridge. The SPE-procedure was repeated until the 200 mL of F1 were extracted. The extracts were evaporated prior to freeze-drying. The total absence of solvents was assessed by headspace solid phase micro extraction using a 75 um Carboxen/PDMS fiber (75 µm at 30 ºC x 10 min) and GC with a MS detector (overall system detection limit 1 ng/sample).

a) Gel permeation chromatography (GPC).

b) Solid-phase extraction (SPE).

c) Freeze-dried fractions (F11, F12, F13, F21, F22, F23) X 4 wine.

Figure II-1. 2. Steps of the preparation of wine fractions.

The six freeze-dried fractions (F11, F12, F13, F21, F22, F23) were stored at 4 ºC prior to sensory and/or chemical analysis (Figure II-1.2c). Fractions (coming from 200 mL of original wine) were dissolved in 100 mL of hydroalcoholic solution (7% ethanol, v/v; 50 mg L^{-1} of SO_2; 80 mg L^{-1} of ascorbic acid) to have fractions twice concentrated in order to facilitate sensory description. The level of ethanol (7%) was selected in preliminary tests, in which the range from 5% to 11% was evaluated. This level fulfilled two criteria: 1) it did not induce a burning effect able to mask other sensations (they are simple fractions with no aroma, which results in an enhanced burning sensation elicited by ethanol in comparison with real wines) and 2) it was as similar as possible to ethanol content in real wines.

2.2.2. Sensory characterization of non-volatile fractions

2.2.2.1. Samples, participants and procedure

Four original wines (HG1-wine 37, HG2-wine 38, LG1-wine 1 and LG2-wine 19) and 24 fractions were sensorily evaluated: six fractions (F11, F12, F13, F21, F22, F23) x 4 wines (HG1, HG2, LG1, LG2) by sixteen wine experts (11 women and 5 men, ranging from 23 to 62 years of age, average = 37). Participants attended two sessions spread over two different days. Each session was split into two parts (ca. 30 min each), which were separated by a break of 15 min. First session included 16 fractions and second session eight fractions and four wines, respectively. The absence or limited availability of reference materials with defined mouthfeel properties makes Rate-all-that-Apply (RATA) methodology an interesting procedure to describe in-mouth properties by wine experts with no specific training phase as described in Sáenz-Navajas et al. (2017). Therefore, participants were presented with a list of 23 terms (four for taste, 18 for mouthfeel and persistence) developed in previous work (Sáenz-Navajas et al., 2017) (See Section I, Table I-1. 8.).

Participants were asked to taste and rate the intensity of exclusively those terms that applied to the sample on a seven-point scale according to RATA methodology. Participants were instructed to sip the samples via a dark straw (to control the volume they had -2 mL each sip- and to limit carry-over effects) and gently spread out the liquid over the whole mouth cavity. After one minute, they were told to expectorate the sample. The use of a sip (rinsing solutions: water and 1 g L^{-1} pectin solution) and spit protocol between each sample was imposed as described elsewhere (Colonna, Adams, & Noble, 2004). Participants tasted samples in a sequential monadic manner. Ten-mL samples were served in dark ISO-approved wine glasses labelled with 3-digit random codes and covered with plastic Petri dishes according to a random arrangement, different for each participant. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC). Participants were informed that samples were not commercial wines but fractions obtained in the laboratory, in case they accepted to continue in the experiment they signed a consent form. Participants were not paid for their participation.

2.2.2.2. Data analysis

To determine discriminant attributes for the four original wines (HG1, HG2, LG1 and LG2) a two-way ANOVA (panellists as random and wines as fixed factors) was calculated for each of the 23 terms on the list. Pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. Next, principal component analysis (PCA) was carried out on the mean intensity scores of the significant terms and the four wines.

For fractions, two-way ANOVAs (panellists as random and fractions as fixed factors) were performed on the intensity ratings of the 24 fractions. Pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. Then, PCA was carried out on the mean intensity scores of the significant terms and the 24 fractions. A hierarchical cluster analysis (HCA) with the Ward criteria was finally

applied to all PCs. Clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centres. The terms that best characteriszed each cluster were identified by using the test-value parameter (Lebart, Morineau, & Piron, 1995). The test-value corresponds to a statistical criterion akin to a standardized variable (zero mean and unit variance). Significance is obtained when the absolute test-value is ≥1.96, which corresponds to an error threshold of 5%.

All statistical analyses were performed using XLSTAT (2015) and SPAD (version 5.5).

2.2.3. Chemical characteriszation of non-volatile fractions and wines

Chemical analyses were performed in four original wines (HG1-wine 37, HG2-wine 38, LG1-wine 1 and LG2-wine 19) and their corresponding fractions (F11, F12, F13, F21, F22, F23). All samples were analysed in duplicate.

2.2.3.1. Conventional oenological parameters

Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and colour intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). For TPI determination, the abs at 280 nm of samples diluted 1:100 in deionized water was measured in 1-cm-quartz cuvettes. For CI, absorbance of undiluted samples was measured in 2-mm-crystal cuvettes.

2.2.3.2. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) in wines and fractions was carried out as described elsewhere (Harbertson, Picciotto, & Adams, 2003). MPs were the group of compounds bleachable with bisulphite, while SPP and LPP were resistant to bisulphite bleaching. SPP did not precipitate with ovoalbumin, different to LPP. Levels of MP, SPP, and LPP were expressed as absorbance at 520 nm.

2.2.3.3. Thiolysis assay

Acid-catalysed degradation in the presence of toluene-α-thiol was performed according to the method described by Gonzalo-Diago et al. (2013). The mean degree of polymerization (mDP) as well as the percentage of procyanidins (%PC), prodelphinidins (%PD), and galloylation (%G) were calculated as the molar ratio of the total units to terminal units.

2.2.3.4. Characteriszation of tannins

Concentration and activity of tannins were estimated by a HPLC-UV-Vis method following the method proposed by Revelette, Barak, and Kennedy (2014).

2.3. Screening for volatile fractions driving green character

2.3.1. Preparation of volatile fractions and sensory characteriszation

2.3.1.1. Samples, participants and procedure

Based on the results of the 1st screening task, one wine with high (HG3-wine 18) and one (LG1-wine 1) with low vegetal aroma and green character were selected. The aroma extract of both wines was obtained and fractionated by semipreparative reversed-phase liquid chromatography using a water–ethanol gradient system as mobile phase as described by Ferreira, Hernández-Orte, Escudero, López, and Cacho (1999). A total of 17 fractions per wine were obtained. Fifteen of them (the first and last fractions were not considered as they were odourless) together with two duplicates were sensory evaluated by sorting task. Therefore, 16 trained subjects (9 women, ranging from 23 to 70 years of age, average = 38), belonging to the laboratory staff and with large experience in aroma analysis, were asked to orthonasally smell the 32 fractions and sort them on the basis of their similarity attending to their aroma properties. They could make as many groups as they wished. Upon completion, they participants recorded the codes of the samples of each group on a paper sheet and described each group with a maximum of three attributes.

One mL of each fraction was presented in 6-mL flasks covered with aluminium foil and labelled with 3-digit random codes according to a random arrangement, different for each participant. Fractions were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC).

2.3.1.2. Data Analysis

For each participant, results were encoded in an individual similarity matrix (fraction x fraction), in which 1 stands for two wines set in the same group and 0 for two wines put in different groups. These individual matrices were summed across subjects; the resulting co-occurrence matrix represents the global similarity matrix

where larger numbers indicate higher similarity between samples. The underlying assumption for this method is that samples plotted together are more similar than samples plotted far away. The resulting co-occurrence matrix was submitted to an MDS analysis in order to derive a spatial representation of fractions. All MDS dimensions were submitted to Hierarchical Cluster Analysis (HCA). All analyses were performed with XLSTAT (2015 version)

2.3.2. Head Space-Gas Chromatography-Olfactometry (HS-GC-O)

2.3.2.1. Samples, participants and procedure

Fractions (F9, F12 and F13) of wines 1-LG1 and 18-HG3, which were scored low and high for green character and vegetal aroma, respectively, were submitted to HS-GC-O. The complete aroma extracts of wines 1 and 19 (low scores for green character) and wines 18, 39-42 (high scores for green character) were also screened by HS-GC-O.

The dichloromethane/methanol extract to be used for GC-O was obtained in a purge and trap system and then screened by GC-O system equipped with a flame ionization detector (FID) and a sniffing port ODO-I from SGE (Ringwood, Australia), connected by a flow splitter to the column exit as described elsewhere (Escudero, San-Juan, Franco-Luesma, Cacho, & Ferreira, 2014).

Sniffing was carried out by a panel composed of six trained subjects, four women and two men belonging to the laboratory staff. Each participant evaluated the sample extract once in two time segments of 30 min to avoid fatigue (one session per day). They were asked to evaluate the time, description and the odour intensity of each aromatic sensation. An intensity scale of 0–3 was used (0 = no odour, 1 = weak odour, low intensity, 2 = clear perception of odour, strong intensity, 3 = extremely strong intensity of odour; intermediate values of 0.5, 1.5 and 2.5 were allowed).

2.3.2.2. Data Analysis

The data processed from GC-O were a mixture of intensity and frequency of detection (labelled as "% modified frequency", %MF), which was calculated with the formula proposed by Dravnieks (1985):

$$\% MF = \sqrt{(\%F \times \%I)}$$

where %F is the detection frequency of an aromatic stimulus expressed as a percentage and %I is the average intensity expressed as percentage of the maximum intensity. The identification of the odorants was carried out by comparison of their odours, chromatographic retention index in both DB-Wax and DB-5 columns, and MS spectra with those of pure reference compounds.

2.3.3. Quantitative analysis of fusel alcohols

Fusel alcohols were quantified in wines 1-LG1 and 19-LG2 (low green character) and wines 18-HG3 and 39-42 (high green character based exclusively on aroma perception). Therefore, major volatile compounds were isolated by liquid-liquid extraction and analysed in a gas chromatograph with flame ionization detector following the method described by (Ortega, Lopez, Cacho, & Ferreira, 2001). Analytes were referred to a selected internal standard, and response factor was the selected method for calibration.

2.3.4. Sensory analysis

Based on the results derived from quantitative studies, the sensory importance of methionol (7.3 mg L^{-1}) and isoamyl alcohol (454 mg L^{-1}) was checked in two different wines with different sensory profile by addition experiments. The wines were one young red wine (YW-Tempranillo 2016; 12.5% ethanol v/v) representative of a neutral red wine in terms of aroma and in-mouth properties (taste and mouthfeel) and an oaked red wine (OW-Tempranillo 2011; 13.5% ethanol v/v) representative of an aged wine with oak, oxidation and ripe fruit aromas and no outstanding taste or mouthfeel properties. Both, isoamyl alcohol and methionol, were firstly purified as described by De-La-Fuente-Blanco, Sáenz-Navajas, and Ferreira (2016) as commercial standards always contain traces of their corresponding aldehydes, which have a very high odorant power. A first set of triangle tests was carried out to evaluate the individual sensory impact of methionol and isoamyl alcohol and in a second set, their sensory interaction was evaluated. Thereafter, a trained sensory panel composed of 20 subjects (11 women, ranging from 24 to 70 years of age) performed tests in duplicate. Samples presented in the triangle tests were the red wine (YW or OW) and the same sample spiked with methionol, isoamyl alcohol or methionol + isoamyl alcohol. Three glasses were presented to each participant and they were asked to select the different sample based exclusively on orthonasal aroma properties. The number of correct answers was compared with tabulated values to evaluate the presence of significant sensory differences between original and spiked samples. In all tests, samples (10 mL, 20 ºC) were presented in random order in coded black tulip shaped wine glasses covered with a Petri dish.

2.4. Evaluation of the sensory impact of fractions and compounds on green character

2.4.1. Samples, participants and procedure

Based on screening steps, the sensory impact of two non-volatile fractions and isoamyl alcohol on the green character was evaluated in two very different wines in terms of sensory properties. These wines were the same young (YW) and oaked red (OW) wines used in the triangle tests (See 2.3.4). Table II-1.1 shows the 16 wines evaluated attending to a full factorial design with three variables (isoamyl alcohol-IA-, F13_HG1 and F22_HG2+F23_HG2) at two levels (not spiked and spiked) in the two different wines. Considering that non-volatile fractions F22_HG2 and F23_HG2 contain similar compounds, basically proanthocyanidins (Sáenz-Navajas et al., 2017), and with the aim of reducing the number of variables, both fractions were pooled and added together in wines (F22+F23_HG2 or F2_HG2).

Table II-1. 1. Samples evaluated in the confirmation task aimed at evaluated the impact of isoamyl alcohol (IA), fractions F22 +F23 of wine HG2 (F2) and/or fraction F13 of wine HG1 (F13) in the green character of two different wines (YW: young and OW: oaked red wines). Y_base and O_base, were not spiked ("0" means not spiked). The rest of samples were spiked ("1" means spiked with the corresponding compound or fraction) with IA, F2 and/or F13.

Spiked wine	Sample	Code	IA (isoamyl alcohol)	F2 (F22_HG2 + F23_HG2)	F13 (F13_HG1)
Young red wine (YW)	1	Y_base	0	0	0
	2	Y_IA	1	0	0
	3	Y_IA_F2	1	1	0
	4	Y_IA_F2_F13	1	1	1
	5	Y_F2	0	1	0
	6	Y_F2_F13	0	1	1
	7	Y_IA_F13	1	0	1
	8	Y_F13	0	0	1
Oaked red wine (OW)	9	O_base	0	0	0
	10	O_IA	1	0	0
	11	O_IA_F2	1	1	0
	12	O_IA_F2_F13	1	1	1
	13	O_F2	0	1	0
	14	O_F2_F13	0	1	1
	15	O_IA_F13	1	0	1
	16	O_F13	0	0	1

Fourteen established winemakers of Somontano region (7 women, from 33 to 56 years, median = 45) carried out the experiment. Participants completed two sessions separated by a break of 30 min. They were presented with the 16 samples (Table II-1.1) simultaneously and asked to taste each sample from left to right. In the first session they had to score the green character of wines on a seven-point scale (1=not intense and 7 = very intense). In the second session participants were asked to describe each sample in for aroma and in-mouth properties by using a list of 9 aroma, 3 taste and 19 mouthfeel terms. These last terms were developed in a previous work as in 2.2.2.1. They were asked to rate the intensity of exclusively those terms that applied to the sample on a seven-point scale according to RATA methodology.

Wines were presented at room temperature, in dark ISO glasses identified only by random three-digit codes (Figure II-1. 3). The poured volume per sample was 10 mL. Samples were presented in random order and different for each participant. Mineral water and unsalted crackers were available for palate rinsing. Participants were asked not to swallow the samples but to expectorate into wine spittoons.

Figure II-1. 3. Sensory evaluation of the impact of fractions carried out in Somontano in 2017.

2.4.2. Data analysis

Two-way ANOVA with assessors as random factor and type of wine (young or oaked red wine) as fixed factor was calculated on the scores of green character to evaluate the effect of wine matrix on green character.

Then, one two-way ANOVA for each wine matrix (young or oaked red wine) was calculated with the scores of green character and aroma and in-mouth attributes (assessors as random factor and wine as fix factor). For significant effects, Fischer post-hoc pairwise comparison (95%) test was performed.

Finally, two principal component analysis (PCA) were calculated (one for each type of wine) with the mean scores (of the 14 winemakers) of the significant in-mouth and/or aroma attributes (active variables) and green character (supplementary variable). All analyses were carried out with XLSTAT (2015 version).

3. Results and discussion

3.1. Screening for wines with different levels of green character

Wine experts were first asked (1st screening task) to score the green character, the preference and 12 additional sensory terms, usually used by experts, referring to aroma and in-mouth properties of the 38 wines included in the first part of the study. All terms were significant and in fact, the factor wine was found to be significant for all of them at $P<0.001$ in all cases except for sourness, for which it was significant at $P<0.05$. The projection of the terms on the two first dimensions of the PCA is shown in Figure II-1. 4.

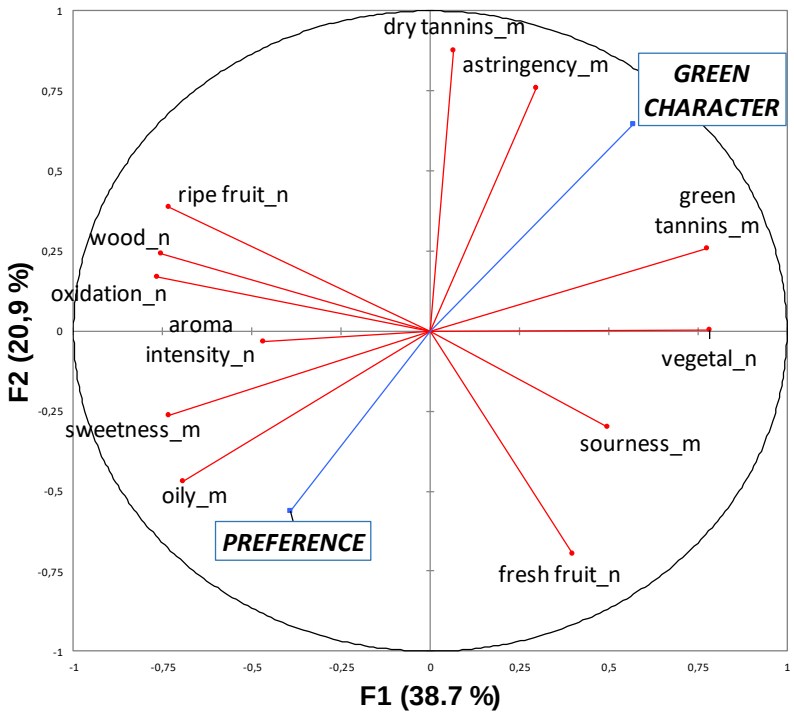


Figure II-1. 4. Projection of active (in red) and supplementary –or illustrative- (in blue) variables on the two first principal components of the PCA calculated with significant attributes scored for the 38 wines evaluated in the 1st screening session (attribute_n: evaluated in nose-orthonasally; attribute_m: evaluated in mouth).

The illustrative variables, green character and preference, are negatively correlated ($r = -0.70$; $P<0.001$) between themselves, which confirms that the multidimensional descriptor green character has a negative valence for wine experts in the region. The green character term seems to be highly multidimensional and it was correlated to both aroma and in-mouth attributes. Green character was positively correlated to the aroma term vegetal and to the in-mouth attributes astringency, green and dry tannins and, and negatively correlated to the aroma term woody and to the in-mouth sensations oily and sweet.

Cluster analysis yielded four groups of wines (Figure II-1. 5) with different aroma and/or in-mouth (taste and mouthfeel) properties. As seen in Table II-1. 2, the first cluster is mainly characterised by the aroma terms oxidation, woody and ripe fruit and by the in-mouth terms sweet and oily, and it is negatively correlated to green character ($P<0.01$). In contrast, cluster 4 which is mainly described with vegetal aroma, astringency and green tannins, is positively correlated to the green character ($P<0.001$) and negatively to preference ($P<0.01$). Clusters 2 and 3 do not show significant correlations with green character. Cluster 2 is mainly defined by terms such as dry tannins and astringency as well as ripe fruit, woody and high aroma intensity. Cluster 3 has mainly fresh fruity aroma and sourness, and is weakly ($P<0.1$) and positively correlated to preference.

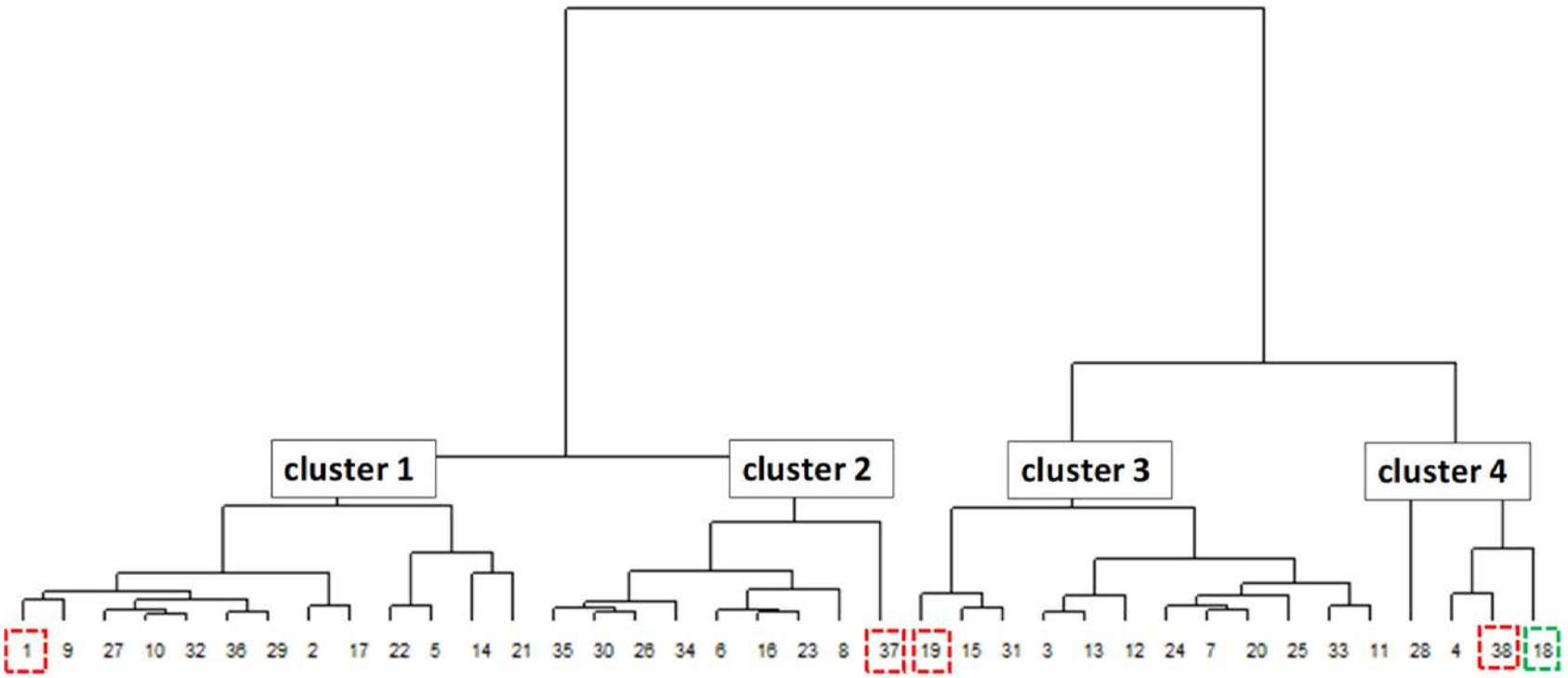


Figure II-1. 5. Four clusters derived from Hierarchical cluster analysis (HCA) calculated with all the PCs obtained from the PCA (performed with significant attributes) derived from the first screening session devoted to select wines with different levels of green character. Wines marked in red (1, 19, 37, 38) were selected for the screening of non-volatile compounds driving different levels of green character and wine 18 for the screening of volatile compounds.

Table II-1. 2. Clusters in which wines were classified by HCA. Descriptors significantly (P<0.05) defining each cluster and correlation to the illustrative variables green character and preference. Wines marked with an asterisk are those closest to the center of gravity of the cluster and were further selected for the study of the non-volatile fraction.

CLUSTER		Attributes positively correlated	Positive correlation with green character and/or preference	Negative correlation with green character and/or preference
1	**1***, 27, 32, 9, 22, 10, 36, 29, 17, 5, 14, 2, 21	oxidation_n, sweetness_m, oily_m, wood_n, ripe fruit_n		Green character (t-value=-2.98; P<0.01)
2	**37***, 23, 30, 6, 16, 35, 26, 34, 8	dry tannins_m, astringency_m, ripe fruit_n, wood_n, aroma intensity_n		
3	**19***, 24, 3, 7, 20, 15, 13, 11, 33, 25, 12, 31	fresh fruit_n, sourness_m	Preference (t-value=+1.38; P<0.1)	
4	**38***, 4, 18, 28	green tannins_m, vegetal_n, astringency_m	Green character (t-value=+3.93; P<0.001)	Preference (t-value=-2.58; P<0.01)

Based on these results, one wine from each cluster was selected (wines 1, 19, 37 and 38) to be screened for non-volatile compounds involved in green character. The selected samples represent the global sensory space of the studied wines and present significant different scores for the green character. As shown in Figure II-1. 6, samples 37 and 38 have, together with sample 18, the highest scores for this attribute, while 1 and 19 the lowest. Sample 37 is representative for the term dry tannins and wine 38 for green tannins.

Regarding aroma terms, wines 1 and 18 were selected for the screening of volatile compounds (semipreparative LC, GC-O and quantitative analysis) involved in green character as they presented the lowest and highest scores for vegetal aroma, respectively, as can be seen in Figure II-1. 6. Besides, in order to expand the space of aroma terms related to the green character, 15 additional wine samples plus sample 18 from the previous task (supplementary material 3) were evaluated for their aroma properties (orthonasal perception only) in an independent second session (2nd screening task). Results confirmed again that the green character is positively correlated ($P<0.001$) to fresh vegetal aroma ($r = 0.88$). Wines 39-42 and 18, with the highest scores for green character (Figure II-1. 7), were further submitted to GC-O and quantitative analysis of volatiles to screen for aroma compounds potentially involved in green character and compared to the composition of non-green wines.

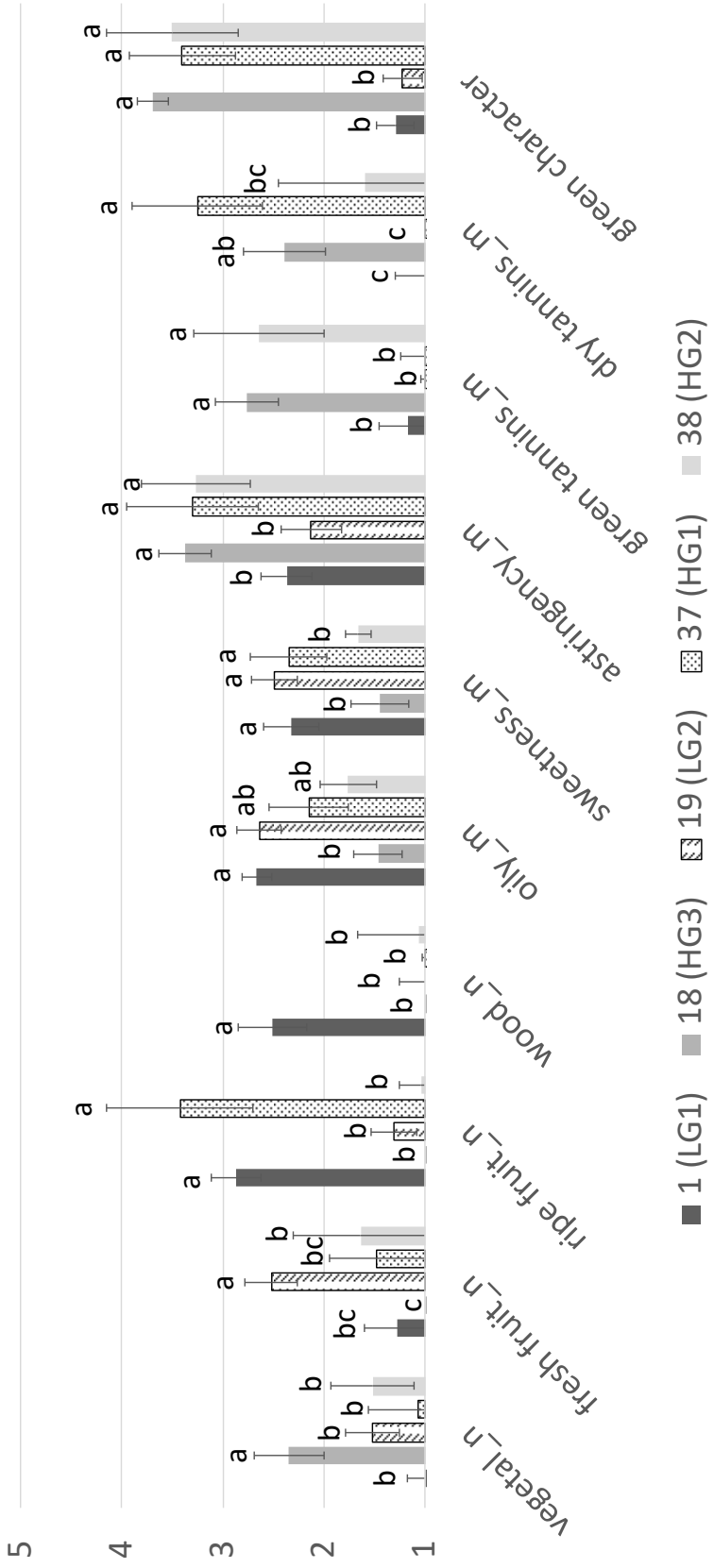


Figure II-1. 6. Mean scores of attributes significantly (P<0.05) discriminating among 5 wines with high (HG1-HG3) and low (LG1 and LG2) green character scores. Error bars are calculated as s/(n)1/2(s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).

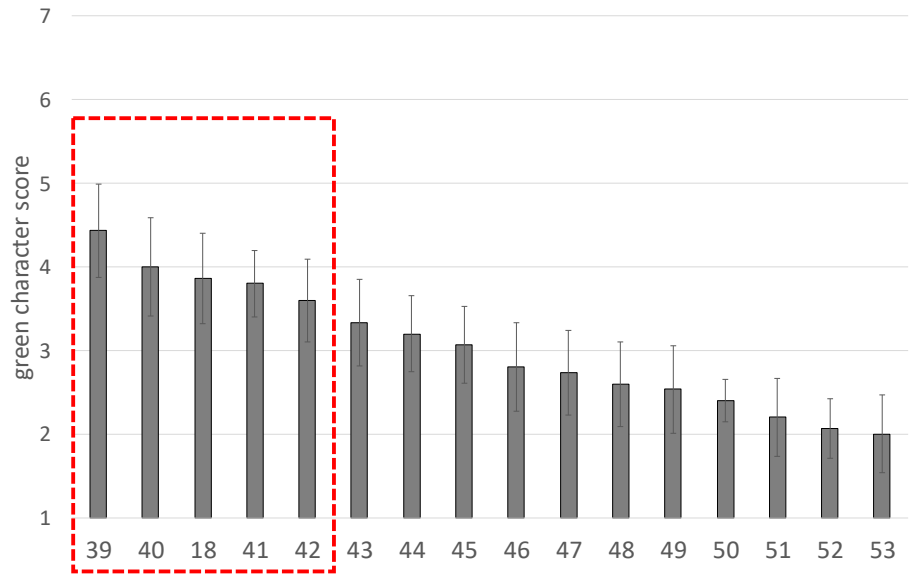


Figure II-1. 7. Green character scores for the 16 wines based on orthonasal aroma in the 2nd screening session. Error bars are calculated as s/(n)1/2(s, standard deviation; n, number of panellists).

3.2. Screening for non-volatile compounds driving green character

3.2.1. Sensory characterisation of fractions

The four wines selected for their different levels of green character (hereinafter coded as 1_LG1, 19_LG2, 37_HG1 and 38_HG2) were sensory described by RATA methodology, using a predefined list of terms related to in-mouth attributes (4 tastes, 18 mouth-feel-related terms and persistence) which was developed in a recent work (Sáenz-Navajas et al., 2017). Significant differences ($P<0.05$) were found in 4 out of the 23 in-mouth attributes: sticky, prickle, dry and dry-on-the tongue side. As shown in Figure II-1. 8, samples with high green character scores (37_HG1 and 38_HG2) had the highest scores for the terms dry and sticky, with 38_HG2 the highest score for dry on the tongue side.

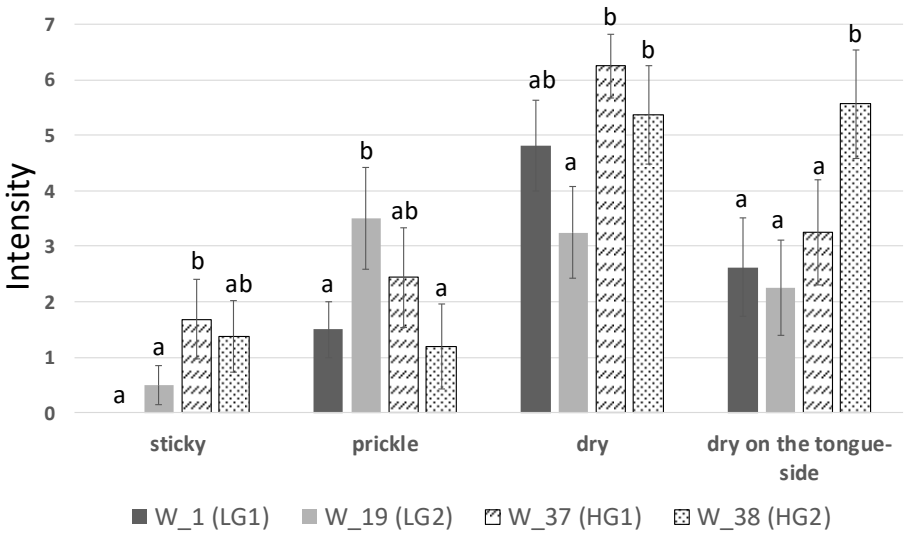


Figure II-1. 8. Mean scores of in-mouth attributes evaluated by RATA and significantly (P<0.05) differing among the 4 selected wines (LG1, LG2, HG1 and HG2). Error bars are calculated as s/(n)1/2(s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).

These four wines were further fractionated by a liquid chromatography procedure to obtain 6 fractions per wine (coded F11, F12, F13, F21, F22, F23). The 24 fractions were sensorily described using the previous methodology. In this case, 14 out of the 23 attributes differed significantly (P<0.05) among fractions. The higher discriminant power of the descriptors must be attributed to the higher precision attainable in the sensory assessment of simplified non-volatile fractions. Results are summarised in Figure II-1. 9, which shows the PCA calculated with the significant attributes and the 24 fractions classified into six clusters derived from cluster analysis. As can be seen in Figure II-1.9.a, the first PC retains nearly 50% of the original variance, and confronts the terms dry, dry on the palate, dry on the tongue side, persistent, coarse, to silky, watery, sweet and greasy. The second PC, explaining almost 15% of the original variance, is mainly negatively correlated to the term sticky, and positively to burning, sour and bitter tastes. Figure II-1.9.b, shows that clusters 1 and 2, which are formed by fractions F22 and F23 of wine 38_HG2 and F13 of wines 1_LG1, 19_LG2 and 38_HG2, respectively, are especially dry, dry on the

palate, dry on the tongue side, persistent and coarse. Conversely, cluster 6 is mainly watery, silky, sweet and greasy. Fractions F11 (cluster 4), mainly sour, F12 (cluster 3), especially bitter, and the fractions within cluster 5, sticky and coarse, are in the centre of the plot. Interestingly, equivalent fractions from the four wines are plotted very close together, indicating that they present similar sensory properties. The exceptions are F22_HG2 and F23_HG2, which are significantly coarser (only for F22_HG2), drier, drier on the palate and more persistent than the equivalent fractions from the other three wines, as well as F13_HG1, which is significantly stickier than the equivalent fractions from the other wines (Annexe II-1.2).

These results strongly suggest that compounds in fractions F22_HG2 and F23_HG2 should be responsible for the high green character of wine 38_HG2, related to astringency and green tannins (Figure II-1. 6), as well as to dry and dry on the tongue-side (Figure II-1. 8). Similarly, compounds eluting in F13_HG1 are suggested to be the drivers of the green character in wine 37_HG1, which is linked to astringency and dry tannins (Figure II-1. 6) and dryness (Figure II-1. 8).

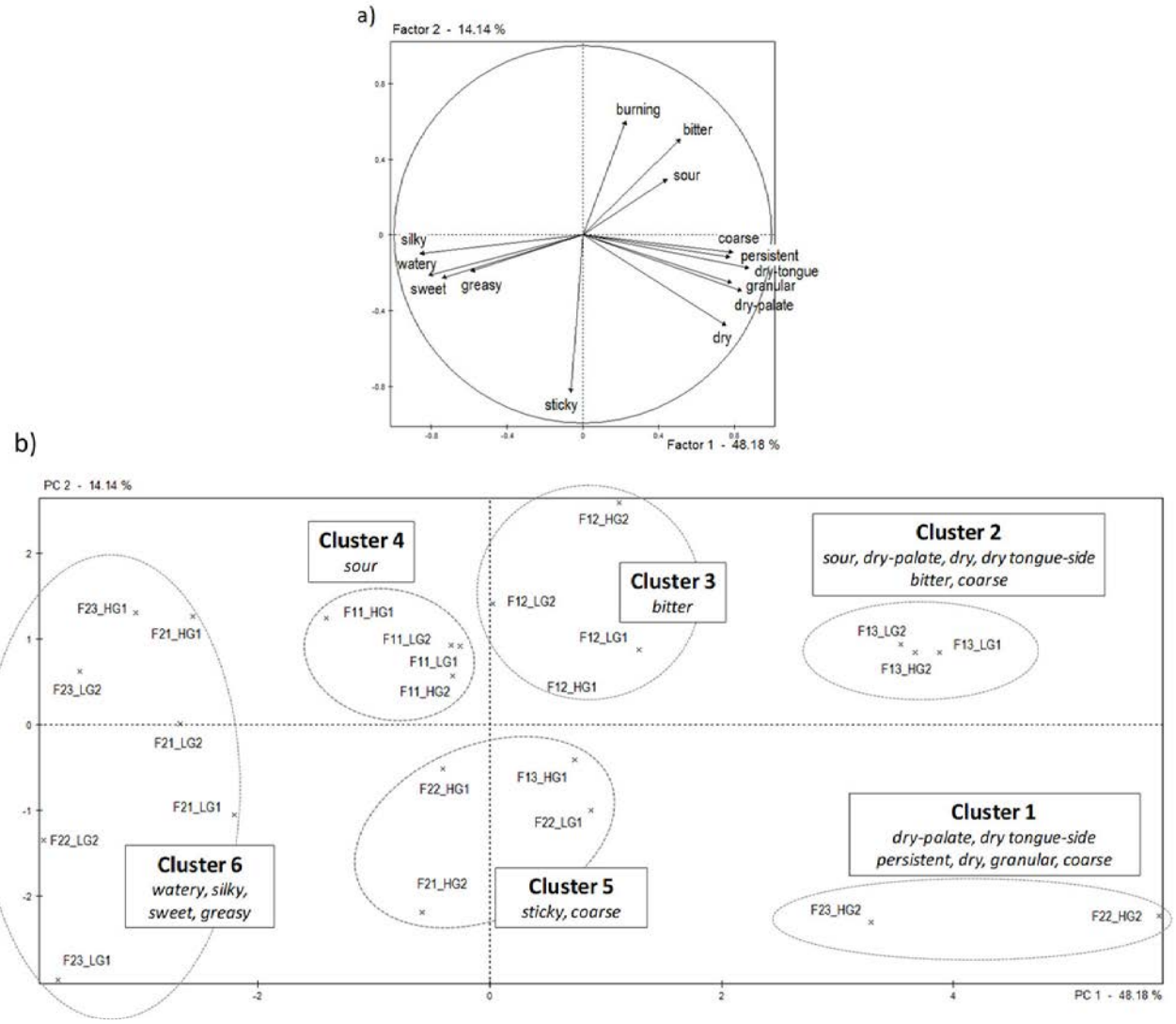


Figure II-1. 9. Projection on the first two principal components of the PCA of a) significant attributes and b) 24 fractions (6 fractions x 4 wines) and the six clusters and significant attributes yielded from the HCA calculated with all dimensions of the PCA.

3.2.2. Chemical characterisation of fractions driving green character

Fractions F13 retain a large proportion of the original colour intensity (at least 66% of CI is retained in F13 in the four wines) and also a large proportion of the phenolic compounds measured by absorbance at 280 nm (at least 50% of TPI is retained in F13 in the four wines) (Annexe II-1.3), in agreement with Section I. Table II-1.3 shows that fraction F13 contains mainly anthocyanin-derivative pigments, including a major part of the monomers bleachable with sulphite (at least 87% of MP are retained in F13 in the four wines), and pigments resistant to sulphite and not precipitable with ovoalbumin (at least 77% of SPP are retained in F13 in the four wines), as well as pigments resistant to sulphite and precipitable with ovoalbumin (between 43% for 38_HG2 and 77% for 19_LG2 of LPP are retained in F13). The mean degree of polymerisation (mDP) of these fractions is below 4 in all cases, which is in accordance with previous works performed with this fractionation method (Gonzalo-Diago et al., 2014; Sáenz-Navajas et al., 2017). This means that F13 is free of tannins and contains small flavanols (dimers and trimers of flavanols) mainly composed of catechin or epicatechin units (%PC up to 68%) (Table II-1.3). Interestingly, F13_HG1, with the highest stickiness, contains the highest levels of anthocyanin-derived pigments resistant to sulphite and able to precipitate proteins (LPP), as shown in Table II-1.3. This is in accordance with previous results suggesting that certain trimers of anthocyanins able to react with proteins could be responsible for certain astringent-related attributes perceived in red wines (Sáenz-Navajas et al., 2017).

Concerning fractions F22+F23, they contain tannins with degrees of polymerisation up to 15 units as well as up to 35% of total LPP in F22+F23_HG2 (Table II-1.3). The tannin concentration in these fractions is given in Table II-1.3, and as can be seen, the highest level of dry and dry on the tongue-side attributes of the fraction F22+F23_HG2 could then be related to its highest concentration of tannins with a mean degree of polymerisation of 10. However, these astringent-related attributes

could not be related either to the activity of tannins as determined as “stickiness” after Revelette et al (2014) or to the level of galloylated units (Table II-1.3) as suggested by other authors (Ferrer-Gallego, García-Marino, Miguel Hernández-Hierro, Rivas-Gonzalo, & Teresa Escribano-Bailón, 2010; Revelette et al., 2014; Soares, Mateus, & De Freitas, 2007).

Table II-1. 3. Mean degree of polymerization (mDP), percentage of flavanols constituted by procyanidins (%PC), prodelphinidins (%PD), gallates (%G) and linked to malvidin (%Mv) calculated by thyolisis. Monomeric anthocyanins (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) analysed as proposed by Harbertson et al. (2003) and expressed in absorbance units. Tannin activity and tannin concentration determined as described by Revelette et al (2014). Different letters for a parameter and the same fraction (F13 or F22+F23) means significant differences (P<0.05) among wine fractions.

Fraction	mDP	%PC	%PD	%PG	%Mv	MP	SPP	LPP	tannin activity (J mol^{-1})	tannin concentration (mg L^{-1})
F13_LG1	3.1±0.0^{a}	75±1^{b}	14±1	2±0	9±0^{b}	0.47±0.03^{c}	0.47±0.02^{b}	0.10±0.03^{bc}	<LD	<LD
F13_LG2	1.7±0.0^{b}	69±0^{b}	14±0	2±0	15±0^{a}	1.22±0.05^{a}	0.57±0.01^{a}	0.08±0.01^{c}	<LD	<LD
F13_HG1	2.8±0.0^{a}	93±0^{a}	12±1	4±0	8±0^{b}	0.68±0.04^{b}	0.60±0.04^{a}	0.25±0.04^{a}	<LD	<LD
F13_HG2	2.6±0.0^{a}	76±0^{b}	13±0	4±0	7±0^{b}	0.61±0.02^{b}	0.60±0.01^{a}	0.12±0.02^{b}	<LD	<LD
F22+F23_LG1	15±1^{a}	71±1^{b}	23±1^{a}	5±0^{a}	1±0^{a}	<LD^{b}	<LD^{b}	<LD^{b}	24526±825^{b}	384±7^{c}
F22+F23_LG2	<LD^{c}	0±0^{b}	nd	nd^{b}	nd^{b}	<LD^{b}	<LD^{b}	<LD^{b}	29499±754^{a}	117±16^{d}
F22+F23_HG1	10±0^{b}	78±1^{a}	15±1^{b}	6±0^{a}	1±0^{a}	<LD^{b}	<LD^{b}	0.08±0.01^{a}	13498±460^{d}	584±26^{b}
F22+F23_HG2	10±0^{b}	70±1^{b}	25±1^{a}	5±0^{a}	1±0^{a}	0.06±0.00^{a}	0.07±0.01^{a}	0.09±0.00^{a}	15732±582^{c}	919±13^{a}

3.3. Screening for volatile compounds driving green character

Cluster analysis (Annexe II-1.4) showed that equivalent aroma fractions from wines 1 (low green score) and 18 (high green score and the highest for vegetal aroma) are plotted very close together, indicating that they present similar aroma properties. The exception is F9, which for wine 1 is mainly sweet and floral, while alcoholic and solvent-like for wine 18. However, the GC-O analysis of these fractions did not show any difference in the aroma zones of both fractions and no relevant compound with vegetal aroma in the green wine (wine 18).

Most surprisingly, the GC-O screening of the samples (wines 18, 39-42) showing vegetal notes did not reveal the presence of any outstanding differences in the aroma profiles of non-green wines (wines 1 and 19) (data not shown). A remarkable difference was, however, that green wines seemed to have smaller GC-O scores in fruity esters and slightly higher scores in fusel alcohols. Significantly higher levels of fusel alcohols, was corroborated by quantitative analysis, as summarized in Table II-1.4. As seen in the table, 1-hexanol in green wines is significantly above the levels found in non-green wines (1 and 19) and in a high-quality reference sample set, but it is anyway well below its odour threshold. Levels of z-3-hexenol, with a clear green-leafy character, in green wines were however, within the range of occurrence observed for the non-green wines 1 and 19 and the reference sample set and also below the sensory threshold. Most differently, isoamyl alcohol, methionol and β-phenylethanol were at significantly higher concentrations ($P<0.001$) in green wines. On the bases of previous research, it can be concluded that those levels of β-phenylethanol are not sensorily significant (De-La-Fuente-Blanco et al., 2016). Thus, the potential sensory role played by methionol and isoamyl alcohol was further tested by simple addition experiments with highly purified standards. In the case of methionol, the standard contained some other non-identified odour impurities which were removed by semipreparative HPLC. As previously found for β-phenylethanol, the reported odour threshold for methionol resulted to be strongly overestimated and the odour threshold for the completely pure standard was found

to be around 9.5 mg L^{-1}, nearly 20 times above the previously reported threshold. Addition experiments at the 7.3 mg L^{-1} maxima levels found in green wines in two different matrices resembling one young and one oaked red wine, did not have any sensory effect, which makes us conclude that methionol levels, per se, are not directly involved in the green character of wines.

Quite differently, the addition of purified isoamyl alcohol to both wine models, bringing their levels to the maxima concentrations found in green wines (454 mg L^{-1}, Table II-1.4) was significantly detected by the test panel ($P<0.01$). Confirming previous results (De-La-Fuente-Blanco, Sáenz-Navajas, & Ferreira, 2017) some individuals resulted to be extremely sensitive to the addition of this chemical and reported it to add a pungent and metallic note to the wine. Furthermore, some of them described it as geranium-like via orthonasal and as harsh and astringent in the mouth, which made us wonder whether this compound could be involved in the formation of green character in red wines.

Table II-1. 4. Concentration, concentration ranges and odour thresholds of fusel alcohols in non-green wines (wine 1 and 19), green wines (wine 18 and 39-42; n=5) and the reference sample set (n=25). For a given compound, different letters mean significant differences ($P<0.001$) among wines.

	concentration ranges (µg L^{-1})				
compound	non-green wine (wine 1)	non-green wine (wine 19)	green wines (n=5)	reference sample set* (n=25)	odour threshold**
isobutanol	42120	45720	33800-43060	21400–61400	40000 [1]
1-butanol	1230	960	892-1294	530–960	150000 [2]
isoamyl alcohol	285270[b]	280550[b]	358220-454190[a]	111000–305000[b]	30000 [1]
1-hexanol	1940[c]	1380[b]	2550-2960[a]	520–1560[b]	8000 [1]
z-3-hexenol	190	90	40-170	<4.47–290	400 [1]
methionol	2560[b]	2900[b]	3870-7310[a]	88.6–1348[c]	500 [1]
benzyl alcohol	1760	4620	390-1360	70–5400	200000 [4]
β-phenylethanol	44850[b]	34850[b]	51610-98150[a]	18700–80500[b]	14000 [3]

*Reference sample set of Spanish Premium red wines (n=25) referred to San-Juan et al. (2012). **Odour thresholds calculated in red wine if available; otherwise threshold in synthetic wine is given. Concentrations are expressed in micrograms per litre. Reference in which the odour threshold value has been calculated is given as superscript. [1]Guth (1997); [2]Etievant (2000); [3]Ferreira et al (2000); [4]Escudero et al (2007)

3.4. Sensory impact of isoamyl alcohol and astringent fractions on green character

Attending to previous results, the compounds present in fractions F22_HG2 (fraction F22 of Wine 38), F23_HG2 (fraction F23 of Wine 38) and F13_HG1 (fraction F13 of Wine 37) are suggested to be involved in green character; isoamyl alcohol could also play some role, not only in the aroma perception, but in the general green character perception. Besides, compounds responsible for woody, ripe fruit and oxidation aroma could also counteract the green perception (Figure II-1. 4). This hypothesis is well in line with the fact that for winemakers in general, and also in some scientific papers, it is generally accepted that problems related to green character are solved or masked by ageing wines with oak (Llaudy, Canals, González-Manzano, Canals, Santo-Buelga, & Zamora, 2006) even if there is no sound scientific evidence for this fact. The present experiment could provide a clue about these assumptions. In order to check these hypotheses, two wine models with different aroma profiles were selected: one young red wine (YW) with fruity aromas, and one oaked aged red wine (OW) described with oaked, ripe fruit and oxidation aromas. These wines were spiked with the fractions F22_HG2/F23_HG2, F13_HG1 and/or with isoamyl alcohol following a factorial design and were further sensory evaluated for their green character by the panel of experts of Somontano region.

Results showed that young wines had significantly ($F=11.8$; $P<0.001$) higher scores (average = 4.9±1.6) for green character than oaked wines (average = 4.0±1.6). This result confirms that the green character is wine dependent and suggests that the oaked, ripe fruit and oxidation aromas of the oaked wine could mask green character.

Interestingly, Figure II-1. 10, shows that the single addition of isoamyl alcohol, proanthocyanidins (F22+F23) or anthocyanin-derivative compounds (F13) to a young or oaked aged red wines (base samples) does not generate a significant increase ($P>0.1$) of the green character. Results indicate that in the young wine model, only

the joint addition of both phenolic fractions (F22+F23 and F13) or the joint addition of F22+F23 and isoamyl alcohol generates a significant increase ($P<0.05$) on the green character of the wine. The maximum green character is observed in the sample containing the three elements (F13, F22+F23 and isoamyl alcohol). In the oaky wine model, all effects are less evident, since the green character is comparatively much reduced. Results clearly demonstrate that there is a significant increase in green character only in the samples containing isoamyl alcohol and one of the phenolic fractions (IA-F13 or IA-F22+F23). Maximum green character is observed in the sample containing the three elements simultaneously.

The panel of experts carried out additionally a quantitative description of the wine models. Results (data not shown) confirmed that the green character of the reconstituted wine models was significantly correlated to mouthfeel sensations such as sticky ($r=0.66$; $P<0.05$) in the young wine model and to green tannins ($r=0.71$; $P<0.01$) in the oaked red wine model. No significant correlation to any aroma term was found, which suggests that aromatic characteristics associated to the green character are due to additional aroma molecules not included in our models.

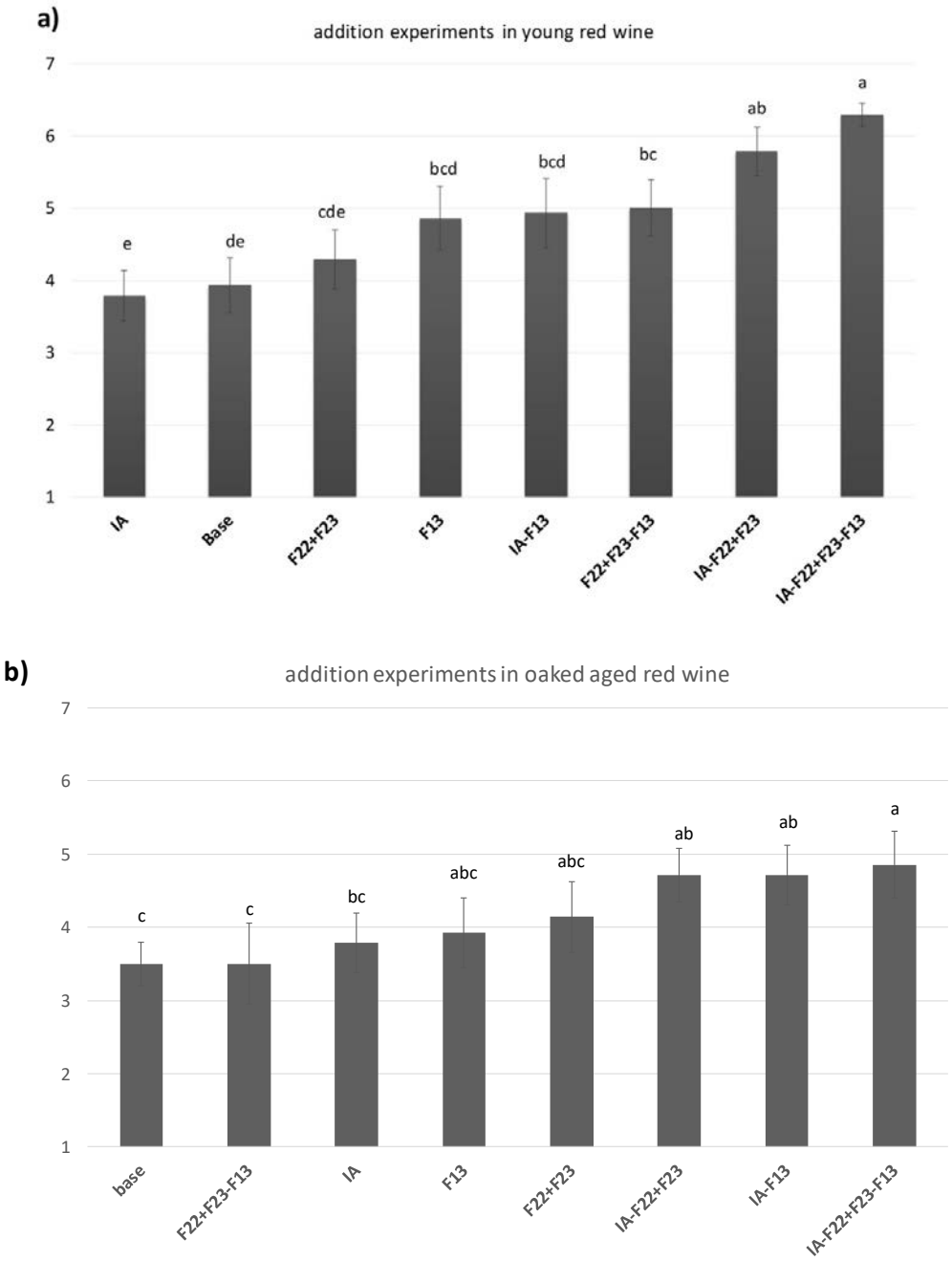


Figure II-1. 10. Green character scores (average of individual scores given by the panel of experts) for a) young red and b) oaked red wines spiked with two phenolic fractions (F13 and/or F22+F23) and/or isoamyl alcohol. Error bars are calculated as s/(n)1/2(s, standard deviation; n, number of panellists). Different letters indicate the existence of a significant difference between samples (P< 0.05) (Fischer posthoc test).

4. Conclusions

The green character is a multivariate character associated to both aroma and mouthfeel descriptors such as vegetal, astringency, green and dry tannins.

Although a direct link between chemicals and sensory perception has not been yet established, wine fractions containing small anthocyanin derivative pigments (<tetramers) and tannins with higher degree of polymerisation (average of decamers) seem to be the most important non-volatile drivers imparting astringent-related sensations. Similarly, fractions containing anthocyanin-derived pigments resistant to SO2 and precipitating with ovoalbumin seem to be related to stickiness (called dry tannins by experts), while tannin fractions seem to be responsible for dry sensations (called green tannins by experts).

The interaction between isoamyl alcohol and the anthocyanin-derivative fraction and/or tannins is suggested to contribute to the green character and to enhance it in red wines. These three elements apparently explain the sticky and dry character, but cannot explain vegetal odour nuances related to the green character. Besides, the intensity of green character is demonstrated to be wine-dependent and it is suggested to be masked by woody, oxidation and/or ripe fruit aromas present in oaked aged red wines.

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SECTION II. CHAPTER 2

Effect of grape maturity on wine sensory and chemical features: The case of Moristel wines

1. Introduction

Grape composition is an important factor influencing sensory characteristics of wines (Niimi, Boss, Jeffery, & Bastian, 2017). Thus, selecting the optimal point to harvest berries with the greatest potential to yield wines with desired sensory properties is a major issue for winemakers. Measuring basic chemical parameters in the grape (such as pH, total acidity, colour intensity, phenolic content) to determine ripeness prior to harvest is a standard industry practice. However, these conventional measurements are not enough to predict wine features (Pérez-Magariño & González-San José, 2006). Phenolic compounds together with sensory-active volatile compounds are generally considered to be major determinants of the quality of red wines (Sáenz-Navajas et al., 2015). However, clear relationships between wine sensory characteristics such as aroma, taste or chemesthesic sensory properties (including thermal, pain related or astringency related sensations) and phenolic composition combined with aroma precursors present in the grape have not been yet established. The potential aromatic quality of the grape is a factor which is still poorly understood. Generally, compared to the resulting wines, vinification grapes present mostly neutral aromas, which is the result of the presence of very low quantities of a long list of aromatic compounds such as furaneol, β-damascenone, terpenols, benzenoids or phenols among others together with a complex series of specific aroma precursors of mainly terpenes, methoxypyrazines or thiols (Ruiz et al., 2019). These odourless non-volatile molecules, known as precursors, contribute the varietal characteristics of wines by generating an aromatic molecule by 1) the break of chemical bonds (including glycoside, S-derivatives of cysteine or glutathione or S-methionine and other precursors of dimethyl sulphur) and/or 2) spontaneous molecular reassembly (by pH effect or esterification) (Parker,

Capone, Francis, & Herderich, 2018). These precursors play an essential role in wine aroma, but how grape maturity affects aroma grape potential is far from clear. Firstly, this is because the analytical tools to quantify these precursors are still being developed and secondly because precursor concentration in juices is not directly correlated to the aroma compounds found in wines (Alegre, Ferreira, & Hernández-Orte, 2019).

Regarding phenolic compounds, tannins (proanthocyanidins) and anthocyanins constitute the most abundant classes in red grapes. Anthocyanins are released from grape skins, whereas proanthocyanidins are released from both skins and seeds. Accumulation of anthocyanins sets in at veraison and declines during over ripening. Proanthocyanidins mainly accumulate before veraison (Fournand et al. 2006). The ripeness of the grape has an important effect on the kind and extractability of phenolic compounds in the resulting wine (Allegro et al., 2018; Allegro, Pastore, Valentini, Muzzi, & Filippetti, 2016). The extractability of proanthocyanidins from seeds decreases with ripeness, probably due to oxidation phenomena and gradual seed lignification that hinders their extraction (Cadot, Miñana-Castelló, & Chevalier, 2006). On the other hand, the extractability of skin polyphenols increases with ripening, which is attributed to the action of enzymes by degrading the wall of skin cells (Gil et al., 2012). In this context, grape maturity represents an important factor determining grape composition; consequently, grape composition determines sensory properties of wines, hence wine quality and acceptability.

In terms of grape cultivars, Moristel is a minor variety which has been suggested to originate in Aragon (north-east Spain), where it is basically found in the Somontano region (Robinson, Harding, & Vouillamoz, 2012). It is a cultivar with reasonably good resistance to drought, pests, and diseases, presenting late ripening with the onset of anthocyanins taking place at low sugar levels (García, Zheng, Balda, & Martinez De Toda, 2017). These characteristics make Moristel an interesting alternative to be grown in warm climates; however scientific literature concerning its potentiality is scarce. In this context, the aim of the current study was to

determine the effect of grape maturity on the sensory attributes of resulting wines and to relate these sensory changes with chemical composition considering Moristel variety as the case study.

2. Material and Methods

2.1. Site location and winemaking

The experiment took place in Barbastro situated in the Somontano region (Huesca, Spain) during the 2017 harvest. Two vineyard blocks (BLA and BLB) with a priori maximum quality diversity were selected based on historical data and criteria derived from the commercial system Dyostem® (Vivelys, France) (Figure II-2.1). According to commercial information, this tool monitors sugar loading and changes in the colour of the fruit to classify grape quality and determines the optimal harvest date.

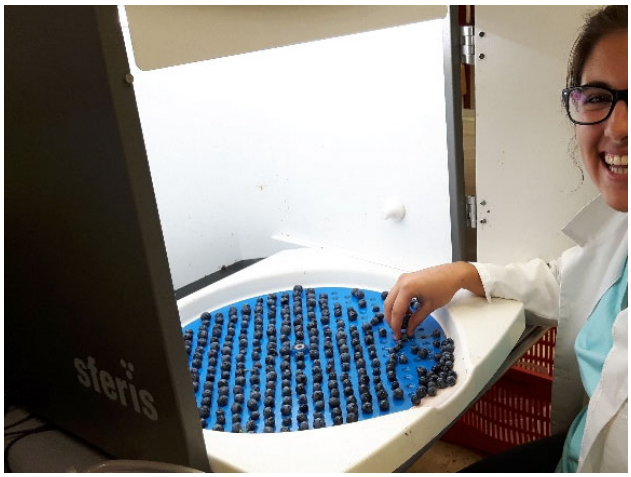

Figure II-2. 1. Dyostem® (Vivelys, France), tool for evaluating the enological potential of plots and determining the harvest date.

Moristel grapes were handpicked at four different maturity points for BLA (BLA_1; BLA_2; BLA_3 and BLA_4) and at three points for BLB (BLB_1; BLB_2; BLB_4); each collection date was separated by one or two weeks. According to the commercial system, the second point of maturity (BLA_2, BLB_2) was the optimal moment to harvest, thus, harvest was carried out one week before (BLA_1, BLB_1) and one (BLA_3) and/or two (BLA_4, BLB_4) weeks after that date to obtain grapes with distinct maturity levels and thus with a priori maximum variability in chemical composition. One hundred and fifty kilograms of fruit were collected at each harvest date following a random design within each block. Grapes were processed

(destemmed/crushed) the same day, and the fruit was divided into three separate lots. Wines were elaborated in 75-litre stain-less steel tanks, in triplicate. To each tank (total of 21), sulphur dioxide was added to reach a total concentration of 50 mg L^{-1}. The day following harvest all tanks were inoculated with Lalvin ICVD 254 (Lallemand) at 106 cells ml^{-1} and pectolytic enzyme at 0.8 mL Hl^{-1}. Alcoholic fermentations (FOH) took place on skins for 10 days, on average. Once alcoholic fermentation was finished wines were inoculated with lactic bacteria (Oenococcus oeni) strain Lalvin VP41 (Lallemand). Malolactic fermentation was completed within 30 and 37 days. Wines were bottled 3 months after FOH (free SO_2 adjusted to 30 mg L^{-1}). Wines were closed with natural cork closures.

2.2. Sensory analysis

2.2.1. Participants

Seventeen panellists from Instituto de Ciencias de la Vid y del Vino (ICVV) and Universidad de La Rioja (Spain) participated in sensory evaluation. They were mainly last-year oenology students and oenologists (60% women, ranging from 22 to 34 years of age, average = 28).

2.2.2. Panel training and generation of sensory attributes

Participants attended a total of 6 training sessions (1.5h each) throughout three weeks. During this period, panellists worked in two subgroups following the same guidelines. The first session was devoted to generating aroma terms differing among samples. Therefore, participants were presented simultaneously with the 21 wines of the study and were asked to sort them based on their aroma similarity according to a sorting task. Once groups were built, they described them with two or three descriptive aroma terms (avoiding hedonic terms). Terms generated were gathered and grouped in categories according to semantic similarities. This process was performed individually by three experienced researchers who, through a triangulation task, (Abric, 2003) achieved a final consensual list of 12 terms that included: “fresh vegetables” (green pepper), “red fruit” (strawberry, cherry,

raspberry), “white fruit” (apple, pear), “black fruit” (blackberry, blackcurrant), “raisin”, “fresh grass”, “oxidation” (acetaldehyde, boiled potato, honey, overripe apple), “roasted/smoky”, “reduction” (cauliflower, rotten eggs), “spicy” (black pepper, nutmeg, clove), “undergrowth” (mouldy, mushroom) and “alcohol” (ethanol, spirit-like). During the successive training sessions, reference standards (prepared at Laboratorio de Análisis del Aroma y Enología of Universidad de Zaragoza) representative of the 12 selected aroma terms, as well as of three taste (“sweet”, “sour”, “bitter”) and three chemesthesic (“astringency”, “alcoholic feeling”, “viscosity/body”) terms were presented. For in-mouth stimuli terms were prepared as follows: solutions containing different concentrations of table sugar (0-7 g L^{-1}) for “sweetness”, tartaric acid (0-3 g L^{-1}) for “sourness”, quinine sulphate (0-40 mg L^{-1}) for “bitterness” and potassium, aluminium sulphate (0-5 g L^{-1}) for “astringency”, absolute alcohol (0-15% v/v) for “alcoholic feeling” and carboxymethylcellulose (0-1.5 g L^{-1}) for “viscosity/body”. During a typical training session, panellists were presented with references illustrating the different aroma, taste, and chemesthetic terms; 2-4 wines were individually described and then ratings were discussed until achieving consensus.

2.2.3. Wine description

The 21 wines (7 distinct wines elaborated in triplicate) were described in duplicate during four sessions (replicated samples were presented in different sessions). Each session was split into two parts (45 min each) (5-6 samples per part), which were separated by an imposed pause of 10 min. Participants were asked to taste and rate the intensity of exclusively those terms (out of 18) that applied to the sample on a seven-point scale according to Rate-all-that-apply (RATA) methodology (Ares et al., 2014). RATA method has shown to be a suitable tool to profile complex products composed of hundreds of odorant compounds such as wine (Franco-Luesma et al, 2016). Terms that did not apply to the sample were allocated a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomised order for each assessor. The use of a sip

(rinsing solutions: water and 1 g L^{-1} pectin solution) and spit protocol between each sample was imposed as described elsewhere (Colonna, Adams, & Noble, 2004). All participants evaluated the 21 samples in duplicate in a sequential monadic manner. Twenty-mL samples were served in dark wine glasses labelled with 3-digit random codes and covered with plastic Petri dishes in a random arrangement which was different for each participant. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at approximately 20 ºC).

2.2.4. Sensory data analysis

A three-way ANOVA for each of the sensory attributes evaluated involving wines (W), judge (J) and replicate (R) as fixed factors and all first order interactions was calculated to confirm panel performance with the 21 wines (in duplicate) of the study.

Then, to find discriminant sensory attributes for the wines within blocks a two-way ANOVA (panellists as random and wines as fixed factors) was calculated for each chemesthesic term of the list. Then, for discriminant terms, pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. All statistical analyses were performed using XLSTAT (2018).

2.3 Chemical analysis

2.3.1. Conventional oenological parameters

Grapes: Sugar content in grapes was analysed by Infrared Spectrometry with Fourier Transformation with a WineScanTM FT 120 (FOSS®, Barcelona, Spain), which was previously calibrated with the official OIV methods.

Wines: Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and colour intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). For TPI determination, the abs at 280 nm of samples diluted 1:100 in deionised water was measured in 1-cm-quartz cuvettes. Reducing sugars, ethanol content, pH, malic and lactic acid as well as titratable and volatile

acidities were analysed by Infrared Spectrometry with Fourier Transformation with a WineScanTM FT 120 (FOSS®, Barcelona, Spain), which was previously calibrated with the official OIV methods.

2.3.2. Chemical characterisation of non-volatile compounds

2.3.2.1. Determination of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP), and large polymeric pigments (LPP) concentrations in the wines and fractions was carried out as described elsewhere (Harbertson, Picciotto, & Adams, 2003). MPs are the group of compounds bleachable with bisulphite, while SPP and LPP are resistant to bisulphite bleaching. SPP do not precipitate with ovalbumin as LPP. Levels of MP, SPP, and LPP were expressed as absorbance at 520 nm.

2.3.2.2. Mean degree of polymerisation (mDP) of tannins

Acid-catalysed degradation in the presence of toluene-α-thiol was performed according to the method described by Labarbe et al. (1999) with some modifications described by Gonzalo-Diago, Dizy, & Fernandez-Zurbano (2013). Quantification was carried out in the negative mode from the extracted ion chromatogram (EIC) for flavan-3-ols and in the positive mode for malvidin-3-O-glucoside. The area under the peaks of malvidin-3-O-glucoside and flavan-3-ol monomers (terminal units) before and after thiolysis, as well as toluene-α-thiol adducts (extension units) released from the depolymerisation reaction were all integrated. Calibration curves were established with malvidin-3-O-glucoside, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, and (−)-epigallocatechin. In the absence of the thiol derivative standards, considering the fact that the thiolytic derivatives were shown to have similar response factors as the correspondent monomeric units, their concentrations were calculated from the respective monomer calibration curves. The mean degree of polymerisation (mDP) was calculated as the ratio of total units (extension + terminal) to terminal units (calculated as the difference between before and after thiolysis). The percentage of tannins linked to malvidin-3-O-glucoside (%T-

M) was calculated as the molar ratio of malvidin-3-O-glucoside linked to tannins (calculated as the difference before and after thiolysis) to the sum of total units of terminal malvidin-3-O-glucoside and extension + terminal units of (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, and (−)-epigallocatechin (i.e. total units of tannins). The percentage of procyanidins (%PC) was calculated as the ratio of total units (extension and terminal) of catechin and epicatechin to total units of tannins. The percentage of prodelphinidins (%PD) and galloylated (%G) were calculated units as the ratio of total units of PD and G to the total units of tannins, respectively.

2.3.2.3. Tannin concentration and activity

Concentration and activity of tannins were estimated by a UHPLC-UV-Vis method following the method proposed by Revelette, Barak, and Kennedy (2014). Tannin activity is related to the thermodynamics of interactions between tannins and a hydrophobic surface (polystyrene divinylbenzene HPLC column).

2.3.3. Chemical characterisation of volatile compounds

2.3.3.1. Determination of Total Odour-Active Carbonyls

The determination by headspace-SPME-GC-MS of total (free plus bound) forms of distinct odour-active carbonyls, such as isobutyraldehyde, 2-methylbutanal, isovaleraldehyde, methional, phenylacetaldehyde, and diacetyl in wine was carried out as described in the method proposed by Bueno, Zapata, and Ferreira (2014). Carbonyls in the headspace were preconcentrated on a PDMS/DVB fibre and further quantified with a GC (Shimadzu GC-2010) coupled to a quadrupole mass detector (Shimadzu QP2010Plus).

2.3.3.2. Determination of major volatile compounds

Major volatile compounds were isolated by liquid-liquid extraction and analysed in a gas chromatograph with a flame ionization detector (GC-FID, CP-2800 Varian) following the method described by Ortega, López, Cacho, and Ferreira (2001). Quantification was performed by interpolation of the analyte/internal standard

(relative area) ratio in the calibration plot built with synthetic wine models containing known concentrations of analytes. Internal standards employed were as follows: 4-hidroxy-4-methyl-pentanone, 2-butanol, 4-methyl-2-pentanol, 2-octanol, heptanoic acid and ethyl heptanoate.

2.3.3.3. Determination of trace aroma compounds

Trace aroma compounds were isolated through solid-phase extraction (SPE) and analysed by gas chromatography coupled to a mass spectrometry detection system (GC-MS, 450-GC and Saturn 2200 GC/MS Varian) as explained by López et al. (2002) yet, with the following modifications. Lower sample volumes (15 mL), smaller LiChrolut EN cartridges (65 mg) and lower cleaning (1.5 mL) and elution (0.6 mL) solvent volumes were employed. SPE cartridges were cleaned up with a solution of 30% methanol in water at pH 3 (1.5 mL) and eluted with dichloromethane with 5% methanol (v/v). Extracts were directly analysed by GC-MS. Selective m/z relation was used for thirty-eight analytes, which were referred to a selected internal standard (2-octanol, 3,4-dimetilphenol and 3-octanone) for the quantification through the response factor method.

2.3.3.4. Determination of alkylmethoxypyrazines and rotundone

Alkylmethoxypyrazines and rotundone were quantified using stir bar sorptive extraction (SBSE), followed by thermal desorption gas chromatography coupled to a mass spectrometry (TD-GCxGC-MS) (Agilent GC 7890A coupled to MS 5975C) (Wen, Ontañon, Ferreira, & Lopez, 2018). Stable isotope dilution analysis was used for quantification (with selective mass fragments). The compounds 2-isobutyl-3-methoxypyrazine (IBMP), 2-isopropyl-3-methoxypyrazine (IPMP), and rotundone were analysed.

2.3.4. Data analysis of chemical data

Quantitative data of the 72 volatile compounds were transformed into Odour Activity Values (OAV) with division by their corresponding sensory thresholds (ST). In the case of concentrations below the limit of detection (LOD) or quantification (LOQ) limits, OAV was calculated as LOD/ST or LOQ/ST, respectively. In order to rank compounds in accordance with their discriminatory ability, the quotients between the maximum and minimum OAV were set for each compound (OAV minimum < 0.2); this value was arbitrary used to avoid quotients which are undetectable from a sensory point of view, especially when an OAVmin is zero.

In order to facilitate the correlation of chemical and sensory spaces, the chemical space of volatile molecules was simplified by creating aroma vectors. Aroma vector is defined as "a perceptual unit constituted by one or several molecules with similar aroma descriptors, which altogether and in an integrated form, are responsible for a specific set of sensory features of a type of products; wine in our case" (Ferreira, Sáenz-Navajas, & de-la-Fuente, 2020). Aroma vectors are built by grouping aroma compounds with similar chemical structure and odour properties based on Ferreira, Sáenz-Navajas, and de-la- Fuente (2020). Therefore, the individual OAV for each compound belonging to each vector is firstly calculated and aroma vectors are the sum of OAVs of compounds within each vector. Table II-2.1 shows the 13 aroma vectors built. Another 11 compounds with known sensory impact were studied individually: β-damascenone (baked apple, dry plum), β-ionone (violets, berry), ethyl dihydrocinnamate (sweet, balsamic), Z-3-hexenal (leaf, grassy), diacetyl (buttery, milky, yogurt), methional (potato, oxidised, overripe), phenylacetaldehyde (honey, oxidized), acetaldehyde (green apple, oxidized), isoamyl acetate (fruit, banana), phenylethyl acetate (floral, rose, sweet) and t-whiskylactone (oaky, coconut).

Table II-2. 1. Limit of detection (LOD), sensory description in isolation, odour thresholds, concentration ranges, median values and maximum to minimum odour activity value rate of volatiles found in the set of the 21 wines of the study (all expressed in micrograms per litre). Compounds found in at least one wine at concentrations above their sensory threshold are marked in bold.

COMPOUNDS	Sensory description	LOD	Odour threshold[a]	Concentration range	Median	OAV_{MAX}/ OAV_{MIN}[b]
β-ionone	**violets, berry**	**0.33**	**0.09[1]**	**0.393-0.752**	**0.499**	1.91
diacetyl	**buttery, milky, yogurt**	**1.59**	**100[2]**	**244-12245**	**1996**	50.1
acetaldehyde (free)	**green apple**	**115**	**500[2]**	**<LD-5437**	**815**	47.3
β-damascenone	**baked apple, dry plum**	**0.187**	**0.05[2]**	**<LD-2.69**	**0.682**	14.4
isoamyl acetate	**banana**	**18**	**30[2]**	**80.9-301**	**163**	3.72
phenylacetaldehyde	**honey**	**1.67**	**1[3]**	**6.68-22**	**13**	3.3
***c*-3-hexenal**	**fresh grass**	**0.059**	**0.12[4]**	**15.1-24.9**	**18.3**	1.65
ethyl dihydrocinnamate	sweet, balsamic	0.026	1.6[1]	<LD-0.491	0.375	1.53
phenylethyl acetate	floral, rose	0.019	250[2]	2.51-8.35	4.88	0.167
t-whiskylactone	oaky, coconut	0.09	67[5]	<LD-0.796	0	0.005
VECTORS	**Sensory description**	**LOD**	**Odour threshold[a]**	**Concentration range**	**Median**	**OAV_{MAX}/ OAV_{MIN}[b]**
ACETATE VECTOR	**fruity, pear**					
butyl acetate		0.167	1830[5]	1.8-15.9	3.39	0.044
isobutyl acetate		0.158	1605[6]	5.42-16	6.73	0.05
ACETIC VECTOR	**vinegar, glue**					
acetic acid		**240**	**300000[2]**	**270496-746363**	**449652**	2.76
ethyl acetate		**10**	**12300[7]**	**1062-81907**	**49833**	33.3
BRANCHED ACIDS VECTOR	**cheese, sweaty**					
isobutyric acid		**101**	**2300[8]**	**2029-3004**	**2574**	1.48
isovalerianic acid		**28**	**33[1]**	**60-3731**	**2211**	62.2
ETHYL ESTER VECTOR	**fruity, apple, strawberry**					
ethyl propanoate		50	5500[9]	<LD-228	0	0.207
ethyl butyrate		**26.3**	**125[9]**	**46-157**	**102**	3.42
ethyl hexanoate		**34.2**	**62[9]**	**145-490**	**277**	3.38
ethyl octanoate		12	580[5]	50.9-173	120	1.49
ethyl decanoate		**17.2**	**200[1]**	**<LD-409**	**33.6**	10.2
ethyl isobutyrate		**0.495**	**15[1]**	**79.9-164**	**106**	2.06
ethyl 2-methylbutyrate		**0.33**	**18[1]**	**10.3-20**	**16.6**	1.94
ethyl isovalerate		**0.33**	**3[1]**	**13.7-61.4**	**20.1**	4.48
ethyl lactate		100	154000[5]	10675-69951	33465	2.27
diethyl succinate		3	200000[5]	1137-8994	7714	0.225

Table II-2. 1. continued

VECTORS	Sensory description	LOD	Odour threshold[a]	Concentration range	Median	OAV_{MAX}/ OAV_{MIN}[b]
ETHYLPHENOL VECTOR	**animal, leather**					
4-ethylguaiacol		0.018	33[1]	<LD-0.289	0.09	0.044
4-ethylphenol		0.023	35[9]	<LD-1.5	0.175	0.215
ISOALDEHYDE VECTOR	**malty, yeasty**					
isobutanal		**0.495**	**6[3]**	**16.5-43.4**	**30.8**	2.63
2-methylbutanal		0.176	16[3]	5.97-14.9	9.45	2.5
3-methylbutanal		**0.206**	**4.6[3]**	**6.85-55.4**	**21.2**	8.1
HIGHER ALCOHOL VECTOR	**spirit, solvent**					
benzyl alcohol		10	200000[10]	44.1-468	199	0.012
1-butanol		2	150000[5]	876-1885	1144	0.063
1-hexanol		14	8000[2]	2556-3348	2921	1.31
t-2-hexenol		0.739	15000[5]	<LD-16.6	3.02	0.006
t-3-hexenol		0.166	1000[11]	33.8-86.1	59.7	0.43
c-3-hexenol		12.2	400[2]	27.9-168	48.4	2.11
isoamyl alcohol		**19**	**30000[2]**	**219088-289686**	**259247**	1.32
isobutanol		**24.3**	**40000[2]**	**28890-40186**	**32461**	1.39
methionol		**26**	**1000[1]**	**169-3882**	**993**	19.4
1-penten-3-ol		7.81	400[12]	<LD-294	97.2	3.68
β-phenylethanol		**5**	**14000[1]**	**2946-43218**	**32360**	14.7
γ -LACTONE VECTOR	**peachy**					
γ -butyrolactone		18.3	35000[14]	1071-17501	10821	2.5
γ-nonalactone		**0.064**	**25[8]**	**9.93-25.1**	**17.1**	2.52
LINEAR FATTY ACID VECTOR	**cheese, soapy**					
butyric acid		**100**	**173[1]**	**162-1170**	**678**	7.24
hexanoic acid		**10**	**420[1]**	**177-1992**	**1546**	11.3
octanoic acid		**10**	**500[1]**	**729-1561**	**975**	2.14
decanoic acid		27	1000[1]	<LD-909	474	4.55

Table II-2. 1. continued

VECTORS	Sensory description	LOD	Odour threshold[a]	Concentration range	Median	OAV_{MAX}/ OAV_{MIN}[b]
METHOXYPHENOL VECTOR	**clove, smoky**					
guaiacol		**0.05**	**9.5[1]**	**<LD-21.5**	**5.67**	11.3
eugenol		0.019	6[1]	1.23-2.99	1.61	2.43
4-vinylguaiacol		0.039	40[2]	5.75-24.7	11	3.09
isoeugenol		**0.073**	**6[13]**	**1.04-14.3**	**3.46**	11.9
2,6-dimethoxyphenol		0.048	570[14]	4.16-23.9	8.65	0.21
4-alyl-2,6-dimethoxyphenol		0.33	1200[8]	1.78-6.15	2.96	0.026
o-cresol		0.33	62[15]	<LD-1.05	0.828	0.17
m-cresol		0.003	20[15]	<LD-0.68	0.078	0.05
4-vinylphenol		0.055	180[16]	6.03-52.4	14.1	1.46
METHOXYPYRAZINE VECTOR	**earthy, green pepper**					
2-isopropyl-3-methoxypyrazine		**0.00007**	**0.001[17]**	**<LD-0.00138**	**0.00007**	19.6
2-isobutyl-3-methoxypyrazine		**0.00002**	**0.002[18]**	**0.00087-0.00222**	**0.00157**	2.56
TERPENOL VECTOR	**jasmine, muscat**					
linalool		0.045	25[1]	2.37-5.05	3.37	1.01
α-terpineol		0.048	250[1]	0.853-1.91	1.46	0.038
β-citronelol		0.779	100[5]	<LD-4.92	2.69	0.246
geraniol		0.33	20[13]	1.38-5.41	3.87	1.35
VANILLIN VECTOR	**vanilla, nutmeg**					
vanillin		0.076	995[13]	3.44-15	6.64	0.075
methyl vanillate		0.041	3000[14]	2.41-5.54	3.52	0.009
ethyl vanillate		0.059	990[14]	67.3-256	133	1.29
acetovanillone		0.136	1000[13]	32-78.1	53	0.391

Reference in which the odour threshold value has been calculated is given in brackets: [1] Ferreira et al. (2000) in 11% water/ethanol, pH=3.4, 7 g/L glycerol, 5 g/L tartaric acid; [2] Guth (1997) in 10% water/ethanol; [3] Culleré et al. (2007) in 10% water/ethanol, pH=3.2, 5 g/L tartaric acid; [4] Sellami et al. (2018) in tap water; [5] Etievant et al. (1991) in 12% water/ethanol, except for t-whiskylactone in 30% ethanol, and t-2-hexenol in beer; [6] Ferreira et al. (2002) in 10% water/ethanol, pH 3.2; [7] Escudero et al. (2004) in 10% water/ethanol, pH 3.2., 5 g/L tartaric acid; [8] Gemert (2003) in water; [9] San Juan et al. (2012) 10% water/ethanol, pH=3.2, 5 g/L tartaric acid; [10] Aznar et al. (2003) in 10% water/ethanol, pH=3.2, 5 g/L tartaric acid; [11] Fariña et al. (2015) matrix not specified; [12] Buttery and Ling (1995) in water; [13] Escudero et al. (2007) in 10% water/ethanol, pH=3.2, 5 g/L tartaric acid; [14] López et al. (2002) in 10% water/ethanol, pH 3.2; [15] Parker et al. (2012) in red wine; [16] Boidron et al. (1988) in 12% water/ethanol, 8 g/L glycerol and different salts; [17] Pickering et al. (2007) in red wine; [18] Buttery et al. (1969) in water. bFor OAVminimum < in 0.2, 0.2 value is considered for calculating the quotient.

Discrimination ability of individual compounds and vectors among wines was evaluated by calculating the ratio OAVmax/OAVmin. Only values >2 were considered discernible among wines. Besides this, only compounds or vectors with OAV> 1 in at least one wine were considered to have a potential sensory impact.

For compounds with OAVmax/OAVmin >2 and OAV>1 in at least one wine, one-way ANOVA (wines as fixed factors) was calculated to find compounds and vectors able to explain the aroma properties of the wines. Pair-wise comparison test (Fischer test) was applied (5% risk) for significant effects. All statistical analyses were performed using XLSTAT (2018).

Finally, a two-way ANOVA (panellists as random factor and wines as fix factor) was calculated for the nine aroma terms and the seven wines considered in the study. With the mean sensory scores of the four significant terms, a principal component analysis (PCA) was calculated with aroma terms as active variables and with significant volatile compounds or vectors as supplementary variables. All analyses were carried out with XLSTAT (2018 version).

2.4. Colour measurement

The absorbance spectra of this set of wines were measured. Measurements were carried out in a Shimazdu UV-1800 (Shimadzu Corporation, Tokyo, Japan), using 0.2-cm path-length crystal cuvettes. Measurements were taken every 1 nm between 380 and 780 nm. Wine samples had been clarified by passing wine through 0.45 µm filters previous to the measurements. From the spectra, the colour coordinates were calculated using the CIE method, with the CIE 1964 10° standard observer and the illuminant D65, according to the OIV. The values correspond to the degree of wine lightness (L_{10}*) and the degree of red (when a_{10}*>0), green (when a_{10}*<0), yellow (when b_{10}*>0), and blue (when b_{10}*<0) colour.

3. Results and discussion

3.1. Effect of grape maturity on conventional parameters of grapes and wines

Overall, grape samples from block A (BLA) reached higher levels of sugars than block B at similar harvest points; this effect was more pronounced in the samples from the first and second points. Both reached 27 g L^{-1} higher in BLA. Significant effects ($P<0.05$) of the maturity point (i.e., harvest point) on sugar content were observed in both blocks; samples from later harvest points (BLA_4 and BLB_4) having the highest levels in both cases (Table II-2.2). This data is well correlated with the ethanol content present in the resulting wines (Table II-2.2), which reached maximal values (15.8 and 13.7%, v/v, in BLA_4 and BLB_4, respectively) at these points. Interestingly, there is a relatively ample variation (3.4 %, v/v) of ethanol content among the studied wines, which is significantly correlated with total polyphenol index (TPI) ($r=0.97$; $P<0.001$). This fact can be related to both the higher accumulation of polyphenols during ripening as well as to the higher ability of ethanol to extract polyphenolic compounds.

A significant effect on titratable acidity due to maturity point was observed in both blocks, with the initial harvest points (BLA_1, BLB_1) having the highest levels (Table II-2.2). This parameter ranged from 5.8 to 6.7 g L^{-1} (expressed as tartaric acid) in the studied wines, which is within the normal values found in Spanish wines (Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2012; Sáenz-Navajas, Fernandez-Zurbano, Tao, Dizy, & Ferreira, 2010). Contrarily, grape maturity was not found to have a significant effect on pH nor volatile acidity in wines from either of the two blocks. Interestingly, the variety presented low pH values (range: 3.2-3.3) in comparison with other Spanish wines elaborated with more common varieties such as Tempranillo, Grenache, Cabernet Sauvignon or Syrah among others, which are reported to range between 3.3 and 4.0, while acetic acid, having ranged from 0.3 to 0.5 g L^{-1} (expressed as acetic acid), was within the range of values reported in literature for Spanish wines (Sáenz-Navajas et al., 2010, 2012).

Based on the content of reducing sugars, which ranged from 1.6 to 2.6 g L^{-1} and confirmed proper alcoholic fermentation, dry wines in all cases were produced (<5 g L^{-1}). Noteworthy is the fact that wines elaborated with grapes from block B, BLB, after having completed malolactic fermentation, provided wines with an average lactic acid of 0.5 g L^{-1}. On the other hand, wines from block A, which were difficult to reach the end of malolactic fermentation, presented lower levels of lactic acid (<0.3 g L^{-1}in all cases).

Table II-2. 2. Conventional oenological parameters of wines and grapes (sugar content) expressed as the average (among replicated tanks) ± standard deviation. Different letters within the same block (BLA or BLB) indicate significant differences (P<0.05 according to pairwise Fischer test) among the maturity points (BLA1-BLA4 or BLB1-BLB4).

	GRAPES	WINES							
	S[a]	pH	VA[b]	TA[c]	RS[d]	MA[e]	LA[f]	ETANOL[g]	TPI[h]
BLA_1	251±3b	3.2±0.0	0.5±0.0	6.7±0.0a	2.0±0.1c	0.6±0.0b	0.2±0.1	14.8±0.2b	45.2±0.0b
BLA_2	254±5b	3.3±0.0	0.5±0.0	6.0±0.0b	2.3±0.0b	0.3±0.0c	0.2±0.1	14.9±0.2b	45.6±0.0b
BLA_3	250±11b	3.3±0.0	0.4±0.0	6.1±0.0b	2.6±0.1a	0.4±0.0c	0.3±0.0	14.4±0.3b	43.8±0.1b
BLA_4	267±3a	3.3±0.0	0.4±0.0	5.8±0.0c	2.5±0.0a	0.8±0.0a	0.1±0.0	15.8±0.0a	53.0±0.0a
BLB_1	224±9b	3.2±0.1	0.5±0.0	6.4±0.1a	1.6±0.0b	0.3±0.1b	0.5±0.0	12.4±0.2b	22.1±1.7b
BLB_2	227±12b	3.2±0.0	0.3±0.0	6.0±0.0b	1.6±0.0b	0.5±0.1a	0.5±0.1	12.7±0.3b	24.2±1.0a
BLB_4	250±6a	3.2±0.0	0.4±0.0	6.0±0.2b	1.9±0.0a	0.3±0.0b	0.5±0.0	13.7±0.2a	25.7±0.6a

a Sugar content (g L^{-1})

b Volatile acidity (g L^{-1} acetic acid)

c Titratable acidity (g L^{-1} tartaric acid)

d Reducing sugars (g L^{-1})

e Malic acid (g L^{-1})

f Lactic acid (g L^{-1})

g Ethanol content (%. v/v)

h TPI: total polyphenol index (a.u.)

3.2. Effect of grape maturity on polyphenolic composition and colour coordinates of wines

Table II-2.3 presents the variables related to the characterisation of wine polyphenolic compounds and colour coordinates. The values of tannin activity, which is measured as the enthalpy of interaction between polyphenols and a hydrophobic surface (Revelette et al., 2014), range between 854 and 2751 –J mol^{-1}, which is relatively low in comparison to other studies with Cabernet Sauvignon (1430-4820 –J mol^{-1}) (Watrelot, Byrnes, Heymann, & Kennedy, 2016) or Merlot wines (3170-4060 –J mol^{-1}) (Sáenz-Navajas et al., 2018). This property has shown to decrease with both barrel ageing and microoxygenation (Watrelot et al., 2016; Sáenz-Navajas et al., 2018) attributed to tannin oxidation (Yacco, Watrelot, & Kennedy, 2016). Concerning tannin concentration and pigmented tannins, which range from 1993 to 4188 mg L^{-1} and 618-1138 mg L^{-1}, respectively in the studied wines, are significantly lower ($P<0.01$) than values found in Cabernet Sauvignon (2750-6160 mg L^{-1}) and Merlot wines (4390-4940 mg L^{-1}) (Watrelot et al., 2016; Sáenz-Navajas et al., 2018) for tannins and in oaked aged Cabernet Sauvignon for pigments (8300-12700 mg L^{-1}). This data illustrates how the Moristel wines studied present relatively low levels of polyphenols when compared to other common varieties such as Cabernet Sauvignon or Merlot. The relatively low concentration of tannins seems to be the responsible for the low b10* values (which measures yellow colour) and high values of the L* coordinate (measures wine luminosity, being higher in clearer wines) in comparison with other young Spanish red wines (Soto Vázquez, Río Segade, & Orriols Fernández, 2010). On the other hand, the red colour of these wines, measured by the a_{10}* coordinate was relatively high (a_{10}*=37-59) in comparison with reported young Mencía wines (a_{10}*=39-46), which could be in part related to the lower pH (average pH=3.3 vs 3.8) of Moristel wines. Lower pH values favour the presence of flavylium cation species, which contributes to red colour.

Table II-2. 3. Chemical characterisation of polyphenolic composition and colour coordinates (a_{10}*, b_{10}*, L*) in wines expressed as the average (among replicated tanks) ± standard deviation. Different letters within the same block (BLA or BLB) indicate significant differences (P<0.05 according to pairwise Fischer test) among the maturity points (BLA1-BLA4 or BLB1-BLB4). Numbers marked in bold are the highest values within a block.

	Tannin activity (-J mol^{-1})	Tannin concentration (mg L^{-1})	Pigments concentration (mg L^{-1})	(mDP)	a_{10}*	b_{10}*	L*
BLA_1	2751±19a	2926±71c	778±25b	1.4±0.1c	**58.6±0.2a**	8.7±0.1b	44.5±0.2b
BLA_2	**2689±96a**	3051±67c	841±120b	1.3±0.0c	56.6±0.1c	8.8±0.2b	45.0±0.1b
BLA_3	1811±15b	**4188±73a**	**1138±160a**	**2.8±0.2a**	46.7±0.1d	0.9±0.4c	**47.0±0.1a**
BLA_4	**2673±32a**	3472±40b	854±80b	1.9±0.0b	57.8±0.1b	**12.5±0.4a**	43.9±0.1c
BLB_1	**1044±47a**	2071±199	618±79	1.6±0.2	42.2±0.6a	0.4±0.1	64.3±0.0b
BLB_2	933±90b	1993±163	646±69	1.7±0.2	43.6±2.0b	1.4±0.9	63.9±2.3b
BLB_4	854±61c	2066±111	687±51	1.8±0.2	36.7±0.6a	0.4±0.4	**70.4±0.4a**

Significant effects of maturity level on all the variables studied were observed for block A, while for block B, only tannin activity, and coordinate L*, changed significantly. Interestingly, tannin activity was inversely correlated with tannin concentration, pigmented tannins and mean degree of polymerisation (mDP) in block A. However, in block B, it decreased with maturity point, while the rest of polyphenolic measurements did not present any significant change. These results suggest that tannin activity is a very interesting parameter that can help control grape maturity, especially because it is independent from other polyphenolic chemical variables including concentration of tannins or pigments and mean degree of polymerisation.

In general, the evolution of the parameters measured are block dependent and no generalisation concerning the effect of grape maturity can be drawn.

3.3. Effect of grape maturity on wine sensory properties

Evaluation of panel performance by three-way ANOVA firstly showed that the replicate effect was only significant (P=0.033) for the term “roasted/smoky”, indicating a global consistent assessment of attributes which reflects the reproducibility of the panel. Thus, the average data between replicated samples was calculated and considered in further analyses. The wine-by-judge interaction (WxJ) was significant for “white fruit”, “roasted/smoky”, “undergrowth”, “reduction”, “oxidation” and “body/viscosity”. A PCA run on these attributes (judges in columns and wines in rows) revealed that judges’ projections were spread over the loading plot for “white fruit”, “undergrowth”, “reduction” and “body/viscosity”, while they were grouped together for the other two attributes. This indicates that there are differences in the use of the scale for “roasted/smoky” and “oxidation”. Yet, for “white fruit”, “undergrowth”, “reduction” and “body/viscosity” there are differences in their interpretation, which suggests that assessors may need more training with respect to these four attributes. These terms were not considered in subsequent analysis. Annexe II-2. 1, shows the average data (for the 3 tanks and 17 panellists) for the remaining 14 of 18 sensory terms evaluated.

Figure II-2.2, shows the flavour (aroma, taste or mouthfeel) descriptors that present significant differences among wines elaborated with grapes harvested at different points of maturation within the two blocks studied: BLA and BLB. Significant effects of grape maturity are observed on the “raisin” ($F=3.41$; $P<0.05$) and “oxidation” ($F=2.93$; $P<0.05$) aromas as well as on “astringency” ($F=6.90$; $P<0.01$) for block A. For block B significant effects on “oxidation” ($F=12.5$; $P<0.001$) and fruity nuances including “raisin” ($F=3.39$; $P<0.05$), “red fruit” ($F=4.32$; $P<0.05$) and “black fruit” ($F=4.82$; $P<0.05$) as well as on “astringency” ($F=4.05$; $P<0.05$) were observed. It is important to note that the sensory effects of grape maturity on most flavour attributes, including fruity aromas as well as “astringency”, are block dependent.

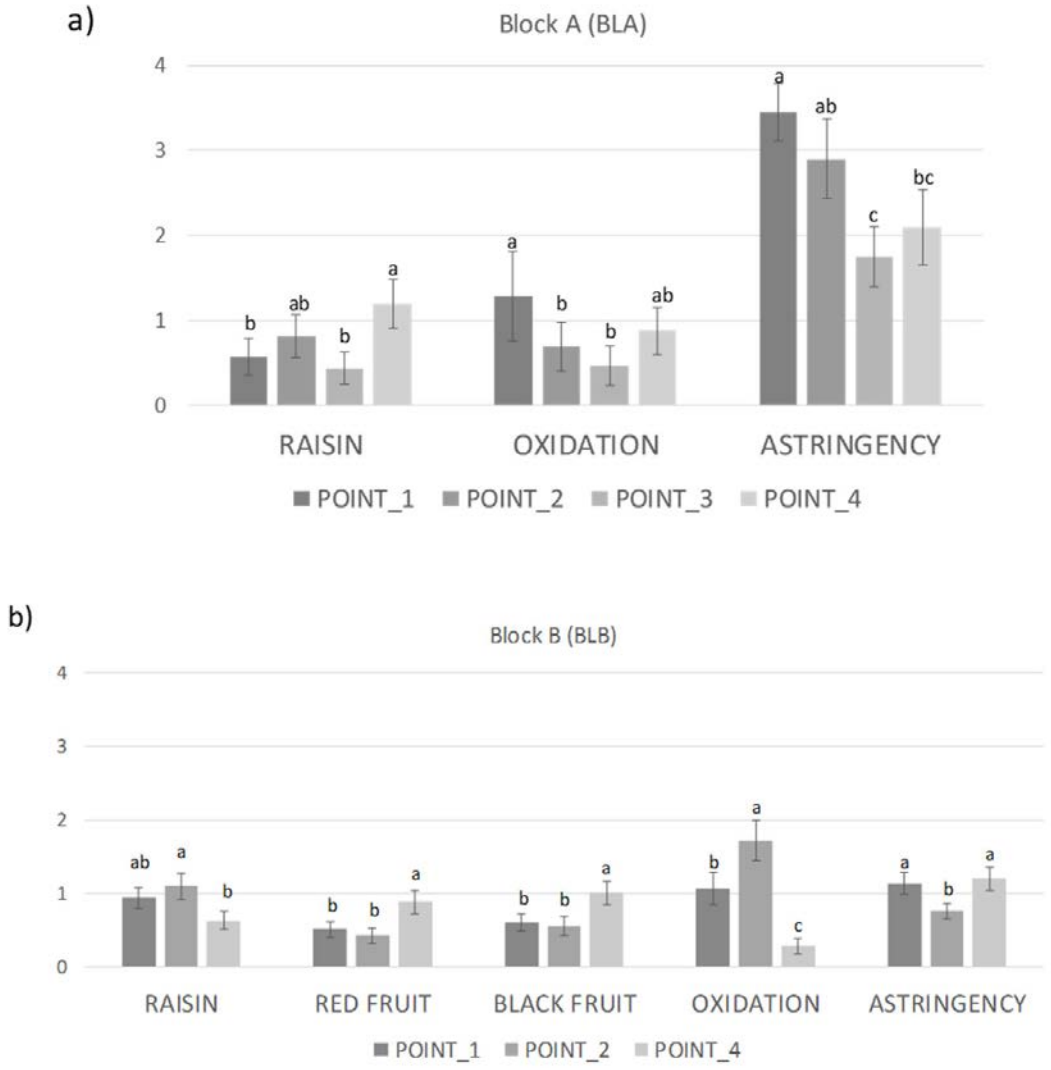

Figure II-2. 2. Sensory attributes (average scores of three tanks and 17 panellists´ evaluations in duplicate; error bars were calculated as s/n1/2: (s) standard deviation; (n) number of panellists) that present significant effects of grape maturity on wines elaborated with grapes within a block: a) Block A harvested at four different points (BLA_1, BLA_2, BLA_3, BLA_4) and b) Block B harvested at three different points (BLB_1, BLB_2, BLB_4).

Further PCA calculated with aroma terms presenting significant sensory differences among the seven wines elaborated in the present study (four from BLA and three from BLB) was calculated (Figure II-2.3). Interestingly, a general pattern of appearance of oxidation nuances in wines elaborated with grapes harvested at earlier points, such as BLA_1, BLB_1, and BLB_2, was observed. These oxidation nuances, change to fresh fruits ("black fruit" or "red fruit") in wines elaborated with

grapes harvested later in both blocks (BLA_3 and BLB_4), and finally, overripe grapes obtained in BLA generated “raisin”-like aromas.

Considering that perceived quality of young red wine is positively linked to fruity aromas and negatively to oxidation and dried fruit (raisin in the present work) nuances (Sáenz-Navajas, Gonzalez-Hernandez, Campo, Fernández-Zurbano, & Ferreira, 2012), results suggest that the optimal point of harvest would be BLA_3 for Block A and BLB_4 for Block B. This result differs from the commercial system employed as it suggested earlier points of maturity: BLA_2 and BLB_2 as optimal points.

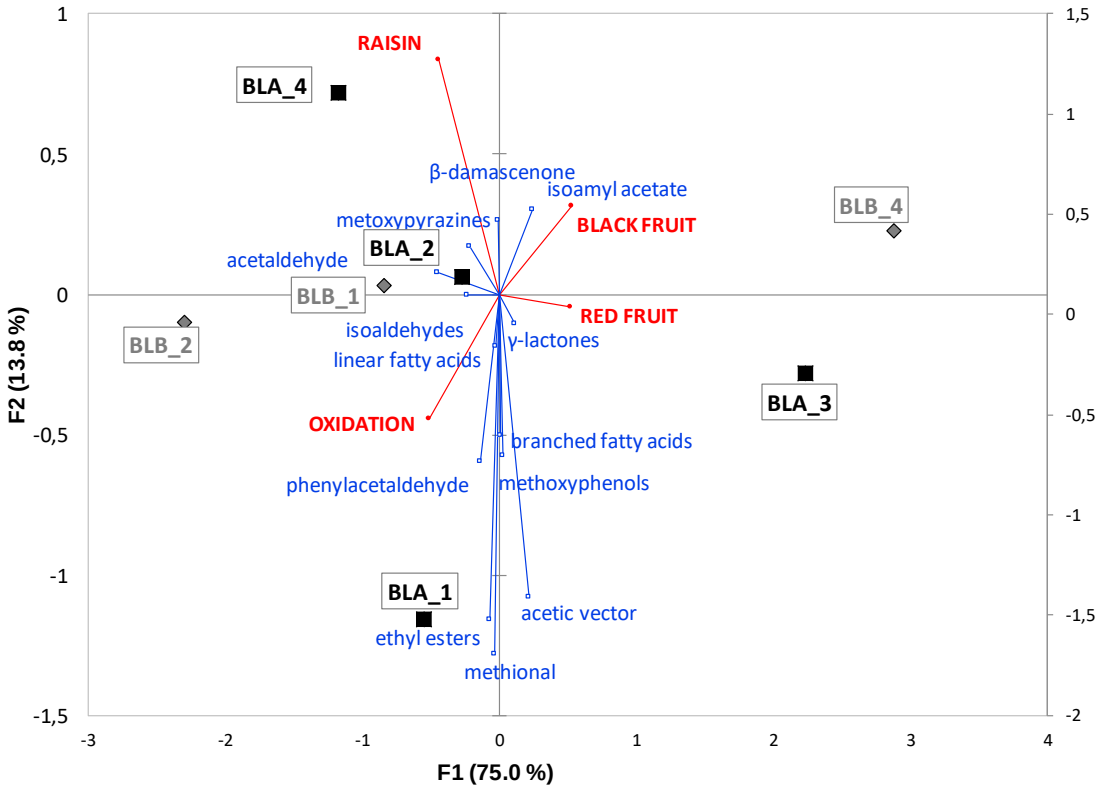

Figure II-2. 3. Principal component analysis plot calculated with the average scores of aroma attributes (of three tanks and 17 panellists evaluated in duplicate) for the 7 different wines elaborated (active variables) and volatile composition (OAV of individual terms or vectors) projected as supplementary variables.

3.3. Relationship between sensory and chemical variables

3.3.1. Aroma properties

The study of the volatile composition of the twenty-one wines has provided quantitative data for 72 compounds. Part of these compounds were grouped into 13 aroma vectors as detailed in Table II-2.1, while the other eleven compounds with known sensory impact by themselves were individually considered following Ferreira, Sáenz-Navajas, and de-la-Fuente (2020). Among these 24 variables, only 13 of them were expected to explain the aroma differences perceived among the 21 wines studied (Table II-2.4).

Table II-2. 4. Individual aroma compounds and vectors with possible sensory impact (Odour Activity Values, OAV>1) and significantly different among the 21 studied wines; maximum and minimum OAVs found in the set of wines, and the ability to differentiate (OAVMax/OAVMin >2) among wines. Significance (P-value): ****P<0.001; ***P<0.01; **P<0.05, *P<0.1.

COMPOUNDS	OAV_{Max}	OAV_{Min}	OAV_{Max}/OAV_{Min}	Significance P-value[a]
acetaldehyde (free)	10.9	0.2	47.3	**
branched fatty acid vector	114.0	3.0	38.7	****
β-damascenone	53.8	3.7	14.4	****
methoxypyrazine vector	5.6	0.7	8.4	****
methoxyphenol vector	5.4	0.9	6.3	****
acetic vector	8.2	1.8	4.5	****
isoaldehyde vector	19.5	5.2	3.8	****
isoamyl acetate	10.0	2.7	3.7	**
linear fatty acid vector	14.0	4.0	3.5	****
phenylacetaldehyde	22.0	6.7	3.3	***
γ-lactone vector	1.3	0.5	2.9	****
methional	55.8	22.5	2.5	*
ethyl ester vector	36.6	15.8	2.3	****

Firstly, because they were above their sensory threshold (OAV >1) and secondly because the difference in odour activity among wines was important enough to induce sensory differences (measured as the ratio of OAVmax/OAVmin). Figure II-2.3, shows the PCA with these 13 volatile-related parameters projected as active variables and four aroma-related variables ("raisin", "red fruit", "black fruit" and "oxidation") projected as illustrative variables. As can be seen in the plot, the first principal component (PC1), explaining 75% of the total variance, confronts samples with "black" and "red fruity" aromas (projected on the right side of the plot) to wines projected on the left part of the plot with "raisin" (BLA_4) and "oxidation" attributes (BLA_1 and BLA_2; BLB_1 and BLB_2). These fresh fruity aromas are positively correlated to the isoamyl acetate compound (with banana-like aroma), which has been described to be an undeniable contributor to fruity nuances in young red wines (Ferreira et al., 2002). Different ethyl ester profiles are suggested to be responsible for the appearance of specific red (higher levels of linear ethyl esters) or black (higher levels of branched ethyl esters) fruit aromas (Pineau, Barbe, Van Leeuwen, and Dubourdieu, 2009). However, such differences were not observed in the studied wines. Contrarily, sample BLA_1, with the highest level of ethyl esters, presented outstanding "oxidation" scores that were also present in samples BLB_1, BLB_2 and BLA_2. These oxidation notes can be easily explained in terms of aldehydes including phenylacetaldehyde, and isoaldehydes, free acetaldehyde and methional. This suggests a possible masking/suppressor effect generated by the aldehydes (Culleré, Cacho, & Ferreira, 2007) as well as the acetic acid vector (San Juan, Ferreira, Cacho, & Escudero, 2011) on the fruity character of ethyl esters, especially in BLA_1.

The appearance of oxidation nuances in wines elaborated with grapes harvested at earlier points can be explained in terms of higher levels of oxidation related aldehydes. Interestingly, this observed wine oxidation seems to be related to the presence of lower polyphenolic contents. More specifically, the levels of both the isoaldehyde vector and phenylacetaldehyde present significant ($p < 0.05$) negative linear correlations with total polyphenol content (TPI) ($r = -0.886$, $r = -0.843$), tannin

concentration (r = -0.782, r = -0.791) and not precipitable anthocyanin-derivative pigments (MP+SPP) (r = -0.810, r = -0.772). This result is well in line with models predicting accumulation of oxidation- related aldehydes calculated by Bueno et al. (2018). In these models, the accumulation of these compounds was negatively correlated to the content in distinct polyphenolic compounds (anthocyanins and tannins), which were denominated aldehyde reactive polyphenols (ARPs). Thus, the lower levels of polyphenolic compounds acting as ARPs found in wines elaborated with prematurely harvested grapes could explain the higher OAVs values observed for both the isoaldehyde vector and phenylacetaldehyde compound, thus the appearance of oxidation nuances in these wines.

Sample BLA_4 presents a specific “raisin” aroma, which could be related to the presence of higher levels of β-damascenone (San Juan et al., 2011). Interestingly, higher levels of this norisoprenoid have been found in wines elaborated with dehydrated grapes compared to those made with fresh grapes (Bowen & Reynolds, 2012; Genovese, Gambuti, Piombino, & Moio, 2007). This is well in accordance with studies that observed the formation of β-damascenone in grapes during the on-vine dehydration process (Lan et al., 2016). This could be the case of sample BLA_4, which was elaborated with the ripest grapes, which were probably overripe.

3.3.2. Astringency

“Astringency” scores range between 0.53 and 3.6 (maximum possible score of 7); significant differences among wines (F = 18.17; P < 0.001) were observed. Table II-2.5 shows 14 variables which could potentially be involved in the formation of astringency perception. All of them were significantly different (P < 0.001) within the 21 wines studied.

“Astringency” scores present significant (p < 0.001) positive linear correlations with six of the 14 chemical variables studied, such as ethanol content and polyphenolic related variables, including total polyphenol index, tannin activity and anthocyanin derivative pigments (such as monomeric and large polymeric pigments).

Table II-2. 5. Conventional parameters and phenolic-related parameters analysed in the 21 wine samples of the study. Maximum, minimum, median, quotient of maximal and minimal level, and Pearson correlation coefficients (r) between sensory astringency and chemical variables. *Chemical variables with significant lineal correlation with astringency are marked in bold (P<0.01)

	max	min	median	max/min	r (astringency)
pH	3.3	3.1	3.3	1.1	0.25
titratable acidity (TA) (g/L)	6.8	5.8	6.1	1.2	0.22
ethanol content (% v/v)	**15.8**	**11.8**	**13.4**	**1.34**	**0.69***
colour intensity (CI) (a.u.)	**14.0**	**4.30**	**6.40**	**3.26**	**0.79***
total polyphenol index (TPI) (a.u.)	**53.0**	**21.0**	**25.5**	**2.52**	**0.75***
tannin activity (TA) (-J/mol)	**2765**	**739**	**1007**	**3.74**	**0.83***
tannin concentration (TC) (mg/L)	4193	1794	2192	2.34	0.57
monomeric pigments (MP) (a.u.)	**0.96**	**0.26**	**0.42**	**3.7**	**0.76***
small and large polymeric pigments (SPP+LPP) (a.u.)	**0.60**	**0.17**	**0.25**	**4.1**	**0.79***
mean degree of polymerisation (mDP)	2.9	1.2	1.7	2.3	-0.17
% of procyanidins in tannins (%PC)	78.0	40.9	67.2	1.90	-0.27
% of galloylated tannins (%G)	2.33	0.639	1.21	3.64	0.39
% of prodelphinidins in tannins (%PD)	10.6	2.65	6.00	3.98	-0.22
% of malvidin in tannins (%M-T)	53.4	9.14	25.3	5.85	0.27

The role played by ethanol content in astringency perception is contradictory since there are previous studies that show a reduction in astringency with increasing ethanol content (Vidal et al. 2004), which is attributed to a decrease of the strength of interaction between tannin and protein in presence of increasing levels of ethanol (McRae et al., 2015). Contrary to these reports, and in accordance with the presented results, other papers establish positive relationships between ethanol content and astringency perception (Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2012; Sáenz-Navajas, Fernandez-Zurbano, Tao, Dizy, & Ferreira, 2010; Sáenz-Navajas et al., 2019; Watrelot, Byrnes, Heymann, & Kennedy, 2016), which can be attributed to an indirect relationship with phenolic content. Grapes harvested at earlier stages present lower levels of extractable polyphenols but also lower sugar content, yielding wines with lower ethanol and polyphenolic concentrations, and resulting in lower astringency. However, it cannot be ruled out that ethanol can induce astringency related sensations by mechanisms other than polyphenol-protein interactions.

Regarding anthocyanin related compounds, both bleachable anthocyanins (or monomeric anthocyanins, MP) and non-bleachable (or polymeric pigments, SPP+LPP) present significant positive correlations with “astringency” (Table II-2.5) ($r = 0.76$; $p < 0.001$ and $r = 0.79$; $p < 0.001$ respectively). Recent studies suggest that certain anthocyanins could be involved in the modulation of taste and/or mouthfeel properties (Ferrer-Gallego et al., 2015; Paissoni et al., 2018; Sáenz-Navajas et al., 2017, 2018b).

Interestingly, tannin activity is highly correlated ($r = 0.91$; $p < 0.01$) with “astringency”. This is the first time that this variable, which measures the affinity of tannins to a hydrophobic surface (polystyrene divinylbenzene HPLC column), has been found to be related to sensory perception. On the other hand, this grape variety from a similar origin, having been processed with the same winemaking protocol, could have helped to establish such an interesting linear relationship between tannin activity and sensory astringency, even though this is in contradiction with other studies in which no significant correlation could be found, and where a possible effect of aroma or even other non-volatile components on astringent perception was suggested (Watrelot et al., 2016).

4.Conclusions

In the present research, the effect of Moristel grape maturity on wine sensory and chemical composition was studied. This variety is an interesting minor variety with potential to be cultivated under warm climates. With the experimental winemaking protocol herein presented Moristel grapes yielded wines with relatively low pH values, high red colour with relatively low tannin activity, tannin and pigmented tannin concentrations that, harvested at the optimal point, are able to yield wines with fresh fruity aroma and moderate astringency. Interestingly, Moristel grapes prematurely harvested yielded “oxidation” aroma nuances. This attribute is related to free acetaldehyde, methional, phenylacetaldehyde and isoaldehydes as well as to low levels of aldehyde reactive polyphenols (tannins, and anthocyanins

that do not precipitate with ovalbumin: MP and SPP). Contrarily, grapes having suffered on-vine dehydration induce the development of “raisin” aroma in wines, which is suggested to be due to the formation of β-damascenone already in the grapes. Astringency is related to ethanol content, tannin activity (measured as the interaction of tannins with a hydrophobic surface) and the content in anthocyanin - derivative compounds.

One potential limitation of the present study is the use of OAVs of individual or groups of compounds to predict aroma intensity, which should be considered only a first and rough approximation. Rather, odour intensity should be used, for which the psychophysical curves of main odorants should be first determined as well as the additivity of the signals as recently suggested (de-la-Fuente-Blanco, Sáenz-Navajas, Valentin, & Ferreira, 2020).

At present, further studies are being carried out to find the grape precursors that yield aldehydes in wines, this which would help to control grape quality and define the optimal point of harvest. Besides this, it would be interesting to perform similar studies in on other grape varieties to elucidate if this tendency to generate oxidised wines with unripe grapes is a general tendency or, on the contrary, is cultivar-dependent.

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SECTION III

STUDY OF MOUTHFEEL PERCEPTION IN RED WINES

SECTION III. CHAPTER 1

Modelling wine astringency from its chemical composition using machine learning algorithms

1.Introduction

Consumption experience and thus wine appreciation is the result of interactions between the consumer and product's properties (Charters and Pettigrew, 2007; Prescott et al., 2002). Product-related factors refer to both intrinsic and extrinsic categories (Jover et al., 2004). Intrinsic cues are related to flavour, while extrinsic cues refer to properties that are not physically part of the wine, such as bottle weight, bottling place, type of wine or appellation, etc. While consumers rely on both types of cues when selecting a product (D'Alessandro and Pecotich ,2013), there is a wide body of work focused on understanding the impact of extrinsic cues on wine appreciation. However, much less is known about the intrinsic cues, i.e. flavour.

A product's flavour is the result of the interaction between sensory modalities including colour, aroma, taste and mouthfeel. In complex systems, the formation of mouthfeel is the least understood overall. This is especially true for astringency perception, which is reported to be mainly driven by alcohol content, polyphenolic compounds, their interaction with oral components (i.e. saliva, mucosa and oral receptors) and brain processing (Canon et al., 2018). There is a lack of consensus in the scientific literature when defining the perceptual phenomenon and mechanisms driving astringency, however, the interactions between phenolics and proteins seem to be the most important driver (García-Estévez et al., 2018). It is important to note that there is great interindividual variability in astringency perception, which has been attributed to differences in saliva composition and buccal microbiota among consumers (Lamy, 2018). This variability in salivary proteome is related to differences in the sensory sensitivity for astringency (Lamy et al., 2017), and has shown to influence the acceptability of phenol-rich foods (Dinnella et al., 2011; Masi et al., 2015).

Tannins have been reported as important drivers of wine astringency, which is mainly understood as dryness perception. These molecules are constituted by catechin or epicatechin (procyanidins), epigallocatechin (prodelphinidins) or epicatechin gallate (gallocatechins) units linked through C4–C8 interflavanoid bonds. Differences in constitutive molecules, concentrations and mean degree of polymerisation (mDP) (Ma et al., 2014) have been reported to have an impact on wine astringency. Recently, tannin activity, measured as the enthalpy of interaction between polyphenols and a hydrophobic surface, has been hypothesised to correlate with wine astringency (Revelette et al., 2014; Watrelot et al., 2016). However, the correlation of sensory astringency with the different chemical variables that characterise phenolic compounds is far from clear. This difficulty in establishing consistent relationships between phenolic compounds and astringency has been attributed to: 1) a lack of enough chemical variability among the samples studied to induce significant sensory differences; 2) a lack of analytical relevant variables analysed in samples; or 3) the presence of cross-modal sensory interactions between aroma or taste properties with astringency and thus the capacity of other sensory modalities to modulate astringent perception (Watrelot et al., 2016). Besides these limitations, in the present work it is hypothesised that relationships between phenolic chemical properties and astringency do not necessarily have to be linear, as most studies have tried to show.

In this context, the present work aimed to firstly generate wines with enough phenolic variability able to induce astringency differences and then model astringency by applying different machine learning algorithms.

The first hypothesis was that grapes from the same variety (Moristel in this case), harvested at different maturity levels and processed with a similar winemaking strategy would yield wines with maximal variability in phenolic composition and most probably astringency, and with minimal aroma variability (as observed among wines from different varieties and winemaking processes). This would reduce the presence of cross-modal interactions. The second hypothesis was that sensory–

chemical relationships between phenolics and astringency do not necessarily have to be linear.

2.Materials and Methods

2.1. Samples and winemaking

Eleven different wines were produced in 2017 with Moristel red grapes (*Vitis vinifera L.*) at the Pirineos winery (Barbastro, Spain). Four different vineyard blocks were selected based on historical data related to maturity evolution as measured by Dyostem® (Vivelys, France). According to commercial information, this tool monitors sugar loading and changes in the colour of the fruit to determine the maturity level of grapes (i.e. polyphenolic and technological maturities related to sugar and acidity levels) and optimal harvest date. Based on this approach, one block did not experience any significant maturity evolution in a four-week period, and thus it was harvested only once. In contrast, the other three blocks were harvested at three or four different points, each separated by one or two weeks. According to the commercial system, the second point of maturity was the optimal point to harvest; therefore, the fruit was harvested one week before and one and/or two weeks after this optimal point, to ensure grapes with different maturity levels and thus, a priori, maximal variability in chemical composition and most probably in astringency.

Grapes were manually harvested from the Somontano region (Huesca, Spain). For each of the 11 selected vineblocks and/or maturity points, 150 kg of fruit was harvested and processed with an automatic crusher/destemmer. Each lot was divided into three stainless steel tanks (75 L capacity), sulphur dioxide was adjusted to 50 mg L^{-1} and the fruit was further inoculated with commercial yeasts (Lalvin ICV D254, Lallemand) at 106 cells mL^{-1}. Alcoholic fermentations (FOH) took place on skins for an average of 10 days. Once FOH was finished, lactic bacteria (Lalvin VP41) were inoculated at a rate of 9 mg L^{-1}. Wines were bottled around 3 months after FOH (free SO2 adjusted to 30 mg L^{-1}). Glass bottles (750 mL capacity) were sealed with natural cork closures.

2.2. Chemical characterisation of wines

2.2.1. Conventional oenological analysis

The total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon 1970) and colour intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories 1984). For TPI determination, the absorbance at 280 nm of samples diluted 1:100 in deionised water was measured in 1-cm quartz cuvettes. For CI, absorbance of undiluted samples was measured in 2-mm crystal cuvettes. Reducing sugars, ethanol content, pH, malic and lactic acid, as well as titratable and volatile acidities, were analysed using infrared spectrometry with Fourier Transformation with a WineScanTM FT 120 (FOSS®, Barcelona, Spain), which was previously calibrated with the official OIV methods.

2.2.2. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) in wines and fractions was carried out as described elsewhere (Harbertson et al., 2003). MPs were the group of compounds bleachable with bisulphite, while SPP and LPP were resistant to bisulphite bleaching. SPP did not precipitate with ovalbumin, in contrast with LPP, which did. Levels of MP, SPP and LPP were expressed as absorbance at 520 nm.

2.2.3. Characterisation of tannins

Acid-catalysed degradation in the presence of toluene-α-thiol was performed according to the method described by Labarbe et al. (1999) but with some modifications as described by Gonzalo-Diago et al. (2013). Quantification was performed in the negative mode from the extracted ion chromatogram (EIC) for flavan-3-ols and in the positive mode for malvidin-3-O-glucosyde. The area under the peaks of malvidin-3-O-glucosyde and flavan-3-ol monomers (terminal units) before and after thiolysis, as well as toluene-α-thiol adducts (extension units) released from the depolymerisation reaction, were integrated.

Calibration curves were established with malvidin-3-O-glucosyde, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, and (−)-epigallocatechin. In the absence of the standards of the thiol derivatives and considering that the thiolytic derivatives were shown to have similar response factors as the correspondent monomeric units, their concentrations were calculated from the respective monomer calibration curves. The mean degree of polymerisation (mDP) was calculated as the ratio of total units (extension + terminal) to terminal units (calculated as the difference before and after thiolysis). The % of tannins linked to malvidin-3-O-glucosyde (%T-M) was calculated as the molar ratio of malvidin-3-O-glucosyde linked to tannins (calculated as the difference before and after thiolysis) to the sum of the total units of terminal malvidin-3-O-glucosyde and extension + terminal units of (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, and (−)-epigallocatechin (i.e. total units of tannins). The % of procyanidins (%PC) was calculated as the ratio of total units (extension and terminal) of catechin and epicatechin to total units of tannins. The % of prodelphinidins (%PD) and galloylated (%G) units was calculated as the ratio of total units of PD and G to the total units of tannins, respectively.

The % of malvidin-3-O-glucosyde linked to tannins (%M-T) was calculated as the molar ratio of the malvidin linked to flavanols (difference of malvidin before and after thiolysis) to total malvidin (before and after thiolysis).

Concentration and activity of tannins were estimated using HPLC-UV-Vis following the method proposed by Revelette et al. (2014). Tannin activity is related to the thermodynamics of interaction between tannins and a hydrophobic surface (polystyrene divinylbenzene HPLC column) as discussed elsewhere (Barak and Kennedy, 2013; Revelette et al., 2014).

2.3. Sensory characterisation of wines

2.3.1. Participants and procedure

The 33 wines produced (11 different wines in triplicate) were evaluated sensory characterised in February and March 2018 by 17 participants at the Instituto de Ciencias de la Vid y del Vino (ICVV) and Universidad de La Rioja (Spain). The participants were mainly oenology students and oenologists (11 women and five men, age range 22–34 years, average age 28) recruited on the basis of interest and availability and were not paid for their participation. They attended a total of 13 sessions spread over four weeks, comprising nine training sessions (90 min each and taking place at 12 p.m.) and four sessions to describe the wines studied (eight or nine samples per session). The participants worked in two subgroups and followed the same guidelines. The first session was devoted to generating aroma terms that differed among samples. During the following training sessions, reference standards (prepared at Universidad de Zaragoza) were presented for the 12 selected aroma terms and six for taste and mouthfeel terms. For in-mouth terms, solutions were prepared containing different concentrations of table sugar (0–7 g L^{-1}) for testing sweetness, tartaric acid (0–3 g L^{-1}) for acidity, quinine sulphate (0–40 mg L^{-1}) for bitterness and potassium, aluminium sulphate (0–5 g L^{-1}) for astringency, absolute alcohol (0–15% v/v) for alcoholic sensation and carboxymethylcellulose (0-1.5 g L^{-1}) for viscosity. During a typical training session, the participants were presented with references illustrating the different aroma, taste and mouthfeel terms. Next, between two and four wines were first individually described and then the ratings were discussed until the participants achieved consensus.

The wines were described in the last four sessions: participants were asked to taste the wines and rate the intensity on a 7-point scale (1 = very low; 7 = very high) using only those terms (out of 18 available terms) that applied to the sample, according to rate-all-that-apply (RATA) methodology (Ares et al., 2014). Terms that did not apply to the wine were allocated a value of 0 when collecting data. To avoid

bias due to order of presentation, terms in the list appeared in different and randomised orders for each assessor. The use of a sip (rinsing solutions: water and 1 g L-1 pectin solution) and spit protocol between each sample was imposed as described elsewhere (Colonna et al. 2004). Participants tasted samples in a sequential monadic manner: 20-mL samples were served in dark wine glasses labelled with random three-digit codes and covered with plastic Petri dishes according to a random arrangement that was different for each participant. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room at around 20 °C.

2.3.2. Data analysis

Only the data for astringency are reported here.

The discriminability potential of chemical variables among wines was calculated as the rate between maximal and minimum level (max/min) for each variable.

Two-way ANOVAs (participants as random and wines as fixed factors) were calculated for the term 'astringency'. Next, a pair-wise comparison test (Fischer test) was applied (5% risk) using XLSTAT (2015).

The first step for modelling was to search for the best simple models and with them ensemble of models was calculated to search for the best model by a machine learning approach specifically designed for the present project. Machine learning algorithms were boosted by SDG using the DataRobot Platform. Therefore, dozens of independent challenger models were developed and validated by cross-validation. Model accuracy was evaluated by root-mean-square error (RMSE), i.e. differences between astringency scores predicted by a model and the scores observed. A robust model k-fold cross-validation framework to test the out-of-sample stability of each model was employed. In addition to the cross-validation partitioning, a holdout sample was calculated to further test out-of-sample model performance and ensure that overfitting did not occur. Therefore, 18% of the training data was set aside as a holdout dataset. This dataset was used to verify that the final model performs well

on data that has not been examined throughout the training process. For further model validation, the remainder of the data was divided into five cross-validation partitions (selected by random sampling). For the best models, five-fold cross-validation training and scoring was completed. Then, the mean score of the complete model cross-validation was calculated across all folds.

The best model for astringency consisted of applying a smooth ridit transformation followed by the calculation of the support vector regressor (SVR; radial kernel). The ridit (or score for a variable) transformation can be interpreted as an adjusted percentile score and extends the Bross ridit method (Bross 1958) by applying the method to numerical values and normalising the score such that the mean calculated for the reference population will always be 0 and the score will be in the interval [-1,1]. The SVR is a generalised version of support vector machine classifiers that uses a special loss function to convert a regression problem into a classification problem. Support vector machines (SVMs) are an extremely robust machine learning model and are very efficient in high-dimensional spaces. In addition, a “kernel” function was used, which allows for a non- linear transformation of the data before fitting the SVM. These kernel functions can be a very useful way to transform a non-linear problem into a linear domain.

The permutated impact of variables on the models is calculated by observing the effect on model scores when altering the input data of a given variable. The algorithm employed normalises the scores so that the values of chemical variables included in the model are normalised to the most important variable (%PC).

3.Results and discussion

The first objective of the present work was to produce Moristel wines with important chemical variabilities, focusing on parameters typically known to be related to astringency perception. Results show that for the 20 chemical variables analysed, highly significant differences (P<0.0001 in all cases) were observed among the 33 wines. Table III-1.1 shows ranges and median values of the parameters measured. These data show important chemical variability among wines, with particular importance placed on the differences, and thus the discriminability potential (measured as the rate max/min) among wines for lactic acid (max/min = 70.8), % of galloylation of tannins (max/min = 55), mean degree of polymerisation of tannins (max/min = 41) and % of prodelphinidins constituting tannins (gallocatechins and epigallocatechins) (max/min = 11).

Table III-1. 1. Chemical variables analysed in the 33 wine samples in the study. Range of occurrence, median, rate of maximal and minimal level, Pearson correlation coefficients (r) between sensory astringency and chemical variables.

	range	median	max/min	r (astringency)
pH	3.1-3.4	3.3	1.1	0.24
volatile acidity (VA) (g/L)	0.3-0.5	0.4	2.1	0.59
total acidity (TA) (g/L)	5.3-7.0	6.0	1.3	0.02
reducing sugars (RS) (g/L)	1.6-2.7	2.3	1.7	0.34
malic acid (MA) (g/L)	0.2-0.8	0.4	5.5	0.16
lactic acid (LA) (g/L)	0.0-0.5	0.3	70.8	-0.45
ethanol content (% v/v)	**12.3-15.8**	**13.2**	**1.3**	**0.71***
colour intensity (CI) (a.u.)	**4.8-15.3**	**11.8**	**3.2**	**0.65***
total polyphenol index (TPI) (a.u.)	**22-53**	**43**	**2.4**	**0.72***
tannin activity (TA) (-KJ/mol)	**853-2751**	**1422**	**3.2**	**0.77***
tannin concentration (TC) (g/L)	1993-4188	2714	2.1	0.51
monomeric pigments (MP) (a.u.)	**0.4-1.0**	**0.8**	**2.9**	**0.65***
small polymeric pigments (SPP) (a.u.)	0.2-0.5	0.4	2.8	0.32
large polymeric pigments (LPP) (a.u.)	0.1-0.4	0.2	4.6	0.25
mean degree of polymerisation (mDP)	0.1-2.8	1.3	28	0.51
% of procyanidins in tannins (%PC)	51-85	69	1.7	-0.62
% of prodelphinidins in tannins (%PD)	**0.6-7.3**	**2.9**	**11.4**	**0.66***
% galloylated tannins (%G)	0.2-10.1	1.9	54.9	-0.40
% of tannins linked to malvidin (%T-M)	10.7-39.5	23.8	3.7	0.61
% of malvidin linked to tannins (%M-T)	11.1-32.2	20.6	2.8	0.34

It is important to note that bitterness and astringency do not present significant linear correlations (r=0.40, P>0.1), which confirms that the participants were not confused and were able to differentiate both sensations (Lea and Arnold, 1978). Astringency scores ranged between 0 and 4 (6 being the maximum possible score) and significant differences among wines (F=15.13; P<0.0001) were observed. These data confirm our first hypothesis related to the strategy followed (selection of grapes from different vineblocks at different maturity points) to generate wines with different chemical compositions, inducing sensory differences in astringency. Table III-1.1 shows that astringency scores present significant (P<0.05) positive linear correlations with six of the 20 chemical variables studied: tannin activity, TPI, ethanol content, % of prodelphinidins in tannins, colour intensity and monomeric pigments.

Our second hypothesis was that sensory and chemical composition do not necessarily follow a linear correlation (i.e. the higher/lower concentration the higher astringency). Thus, astringency scores were modelled from the 20 chemical variables using machine learning algorithms. A highly satisfactory model was obtained. Residual error (measured through root-mean-square deviation, RMSE, by full cross-validation) was 0.19. The best algorithm was SVR (radial kernel). Figure III-1.1 shows the lift chart, which confirms model performance and thus its capability to predict astringency. Model performance was evaluated by calculating possible predictions partitioned into subsegments, deciles or bin. For each bin, average predicted astringency scores (blue line) were compared to average actual values (orange value). Both predicted and actual scores were very closely projected and lines consistently increased, both being indicators of satisfactory model performance and the accuracy of the model.

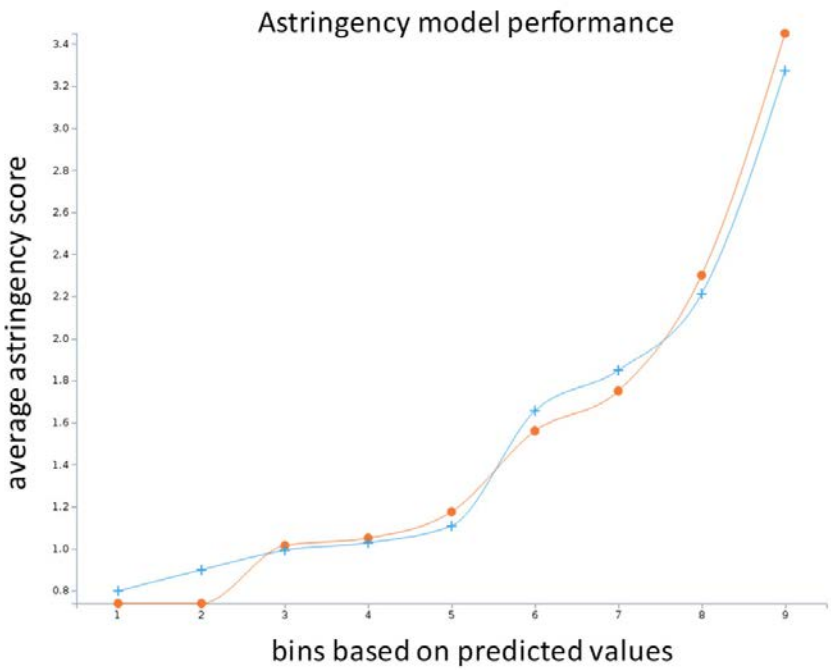

Figure III-1. 1. Lift charts for astringency model. Lines in orange and blue displays the average actual and predicted scores for a given bin.

Figure III-1.2 shows the permutated impact of chemical variables on the model. Half of the chemical variables (10/20) were included in the model, with the most important of these the % of procyanidins constituting tannins (%PC) and ethanol content (1.0 and 0.98 of normalised impact, respectively). The next important variables, all with normalised impact factors lower than 0.5, were TPI, % of prodelphinidins constituting tannins (%PD), mean degree of polymerisation of tannins (mDP), volatile acidity, tannin activity, % of tannins linked to malvidin (%T-M) and % of galloylated (%G) flavanols constituting tannins.

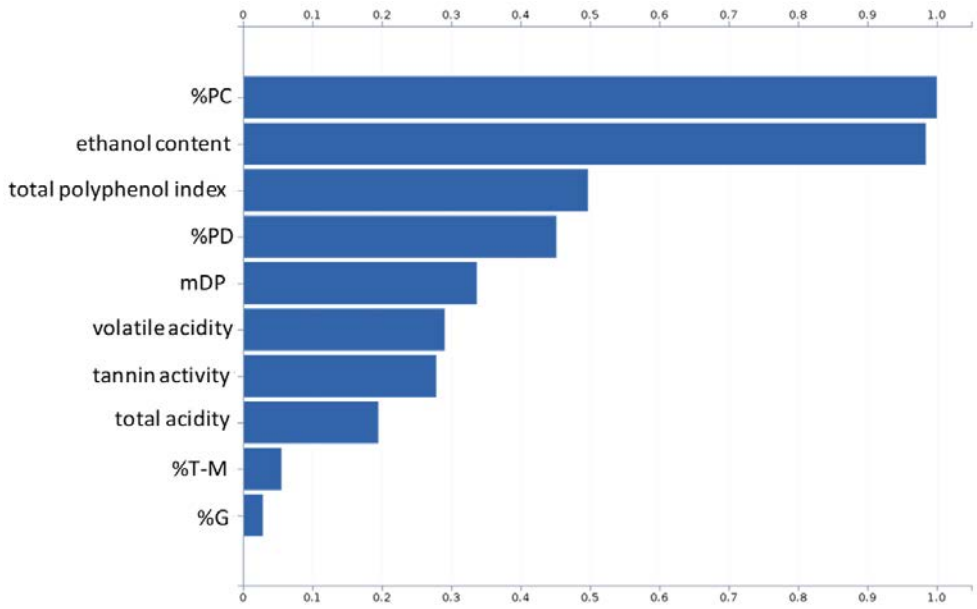

Figure III-1. 2. Permutated impact factor of chemical variables included in the astringency model.

These results confirm the importance of tannin structure, ethanol content and acids (measured as titratable acids or total acidity and volatile acidity) on the modulation of astringency perception, which is not surprising given the many publications that mention these variables as important drivers of this sensory perception in wine (Ma et al., 2014; Sáenz-Navajas et al., 2012; Soares et al., 2017; Watrelot et al., 2016). However, most of the existing literature tries to establish linear correlations between astringency and chemical composition (i.e., higher/lower levels of a component generate higher astringency), which could be the main source of contradictory results reported when establishing sensory-chemical relationships. In the present work, different tendencies in astringency perception can be observed depending on the levels of a given chemical variable Figure III-1.3. (More details Annexe III-1.1).

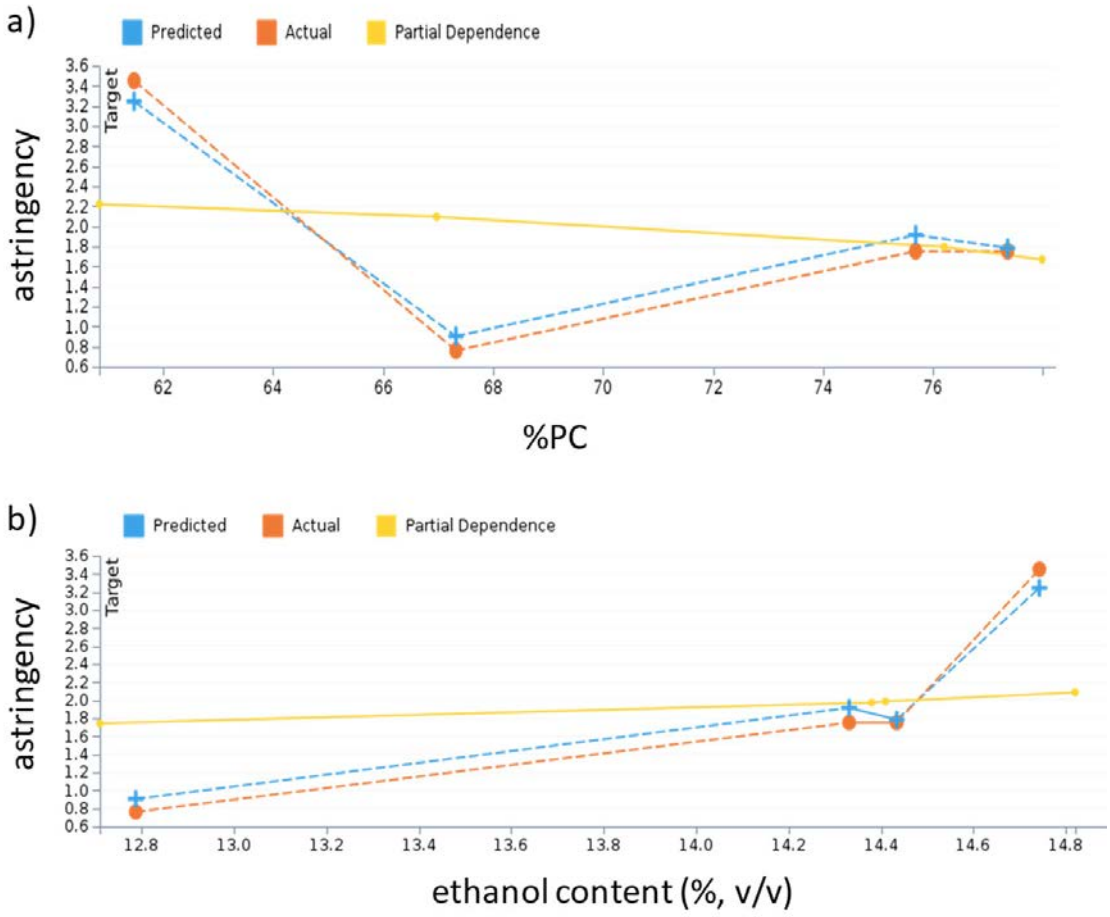

Figure III-1. 3. Partial dependence plot of astringency and a) %PC and b) ethanol content.

Figure III-1.3a shows the partial dependence plot of astringency and % procyanidins (%PC) in tannin structures. Three main tendencies could be identified depending on the %PC: 1) a steep negative linear relationship for %PC< 68%; 2) a moderate positive linear relationship for the 68–76% range, and 3) no change in astringency associated with different %PC for %PC>76%. Interestingly, the % of total (extension + terminal) catechins and epicatechins (%PC) presents a significant correlation with the % of epicatechin units ($r = 0.80$; $P < 0.001$). Thus, this modulation of astringency with %PC could be attributed to changes in the stereochemistry of tannins related to the % of epicatechins in procyanidins. Thus, at low levels of epicatechin subunits, astringency decreases with increases in terminal and extension epicatechins in tannins. However, at intermediate levels (68–76%) of epicatechin units in tannins, increasing epicatechins generate higher astringency, but at higher

levels no effect is observed. Results observed for intermediate levels (68–76%) are in agreement with results reported in the literature (Quijada-Morin et al., 2012), in which higher astringency is observed for tannins with higher proportions of epicatechin than catechin subunits. However, in the present non-linear model, two further trends could be identified depending on the % levels of PC (one with opposite negative effect for low levels of PC and other with no effect for higher levels of PC). These results could be explained in terms of structural/conformational differences of tannins with different structural properties that have different site-specific bindings with tannin (De Freitas and Mateus, 2001; Thorngate and Noble, 1995). Further research in this topic should be carried out to find a plausible explanation.

Figure III-1.3b shows a general positive correlation of astringency and ethanol content with two main tendencies: 1) <14.4% of ethanol (moderate positive linear correlation) and 2) >14.4% of ethanol (steep positive linear correlation). This is well in accordance with data reported for commercial wines containing ethanol levels of 13–17% (v/v) (Saenz-Navajas et al., 2010; Sáenz-Navajas et al., 2012; Watrelot et al., 2016) but contradicts studies carried out with model wines at typical wine ethanol levels of 11–15% (Fontoin et al., 2008; Vidal et al., 2004). These studies report a decrease in astringency with ethanol content, which has been attributed to a decrease of the interaction power between tannins and proteins from 10% of ethanol (hydrophobic + hydrogen-bond interactions) to 15% (hydrogen-bond interactions) (McRae et al., 2015). Thus, the positive correlation between ethanol content and astringency observed in the present work could be attributed to an indirect relationship with phenolic content. Grapes harvested at earlier stages present lower levels of extractable polyphenols but also lower sugar content, yielding wines with lower ethanol levels and polyphenolic concentration and resulting in lower astringency. However, it cannot be ruled out that ethanol can induce astringency-related sensations by mechanisms other than polyphenol-protein interactions. This would be supported by an important number of papers that have established positive relationships between ethanol content and

astringency perception (Saenz-Navajas et al., 2010; Sáenz-Navajas et al., 2012; Watrelot et al. 2016). Additional investigation is needed to understand the relationship between ethanol and astringent sensations.

For the rest of chemical variables included in the model, three different tendencies were globally identified (Annexe III-1.1).

The first trend is observed for the mean degree of polymerisation. For low mDP values (up to approximately 1.4) astringency increases with mDP, while for higher values astringency decreases. This result is well in accordance with the positive linear relationships observed between astringency and DP with low molecular tannins by Peleg et al. (1999). Interestingly, Chira et al. (2009) also found significant (P = 0.04) positive correlations, but only for skin tannins in one of the two years studied. This sample set presented an average mDP of 21 (range 4–49), which is far out of the range for the wines studied here. This lack of significance for the rest of sample sets (year 2016 and seed tannins of 2006 and 2007) could be attributed to the presence of different relationships depending on the level of mDP as observed in the present work. The effect of the size of tannins (measured through the mDP) on astringency could be explained in terms of tannin hydrophobicity. Thus, even if higher tannin polymerisation can bring more hydrophobic parts, and thus higher astringency (due to higher tannin-protein interactions), this relationship is thought not to be linear and is attributed to conformational arrangements and aggregation processes (Ma et al., 2014).

The second trend is observed for total acidity, volatile acidity, % of prodelphinidins (%PD), total polyphenol index (TPI) and tannin activity (measured as the enthalpy of interaction of tannins with a hydrophobic surface). These present positive linear relationships with astringency, with this relationship more pronounced at higher values of the corresponding chemical variable and astringency perception. It has been shown that the effect of acidity on astringency is attributed to changes in pH. Thus, for similar pH values, changes in acidity do not have

significant effects on astringency (Fontoin et al., 2008). This increase of astringency is attributed to the presence of more phenolate forms and an augmentation of charged molecules, susceptible to participating in protein binding (Siebert and Euzen, 2008). Concerning the positive relationship observed between astringency and %PD, it is interesting to note that this is more important at higher levels (range 1.8–3.2) of astringency. This result is in apparent contradiction with other studies, which have shown in simple model solutions that procyanidins present a faster and stronger interaction with salivary proteins than prodelphinidins (Ferrer-Gallego et al., 2015). At present it is difficult to explain such a relationship, because it is likely that astringency differences related to polyphenolic structures are the result of conformational differences among tannins that cannot yet be measured in such a complex mixture such as wine. To this regard, the measure of tannin activity by HPLC seems to be a promising index. Tannin activity is a parameter that measures the enthalpy of interaction of tannins with a hydrophobic surface. It appeared as an interesting measure of tannin affinity to proteins and thus of wine astringency (Revelette et al., 2014). However, until now no direct relationship with tannins could be established that was attributed to the presence of strong interactions (with polysaccharides or aroma perception) appearing in wines with very different chemical and sensory spaces (i.e., different varieties, winemaking processes, origins, etc) (Watrelot et al., 2016). Thus, working with the same grape variety from a similar origin and processed with the same winemaking protocol could have helped establish such interesting linear relationships between tannin activity and sensory astringency (i.e., drying sensation). It is interesting to note that this is the first time a relationship of this chemical variable with sensory perception has been established.

The third trend is related to the % of galloylated tannins (%G) and tannins linked to malvidin (%T-M), which show a similar relationship with astringency as % PC. Thus, two segments are observed: one for low levels of the chemical variable with a negative linear relationship with astringency and a second for high values with a

positive linear correlation. As explained above, the constitutive units of tannins as well as their polymerisation degree play an important role in tannin conformational structures, which drives tannin hydrophobicity, tannin-protein interactions and thus perceived astringency.

4.Conclusions

The present work has successfully modelled the perception of wine astringency from its chemical composition by applying machine learning approaches. This strategy has explained non-linear relationships by means of the SVR (radial kernel) algorithm, which showed a very low residual error between actual and predicted astringency scores. This and the fact that sensory perception is distinctly non-linear show the necessity of considering non-linear models to explain sensory precepts from chemical composition.

The main drivers of the astringency model were related to ethanol content (potentially elicited by a mechanism different from polyphenol-protein interactions), acidity (related to pH variations), as well as to effects of chemical variables linked to tannin structure, such as 1) the constitutive subunits of tannins (%PC, %PD, %G and %T-M), 2) tannin activity measured as the enthalpy of interaction with a hydrophobic surface, and 3) the size of tannins measured by the mean degree of polymerisation.

The results presented here increase understanding of astringency perception and provide wine producers with objective tools to help them control and optimise grape and wine production stages for further modulating astringency and thus maximising the quality and consumer appeal of their wines.

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SECTION III. CHAPTER 2

Effect of aroma perception on taste and mouthfeel dimensions of red wines: Correlation of sensory and chemical measurements

1.Introduccion

Product flavour is the result of the interaction between sensory modalities including perceptions of colour, aroma, taste and mouthfeel (Prescott, 2012). In complex systems, the sensation of mouthfeel is overall the least understood, and this is especially true in the case of red wine. Indeed, studies that relate wine appreciation of either experts or consumers with sensory properties tend to be limited to measuring a few mouthfeel-related terms such as overall astringency, hot sensation, body or viscosity (Hopfer & Heymann, 2014; Sáenz-Navajas et al., 2016; Sáenz-Navajas, Ballester, Pêcher, Peyron, & Valentin, 2013), in contrast to the array of aroma terms employed to characterise wines (Noble et al., 1987). However, wine experts, communicators and regular consumers use a wide range of terms related to mouthfeel cues to describe wines, and even relate them to wine judgements (e.g., rough, dry, strong, astringent, harsh, body, hard or tannin) (Gawel, Oberholster, & Francis, 2000; Sáenz-Navajas et al., 2016; Vidal, Giménez, Medina, Boido, & Ares, 2015). Even if it is well recognised that overall astringency is related to tannin content in red wines, this disconnect between scientific literature and popular use of mouthfeel-related terms can be attributed to the fact that other specific compounds and structures aside from tannins driving such properties have not been adequately defined. This generates a situation where there are no appropriate reference materials illustrating the different mouthfeel attributes, which results in the lack of a stable lexicon to characterise the whole continuum of mouthfeel perceptions for red wines.

In the last decades, different authors have worked on developing mouthfeel vocabulary for wines based either exclusively on consumers´ memory (Vidal et al., 2015) or on perception (i.e., with product exposure) (Gawel, Iland, & Francis, 2001; Gawel et al., 2000; Kang, Niimi, Muhlack, Smith, & Bastian, 2019; Pickering & Demiglio, 2008; Sáenz-Navajas et al., 2017). The studies involving perception have succeeded in finding mouthfeel differences among wines by both static or dynamic approaches, and acknowledged the fact that astringency and its subqualities change with time (Kang et al., 2019; Watrelot, Heymann, & Waterhouse, 2019). Most of these descriptive works have been carried out by trained participants with the aim of achieving consensus among them by presenting specific reference materials or definitions illustrating each attribute. The fact that the specific molecule or defined group of compounds eliciting mouthfeel-related sensations is still relatively unknown means that the reference materials may in some cases be confusing for panellists. Such confusion can be attributed in part to the fact that some reference standards are multidimensional and elicit more than one sensory property, including different astringent subqualities such as roughing, drying, and constricting or puckering (Kershaw & Running, 2019). For example, alum generates a dryness sensation together with sweet and sour tastes, and proanthocyanidin extracts elicit mouthfeel sensations due to different classes of flavan-3-ols, and have residual aromas, are bitter, and in some cases sour too.

As an alternative to conventional descriptive analysis and to overcome the limitation of the absence of appropriate reference materials, a variant of the rate-all-that-apply (RATA) methodology has been proposed to describe wines using an expert panel (Sáenz-Navajas et al., 2018; Sáenz-Navajas et al., 2017). This methodology can be called rate-K attributes by analogy to pick-K attributes, which is a variant of check-all-that-apply (CATA) (Coomb, 1964; Valentin, Chollet, Lelievre, & Abdi, 2012). In the rate-K attributes or pick-K attributes methods, a lower number of attributes have to be rated or selected to describe samples. These variants underline the salient attributes of the product, while RATA or CATA generate a more complete

characterisation (Valentin, Chollet, Lelievre, & Abdi, 2012). Globally, these kinds of consumer-based methods are not aimed at achieving consensus among participants, and consequently, participants are not trained: it is assumed that they are able to describe and find differences among products (Valentin, Chollet, Lelievre, & Abdi, 2012; Varela & Ares, 2012). RATA and rate-K attributes in particular have the advantage of requiring a smaller number of panellists compared to other consumer-based approaches such as check-all-that-apply (CATA) or pick-K attributes – which generate counts rather than rankings or intensities – especially if the panellists are trained or experts (Giacalone & Hedelund, 2016). These approaches work under the assumption that wine experts have constructed a specific prototype-based language to describe wines (Brochet & Dubourdieu, 2001).

Astringency is an important property of red wine and is arguably the most studied mouthfeel attribute (Cheynier et al., 2006; García-Estévez, Pérez-Gregorio, Soares, Mateus, & De Freitas, 2017; Gawel, 1998; Soares, Brandão, Mateus, & de Freitas, 2017). The perceptual phenomenon driving astringent-related mouthfeel is a source of controversy in the scientific literature, but interactions between various phenolics and salivary proteins seem to be the most accepted mechanism (García-Estévez, Ramos-Pineda, & Escribano-Bailón, 2018; Soares et al., 2017). As an alternative to sensory methods, different chemical measures have been proposed for assessing astringency. These include simple measures such as the classical absorbance at 280 nm or the more recently proposed wavelength at 230 nm (Boulet, Ducasse, & Cheynier, 2017; Boulet et al., 2016), measures based on precipitation with proteins or methylcellulose (Aleixandre-Tudo, Buica, Nieuwoudt, Aleixandre, & Du Toit, 2017), or more sophisticated measurements such as tannin activity based on the enthalpy of interaction between tannins and a hydrophobic surface (Revelette, Barak, & Kennedy, 2014; Sáenz-Navajas et al., 2019). Notwithstanding, the sensory percept of astringency has been reported to be influenced by other wine components such as alcohol, pH or acidity (Fontoin, Saucier, Teissedre, & Glories, 2008), polysaccharides (Watrelot, Schulz, & Kennedy, 2017), polyphenolic profile via

interaction with oral components (i.e., saliva or mucosa), and cognitive processing (Canon, Neiers, & Guichard, 2018) through aroma-astringency or taste-astringency interactions (Sáenz-Navajas, Campo, Avizcuri, et al., 2012; Sáenz-Navajas, Campo, Valentin, Fernández-Zurbano, & Ferreira, 2012; Saenz-Navajas, Ferreira, Campo, Fernandez-Zurbano, & Valentin, 2010; Sereni, Osborne, & Tomasino, 2016). However, the effect of aroma on taste or mouthfeel perception has been limited to the study of a reduced number of real wines or the exclusive evaluation of the global term astringency (Arvisenet, Guichard, & Ballester, 2016; de-la-Fuente-Blanco, Fernández-Zurbano, Valentin, Ferreira, & Sáenz-Navajas, 2017; Sáenz-Navajas, Campo, Avizcuri, et al., 2012).

Considering the importance of mouthfeel to red wine quality, the present study aimed to increase the understanding of red wine mouthfeel by exploring the hypotheses that interactions exist between aroma and mouthfeel perceptions and that tannin measures would correlate with mouthfeel characters. Addressing this, the effect of aroma on taste and mouthfeel properties of a set of 42 red young wines was investigated by 18 wine experts who described them by means of a rate-K attributes method (Sáenz-Navajas et al., 2017) under two conditions: with and without nose clips. In addition, relationships were assessed between mouthfeel and chemical measures including activity and concentration of tannins, and spectroscopic measures at both 280 nm and 230 nm.

2. Material and methods

2.1. Wines

A total of 42 non-wooded commercial and non-commercial red wines were selected. Commercial wines were purchased online, and non-commercial (still unblended) wines were provided by wineries. These were monovarietal wines elaborated from one of 14 different varieties, and were of different origins (mainly from Spain, but also from Argentina and France) and vintages. A detailed list of wine characteristics (origin, variety, vintage and conventional oenological parameters) is presented in Annexe III-2.1.

2.2. Sensory analysis

2.2.1. Participants

A volunteer sensory panel was comprised of 18 established winemakers from the Rioja area, Spain (15 women and 3 men, ranging in age from 23 to 54, with an average of 38 years old). They had extended experience in wine production and tasting (average of 13 years) and were considered to be experts according to Parr, Heatherbell, & White (2002).

2.2.2. Procedure

Samples were characterised by a rate-*K* attributes methodology, which is a variant of rate-all-that-apply (RATA), with a list of 23 taste and mouthfeel-related attributes (See Section I, and/or Table III-2.1) that were developed previously (Sáenz-Navajas et al., 2017) and another 20 aroma attributes (Table III-2. 2) obtained from the literature (Noble et al., 1987; Sáenz-Navajas, Gonzalez-Hernandez, Campo, Fernández-Zurbano, & Ferreira, 2012).

Participants attended four sessions on four different days, either at 12:00 pm or 6:00 pm. Each session was split into two sub-sessions (ca. 40 min each), which were separated by an imposed pause of 20 min. In each sub-session, panellists were presented with 10 or 11 wine samples with a 10-min break every 5 samples. In the

first sub-session, nose-clips were employed and only taste and mouthfeel (list of attributes in Table III-2.1) were characterised for the wines. In the second sub-session, orthonasal aroma (list of attributes in Table III-2. 2) was evaluated for the same wines as well as taste and mouthfeel attributes.

All participants evaluated the 42 wine samples. In the first sub-session (with nose clips, Figure III-2.1), they were instructed to take a sip of wine and slightly distribute it all over the mouth cavity, including palate and gums. Then, they had to select a maximum of 5 terms that applied to the sample and rate their intensity on a seven-point scale (1 = not intense; 7 = very intense) according to a methodology based on RATA (Ares et al., 2014; Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014). In the second sub-session, they were instructed to firstly smell samples orthonasally and describe each sample by the rate-*K* attributes method, then proceed as explained above for taste and mouthfeel properties. Terms that were not selected (maximum of five had to be rated for a sample) were allocated a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomised order for each assessor.

Figure III-2. 1. Sensory evaluation with nose-clips

Participants tasted samples in a sequential monadic manner and had to follow a sip (rinsing solutions: water and 1 g L^{-1} pectin solution) and spit protocol between each sample (Colonna, Adams, & Noble, 2004). Twenty-mL samples were poured 30 minutes prior to the session and served in normalised (German Institute for Normalisation, DIN) dark wine glasses (Sensus, Schott Zwiesel, Germany) labelled with random three-digit codes (different for each condition) and covered with plastic Petri dishes. The order of presentation of wines was the same under the two conditions (with and without nose clips) but different for each participant attending to a randomised order. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room at around 20 °C under ambient light. Participants were not informed about the nature of the samples nor the objective of the study.

2.3. Chemical analysis

Reducing sugars, ethanol content, pH and titratable acidity were analysed using Fourier Transform infrared spectrometry with a WineScanTM FT 120 (FOSS®, Barcelona, Spain), which was previously calibrated with the official OIV methods (International Organisation of Vine and Wine, 2019).

Absorbances at 280 and 230 nm of wines diluted between 1:100 and 1:400 in deionised water (to obtain absorbance units in the range of 0.2-0.9) were measured in 1-cm quartz cuvettes with a Shimadzu UV-spectrophotometer (UV-1800).

Concentration and activity of tannins were determined using HPLC-UV-vis following the method proposed by Revelette et al. (2014).

All chemical analyses were carried out in duplicate.

2.4. Data analysis

2.4.1. Effect of aroma perception on taste and mouthfeel attributes

To evaluate the effect of aroma on taste and mouthfeel attributes, a three-way ANOVA (participants as random factor and wines and evaluation condition as fixed factors), considering all main effects and interactions, was calculated on the 23 taste and mouthfeel attributes. The degree of similarity between the mouthfeel characterisation carried out under the two conditions (with and without nose clips) was assessed by calculating RV coefficients (Robert & Escouffier, 1976). The significance of the RV coefficient was estimated by an approximation of the exact distribution of the RV statistic with the Pearson type III approximation (Kazi-Aoual, Hitier, Sabatier, & Lebreton, 1995).

For the characterisation of wines, a two-way ANOVA (participants as random factor and wines as fixed factor) was calculated on the aroma, taste and mouthfeel attributes evaluated under the condition with access to aroma (WA). Principal component analysis (PCA) was conducted on the correlation matrix of mean ratings over the 18 panellists for significant aroma, taste and mouthfeel attributes obtained under the condition without nose clips with the 42 wines of the study. Further hierarchical cluster analysis with the Ward criteria was performed on all the dimensions of the PCA. The three clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centres. To identify the terms that best characterised each cluster, firstly a two-way ANOVA with panellists as random factor and clusters as fixed factors was calculated, then for significant attributes, a pair-wise comparison (Fischer) test was applied ($\alpha = 0.05$).

To identify the aroma attributes possibly involved in the oily perception, a two-way ANOVA for aroma terms was calculated with the five wines (SM4, SM8, SM37, SM38, SM40) that presented a significant effect of the evaluation condition for the term oily. For significant ($P<0.05$) aroma terms (earthy and alcoholic) pair-wise comparison (Fischer) tests were applied.

Analyses were carried out using XLSTAT (version 19.03).

2.4.2. Correlations between physicochemical variables and mouthfeel attributes

Pearson correlation coefficient (r) and its significance were calculated between significant mouthfeel-related attributes (mean sensory scores of the 18 wine experts) and physicochemical variables (ethanol content, pH, titratable acidity, tannin activity, tannin concentration, and absorbance at 280 and 230 nm). Analyses were carried out using XLSTAT (version 19.03).

Partial least squares regression models (PLSR1) were calculated to predict the position of samples on dimension 1 (derived from PCA calculated with scores derived from the rate-*K* attributes sensory method) from chemical variables (tannin activity, TPI-280, TPI-230, tannin concentration, pH, titratable acidity, ethanol content). Analyses were carried out using Unscrambler (version 9.7).

3. Results and discussion

3.1. Effect of aroma on taste and mouthfeel properties

The first aim of the study was to evaluate the effect of aroma on taste and mouthfeel properties of a variety of red wines. This was addressed with expert panellists using a rate-*K* attributes method based on RATA, i.e., the number of attributes to rate was restricted to five, on a broad selection of wines obtained with the intention of having samples with important sensory variability in terms of both aroma and palate properties (taste and mouthfeel). Notably, this appeared to be the first time that this sensory methodology has been applied for mouthfeel description of a relatively wide range of diverse red wines.

Table III-2.1 shows significant main effects ($P<0.05$) for wine in three out of the four tastes evaluated (salty, bitter and sour), with a tendency ($P<0.1$) observed for sweetness. Eight out of the 19 mouthfeel-related terms were also significant ($P<0.05$), with the three dryness-related terms among the most significant, together with sticky and prickly, followed by unctuous, grainy and oily. A tendency was observed ($P<0.1$) for the term silky. Table III-2.2 shows that among the 20 aroma terms evaluated, 15 presented significantly different scores among the wines. Significant terms included those related to a range of fruit nuances along with floral, spice, vegetal and developed attributes.

Table III-2. 1. List of taste and mouthfeel attributes obtained from Sáenz-Navajas et al. (2017) used to sensory evaluate the wines. Three-way ANOVA (participants as random factor, wine and condition of evaluation as fixed factors) calculated to evaluate the effect of evaluation condition (C) (with and without aroma perception) on taste and mouthfeel sensory properties of the 42 red wines studied (F values; P-significance: *P<0.1, **P<0.05, ***P<0.01, ****P<0.001; fd: freedom degrees; ns: not significant).

	wine (W) fd = 41		condition (C) fd = 1		wine (W) × condition (C) fd = 41	
	F	P	F	P	F	P
salty	1.453	**	2.473	ns	1.021	ns
bitter	1.873	***	6.350	**	1.100	ns
sweet	1.523	*	0.376	ns	0.987	ns
sour	2.618	****	3.712	*	0.947	ns
dry	3.804	****	0.161	ns	1.171	ns
dry on tongue side	2.132	****	0.346	ns	0.683	ns
dry on palate	3.480	****	1.905	ns	0.742	ns
sticky	1.633	****	0.782	ns	0.743	ns
dusty	0.863	ns	0.888	ns	1.212	ns
grainy	4.439	**	9.230	***	0.769	ns
sandy	1.240	ns	1.227	ns	0.697	ns
coarse	1.130	ns	7.178	***	0.737	ns
unctuous	1.828	***	0.835	ns	1.020	ns
oily	1.576	**	0.800	ns	1.523	**
fleshy	1.011	ns	0.238	ns	0.635	ns
mouthcoating	1.178	ns	1.280	ns	0.935	ns
silky	1.388	*	1.316	ns	0.935	ns
gummy	1.164	ns	0.092	ns	1.093	ns
watery	1.190	ns	0.052	ns	1.263	ns
burning	0.941	ns	1.702	ns	1.057	ns
hot	1.204	ns	2.219	ns	0.981	ns
prickly	1.616	****	0.509	ns	1.123	ns
persistent	0.824	ns	0.510	ns	1.070	ns

Figure III-2.2 shows the cluster analysis calculated with significant attributes. Three main clusters of wines with important sensory differences could be identified among the 42 wines studied. Cluster 1 was mainly characterised by aroma terms linked *a priori* to low quality according to expert and consumer judgements (Sáenz-Navajas et al., 2013), such as vegetal and animal, as well as bitter taste and dry and sticky mouthfeel. The second cluster presented *a priori* quality-related attributes such as fruity (yellow, red and black fruits) and floral aromas. Interestingly, the three

Bobal wines belonged to this cluster, which was well in accordance with previous works that found red and cassis aromas in wines elaborated with this variety and also coming from East-central Spain (García-Carpintero, Sánchez-Palomo, & González Viñas, 2010). The third and largest cluster, containing more than 50% of wines studied, presented dried fruit/compote, roasted and alcoholic aromas, and unctuous mouthfeel. Wines elaborated with Bonarda (Argentina), Carignan (Northeast Spain), Prieto Picudo (Northwest, Spain), Syrah (Northeast, Spain) and Grenache (4 out of 5, Northeast Spain) were included in this last cluster, which provides information about the sensory profile of wines elaborated with these specific varieties and origins. Notably, even though the literature highlights that both the variety and origin of grapes are important factors affecting sensory profile and thus typicality of wines (Parr, 2009; Picard, Tempere, de Revel, & Marchand, 2015; Picard, Thibon, et al., 2015), to the best of our knowledge there was no systematic scientific study aimed at describing the sensory profiles of wines as a function of both variety and origin. Thus, further studies should be carried out to evaluate such effects on wine sensory profiles. The present results also revealed that a rate-*K* attributes methodology (Sáenz-Navajas et al., 2017), carried out by a non-specifically trained panel of wine experts who described wines with a pre-established list of aroma, taste and mouthfeel terms, was successful in discriminating among the set of wines studied.

Table III-2. 2. List of aroma attributes employed during wine sensory description by "rate-K attributes" methodology and two-way ANOVA (participants as random factor, and wine as fixed factor) calculated to evaluate the discrimination effect of aroma attributes among the 42 red wines studied (F values; P-significance: *P<0.1, **P<0.05, ***P<0.01, ****P<0.001; ns: not significant).

Aroma attribute	F	P
white fruit (apple, pear, quince)	1.763	***
yellow fruit (peach, apricot)	1.805	***
tropical fruit (banana)	1.996	***
red fruit (strawberry, raspberry, cherry)	2.557	****
black fruit (blackberry, blueberry)	1.358	*
dried fruit/compote (raisin, prune)	1.341	*
floral (violet, rose, acacia)	2.519	****
alcohol	1.612	**
spices (clove, black pepper, cinnamon, vanilla)	1.631	***
roasted (coffee, toast, toffee, cacao)	2.051	***
vegetal (green pepper, fresh grass)	1.453	**
cooked vegetables (artichoke, asparagus, olive)	2.430	****
earthy (mushroom, truffle)	1.629	***
reduction (rotten egg, cauliflower)	1.730	***
animal (leather, stable)	2.820	****
citrus (lemon, orange)	0.942	ns
nuts (walnut, hazelnut, almond)	1.189	ns
balsamic (eucalyptus, menthol)	1.143	ns
mouldy (cork, mould)	0.720	ns
lactic (milk, yogurt, cream)	1.249	ns

A significant effect of the evaluation condition (with (WA) and without (NA) aroma perception, using a nose clip for the latter) was evident for 4 out of the 23 taste and mouthfeel-related terms evaluated (Table III-2.1). Evaluation condition was especially significant for grainy (average scores for the 42 wines and the 18 participants under the two conditions: WA = 0.08; NA = 0.22) and coarse (WA: 0.44; NA: 0.64) (P<0.01) mouthfeel attributes, followed by bitter taste (WA = 1.11; NA = 1.35) (P<0.05). A tendency was also observed for sour taste (WA = 1.32; NA = 1.48)

(P<0.1). It is noteworthy that the scores of these four attributes were higher under the nose-clip condition regardless of the wine evaluated. This could be attributed to the fact that in the absence of aroma perception, participants were exposed to a lower number of stimuli, which could have made them to focus their attention on mouthfeel and taste attributes leading to check and rate a higher number of these terms. This hypothesis is supported by the fact that the number of citations is significantly higher (P<0.05 based on chi-square test) under the nose clip condition than for the condition that permitted olfaction, especially for sour taste (WA = 265; NA = 332; difference = 67), followed by bitter taste (WA = 253; NA = 295; difference = 42), coarse (WA = 89; NA = 126; difference = 37), and silky (WA = 61; NA = 88; difference = 27). Thus, the increase of the scorings for these four palate-related attributes in the absence of aroma could be attributed to an attentional effect produced by the tasting condition. Similar cross-modal interactions have been reported in the literature (Arvisenet et al., 2019; Bult, de Wijk, & Hummel, 2007; Sáenz-Navajas, Campo, Avizcuri, et al., 2012), where an increase in the complexity of the sensory stimuli (i.e., palate stimuli only *vs* palate and aroma simultaneously) increased the level of cross-modal interactions. This has been attributed to the difficulty of even trained panellists to disentangle unitary flavour stimuli when those occur simultaneously. However, the present results cannot be attributed to other cross-modal interactions more usually observed in other works and related to the enhancement of tastes by congruent aromas (e.g., sweet taste-fruity aroma, sour taste-citrus aroma, or bitter taste-animal aroma) (Arvisenet et al., 2016; de-la-Fuente-Blanco et al., 2017; Saenz-Navajas et al., 2010). These last interactions can be ruled out in the present work because if this had been the case, significant effects of condition × wine would have been observed for taste or mouthfeel attributes, given the high aroma variability presented by the studied wines.

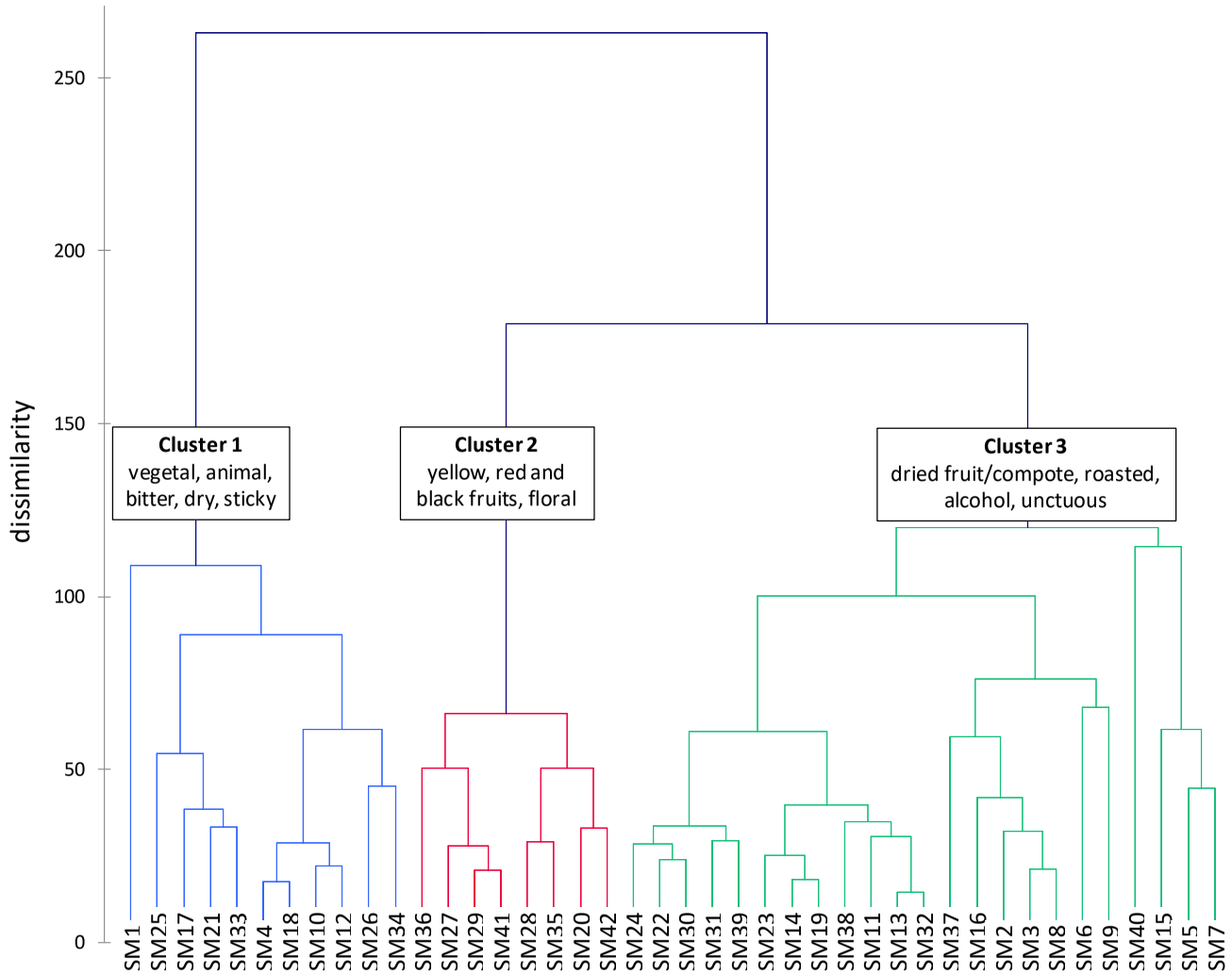


Figure III-2. 2. Tree diagram showing the three wine clusters derived from hierarchical cluster analysis calculated on all dimensions of the PCA performed with the 42 studied wines and using significant aroma, taste and mouthfeel attributes. Attributes describing each cluster are those with significantly higher scores in the given cluster.

Concerning interactions between wine and evaluation condition, only the term oily was significantly different when comparing the sensory results with and without aroma perception. Pair wise *post-hoc* tests of significance showed significant effects ($P<0.05$) of evaluation condition only for 5 out of the 42 wines studied (SM4, SM8, SM37, SM38, SM40). Figure III-2.3 shows that for SM4 and SM8, the term oily is higher under the nose clip condition, thus the presence of aroma stimuli seems to mask the oily perception. The contrary is observed for wines SM37, SM38 and SM40, in which aroma stimulus seems to significantly ($F=2.22$; $P<0.05$) enhance oily mouthfeel. Further ANOVA calculated considering these two groups of wines as fixed factors showed that wines SM4 and SM8 presented significantly higher scores for earthy aroma ($F=7.98$; $P<0.01$), whereas SM37, SM38 and SM40 had higher scores for the term alcohol ($F=5.55$; $P<0.05$). These results would suggest that the presence of earthy aromas could be masking the oily mouthfeel whereas alcoholic nuances can enhance the attribute in the studied wines. The use of a nose clip avoided any effect of volatile molecules on in-mouth sensory perceptions, and consequently the results should be exclusively attributable to cognitive interactions (Poinot, Arvisenet, Ledauphin, Gaillard, & Prost, 2013), and thus to effects related to top-down processing such as those related to cognitive processes. Such cognitive interactions include attention, memory, leraning, or metacognition processes as recently reviewed by White and colleagues (White, Thomas-Danguin, Olofsson, Zucco, & Prescott, 2020). In this regard, similar trigeminal-odour interactions have been reported (Labbe, Gilbert, & Martin, 2008; Spence, 2016) and certain aromas have been shown to enhance mouthfeel properties such as viscosity through associative learning (Stevenson & Mahmut, 2011). Thus, it can be hypothesised that the oily sensation perceived for some wines may be associated with a congruent effect between mouthfeel and aroma stimuli. Notwithstanding, further research experiments should be carried out to confirm the hypothesis that has arisen from the presented data set.

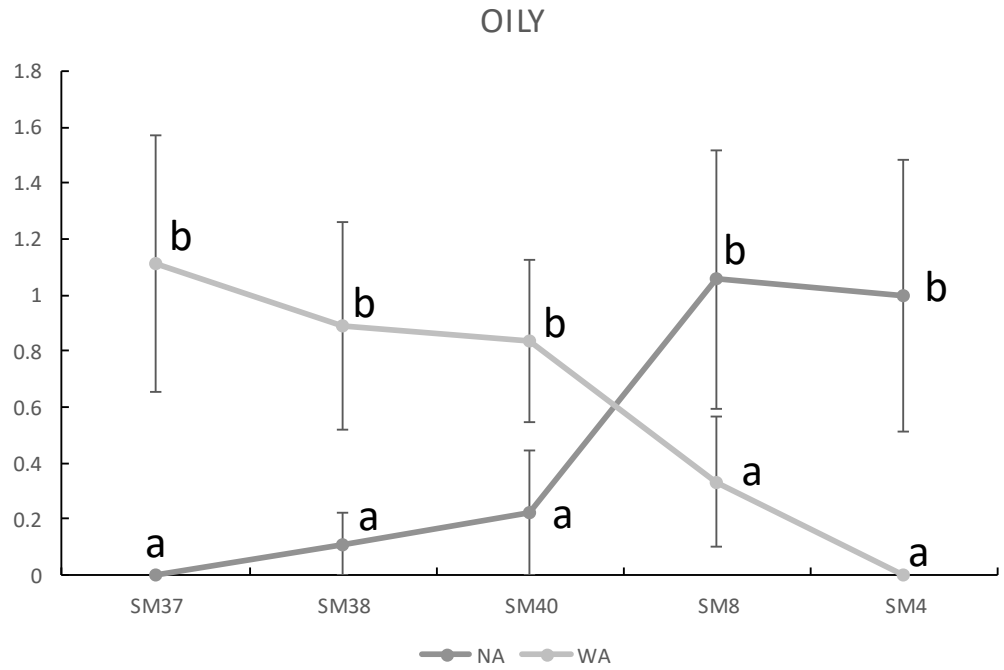


Figure III-2. 3. Average sensory scores of 18 participants for the oily attribute, evaluated with no aroma perception (NA) using nose clips compared to aroma perception (WA), for wines presenting a significant effect of aroma perception on oily scores. Error bars represent the standard error of the mean. Different letters indicate the existence of significant differences between samples ($P<0.05$, pairwise Fischer post hoc test).

The RV coefficient, which can be understood as a correlation coefficient between two individual sample spaces and varies from 0 (full disagreement) to 1 (perfect agreement), was calculated between the sensory space generated by taste and mouthfeel properties under the two conditions (with and without nose clip) and found to be 0.617 ($P<0.01$). This suggested that the sensory spaces were not significantly different, which was in good accordance with the ANOVA results, in which only a significant effect of condition × wine was observed for oily mouthfeel. Notably, this contrasted with previous work carried out with reconstituted wines, where a significant effect of the fruity aroma extract of a white wine was observed for astringent perception elicited by the non-volatile matrix of both white and red wines (Saenz-Navajas et al., 2010). However, the lack of effect of aroma on the

astringency of red wines observed in the present work is well in line with previous results obtained with model red wines (de-la-Fuente-Blanco et al., 2017; Sáenz-Navajas, Campo, Avizcuri, et al., 2012). All this suggested that aroma seems to have a significant effect on palate properties, but such an effect would be dependent on the different sensory properties of the sample set that was studied. Based on the scarce results found in scientific literature, it can be hypothesised that the effect of aroma would be predominant in white wines, whereas in red wines the effect would be secondary, if any. However, further studies should be carried out to confirm such a hypothesis.

3.2. Taste and mouthfeel dimensions

Table III-2.3 shows the correlation matrix calculated with the significant palate-related attributes among the studied wines. Some mouthfeel terms were highly correlated, such as dry and dry on the palate ($r = 0.64$, $P<0.001$) and both terms negatively correlated with silky ($r = -0.52$, $P < 0.01$ and $r = -0.42$, $P<0.01$, respectively). This would suggest that the terms dry and dry on the palate present similar meanings for wine experts and were redundant. The highly significant ($P<0.001$) negative correlation of silky and dry (and dry on the palate) was notable, and suggested that these terms could be part of the same mouthfeel dimension and that probably panellists used them to score high or low intensity of that perceived characteristic. This may mean that in future these terms could be denoted dry-sticky/silky to avoid redundancy among the terms in a list. However, it is important to highlight that this suggestion is based exclusively on statistical correlations, thus further cognitive experiments should be carried out to confirm the hypothesis. Also of interest was the significant correlation observed for the terms unctuous and oily ($r = 0.36$, $P<0.05$), which could suggest that both terms are part of the same sensory dimension. They are most probably redundant terms that belong to the same category and thus it can be suggested to unify both terms under the same name, for example unctuous.

Table III-2. 3. Correlation matrix calculated for average scores (of 18 panellists) of taste and mouthfeel attributes for the 42 wines evaluated under the two conditions with and without noseclips. Significant correlations (P<0.05) are marked with an asterisk (P-significance: *P<0.05, **P<0.01, ***P<0.001).

	Salty	Bitter	Sweet	Sour	Dry	Dry on tongue side	Dry on palate	Sticky	Grainy	Unctuous	Oily	Silky	Prickly
Salty	**1.00**	-0.31	0.01	0.07	-0.24	-0.27	-0.34	-0.12	-0.12	0.24	0.12	0.25	0.12
Bitter	-0.31	**1.00**	-0.22	-0.30	**0.40****	0.32	0.24	0.08	0.32	-0.18	0.01	-0.18	0.03
Sweet	0.01	-0.22	**1.00**	-0.07	-0.20	-0.02	-0.01	-0.05	0.20	0.19	0.04	0.07	0.12
Sour	0.07	-0.30	-0.07	**1.00**	-0.27	-0.33	-0.32	-0.14	-0.09	0.12	0.06	0.25	0.07
Dry	-0.24	0.40	-0.20	-0.27	**1.00**	**0.41****	**0.55*****	0.32	0.28	-0.33	-0.33	**-0.52*****	-0.14
Dry on tongue side	-0.27	0.32	-0.02	-0.33	0.41	**1.00**	**0.44****	0.19	0.28	0.05	-0.18	-0.26	-0.08
Dry on palate	-0.34	0.24	-0.01	-0.42	0.55	0.44	**1.00**	0.32	**0.43****	-0.28	-0.11	**-0.42****	-0.15
Sticky	-0.12	0.08	-0.05	-0.14	0.32	0.19	0.32	**1.00**	0.11	-0.30	-0.21	-0.22	-0.11
Grainy	-0.12	0.32	0.20	-0.09	0.28	0.28	0.43	0.11	**1.00**	0.02	0.07	-0.14	0.18
Unctuous	0.24	-0.18	0.19	0.12	-0.33	0.05	-0.28	-0.30	0.02	**1.00**	**0.36***	0.32	0.29
Oily	0.12	0.01	0.04	0.06	-0.33	-0.18	-0.11	-0.21	0.07	0.36	**1.00**	0.30	0.16
Silky	0.25	-0.18	0.07	0.25	-0.52	-0.26	-0.42	-0.22	-0.14	0.32	0.30	**1.00**	0.15
Prickly	0.12	0.03	0.12	0.07	-0.14	-0.08	-0.15	-0.11	0.18	0.29	0.16	0.15	**1.00**

Notwithstanding, similar to that noted for dry and silky, this hypothesis should be further evaluated to confirm the similarity between both percepts. The positive correlations of bitter taste with dry ($r = 0.40$, $P<0.01$) could be attributed to an indirect effect related to the fact that wine polyphenols elicit both sensations simultaneously (Ma et al., 2014; Soares et al., 2017).

In other to further understand the sensory dimensions arising from the rate-*K* attributes methodology employed in the present work, PCA was conducted with significant attributes (4 for taste and 9 for mouthfeel, Table III-2.1) for the 42 wines under the two evaluation conditions (Figure III-2.4). Among the 13 PCs explaining 100% of the original variability, the first six dimensions were mostly contributed (based on highest squared cosines of the variable for each PC) by at least one of the 13 significant sensory attributes and explained 72% of the variance. The necessity of a relatively high number of PCs to explain the original variance suggests that the sensory approach employed was able to disclose a wide range of non-correlated and independent palate sensations, mainly involving six sensory dimensions (or PC) that are built from different taste or mouth-feel related attributes. Figure III-2.4.a shows that the first sensory dimension, PC1 (explaining 28% of variance), was mainly contributed by three attributes associated with dryness along with stickiness, and the term silky (along with salty and sour to a lesser extent). As suggested above, this dimension could be denoted as dry-sticky/silky. PC2 (explaining 13%) was mainly built by the terms grainy and unctuous in the positive direction, which were non-correlated and thus independent terms ($r = 0.020$). Both PC3 and PC4, which respectively explained 9% and 8% of original variance, were mainly contributed by tastes: sweet opposing bitter for PC3 and sour opposing salty for PC4 (Figure III-2.4.b). On the other hand, PC5 and PC6, both explaining 7% of variance, were mostly related to the terms prickly and oily, respectively (Figure III-2.4.c).

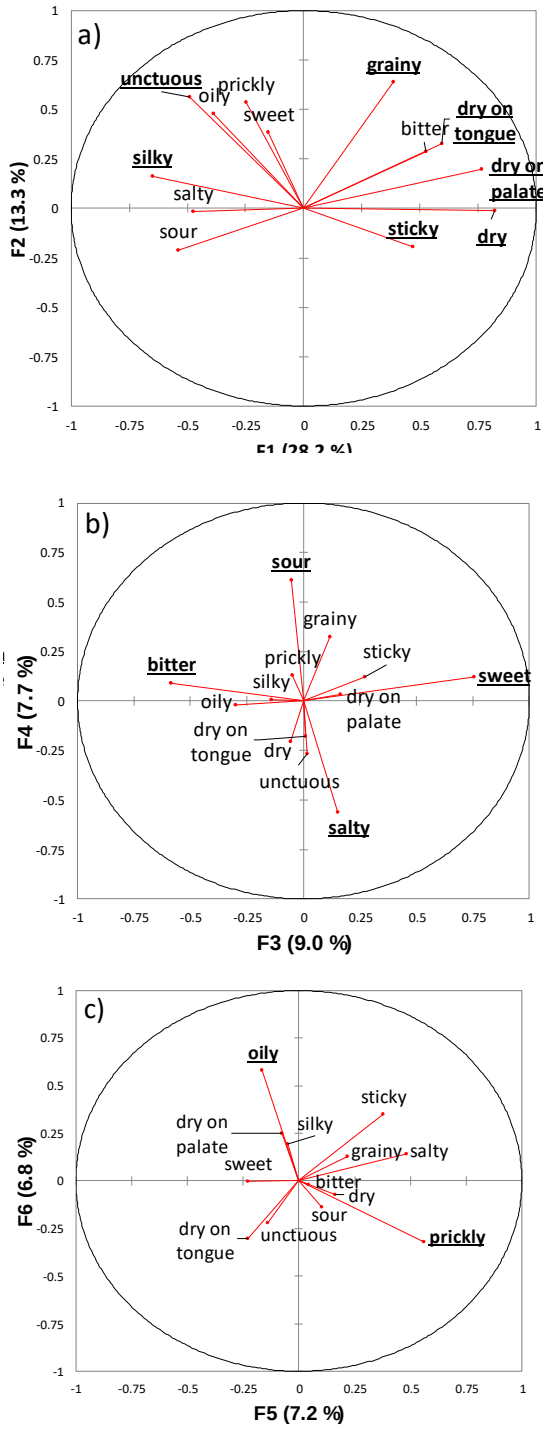


Figure III-2. 4. Two-dimensional loadings plots derived from PCA of significant taste and mouthfeel attributes that were calculated with the average scores of 18 participants who evaluated 42 wines with and without nose clips, showing a) PC1 vs PC2, b) PC3 vs PC4, and c) PC5 vs PC6. Underlined attributes are significantly correlated with one of the PCs (i.e. independent sensory dimensions) represented.

Leaving aside the four tastes, these results suggest that the chosen sensory approach was able to identify four independent and non-correlated mouthfeel dimensions for this set of red wines (PC1, PC2, PC5 and PC6), which represented sensory aspects that go beyond the most common mouthfeel term astringency, including: dry-sticky/silky, grainy, prickly and unctuous. This valuable information will help to open a new path of experiments aimed at identifying the molecules responsible for these mouthfeel dimensions. The first insight into the chemical drivers of these sensory properties is described next, and together with the rate-*K* attributes results, will contribute to the ultimate goal of obtaining reference materials that will enable the training of sensory panels.

3.3. Relationships between mouthfeel properties and physicochemical measurements

The second aim of the work was to further understand the relationships between some chemical variables and the specific sensory mouthfeel descriptors and/or the mouthfeel dimensions identified. Addressing this aim, different chemical and spectrometric variables including ethanol content, pH, titratable acidity, absorbance at 280 nm (TPI-280) and 230 nm (TPI-230), tannin concentration, and tannin activity were measured and correlated with the sensory data. It is important to highlight that the highest correlation between chemical variables was observed between tannin concentration and TPI-280 ($r = 0.92$; $P < 0.001$), followed by TPI-230 ($r = 0.86$; $P < 0.001$). Besides, tannin activity was significantly correlated to TPI-280 ($r = 0.56$; $P < 0.01$) and TPI-230 ($r = 0.51$; $P < 0.05$) (Table III-2.4). This result suggests that TPI-280 would be a better predictor of tannin concentration than TPI-230 for the present set of wines studied, in contrast to data reported elsewhere (Boulet, Ducasse, & Cheynier, 2017; Boulet et al., 2016). Thus, the replacement of meaurement at 280 nm by 230 nm to predict tannin concentration should be undertaken with caution because such dependency seems to rely on the sample set under consideration.

Table III-2. 4. Correlation matrix for chemical variables. Significant correlations (P<0.05) are marked with an asterisk (P-significance: *P<0.05, **P<0.01, ***P<0.001). *VA: volatile acidity; TA: titratable acidity; RS: reducing sugars; MA: malic acid; LA: lactic acid; TPI: total polyphenol index

	Activity	Tan-Con	TPI-280	TPI-230	Ethanol	pH	VA	TA	RS	MA	LA
Tannin activity	1										
Tannin concentration	0.66***	1									
TPI-280	0.56**	0.92***	1								
TPI-230	0.51*	0.86***	0.89	1							
ethanol	0.17	0.43	0.46	0.35	1						
pH	0.29	0.44	0.42	-0.47	0.22	1					
VA	0.00	0.07	0.03	0.19	-0.19	0.41	1				
TA	0.17	0.19	0.23	0.14	0.23	-0.58**	-0.18	1			
RS	0.08	0.30	0.24	0.25	0.07	-0.06	-0.04	0.26	1		
MA	0.22	0.33	0.37	0.30	0.47	-0.30	-0.32	0.78***	0.06	1	
LA	-0.01	0.06	0.08	0.18	-0.48	0.48	0.69***	-0.43	-0.14	-0.50*	1

The highest significant correlation (r = 0.70, P < 0.001, Table III-2.5) was found between the chemical variable tannin concentration and the first sensory dimension (dry-sticky/silky) of the PCA (PC1, Figure III-2.4.a). Accordingly, tannin concentration was significantly (P<0.001) and equally correlated with both dryness and dryness on the palate (r = 0.65). These results suggested that the first PC, which was mainly positively contributed by the terms dry, dry on the palate and sticky, and negatively by silky, was most probably related to the global attribute astringency. This percept is accepted to be mainly driven by the interaction between tannins and salivary proteins (García-Estévez et al., 2017; Soares et al., 2017). In this regard, different strategies have been developed to predict overall astringency from chemical measures of tannins (Kennedy, Ferrier, Harbertson, & Peyrot Des Gachons, 2006; Umali et al., 2015), showing that tannin concentration is only one of the tannin features involved in astringency. Thus, tannin activity has been defined in an attempt to identify other properties of tannins involved in the perception of astringency (Revelette et al., 2014). Tannin activity, which is related to the thermodynamics of interaction between tannins and a hydrophobic surface (polystyrene divinylbenzene HPLC column) (Barak & Kennedy, 2013), was found to have a significant (p<0.001) correlation with dry on palate (r = 0.64, Table III-2.5).

Table III-2.5 shows that both spectroscopic measurements at 280 nm and 230 nm presented significant (P<0.01) but weaker correlations (r ≤0.60 in all cases) with PC1 (dry-sticky/silky) and the individual terms dry and dry on palate than did tannin concentration. Besides, the spectroscopic measures did not correlate with any other mouthfeel-related attribute, although this was not too surprising. Even if these global measures are often used to evaluate polyphenol content in wines and are further related to astringency perception, there is a wide range of phenolics that absorb in the UV-region that are not involved or have a limited role in the modulation of wine astringency. These include low molecular weight flavan-3-ols (monomers or dimers), phenolic acids, flavonols, and anthocyanins among others components.

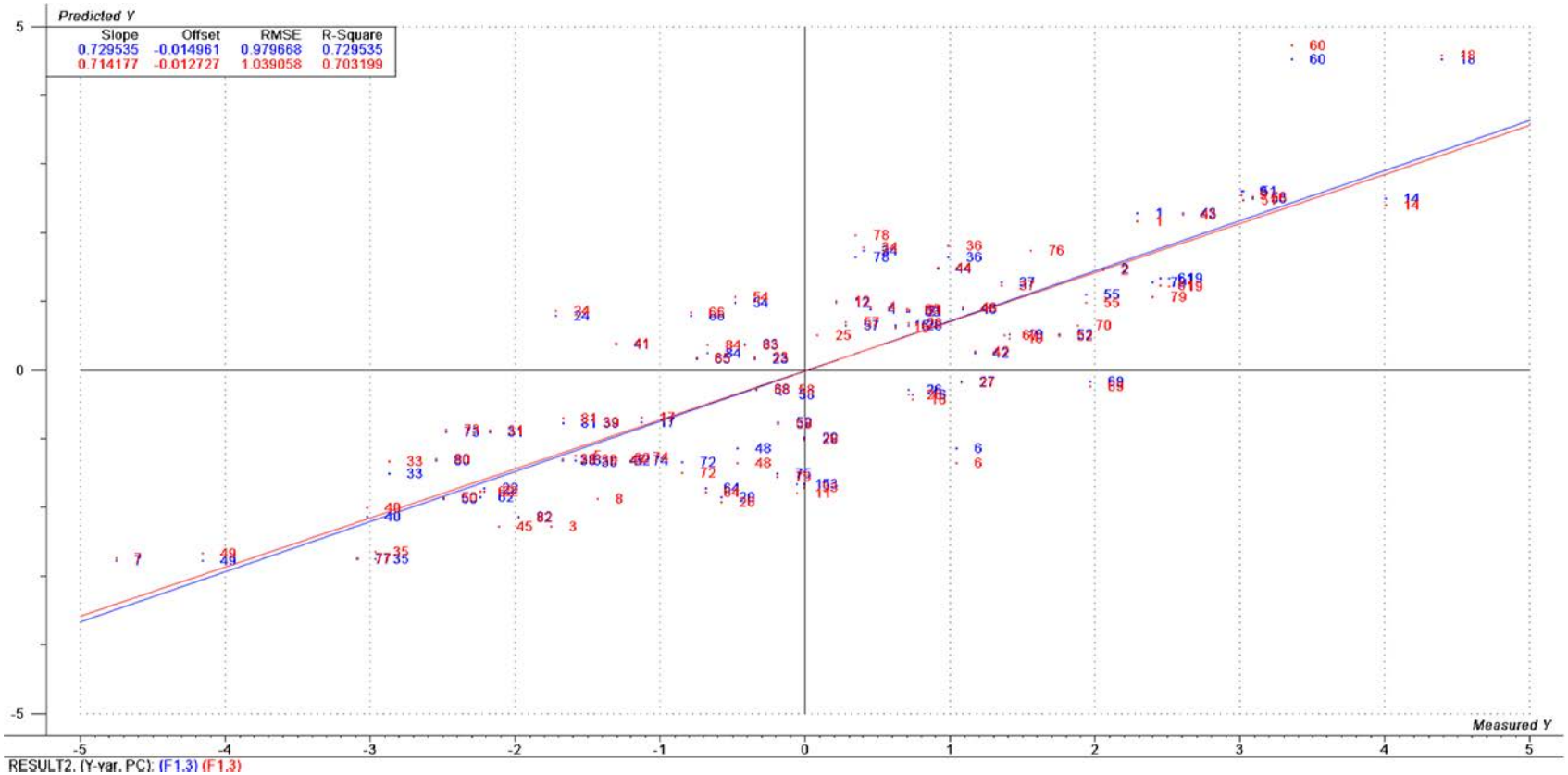
Table III-2. 5. Pearson correlation coefficient (r) calculated between chemical variables and tastes, mouthfeel attributes and sensory dimensions (i.e., principal components derived from PCA). Significant correlations (P<0.05) are marked with an asterisk (P-significance: *P<0.05, **P<0.01, ***P<0.001).

	Tannin activity	Tannin conc.	TPI-280 (au)	TPI-230 (au)	Ethanol (%v/v)	pH	Titratable acidity
Salty	-0.20	-0.31	-0.28	-0.18	-0.27	-0.11	0.11
Bitter	0.07	0.32	0.27	0.30	0.27	0.30	-0.34
Sweet	0.10	-0.07	-0.04	-0.06	0.04	0.05	0.02
Sour	-0.39*	-0.50**	-0.40*	-0.42	-0.20	-0.52	0.34
Dry	0.51**	0.65***	0.51**	0.56**	0.15	0.38	-0.17
Dry on tongue side	0.28	0.43*	0.36	0.30	0.18	0.10	0.01
Dry on palate	0.64***	0.65***	0.57**	0.59**	0.02	0.26	-0.08
Sticky	0.39*	0.36	0.31	0.28	0.08	-0.03	0.13
Grainy	0.22	0.26	0.18	0.21	-0.09	0.00	-0.07
Unctuous	-0.21	-0.24	-0.24	-0.24	-0.17	0.01	-0.04
Oily	-0.29	-0.20	-0.18	-0.16	-0.15	0.09	-0.19
Silky	-0.36	-0.34	-0.30	-0.30	-0.28	-0.04	-0.05
Prickly	-0.18	-0.06	-0.09	0.01	-0.07	-0.02	-0.04
PC1 (dry-sticky/silky)	0.59**	0.70***	0.60**	0.59**	0.26	0.28	-0.12
PC2 (grainy)	0.00	0.12	0.06	0.11	-0.07	0.19	-0.22
PC4 (prickly)	0.32	0.04	0.05	0.02	-0.05	-0.12	0.26
PC5 (unctuous)	-0.09	-0.12	-0.07	-0.12	0.01	-0.32	0.15

Besides tannin properties, other factors such as pH, acidity or ethanol content have been shown to modulate astringency (Fontoin et al., 2008). Even if no direct linear correlation was observed between these three variables and the sensory properties evaluated (Table III-2.5), a PLSR1 model (with 3 PCs and explaining 71% of the original variance by cross-validation, Figure III-2.5) was able to predict the position scores of samples on the first sensory dimension derived from PCA (which mainly referred to global astringency) in part from these chemical measures. Tannin concentration was the main variable contributing to the model (weighted regression coefficient (wrc) = 0.928), followed by a negative contribution of pH (wrc = −0.597), and then tannin activity (wrc = 0.263). The involvement of tannin activity in astringency perception is of interest, but outcomes can be variable and more understanding is required. A previous study could not establish a link in Cabernet Sauvignon wines (Watrelot, Byrnes, Heymann, & Kennedy, 2016), which was attributed to the presence of other compounds such as polysaccharides or even to the presence of cross-modal interactions of astringency with aroma.

In contrast, a relationship between tannin activity and astringency was recently confirmed by a machine learning modelling approach (Sáenz-Navajas et al., 2019). As for the effect of wine pH, the increase of astringency with decreases in pH was previously observed with model wines (Fontoin et al., 2008) and was attributed to an effect of pH on rheological properties of saliva. Low pH values have been suggested to decrease viscosity and thus increase astringent perception (Luck et al., 1994).

Figure III-2. 5. PLS regression plot of predicted vs measured Y, i.e., position scores of samples on PC1 derived from PCA (mainly related to dry-sticky/silky dimension).



4. Conclusions

A rate-*K* attributes-based sensory methodology employed with wine experts was successful in discriminating among the taste and mouthfeel sensory properties of a relatively wide range of red wines. Different independent and non-correlated mouthfeel dimensions could be identified, encompassing perceptions associated with dry-sticky/silky, grainy, prickly and oily. Moreover, the effect of aroma on palate sensations was studied using the rate-*K* attributes methodology with and without nose clips, which confirmed in the main that aroma did not have an effect on taste or mouthfeel perception of red wines. However, an aroma-mouthfeel interaction was identified for the term oily, which was hypothesised to be masked by earthy aromas and enhanced by alcoholic nuances.

Concerning the correlation of mouthfeel properties and chemical variables, tannin activity was shown to be a good predictor of wine dryness on the palate, and tannin concentration correlated with both dry (in general) and dry on the palate terms (as well as with PC1, which was driven by these attributes). However, the results provided evidence that there are (at least three) other independent mouthfeel dimensions (related or not to drying) identified in the present work that are not linked to the other chemical variables studied, which highlights the need for deeper investigation. The possible chemical compounds involved in the other mouthfeel dimensions could include polysaccharides or other families of polyphenols aside from tannins. Furthermore, although simple spectroscopic measurements (280 nm and 230 nm) were positively correlated to wine dryness, the correlations were poor compared to tannin specific estimations.

Overall, the study has increased knowledge of the mouthfeel perception of red wines, which is valuable for both wine researchers and the wine industry. The variant of RATA, rate-*K* attributes, was shown to be a useful sensory tool to be directly used in the wine industry to evaluate differences in taste and mouthfeel properties of wine. Besides, this work provides the basis for an important field of research aimed

at identifying the molecules or groups of molecules that elicit the different sensory dimensions identified in the present work. This is not a trivial undertaking and is part of a longer term strategy, where the most different wines from among the selected 42 are being fractionated and further characterised, to identify sensory-active fractions related to different mouthfeel dimensions.

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SECTION III. CHAPTER 3

Application of UPLC-QTOFMS-based untargeted analysis for understanding wine mouthfeel: a sensometabolomic approach

1.Introduccion

In the last decades, there has been an increasing number of works focused on the development of methodologies for determining the compounds responsible for in-mouth sensations. Most of them have been focused on analysing phenolic compounds involved in astringency perception of red wines. To accomplish such goal most literature quantifies known phenolic compounds following instrumental targeted approaches and relate them with sensory properties (mainly with astringency) as reported in the rest of the chapters of the present Doctoral Thesis. The main limitation of this classical approach is that it does not collect enough information, as only compounds with known sensory activity or highly concentrated (i.e., those that are easier to analyse) are analysed. This approach neglects the importance of unknown metabolites as well as those present at low concentrations, which, with no doubt, can play a major role in the formation of mouthfeel properties.

Not long ago, the development of high-resolution Liquid-Mass techniques has revolutionised the food chemistry. The application of non-targeted analytical methods has been revealed to be a powerful and promising alternative tool to targeted methods when identifying chemical markers involved in different chemical and biological phenomena. Untargeted approaches have been used to identify hundreds of metabolites simultaneously with the aim to explore relationships between chemical composition and sensory properties (Cevallos-Cevallos & Reyes-De-Corcuera, 2012; Gika, Wilson, & Theodoridis, 2014; Hufnagel & Hofmann, 2008; Pérez-Jiménez, Sherman, Pozo-Bayón, & Pinu, 2021; Sherman, Coe, Grose, Martin, & Greenwood, 2020; Vallverdú-Queralt et al., 2017).

In spite of the potential of using metabolomics to unravel the sensory activity of compounds (sensometabolome as defined by Hufnagel and Hofmann), this sensometabolomic approach has barely been explored before now. Thus, the present work offers an unprecedented opportunity to apply a sensometabolomic approach to elucidate palate drivers and predict wine taste and mouthfeel from wine composition.

Objectives

In this context, being aware of the need for exploring new analytical approaches for understanding the chemical basis of mouthfeel, the goal of this work is to explore a sensometabolomic approach to establish relationships between wine composition and mouthfeel properties considering the whole wine metabolome (obtained by an instrumental untargeted method). More specifically, the main objectives of the present chapter are: 1) to define the mouthfeel dimensions of a relatively wide range of red wines with very different chemical composition, 2) to identify molecular markers of the mouthfeel dimensions defined in the first aim following an untargeted metabolomic approach, and 3) to generate mathematical models able to predict mouthfeel dimensions from chemical markers identified by both untargeted and targeted methods. This last aim working under the assumption that untargeted methods can provide a more complete information than targeted approaches.

2. Material and methods

2.1. Chemicals

Methanol of LC-MS LiChrosolv grade used for the preparation of mobile phase was purchased from Fluka Sigma-Aldrich and the LC-MS grade formic acid used as the mobile phase additive was obtained from Sigma-Aldrich. Water was purified by a Milli-Q Water Purification System for chromatography.

2.2. Wine Samples

A total of 42 non-wooded wines, all of them monovarietal, were selected in previous work (Section III-Chapter 2). Wines came from 14 different varieties, from different origins (Spain, Argentina and France) and from different vintages. The wine sample set included commercial and non-commercial red wines. A detailed list of wine characteristics is presented in the corresponding Annexe III-2.1 of chapter 2.

2.3. Sensory characterisation

The samples, 42 red wines, were characterised following the sensory strategy rate-k-attributes (i.e., a variant of rate-all-that-apply-RATA-) developed in Section I-Chapter 1 and applied in Section III-Chapter 2 under two conditions: (1) with no aroma perception (using nose clips), and (2) with aroma perception. In the present work, only the results from the analysis employing nose clips are considered. Briefly, sensory analysis was carried out by 18 wine experts from Rioja area, Spain (15 women and 3 men, ranging in age from 23 to 54, with an average of 38 years old). Samples were served in normalised dark approved wine glasses (German Institute for Normalization, DIN) labelled with 3-digit random codes, in a randomised distinct order of presentation for each participant. Samples were served at room temperature and evaluated in a ventilated, air-conditioned tasting room (approximately 20 ºC). All participants evaluated the 42 wine samples with nose clips in a sequential manner. They were instructed to distribute the sample throughout the oral cavity (as a mouthwash) to reach the entire surface of the mouth. After tasting each sample, they had to follow a mandatory rinsing protocol with mineral

water and pectin (1 g L^{-1}) before tasting the next sample (Colonna, Adams, & Noble, 2004).

The list of terms consisted of 23 taste and mouthfeel-related attributes generated previously (Section I-Chapter 1). To avoid bias, attributes on the list appeared in a distinct randomised order for each participant. They were asked to taste and rate the intensity of a maximum of five attributes appearing in each sample on a 7-point scale (1 = not intense; 7 = very intense). Attributes that were not rated were allocated a value of zero when collecting data.

Participants attended four sessions on four different days. In each day, panellists had to evaluated between 10 or 11 wine samples (42 wine samples in total). They were not informed about the objective of the study neither paid for their participation.

2.4. Chemical analysis

2.4.1. Conventional oenological parameters

Reducing sugars, ethanol content, pH and titratable acidity were analysed using Fourier Transform infrared spectrometry with a WineScanTM FT 120 (FOSS®, Barcelona, Spain). Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970). Wines were diluted 1:200 in deionised water (to obtain absorbance units in the range of 0.2-0.9) and were measured in 1cm quartz cuvettes with a Shimadzu UV-spectrophotometer (UV-1800). Colour intensity (CI) was estimated as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). Wines without dilution were measured in 2 mm crystal cuvettes. Data are shown in Annexe III-2.1 of chapter 2.

2.4.2. Ultra-Performance Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (UPLC–QTOF MS) Analysis.

Sample preparation was performed as follows, 1 mL of each wine sample was diluted with 2 mL of Milli-Q sonicated water, and filtered with 0.22 µm polytetrafluoroethylene (PTFE) filters into a 2 mL MS vials for LC–MS analysis. A pooled quality control (QC) sample was prepared by pooling 1 mL of each wine of the 42 wines analysed. The samples were prepared and analysed according to a randomised order (https://www.rando m.org/sequences/). At the beginning of each batch of analysis, one blank sample (Milli-Q water instead of wine) and five QC samples were analysed, and then samples were injected, with one QC after every six samples. Finally, at the end of each batch, one QC and one blank were injected.

The liquid chromatography system used was Waters Acquity UPLC coupled via an electrospray ionization (ESI) interface to the mass Spectrometer system, Waters Synapt HDMS QTOF MS (Waters, Manchester, U.K.) operating in W mode and controlled by MassLynx 4.1 software (Figure III-3.1). The column used was a Waters Acquity UPLC 1.8 µm 2.1 × 150 mm HSS T3 (Waters) at 40 °C and the injection volume was 10 µL and the samples were kept at 4 °C throughout the analysis.

Figure III-3. 1. Chromatography system. Waters Acquity UPLC coupled Synapt HDMS QTOF MS (Waters, Manchester, U.K.)

The LC−MS conditions were in accordance with the method described in Arapitsas, Speri, Angeli, Perenzoni, & Mattivi, (2014) and used by Arapitsas et al. (2016) and Arapitsas & Mattivi, (2018). Briefly, mobile phase flow rate was 0.28 mL/min and the eluents were water (A) and methanol (B) both with 0.1% v/v formic acid as an additive; the gradient used was as follows: 0–1 min, 100 % A isocratic; 1–3 min, 100–90 %A; 3–18 min, 90–60 %A; 18–21 min, 60–0 % A; 21–25.5 min, 0 % A isocratic; 25.5–25.6 min, 0–100 %A; 25.6–28 min 100 % isocratic; Mass spectrometric data were collected by separate runs in positive and negative ESI mode over a mass range of 50–2,000 amu with scan duration of 0.4 s in centroid mode. The transfer collision energy and trap collision energy were set at 6 and 4 V, respectively. The source parameters were set as follows: capillary, 3 kV for positive scan and 2.5 kV for negative scan; sampling cone, 25 V; extraction cone, 3 V; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 1000 L/h; and nebuliser gas, 50 L/h.

The UPLC–MS/MS data acquisition was performed using the same conditions described above, but in V mode; the data were acquired in centroid mode with a scan duration of between 0.2 and 0.5 s. For MS/MS experiments the molecular ions were fragmented by setting the transfer collision energy to 20 and 40 V.

External calibration of the instrument was performed at the beginning of each batch of analysis by direct infusion of a sodium formate solution (10 % formic acid/0.1 M NaOH/Acetonitrile at a ratio of 1/1/8), controlling the mass accuracy from 40 to 2,000 m/z (less than 5 ppm), and mass resolution (over 14,000 FWHM). LockMass calibration was applied using a solution of leucine enkephalin (0.5 mg/L, m/z 556.2771 for positive and 554.2620 for negative ion mode) at 0.1 mL/min.

2.5. Data analysis

2.5.1. Sensory analysis: Rate-k-attributes

According to Section III-Chapter 2, the results derived from rate-k-attributes methodology evaluation with nose clips were considered. To find discriminate attributes a two-way ANOVA (panellists as the random and samples as the fixed factors) was calculated for each of the 23 attributes of the list. A principal component analysis (PCA) was carried out with the correlation matrix of mean intensity scores (n = 18 judges) of the significant attributes. A hierarchical cluster analysis (HCA) with the Ward criteria was applied to all dimensions derived from the principal component analysis (PCA) calculated with significant attributes. To identify the attributes that best defined clusters, a two-way ANOVA with the scores of attributes was calculated with panellists as the random factor and cluster as the fixed factor. For significant attributes ($P < 0.05$), pair-wise comparison test (Fischer test) was applied (5% risk).

All analyses were carried out with XLSTAT (2019.3.1.60623 version)

2.5.2. UPLC–QTOF MS analysis

For quality control during the runs and data analysis, principal component analysis plots generated by Progenesis QI (version 2.4, nonlinear dynamics) were employed, by importing the raw files directly into the software, and checking the distribution/clustering of the QC injections (Arapitsas et al., 2018). Progenesis QI parameters used for alignment were performed in software default mode, with peak picking performed at the maximum level, and the first minute, and the last 6 min of the run were excluded from data processing (only the 1–22 min range was used). By default, the Progenesis QI software considers a group of isotopic and adduct features coming from the same metabolite as a “compound”. Presumed markers were considered the “compounds” that according to the Progenesis QI statistical analysis had a maximum fold range of ≥2 and analysis of variance (ANOVA) p value ≤0.05, employing the wine classification according to their sensory similarity. The pattern

of the selected features was visually inspected one by one. Subsequently, a semi manual integration of visually inspected "putative markers" by the TargetLynx tool from MassLynx 4.1 software was performed. Finally, in order to filtrate "putative markers", pearson correlation coefficients between sensory and semi-quantitative data (area) were calculated.

Annotation was performed manually by comparing retention times and mass spectra accuracy with a mass tolerance of 5 ppm based on the previous experience of the group with the specific instrumentation mass resolution and in accordance with the four levels of annotation described by Sumner et al., (2007). MS/MS data was also registered to support the annotation of selected tentative biomarker.

Besides this approach in which all tentative chemical markers were considered, a second approach was followed. Based on the internal standard data set of the laboratory of Food Quality and Nutrition Department of Fondazione Edmund Mach (FEM) (chromatographic and spectral libraries of over 400 compounds obtained in the same condition of the experiment), it was possible to tentatively identify well-known wine metabolites previously annotated using the same protocol and therefore under the same conditions of the experiment (i.e., target compounds). The known wine metabolites annotated were integrated using the TargetLynx tools of Waters MassLynx 4.1 software (Milford, MA, U.S.A.). The parameters of the integration were set at chromatogram mass window of 0.08 Da, retention time window of ±0.2 min, smoothing iterations of 1, and smoothing width of 2.

2.5.3. PLS-Regression models

PLS-Regression models were calculated to predict sensory variables from chemical compounds (i.e., tentative chemical markers and target compounds) identified following the two approaches indicated above (i.e., untargeted and targeted).

Prediction models of sensory attributes were calculated by PLS-regression attending to the following model:

$$Y = XB + F$$

where, for a sample size n ($n = 42$), $X_{(42,61)}$ for untargeted and $X_{(42,108)}$ for target represent the input matrix, $Y_{(42,23)}$ the output matrix with the chemical variables, $B_{(61,23)}$ and $B_{(108,23)}$ are the matrix of regression coefficients and $F_{(42,23)}$ the matrix of residuals. Single response models are analysed. Then, single Y - variable Partial Least Square regression method is used for every significant sensory variable (Y) and the 61 and 108 chemical variables (X). Therefore, the prediction by regressing for one single y data on X was as follows:

$$y_i = Xb_i + f_i,$$

where, $y_{i\ (42,1)}$ are the vectors that represent every one of the sensory variables $1 \leq i \leq 23$ and $b_{i\ (61,1)}$ for target and $b_{i\ (108,1)}$ for untarget and $f_{i\ (42,1)}$ are respectively, the vectors of regression coefficients and residuals.

Input variables X have been standardised to comparable noise levels. Likewise, sensory variables $y_{i;1\leq i\leq 23}$ have been standardised.

With these considerations, a first PLS model was computed. Taking the ratio between sample size and number of variables into account, variable selection has not been considered to avoid the problem of overfitting. Models were validated using full cross validation.

Then, those models with validated explained variance greater than 39% were considered (i.e., regression coefficient, r > 0.6). The analyses have been carried out with Unscrambler X 10.5.1, Matlab R2018a, R 4.0 and XLStat v2018, XLStat 2020 version.

3. Results and discussion

3.1. Sensory characterisation of wines

The sensory data reported in the present chapter were collected in the experiment described in Section III-Chapter 2. These sensory data refer to taste and mouthfeel properties significantly differing among the 42 red wines. The description of wines was carried out using rate-k-attributes methodology (method developed in previous chapter, Section III-Chapter 2) and employing a nose clip and dark glasses. Thereby, sensory description was focused on taste and mouthfeel attributes.

Two-way ANOVA calculated with sensory data yielded eight significantly different ($P < 0.05$) attributes among the 23 attributes evaluated. The eight significant attributes were “bitter”, “sweet”, “dry”, “dry on tongue side”, “dry on palate”, “unctuous”, “oily” and “watery”, and another four attributes (“sour”, “silky”, “burning” and “prickly”) when relaxing the criteria for significance ($P < 0.1$). The PCA conducted with the projection of the average scores of the 12 significantly different attributes for the 42 wines yielded four independent and non-correlated sensory dimensions, which explained 71% of the original variance and were identified as significant according to the Kaiser criterion (eigenvalue ≥ 1). The first PC (31.4% explained variance) was positively contributed by the terms “dry on palate” ($r = 0.80$), “dry” ($r = 0.80$) and “bitter” ($r = 0.57$), and negatively by “silky” ($r = -0.72$), and “unctuous” ($r = -0.66$). The second PC (18.9% of original variance) was positively contributed by “oily” ($r = 0.70$), and negatively by “sour” taste ($r = -0.66$). The third dimension (11.3% explained variance) was mainly influenced by “sweet” taste ($r = 0.79$) and negatively by “burning” taste ($r = -0.61$). Finally, the fourth PC (9% of variance) was positively contributed by “dry on tongue” ($r = 0.63$) and negatively by “prickling” ($r = -0.60$) (Annexe III-3.1). Subsequently, the hierarchical cluster analysis (HCA) calculated on all the PCA dimensions (Figure III-3.2) showed two main clusters of wines. Cluster 1 formed by 23 samples, and cluster 2 formed by 19 samples. ANOVA results showed 12 significant attributes to differ among clusters: “dry” ($F =$

41.53; P < 0.0001), “dry on palate” (F = 38.56; P < 0.0001), “silky” (F = 18.05; P < 0.0001), “watery” (F = 17.45; P < 0.0001), “dry on tongue” (F = 17.05; P < 0.0001), “unctuous” (F = 12.87; P < 0.001), “sour” (F = 11.95; P < 0.001), “bitter” (F = 10.66; P = 0.001), “oily” (F = 10.36; P = 0.001), “gummy” (F = 7.60; P < 0.05), “prickly” (F = 5.49; P < 0.05) and “grainy” (F = 5.03; P < 0.05). Cluster 1 was mainly described as “dry” (average = 2.6), “dry on palate” (average = 1.9), “dry on tongue” (average = 1.0), “bitter” (average = 1.5) and “grainy” (average = 0.30). Regarding cluster 2, it presented the higher score values for the terms “sour”, “watery”, “unctuous, “silky”, “oily”, “prickly”, and “gummy”.

The two principal groups of wines classified according to their sensory similarity were employed in the Progenesis QI supervised data analysis for untargeted metabolome profile.

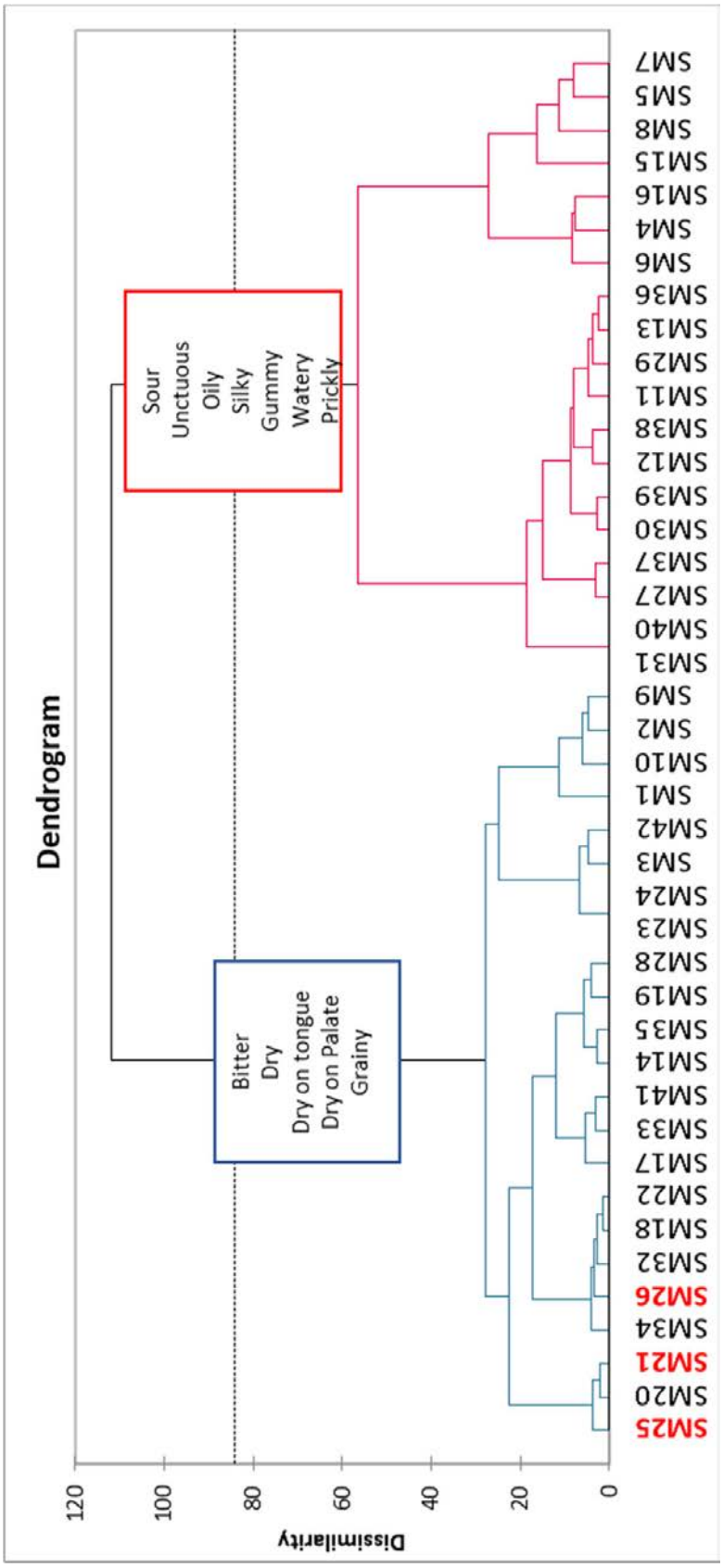

Figure III-3. 2. Dendrogram of the 42 wines derived from the sensory descriptions of samples by rate-k-attribute method. Attributes describing clusters refer to significant terms coming from ANOVA analysis with clusters as fix factor.

3.2. UPLC–QTOF MS analysis.

3.2.1. Metabolomic untargeted analysis

Before carrying out a detailed metabolomic data processing, it is necessary to ensure about the quality of the data set. The reliability and quality of the data acquired in a single batch were assessed by principal component analysis (PCA) for each ionisation mode. This is fundamental in order to guarantee the robustness of the data set.

Thus, the PCA performed (both for ESI+ and ESI-) showed the pool of QC samples in the middle of all samples at the PCA plots demonstrating the data set quality. Different sample injections did not show variations, which provide the reliability of the measure at different time points. Nevertheless, according to this unsupervised analysis, it was possible to notice that three out of 42 samples had a metabolomic profile especially different from the rest of wines (Figure III-3.3).

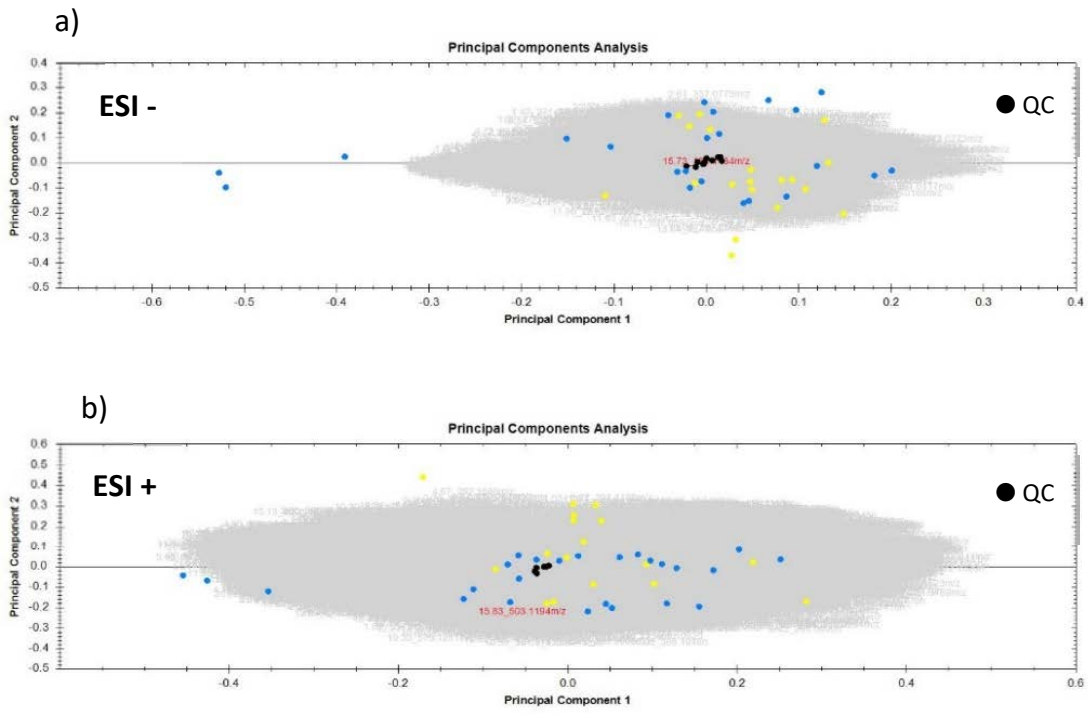

Figure III-3. 3. PCA plots considered all chemical features registered in a) ESI – and b) ESI+

We realised that these samples had a different origin, in the sense of how wines had been made. These 3 samples (SM21, SM25, SM26), proceeded from strongly pressed wine. The wine changes with the increase of the pressure. While soft pressing extracts less solids, harsh pressing, damage the grape skins and seeds and extracts unwanted chemical and sensory characteristics. Therefore, these samples were considered outliers and further not considered. As it can be seen, main sample distribution is maintained with the remaining 39 samples (Figure III-3.5). The unsupervised analysis did not show a clear separation between other samples apart from SM21, SM25, SM26. These results indicate that although sensory differences were found among the 39 wines samples showed similar chemical spaces.

The PCA performed for the 39 samples is shown in Figure III-3.4 and the QC injections, for both injections modes ESI+ and ESI- clustered together.

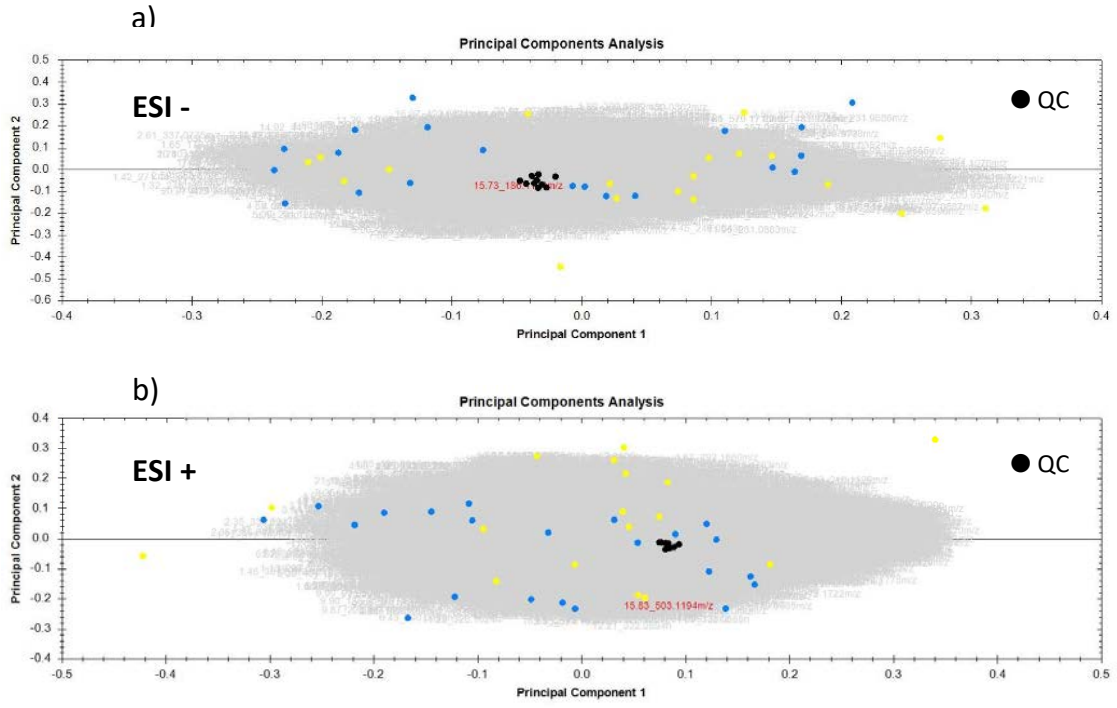

Figure III-3. 4. PCA plots of all features registered in a) ESI – and b) ESI+, for 39 out of 42 samples. Yellow and blue dots represent samples of cluster 1 and 2, respectively, and black dots are the QC samples.

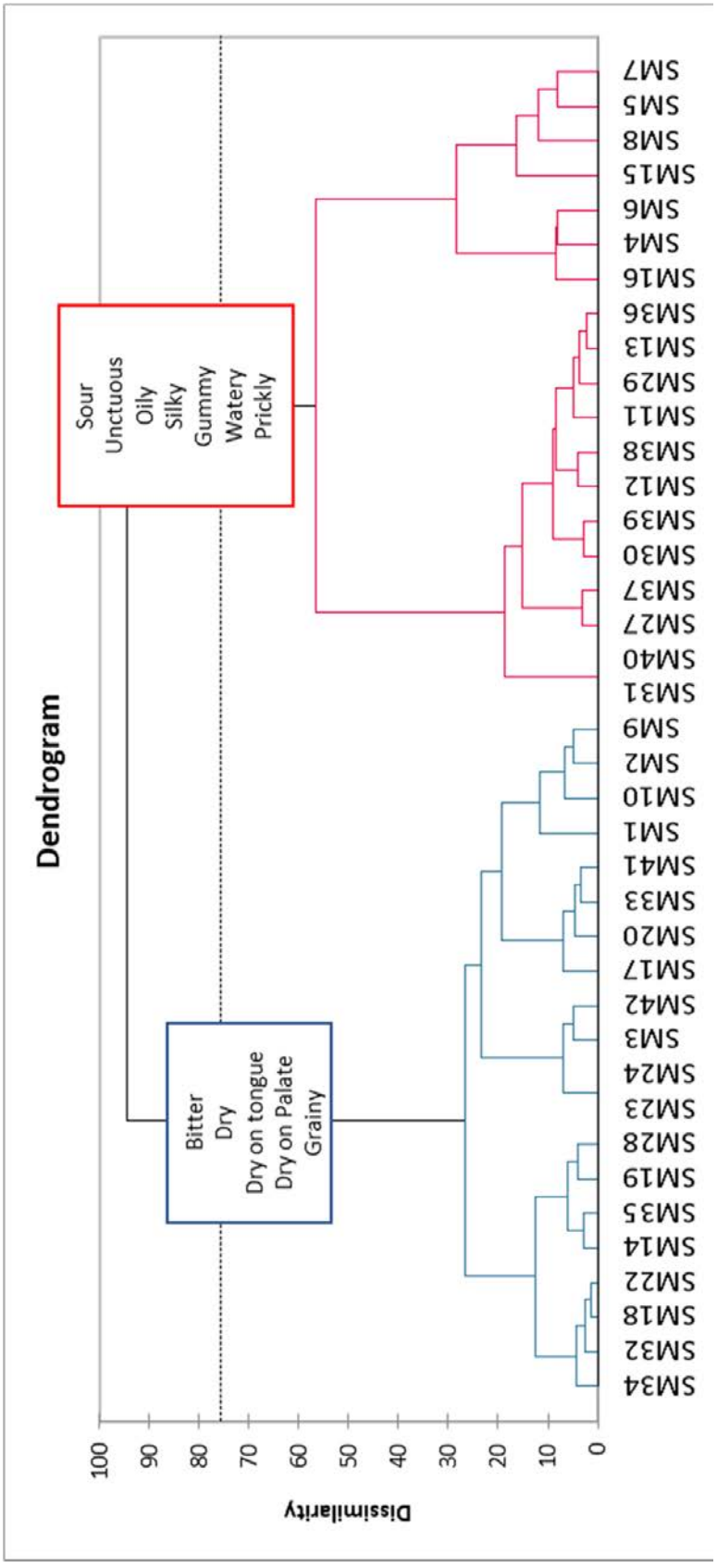

Figure III-3. 5. Dendrogram of the 39 wines derived from the sensory descriptions of samples by rate-k-attribute method. Attributes describing clusters refer to significant terms coming from ANOVA analysis with clusters as fix factor.

3.2.1.1. Tentative markers

The number of chemical features registered in the unsupervised analysis revealed 14280 and 6012 features in ESI positive and negative mode, respectively (summarised in the PCA plots given in Figure III-3.4). To investigate the differences between the metabolomic fingerprints of the wine groups with different sensory properties, and select putative compounds differing between them, an ANOVA analysis was performed. The "putative compounds" selected satisfy two criteria: 1) only features that according to the Progenesis QI statistical analysis had a maximum fold range of ≥2 (i.e., minimum ratio response between two values) were considered, and 2) abundance of features significantly differing among sensory groups (according to ANOVA; $p \leq 0.05$) were selected. Among these 20292 features, 1960 in ESI positive mode (14% of original features), and 410 in negative mode (6% of original features) were identified as tentative markers possibly involved in the sensory differences among wines.

The pattern of the selected features (i.e., their correct assignment to any of the sensory groups) was visually inspected one by one. Finally, in order to filtrate "putative markers" Pearson correlation coefficients between sensory and semi-quantitative data (area) were calculated. Accordingly, the final list of markers tentatively involved in the sensory (i.e., taste and mouthfeel) differences observed among wines was constituted by 38 tentative features for the ESI + mode and 23 for the ESI - mode. Among the 61 putative markers, only 23 in the ESI + and 16 in the ESI - mode could be annotated. Eight were identified (= first level annotation), fourteen were putatively annotated (= second level annotation), and seventeen were putatively characterised (= third level annotation) (Annexe III-3.2).

Since the main purpose was the prediction of sensory variables from chemical parameters, the 61 chemical variables annotated by UPLC−QTOF MS untargeted analysis, and the 12 significant attributes that differentiate between samples were employed to build predictive PLS models (models based on untargeted data).

3.2.2. Targeted analysis

In parallel, 108 target compounds (Annexe III-3.3) were selected based on the standard data set of the laboratory, and their area was employed to build predictive PLS models (models based on targeted data).

According with the currently knowledge, classical approaches following targeted analysis have a great limitation in that they are not able to collect enough information to be able to explain taste and mouthfeel attributes. Therefore, untargeted methods are suggested to provide a more complete information than targeted approaches. In this context, with the objective to evaluate relationships and predict mouthfeel attributes from chemical variables results coming from the targeted and untargeted method have been considered in parallel.

3.2.3. PLS-Regression models

Five sensory variables out of 12 could be satisfactorily predicted from chemical parameters by PLS-regression. Three fully validated PLS-models predicting sensory properties (sour, prickly, dry) from targeted and another three (oily, unctuous, dry) from untargeted chemical variables were obtained (Table III-3.1.).

Regarding models derived from the targeted approach (Annexe III-3.4.), three models predicting “dry”, “sour” and “prickly” attributes were obtained. Seventy-five out of 108 chemical features analysed contributed to construct the predictive models. Validated models explain at least 53% of original variance by full-cross validation, which corresponds to high correlation coefficients ranging from 0.7 to 0.8 (average = 0.75). The explained variances by calibration reach values at least of 70% (corresponding to correlation coefficients between 0.8 and 0.9).

Table III-3.1. Variables successfully modelled by PLS regression, % of explained variance by full cross validation (and the % of explained variance), the number of PLSs included in each model and the root mean squared error of prediction.

variable	*% explained variance P (number of PLSs) [% explained variance C]*	*RMSEP [RMSE C][1]*
Targeted approach		
sour	53% (3) [74%]	0.70 [0.54]
prickly	54% (2) [69%]	0.71 [0.54]
dry	66% (4) [88%]	0.58 [0.34]
Untargeted approach		
unctuous	55% (4) [76%]	0.68 [0.48]
oily	39% (2) [60%]	0.7 [0.60]
dry	74% (3) [87%]	0.48 [0.34]

[1]RMSE is given in z-units for a normal distribution. Given that 99.7% of normal values are between z=-3 and z=3, a RMSE of 0.6 represents around 10% of the range.

Regarding the "sour" attribute, it is usually observed that it presents a very well correlation with total acidity in red wines, however this relationship could not be observed in the present data set. This justifies the prediction of sour perception from other chemical compounds. The obtained model includes 47 significant variables, however most of them present low contribution coefficients. This result seems logical considering that most of the compounds included in the model are mainly astringent/dry or bitter, while its activity for sour taste seems to be secondary. Notwithstanding, it could be observed a negative highly significant correlation between "sour" and "dry" attributes ($p<0.001$), which would suggest the ability of dryness to mask sour perception as a result of a cognitive interaction between both sensations related to attentional deviation. Thus, the attention to a specific attribute such as "sour" presents difficulties, when other sensory property such as "dry" is present at considerably higher perceived intensity (de-la-Fuente-Blanco, Fernández-Zurbano, Valentin, Ferreira, & Sáenz-Navajas, 2017).

Concerning the “prickly” attribute, it could be naïvely associated to higher total acidity and/or ethanol content, but no significant correlations observed. Differently, a significant positive correlation was found for volatile acidity ($p<0.001$). Besides this simple correlation, a very significant validated PLSR model was built, which involves 23 out of the 108 features considered. As expected, acids such as vanillic, coumaric or cinnamic acids contribute positively to prickly sensation, together with other phenolic compounds such as malvidin 3-glucoside 4-vinylcatechol, ethyl-coumaric ester, and epicatechin malvidin-3-*O*-glucoside. Contrary to protocatequic acid, which contributes negatively to the model. The effective role of these compounds should be confirmed by addition experiments at concentrations observed in real wine samples.

A very significant PLS-model was obtained for “dry”. It includes 4PLSs, and explains 66% of the original variance by full-cross validation, which corresponds to a very high correlation coefficient of 0.8 (RMSE = 0.58). The explained variance by calibration reaches 88% (correlation coefficient of 0.9) with a RMSEC of 0.34. Regarding the features negatively correlated with this term, it is very important to remark the role of two sulfonated phenolic derivatives (procyanidin type B 4β-sulfonate and epicatechin 4β-sulfonate), which present the highest coefficients in the model together with tartaric acid. The role played by tartaric acid could be attributed to a masking effect of sour taste elicited by this acid over dryness, as explained above. Concerning the sensory contribution of sulfonated flavanol-derivative compounds, it was already suggested in the literature. The authors pointed out that these compounds could be associated to a decline of astringency (dryness in our case) by tannin alteration profile (Ma, Watrelot, Addison, & Waterhouse, 2018), which is well in line with our results. This result is also supported by the predictive model obtained for the “dry” attribute built considering chemical variables coming from the untargeted approach (Annexe III-3.5.). Interestingly, the predictive power of this model was higher than the model with target compounds. It included 3 PLSs and explained 74% of the original variance by full-cross validation

with a RMSE of 0.48 (correlation coefficient of 0.86). The explained variance by calibration reaches 87%, corresponding to a correlation coefficient of 0.9, with a RMSEC of 0.34. Among the 61 features considered, twenty-eight contribute to the predictive ability of dryness. A relative low number of variables (7 out of 28) contributed negatively to the model, while most of them contributed positively.

Another two sensory variables “oily” and “unctuous” could be satisfactorily predicted by the untargeted approach. The PLS-model obtained for “oily” includes 2PLSs, and explains 39% of the original variance by full-cross validation (correlation coefficient of 0.6) with a RMSE of 0.7. The explained variance by calibration reaches the value of 60%, which corresponds to a correlation coefficient of 0.77 (RMSE = 0.6). Fifteen out of the 61 considered chemical variables contribute to the model, however, we were unable to identify the features with the highest contribution to the model. The last sensory variable successfully modelled was “unctuous”. The validated model explains 55% of original variance by full-cross validation, and explained variance by calibration reaches the value of 76% (correlation coefficients of 0.74 and 0.87, respectively). Four PLSs are included in the model and the root mean squared error by cross-validation is 0.68, being the root mean squared error for calibration 0.48. An important number of features (28 out of 61, i.e., 46%) presented high contribution coefficients, indicating that the studied chemical composition has an important impact on this attribute. Fourteen variables positively contributed to the model. Among them, two were putatively characterised as an stilbene derivate, and dihydroxycinnamic acid sulphate. Variables with the highest coefficients and negatively contributing to the model could not be identified.

Considering the models obtained, among the most relevant results is the contribution of amino acids and peptides to the models. For example, L-Amino-l-phenylalanine as well as the peptide Leu-Leu-Tyr positively contribute to the “dry” model, while the L-Amino-l-phenylalanine contributes negatively to “oily”. Similarly, valine shows a positive contribution to the “dry” model (target approach), while proline contributes negatively. The sensory activity of amino acids is usually related

to bitter taste (Hufnagel & Hofmann, 2008), even if Castro-Alves et al. (2021) also reported that increases in amino acids along with organic acids were involved in the increase of bitter taste as well as in pungent sensations in dill. Besides, an ancient paper demonstrated that peptides and proteins were involved in the tactual and astringent properties of aqueous solutions (Solms, 1969). More recently, it has been suggested that proteins were able to modulate astringent perception in plant-protein-based solutions. The results showed that the difference in texture perception was related to the protein type rather than to their concentration. This indirect effect was attributed to interactions occurring between certain proteins and polyphenols, which generated a decrease in astringency perception (Cosson, Blumenthal, Descamps, Souchon, & Saint-Eve, 2021). In this context, the present results suggest that amino acids or peptides could be involved in the modulation of different mouthfeel properties of red wines, namely dry and oily sensations. However, this hypothesis should be confirmed with further experiments.

With regard to anthocyanidins and anthocyanidin derivates, results derived from previous chapters as well as the models derived from the present work suggest that they play an unquestionable role in taste and mouthfeel perception of red wines, however further studies are required to elucidate the role of their different structures in wine mouthfeel.

4. Conclusions

The present chapter shows an interesting chemosensory strategy for characterising taste and mouthfeel properties of wine. Untargeted analytical methods combined with multivariate analysis considering sensory results obtained by a verbal sensory task (i.e., rate-k-attribute) have demonstrated to be a useful tool to elucidate the chemical basis of taste and mouthfeel in wines.

Significant correlations and very satisfactory PLS-models could be built to predict sensory variables from chemical parameters, derived from both targeted and untargeted instrumental analysis. Remarkable are the results obtained for sulfonated-flavanol derivatives, which are suggested to be involved in the reduction of wine dryness. Similarly, amino acids or peptides are hypothesised to be involved in the modulation of dryness and oily sensations. Further, the sensory role of anthocyanidins and anthocyanidin derivates in taste and mouthfeel perception of red wines, could be confirmed. These results lay the foundations for different hypotheses related to the sensory (i.e., taste and mouthfeel) activity of different compounds in red wines. Further reconstitution studies are required to confirm the role of the different compound and their structures in wine mouthfeel and taste.

It has also to be highlighted that only five out of the 12 significant variables could be modelled. Thus, it has to be recognized that these approaches, following both sensory-targeted and sensory-untargeted (sensory targeted) methods, have still limitations and the development of new analytical tools is a key factor to success in the understanding of the evolution during aging of taste and mouthfeel properties driven by sensory-active compounds. The main limitation is the difficulty of analysing polymerised polyphenolic compounds (>3 units) including proanthocyanidins and polymeric pigments by HPLC methods. Developing methods to obtain information related to their molecular structure seems fundamental to fully understand wine mouthfeel and it remains a major challenge. In this context, information-rich

methodologies such as voltammetric and spectrofluorometric techniques could increase the array of sensory attributes satisfactory modelled.

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SECTION IV

STUDY OF MOUTHFEEL IN GRAPE AND WINE FRACTIONS.

SECTION IV. CHAPTER 1

Modelling grape taste and mouthfeel from chemical composition

1.Introduccion

Perceived intrinsic quality of wine is driven by volatile and non-volatile compounds involved in the formation of aroma, taste, mouthfeel and colour (Sáenz-Navajas et al., 2015). Wine aroma is the result of aroma and aroma precursors in the raw grape material, the action of microorganisms during alcoholic and malolactic fermentation, and aging. Wine taste, mouthfeel, and colour are driven principally by phenolic compounds present in grapes and their interaction with other wine components (e.g., polysaccharides, acids, alcohol or aroma among others). Grape phenolic compounds are extracted mainly from skins and seeds during the maceration and fermentation processes. The underlying aromatic and phenolic composition of the grape berry has an important impact on the final intrinsic quality of wines. Grape growers, winemakers, and researchers seek to infer wine quality from both the sensory and the chemical properties of wine grapes. For this purpose, classical oenological measures are traditionally employed in addition to berry-tasting with the specific aims of evaluating both the evolution of grape properties during ripening and the grape´s potential with which to elaborate wines. The determination of chemical parameters is usually limited to a reduced number of variables (total acidity, colour intensity, total polyphenolic index or pH among others), which does not produce an overall quality potential classification of the grape lot. The sensory assessment of grapes is not a generalized practice probably because the proposed berry sensory assessment method (Mantilla et al., 2010; Rousseau and Escoufier, 2000) has two main limitations that make the generation of reliable results difficult. The first is the use of a reduced number of panellists to carry out the sensory evaluation and second the lack of grape representativeness, because generally, in each evaluation one expert analyses a relatively reduced number of berries. These limitations, related to the sensory characterization of grapes, could be overcome by

extracting the main sensory-active compounds of grapes, mainly aroma, aroma precursors and phenolic compounds from a representative sample of grapes and characterize their sensory attributes with a sufficient number of panellists.

Recently, a study published by Alegre et al. (2020) shows a promising strategy for evaluating grape quality by focusing on the study of the aroma potential of wine grapes. The authors subjected reconstituted polyphenolic and aromatic fractions of grapes to accelerated hydrolysis in strict anoxia which yielded strong and differentiated aromas. This approach has proven useful for characterizing the potential aroma of grapes and thus, the evaluation of the potential aroma quality of grapes. The present research is focused on taste and mouthfeel induced by the phenolic fraction. Taste quality refers to the classical percepts of sweetness, sourness, bitterness, saltiness and umami which are the sensations that occur when the receptors present in the taste buds of the oral cavity are activated. Mouthfeel is related to tactile sensations generated in the oral cavity by the activation of the trigeminal nerve. The mechanisms modulating mouthfeel are the least understood; the study of "astringency" and its sub-qualities elicited by wine phenolic compounds have been scarcely found in the literature (Gawel, Oberholster, & Francis, 2000; Piombino, Pittari, Gambuti, Curioni, Giacosa, Mattivi, et al., 2020; Sáenz-Navajas, Avizcuri, Ferrero-del-Teso, Valentin, Ferreira, & Fernández-Zurbano, 2017).

Little is known about the sensory properties elicited by the phenolic fraction of grape berries and their relationship with chemical variables. This knowledge would be valuable for inferring grape quality. Despite the large amount of instrumental chemical strategies currently available, perceived taste and mouthfeel cannot be predicted from chemical composition and consequently, these percepts can only be measured by sensory evaluation strategies. In this context, the present work aims at identifying chemical markers that allocate the inference of sensory properties driven by the phenolic fraction of grapes. One of the main challenges is to differentiate the phenolic fraction of grapes based on their mouthfeel properties. This could be attributed to the lack of reference materials illustrating these sensory

characteristics. New strategies have been developed and successfully applied for describing wine mouthfeel. These strategies are alternative to classical descriptive analysis and do not require consensus among participants and thus do not need reference materials (Ares et al., 2014; Valentin et al., 2012; Varela & Ares, 2012). These strategies include non-verbal and verbal approaches. Among the first, similarity based methods such as "free sorting task" (Chollet, Valentin, & Abdi, 2014) have shown to be interesting approaches that highlight the most salient sensory differences among wines in terms of mouthfeel and taste perceptions (Sáenz-Navajas, Ferrero-del-Teso, Jeffery, Ferreira, & Fernández-Zurbano, 2020). Verbal-based strategies such as "rate-all-that-apply" (RATA) or its variant rate-k-attributes have been successfully applied to differentiate red wines in terms of mouthfeel and taste by wine experts without previous training (Sáenz-Navajas et al., 2020). In this verbal-based strategy, the list of specific attributes of each product is a main concern to discriminate among samples. To this end, a relatively ample list of mouthfeel and taste properties elicited by phenolic fractions was recently developed (Sáenz-Navajas et al., 2017) and its use in discrimination trials was confirmed to be valuable with a wide range of wines (Sáenz-Navajas et al., 2020) and phenolic fractions (Ferrero-del-Teso et al., 2020). However, the efficacy of this strategy to discriminate phenolic fractions directly from grapes has remained unevaluated.

In this context, the ability to differentiate the phenolic fraction of different grape lots based on their sensory properties following alternative sensory descriptive methods such as sorting task or rate-k-attributes method was the first hypothesis of the present study. The second hypothesis was the ability to predict sensory differences elicited by grape phenolic fractions from chemical measurements. To test these hypotheses a wide range of Garnacha Tinta and Tempranillo Tinto grapes harvested at different maturation points from distinct geographic origins were selected in order to represent a large variability in their chemical composition. Tempranillo Tinto typically presents higher levels of phenolic compounds than Granacha Tinta (Santesteban, Miranda, & Royo, 2011); the variability endowed by

the range of harvests should also provide a wide array of taste and mouthfeel sensory attributes. The phenolic fraction (PF) of 31 grape batches (15 for Tempranillo Tinto and 16 for Garnacha Tinta) was obtained following the strategy proposed by Alegre et al. (2020). Reconstituted PFs were sensory evaluated following both verbal and non-verbal strategies and sensory descriptors derived from the verbal task were predicted by PLS-regression models from chemical variables.

2.Material and methods

2.1. Samples

2.1.1. Grapes

During the 2017 harvest, two different varieties, Tempranillo Tinto and Garnacha Tinta from different regions (DO Ribera del Duero and DOCa Rioja for Tempranillo Tinto and DOCa Rioja and DO Somontano for Garnacha Tinta) were harvested by hand from distinct blocks in different dates. For the Tempranillo Tinto variety, a total of 15 samples from five different blocks and in three different weeks were collected. For Garnacha Tinta, 16 samples from eight blocks were harvested in two different dates, each separated by one week (Table IV-1.1 and Table IV-1.2).

Table IV-1. 1. Detailed list of Tempranillo Samples employed in the study.

Sample	Variety	Block	Date of Harvest	pH	TA	ºBrix	Origin
T-A-1	Tempranillo	A	28-08-2017	-	-	-	D.O. Ca Rioja
T-A-2			06-09-2017	3.5	4.3	24.6	
T-A-3			12-09-2017	3.8	4.5	23.6	
T-B-1	Tempranillo	B	28-08-2017	-	-	-	D.O. Ca Rioja
T-B-2			12-09-2017	3.8	4.3	25.7	
T-B-3			03-10-2017	3.5	4.2	25.1	
T-C-1	Tempranillo	C	07-09-2017	3.2	5.5	19.8	D.O. Ca Rioja
T-C-2			21-09-2017	3.2	5.9	24.5	
T-C-3			26-09-2017	3.3	4.9	23.8	
T-D-1	Tempranillo	D	06-09-2017	3.6	4.0	25.0	D.O Ribera del Duero
T-D-2			13-09-2017	3.7	4.3	24.2	
T-D-3			21-09-2017	3.5	4.1	23.6	
T-E-1	Tempranillo	E	13-09-2017	3.9	3.8	24.6	D.O Ribera del Duero
T-E-2			19-09-2017	3.3	4.7	23.6	
T-E-3			26-092017	3.6	3.8	23.8	

Table IV-1. 2. Detailed list of Garnacha Tinta Samples employed in the study.

Sample	Variety	Block	Date of Harvest	pH	TA	ºBrix	Origin
G-A-1	Garnacha Tinta	A	07-09-2017	3.6	3.5	23.6	D.O. Ca Rioja
G-A-2			18-09-2017	3.2	5.7	23.8	
G-B-1	Garnacha Tinta	B	19-09-2017	2.9	7.9	23.6	D.O. Ca Rioja
G-B-2			27-09-2017	3.19	7.8	25.8	
G-C-1	Garnacha Tinta	C	30-08-2017	-	-	-	D.O. Ca Rioja
G-C-2			07-09-2017	3.3	4.7	25.6	
G-D-1	Garnacha Tinta	D	30-08-2017	-	-	-	D.O. Ca Rioja
G-D-2			07-09-2017	3.4	4.2	20.0	
G-E-1	Garnacha Tinta	E	11-09-2017	3.2	4.8	26.0	D.O Somontano
G-E-2			27-09-2017	3.5	4.3	27.6	
G-F-1	Garnacha Tinta	F	11-09-2017	3.1	5.7	23.0	D.O Somontano
G-F-2			18-09-2017	3.6	4.5	26.0	
G-G-1	Garnacha Tinta	G	11-09-2017	3.5	3.9	25.2	D.O Somontano
G-G-2			18-09-2017	3.4	4.9	27.2	
G-H-1	Garnacha Tinta	H	11-09-2017	3.3	4.9	26.0	D.O Somontano
G-H-2			21-09-2017	3.4	5.8	24.4	

2.1.2. Preparation of grape extracts

For each sample, ten kilograms of grapes were first destemmed, and 8.5 Kg of the destemmed grapes were macerated with 5 g/hL of potassium metabisulfite and ethanol 15 % (p/p). After one week of maceration at 5 °C samples were pressed with a hydraulic wine press of 15 Kg of capacity and stored at 5 °C in the dark. Two weeks later, when the solids were precipitated, samples were bottled. For each grape batch, eight 750 mL-bottles were obtained as average (corresponding to a yield of 70%). Details of the process can be seen in Figure IV-1.1.

Figure IV-1. 1. Diagram of grape extracts preparation.

2.1.3. Preparation of phenolic fractions (PF)

A volume of 750 ml of each grape extract was centrifuged at 4500 rpm, 10 °C for 20 min after which were separated from the alcohol in a rotary evaporator system (8 mbar, 28 ºC, 30 min). The resulting dealcoholized extracts (containing no more than 2% ethanol) were passed through a 10g C18 prepared cartridge (Waters- Sep Pak-C18 35 cc, Figure IV-1.2). For cartridge conditioning, methanol followed by milli-Q water with 2% ethanol were employed. Then, the whole sample was loaded, and washed with milli-Q water pH 3.5 to remove sugars, amino acids, acids and ions. Cartridges were finally dried by letting air pass through them and phenolic fractions (PF) were recovered with 100 mL of ethanol as described by Alegre et al. (2020).

Figure IV-1. 2. *System of* phenolic fractions (PF) *preparation*

2.1.4. Preparation of samples for sensory analysis

PFs (coming from 750 mL of grape extract and eluted with 100 mL of ethanol in the SPE system) were twice concentrated by rotatory evaporator system (i.e., the resulting PFs were 15 times concentrated). Then, PFs were reconstituted to their original volume in a solution prepared with mineral water, 1g L^{-1} of tartaric acid reaching 7% of ethanol concentration and pH was adjusted to 3.7.

The level of ethanol in the hydroalcoholic model solution (7%) was selected in preliminary bench top tastings. It corresponds to the minimal level of ethanol able to induce the lowest “burning” and “hot” effect that is able to mask other sensations.

2.2. Chemical analysis

2.2.1. Chemicals

Preparation of grape extracts: LiChrosolv quality ethanol and potassium metabisulfite were purchased from Merck (Darmstadt, Germany).

Extraction of (PFs): Sep Pak-C18 resins, prepacked in 10 g cartridges, were obtained from Waters (Ireland) and LiChrosolv quality methanol and ethanol were purchased from Merck (Darmstadt, Germany). Deionized water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use.

Reconstitution of phenolic fractions (PFs): L (+)-tartaric acid (E334) was supplied by Enartis (Trecate, Italy).

Spectrophotometric and chromatographic analysis: ovalbumin and (−)-epicatechin (purity ≥ 90%) were purchased from Sigma-Aldrich (St Louis, MO). Phloroglucinol and all the solvents for the phloroglucinolysis reactions, extraction, isolation and analysis were purchased from FLUKA Sigma-Aldrich (St. Louis, MO, USA). Highest purity (> 98%) grade (+)-catechin, (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), procyanidin B1 and procyanidin B2 were obtained from TransMIT PlantMetaChem (Gießen, Germany). The phloroglucinolated derivatives epicatechin 4-phloroglucinol, epicatechin-gallate 4-phloroglucinol and epigallocatechin 4-phloroglucinol were prepared according to Arapitsas et al (2021). Malvidin 3-*O*-glucoside was obtained from Sigma-Aldrich (St Louis, MO). HPLC-grade acetonitrile and *o*-phosphoric acid were purchased from Scharlab (Sentmenat, Spain), methanol of LC-MS LiChrosolv grade used for the preparation of mobile phases was purchased from Fluka Sigma-Aldrich and the LC-MS grade formic acid used as the mobile phase additive was obtained from Sigma-Aldrich.

2.2.2. Conventional oenological parameters

The concentrated PFs were reconstituted in a 3.7 pH solution prepared with 5 g L^{-1} tartaric acid, milli-Q water, and hydroalcoholic solution (12%, v/v).

Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and color intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984).

2.2.3. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP), and large polymeric pigments (LPP) in wines and fractions was carried out as described by Harbertson, Picciotto, & Adams (2003).

2.2.4. Characterization of tannins

Tannin activity was calculated as the specific enthalpy of interaction between tannins and a hydrophobic surface (polystyrene divinylbenzene HPLC column), as proposed by Revelette, Barak, and Kennedy (2014).Briefly, the analyses were carried out with an Ultra High Pressure Liquid Chromatography system (Shimadzu Nexera, KIOTO, JAPAN) coupled to a Photodiode Array Detector - SPD-M30A from Shimadzu (KIOTO, JAPAN). It was operated with Labsolutions software, using a PLRP-S 100 Å 3µm, 2.1 × 50 mm column (Agilent) protected with a PLRP-S 100Å 3µ × 5 mm guard column (Agilent). The samples were run at four column temperatures (30, 35, 40, and 45 °C). The concentration of tannin and concentration of pigments were also determined, and were reported in (-)-epicatechin equivalents.

2.2.5. Mean degree of polymerization by phloroglucinol reaction

The protocol was made according to a previously described method (Arapitsas et al., 2021). Briefly, a solution of 0.1 N HCl in MeOH, containing 100 g L^{-1} phloroglucinol and 20 g L^{-1} ascorbic acid was prepared. Then, 100 µL of the PFs

samples were reacted individually with 100 µL of the phloroglucinol solution at 50 °C for 30 min. In order to stop the reaction 1 mL of 40 mM aqueous sodium acetate was added. Samples were filtered by 0.22 µm before injection. Calibration curves were prepared with (+)-catechin, (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), procyanidin B1, procyanidin B2, epicatechin 4-phloroglucinol, epicatechin-gallate 4-phloroglucinol and epigallocatechin 4-phloroglucinol for the quantification before the reaction and with the corresponding phloroglucinol adducts after reaction (Figure IV-1.3).

The quantification was carried out with an Acquity Ultra Performance Liquid Chromatographic system (Waters, MA, USA) coupled to a Xevo TQ MS System (Waters, UK) operating under MassLynx XS software, using a Waters Acquity HSS T3, 1.8 µm, 2.1 × 150 mm column (Waters), at 40 ºC. The compound detection was based on specific MS transitions in Multiple Reaction Monitoring (MRM) mode. The injection volume of 2 µL was employed for both the standard solutions and the samples.

The mean degree of polymerization (mDP) was calculated as the ratio between total units (extension + terminal) to terminal units (calculated as the difference between monomers before and after the phloroglucionolysis nucleophilic reaction).

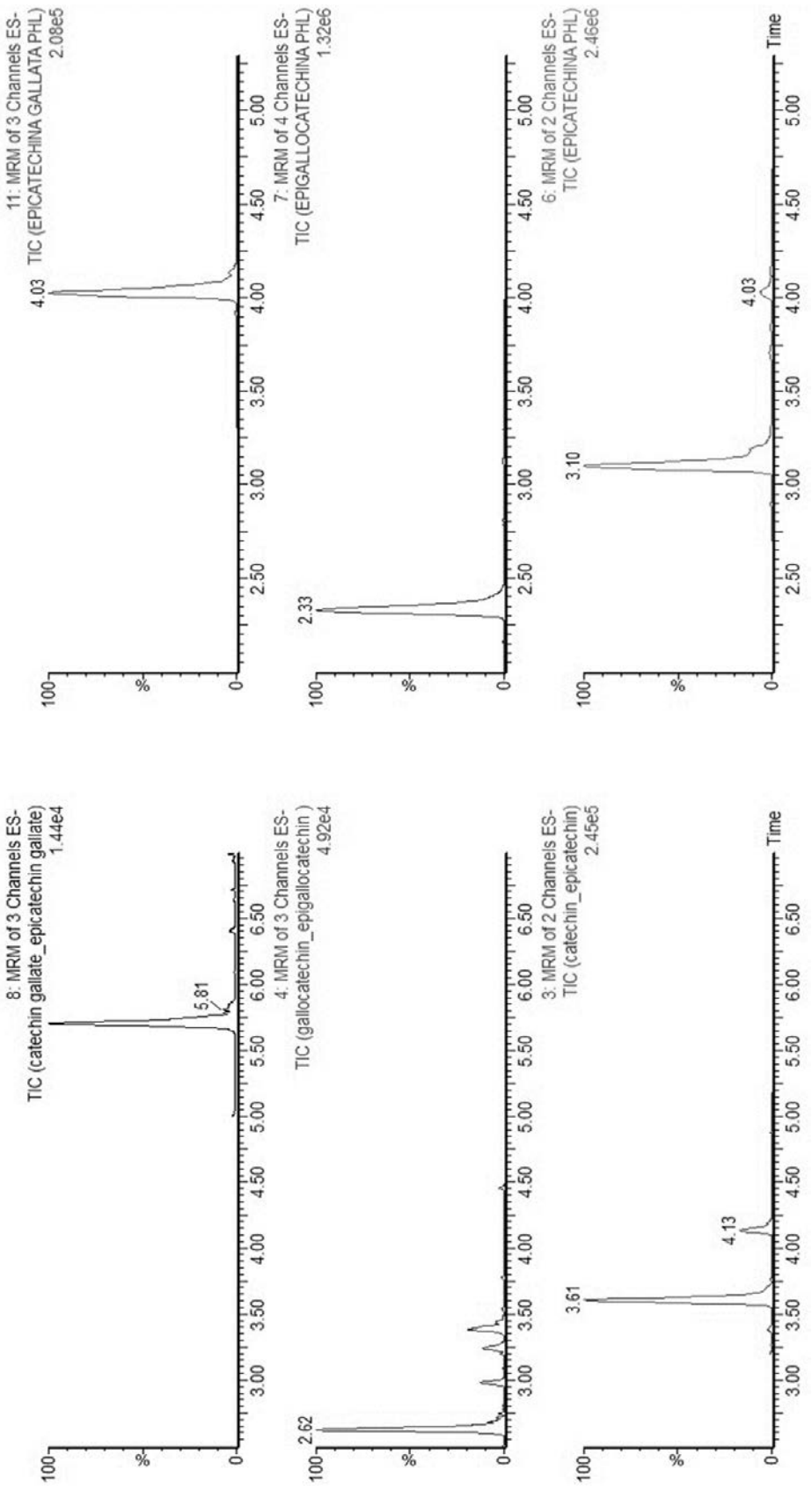


Figure IV-1. 3. Some chromatograms derived from the analysis of the mean degree of polymerization by phloroglucinol reaction, according to Arapitsas et al. (2021). a) Epicatechin gallate (ECG), b) Gallocatechin (GC), Epigallocatechin (EGC) and c) catechin (C), Epicatechin (EC), and the corresponding phloroglucinol adducts after reaction a') b') c').

2.2.6. UHPLC-MS/MS determination of anthocyanins

The samples were analyzed with an Acquity Ultra Performance Liquid Chromatographic system (Waters, MA, USA) coupled to a Xevo TQ MS System (Waters, UK) operating under MassLynx XS software. A reverse phase (RP) Acquity UPLC BEH C18, 1.7 µm, 2.1 × 150 mm column (Waters), protected with an Acquity UPLC BEH C18, 1.7 µm, 2.1 × 5 mm precolumn (Waters) was employed. The quantification was carried out using the method described by Arapitsas et al. (2012). All the compounds were quantified as equivalents of malvidin-3-O-glucoside. Some chromatograms can be seen in Figure IV-1.4.

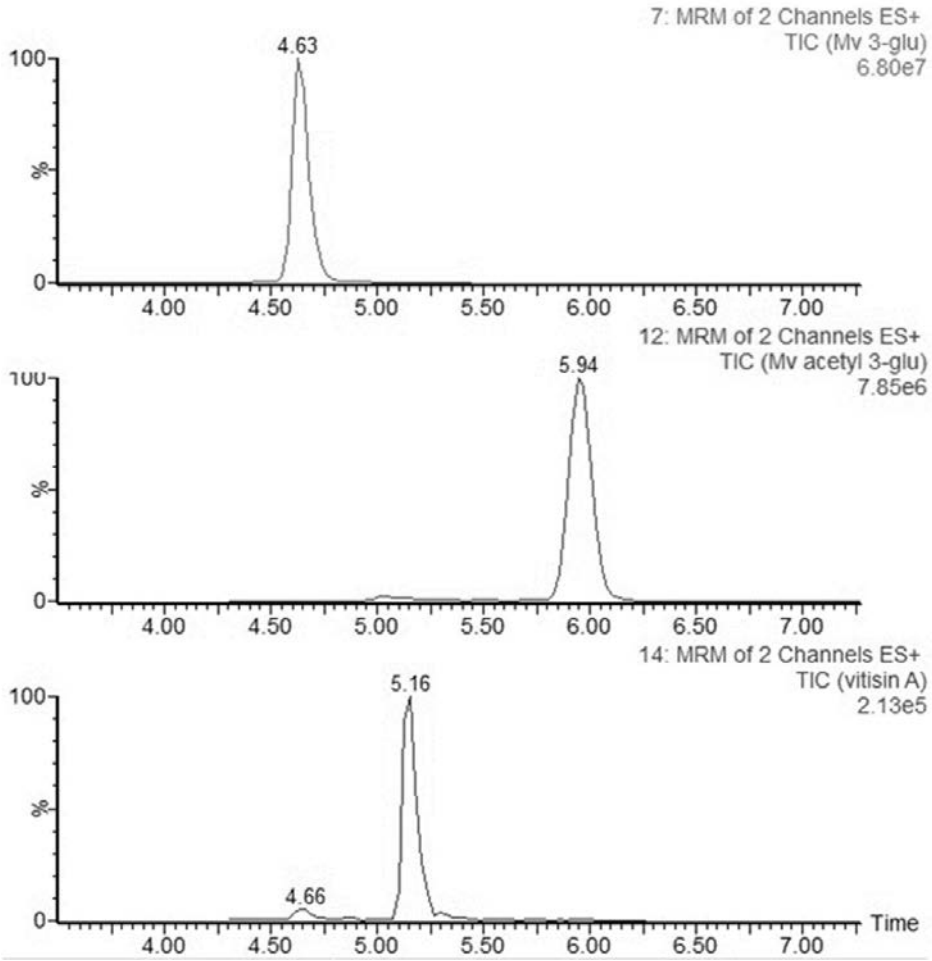


Figure IV-1. 4. Sample chromatograms of anthocyanins obtained by Arapitsas et al. (2012) method. a) Malvidine-3-glucoside, b) Malvidin 3-(6”-acetyl)-glucoside and c) Vitisine A.

2.2.7. UHPLC-MS/MS determination of polyphenols

For the determination of flavanols, flavonols and hydroxycinnamic acids the method described by Vrhovsek et al. (2011) was employed. Samples were analyzed with an Acquity Ultra Performance Liquid Chromatographic system (Waters, MA, USA) coupled to a Xevo TQ MS System (Waters, UK) operating under MassLynx XS software. The chromatographic separation of the phenolic compounds was carried out in a Waters Acquity HSS T3 column, 1.8 µm, 2.1 × 150 mm (Waters). Some chromatograms can be seen in Figure IV-1.5.

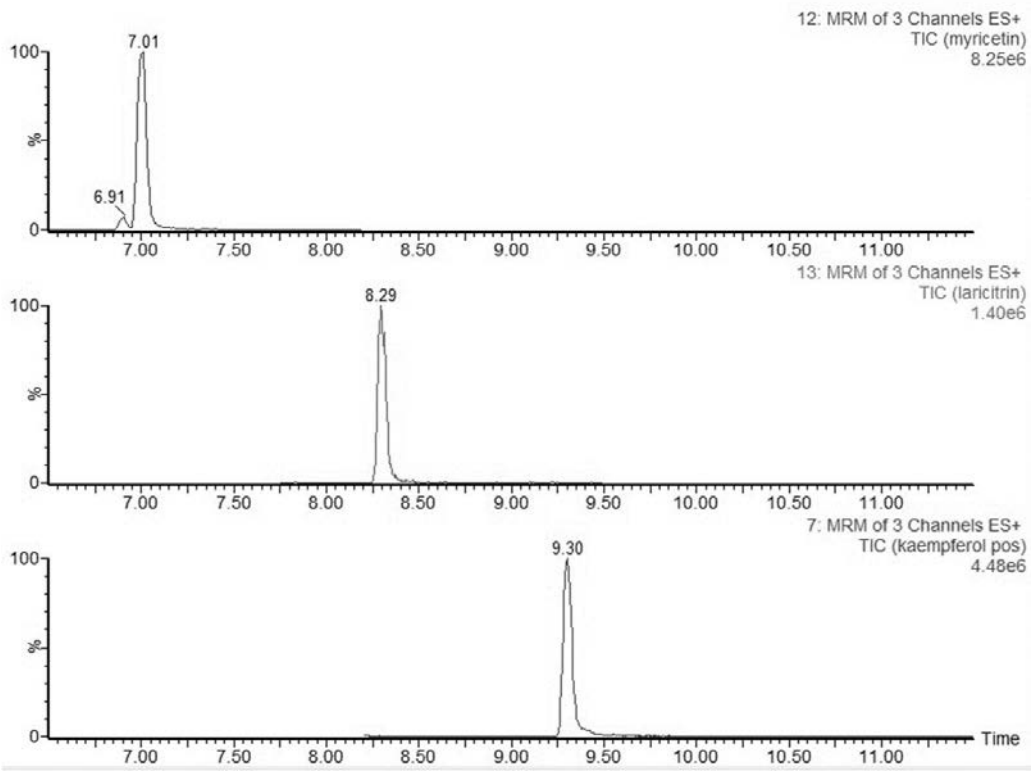


Figure IV-1. 5. Sample chromatograms derived from the UHPLC-MS/MS analysis of low molecular weight polyphenols. a) Myricetin b) Laricitrin and c) Kaempferol.

2.3. Sensory characterization

Two different sensory strategies were followed: sorting task (non-verbal) and rate-k-attributes (verbal). Both tasks were carried out by 21 wine experts from Rioja area, Spain (18 women and 3 men, ranging in age from 26 to 55, with an average age of 39). They were all established winemakers with extended experience in wine production and tasting. Samples were served in normalized dark approved wine glasses (German Institute for Normalization, DIN) labeled with 3-digit random codes, in a randomized distinct order of presentation for each participant. Samples were served at room temperature and evaluated in a ventilated, air-conditioned tasting room (approximately 20 ºC).

Panelists were instructed to put the sample in the mouth and to gently distribute it during five seconds throughout the oral cavity (as a mouthwash) to reach the entire surface of the mouth (including the mouth wall, gums, back palate and tongue). After expectorating the sample, panelists had to wait one minute before rating the sample. After each sample, they were required to follow a mandatory rinsing protocol with mineral water and pectin (1 g L^{-1}) before tasting the next sample (Colonna, Adams, & Noble, 2004). Although samples were odorless, they were instructed not to smell samples in an orthonasal manner.

Participants were informed that samples had been prepared in the laboratory and were not commercial wines. They were also required to sign a consent form prior to undertaking the sensory testing. They were neither informed about the objective of the study nor paid for their participation.

2.3.1. Sorting task

Two sorting task sessions (30 min each) were held on the same day (separated by at least 15 min), devoted to Tempranillo Tinto and Garnacha Tinta Garnacha sample sets, respectively.

In each session, participants were simultaneously presented with all samples (15 for Tempranillo Tinto and 16 for Garnacha Tinta) and were asked to sort them on the table according to similarities in the sensations perceived in mouth (mouthfeel and taste). Participants could form as many groups as they wished (minimum of two groups) and put as many samples as they wanted in each group (groups could be formed by only one sample). After that, they were asked to note the three-digit codes of the samples belonging to each group on a paper sheet and were asked to describe the groups they formed with their own words (maximum of three terms per group).

2.3.2. Rate-k-attributes

Panelists attended two sessions (35 min each) held on different days; one for each variety. Each session was split into two parts separated by an imposed pause of 15 min. Samples were characterized following a rate-k-attribute method with a list of 23 taste and mouthfeel related attributes (Section I, Table I-1.8) that had been previously developed (Sáenz-Navajas et al., 2017). Participants were asked to taste and rate the intensity of a maximum of five attributes appearing in each sample on a 7-point scale (1 = not intense; 7 = very intense). Attributes that were not rated were allocated a value of zero when collecting data. To avoid bias due to order of presentation, attributes on the list appeared in a distinct randomized order for each participant.

2.4. Data analysis

2.4.1. Sorting Task

For each participant, results were encoded in an individual similarity matrix (wines x wines) with each cell indicating weather two wines were put in different groups or in the same group (0 and 1, respectively). These individual matrices were summed across participants; the resulting co-occurrence matrix represents the global similarity matrix, where larger numbers indicate higher similarity between samples and the main diagonal accounts for the number of participants. The

resulting co-occurrence matrix was submitted to a non-parametric MDS analysis in order to obtain a spatial representation of the samples. Hierarchical cluster analysis with the Ward criterion was performed on all the MDS dimensions.

Terms derived from the description of the groups were analyzed. First an initial list was built with all the terms elicited by participants. This list was reduced by omitting adverbs and words with hedonic or emotional character. Then, a lemmatization process was performed; words sharing the same lemma or root were grouped. Finally, a triangulation process was followed individually by three experienced researchers to achieve a final consensual list of terms. Terms belonging to the same semantic category were grouped, the frequency of citation of each consensual term was calculated, and only those cited by at least three panelists (15% of the panel) were considered. Chi-square ($\chi 2$) test was applied for calculating significance differences ($P < 0.05$) among clusters. In addition, two-way ANOVAs (panelists as the random and cluster as the fixed factors) were calculated with the scores of the 23 terms obtained by rate-K-attribute method to get an alternative characterization of clusters derived from the sorting task. For significant attributes ($P < 0.05$), pair-wise comparison test (Fisher test) was applied (5% risk).

2.4.2. Rate-k-attributes

To find discriminate attributes a two-way ANOVA (panelists as the random and samples as the fixed factors) was calculated for each of the 23 attributes of the list. Then, for discriminate attributes, pair-wise comparison test (Fisher test) was applied (5% risk) for significant effects. A principal component analysis (PCA) based on the correlation matrix was carried out with the mean intensity scores ($n = 21$) of the significant attributes. A hierarchical cluster analysis (HCA) with the Ward criteria was finally applied to all dimensions derived from the PCA. To identify the attributes defining clusters, a two-way ANOVA with the scores of attributes was calculated with panelists as the random factor and cluster as the fixed factor. For significant attributes ($P < 0.05$), pair-wise comparison test (Fisher test) was applied (5% risk).

2.4.3. Comparison of sensory strategies

The degree of similarity between the two sensory spaces derived from the sorting task and the rate-k-attributes was calculated employing an RV coefficient (Robert & Escouffier, 1976) and the Pearson correlation coefficients between the dimensions of both sensory spaces.

2.4.4. Relationship between chemical and sensory variables

To establish relationships between mouthfeel related attributes and chemical variables, the 31 samples (15 Tempranillo Tinto and 16 Garnacha Tinta) were considered.

Firstly, to find discriminate attributes among the sample set, two-way ANOVAs (panelists as the random and samples as the fixed factors) were calculated for each of the 23 attributes of the list. Then, for discriminate attributes, pair-wise comparison test (Fisher test) was applied (5% risk) for significant effects. On the first three PCs a PCA was carried out with the mean intensity scores (n = 31) of the significant sensory attributes with VARIMAX rotation. Rotation eases the interpretation of results by maximizing high- and low-value factor loadings and minimizing intermediate values. Further Pearson correlation coefficients (r) and their significance were calculated between the significant mouthfeel related attributes among samples (n = 6), PCA dimensions (n = 3), and the calculated chemical variables (n = 45).

The six sensory attributes and the three PCA sensory dimensions were predicted by regressing calibration from chemical variables by PLS-regression attending to the following model:

$$Y = XB + F$$

where, for a sample size n ($n = 31$), $X_{(31,45)}$ represents the input matrix, $Y_{(31,6)}$ the output matrix with the chemical variables, $B_{(45,6)}$ is the matrix of regression coefficients and $F_{(31,6)}$ the matrix of residuals. Single response models are analyzed.

Then, single Y - variable Partial Least Square regression method is used for every sensory variable and the 45 chemical variables (X).

Input variables X have been filtered applying a 7 points window Stavizki-Golay smoothing; and, they have been standardized to comparable noise levels. Likewise, sensory variables $y_{i;1\leq i\leq 6}$ have been standardized.

Variable selection has not been considered to avoid the problem of overfitting. The model was validated using full cross validation.

All the analyses have been carried out with Unscrambler X 10.5.1, Matlab R2018a, R 4.0 and XLStat v2018.

3.Results and discussion

3.1. Sensory characterization of Tempranillo Tinto and Garnacha Tinta sample sets

The first objective of the present research was to evaluate the capacity of the proposed chemosensory strategy to differentiate among distinct sample sets. Therefore, 15 and 16 phenolic fractions of the Tempranillo Tinto and Garnacha Tinta sample sets, respectively, were obtained and characterized with the two sensory strategies.

3.1.1. Tempranillo Tinto sample set

In the sorting task carried out with the 15 Tempranillo Tinto samples, participants formed 2 to 8 groups; 5 on average. Two samples were grouped together a maximum of nine times (43% of participants). Figure IV-1.6 presents the dendrogram derived from the cluster analysis calculated with all the MDS dimensions that the sorting task data yielded. Four main groups were identified containing three, four, two and six samples, respectively. Nine attributes including “dry” (62% of maximum citations for a given wine), “bitter” (52%), “astringent” (52%) and “sour” (43%), followed by “sweet” (19%), “watery” (19%), “persistent” (14%), “sticky” (14%) and “coarse” (14%) were among the most cited to describe the groups. Based on the highest frequency of citation for each cluster, cluster 1 was mainly described as “dry” (48%) and “bitter” (41%), cluster 2 as “dry” (50%), cluster 3 as “dry” (40%) and “astringent” (40%), and cluster 4 was mainly characterized as “astringent” (44%) and “sour” (31%). These results suggest that the most salient attributes of the set of Tempranillo Tinto PFs are “dry”, “bitter”, “astringent” and “sour”. However, no significant effect was observed among clusters according to Chi-square (χ^2) test. Similarly, no significant effect of cluster was found for any of the attributes derived from the rate-k-method. Clusters obtained from MDS-HCA illustrate important sensory differences among the Tempranillo Tinto PFs herein

studied. However, the specific attributes explaining such significant differences could not be identified. This, firstly suggests that differences among samples could not be verbalized in the description step of the sorting task and secondly, that the list of terms of the rate-k-attributes method did not contain the specific term differentiating among samples.

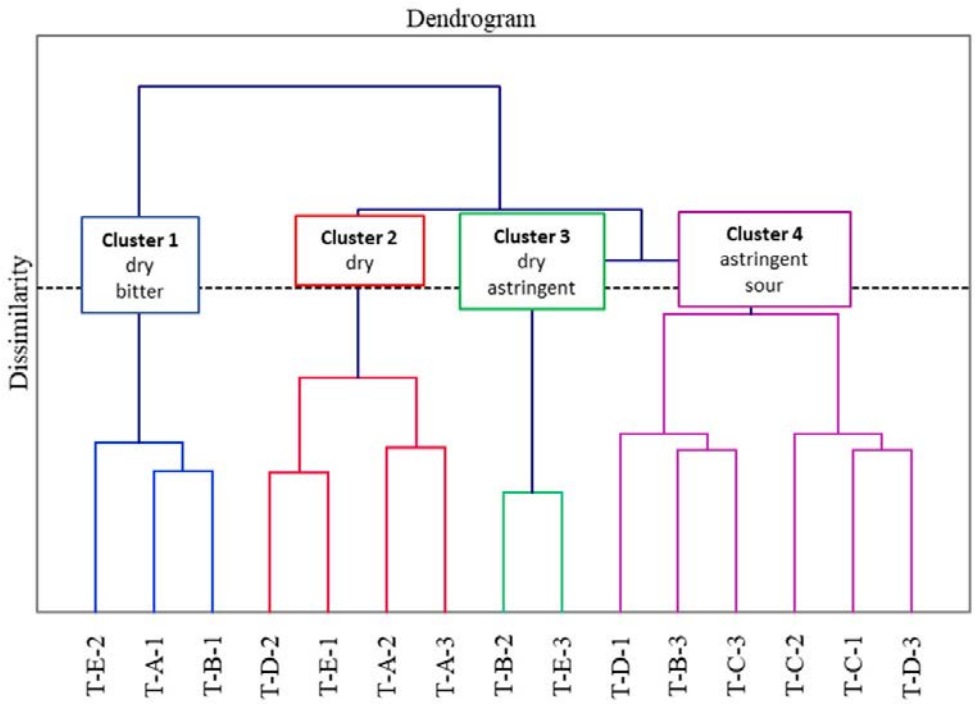


Figure IV-1. 6. Dendrogram of the polyphenolic fractions of Tempranillo Tinto derived from the sensory descriptions of samples by sorting task. Attributes describing clusters refer to terms with highest average frequency of citation- FC- calculated with individual FC of wines which belong to each cluster

Regarding the results derived from rate-k-attributes methodology, ANOVA results showed six significant attributes to differ among clusters: “coarse” ($F = 7.35$; $P < 0.0001$), “dusty” ($F = 6.50$; $P < 0.001$), “burning” ($F = 6.23$; $P < 0.001$), “bitter” ($F = 4.56$; $P < 0.005$) and “fleshy” ($F = 3.91$; $P < 0.05$), as well as “sticky” ($F = 2.54$; $P < 0.1$) when relaxing the criteria for significance. Further cluster analysis calculated on all PCA dimensions (PCA computed with the six significant terms) showed four main clusters of samples (Figure IV-1.7) with different sensory properties. Cluster 1, formed by two samples was mainly described as “sticky” (average = 1.26) and

"dusty" (average = 0.98), cluster 2, formed by three samples, presented the lowest score value for the term "bitter" (average = 1.36) (Figure IV-1.8). Cluster 3, including two samples, was significantly characterized by "burning" (average 0.76) and reached the highest score for "bitter" (average = 2.43). Cluster 4, which is formed by eight samples, presented the lowest scores for the attributes "coarse" and "fleshy".

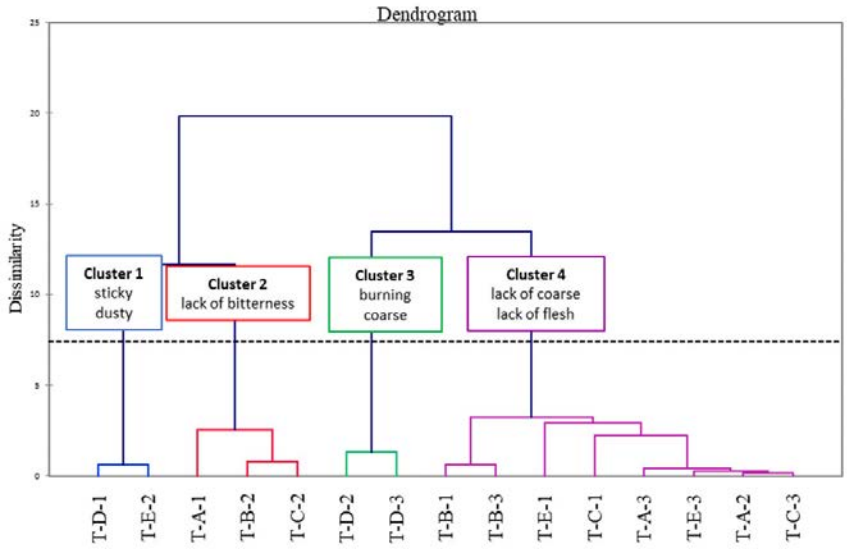


Figure IV-1. 7. Dendrogram of the polyphenolic fractions of Tempranillo Tinto derived from the sensory descriptions of samples by rate-k-attribute methods. Attributes describing clusters refer to terms with highest average scores calculated with wines that belong to each cluster.

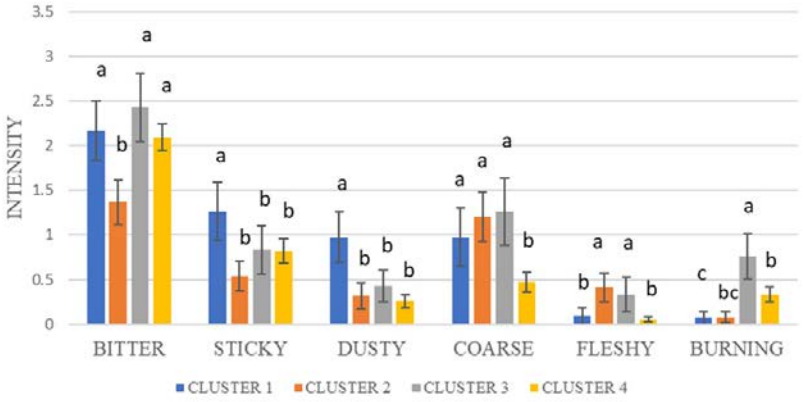


Figure IV-1. 8. Mean intensity scores of discriminant attributes among cluster derived from rate-k-attribute, scored by rate-k-attribute method, for Tempranillo sample set. Error bars are calculated as the ratio of standard deviation (sd) and the squared root of the number of panelists (n): $sd/n^{\wedge 0.5}$. For each attribute, different letters indicate significant differences according to post-hoc Fisher test ($P<0.05$).

The two sensory spaces obtained by two different sensory strategies were compared. Therefore, MDS dimensions obtained from sorting task and the PCs derived from sensory characterization by rate-k-attributes method were employed to determine the RV coefficient. The RV coefficient was found to be 0.398 ($P > 0.1$), which indicates that the configurations of the two sensory spaces were different. A possible explanation for this result is that these two approaches, verbal (rate-k-attributes) and non-verbal (sorting task), induce participants to adopt different strategies when characterizing samples. In the sorting task panelists follow a holistic strategy in which overall and most salient differences among samples (Valentin, Chollet, Nestrud, & Abdi, 2017) are identified. While the rate-k-attribute methodology, which follows an analytical approach, is able to identify subtler and specific sensory differences among the sample set (i.e., "coarse", "dusty" "burning", "bitter", "fleshy" and "sticky").

3.1.2. Garnacha Tinta sample set

Based on the sorting task, the 16 Garnacha Tinta samples were grouped into 3 to 8 groups, 5 on average, similar to the Tempranillo Tinto sample set; illustrating the sensory variability associated with Garnacha Tinta PFs. Any two samples were grouped together a maximum of 10 times (48% of participants). As Figure IV-1.9 shows, four main groups were identified. In this case, cluster 1 was formed by six samples, cluster 2 and 3 were built by three and five samples, respectively, and cluster 4 was composed of only two samples. The analysis of the attributes employed to describe the groups led to a final consensual list of 8 attributes including "dry" (62% of maximum citations for a given wine), "bitter" (52%), "sour" (48%), "astringent" (38%), and "watery" (24%), followed by "sticky" (19%), "sweet" (19%) and "alcoholic" (14%). Based on the highest frequency of citation for each cluster, all clusters were mainly described with the attributes "bitter" and "dry": 44% and 41% of citations for cluster 1, 41% and 51% for cluster 2, 43% and 48% for cluster 3, and 38% and 55% for cluster 4. Notably, cluster 2 reached the highest frequency of citation with "sour" (40%) and cluster 4 with "astringency" (31%). However, there

was no significant attribute that differed among clusters according to chi-square (χ^2) tests.

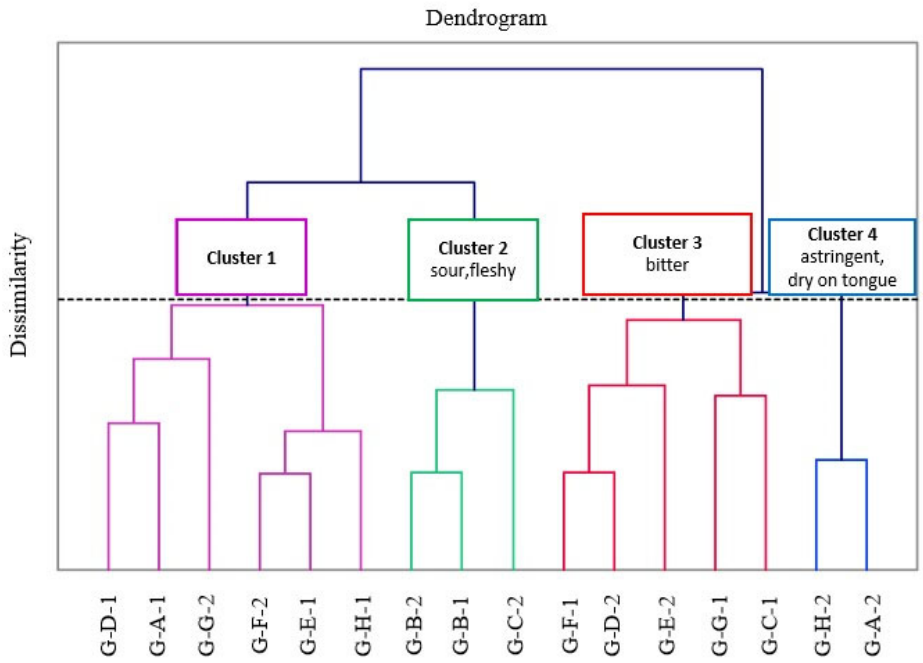


Figure IV-1. 9. Dendrogram of the polyphenolic fractions of Garnacha Tinta derived from the sensory descriptions of samples by sorting task. Attributes describing clusters refer to terms with highest average frequency of citation- FC- calculated with individual FC of wines which belong to each cluster.

Distinctly, three significant attributes evaluated by rate-k-attribute method appeared to be significant among clusters derived from sorting task: “dry on the tongue” ($F = 3.956$; $P < 0.05$), “fleshy” ($F = 4.648$; $P < 0.05$), and “bitter” ($F = 2.467$; $P < 0.1$) (Figure IV-1.10). Cluster 3, which included five samples, was significantly characterized by “bitter” (average 2.05). Cluster 4, which was formed by two samples, presented the highest score for the attribute “dry on tongue” (average 1.26) and cluster 2, formed by three samples, was significantly characterized by “fleshy (average 0.37).

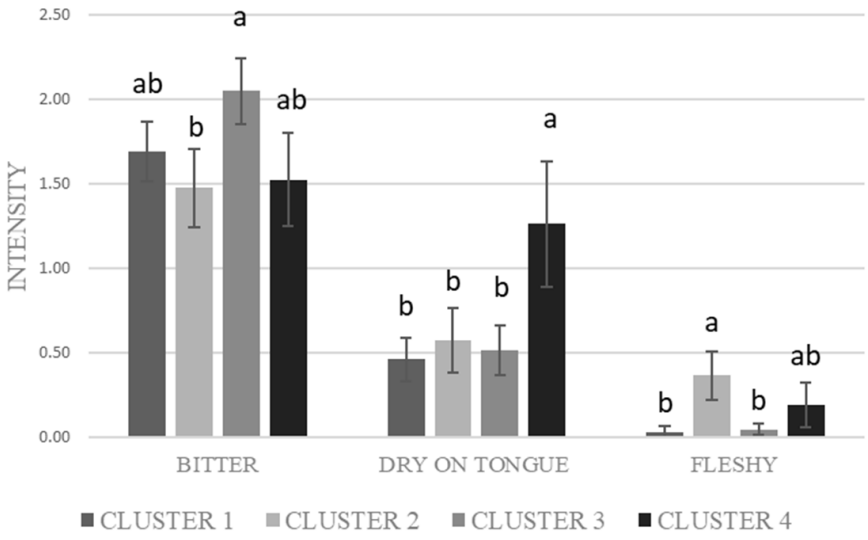


Figure IV-1. 10. Significant terms derived from rate-k-attributes method describing Garnacha Tinta clusters derived from sorting task. Error bars are calculated as the ratio of standard deviation (sd) and the squared root of the number of panelists (n): sd/n^0.5. For each attribute, different letters indicate significant differences according to post-hoc Fisher test (P<0.05).

In regards to the results derived from rate-k-attributes, as occurred for the Tempranillo Tinto samples, three out of 23 attributes presented the highest scores among the 16 PFs studied: “dry” (max = 4.00), “bitter” (max = 2.33) and “dry on palate” (max = 2.14).

Figure IV-1.11, illustrates the four clusters obtained from the hierarchical cluster analysis calculated on all the PCA dimensions. Even if this option (two clusters) was the most natural partition of the tree diagram, we chose the partition containing a total of four clusters as it permitted more precise descriptions of samples belonging to each of the groups. The ANOVA results identified significant differences among clusters for 8 out of the 23 attributes evaluated, “dry” ($F = 9.577$; $P < 0.0001$), “bitter” ($F = 6.705$; $P < 0.001$), “dry on tongue” ($F = 6.363$; $P < 0.001$), “coarse” ($F = 5.445$; $P < 0.005$), “watery” ($F = 3.827$; $P < 0.05$), “grainy” ($F = 3.097$; $P < 0.05$), “sticky” ($F = 2.851$; $P < 0.05$) and “dry on palate” ($F = 2.480$; $P < 0.1$) when relaxing the criteria for significance (Figure IV-1.12).

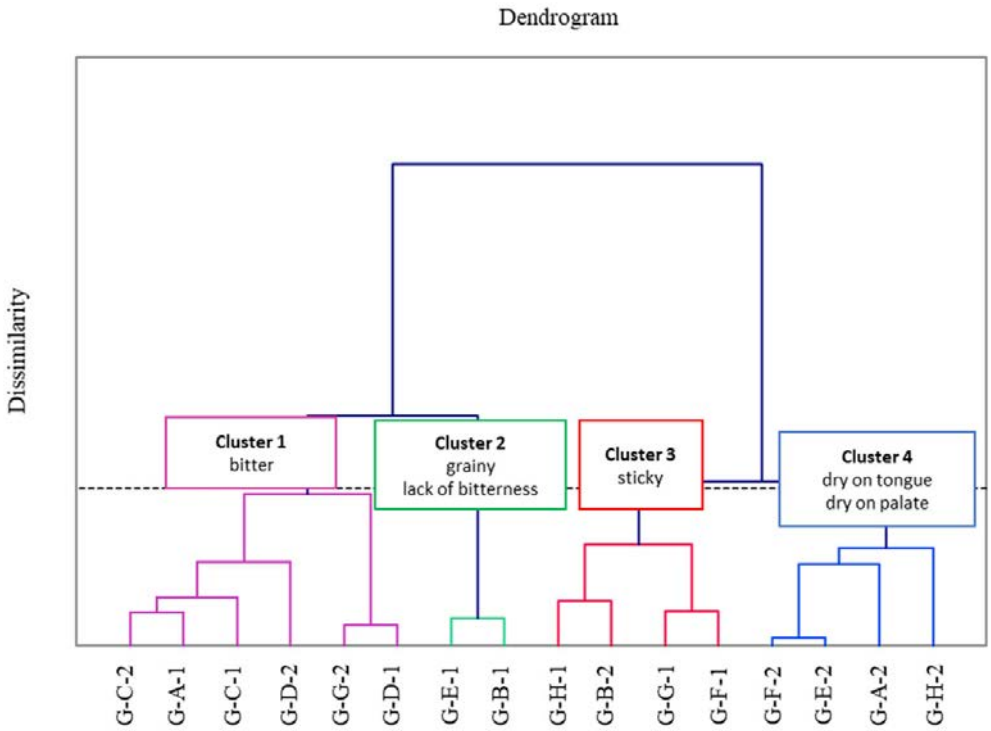


Figure IV-1. 11. Dendrogram of the polyphenolic fractions of Garnacha Tinta derived from the sensory descriptions of samples by rate-k-attribute method. Attributes describing clusters refer to terms with highest average scores calculated with wines that belong to each cluster.

Clusters 1 and 2 (formed by six and two samples, respectively) present the lowest scores for “dry”, “dry on tongue”, “sticky” and “coarse”, yet the highest for “watery”. Cluster 2 presents the lowest value for the attribute “bitter” (average = 0.86), and the highest for “grainy” (average = 0.26). Clusters 3 and 4 (formed by four samples each) presented the highest scores for the “dry” and “coarse” attributes. Cluster 3 was characterized by “sticky” (average = 0.96); while, cluster 4 was mainly described as “dry on tongue” (average = 1.12) and “dry on palate” (average = 1.67).

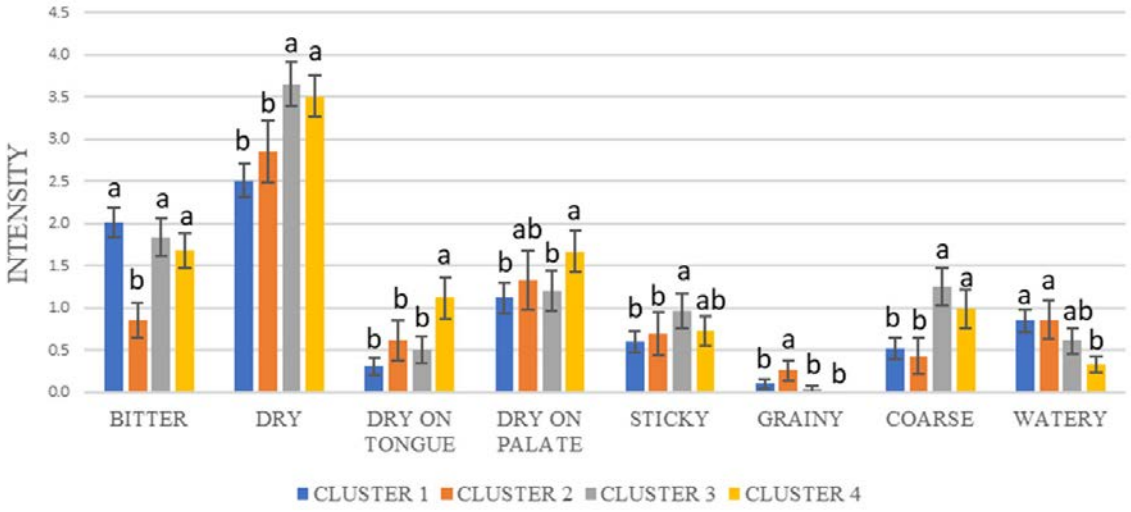


Figure IV-1. 12. Significant terms derived from rate-k-attributes method describing Garnacha Tinta clusters derived from rate-k-attributes. Error bars are calculated as the ratio of standard deviation (sd) and the squared root of the number of panelists (n): sd/n^$^{0.5}$. For each attribute, different letters indicate significant differences according to post-hoc Fisher test (P<0.05).

As in the Tempranillo Tinto sample set, when comparing the two sensory spaces derived from sorting and rate-k-attributes, differences are found according to the RV coefficient, which was not found to be significant (RV = 0.420; P > 0.1). This is consistent with the fact that although the sorting task approach can overcome the difficulties in verbalizing mouthfeel features of wine reported by various authors (Piombino et al., 2020; Sáenz-Navajas et al., 2017), only evident sensory differences among the studied samples can be identified. Distinctly, panelists follow an analytical strategy under the rate-k-attribute, in which more specific and subtle differences can be identified.

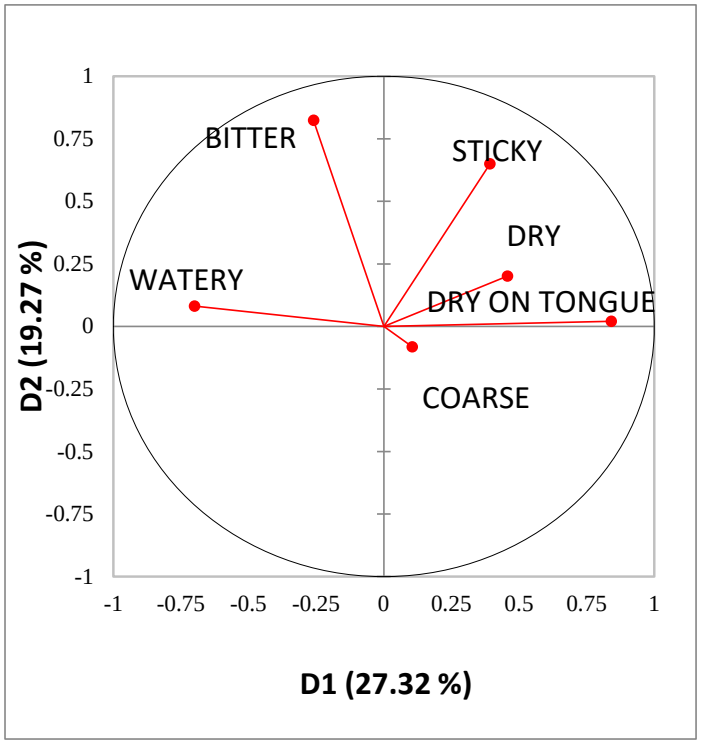
Overall, these results confirm our first hypothesis related to the appropriateness of the proposed chemosensory strategy to identify intra-varietal sensory variability associated with phenolic fractions of grapes. The RATA methodology was more efficient in identifying subtle and specific sensory differences among PFs derived from the same variety.

3.2. Relationships between mouthfeel properties and chemical measurements

The main objective was to establish relationships and predict mouthfeel attributes from chemical variables of grape phenolic fractions. Therefore, the sensory data derived from the rate-k-attributes method and chemical variables for the two varieties were pooled together (total of 31 samples) in order to increase the robustness of the statistical tests.

3.3.1. Sensory dimensions and significant taste and mouthfeel properties

Six significant attributes among the 31 samples differed based on ANOVA results; “dry” ($F = 2.382$; $P < 0.0001$), “coarse” ($F = 2.277$; $P = 0.0002$) “bitter” ($F = 1.538$; $P = 0.035$), “dry on tongue” ($F = 1.485$; $P = 0.048$), “sticky” ($F = 1.477$; $P = 0.050$) and “watery” ($F=1.374$; $P = 0.090$) when relaxing the criteria for significance. Figure IV-1.13 shows the PCA calculated with these significantly different attributes. The first three dimensions, which explain 70% of the original variance, are considered significant according to Kaiser criterion (eigen value > 1). These three dimensions were rotated with VARIMAX algorithm to facilitate the interpretation of the results. The first dimension, after rotation (D1), explains 27% of the variance and is mainly positively contributed by the attributes “dry on tongue” (43% of contribution, $r = 0.842$) and negatively by “watery” (30%, $r = -0.700$). The second dimension, D2, presents 19 % of the variance, and is mainly formed by “bitter” (59%, $r = 0.824$) and “sticky” (37%, $r = 0.650$). The third dimension explains 24% of the variance; it is mainly built by the attributes “coarse” (54%, $r = 0.881$) and “dry” (31%, $r = 0.673$). These results identify the presence of three main independent and non-correlated mouthfeel and taste dimensions defining the sensory space of PFs.



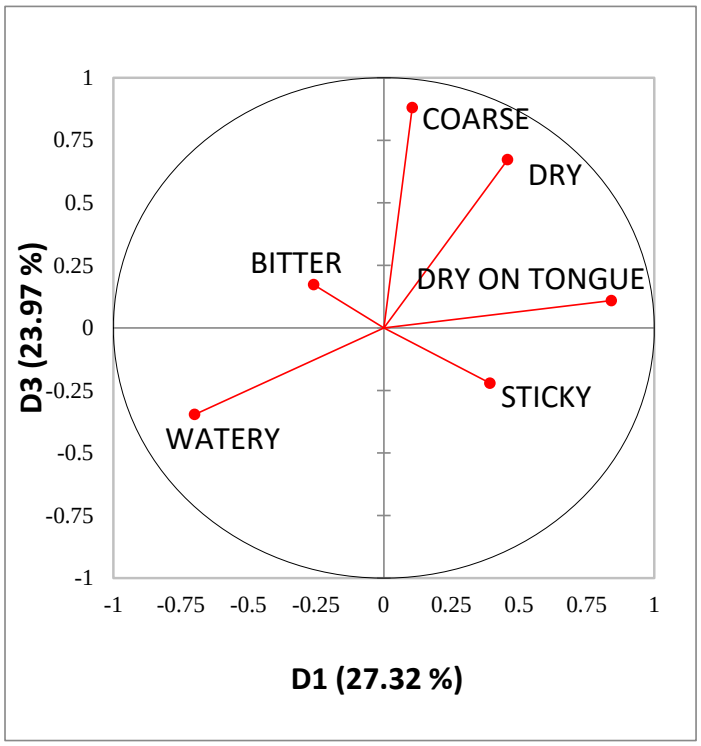


Figure IV-1. 13. PCA plots with the projection of rotated dimensions a) D1–D2 and b) D1-D3 performed for the significant terms of the 31 samples

3.3.2. Correlation between taste and mouthfeel dimensions and chemical parameters: correlation coefficients and PLS-modeling

Table IV-1.3 presents the correlation coefficients (r) between sensory attributes and dimensions and chemical variables. Table IV-1.4 contains the models that satisfactorily predict the sensory parameters (4 out of 9). Validated models explain more than 60% of original variance by full-cross validation which corresponds to high correlation coefficients (r) between predicted and measured values of at least 0.77. Explained variances by calibration, reach values at least of 80% ($r > 0.90$). Figure 4 lists the chemical variables included in models and the sign and magnitude of their coefficients following a color code. Interestingly, two out of three independent, non-correlated sensory dimensions identified (D1 and D2 of the PCA calculated with Varimax rotation) could be successfully modeled.

The highest significant linear correlations ($P < 0.0001$) (Table IV-1.3) were found between two chemical variables (tannin activity and tannin concentration) and the attribute “dry” ($r = 0.68$ for both variables). The tannin activity was measured as the interaction of tannins with a hydrophobic surface (Barak & Kennedy, 2013). Interestingly, “dry on tongue” was also positively correlated with both chemical variables ($r = 0.55$ for tannin activity, $r = 0.50$ for tannin concentration), but to a lesser extent than for “dry”. This is well in line with the PLS-models obtained for both the “dry” attribute and the PCA dimension D1 (related to dry on the tongue). Both models highlight the importance of tannin activity because this chemical variable presents the highest positive correlation coefficients, followed by tannin concentration (Figure IV-1.14).

Table IV-1. 3. Pearson correlation coefficients (r) calculated between chemical variables, significant taste and mouthfeel attributes and sensory dimensions (i.e., principal components derived from PCA of 31 samples). Significant correlations (P < 0.05) are marked in bold.

	Sensory attribute						Sensory dimensions		
	Bitter	Dry	Dry on tongue	Sticky	Coarse	Watery	D1*	D2†	D3‡
Tannin characterization§									
TA	0.05	**0.68**	**0.55**	0.25	0.2	-0.27	**0.54**	0.23	0.28
Tannin concentration	0.24	**0.68**	**0.50**	0.23	0.15	-0.12	**0.40**	**0.37**	0.29
Pigmented tannin	0.20	**0.57**	0.34	0.10	0.20	-0.09	0.24	0.25	0.34
mDp	0.1	**0.62**	**0.49**	0.15	0.19	-0.20	**0.42**	0.21	0.30
%PD	0.02	-0.21	-0.04	0.03	-0.23	**0.47**	-0.19	0.07	-0.28
%G	-0.34	-0.13	-0.18	-0.13	0.19	-0.26	0.00	**-0.38**	0.09
Conventional oenological parameters§									
TPI	0.21	**0.40**	0.17	0.17	0.19	-0.04	0.12	0.28	0.27
IC	0.31	**0.53**	0.29	0.19	0.11	-0.03	0.20	**0.40**	0.26
Anthocyanin-derived pigments§									
MP	0.18	**0.54**	0.34	0.21	0.15	-0.09	0.28	0.30	0.25
LPP	0.10	0.27	0.25	**0.58**	-0.07	-0.27	**0.46**	**0.40**	-0.11
Anthocyanins§									
Acylated	0.29	**0.48**	0.21	0.08	0.18	-0.05	0.11	0.31	0.33
Glycosylated	0.01	**0.51**	0.01	-0.04	0.3	-0.07	0.02	0.03	**0.44**
Vitisine A	-0.36	-0.02	0.00	-0.25	0.14	-0.25	0.09	**-0.44**	0.11
Flavonols§									
Kaempferol	**0.43**	0.28	0.15	0.18	0.04	0.07	0.03	**0.45**	0.16
Myricetin	**0.47**	0.34	0.19	0.25	-0.1	0.00	0.12	**0.54**	0.07
Laricitrin	**0.39**	0.12	0.03	0.30	-0.12	-0.18	0.12	**0.46**	-0.03

* Mainly built by dry on tongue and watery.
† Mainly built by bitter and sticky.
‡ Mainly built by dry and coarse.

§ **TA**: Tannin activity (-J/mol), **Tannin concentration** (mg/L), **Pigments** (mg/L), **TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.), **MP**: Monomeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **mDp**: mean degree of polymerization, **%PD**: percentage of prodelphinidins, **%G** percentage of galloylation, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside , **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, Delphinidin 3,5-diglucoside, Cyanidin 3,5-diglucoside, Petunidin 3,5-diglucoside, Peonidin 3,5-diglucoside, Malvidin 3,5-diglucoside, **Vitisine A** (mg/L), **Kaempferol** (mg/L), **Myricetin** (mg/L), **Laricitrin** (mg/L).

These results partly differ from previous studies in which no significant correlation between the tannin concentration or tannin activity and wine dryness perception was found (Watrelot, Byrnes, Heymann, & Kennedy, 2016). However, the results observed in the present research with grape PFs are well in line with other studies carried out in our laboratory with red wines, where linear (Ferrero-del-Teso et al., 2019) and non-linear (Sáenz-Navajas et al., 2019) relationships between tannin activity and the global "astringency" attribute were reported.

Table IV-1. 4. Variables successfully modeled in the set by PLS regression, % of explained variance by full cross validation (and the % of explained variance), the number of PLSs included in each model and the root mean squared error of prediction.

variable	*% explained variance P (number of PLSs) [% explained variance C]*	*RMSEP [RMSE C][1]*
Dry	64% (3) [82%]	0.63 [0.4]
Watery	66% (3) [86%]	0.58 [0.38]
D1 (dry on the tongue-watery)	66% (3) [81%]	0.67 [0.43]
D2 (dry/coarse)	63% (2) [80%]	0.57 [0.4]

[1]RMSE is given in z-units for a normal distribution. Given that 99.7% of normal values are between z=-3 and z=3, a RMSE of 0.6 represents around 10% of the range.

Regarding the mean degree of polymerization of tannins (mDP), significant positive correlations were found with "dry" (r = 0.62) and "dry on tongue" (r = 0.49). Accordingly, the coefficient for mDP is very high for the D1 model, and especially for "dry". Thus, this data is in agreement with other studies (Arnold, Noble, & Singleton, 1980; Gawel, 1998; Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003) where an increase in mDP resulted in an increased in the perceived overall astringency. A positive correlation was found between %PD and "watery" which is in line with the high positive correlation coefficient observed in the PLS-model. This could be explained in terms of decreases in the perception of astringency with higher %PD shown in previous research (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; Lisjak et al., 2020; Vidal et al., 2003).

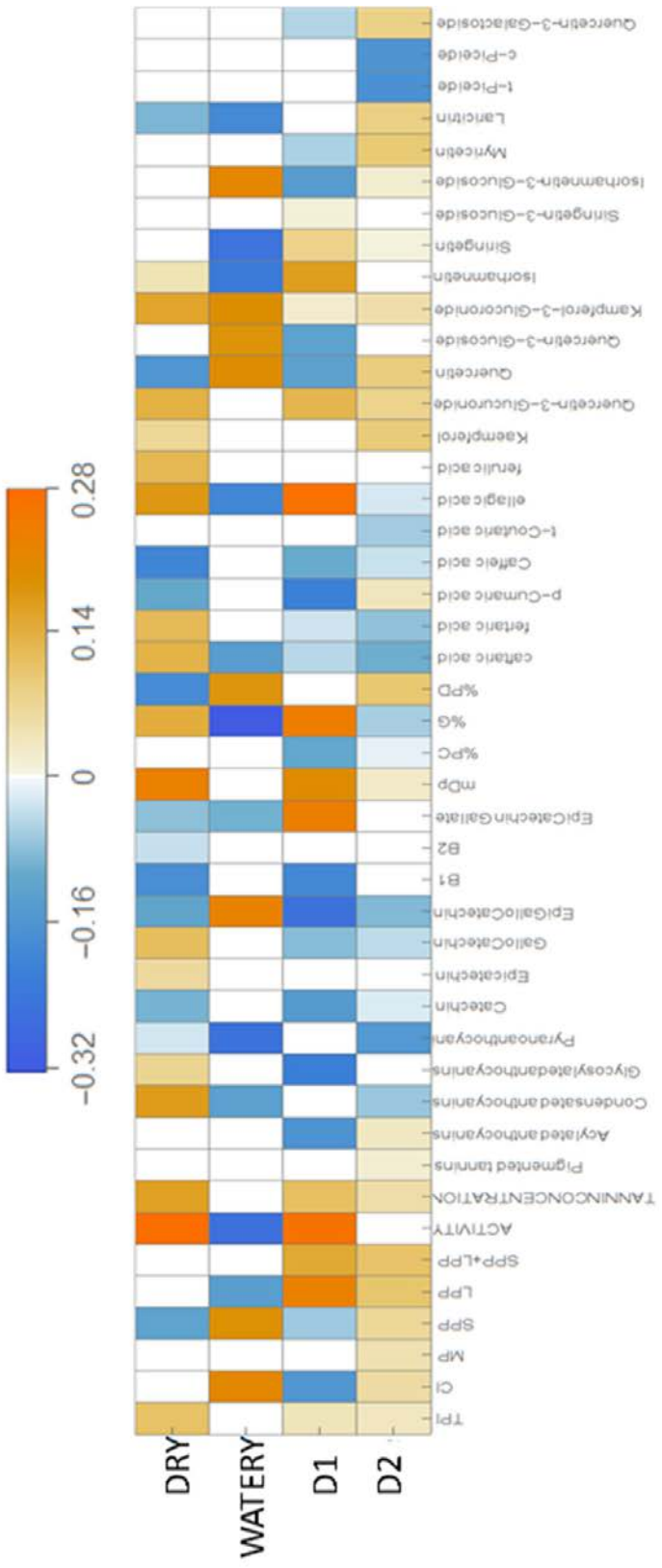


Figure IV-1. 14. Map with regression coefficients of variables included in validated PLS-models predicting sensory attributes or dimensions from chemical variables.

The term “sticky” (r = 0.58) as well as the second dimension (D2 contributed mainly by the attributes “sticky” and “bitter”) of the PCA (r = 0.40) are correlated with the level of large polymeric pigments (LPP), which present high positive correlation coefficients in the D2 of the PLS-model (Figure IV-1.14). Besides this, low molecular weight anthocyanins measured by both Harbertson and Adams assay (MP, r = 0.54) as well as glycosylated (r = 0.51) and acylated (r = 0.48) anthocyanins, measured by chromatography, show significant positive correlations with “dry”. While the highest coefficient in the PLS-models was that of the condensed anthocyanins (mainly dimers of anthocyanin-flavanol), which contribute significantly to the “dry” sensation. The high positive coefficients of MP presented by pigmented tannins and acylated anthocyanins in the D2 dimension model was a notable outcome. These results suggest the sensory importance of anthocyanins and anthocyanin-derived pigments in taste and mouthfeel sensations; they reinforce the results recently reported by other authors (Ferrer-Gallego et al., 2015; Ferrero-del-Teso et al., 2020; Paissoni et al., 2018).

Bitter perception is related to phenolics with low molecular weights such as flavonol, aglycones, and monomeric flavanols (Preys et al., 2006; Sáenz-Navajas et al., 2010). Results of this research are well in line with the previous research in the literature as significant positive correlations between flavonols such as kaempferol, myricetin, and laricitrin and the “bitter” attribute (r = 0.43, r = 0.47 and r = 0.39, respectively) were observed. Similarly, the model for the D2 dimension, to which bitterness greatly contributes, shows positive, high correlation coefficients for flavonols.

These results partly confirm our second hypothesis related to the relationships between taste and mouthfeel properties and chemical variables. Two (mainly D1 and D2) out of the three sensory dimensions representing taste and mouthfeel variability among the studied FFAs presented high and significant relationships with some of the chemical variables measured. Both sensory dimensions related to dryness and sticky/bitterness could be satisfactory modeled from the chemical variables studied.

The exception was the third dimension (D3), in which “coarse” is the main contributor, from grape fractions. The individual term “coarse” presented no significant correlation with chemical variables; neither it, nor D3 could be satisfactorily modeled. It cannot be ruled out that this percept is driven by other molecules present in wine such as aroma or mannoproteins or their sensory or physical interactions with polyphenols which were not examined in the present research.

4. Conclusions

The current research presents a new chemosensory strategy for characterizing the sensory properties of phenolic fractions of grapes. This approach has shown to be efficient in differentiating grape phenolic fractions based on mouthfeel and taste properties; both inter- and intra-varietal.

The non-verbal and holistic sensory task, i.e., sorting task, highlighted the salient sensory differences among samples. While the more specific descriptors and subtle differences varying among Tempranillo Tinto ("coarse", "dusty", "burning", "bitter", "fleshy", "sticky") or Garnacha Tinta ("dry", "bitter", "dry on tongue", "coarse", "watery", "grainy", "sticky", "dry on palate") PFs could be identified by the rate-k-attribute methodology; a methodology which follows an analytical verbal strategy.

Three distinct, independent, non-correlated, sensory dimensions could be identified for the overall sample set: 1) "dry on tongue/watery", 2) "sticky/bitter" and 3) "dry/coarse".

Significant correlations and very satisfactory PLS models could be built to predict sensory variables from chemical parameters. Tannin activity and tannin concentration along with mDP of tannins proved to be good predictors of PF perceived dryness. Flavonols could have a good prediction power for the "bitter" attribute and the "sticky/bitter" dimension. In addition, low molecular weight anthocyanins seem to be involved in the formation of the "dry" attribute, whereas large polymeric pigments in the "sticky" attribute and the "sticky/bitter" dimension.

Distinctly, the "coarse" dimension could not be modeled which suggests that there are other macromolecules involve in the formation of this percept. Examination of the "coarse" dimension should be tackled in future research.

With these results, grape properties and intrinsic quality could be inferred with the measurement of chemical variables. This approach provides an interesting tool to assess grape quality.

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SECTION IV. CHAPTER 2

Sensory variability associated with anthocyanic and tannic fractions isolated from red wines

1.Introduccion

Polyphenols are important bioactive molecules present in grapes and wines, with red wines being especially rich in these compounds. Their significance is based on their effect on human health (antimicrobial, antiviral, anti-inflammatory and antioxidant activity) (Arranz et al., 2012; Nash et al., 2018) as well as on their ability to modulate intrinsic quality of red wines (colour, aroma, taste and mouthfeel) (Soares, Brandão, Mateus, & de Freitas, 2017), thus consumer preference and appreciation. In this context, the most relevant polyphenols are tannins (oligomers and polymers of flavan-3-ols, dimers) and anthocyanins (and their derivative pigments).

Tannins can be classified as hydrolysable, such as those coming from oak wood or oenological tannin addition, or condensed (i.e., proanthocyanidins, PAs), coming from grape skin and seed and extracted during fermentation. The concentration and structure of PAs in wine are dependent on the grape variety, degree of ripening and interventions during winemaking (Harrison, 2018), researchers still seek to understand how these variables influence the composition and sensory perception of red wines. In a recent study (Sáenz-Navajas et al., 2017), the sensory properties of an isolated PA fraction (mean degree of polymerisation (mDP) in the range of 3-10), obtained from three red wines that differed in mouthfeel, were described with terms such as “coarse”, “grainy, “dry on the tongue” and “dry on the palate”. Interestingly, in another study of four wines with very different mouthfeel properties (Sáenz-Navajas et al., 2018), it was determined that the mouthfeel characteristics of the PA fraction isolated as per the previous study were more diverse, appearing to resolve into three types of sensory-active fractions: 1) “silky”, “sweet” or “greasy”, 2) “sticky” and “coarse”, and 3) “dry”, “granular” and “coarse”. These fractions were

characterised by measuring the usual chemical parameters linked to astringent mouthfeel sensations: concentration and activity of tannins (Watrelot, Byrnes, Heymann, & Kennedy, 2016), mean degree of polymerisation (mDP), and % of galloylation (Kennedy & Jones, 2001). However, the analysed chemical variables were not able to explain such important sensory differences. This might be attributed to the low number of assessed fractions (four) hindering the calculation of significant statistical correlations or to the limited number of chemical parameters currently available for characterising polymerised flavan-3-ols isolated from wine. Nonetheless, the studies revealed the existence of an array of astringent sub-qualities arising from PA fractions obtained from different wines, which gives insight into further studies that are required to better understand the mouthfeel phenomenon of red wines.

As for the other important class of polyphenols in red wines, anthocyanins present as monomers, oligomers and polymers are conventionally thought to be responsible exclusively for the colour of red wines (i.e., had no taste or mouthfeel properties). This understanding is based on studies carried out with grape anthocyanins (containing mainly anthocyanin glucosides along with their monoglucoside acetate and coumarate counterparts) (Vidal, Francis, et al., 2004). The authors concluded that free anthocyanins, like the coloured tannin-like polyphenolic compounds from red wine and pomace, do not contribute astringency nor bitterness to wine. The same authors found in other work that grape anthocyanins could contribute to fullness, chalkiness and coarseness when assessed in model wine (Vidal, Courcoux, et al., 2004). However, they questioned their results and concluded that such sensory properties were attributed to other phenolics present in the studied fraction.

Most recently, in their review of astringency perception, García-Estévez, et al., 2018 summarised that the effect of anthocyanins was indirect because they hinder the interaction between proteins and other phenolics such as flavanols, which are thought to be chiefly responsible for astringency in wines. However, these authors

rightfully acknowledged the lack of research and inconclusive understanding of the role of anthocyanins in the development of astringency in relation to protein interactions. Indeed, new studies correlating surface plasmon resonance response and wine composition have suggested that anthocyanins are important drivers of pigmentation and mouthfeel properties (Guerreiro, Teixeira, De Freitas, Sales, & Sutherland, 2017), and they have also been implicated in bitterness (Soares et al., 2013). This proposition regarding the mouthfeel properties of anthocyanins is supported by studies demonstrating the formation of complexes of malvidin-3-*O*-glucoside (Ferrer-Gallego et al., 2015) and pyranoanthocyanins (García-Estévez et al., 2017) with proteins, combined with knowledge of the sensory activity of certain anthocyanic fractions (Sáenz-Navajas et al., 2018; Sáenz-Navajas et al., 2017). The sensory variability of a discrete anthocyanin-containing fraction obtained from red wines pertained to descriptions such as “sour”, “dry”, “bitter”, “persistent” and “coarse” for three wines or “sticky” and “coarse” for a fourth wine (Sáenz-Navajas et al., 2018). In another set of three wines, the corresponding fraction was “dry”, “bitter”, and “persistent” for two wines and “silky” for the third. These results reinforced the uncertainty about the sensory role of anthocyanic fractions, and it is of paramount importance for the production of quality wines to better understand the sensory impact of different classes of wine polyphenols.

Having highlighted the lack of knowledge about the sensory variability associated to both red wine tannins and anthocyanins (and derived pigments), the main aim of the present study was to identify taste and mouthfeel dimensions related to red wine polyphenols, particularly in relation to tannins and anthocyanins. Specifically, the work tested the hypotheses that 1) the anthocyanic fractions present a sensory variability that differs from the tannic fractions, and 2) both the anthocyanic and tannic fractions contribute taste and mouthfeel properties of the original wines. In this context, a previously developed chemosensory strategy (Sáenz-Navajas et al., 2017) was used to isolate a wide range of fractions, mainly containing tannins and anthocyanins, from wines differing largely in their taste and

mouthfeel properties, and then to characterise their sensory properties using both nonverbal (sorting task) and verbal (rate-*K* attributes) sensory strategies. The sensory activity of specific fractions and the impact of introducing an anthocyanic fraction back into wine were also evaluated.

2. Material and methods

2.1. Chemicals

Solvents used for isolation of fractions: HPLC-grade acetonitrile, ethanol and methanol were obtained from Scharlab (Sentmenat, Spain). Formic acid (98-100%), acetone and ethyl acetate of reagent grade were purchased from Scharlab (Sentmenat, Spain). Deionised water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use.

Chemicals for reconstitution of fractions: food-grade potassium metabisulfite (E224), L (+)-tartaric acid (E334) and ascorbic acid were obtained from Enartis (Trecate, Italy). Gradient-grade absolute ethanol for liquid chromatography was purchased from Merck (Darmstadt, Germany).

2.2. Selection of wines

Twenty wines with a wide difference in taste and mouthfeel properties were selected (Annexe IV-2.1) from an original sample set of 42 red wines characterised by rate-*K*-attributes methodology in previous work (Section II, Chapter 2) (Sáenz-Navajas, Ferrero-del-Teso, Jeffery, Ferreira, & Fernández-Zurbano, 2020). Principal component analysis (PCA) was calculated with the correlation matrix of mean scores (n = 18 participants) of the 42 wines for significant ($P < 0.05$) and close to significant ($P < 0.1$) mouthfeel and taste attributes. Further hierarchical cluster analysis (HCA) with the Ward method was calculated on all dimensions derived from PCA. The number of clusters was constrained to 20 to have the maximal number of wines with the widest possible sensory variability and to coincide with the number of samples in the subsets of fractions being evaluated in the subsequent sensory tests. From each of the 20 clusters, one wine was selected and further PCA was calculated followed by HCA with the Ward method with all dimensions of the PCA. The clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centres. The terms that best characterised each of the clusters were identified by using the test-value parameter (Lebart, Morineau, & Piron, 1995).

All analyses were carried out with XLSTAT (19.3 version) and SPAD (version 5.5, CISIA-CESRESTA, Montreuil, France) software.

2.3. Fractionation procedure

The 20 wines selected via sensory analysis underwent a two-step protocol (Sáenz-Navajas et al., 2017) to isolate anthocyanic (F_{antho}) and tannic (F_{tannin}) fractions (Section I). Accordingly, a first preparative fractionation approach was followed to obtain two fractions (F1 and F2, see Figure IV-2.1) based on previous work (Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000). To begin, 400 mL of wine were freeze-dried (Coolsafe freeze-drier, Labogene, Denmark) and the residue was redissolved in 40 mL of hydroalcoholic solution (12% ethanol, v/v). The entire 40 mL was injected into a preparative column packed with Toyopearl gel (HW 50F) in a Millipore (Bedford, MA) Vantage L column (280 mm × 44 mm id). F1 was eluted with ethanol/water/formic acid (55:44:1, v/v/v) during 255 min at a flow rate of 10 mL min^{-1} and reserved for further fractionation to obtain F_{antho}, as described below. F2 (F_{tannin}) was eluted with 840 mL of acetone/water (60:40) at a flow rate of 14 mL min^{-1}. Fractions were evaporated under vacuum (8 mbar, 28 ºC, 30 min) with a rotary evaporator (KNF, Freiburg, Germany) to remove organic solvents, freeze-dried (yielding 0.06–1.01 g from 400 mL of original wine), and stored at 5 ºC until SPE fractionation of F1 and sensory analysis in the case of F2.

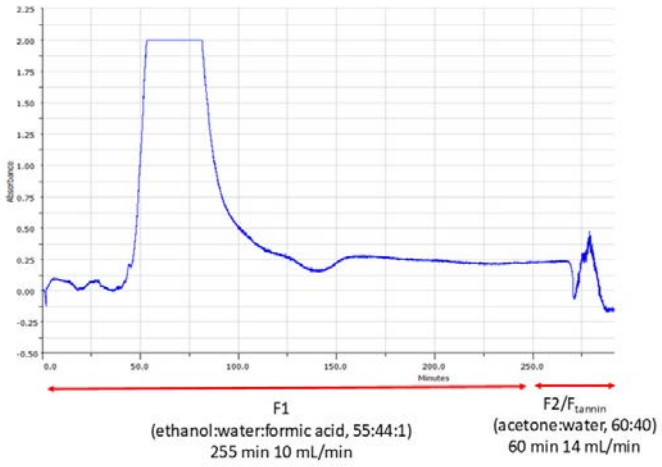

Figure IV-2. 1. Preparative chromatogram of sample SM29 recorded at 520 nm.

F1 was subsequently reconstituted in 400 mL of hydroalcoholic solution (12%, v/v) and submitted to solid-phase extraction (SPE) using an extraction unit (VAC ELUT 20 Station from Varian, USA). SPE cartridges filled with 500 mg of Bond Elut LRC-C18 resin (Agilent Technologies, USA) were firstly conditioned by passing 5 mL of methanol followed by 10 mL of an aqueous solution of tartaric acid (5 g L^{-1}) adjusted to pH 2.5 with 0.1 M NaOH. Reconstituted F1 (5 mL) was then loaded and eluted with 5 mL of ethyl acetate. This eluate, which contains sugars, organic acids, flavanols, flavonols and hydroxycinnamic acids, was discarded. F_{antho} was recovered with 10 mL of methanol. Cartridges were re-conditioned) and re-used a maximum of five times (with being allowed to dry out in between uses). The SPE procedure was repeated until the 400 mL of F1 were extracted. Recovered F_{antho} (recoveries of >80% in relation to malvidin-3-*O*-glucoside) was evaporated under vacuum (8 mbar), freeze-dried (yielding 0.20-1.25 g from 400 mL of original wine) and stored at 5 ºC until sensory analysis.

The day before sensory evaluation tasks, proportionate amounts of the target fractions (F_{antho} and F_{tannin}) were redissolved in a hydroalcoholic solution (4 % ethanol, v/v and 50 mg L^{-1} of SO_2; 80 mg L^{-1} of ascorbic acid as antioxidants) based on natural mineral water (Acquajet, Tomares, Spain) to obtain samples that were approximately twice as concentrated as the original wine (based on previous determinations of recovery), (Sáenz-Navajas et al., 2017), to facilitate the sensory characterisation of fractions. The level of ethanol (4%) was selected in preliminary bench top tastings (2%-7% range was evaluated) and corresponded to the minimal level of ethanol able to correctly dissolve freeze-dried fractions and to induce the lowest burning effect able to mask other sensations. Fractions were deemed to be odourless, and the total absence of solvents was confirmed by headspace solid-phase micro extraction (Carboxen/PDMS 75 µm at 30 °C × 10 min) and analysis with a GCMS-QP2010 system (Shimadzu, Tokyo, Japan) having an overall system detection limit of 1 ng/injection.

2.4. Sensory characterisation of fractions

Reconstituted wine fractions (20 each for F_{antho} and F_{tannin}) were evaluated following two different sensory strategies during four sessions held on separate days during two weeks at the Instituto de Ciencias de la Vid y el Vino. Sessions 1 (20 fractions of F_{antho}) and 2 (20 fractions of F_{tannin}) were devoted to nonverbal characterisation (i.e., sorting task) and sessions 3 (20 fractions of F_{antho}) and 4 (20 fractions of F_{tannin}) to sensory characterisation by rate-*K* attributes methodology. The order of presentation of samples was different for each participant attending to a randomised order and in a monadic manner. Five-mL samples were served in normalised (German Institute for Normalisation, DIN) dark wine glasses (Sensus, Schott Zwiesel, Germany) labelled with 3-digit random codes. Participants were instructed to take a small sip of the sample (reserving some to describe groups in the labelled sorting task and to re-evaluate if necessary) and to gently distribute it throughout the oral cavity (as a mouthwash) to reach the entire surface of the mouth (including the mouth wall, gums, back palate and tongue). After expectorating the sample, panellists had to wait one minute before grouping or rating the sample. Finally, they followed the rinsing protocol described by Colonna, Adams, and Noble (2004) with mineral water and pectin (1 g L^{-1}, Sosa, Barcelona, Spain) before tasting the next sample. Fractions were served at room temperature and evaluated in individual booths in a ventilated and air-conditioned tasting room (at around 20 °C) under ambient light. Participants were aware that samples were not commercial wines but fractions obtained in the laboratory, and their informed consent was gained prior to undertaking the sensory testing. They were not informed about the objective of the study nor paid for their participation.

2.4.1. Nonverbal characterisation by wine experts: Sorting task

Participants. Twenty winemakers from Rioja area, Spain (16 women and 4 men, ranging from 28 to 57 years of age, average = 40) participated in the study.

Procedure. Working with F_{antho} (session 1) and F_{tannin} (session 2), participants were presented in the separate sessions with the 20 samples simultaneously and asked to sort them on the basis of mouthfeel and taste properties by arranging wine glasses on the table. Participants could form as many groups as they wished (minimum of two) and groups could contain from 1 to 19 samples. Upon completion, panellists recorded the three-digit codes of the samples of each group on a sheet of paper and were asked to describe the groups they formed with their own words (maximum of two terms per group). At this stage, participants were allowed to taste again, but not to modify the groups they had already established.

Data analysis. For each participant, results were encoded in an individual similarity matrix (wines × wines), in which 1 stands for two samples allocated in the same group and 0 for different groups. The three-way distance table was analysed by DISTATIS methods (Abdi, Valentin, Chollet, & Chrea, 2007). The quality and the reliability of representations were evaluated by Shepard diagrams and Kruskal´s stress value. HCA with the Ward criterion was performed on the matrix consisting of wines x-coordinates of the retained DISTATIS dimensions. DISTATIS analyses were carried out with R software (version 3.6.0 for Windows) with the additional R packages DistatisR and FactoMineR. HCA was undertaken with XLSTAT (19.3 version).

For the terms derived from the description of the groups, an initial list was built with all the terms elicited by participants. This list was first reduced by omitting words with hedonic or emotional character (e.g. pleasant, easy, classic, different…) and adverbs (e.g., very, barely, extremely…). For remaining words, a lemmatisation process was performed, i.e., words sharing the same lemma or root (e.g., sour, sourness) were grouped in the same category. Finally, all terms were grouped in categories according to semantic similarities. This process was performed

individually by three experienced researchers, who through a triangulation task (Abric, 2003) achieved a final consensual list of terms. The frequency of quotation of each term was calculated and only terms cited by at least by 20% of the panel (>4 participants) were considered. Chi-square (χ^2) tests were applied for calculating significance differences ($P < 0.05$) for attributes among clusters. Marascuilo post-hoc pairwise comparison (95%) was carried out for significant effects.

2.4.2. Verbal characterisation by wine experts: Rate-K attributes

Participants. Seventeen winemakers from Rioja area, Spain (14 women and 3 men, ranging from 28 to 57 years of age, average = 37) participated.

Procedure. As with the procedure in Section 2.4.1, participants evaluated the 20 fractions of F_{antho} (session 3) and 20 fractions of F_{tannin} (session 4) on separate days using the rate-*K* attributes methodology described elsewhere (Sáenz-Navajas et al., 2020).

Data analysis. Two-way ANOVAs with assessors as random factors and fractions as fixed were calculated on the scores of each sensory attribute. For significant effects, Fischer post-hoc pairwise comparison (95%) test was performed.

Principal component analysis (PCA) was calculated with mean scores (n = 17) of the significant mouthfeel and taste attributes. Further HCA with the Ward method was calculated on all dimensions derived from PCA. The attributes best defining the resulting clusters were identified by calculating an ANOVA with clusters as fixed factors. For significant attributes ($P < 0.05$), the highest score for a given attribute among clusters was selected to be the attribute best defining the cluster(s). Analyses were carried out with SPAD software (version 5.5, CISIA-CESRESTA, Montreuil, France) and XLSTAT (19.3 version).

The degree of similarity between the sensory spaces of 1) fractions (F_{tannin} or F_{antho}) derived from both sorting tasks and the rate K-attributes analysis, and 2) wines

(rate K-attributes) and fractions (rate-*K* attributes and sorting task), was assessed by calculating RV coefficients (Robert & Escouffier, 1976).

In order to evaluate the level of contribution of fractions to wine sensory spaces, Pearson correlation coefficients were calculated between the 1) scores of similar descriptors for wines (Annexe IV-2.2) and fractions (F_{antho} or F_{tannin}) (Annexe IV-2.3 and Annexe IV-2.4), 2) significant PCs derived from the PCA calculated with the significant wine and fraction attributes scored by the rate-*K* attributes method, and 3) significant PCs derived from the PCA calculated with the significant wine attributes scored by the rate-*K* attributes method and the significant factors derived from the sorting task of fractions. Partial least squares regression models (PLSR1) were calculated for each sensory attribute to predict wine scores from fraction scores. Analyses were carried out using Unscrambler (version 9.7).

2.5. Evaluation of the sensory activity of anthocyanic fractions (F_{antho})

2.5.1. Calculation of orosensory threshold of fractions

F_{antho} derived from two different wines (F_{antho}SM5 and F_{antho}SM25) was separately prepared at 6 concentration levels relative to the original wine: 1) twice the concentration (C1), 2) the same concentration (C2), 3) dilution 1:2 (C3), 4) dilution 1:4 (C4), 5) dilution 1:8 (C5), and 6) dilution 1:16 (C6). Weighed amounts of the lyophilised fractions were dissolved in an ethanolic solution (4% ethanol v/v, 50 mg L^{-1} of SO_2, 80 mg L^{-1} of ascorbic acid) to arrive at the desired concentrations.

Ten wine experts (two men and eight women, ranging from 27 to 55 years of age, average = 37) carried out six ascending forced choice triangle tests for each of the two fractions studied (F_{antho}SM5 and F_{antho}SM25), in duplicate. The samples (4 mL, 20 ºC) assessed in each triangle test were the fraction at one of the six concentration levels (two identical samples) and the ethanolic solution used to prepare the samples. Testing was conducted in random order in coded black tulip shaped wine glasses under ambient light in individual booths in a ventilated and air-conditioned tasting room (20 ± 2 °C).

Each participant was presented with three glasses and asked to select the sample that presented different oral properties. The number of right answers was compared with tabulated values to evaluate the presence of significant sensory differences between ethanolic solution and reconstituted fraction at different concentration levels. If obvious differences were detected, panellists were asked to freely cite 2-3 descriptors inducing the sensory difference.

2.5.2. Effect of wine matrix on the sensory activity of fraction $F_{antho}SM5$

Five of the original wines were spiked with $F_{antho}SM5$ at the concentration present in the original wine (SM5). A total of ten wines (the five original wines: SM5, SM8, SM16, SM23, SM25, and the five spiked with $F_{antho}SM5$: SM5+$F_{antho}SM5$, SM8+$F_{antho}SM5$, SM16+$F_{antho}SM5$, SM23+$F_{antho}SM5$, SM25+$F_{antho}SM5$) were described in duplicate by ten wine experts (two men and eight women, ranging from 27 to 55 years of age) according to the rate-*K* attributes methodology described previously while wearing nose clips (Sáenz-Navajas et al., 2020).

A three-way ANOVA was calculated for each attribute with judges as random factor and wine and type of wine (i.e., spiked or non-spiked) as fixed factors including “wine” x “type of wine” interaction.

3. Results and discussion

3.1. Sensory description of wines. Sample selection

From an expert assessment of 42 wines using rate-*K* attributes (Sáenz-Navajas et al., 2020), 8 of 23 descriptors were found to differ significantly according to two-way ANOVA (Table IV-2.1). They were “bitter”, “sweet”, “dry”, “dry on tongue side”, “dry on palate”, “unctuous”, “oily” and “watery”, together with another four (“sour”, “silky”, “burning” and “prickly”) when relaxing the criteria for significance ($P < 0.1$) to avoid missing a true difference. Based on these 12 attributes, the PCA scores plot in Figure IV-2.2 shows the projection of the 42 wines on the first two PCs, which explained 50% of original variance. From the 20 clusters derived from HCA, one wine was chosen at random from each with the aim of selecting the 20 most different wines in terms of both taste and mouthfeel. These wines included samples elaborated with 13 different varieties and from 12 different regions of origin.

PCA conducted with the 12 significantly different attributes for the 20 wines yielded four independent and non-correlated sensory dimensions (data not shown), which explained 79.3% of the original variance and were identified as significant according to the Kaiser criterion (eigenvalue ≥ 1). The first PC (35.3% explained variance) was positively contributed by the terms “unctuous” ($r = 0.73$), “oily” ($r = 0.50$) and “silky” ($r = 0.90$), and negatively by “dry” ($r = -0.85$) and “dry on palate” ($r = -0.85$). The second PC (22.8% explained variance) was positively contributed by “bitter” ($r = 0.71$), “dry on tongue sides” ($r = 0.61$), “burning” ($r = 0.62$) and “prickling” ($r = 0.57$), and negatively by “sour” taste ($r = -0.69$). The third dimension (11.7% explained variance) was mainly influenced by “sweet” taste ($r = 0.79$) and the fourth (9.5% of variance) by “watery” ($r = 0.68$).

Table IV-2. 1. Two-way ANOVA (participants as random factors, wine as fixed factor) calculated to evaluate the effect of wine on taste and mouthfeel sensory properties (F values; P-significance: *P<0.1, **P<0.05, ***P<0.01, ****P<0.001).

ATTRIBUTES	F	P
Salty	0.987	ns
Bitter	1.754	***
Sweet	1.426	**
Sour	1.384	*
Dry	2.225	****
Dry on tongue side	1.539	**
Dry on palate	2.410	****
Sticky	1.233	ns
Dusty	1.093	ns
Grainy	1.104	ns
Sandy	0.961	ns
Coarse	1.100	ns
Unctuous	1.692	***
Oily	1.791	***
Fleshy	0.929	ns
Mouth coating	1.129	ns
Silky	1.324	*
Gummy	0.882	ns
Watery	1.486	**
Burning	1.366	*
Hot	1.190	ns
Prickly	1.348	*
Persistent	1.188	ns

Table IV-2.2 shows the six main groups of wines identified by HCA. Cluster 1 was composed of four wines, including two Bobal from La Mancha, one Merlot from Somontano and one Gamay from Côte du Puy (France) (Annexe IV-2.1), and was significantly described with terms such as “sweet”, “dry on palate” and “dry” based on test-value (Lebart et al., 1995). Cluster 2 was the most numerous group, having five wines elaborated with four different varieties: Merlot, Cabernet Sauvignon, Mencía and Tempranillo all from the north of Spain. This is characterised with the terms “bitter”, “dry on the tongue side”, “burning” and “dry on palate”. Cluster 3 included four wines from very diverse origins (Argentina, France, Castilla and Bierzo) and varieties (Bonarda, Pinot Noir, Prieto Picudo and Mencía), and was mainly “watery” and “oily”. Cluster 4, formed by three wines elaborated in very close

regions (DO Campo Borja and DOCa Rioja, Spain) but with different varieties (Grenache, Syrah, Tempranillo) was basically “sour”. Clusters 5 and 6 were formed by two wines each elaborated with four different varieties in four different regions. Cluster 5 was characterised with terms such as “silky”, “unctuous” and “sour”, and cluster 6 with “unctuous”, “burning”, “prickly” and “oily”. These results reflect the wide sensory variability in terms of taste and mouthfeel associated to the 20 selected wines Figure IV-2.2 with very different origins and varieties (Annexe IV-2.1).

Table IV-2. 2. Clusters yielded by HCA calculated with the 20 selected wines and 12 significant sensory terms.

Cluster	Wines	Attributes
1	SM42_BOB†, SM23_MER, SM41_BOB, SM1_GAM	sweet, dry on the palate, dry
2	SM26_CS†, SM25_MER, SM10_MEN, SM35_TEM, SM31_MER	bitter, dry on tongue side, burning, dry on palate
3	SM6_BON†, SM12_PN, SM16_PP, SM4_MEN	watery, oily
4	SM29_GRE†, SM30_SY, SM37_TEM	sour
5	SM15_CAR†, SM7_MEN†	silky, unctuous, sour
6	SM5_MAL†, SM8_GAM†	unctuous, burning, prickly, oily

†Wines closest to the centre of gravity of the cluster. Attributes contributing most to the building of the cluster (test-value ≥ 1.96; $P < 0.05$) and wines belonging to each cluster are listed.

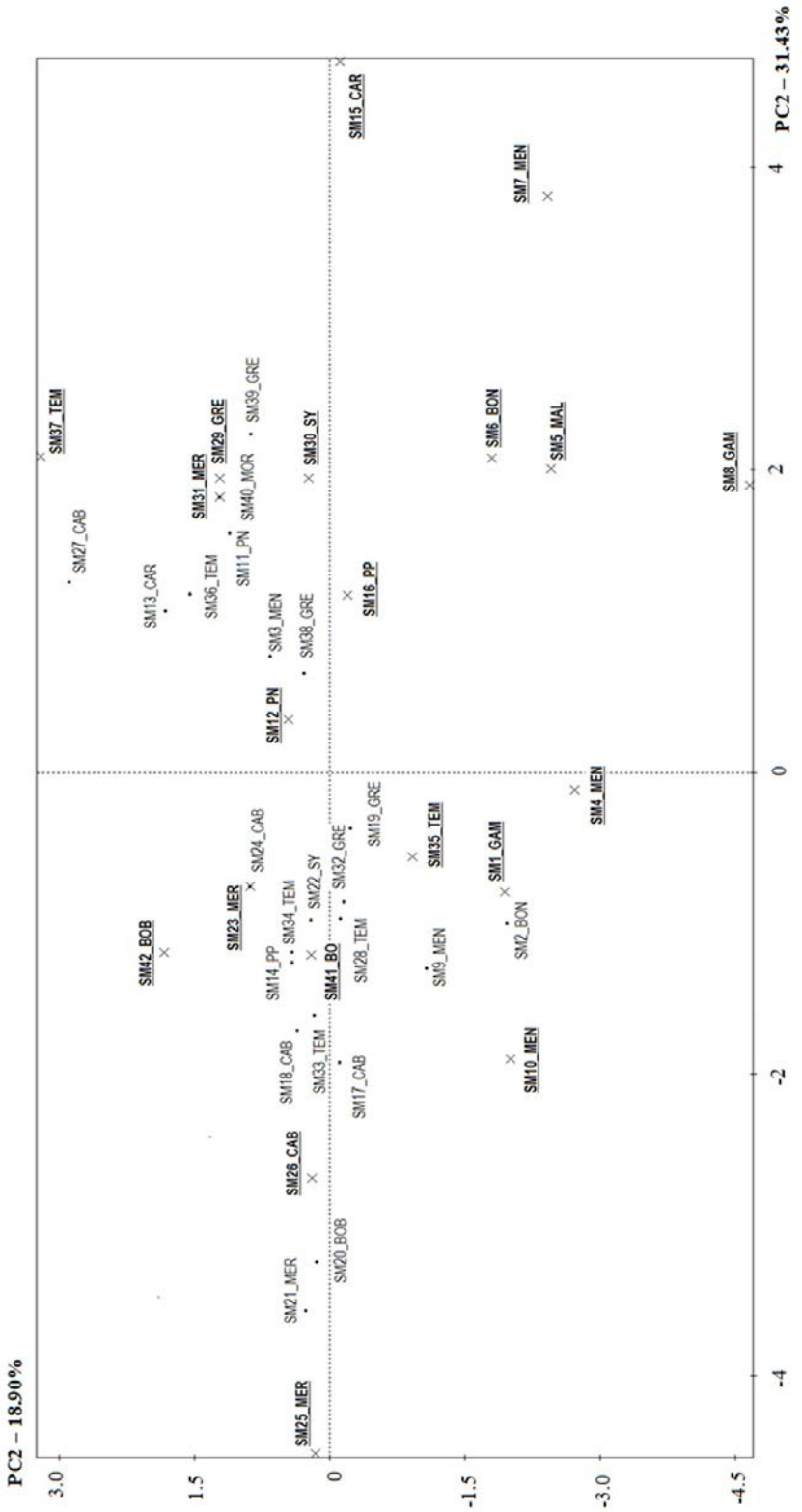

Figure IV-2. 2. PCA scores plot calculated with the average scores (n = 20) of 12 significant taste and mouthfeel attributes derived from the sensory characterisation of 42 wines by rate-K attributes using nose clips. The 20 wines selected for fractionation and sensory characterisation of their anthocyanic and tannic fractions are marked in bold. The three letters going with sample code correspond to the abbreviation of the wine variety

3.2. Sensory characterisation of anthocyanic fractions (F_{antho})

3.2.1. Sorting task

The number of groups formed by participants with the 20 F_{antho} samples ranged from 4 to 11, being 7 the average. The maximum number of times two samples were grouped together was 9 (45% of participants). The individual similarity matrices obtained from the sensory sorting task were submitted to DISTATIS and based on Kruskal's stress, three dimensions were required to reach a Kruskal value < 0.2, which indicated a good fit to the original data (Cox & Cox, 2000). Figure IV-2.3a shows the dendrogram derived from the cluster analysis calculated on the DISTATIS dimensions. Three main groups of F_{antho} samples with different sensory properties can be identified. Based on the verbal description of groups carried out after the sorting task, the most cited terms were "bitter" (maximum of 90% citations for F_{antho}SM5 and F_{antho}SM25) and "dry" (maximum of 55% citations for F_{antho}SM37), but only "bitter" was significantly different ($\chi^2 = 5.2$; $P < 0.05$) among the three clusters: cluster 3 was the most bitter (average of 67% of citations), followed by cluster 2 (43%) and cluster 1 (34%). These results show that the F_{antho} fractions were mainly "dry" and "bitter", and that participants grouped samples mainly based on their variability in terms of bitter intensity.

3.2.2. Rate-K attributes

According to sensory description following rate-*K* attributes methodology, "bitter" ($F = 11.4$; $P < 0.0001$), "persistence" ($F = 11.2$; $P < 0.0001$), "watery" ($F = 2.4$; $P = 0.001$), "sweet" ($F = 2.2$; $P = 0.04$), and "dry" ($F = 2.1$; $P = 0.006$) were the attributes presenting higher sensory differences among the F_{antho} fractions, followed by "grainy" ($F = 1.8$; $P = 0.025$), "sour" ($F = 1.8$; $P = 0.028$), "silky" ($F = 1.7$; $P < 0.033$), "mouthcoating" ($F = 1.6$; $P = 0.047$), and "unctuous" ($F = 1.6$; $P = 0.056$). PCA calculated on significant attributes showed that the first three dimensions (accounting for 73% of the original variance) presented eigenvalues higher than one and thus could be considered significant based on the Kaiser criterion. Of note,

identifying three main independent and non-correlated sensory dimensions as represented by these three PCs highlighted the utility of the rate-*K* attributes sensory methodology. The first PC (43.7% explained variance) was significantly ($P < 0.05$) and positively correlated to “watery” ($r = 0.85$), “silky” ($r = 0.81$), “sweet” ($r = 0.68$) and “unctuous” ($r = 0.64$), and negatively to “bitter” ($r = -0.89$), “dry” ($r = -0.75$) and persistent ($r = -0.68$). The second PC (20% explained variance), was positively correlated to “grainy” ($r = 0.87$) and “mouthcoating” ($r = 0.66$), and the third PC (9.6% explained variance) was positively correlated to sour taste ($r = 0.68$).

HCA calculated on the PCA dimensions yielded five main clusters of F_{antho} fractions (Figure IV-2.3b). Cluster 1, formed by four samples, was significantly ($P < 0.05$) described as “watery” (average = 1.4), “silky” (average = 1.3) and “sweet” (average = 0.8), and cluster 2, which included only sample F_{antho}SM5, was the most bitter (score = 6.1), and most persistent (score = 4.8). Cluster 3 (3 samples) was characterised by the terms “mouthcoating” (average = 0.7) and “grainy” (average = 0.6), cluster 4 (7 fractions) was especially “bitter” (average = 3.0) and “dry” (average = 1.8), and cluster 5 was mainly “sour” (average = 0.7). Notably, “bitter” and “persistent” reached the highest scores by far for F_{antho}SM5 among the fractions studied, with values of 6.1 and 4.8, respectively, followed by “dry” with an average value of 2.7 for F_{antho}SM26. Further, considering the difference between the maximum and minimum score for a given attribute as a discriminatory value, the highest discriminative ability was found for the terms “bitter” (max-min = 5.6) and “persistent” (max-min = 4.8) followed by “dry” (max-min = 2.4) for the 20 F_{antho} fractions studied (Table A.3.).

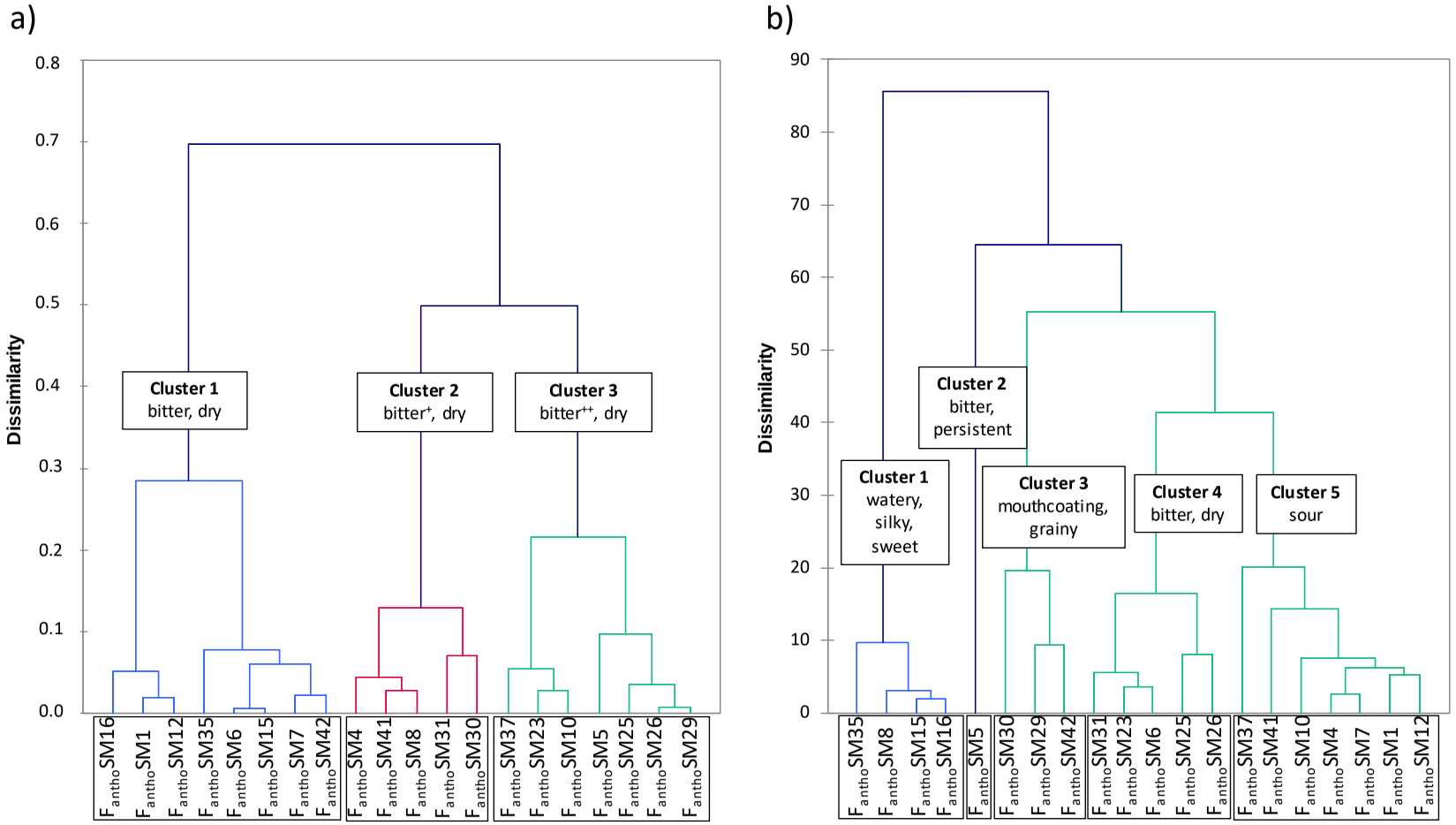

Figure IV-2. 3. Dendrograms showing the sensory clusters of the 20 Fantho derived from hierarchical cluster analysis calculated on dimensions of a) DISTATIS obtained by sorting task and b) PCA derived from rate-K attributes. Attributes describing each cluster are those with significantly higher scores in the given cluster (significant differences in the frequency of citation for the term "bitter" are expressed as follows: bitter < bitter+ < bitter++).

3.3. Sensory characterisation of tannic fractions (F_{tannin})

3.3.1. Sorting task

The number of groups formed by participants with the 20 F_{tannin} samples ranged from 3 to 12, with an average of 6. The maximum number of times two samples were grouped together was 13 (65% of participants). The similarity/proximity of fractions evaluated by DISTATIS algorithms showed a Kruskal stress value lower than 0.2 for two dimensions. Attending to cluster analysis, three main clusters of F_{tannin} samples could be identified (Figure IV-2.4a). They were mainly described with the terms "dry" and "bitter", with both terms differing ($P < 0.1$) among the three clusters ($\chi^2 = 4.5$ and 4.9, respectively). Clusters 2 and 3 were drier (average of 75% and 70% of citations, respectively) than cluster 1 (45%), and cluster 2 was most bitter (70%) followed by cluster 3 (50%) and cluster 1 (35%). These results showed that the F_{tannin} fractions studied were mainly "dry" and "bitter", and that participants grouped samples based on the intensity of both perceptions.

3.3.2. Rate-K attributes method

Eleven out of the 23 evaluated attributes were significantly different among the fractions, which was comparable with the 10 significant attributes found for F_{antho}.The 11 attributes were "bitter" ($F = 8.2$; $P < 0.0001$), "watery" ($F = 3.6$; $P < 0.0001$), "dry on palate" ($F = 3.3$; $P < 0.0001$), "dry" ($F = 2.8$; $P = 0.000$), and "dry on the tongue side" ($F = 2.8$; $P = 0.000$), which were the most different, followed by "silky" ($F = 2.5$; $P = 0.001$), "persistence" ($F = 2.1$; $P = 0.005$), "sweet" ($F = 2.1$; $P = 0.006$), "oily" ($F = 1.8$; $P = 0.021$), "sticky" ($F = 1.6$; $P = 0.059$), and "gummy" ($F = 1.5$; $P < 0.077$).

PCA calculated with the 11 different terms yielded two significant PCs (77% variance explained) according to Kaiser criterion. Thus, two main independent and non-correlated sensory dimensions can be identified by the rate-*K* attributes methodology. The first PC (66.9% explained variance) was composed of "bitter" (correlation coefficient between PC1 and "bitter": $r = 0.95$), "dry" ($r = 0.87$), "dry on

tongue side" (r = 0.89), "dry on palate" (r = 0.95), "sticky" (r = 0.81) and "persistent" (r = 0.74) to terms such as "sweet" (r = -0.65), "oily" (r = -0.84), "silky" (r = -0.86), and "watery" (r = -0.89). PC2 (10.1% variance explained) was positively correlated only to the term "gummy" (r = 0.84). Figure IV-2.4b shows the four clusters derived from HCA calculated on the PCA dimensions. Cluster 1, formed by eight fractions, was mainly described as "watery" (average among wines of the cluster = 1.1), "sweet" (average = 0.9), "silky" (average = 0.9) and "oily" (average = 0.5). Cluster 2, which included two fractions (F_{tannin}SM25 and F_{tannin}SM26), was mainly "bitter" (average = 4.2) followed by "dry" (average = 3.4), "persistent" (average = 2.2), "dry on palate" (average = 2.2), "dry on tongue sides" (average = 1.7), and "sticky" (average = 1.1). Cluster 3 (3 fractions) was characterised by the term "gummy" (average = 0.4), and cluster 4 (7 fractions) was mainly "sticky" (average = 1.0).

Interestingly, "bitter" and "dry" reached the highest scores among the fractions studied with values of 4.5 and 3.8, for fraction F_{antho}SM25 and F_{antho}SM26, respectively. Besides, similar to F_{antho} fractions, the term "bitter" seems to be the most discriminant among the studied fractions based on the difference between maximal and minimal values (max-min = 4.2 for F_{tannin} and 5.6 for F_{antho}), followed by "dry" (max-min = 2.9 for F_{tannin} and 2.4 for F_{antho}) (Tables A.3. and A.4. of Supporting information).

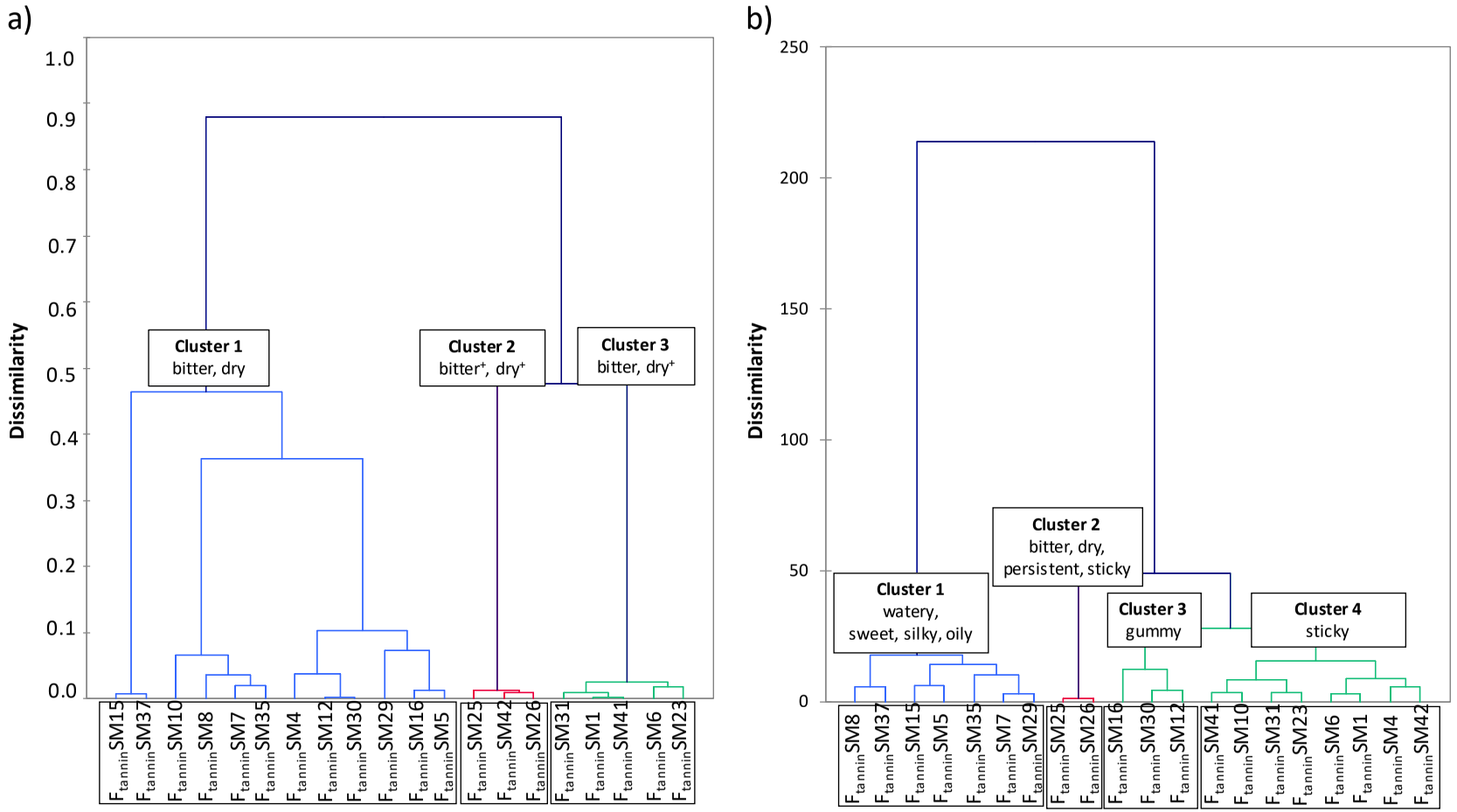

Figure IV-2. 4. Dendrograms showing the sensory clusters of the 20 F_{tannin} derived from hierarchical cluster analysis calculated on dimensions of a) DISTATIS obtained by sorting task and b) PCA derived from rate-K attributes. Attributes describing each cluster are those with significantly higher scores in the given cluster (significant differences in the frequency of citation for the terms "bitter" and "dry" are expressed as follows: bitter < bitter$^+$ and dry < dry$^+$).

3.4. Comparison of the sensory spaces of F_{antho} and F_{tannin}

Following verbal and nonverbal approaches, bitterness followed by dryness arose as the salient attributes describing both groups of fractions. Besides, the RV coefficient calculated on the sensory spaces derived from both sensory tasks was significant, with a moderate value (RV = 0.448; $P < 0.01$) for F_{antho} and moderate to strong (RV = 0.640, $P < 0.001$) for F_{tannin}. These results suggested that both nonverbal (sorting task) and verbal (rate-*K* attributes) sensory techniques generated overall similar sensory spaces, especially for F_{tannin} fractions. This can be attributed to the fact that in both strategies, the differences among the most salient sensory properties were represented. In the sorting task, all samples were presented simultaneously and groups built based on similarity among samples, thus memory played an important role (Valentin, Chollet, Lelievre, & Abdi, 2012), especially considering that there was a relatively high number of samples (n = 20). In this scenario, participants had to adopt a strategy that allowed them to compare among samples, and thus a reduced number of the most salient properties (dryness and bitterness) were selected to form the groups in both sample sets (F_{antho} and F_{tannin}). In the rate-*K* attributes approach, the number of selected and rated attributes was limited to five descriptors, which leads to underlining the salient attributes of the product (Valentin et al., 2012). Notwithstanding, even if both tasks yielded close sensory spaces, the rate-*K* attributes approach was able to find differences among samples based on less salient dimensions such as “grainy/mouthcoating” and “sour” taste for F_{antho} and “gummy” for F_{tannin}.

In summary, the anthocyanic fractions (F_{antho}) could be represented by three independent and non-correlated dimensions in both sensory tasks, and for tannic fractions (F_{tannin}), the sensory space showed a relatively lower variability than F_{antho}, with two main sensory dimensions conforming the sensory space. In this context, the first hypothesis related to anthocyanic fractions presenting a sensory variability that differs from tannic fractions was demonstrated to be correct. This is a relevant result because until now, anthocyanic fractions of wines have been mainly reported

to be involved in wine colour, and their sensory activity in terms of taste and/mouthfeel was far from clear (García-Estévez et al., 2018).

3.5. Contribution of fractions to wine sensory properties

Results showed significant positive correlations between "dry" ($r = 0.72$; $P < 0.01$), "dry on palate" ($r = 0.65$; $P < 0.01$), and "silky" ($r = 0.51$; $P < 0.05$) terms scored for wines and F_{tannin} samples, and the term "dry" ($r = 0.40$; $P < 0.05$) for F_{antho} samples. RV coefficients calculated between the sensory spaces of wines derived from the rate-*K* attributes analysis and fractions obtained by both the rate-*K* attributes and sorting task showed significant correlations ($P < 0.001$) for wine and F_{tannin} sensory spaces (RV = 0.520 and 0.539, for rate-*K* attributes and sorting task, respectively), but no significant correlation was observed with F_{antho} samples (RV = 0.276 and 0.200 for the sensory spaces derived from sorting and the rate-*K*-attributes method, respectively). A moderate negative correlation ($r = -0.5$; $P < 0.1$) was observed between PC1 derived from the sensory spaces of wine and Factor 1 derived from the sorting task of F_{antho} samples. This result partially confirms our second hypothesis by highlighting the direct sensory importance of the F_{tannin} fraction on the final sensory properties of wines, contrary to the F_{antho} fractions, which is suggested to have a secondary role related to the overall dryness perception in wines.

In other to verify this hypothesis and considering that the terms "bitter" and "dry" were the most salient sensory properties differing among the 20 fractions of both F_{antho} and F_{tannin}, linear models to predict these sensations in wines were built. Interestingly, the dryness of wines could be predicted ($F = 11.41$; $P < 0.01$; $R^2 = 0.59$; RMSE = 0.449) from the "dry" scores of F_{tannin} and F_{antho}, with the tannic fraction contributing double that of the anthocyanic fraction (coefficients of 0.53 for F_{tannin} and 0.25 for F_{antho}). On the other hand, bitterness in wines could not be predicted from the bitter scores of any of the two fractions studied, even if they were especially bitter in isolation (especially F_{antho}). The reason the high bitterness of these fractions was not transmitted to wines could have two plausible explanations: 1) the sensory

threshold of these fractions could have been below their concentration in wines, or 2) there were other wine components masking bitterness and acting as suppressors in wines.

The relative orosensory threshold of fractions F_{antho}SM5 and F_{antho}SM25 (with scores for bitterness of 6.1 and 4.6, respectively, see Annexe IV-2.3) was estimated to further explore their sensory impact. These fractions were the most bitter among the 40 studied and the intensity of other sensory attributes such as dryness (1.5 and 2.2, respectively) were relatively low in comparison with bitterness, especially for F_{antho}SM5. Figure IV-2.5a shows that for the F_{antho}SM5, which was mainly "bitter", the sensory threshold in model solution was slightly below the relative concentration of F_{antho}SM5 if wine SM5 was diluted four-fold. For F_{antho}SM25, which was mainly "bitter" but also "dry" (2.2±0.7), the sensory threshold was somewhat below the relative F_{antho}SM25 concentration if wine SM5 was diluted two-fold (Figure IV-2.5b). These results confirmed the sensory activity of these fractions in isolation and suggested that they could be important contributors to the sensory profile of wines.

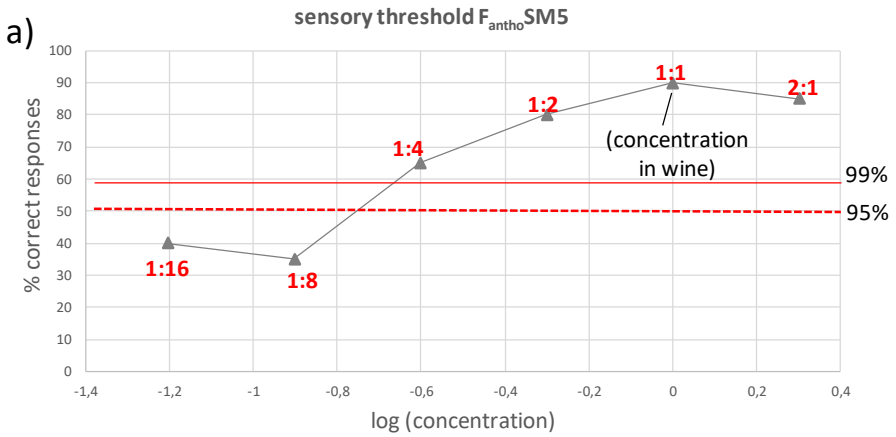

Figure IV-2. 5. Percentage of correct responses (n = 20) derived from triangle tests comparing wine model to wine model spiked with different concentrations of the Fantho obtained from wines a) SM5. Lines indicate the 5% (lower) and 1% (upper) significance criterion, respectively.

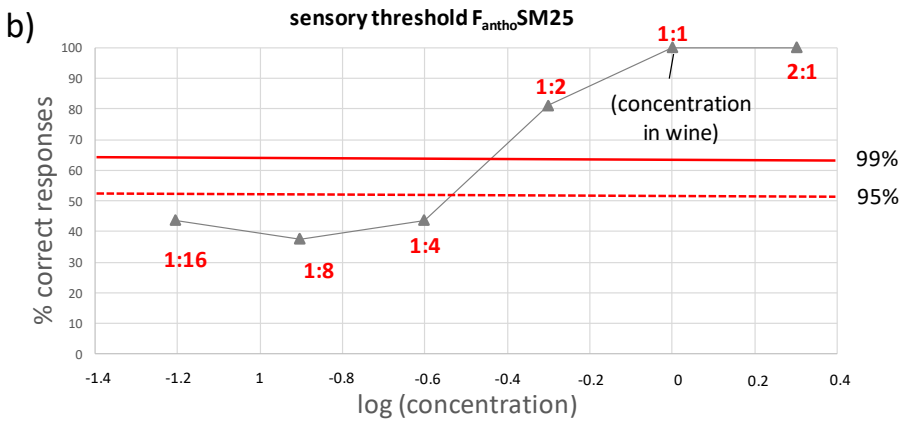

Figure IV-2. 6. Percentage of correct responses (n = 20) derived from triangle tests comparing wine model to wine model spiked with different concentrations of the Fantho obtained from wines b) SM25. Lines indicate the 5% (lower) and 1% (upper) significance criterion, respectively.

The presence of sensory interactions at either perceptual (cognitive or receptor level) or physicochemical level was investigated by assessing the impact of F_{antho}SM5 on wine sensory properties in five of the original wines (SM5, SM8, SM16, SM23, SM25). Wine SM5 was included along with two pairs/groups of wines with different intergroup scores for bitterness but similar intragroup scores: SM23 and SM16 (average = 0.7) and SM8 and SM25 (average = 1.7). Besides, bitter scores for their respective F_{antho} varied largely intragroup. This selection was aimed at having wines with ample bitter scores and with different contributions of F_{antho} to the overall bitterness perceived in original wines. Five original wines and these same samples spiked with F_{antho}SM5 were described with the rate-*K* attributes approach. ANOVA showed a significant effect for bitter scores due to the spiking ($F = 5.05$; $P < 0.05$), whereby wines spiked with fraction F_{antho}SM5 presented significantly higher scores for bitterness (average = 3.5 ± 0.6, 7-point scale) than non-spiked wines (average = 2.7 ± 0.5). This result confirmed the sensory contribution of F_{antho} to the sensory properties of wines. Interestingly, the increase in bitterness with F_{antho}SM5 was significant ($P < 0.05$) for three wines (SM8, SM5, SM23), whereas for wines SM16 and SM25, no sensory change was evident. This suggested that there were components

in wines SM16 and SM25 that were able to mask the bitter perception associated with F_{antho}SM5, thus acting as bitterness suppressors.

It is important to remark that fractions were dissolved in a model solution with a minimal concentration of ethanol (4% v/v) and pH value of 6.5 ± 0.5 to avoid the saliency of burning sensations related to ethanol content and of sour taste due to the presence of acids needed to reach normal pH values found in wines. Ethanol is reported to have a significant effect on wine astringency, even if reported results are contradictory. On the one hand, there are authors that confirm that ethanol hinders protein-tannin interactions and consequently decrease "astringency" (Fontoin, Saucier, Teissedre, & Glories, 2008; McRae, Ziora, Kassara, Cooper, & Smith, 2015), but on the other hand, ethanol content is also suggested to increase this precept driven by other mechanisms different from tannin-protein interaction (Sáenz-Navajas et al., 2019; Watrelot et al., 2016). Concerning the effect of pH, it has been observed that decreases in pH can increase astringency (Fontoin et al., 2008; Luck et al., 1994; Sáenz-Navajas et al., 2020), attributable to decreases in saliva viscosity. This suggested that dryness perceived in the fractions of the present work could be drier at wine pH. In this context, it has to be considered that differences in ethanol and pH between fractions and wines could be related to certain discrepancies between the sensory properties of both matrices.

4. Conclusions

The present work has successfully described the sensory space of two families of wine polyphenols, namely anthocyanins and tannins isolated from a sample set of 20 wines that exhibited distinct variability in terms of taste and mouthfeel properties. The anthocyanic (F_{antho}) and tannic (F_{tannin}) fractions of these 20 wines underwent sensory evaluation by nonverbal (sorting task) and verbal (rate-*K* attributes) sensory techniques, with both approaches generating overall similar sensory spaces as determined by RV values, especially for F_{tannin} fractions. Results showed that the sensory space of the F_{antho} studied could be represented by three independent and non-correlated dimensions in both sensory tasks, and related to taste and mouthfeel related terms, with “bitter” and “dry” appearing as the most salient and differential sensory properties among both groups of fractions.

Interestingly, dryness of wines was satisfactorily predicted from “dry” intensity of F_{antho} and $F_{tannin,}$ which confirmed that tannins have a major role in the perception of dryness, and more importantly, unequivocally demonstrated a relevant implication of certain anthocyanins in the “dry” attribute. In contrast, bitterness of the wines could not be directly related to the bitterness perceived in any of the two fractions. Furthermore, the addition of extremely bitter anthocyanic fractions to wines increased bitterness only in certain wines, purportedly due to the presence of masking agents in wines.

The results contribute to the understanding of red wine taste and mouthfeel perception by suggesting that the anthocyanic fraction contributes to orosensory properties more than previously thought. Gaps in knowledge regarding the fractions and wines will involve comprehensive chemical characterisation by both targeted and untargeted metabolomics to identify the specific molecules and groups of molecules involved in “sour” and especially “bitter” tastes, as well as in the different mouthfeel dimensions identified.

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SECTION IV. CHAPTER 3

Evolution of different wine phenolic fractions in presence of oxygen

1.Introduccion

Wine ageing usually consists of two stages, the first that takes place in the barrel (oxidative ageing) and the second in the bottle (reductive ageing). In both periods, the amount of oxygen supplied to the wine and the time are critical factors able to modulate the sensory properties (positive and negatively) and consequently determine its quality and longevity. This is a direct consequence of the complex chemical reactions that wine compounds experience due to both oxygen contact and storage time. Among wine compounds, phenolic composition plays a very important role and it has been object of lot of studies (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006; Bueno et al., 2018; Danilewicz & Standing, 2018).

The oxygen consumption rate (OCR) of wine has been widely studied (Jeremic, Vongluanngam, Ricci, Parpinello, & Versari, 2020; Marrufo-Curtido, Carrascón, Bueno, Ferreira, & Escudero, 2018; Navarro et al., 2016; Nevares et al., 2017; Pascual et al., 2017. It has been shown to be strongly wine-dependent and be affected by factors such as temperature, composition of the wine in phenolic compounds, sulphur dioxide or metal ion content (Cu, Fe and Mn) (Danilewicz, 2003, 2007; Danilewicz & Standing, 2018; Ferreira, Carrascon, Bueno, Ugliano, & Fernandez-Zurbano, 2015; Kontoudakis & Clark, 2020).

Phenolic compounds as tannins and anthocyanins, which are chiefly involved in the formation of taste and mouthfeel attributes, are unstable and undergo multiple reactions. Tannins undergo polymerisation reactions, but also the interflavanic bond cleavage occurs in an acidic context as wine. This last reaction releases a carbocation that can react with wine nucleophiles such as other flavanols, anthocyanins or sulfites (Arapitsas et al., 2016; Salas et al., 2004; Vidal, Cartalade, Souquet, Fulcrand,

& Cheynier, 2002). In addition, the oxidation of proanthocyanidins or flavanols generates quinones, highly reactive species, which can either be reduced by oxidising other compounds (e.g., SO_2, ascorbic) or can react with nucleophiles such as flavanols (yielding an increased degree of polymerisation). Anthocyanins can react either with tannins forming polymeric pigments or through cycloaddition reactions with other compounds such as acetaldehyde or pyruvic acid to form pyranoanthocyanins. These compounds react with cations formed by the cleavage of interflavane bonds to form pyranoanthocyanic derivatives, being these new pigments more stable than the native anthocyanins. In addition, both tannins and anthocyanins can react with acetaldehyde since when it is protonated in the acidic pH of the wine, it is a powerful electrophile that can react with the nucleophilic positions of the A ring of these flavonoids forming covalent bonds (Escribano-Bailón, Álvarez-García, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001). These reactions experienced by the polyphenolic compounds modify the molecular weight and structure of the original phenolic compounds, thus it seems logical to think that not only wine colour is modified, but also their ability to interact with oral receptors and saliva proteins and consequently the gustatory (mainly bitterness) and mouthfeel properties of the original wines.

Concerning wine colour, studies of micro-oxygenation and nano-oxygenation have shown that in both cases the reaction between tannins and anthocyanins is favoured, yielding to increases in colour intensity (CI) (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Wirth et al., 2010).

Regarding taste and mouthfeel, on the one hand, the supply of oxygen in the maturation stage, can generate desirable effects on taste and mouthfeel sensory properties such as decreased bitterness and astringency. In red wines, tannins are the main inducers of astringency, the decrease in astringency observed in micro-oxygenated wines has been attributed to their reactivity and to increases in the % of polymeric pigments, while the observed increases in astringency in micro-oxygenated wines is attributed to increases in the mean degree of polymerisation of

tannins. (Cejudo-Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2011; Cejudo-Bastante, Hermosín-Gutierrez, & Pérez-Coello, 2012; Oberholster et al., 2015; Sáenz-Navajas et al., 2012).

On the other hand, the ageing phase in the bottle (i.e., nano-oxygenation) seems to have a greater effect on aroma properties than on the in-mouth properties. In relation to the effects that ageing produced on the bitter taste, Sáenz-Navajas et al., (2014) have not found any general model of evolution in the wines since in some of them, bitterness increased, while in others it decreased, indicating that the changes depend on the overall composition of the wine as we have subsequently observed. As for astringency, the effect of ageing observed was very limited.

In summary, a clear correlation between the involved mechanisms of phenolics transformation and mouthfeel changes occurring during ageing are far from being understood.

In this context, the aims of this study were: 1) determine the rate of oxygen consumption of different wine polyphenolic fractions, 2) evaluate the effect of oxygen consumption on taste and mouthfeel properties, 3) evaluate the effect of oxygen consumption on chemical parameters. To reach these objectives, firstly, wine and its phenolic fractions were submitted to oxygen exposure. The oxygen consumption was measured using a non-invasive method based on luminescence, in order to increase the knowledge about the oxygen consumption of different wine compounds. Secondly, the same samples were kept in anoxia (non-oxygenated). The analyses were held in two different time periods, spaced eighteen weeks apart in time, which allowed to assess oxygen impact.

2. Material and methods

2.1. Chemicals

For wine model: copper (II) chloride dihydrate, iron (II) chloride tetrahydrate, acetaldehyde (≥99.5%), L (+)-tartaric acid and potassium disulfite were purchased from Sigma-Aldrich (St. Louis, MO, USA); Ethanol LiChrosolv quality were purchased from Merck (Darmstadt, Germany). Deionized water was purified with a Milli-Q water system (Millipore, Molsheim, France) prior to use, and pH 3.7 adjusted with diluted NaOH (0.1 N).

For fractionation: Toyopearl gel (HW 50F) (TOSOH CORPORATION, Japan) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and Bond Elut LRC C18 cartridges (500 mg, 10 ml) from Agilent Technologies. HPLC -grade acetonitrile and formic acid, methanol, diethyl ether and absolute ethanol all of them of reagent grade were obtained from Scharlab (Sentmenat, Spain).

For chemical characterization: *for Tannin activity,* HPLC-grade acetonitrile and o-phosphoric acid were purchased from Scharlab (Sentmenat, Spain), *for Acid-catalysed reaction and anthocyanins and low molecular polyphenols determination,* (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, (−)-epigallocatechin, Oenin-chloride, caffeic acid and quercetin-3-O-glucoside, were provided by Extrasynthese (Genay, France). Purity of chemical standards was over 95% in all cases and most of them over 99%.

2.2. Samples

2.2.1. Wine

Detailed of selected wine characteristics is presented in Annexe IV-3.1. Ethanol content, pH, reducing sugars, malic acid, lactic acid, titratable (total) and volatile acidities were determined in the original wine by Infrared Spectrometry with Fourier Transformation (IRFT) with a WineScanTM FT 120 (FOSS®), which was calibrated with wines analysed in accordance with official OIV methods. Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and color intensity

(CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). Exhaustive analyses included metals (Fe, Cu, Mn, Zn) were determined by plasma–mass spectrometry with collision/reaction cell technology (CCT–ICP–MS) (Grindlay, Mora, De Loos-Vollebregt, & Vanhaecke, 2014) and acetaldehyde were determined by HPLC-DAD/MS (Han, Wang, Webb, & Waterhouse, 2015).

2.2.2. Preparation of fractions

In the first step, two fractions (F1 and F2), as can be seen in Figure IV-3. 1, were collected by a preparative LC method adapted from Remy, Fulcrand, Labarbe, Cheynier, and Moutounet (2000) and Gonzalo-Diago, Dizy, & Fernández-Zurbano (2013).

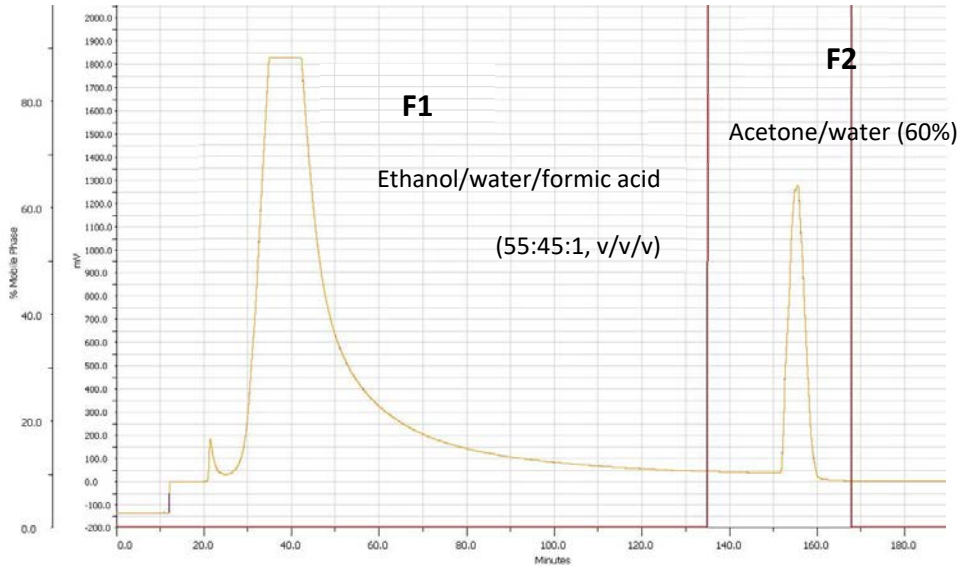

Figure IV-3. 1. First step Preparative LC method, F1 and F2 fractions.

For this, the ethanol content of three hundred fifty milliliters of wine was removed in a rotary evaporator (15 min at 25 ºC). Then, the sample was further freeze-dried in 500 mL-rounded flasks. The extract was redissolved in the minimum amount of hydroalcoholic solution (12% ethanol, v/v) possible and the whole volume was injected into a preparative Millipore LC column (280 mm x 32 mm) filled with Toyopearl HW-50F gel; flow rate: 10 mL min^{-1}). A first fraction (F1) was eluted with 1350 mL of ethanol/water/formic acid (55:45:1, v/v/v). The second fraction (F2) was

recovered by elution with 400 mL of acetone/water (60%). Solvents present in the fractions were evaporated under vacuum and samples were further freeze-dried.

Then, according to Sáenz-Navajas et al., (2017), with some modifications, each fraction F1 was redissolved in 350 mL of hydroalcoholic solution (12%, v/v) and further submitted to solid-phase extraction (SPE) using an extraction unit (VAC ELUT 20 Station from Varian, USA). SPE cartridges filled with 500 mg of Bond Elut LRC-C18 resins (Agilent Technologies, USA) were firstly conditioned by passing five mL of methanol and ten mL of an aqueous solution at pH 2.5 (5 g L^{-1} of tartaric acid, pH adjusted to 2.5 with 0.1 M NaOH). After this, 5 mL of F1 were loaded in each cartridge and sugars and organic acids were washed with 10 mL of aqueous solution at pH 2.5 in order to obtain F1L. For the rest of the samples, the protocol was as follow, F11+F12 was eluted with 5mL of ethyl acetate and F1.3 with 10 mL of methanol. Each cartridge was used a maximum of 5 times. The SPE procedure was repeated until the 350 mL of F1 were extracted. Fractions F1L, F11+F12 and F1.3 were gathered in a round flask and further evaporated prior freeze-drying.

Five different fractions (F1, F1L, F2, F13 y F11+F12) of wine were obtained and finally, eight different samples were considered (F1, F1L, F2, F13 y F11+F12) plus F2+F13 and two control samples, these lasts to monitor the effect of wine fractionation. These samples were one sample constituted by freeze dried wine (W) and dissolved in model wine, and the other containing all the fractions (F1+F2). A detailed list of fractions used, is presented in Table IV-3.1. For each of the eight samples, four replicates were prepared, two of them were not oxygenated, while the other two were oxygenated.

Table IV-3. 1. Detailed list of fractions employed.

	Samples	Description
1	W	Original wine freeze-dried and reconstituted in model wine.
2	F1+F2	Reconstituted wine by sum of fractions F1 + F2
3	F1	First fraction by preparative LC Ethanol/water/formic acid (55:45:1, v/v/v)
4	F1L	First fraction, without acids. F1 submitted to SPE and washed with 10 mL of aqueous solution at pH 2.5
5	F2	Second fraction by preparative LC Acetone/water (60%)
6	F11+F12	F1 submitted to SPE and eluted with 5 mL of ethyl acetate
7	F13	F1 submitted to SPE and eluted with 10 mL of methanol.
8	F13+F2	Reconstituted fraction by sum of individual fractions F13 + F2

2.2.3. Preparation of model wine solutions.

To prepare the samples for the oxygen evaluation experiment, the dried simple fractions (coming from 350 mL of wine) were reconstituted in different hydroalcoholic solutions prepared with distilled water, and 7% of ethanol. The level of ethanol (7%) was selected, in order to prepared samples for the subsequently sensory analysis. This level was determined in preliminary tastings (data not shown) and corresponded to the minimal level of ethanol that induce the lowest burning effect, to avoid mask other sensations but it´s able to dissolve freeze-dried fractions.

According to initial chemical analysis of wine and fractions showed in Table IV-3.2, the final composition of the wine model solution employed is described as follow: 7 % ethanol; 5 mg/L Fe; 0.2 mg/L Cu; 15 mg/L acetaldehyde; 1 g/L a tartaric acid; pH: 3.7 and 30 mg/L SO_2. In order to reach that composition for W sample, F1+F2 sample and F1 fraction, it was not necessary adjusted acids and pH, due to the acids belonging to the wine are included in these fractions, reaching an adequate pH.

To reach the normal concentration in wine Fe, Cu, acetaldehyde and SO_2 were adjusted. However, for the rest of samples, F1L, F2, F11+F12, F13 and F13+F2, it was necessary also adjusted acids and pH, thus $1gL^{-1}$ tartaric acid and pH 3.7 along with Fe, Cu, acetaldehyde and SO_2 were adjusted, in order to reach the proposed composition of model wine.

Table IV-3. 2. Initial composition of wine and fractions

	Original Wine	F1	F1L	F2	F11+F12	F13
Acetaldehyde	6.4 ± 0.33	4.81 ± 0.33	< LC	1.78 ± 0.34	<LOQ	<LOQ
Fe 56	1661.6 ± 18.3	2485.0 ± 55.0	<LOQ	<LOQ	<LOQ	<LOQ
Cu 65	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Cu 63	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mn 55	1094.0 ± 6.5	1111.5 ± 26.8	<LOQ	<LOQ	<LOQ	<LOQ
Zn 66	948.6 ± 25.6	<LOQ	1033.9 ± 12.2	<LOQ	<LOQ	<LOQ
Zn 68	966.6 ± 31.5	<LOQ	1043.5 ± 6.1	<LOQ	<LOQ	<LOQ
pH	3.7	3.7	-	5.8	-	-
SO_2	30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Acetaldehyde, expressed as mg L^{-1}
Metals analyzed (Fe, Cu, Mn, Zn), expressed as µg L^{-1}.
SO_2, expressed as mg L^{-1}

2.3. Experimental design

2.3.1. Assessment of oxygen permeability

The assessment of oxygen permeability was carried out by compare the oxygen dissolved in model wine in different material bottles along the time (Figure IV-3.2). The systems, were sterile 372 mL (total volume) bottles hermetically closed and made with a permeable material. Bottles were supplied by VWR International Eurolab, S.L (Spain).

Figure IV-3. 2. Assessment of bottle`s O2 permeability

The assessment of bottle`s O_2 permeability, was carried out in a short experiment. To reach this aim, bottles made of three different materials (polystyrene, polycarbonate and polyethylene glycol terephthalate), four bottles of each material and containing a model wine solution of 7% ethanol (v/v), 1 g/L tartaric acid and pH= 3.7 in water were stored at 20°C (Compounds with oxygen consumption ability were avoided in wine model). Each bottle, contained one PSt6 (0-1.8 mg/L and limit of detection 1µg/L) (PreSens Precision Sensing GmbH, Ordering Code: SP-PSt3-NAU-D5-CAF; Batch number: 161019-004_PSt6-0834-01, Regensburg, Germany) O_2 sensor (Nomasense^{TM}) and one PSt3 (0-22 mg/L and limit of detection

15 µg/L) (PreSens Precision Sensing GmbH, Ordering Code: SP-PSt3-NAU-D5-CAF; Batch number: 151210-005_PSt3-1208-02, Regensburg, Germany) O_2 sensor (Nomasense™) from Wine Quality Solutions, Vinventions S.A, (Thimister-Clermont, Belgium) and the oxygen level was determined by measuring the dissolved oxygen. The level of O_2 was monitored in all bottles using an oxygen analyzer (Nomasense™) from Wine Quality Solutions, Vinventions S.A, (Thimister-Clermont, Belgium), during ninety-one days. The objective was to measure the permeabilities of different materials until reach the saturated concentration of oxygen (8.0 mg/L) (Singleton, 1987). (Singleton, 1987).

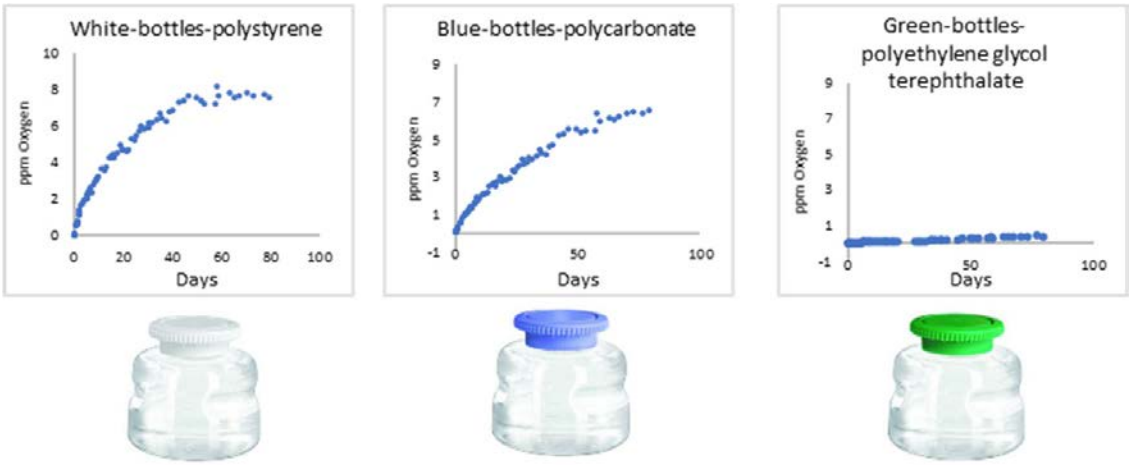

Figure IV-3. 3. Monitoring of assessment of bottle`s O2 permeability

As result of this approach, bottles of polystyrene material (white bottles) were selected, since the saturation was reached first Figure IV-3. 3, which means that the permeability is higher. Furthermore, considered that in a previous work (Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2014), where commercial red wines were stored at 25 °C for 6 months in airtight containers under different oxygen doses (0–30 mg L^{-1}), and results showed just discrete increases ($p<0.01$) in astringency, we are interesting on increase that oxygen doses.

2.3.2. Experimental protocol

In each bottle were placed one PSt6 and one PSt3 O_2 sensor (Nomasense^{TM}) from Wine Quality Solutions, Vinventions S.A, (Thimister-Clermont, Belgium) (Figure IV-3.2) and the oxygen level was determined by measuring the dissolved oxygen, using the non-invasive method based on luminescence, similarly to the previous short experiment.

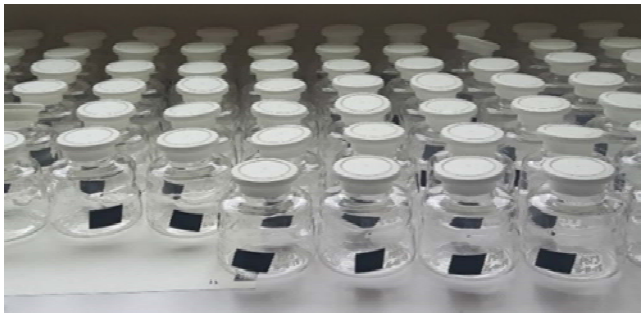

Figure IV-3. 4. Experimental bottles containing O_2 sensor (Nomasense^{TM})

The control and reproducibility of O_2 permeability of the systems assessed in the previous study, was rechecked along to the experiment with the introduction of control samples. This samples only contained model wine (MW) (7 % ethanol; 5 mg/L Fe; 0.2 mg/L Cu; 15 mg/L acetaldehyde; 1 g/L a tartaric acid; pH: 3.7 and 30 mg/L SO_2). In that case, SO_2 was considered to avoid that oxygen consumption by SO_2 in samples, was assumed as oxygen consumption by phenolic fraction (Ferreira et al., 2015). A detailed list of samples, is presented in Table IV-3.3.

Table IV-3. 3. List of 64 samples (oxygenated and non-oxygenated) and 3 wine models (WM).

Non-Oxygenated (A)				Oxygenated (O)			
A-I		A-II		O-I		O-II	
+ 6 weeks anoxia		+ 24 weeks anoxia		+ 6 weeks anoxia		+ 24 weeks anoxia	
W-A1	W-A2	W-A3	W-A4	W-O1	W-O2	W-O3	W-O4
F1+F2-A1	F1+F2-A2	F1+F2-A3	F1+F2-A4	F1+F2-O1	F1+F2-O2	F1+F2-O3	F1+F2-O4
F1-A1	F1-A2	F1-A3	F1-A4	F1-O1	F1-O2	F1-O3	F1-O4
F1L-A1	F1L-A2	F1L-A3	F1L-A4	F1L-O1	F1L-O2	F1L-O3	F1L-O4
F2-A1	F2-A2	F2-A3	F2-A4	F2-O1	F2-O2	F2-O3	F2-O4
F11+F12-A1	F11+F12-A2	F11+F12-A3	F11+F12-A4	F11+F12-O1	F11+F12-O2	F11+F12-O3	F11+F12-O4
F13-A1	F13-A2	F13-A3	F13-A4	F13-O1	F13-O2	F13-O3	F13-O4
F13+F2- A1	F13+F2-A2	F13+F2-A3	F13+F2-A4	F13+F2-O1	F13+F2-O2	F13+F2-O3	F13+F2-O4
MW-1 MW-2 MW-3							

The (64 sample + 3 control wines) bottles and prepared dissolutions containing the samples, were introduced in the anoxic glove chamber from Jacomex (Dagneux, France) in which oxygen in the gas phase was below 0.002% (v/v) two days before filling them with the samples, in order to eliminate any O_2 contained in the walls of the material, caps or liquid.

Figure IV-3. 5. Experimental process: Left, caps sealing with glue to ensure complete airtightness; Right, fractions keeping in the anoxic glove chamber from Jacomex (Dagneux, France).

The real capacity of polystyrene bottles, was 372 mL, thus, bottles were filled up to 300 mL allowing a 72 mL headspace. All the bottles were capped and then, the caps were sealed with glue to ensure complete airtightness through the cap Figure IV-3.5. Three days inside the chamber were further allowed to ensure the glue runs dry. Along with the half of the samples (O1, O2, O3, O4), the three controls (MW-1, MW-2, MW-3) were then taken out of the chamber and let in an incubator (ClimasLab, Barcelona, Spain) at 20 °C. The level of O_2 was daily monitored in all bottles using an Oxygen analyzer from Nomacorc (Thimister-Clermont, Belgium). When the total package oxygen or oxygen equivalent (Dissolved Oxygen + Head Space Oxygen) in the control reach approximately 50 ppm, and the calculated wine sample oxygen consumption was approximately 40 ppm (upper to Sáenz-Navajas et al. (2014) the experiment was finished by introducing again the bottles in the glove chamber. Samples were keeping inside until the corresponding sensory analysis. All the experiments were performed in duplicate, A1, A2, O1 and O2 samples were sensory and chemically analysed first (after 6 weeks in anoxic chamber), and A3, A4, O3 and O4 samples (after 24 weeks in anoxic chamber) were subsequently sensory and chemically analysed.

2.3.3. Total oxygen dissolved and estimation of oxygen consumption rate

The level of O_2 was daily monitored in all the samples (Annexe IV-3.2). The measured was directly the O_2 dissolved in solution. However, inside bottles exist an equilibrium between the dissolution and the head space Figure IV-3.6.

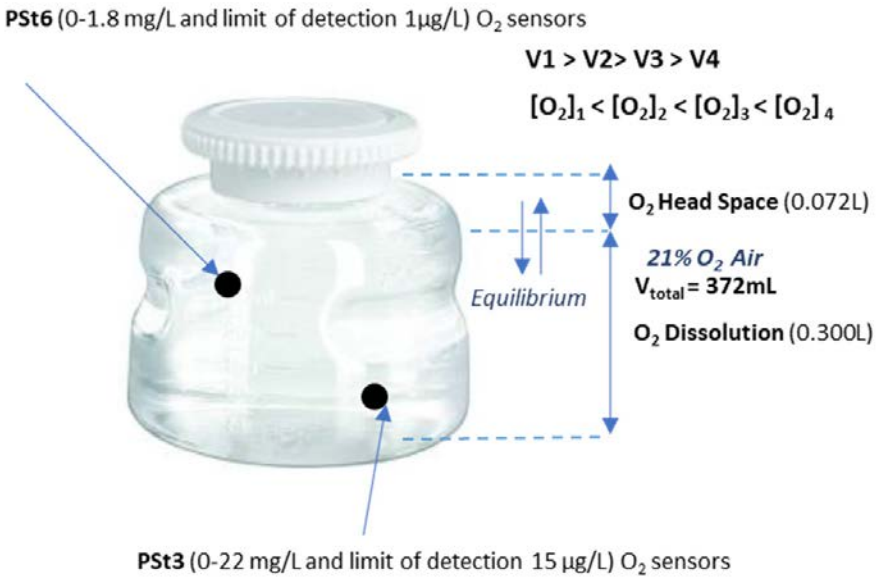

Figure IV-3. 6. Experiment design scheme

To calculate the O_2 consumed in the samples, the following has been taken into account:

Control sample containing only model wine (MW) and thus, free of phenolic compounds, results the sample with high oxygen accumulation. The rate of oxygen diffusion through polystyrene depends on the concentration of dissolved oxygen, this rate is lower when more oxygen is dissolved in the samples.

The different phenolic composition of the samples induces different oxygen consume rates.

The real dissolved oxygen in each sample, was calculated as the sum of dissolved oxygen (DO) and headspace oxygen (HSO) which we call oxygen equivalent.

$$(O_2)_{Equivalent} = DO + \mathrm{HSO}$$

$$(O_2)_{Equivalent} = (O_2)_{Dissolution} + (O_2)_{Head\ Space}$$

Therefore, the real dissolved oxygen in each sample, was calculated as oxygen equivalent (DO+HSO), considering the velocity of oxygen diffusion different in each sample. To estimate it, the zone in which the velocity of model wine and the corresponding sample was similar was considered as the velocity of diffusion for the corresponding sample (Figure IV-3.7), avoiding the date from the first day in which case the velocity is faster as previously noted (Ferreira et al., 2015).

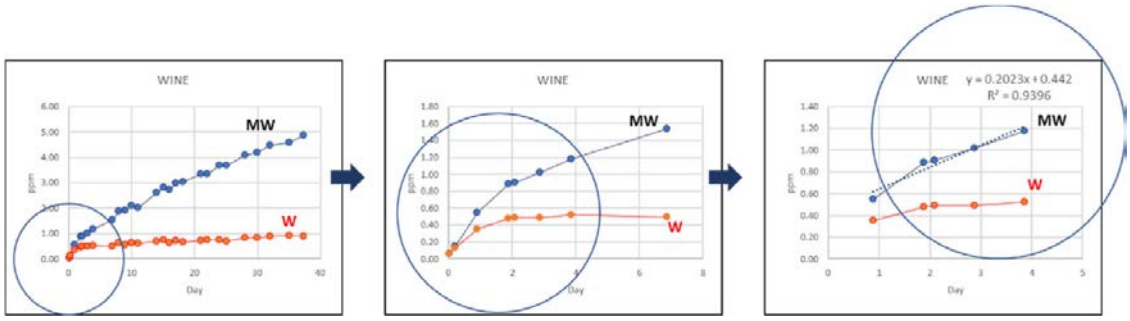

Figure IV-3. 7. Estimation of diffusion velocity to estimate the total oxygen dissolved. Case of wine sample.

$$Oxygen\ consumed = (O_2 Model\ Wine)_{Equivalent} - (O_2\ Sample)_{Equivalent}$$

In this context, the oxygen consumed and the corresponding velocity of oxygen consumption is different that the total oxygen that has been able to permeate inside the bottle.

2.4. Chemical analysis

2.4.1. Conventional oenological parameters

Spectrophotometric measures were carried out in a Shimazdu UV-1800 (Shimadzu Corporation, Tokyo, Japan). Total polyphenol index (TPI) was estimated as absorbance at 280 nm (Ribéreau-Gayon, 1970) and colour intensity (CI) as the sum of absorbance at 420, 520 and 620 nm (Glories, 1984). For TPI determination, samples diluted 1:100 in deionised water, the absorbance at 280 nm of samples, was measured in 1-cm-quarz cuvettes. For CI, absorbance of undiluted samples was measured in 2-cm-crystal cuvettes.

CIElab coordinates were evaluated according to the OIV methods (OIV, 2007). The absorbance spectres were measured using 0.2-cm path-length crystal cuvettes. Measurements were taken every 1 nm between 380 and 780 nm. Wine samples had been filtred by passing wine through 0.45 µm filters previous to the measurements. From the spectra, the colour coordinates were calculated using the CIE method, with the CIE 1964 10° standard observer and the illuminant D65, according to the OIV. Since its publication in 1976, CIELAB (CIE L*a*b* and CIEL*C*h*) has been extensively employed to describe colour space. Thus, The CIE L*a*b* system describes colours based on the opponent theory, it means that colours cannot be perceived as both red and green at the same time, or yellow and blue at the same time. The values correspond to the degree of wine lightness (L_{10}*) and the degree of red (when a_{10}*>0), green (when a_{10}*<0), yellow (when b_{10}*>0), and blue (when b_{10}*<0) colour.

CIELab colour difference, which numerically quantifies the colour perception difference between two samples was calculated as follow:

$$\Delta E^*_{a,b} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

2.4.2. Analysis of anthocyanin-derived pigments

Determination of monomeric (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP) in wines and fractions was carried out as described elsewhere (Harbertson, Picciotto, & Adams, 2003). MPs were the group of compounds bleachable with bisulfite, while SPP and LPP were resistant to bisulfite bleaching. SPP did not precipitate with ovoalbumin, different to LPP. Levels of MP, SPP, and LPP were expressed as absorbance at 520 nm

2.4.3. Tannin activity

Tannin activity is related to the thermodynamics of interactions between tannins and a hydrophobic surface (polystyrene divinylbenzene HPLC column). Analyses were carried out with an Ultra High Pressure Liquid Chromatography system (Shimadzu Nexera, KIOTO, JAPAN) coupled to a Photodiode Array Detector - SPD-M30A from Shimadzu (KIOTO, JAPAN) operating under Labsolutions software, using a PLRP-S 100 Å 3µm, 2.1 × 50 mm column (Agilent) protected with a PLRP-S 100Å 3µ × 5 mm guard column (Agilent). To determine tannin activity, samples were run at four column temperatures (30-35-40-45 °C) following the method proposed by Revelette et al. (2014).

2.4.4. Mean degree of polymerization by acid-catalysed reaction

Acid-catalysed degradation in the presence of toluene-α-thiol was performed according to the method described by Labarbe, Cheynier, Brossaud, Souquet, & Moutounet (1999) but with some modifications as described by (Gonzalo-Diago et al., 2013. Quantification was carried out in the negative mode from the extracted ion chromatogram (EIC) for flavan-3-ols. The area under the peaks of flavan-3-ol monomers (terminal units) before and after thiolysis, as well as toluene-α-thiol adducts (extension units) released from the depolymerisation reaction were all integrated. Calibration curves were established with (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, and (−)-epigallocatechin. In the absence of the thiol derivative standards, considering the fact that the thiolytic derivatives were shown

to have similar response factors as the correspondent monomeric units, their concentrations were calculated from the respective monomer calibration curves. The mean degree of polymerisation (mDP) was calculated as the ratio of total units (extension + terminal) to terminal units (calculated as the difference between before and after thiolysis). The percentage of procyanidins (%PC) was calculated as the ratio of total units (extension and terminal) of catechin and epicatechin to total units of tannins. The percentage of prodelphinidins (%PD) and galloylated (%G) were calculated units as the ratio of total units of epigallocatechin and gallocatechin and flavanol-gallates to the total units of tannins, respectively.

2.4.5. UHPLC-MS determination of anthocyanins

Anthocyanins were identified by UHPLC-DAD-MS and quantified by UHPLC-DAD following the method described by Gonzalez-Hernandez, Avizcuri, Dizy, and Fernandez-Zurbano (2014) with some modifications.

The samples studied were analysed with an ultra-high pressure liquid chromatography system (Shimadzu Nexera) with diode array photodetector and coupled to ABSciex 3200QTRAP® system, operating under Analyst software. A reverse phase (RP) Acquity UPLC BEH C18, 1.7µm, 2.1 × 150 mm column (Waters), protected with an Acquity UPLC BEH C18, 1.7 µm, 2.1 × 5 mm precolumn (Waters), was employed. The mobile phase was a mixture of solvent A (water–formic acid, 95:5, v/v) and solvent B (acetonitrile–formic acid, 95:5, v/v). The quantification was carried using malvidin-3-glucoside compound for quantified all the compounds as equivalents.

2.4.6. UHPLC-MS determination of polyphenols

Flavonols, flavanols and acids were identified by UHPLC-MS following the method described by Gonzalez-Hernandez, Avizcuri, Dizy, and Fernandez-Zurbano (2014). Samples were analysed with an ultra-high pressure liquid chromatography system (Shimadzu Nexera) with diode array photodetector and coupled to ABSciex 3200QTRAP® system, operating under Analyst software. A reverse phase (RP)

Acquity UPLC BEH C18, 1.7µm, 2.1 × 150 mm column (Waters), protected with an Acquity UPLC BEH C18, 1.7 µm, 2.1 × 5 mm precolumn (Waters), was employed. The mobile phase was a mixture of solvent A (water–formic acid, 99:1, v/v) and solvent B (acetonitrile–formic acid, 99:1, v/v).

2.5. Sensory evaluation

Two different sensory sessions were carried out employing rate-all-that-apply (RATA) methodology (Ares et al., 2014) and were separated in time by 4 months. Both sessions were held in the Institute of Grapevine and Wine Sciences (ICVV), Logroño (Spain). Each session was split into two parts and were held in two different days. Each day 14 samples were assessed, separated by an imposed pause of 15 min. Both sessions included seven out of the eight kind of samples and their corresponding replicates. According to previous work, (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014) fraction F1, was not sensory evaluated due to its high acidity, that make it impossible to evaluate correctly this sample. Another taste apart from sour and mouthfeels are mask by acidity, so F1 was not employed in the sensory analysis. Instead F1L, was evaluated.

First session was carried out by sixteen wine experts (10 women and 6 men, ranging in age from 29 to 66 years, with an average of 37 years old) and second session was carried out by seventeen wine experts (12 women and 5 men, ranging in age from 26 to 67 years, with an average of 36 years old). They were from the Rioja area, (Spain), and had extended experience in wine production and tasting. Samples were served in dark approved wine glasses (ISO 3591, 1977) labelled with 3-digit random codes, and the order of presentation of samples was different for each participant attending to a randomized order. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room (at around 20 ºC).

Participants were informed that samples were not commercial wines. They were not informed about the objective of the study and were not paid for their participation.

2.5.1. Rate-all-that-apply

Samples were characterized by rate-all-that-apply (RATA) method employing a list of 23 taste and mouthfeel-related attributes, described in Section I-Chapter1. The detailed list is presented in Table IV-3. 4. Participants were asked to rate the intensity of the terms, that appear in the list, in a 7-point scale (1 = not intense; 7 = very intense), according to all-that-apply methodology. Terms that did not appear in the samples were rate with a value of zero when collecting data. To avoid bias due to order of presentation, terms in the list appeared in different and randomized order for each assessor.

Table IV-3. 4. Taste and mouthfeel-related attributes employed in RATA methodology.

CATEGORY	INDIVIDUAL TERMS			
Taste	1	Bitter (*amargo*)	3	Salty (*salado*)
	2	Sour (*ácido)*	4	Sweet (*dulce*)
Mouthfeel	5	Burning (*ardiente*)	6	Coarse (rugoso)
	7	Dusty (*polvoriento*)	8	Feeling of dryness (*secante*)
	9	Feeling of dryness on palate (*secante en el paladar*)	10	Feeling of dryness on tongue side (*secante en el lateral de la legua*)
	11	Fleshy (*carnoso*)	12	Grainy (*granuloso*)
	13	Gummy (*gomoso*)	14	Hot (*picante*)
	15	Mouthcoating (*envolvente*)	16	Oily (*graso*)
	17	Prickly (*punzante*)	18	Sandy (*arenoso*)
	19	Silky (*sedoso*)	20	Sticky (*pegajoso*)
	21	Unctuous (*untuoso*)	22	Watery (*aguado*)
Other	23	Persistent (*persistence*)		

2.6. Data analysis

2.6.1. Data analysis for Rate-all-that- apply

To study the oxygen effect on the sensory properties of samples, a two-way ANOVA with the intensity of sensory attributes was calculated for each sample considering panellists as random factor and oxygen supply (oxygenated vs non-oxygenated samples) as fixed factor.

All analyses were carried out with XLSTAT (2019.3.1.60623 version).

2.6.2. Data analysis for chemical parameters

The effect of oxygen consumption in chemical parameters, was evaluated by one-way ANOVA analysis. Discriminant chemical parameters were calculated for each of the samples.

Analyses were carried out using XLSTAT (2019.3.1.60623 version).

3. Results and discussion

3.1. Chemical characterisation of samples

From the five different fractions (F1, F2, F1L, F13 y F11+F12) obtained by different steps, eight different samples were prepared (W, F1+F2, F1, F1L, F2, F11+F12, F13, F2+F13) as described in material and methods section (Table IV-3. 1).

It should be expected, that W and F1+F2 samples, reach the same characterization. Results obtained from the one-way ANOVA analysis (Table IV-3. 5), showed that the analysed phenolic composition of W sample (corresponding to the freeze-dried wine and reconstituted in model wine) and F1+F2 sample (coming from the sum of F1 and F2 fractions obtained by semipreparative liquid chromatography and reconstituted in model wine) did not show great differences. Both samples differ in the concentration of flavanols and proanthocyanidins (<3 units), being the difference 0.9 and 3.1 ppm, respectively. This result suggests that the fractionation method did not considerably affect the original wine composition.

F1 and F2 samples contain a very different polyphenolic composition (Table IV-3. 6). Thus, F1 contains compounds of low molecular weight including anthocyanins, small pigments, phenolic acids, flavonols, flavanols and PAs (< 3 units). This fraction contributes the most to colour (as it can be seen on CI value). While, F2 is formed by compounds of higher molecular weight (mainly proanthocyanidins > 3 units). F1 presents a higher value of TPIs than Fraction F2, however the phenolic composition contained in this fraction presents lower tannin activity than F2. It is important to note that tannin activity is an interesting parameter that has been related to the perception of astringency in mouth (Section III-Chapter 1).

Table IV-3. 5. Chemical parameters for F1+F2 and W. Chemical variables with significant differences are marked in bold* (P<0.05)

	W	F1+F2	Pr > F
Conventional oenological parameters[1]			
TPI	68,825 a	67,500 a	0,529
CI	13,639 a	13,336 a	0,828
Anthocyanin-derived pigments[2]			
MP	1,093 a	1,035 a	0,561
SPP	0,468 a	0,488 a	0,685
LPP	0,355 a	0,353 a	0,976
Glycosilated	49,780 a	43,971 a	0,068
Acylated	8,002 a	7,373 a	0,550
Pyranoanthocyanins	9,640 a	10,171 a	0,369
Tannin characterization[3]			
Activity	3362,036 a	3535,799 a	0,416
mDP	4,675 a	4,450 a	0,845
%PC	82,620 a	81,330 a	0,091
%G	**1,580 b**	**2,665 a**	**0,040***
%PD	15,795 a	16,000 a	0,690
Low molecular phenolic compounds[4]			
Cafeic acid	2,655 a	2,800 a	0,217
Caftaric acid	1,068 a	1,048 a	0,257
Galic acid	15,175 a	14,625 a	0,501
Flavanols	**8,175 a**	**7,354 b**	**0,035***
PAs (PBs + PCs)	**19,838 a**	**16,757 b**	**0,030***
Flavonols	27,990 a	28,334 a	0,790

[1]**TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); [2]**MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6”-acetyl)-glucoside, Cyanidin 3-(6”-acetyl)-glucoside, Petunidin 3-(6”-acetyl)-glucoside, Peonidin 3-(6”-acetyl)-glucoside, Malvidin 3-(6”-acetyl)-glucoside, Delphinidin 3-(6”-p-coumaroyl)-glucoside, Cyanidin 3-(6”-p-coumaroyl)-glucoside, Petunidin 3-(6”-p-coumaroyl)-glucoside, Peonidin 3-(6”-p-coumaroyl)-glucoside, Malvidin 3-(6”-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; [3]**TA**: Tannin activity (-J/mol), **mDp**: mean degree of polymerization, **%PC**: percentage of procianidins, **%PD**: percentage of prodelphinidins, **%G** percentage of galloylation; [4] **Low molecular phenolic compounds** (mg/L), **Flavanols:** gallocatechin, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, (−)-epigallocatechin (mg/L), **Flavonols:** myricetin, quercetin, myricetin 3-glucoside, quercetin-3-glucuronide (mg/L).

As result of further fractionated F1 by Solid-Phase Extraction (SPE) FIL, F11+F12 and F13 were obtained (Table IV-3. 7). F1L is the result of subjecting F1 to SPE, removing sugars and organic acids, responsible of its high acidity, as well as mineral compounds during washing stage that is carried out in the fractionation.

Table IV-3. 6. Chemical parameters for F1 and F2. Chemical variables with significant differences are marked in bold (P<0.05)

	F1	F2	Pr > F
Conventional oenological parameters[1]			
TPI	51,075 a	18,575 b	**0,000**
CI	11,141 a	1,395 b	**0,003**
Anthocyanin-derived pigments[2]			
MP	0,913 a	0,170 b	**0,004**
SPP	0,458 a	0,000 b	**0,005**
LPP	0,158 a	0,065 a	0,348
Glycosilated	47,192 a	2,987 b	**< 0,0001**
Acylated	7,762 a	0,355 b	**0,000**
Pyranoanthocyanins	9,805 a	0,140 b	**< 0,0001**
Tannin characterization[3]			
Activity	1105 b	9652 a	**0,002**
mDP	1.88 b	9,775 a	**0,032**
%PC	72.41 b	79,780 a	**0,020**
%G	4.47 a	2,440 a	0,597
%PD	23.12 b	17,785 a	**< 0,0001**
Low molecular phenolic compounds[4]			
Cafeic acid	2,935 a	0,000 b	**0,001**
Caftaric acid	1,010 a	0,000 b	**0,000**
Galic acid	15,075 a	0,454 b	**< 0,0001**
Flavanols	7,944 a	2,563 b	**0,000**
PAs (PBs + PCs)	14,674 a	6,065 b	**0,002**
Flavonols	27,893 a	0,121 b	**< 0,0001**

[1]**TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); [2]**MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; [3]**TA**: Tannin activity (-J/mol), **mDp**: mean degree of polymerization, **%PC**: percentage of procianidins, **%PD**: percentage of prodelphinidins, **%G** percentage of galloylation; [4] **Low molecular phenolic compounds** (mg/L), **Flavanols:** gallocatechin, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, (−)-epigallocatechin (mg/L), **Flavonols:** myricetin, quercetin, myricetin 3-glucoside, quercetin-3-glucuronide (mg/L).

After that, F11+F12 and F13 were obtained, by different solvents. The fraction F11+F12 contains uncoloured low molecular weight phenolic compounds (benzoic and hydroxycinnamic acids and their esters, monomers of flavanols and flavonols). Fraction F13 retain chiefly compounds responsible for colour, i.e., anthocyanins and anthocyanin-derived compounds, more than 85% of colour intensity (CI) and more than 65% of TPI.

Anthocyanin derivatives, including those non-resistant to SO_2 bleaching (MP), resistant to SO_2 bleaching but not precipitating with albumin (SPP), and those resistant to SO_2 and able to precipitate with protein (LPP), are present in F13. It is worth to highlight, that even though flavavols are in a greater proportion in sample F11+F12 (6.4 ppm), they are also present in sample F13 (4.5 ppm), as gallic acid (0.32ppm). The proanthocyanidins (<3 units) have been found mainly present in sample F13.

It is noteworthy, that while W and F1+F2 samples reach values of approximately 3400 (-J·mol^{-1}) for tannin activity, their corresponding independent fractions, F1 and F2, reach values of 1100 and 9652 (-J·mol^{-1}), respectively. Thus, the tannin activity measured as the hydrophobic interaction resulted to be three times lower for F1 than for wine. While for F2, resulted to be three times higher. Hence, it seems to indicate that there is a modulator effect of F1 on F2 in terms of tannin activity, and as a result this parameter is modified for F2 by the presence of F1. This effect is also observed in sample F13+F2, which reaches the value of 5300 (-J·mol^{-1}), being almost the half of F2. The changes that the compounds constituting F1 and F13 induce in the tannic activity of sample F2 (tannic fraction), do not appear to be the result of chemical reactions occurring between the compounds of both fractions, rather seems to be a “modulator” effect due to the presence in the same matrix of the compounds involved in F1 and tannins involved in F2, which induce a decrease on the tannin activity.

Table IV-3. 7. Chemical parameters for F1L, F13 and F11+F12. Chemical variables with significant differences between F13 and F11+F12 are marked in bold (P<0.05)

	FIL	F13	F11+F2	Pr > F
Conventional oenological parameters[1]				
IPT	28,538 a	**19,263 b**	6,700 c	**< 0,0001**
IC	9,183 a	**7,209 b**	0,311 c	**0,00**
Anthocyanin-derived pigments[2]				
MP	0,703 a	**0,603 a**	0,040 b	**0,01**
SPP	0,278 a	**0,240 a**	0,010 b	**0,00**
LPP	0,118 a	**0,100 a**	0,000 b	**< 0,0001**
Glycosilated	59,727 b	**64,313 a**	4,745 c	**< 0,0001**
Acylated	12,173 a	**11,614 a**	3,583 b	**< 0,0001**
Pyranoanthocyanins	7,246 a	**5,587 b**	0,143 c	**< 0,0001**
Low molecular phenolic compounds[3]				
Cafeic acid	2,593 b	0,000 c	**3,403 a**	**0,00**
Caffeic acid ethyl ester	1,930 a	0,000 b	**2,275 a**	**0,00**
Galic acid	0,680 a	0,323 c	**0,462 b**	**0,01**
Flavanols	9,231 a	4,518 c	**6,413 b**	**0,00**
PAs (PBs + PCs)	13,568 a	**9,349 b**	4,523 c	**0,01**
Flavonols	40,522 a	8,107 c	**19,619 b**	**0,00**

[1]**Conventional oenological parameters: TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); [2]**Pigments: MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; [3]**Low molecular phenolic compounds** (mg/L), **Flavanols:** gallocatechin, (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, (−)-epigallocatechin (mg/L), **Flavonols:** myricetin, quercetin, myricetin 3-glucoside, quercetin-3-glucuronide (mg/L).

3.2. Oxygen consumption rates

Figure IV-3. 8 shows the O_2 dissolved (in solution) in all experimental samples during the time in which samples were kept in contact with atmospheric oxygen. It should be noted that MW (model wine or control) and F11+F12 samples, shows similar oxygen dissolved amounts. This result suggests either there is no oxygen consumption or the oxygen consumed is the same in both samples despite the phenolic compounds present in the sample F11+F12. The rest of samples showed lower dissolved oxygen levels than the MW sample, indicating that all of them consumed oxygen.

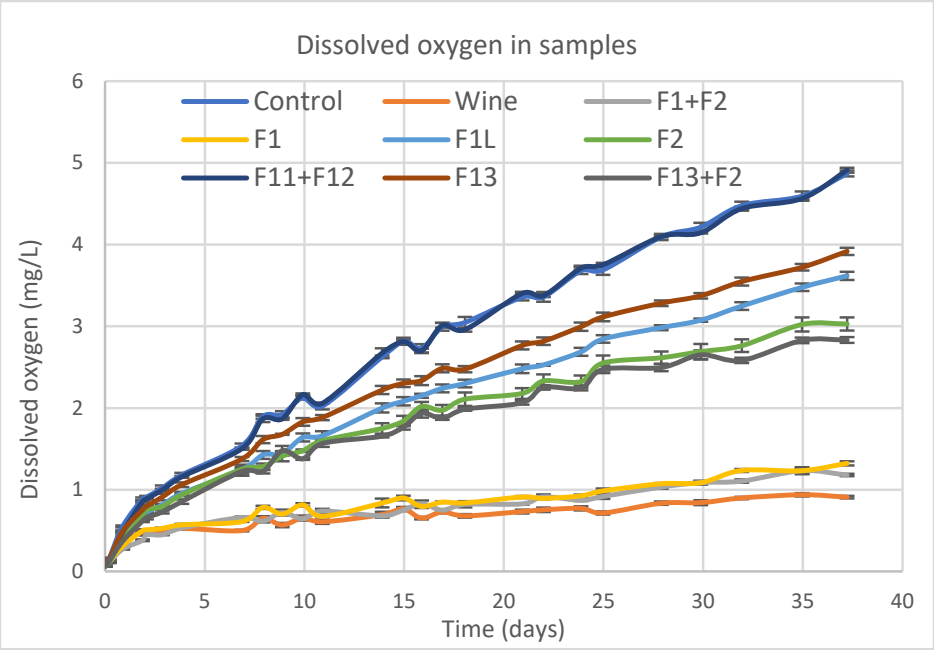

Figure IV-3. 8. Register data of oxygen in dissolution. Data got directly from Oxygen analyzer from Nomacorc (Thimister-Clermont, Belgium). Each point i*correspond to average* of 4 replicates (3 in the case of the control sample) and the bars *are* indicat*ive of* the error.

The oxygen consumption rates (OCRs) are shown in Table IV-3. 8 and its calculation previously described in material and methods (2.3.3 section).

W, F1+F2 and F1 samples showed significantly higher oxygen consumption than F1L, F11+F12, F13, F13+F2 and F2, which corresponds to lower dissolved oxygen levels (Figure IV-3. 8). Curiously, these last samples have in common the absence of acids in their composition and all presented lower oxygen consumption. The highest

oxygen consumption rate has been observed for W sample, where the oxygen consumption resulted significantly higher than in sample F1+F2 and F1 sample (40, 37 and 36 mg/L, respectively). According to the phenolic characterisation of samples, as it has been mentioned above, there is not a big difference between W and F1+F2 samples, but it could be enough to explain the slight difference found in OCRs.

It is noteworthy, that F1 surprisingly shows comparable OCR to W and F1+F2, but very different from F1L. F1 and F1L differ in that F1L is the result of subjecting F1 to SPE, removing sugars, organic acids and mineral compounds. The aim was obtaining a fraction free of the characteristic high acidy of F1, which allowed to evaluate the sensations in mouth generated by low molecular phenolic compounds (non-PAs) contained in F1. As result, the OCR has been reduced approximately three times respect to F1 (from 0.96 to 0.34 mg/L/day).

Table IV-3. 8. Register data of oxygen consumption. Results are expressed as mean of the four replicates in each case. Different lowercase letters in a column indicate a statistical difference ($p < 0.05$) between different samples.

	Average velocity of O_2 consumption mg/L/day	Total dissolution of O_2 mg/L	Oxygen consumed mg/L	Oxygen not consumed mg/L
W	1.071a	74.9	39.6a	35.3
F1+F2	0.997b	56.3	36.9b	19.4
F1	0.959b	56.3	35.5b	20.8
F13+F2	0.551c	53.1	20.4c	32.7
F2	0.498c	52.2	18.4c	33.8
F1L	0.339d	46.6	12.5d	34.1
F13	0.257e	45.5	9.5e	36.0
F11+F12	0f	42.8	0f	42.8

The reconstitution process of all the samples has required the use of a model wine (previously described), in which Cu and Fe were adjusted. However, cations such as Mn did not. It should be noted, that W, F1+F2, and F1 contain Mn originally present in the wine (Table IV-3.2), unlike the rest of samples, which could explain

their similarity in terms of OCR. This metal has been mentioned in several articles as an element involved in the oxidation process of wines (Cacho et al., 1995; Ferreira et al., 2015; Guo, Kontoudakis, Scollary, & Clark, 2017; Marrufo-Curtido et al., 2018).

Therefore, the lower oxygen consumption rate shown by the F1L, F11+F12, F13, F13+F2 and F2 samples compared to W, F1+F2 and F1 samples could be attributed to differences in the content of this element. Further analysis should be considered to understand the role of manganese in wine oxidation.

Among samples presenting lower oxygen consumption rates F13+F2, F2, F1L, F13 and F11+F12, those containing the proanthocyanic fraction of wine (i.e., F2 and F13+F2) show the highest OCRs. Differently, samples that contain phenolic compounds of low molecular weight show lower OCRs with significant differences among them. The fact that F2 and F13+F2 samples do not show significant differences (18.4 y 20.4 mg/L respectively) suggests that the presence of the fraction containing anthocyanins and derivate pigments (i.e., F13) does not significantly increase the O_2 consumption of the tannic fraction. This fraction in isolation consumed 9.5 mg/L of O_2, half of the O_2 consumed by the tannic fraction.

3.3. Effect of time on colour and phenolic composition of non-oxygenated samples

The oxygen effect has been studied considering the oxygenated and non-oxygenated samples (control samples kept in anoxic glove chamber). To find variables able to explain the effect of oxygen consumption, one-way ANOVA with condition oxygenate vs non-oxygenated as factor was carried out, for each of the samples. The analysis was carried out for both time periods (T6W and T24W).

On the other hand, the differences between the variable measured in the oxygenated sample and the variable measured in the non-oxygenated sample were calculated. Positive results show an increase with oxygen consumption while negative results indicate a decrease in the value of the variable with oxygen consumption. Further, the influence of sample in the processes (i.e., W and F1+F2), was analysed by ANOVA. When significant differences were revealed ($p < 0.05$), mean intensities were compared using the Tukey (HSD) multiple comparison test.

3.3.1. Case of W and F1+F2 samples

After 6 weeks (T6W). Results displayed in Table IV-3. 9, indicate that W and F1+F2 sample, have shown a very similar behaviour after 6 weeks, since the differences calculated do not present significant differences. On the other hand, as result of oxygen consumption, the spectrophotometric measurements revealed a decreased in colour intensity (CI), which is higher for F1+F2. This result, are in accordance with a general decrease in all variables related to pigments as MP, SPP and anthocyanins concentration. While the red coordinate (a*) decrease an increase on yellow coordinate (b*) is observed. Interestingly, anthocyanin derivatives, resistant to SO_2 and able to precipitate with protein (LPP) suffer an increase.

It is remarkable, that tannin activity, measured as thermodynamic interactions between tannins and a hydrophobic surface, showed an increased by effect of oxygen consumption. This increase in tannin activity, in oxidized samples, has also been described by Bueno-Aventín, Escudero, Fernández-Zurbano, & Ferreira (2021).

Table IV-3. 9. Difference (T6W) between the variables measured in the control sample (A1+A2) and the variable measured in the sample exposed to oxygen (O1+O2). Positives values indicate an increase due to oxygen effect while negative values mean a decrease. Chemical variables with significant differences between W and (F1+F2) are marked in bold (P<0.05).

(T6W)	W_O - W_A	(F1+F2_O) - (F1+F2_A)	Pr > F
Conventional oenological parameters[1]			
TPI	0,875 a	-4,900 a	0,37
CI	-0,951 a	-1,845 a	0,58
Anthocyanin-derived pigments[2]			
MP	-0,353 a	-0,425 a	0,27
SPP	-0,090 a	-0,168 a	0,41
LPP	0,100 a	0,050 a	0,39
a*	-12,148 a	-16,050 a	0,25
b*	10,645 a	7,278 a	0,13
Glycosilated	-41,025 a	-38,681 a	0,23
Acylated	-5,862 a	-5,162 a	0,50
Pyranoanthocyanins	-2,125 a	-2,033 a	0,69
Tannin characterization[3]			
Activity	797 a	1170a	0,37
mDP	**7,695 a**	**-1,120 b**	**0,01**
%PC	-0,200 a	-0,250 a	0,92
%G	**-1,580 b**	**0,045 a**	**0,02**
%PD	1,785 a	0,210 a	0,071

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2]MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*)**(a.u.), **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; **[3]TA**: Tannin activity (-J/mol), **mDp**: mean degree of polymerization, **%PC**: percentage of procianidins, **%PD**: percentage of prodelphinidins, **%G** percentage of galloylation;

The main difference in oxygen effect suffered by these samples, corresponding to values obtained for the mean degree of polymerization (mDp), which seems to increase in fraction W, nevertheless the reconstituted sample F1+F2 shows a decreased. Maybe it has involved in the decreased observed in Total Polyphenol Index (TPI), measured as concentration at 280 nm. Since these fractions are obtained by different ways, a further evaluation in needed to understand this behaviour.

After 24 weeks (T24W). Results displayed in Table IV-3. 10, indicate that after 24weeks, sample W and F1+F2, have shown a similar behaviour. Regarding to colour intensity (CI), it has been a widespread decreased, which is significatively higher in

F1+F2. In the same way, b* CIELAB coordinate shows an increment, as it was observed on T6W. The colour of a wine provides relevant information, thus, the change of wine colour from red to yellow hues (b* coordinate > O indicates yellow colour), is an index of aging and oxidative deterioration of red wine and it is due to the action of oxygen (Picariello et al., 2019).

It is noteworthy that the activity of tannins again showed an increase due to the effect of oxygen consumption.

Table IV-3. 10. Differences (T24W) between the variables measured in the control sample (A3+A4) and the variable measured in the sample exposed to oxygen (O3+O4). Positives values indicate an increase due to oxygen effect while negative values mean a decrease. Chemical variables with significant differences between W and (F1+F2) are marked in bold (P<0.05).

(T24W)	W_O - W_A	(F1+F2_O) - (F1+F2_A)	Pr > F
Conventional oenological parameters[1]			
IPT	-10,225 a	-12,925 a	0,147
IC	**-2,165 a**	**-3,555 b**	**0,015**
Anthocyanin-derived pigments[2]			
MP	-0,265 a	-0,335 a	0,421
SPP	-0,080 a	-0,140 a	0,051
LPP	-0,015 a	-0,105 a	0,245
a*	**-9,250 a**	**-14,425 b**	**0,003**
b*	**6,540 a**	**1,355 b**	**0,002**
Glycosilated	**-11,420 b**	**-7,560 a**	**0,004**
Acylated	-1,570 a	-1,115 a	0,074
Pyranoanthocyanins	-2,495 a	-2,995 a	0,106
Tannin characterization[3]			
Activity	449 a	382 a	0,799
mDP	-0,151 a	-0,014 a	0,492
%PC	0,266 a	0,155 a	0,943
%G	-0,023 a	0,357 a	0,178
%PD	-0,244 a	-0,513 a	0,853

[1]**TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); [2]**MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*)**(a.u.), **Glycosylated** (mg/L):): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; [3]**TA**: Tannin activity (-J/mol), **mDp**: mean degree of polymerization, **%PC**: percentage of procianidins, **%PD**: percentage of prodelphinidins, **%G** percentage of galloylation;

3.3.2. Case of F2 sample (tannin fraction)

After 6 weeks (T6W). Significant effects of oxygen consumption, on the variables studied were observed in Table IV-3. 11. Pigments compounds are mainly affected by the oxygen consumption. In spite of F2 contain high molecular weight compounds (PAs), it also contains small amounts of monomeric anthocyanins (glucosides), which showed a significant decrease after oxygen consumption well in line with Ćurko et al. (2021) and references, who describes a decrease of total and free anthocyanins (glucosides and acyl derivatives) during aging in barrels and bottles. However, the colour intensity (CI), has showed a significant increased, as well as, the red colour measured by the a* coordinate showed a slight increase, although it was not significative. A possible explanation of this colour increment is the observed increased on large polymeric pigments in oxygenated samples.

Regarding to CIElab coordinates, the increased of tawny tonality is shown by the increase of b* (yellow), and hue (angle), already observed in other works. Consequently, the colour variation between oxygenated and non-oxygenated samples is higher than three CIElab units (ΔE > 3; 15.6), showing a difference detectable to the human eye. Visual differences were observed between samples.

It is noteworthy, that unlike what happened after T24W (Figure IV-3. 9b) and in other fractions (Figure IV-3. 9a), fraction F2 after oxygen consumption and six weeks in anoxia suffers a significant decreased in tannin activity as well as a decreased in tannin concentration measured as the interaction between tannins and a hydrophobic surface at 280nm at 30 °C (Table IV-3. 11).

Regarding to percentage of prodelphinidins (%PD), a decrease is observed, which is reflected in an increase in the percentage of procyanidins (%PC)

Table IV-3. 11. Chemical variables with significant differences are marked in bold (P<0.05).

	F2_A	F2_O	Pr > F	ΔT6W
	T6W	T6W		O_{T6W}-A_{T6W}
Conventional oenological parameters[1]				
TPI	18,575 a	23,663 a	0,217	5.09
CI	**1,395 b**	**3,123 a**	**0,044***	1.73
Anthocyanin-derived pigments[2]				
LPP	**0,065 b**	**0,438 a**	**0,022***	0.37
a*	8,370 a	9,723 a	0,307	1.35
b*	**2,830 b**	**16,953 a**	**0,008***	14.12
L*	91,325 a	84,850 a	0,062	-6.48
C*	**8,835 b**	**19,543 a**	**0,023***	10.71
h*	**18,688 b**	**60,205 a**	**0,000***	41.52
Glycosilated	**2,987 a**	**0,182 b**	**0,001***	-2.81
Tannin characterization[3]				
Activity	**9652 a**	**3397b**	**0,006***	-6255
conc 280nm	**1166 a**	**789 b**	**0,001***	-377
mDP	9,775 a	11,490 a	0,074	1.72
%PC	**79,780 b**	**93,560 a**	**< 0,0001***	13.78
%G	2,440 b	2,890 a	0,038	0.45
%PD	**17,785 a**	**3,550 b**	**< 0,0001***	-14.24

[1]**TPI**: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); [2]**LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)** (a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, [3]**TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L), **mDp**: mean degree of polymerization, **%PC**: percentage of procyanidins, %**PD**: percentage of prodelphinidins, **%G** percentage of galloylation.

After 24 weeks (T24W). In the same way that it happened only after 6 weeks, CI has shown a significant increased, as well as has shown an increase of b* CIElab coordinate (yellow) and LPP (Table IV-3. 12). According to Harbertson et al. (2003) large polymeric pigments, are recognized as the stable form of colour in red wines. LPP, are formed for anthocyanins that have reacted with polymeric flavan-3-ols extracted from skins and seeds or that have formed by acetaldehyde cross-linking and from direct anthocyanin-tannin reaction to give large polymeric pigments (Dallas, Ricardo-da-Silva, & Laureano, 1996; Remy et al., 2000; Erika Salas, Fulcrand, Meudec, & Cheynier, 2003; Erika Salas, Guernevé, Fulcrand, Poncet-Legrand, & Cheynier, 2004).

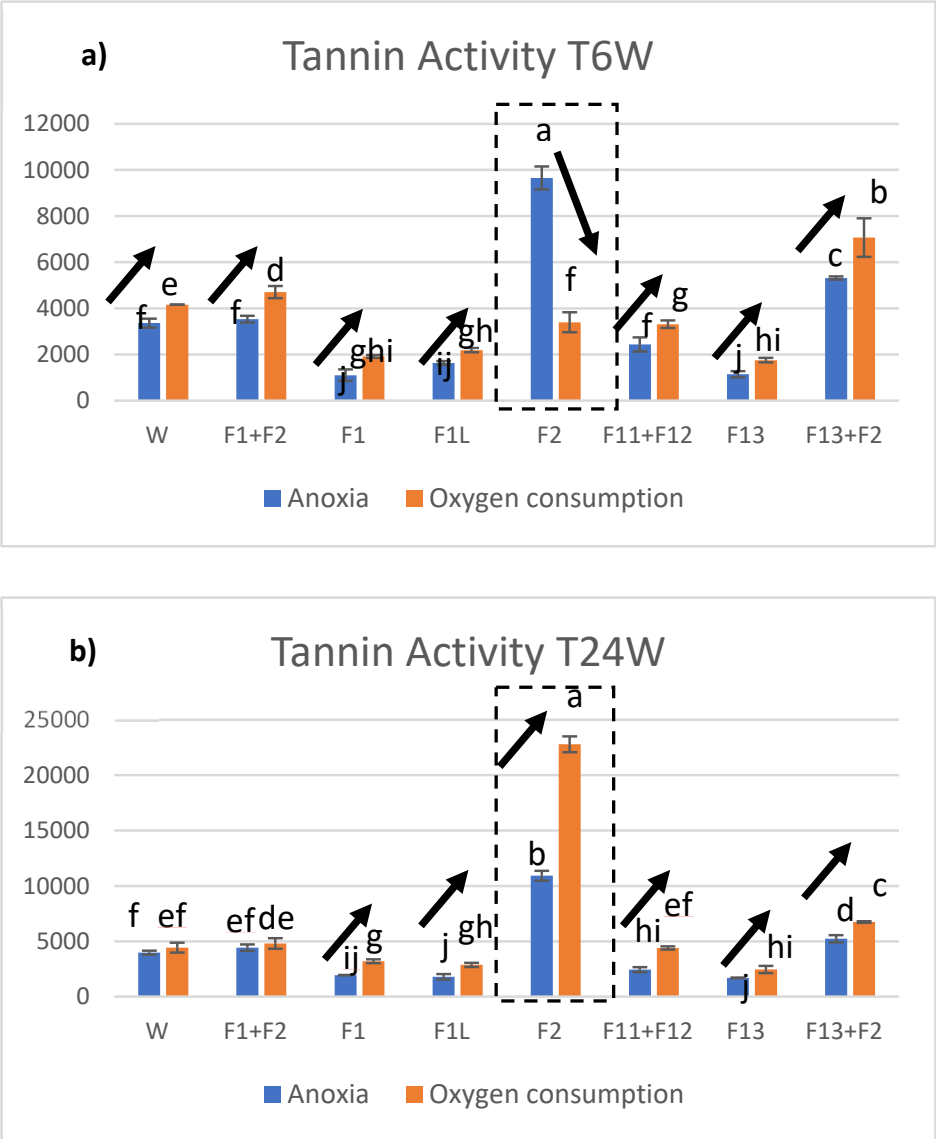

Figure IV-3. 9. Mean value of tannin activity, Test-ANOVA calculated to evaluate the effect of condition and fraction. a) after 6 weeks, b) after 4 months. Different letters indicate significant differences according to post-hoc Fisher test (P<0.05).

The colour variation between samples oxygenated and non-oxygenated is higher than three CIElab units (ΔE = 16.5), thus, visual differences were observed between samples.

As mentioned before, sample F2 increase in tannin activity after 24 weeks kept in anoxia conditions after being subjected to oxygen conditions. A possible explanation would be that the reactions that take place by the consumed oxygen are

slow. While this increase could not be explained by the measured chemical variables, further studies would be necessary.

Regarding to the percentage of prodelphinidins (%PD), it experiences a decrease reflected in an increase in the percentage of procyanidins (%PC). No changes in the mean degree of polymerization (mDP) have been observed.

Table IV-3. 12. Chemical variables with significant differences are marked in bold (P<0.05).

	F2_A	F2_O	Pr > F	ΔT24W
	T24W	T24W		O_{T24W}-A_{T24W}
Conventional oenological parameters[1]				
IPT	**17,915 b**	**20,455 a**	**0,026***	2.54
IC	**1,360 b**	**2,855 a**	**0,005***	1.495
Anthocyanin-derived pigments[2]				
LPP	**0,115 b**	**0,585 a**	**0,002***	0.47
a*	8,270 a	9,180 a	0,088	0.910
b*	**2,925 b**	**18,830 a**	**0,000***	15.905
L*	**91,550 a**	**86,575 b**	**0,011***	-4.975
C*	**8,770 b**	**20,945 a**	**0,001***	12.175
h*	**19,490 b**	**64,025 a**	**0,000***	44.535
Glycosilated	**1,635 a**	**0,000 b**	**0,010***	-1.635
Tannin characterization[3]				
Activity	**10914 b**	**22784 a**	**0,003***	11870
conc 280nm	**1249 a**	**1018 b**	**0,016***	-231
mDP	4,657 a	3,750 a	0,109	-0.907
%PC	**58,027 b**	**87,927 a**	**0,000***	29.9
%G	2,782 a	4,280 a	0,051	1.498
%PD	**39,191 a**	**7,793 b**	**< 0,0001***	-31.398

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2]LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)** (a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **[3]TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L), **mDp**: mean degree of polymerization, **%PC**: percentage of procyanidins, %**PD**: percentage of prodelphinidins, **%G** percentage of galloylation.

3.3.3. Case of F1 and F1L samples

The effect of oxygen consumption, with respect to CIElab coordinates, for both F1 and F1L, undergo the increase of b* (yellow) and decrease of a*(red) coordinates in both times, Table IV-3.13 and Table IV-3.14. Consequently, the colour variation between samples before and after oxygen consumption is higher than three CIElab units being ΔE=15.9 for F1 and ΔE=19.8 for F1L showing a difference detectable to the human eye after six weeks (T6W). Likewise, ΔE=12.4 for F1 and ΔE=17.7 for F1L after four months (T24W). F1L, experiences higher colour variation, so it can be inferred that it is less stable.

After 6 weeks (T6W). The colour intensity (CI), has shown a decreased (Table IV-3. 13), in both cases being significatively higher for FIL.

MP and SPP, experience a decreased for both (F1, F1L), while LPP undergo an increment. Anthocyanidins measured as free compounds has experienced a decreased.

As it has been shown in Table IV-3. 13, tannin activity, showed an increased by effect of oxygen consumption both F1 and F1L. This increase in tannin activity, in oxidized samples, has also been mentioned above.

After 24 weeks (T24W). As it can be seen in Table IV-3. 14, the changes introduced by the oxygen consumption are maintained after 4 months of storage in anoxia, for both samples. It is noteworthy, that in both samples there is a decrease in anthocyanins and pyranoanthocyanins, with the decrease in glucosides and acyl anthocyanins being significantly greater in F1L.

Tannin activity, showed an increased higher after four months than after six weeks, notwithstanding, no significative differences has been found between the behaviour of both samples.

Table IV-3. 13. Chemical variables with significant differences are marked in bold (P<0.05).

T6W	F1_O – F1_A	F1L_O – F1L_A	Pr > F
Conventional oenological parameters[1]			
IPT	-0.450 a	-4.875 a	0.398
IC	-0.446 a	-2.304 b	**0.005***
Anthocyanin-derived pigments[2]			
MP	-0.248 a	-0.190 a	0.511
SPP	-0.038 a	-0.055 a	0.477
LPP	0.120 a	0.010 a	0.094
a*	-10.550 a	-12.760 a	0.069
b*	11.225 a	10.438 b	**0.034***
L*	3.975 b	11.025 a	**0.011***
C*	-5.978 a	-9.653 b	**0.027***
H*	16.988 a	19.568 a	0.116
Glycosilated	-40.805 a	-44.071 a	0.077
Acylated	-5.588 a	-9.421 b	**0.003***
Pyranoanthocyanins	-2.125 a	-3.156 a	0.101
Tannin characterization[3]			
Activity	810 a	556 a	0.196
Conc-280nm	-25 a	-196 a	0.311

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2] MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)**(a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; **[3]TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L).

3.3.4. Case of F13 sample

F13 was obtained, as result of F1 submitted to solid-phase extraction. As shown in Table IV-3. 7, fraction F1.3 retain chiefly more than 85% of CI and more than 65% of phenolic compounds. As result of oxygen exposition, F13, together with F11+F12 present the lowest oxygen consumption rates. Notwithstanding, significant oxygen effects on the variables studied have been observed.

The effect of oxygen consumption, with respect to CIElab coordinates, supposed an increase of b* (yellow) and decrease of a*(red) coordinates in both times, Table IV-3.15 and Table IV-3.16. The colour variation between oxygenated and non-oxygenated samples is higher than three CIElab units being ΔE=17.1 after six weeks

and ΔE=16.6 after four months, indicating that those colour differences can be visually discriminated.

Table IV-3. 14. Chemical variables with significant differences are marked in bold (P<0.05).

T24W	F1_O – F1_A	F1L_O – F1L_A	Pr > F
Conventional oenological parameters[1]			
IPT	-2.963 a	-5.013 a	0.280
IC	-1.671 a	-1.868 a	0.300
Anthocyanin-derived pigments[2]			
MP	-0.218 a	-0.145 a	0.285
SPP	-0.113 a	-0.103 a	0.515
LPP	0.033 a	0.025 a	0.609
a*	-9.190 a	-12.080 b	**0.032***
b*	3.995 b	8.525 a	**0.000***
L*	7.275 b	9.800 a	**0.045***
C*	-6.657 a	-8.945 a	0.058
H*	10.063 b	19.463 a	**0.000***
Glycosilated	-9.933 a	-28.354 b	**0.001***
Acylated	-1.024 a	-4.666 b	**0.005***
Pyranoanthocyanins	-2.719 b	-2.156 a	**0.003***
Tannin characterization[3]			
Activity	1247 a	1067 a	0.640
Conc-280nm	-150 a	-229 a	0.607

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2] MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)**(a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; **[3]TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L).

After 6 weeks (T6W). It is remarkable, that tannin activity showed an increase. Furthermore, as it is indicated by Barak & Kennedy (2013) this parameter is concentration independent, how it can be seen in Table IV-3. 15, where phenolic compounds (measured as absorbance at 280nm: TPI) and tannin concentration measured as the interaction between tannins and a hydrophobic surface at 280nm at 30 °C, show a significative decrease.

Pyranoanthocyanins show a significant decrease, as it has been seen in W and F1+F2 samples (Table IV-3. 15 and Table IV-3. 16) with respect of the control samples (non-oxygenated). Although, a drop in pyranoanthocyanins during ageing, has been

observed, and, it is true, that previous works shown a downward trend on anthocyanin-pyruvic derivatives along the time, however its oxygenated wines showed higher levels than their respective control (nonmicro-oxygenated) wines (Cano-López, Pardo-Minguez, López-Roca, & Gómez-Plaza, 2007; Ćurko et al., 2021; Sánchez-Iglesias, González-Sanjosé, Pérez-Magariño, Ortega-Heras, & González-Huerta, 2009)

Table IV-3. 15. Chemical parameters for F13 after 6 weeks. Chemical variables with significant differences are marked in bold* (P<0.05)

T6W	F13 A	F13 O	Pr > F	ΔT6W
	T6W	T6W		O_{T6W}-A_{T6W}
Conventional oenological parameters[1]				
TPI	19.263 a	16.100 b	**0.001**	-3.163
IC	7.209 a	6.080 b	**0.007**	-1.129
Anthocyanin-derived pigments[2]				
MP	0.603 a	0.470 a	0.061	-0.133
SPP	0.240 a	0.185 a	0.257	-0.055
LPP	0.100 a	0.118 a	0.804	0.018
a*	39.175 a	28.433 b	**<0.0001**	-10.742
b*	0.515 b	11.818 a	**<0.0001**	11.303
L*	61.500 b	68.500 a	**0.001**	7.000
C*	39.180 a	30.795 b	**<0.0001**	-8.385
H*	0.763 b	22.583 a	**<0.0001**	21.820
Glycosilated	64.31 a	15.23 b	**<0.0001**	-49.08
Acylated	11.61 a	1.97 b	**<0.0001**	-9.64
Pyranoanthocyanins	5.59 a	3.10 b	**<0.0001**	-2.49
Tannin characterization[3]				
Activity	1143 b	1757 a	**0.000**	614
Conc-280nm	1093 a	994 b	**<0.0001**	-99

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2] MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)**(a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; **[3]TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L).

A decrease of total and free anthocyanins (glucosides and acylderivatives) by oxygen consumption effect has been observed. These changes can be due to the formation of anthocyanin-derived pigments, which at T24W, shows a significant increase. These changes are responsible for changes in colour from purple-red in

young wine toward redder–orange hues in aged ones. Although a decrease of CI is showed, an increase in yellow colour (from 32% to 40%) and a decrease in the contribution of red colour (from 55% to 48%) is produced.

After 24 weeks (T24W). As it can be seen in Table IV-3.16, the changes introduced by the oxygen consumption are maintained after 4 months of storage in anoxia with respect to 6 weeks. It is noteworthy, that in T24W, the decrease on MP and SPP, become significative as well as the increase on LPP, which means that the time it is an important factor.

Table IV-3. 16. Chemical parameters for F13 after 4 months. Chemical variables with significant differences are marked in bold* (P<0.05)

T24W	F13 A	F13 O	Pr > F	ΔT24W
	T24W	T24W		O_{T24W}-A_{T24W}
Conventional oenological parameters[1]				
IPT	17.95 a	14.25 b	**<0.0001**	-3.7
IC	6.27 a	5.04 b	**<0.0001**	-1.23
Anthocyanin-derived pigments[2]				
MP	0.508 a	0.358 b	**<0.0001**	-0.15
SPP	0.278 a	0.175 b	**0.000**	-0.103
LPP	0.135 b	0.185 a	**<0.0001**	0.05
a*	35.63 a	24.76 b	**<0.0001**	-10.87
b*	1.82 b	11.81 a	**<0.0001**	9.99
L*	65.55 b	73.20 a	**<0.0001**	7.65
C*	35.68 a	27.43 b	**<0.0001**	-8.25
H*	2.92 b	25.50 a	**<0.0001**	22.58
Glycosilated	36.85 a	4.66 b	**<0.0001**	-32.19
Acylated	4.58 a	0.61 b	**<0.0001**	-3.97
Pyranoanthocyanins	3.59 a	1.46 b	**<0.0001**	-2.13
Tannin characterization[3]				
Activity	1689 b	2452 a	**0.002**	763
Conc-280nm	1074 a	922 b	**<0.0001**	-152

[1]TPI: Total polyphenol index (a.u.), **IC**: Colour intensity (a.u.); **[2] MP**: Monomeric pigments (a.u.), **SPP**: Small polymeric pigments (a.u.), **LPP**: Large polymeric pigments (a.u.), **CIELAB coordinates (a*, b*, L*)**(a.u.), **Glycosylated** (mg/L): Delphinidin 3-glucoside, Cyanidin 3-glucoside, Petunidin 3-glucoside, Peonidin 3-glucoside, Pelargonidin 3-glucoside, Malvidin 3-glucoside, **Acylated** (mg/L): Delphinidin 3-(6"-acetyl)-glucoside, Cyanidin 3-(6"-acetyl)-glucoside, Petunidin 3-(6"-acetyl)-glucoside, Peonidin 3-(6"-acetyl)-glucoside, Malvidin 3-(6"-acetyl)-glucoside, Delphinidin 3-(6"-p-coumaroyl)-glucoside, Cyanidin 3-(6"-p-coumaroyl)-glucoside, Petunidin 3-(6"-p-coumaroyl)-glucoside, Peonidin 3-(6"-p-coumaroyl)-glucoside, Malvidin 3-(6"-p-coumaroyl)-glucoside, **Pyranoanthocyanins** (mg/L): Vitisine A, Carboxypyrano Delfinidine 3-glucoside, Carboxypyrano petunidine 3-glucoside, Vitisina A-3(6"Acetyl)-glucosido, Malvidine-3-glucoside-4-vinilfenol; **[3]TA**: Tannin activity (-J/mol), **conc-280nm** (mg/L).

In the same way that happened at T6W a decrease of CI, an increase in yellow colour (from 33% to 40%) and a decrease in the contribution of red colour (from 54% to 48%) is produced, according with CIELab coordinates. The contribution of blue colour presents a slightly decrease from 13% to 12%.

3.4. Effect of oxygen in sensory properties

Discriminant attributes between oxygenated and non-oxygenated samples, at the two time periods (six weeks-T6W and 24 weeks-T24W), were found by two-way ANOVA analysis, with the intensity of sensory attributes, considering panellists as random and oxygen supply (oxygenated vs non-oxygenated samples) as fixed factor. The data treatment was carried out individually for each sample. A general assertion regarding the oxygen effect on sensory properties of the studied samples could not be done, because there are high significant differences induced by fraction composition. Results can be seen in Annexe IV-3.3.

3.4.1. Sensory analysis at T6W

W and F1+F2 Samples are mainly characterized by the attributes: "sour", "dry", "dry on palate", "persistent", “dry on tongue" and to a lesser extent by the attributes, "bitter", "coarse", “sandy” and "dusty" (Annexe IV-3.3a and Annexe IV-3.3b).

Regarding to W samples, the effect of oxygen consumption, showed a significant effect for “sweet” ($F=7.268$; $P<0.05$) and “hot” ($F=4.813$; $P<0.05$) as well as “sticky” ($F=3.019$; $P<0.1$) when relaxing the criteria for significance. However, only one out of the twenty-three terms rated was significantly different among “F1+F2” fractions, “silky” ($F=3.190$; $P<0.1$), if relaxing the criteria for significance. These differences between “W” and “F1+F2” maybe are due to the slight difference in fractions compositions, being that minimal.

F1L, is mainly characterized by the attributes: "bitter", "dry” and to a lesser extent by the attributes, "dry on tongue", "dry on palate", “watery” and "persistent" (Annexe IV-3.3c). F1 was not considered for sensory analysis due its high content on acids compounds, which hinder its evaluation. Regarding to FIL, it should be considered that presents one of the lowest oxygen consumption rates and it does not suffer significative sensory changes in attributes analysed.

The most salient changes due to oxygen effect (i.e., comparing non-oxygenated vs oxygenated samples) were observed for the tannic F2 sample (with differences higher than 1 point, in a 7 points scale). The highest scores for F2 sample, among the studied terms, were reached for “dry”, “dry on tongue” and “dry on palate” as well as “persistent” (Annexe IV-3.3d). This fraction experienced a decrease in oxygenated samples of the terms “dry” ($F=3.257$; $P<0.1$), “dry on tongue” ($F=8.42$; $P<0.01$) and “coarse” ($F=3.412$; $P<0.1$), while an increase in “sour” ($F=6.040$; $P<0.05$). This last effect could be attributed to a cross-modal interaction between dry and sour percepts between dry and sour percepts, it has already been observed in previous chapters and in the literature (de-la-Fuente-Blanco et al., 2017). Curiously, the most significant sensory changes in the mouth occur for F2 samples evaluated after 6 weeks of oxygen consumption (Annexe IV-3.3d). These sensory differences between non-oxygenated and oxygenated samples seem to disappear when both samples are evaluated after 24 weeks of anoxic ageing (Annexe IV-3.4d). These results show that in the present study, the time effect minimises the sensory differences introduced by the oxygen in the samples.

With regard to F11+F12 sample, this fraction, is mainly characterized by the attributes with highest scores, like: "bitter", "sweet”, “unctuous” and “watery” (Annexe IV-3.4e). It showed similar oxygen consumption rates as model wine control sample; thus, it is not surprising that oxygen consumption effects were not found.

Sample F13, is mainly characterized by the attributes "bitter", and “watery”, attributes (Annexe IV-3.3f). It belongs to the group of samples with lowest oxygen consumption rates, and oxygen consumption effects were not significative in sensory attributes.

The F13+F2 sample, is mainly characterized by the attributes for “dry”, “dry on tongue” and “dry on palate” as well as “persistent” and “bitter” (Annexe IV-3.3g). It is noteworthy, that, among samples presenting lower oxygen consumption rates (OCR), those containing the proanthocyanic fraction of wine, F2 and F13+F2, have

shown the highest OCR. In this context, higher changes in sensory properties could be expected. While the sample F2, experienced significative oxygen effect for four out the twenty-three terms rated, only one resulted significative for F13+F2 sample, “bitter” with a significant decrease from 3.1 to 1.8 points of intensity ($F=8.389$; $P<0.01$). It can be inferred that the addiction of F13 to F2, introduce sensory changes.

3.4.2. Sensory analysis at T24W

It was hypothesised that time is needed to consume oxygen and react, thus major changes were expected. After 24 weeks of reductive ageing, W sample experience a significant decrease on “coarse” ($F=8.474$; $P<0.01$) attribute due to oxygen consumption effect (Annexe IV-3.4a). Interestingly results show a significative increase on “coarse” ($F=8.474$; $P<0.01$) term for F1+F2 sample (Annexe IV-3.4b). Chemical analysis showed a decreased in Total Polyphenol Index (TPI) measured as concentration at 280 nm and in colour intensity (CI) for both samples, which could explain the decrease on “coarse” in W, however, the increase observed for “coarse” in the case of F1+F2 could not been explained.

Regarding to F1L, one out of the twenty-three terms rated were significantly different among samples (oxygenated vs non-oxygenated, at T24W), “oily” ($F=4.558$; $P<0.05$) and “bitter” ($F=3.793$; $P<0.1$), when relaxing the significance criteria (Annexe IV-3.4c). The significative increment observed for “oily” term can not be directly explained by chemical results, where it has been shown a high colour difference between samples oxygenated and non-oxygenated, showing a difference detectable to the human eye $\Delta E=17.7$ (T24W) and decrease in anthocyanidins measured as free compounds. Notwithstanding, accordingly with Ferrer-Gallego et al. (2015), anthocyanins were able to interact with saliva proteins, forming soluble aggregates, which could contribute to astringency perception, which is in accordance with observed results, since, a decrease on free anthocyanidins was found. Paissoni et al. 2018, described as astringency and bitter all the anthocyanins tasted. Furthermore,

a non significative decrease has been observed for the terms related to dryness and persistency, as well as a non significative increase for “watery”, “unctuous” and “silky” terms (Annexe IV-3.4c). In accordance, the significative decrease on “bitter” attribute could be induce by the loss of free anthocyanidins, related with this term.

As previously said, the most significant sensory changes in the mouth occur for F2 samples (Annexe IV-3.4d). The results show that two out of the twenty-three terms rated were significant differing among samples, “burning” ($F=8.525$; $P<0.01$) and “sandy” ($F=4.159$; $P<0.05$). As well as “fleshy”, “unctuous” and “coarse”, when relaxing the significance criteria. Results show, that, after 24 weeks oxygen consumption has induced a significative decrease on the scores for “sandy” and “coarse”. It is noteworthy, that “burning” experienced a significative increase, what can be attributed to an indirect effect, due to the loss of “dryness”, similarly as it was observed after 6 weeks for “sour” term. In accordance, “unctuous” has experienced an increase.

With regard to the anthocyanic fraction (F13) (Annexe IV-3.4f), did not show significant differences between oxygenated vs non-oxygenated samples after 24 weeks of reductive ageing. It should be noted, that the significant increase on tannin activity, has not induced sensory changes, thus, major differences are required to induce sensory changes.

While F2, experienced significative oxygen effect for two out the twenty-three terms rated, one resulted significative for F13+F2 sample, “sweet” ($F=4.360$; $P<0.05$). As above, it can be inferred that the addiction of F13 to F2, introduce sensory changes (Annexe IV-3.4g).

4. Conclusions

One of the main findings regarding to sample characterisation, refers to tannin activity. Results suggest that there is a modulator effect of the compounds constituting F1 and F13 in terms of tannin activity of sample F2 (tannic fraction), which induce a decrease on the tannin activity.

According to the results obtained from these samples, the oxygen consumed rate depends extensively on the phenolic composition of the samples and it is suggested on the presence or absence of Mn. The presence of this metal resulted in higher oxygen consumption, which should be evaluated in subsequent works.

With regard to the effect of oxygen consumption on the chemical features the colour variation between oxygenated and non-oxygenated samples resulted in a significant colour change according to the CIELab space ($\Delta E > 3$).

It is outstanding that the effect of oxygen consumption induced a significant increase of the tannin activity, as has been suggested recently by other authors. An exception was observed for fraction F2 after 6 weeks for which the sample in contact with oxygen experienced a significant decrease. Such an unexpected result could not be explained with the data available, thus, further studies are required.

Regarding the oxygen effect on sensory properties, a general assertion could not be done, due to the high significant differences induced by fraction composition. The most salient changes due to oxygen effect were observed for the tannic F2 sample, which experienced a decrease of the terms “dry”, “dry on tongue” and “dry on palate”, while an increase in “sour”. This last effect could be attributed to a cross-modal interaction between dry and sour percepts already observed in previous chapters and in the literature

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GENERAL CONCLUSIONS/ CONCLUSIONES GENERALES

GENERAL CONCLUSIONS

Due to the lack of knowledge about the formation of mouthfeel properties in red wines and the compounds and parameters involved in it, the development of this Thesis arises to shed light on the unknown world of this percept. To progress in the finding of single chemical compounds or group of compounds responsible for such properties, different sensory approaches in combination with chemical strategies have been developed and applied to wines and grapes. The results provide useful information for the wine industry, since it has been widely recognised that mouthfeel properties play an important role in quality perception and thus consumer´s acceptability. In this context, the results are of major interest for the wine sector, and can be employed in new product development and quality control strategies among others, which ultimately will add value to the product.

Previous to this work the lack of adequate lexicon and references made it difficult to describe and identify differences among wines in terms of mouthfeel properties. One of the main advances of this Doctoral Thesis is the development and validation of a sensory method which has been proven to be successful in describing and identifying taste and mouthfeel differences among wines, fractions and grapes. This method is based on an alternative verbal-based sensory approach (RATA) using a list of 23 terms related to in-mouth properties (18 to mouthfeel). This list of terms was generated following different sensory strategies and using simple and odourless fractions. These simple fractions were obtained by a semipreparative method followed by SPE, which was able to isolate groups of non-volatile compounds eliciting different in-mouth properties. The use of fractions definitely simplifies the study of wine sensometabolome. In this first study it was observed that fractions containing oligomers of flavanols (from tetramers up to decamers) resulted mainly “coarse”, “grainy”, “dry on the tongue”, and “dry on the palate”. Interestingly, the fraction containing anthocyanin-derivative pigments, resulted especially “dry”, “bitte”r and “persistent” as was the original wine. A series of oligomeric anthocyanins (trimers

glycosylated and/or acylated) was tentatively suggested to be involved in the feeling of dryness perceived in anthocyanic fraction.

The identification of independent and non-correlated different mouthfeel dimensions certainly sheds light on the understanding of mouthfeel, since it has further allowed to relate the different sensory dimensions perceived with the analysed chemical composition. Interestingly, taste and mouthfeel dimensions could be successfully predicted from chemical composition by the generation of very satisfactory and validated mathematical PLS-models.

This sensory methodology was applied to a wide range of red wines (44 wine samples). Four independent and non-correlated mouthfeel dimensions including “dry/silky” and “sticky”, “grainy”, “prickly” and “oily” were identified. Concerning the results from partial least squares regression, tannin activity was shown to be a good predictor of wine dryness on the palate, and tannin concentration of both “dry” (in general) and “dry on the palate” terms. However, the results provided evidence that the other independent mouthfeel dimensions identified could not be linked to the other chemical variables studied, which highlights the need for quantifying other sensory-active chemical variables. To this regard, the possible chemical compounds involved in the other mouthfeel dimensions could include polysaccharides or other families of polyphenols aside from tannins.

To further study the mouthfeel properties of isolated fractions, forty fractions containing tannins or anthocyanins were chemically and sensory analysed in terms of taste and mouthfeel properties. Bitterness and dryness were the salient attributes differing among the sensory spaces of both families of wine polyphenols. The results confirmed the sensory activity of the studied anthocyanic fractions, which unequivocally demonstrated a relevant implication of certain anthocyanins in dryness. It is noteworthy that, the addition of an extremely bitter anthocyanic fraction to wines only increased bitterness in certain wines, suggesting that

bitterness in wines may result from perceptual interactions and that some wines contain strong bitterness suppressors.

Moreover, the application of chemosensory strategies to grape fractions has shown to provide an interesting tool to assess grape quality. Therefore, the main sensory-active compounds of grapes were extracted and further sensory characterised. This approach overcomes the main limitations that classical berry assessment presents (reduced number of panellists involved in sensory evaluation and lack of grape representativeness). Three distinct, independent, and non-correlated sensory dimensions could be identified for the overall sample set: 1) “dry on tongue/watery”, 2) “sticky/bitter” and 3) “dry/coarse”. Tannin activity and tannin concentration along with mDP of tannins proved to be good predictors of the perceived dryness. Flavonols had a good prediction power for the “bitter” attribute and the “sticky/bitter” dimension. In addition, low molecular weight anthocyanins seem to be involved in the formation of the “dry” attribute, whereas large polymeric pigments in the “sticky” attribute and the “sticky/bitter” dimension. Distinctly, the “coarse” dimension could not be modelled which suggests that there are other (macro)molecules involve in the formation of this percept.

Besides PLS regression, the present work has effectively applied non-linear models derived from machine learning approaches to predict wine astringency from its chemical composition. The main variables of the astringency model were the % of procyanidins constituting tannins and ethanol content, followed by other eight variables related to tannin structure and acidity.

Further, different oenological concepts including "green character", maturity and oxidative aging have been studied in the present work applying different chemo-sensory approaches.

The "green character" was confirmed to be a multidimensional term associated to both aroma and mouthfeel descriptors such as vegetal, astringency, green and dry tannins according to experts of the Somontano region, which was able to induce a

decrease in the acceptability of the product by consumers. Besides, two non-volatile fractions imparting astringency-related sensations (dryness and stickiness) were suggested to be responsible for the high "green character" of their respective original wines. These two fractions were a fraction containing tannins with mean degree of polymerisation of ten and eliciting dryness, and a second fraction with small anthocyanin-derivative pigments (< tetramers) described as sticky. Although no specific aroma compounds were identified, green wines contained significantly higher levels of fusel alcohols. The interaction between isoamyl alcohol and the anthocyanin-derivative fraction and/or tannins is suggested to contribute to the "green character" and to enhance it in red wines. Besides, "green character" was demonstrated to be wine-dependent and it was suggested to be masked by woody, oxidation and/or ripe fruit aromas present in oaked aged red wines.

As far as the maturity of the grape is concerned, grape ripeness induced significant sensory effects on wine astringency and fruity aromas including "raisin", "black fruit" and "red fruit". It is worth noting that wines elaborated with grapes prematurely harvested presented higher oxidation aromas. This attribute was related to higher concentrations of free acetaldehyde, methional, phenylacetaldehyde and isoaldehydes as well as low levels of certain polyphenols capable of reacting with the aforementioned aldehydes, called in previous works ARPs (aldehyde-reactive polyphenols). Astringency was related to ethanol content, tannin activity (measured as the interaction of tannins with a hydrophobic surface) and the content in anthocyanin -derivative compounds.

Concerning oxidative ageing, it is outstanding that the effect of oxygen consumption induced a significant increase of the tannin activity, which was measured as the variation in the enthalpy of interaction between tannin and a hydrophobic surface. Furthermore, phenolic fractions of wines containing manganese presented a significantly higher oxygen consumption. Interestingly, this metal has been mentioned in several works as an element involved in the oxidation process of wines.

Taking a step forward, the use of untargeted analysis is presented as a valuable tool to overcome the limitations of classical directed instrumental techniques, which do not collect enough information, since only compounds with known sensory activity or present in high concentrations (i.e., those that are easier to analyse) are analysed. This classical approach does not consider the importance of unknown metabolites, as well as those present in low concentrations, which can certainly play a major role in the formation of mouthfeel properties. The results of this study allowed to obtain very satisfactory PLS models predicting sensory variables from chemical features. Remarkable are the results obtained for the sulphonated flavanols derivatives, from which it is inferred that they are involved in the reduction of the tactile sensation of dryness caused by the wine. Similarly, the results suggest that amino acids or peptides are possible candidates involved in modulating dryness and oily perception in wines. In addition, it was possible to confirm the sensory role of anthocyanidins and anthocyanidin derivatives in the perception of taste and mouthfeel properties of red wines. Reconstitution studies to confirm the role of different compounds and their structures in mouthfeel and taste properties are still required.

In the present work the strategies employed following targeted sensory methods and targeted and untargeted chemical methods have shown very satisfactory results. Notwithstanding, it has to be recognised that they still have certain limitations. Thus, the difficulty of analysing polymerised polyphenolic compounds (>3 units) including proanthocyanidins and polymeric pigments by HPLC methods is well recognised. In this context, the development of new analytical tools is a key factor for success in understanding taste and tactile properties driven by sensory-active compounds. The determination of the molecular structure of the compounds involved in taste and mouthfeel properties is fundamental to be able to fully understand their role. In this context, techniques such as voltammetry and spectrofluorometry could increase the variety of sensory attributes satisfactorily modelled.

CONCLUSIONES GENERALES

Debido a la falta de conocimiento sobre la formación de las sensaciones táctiles en la boca generadas por los vinos tintos y los compuestos y parámetros que intervienen en ellas, el desarrollo de esta Tesis surge para arrojar luz sobre el desconocido mundo de esta percepción. Para avanzar en la búsqueda de compuestos químicos individuales o grupos de compuestos responsables de tales propiedades, se han desarrollado y aplicado a vinos y uvas diferentes estrategias sensoriales en combinación con estrategias químicas. Los resultados proporcionan información útil para la industria del vino, puesto que, la percepción de las sensaciones táctiles en boca juega un papel importante en la percepción de la calidad, especialmente en situaciones de recompra, y, por lo tanto, en la aceptabilidad del producto por parte del consumidor. En este contexto, los resultados son de gran interés para el sector vitivinícola, y pueden emplearse en el desarrollo de nuevos productos y estrategias de control de calidad, y así añadir valor a los mismos.

Antes del desarrollo de este trabajo, la falta de léxico y referencias adecuadas dificultaba la descripción e identificación de diferencias entre vinos en términos de propiedades táctiles en boca. Uno de los principales avances que ha permitido esta Tesis Doctoral es el desarrollo y validación de un método sensorial que ha demostrado ser útil en la descripción e identificación de diferencias de gusto y sensaciones táctiles en boca entre vinos, fracciones y uvas. Este método se basa en una estrategia sensorial alternativa de base verbal (RATA) empleando una lista de 23 términos relacionados con las propiedades en la boca (18 relacionadas con sensaciones táctiles). Esta lista de términos se generó siguiendo diferentes estrategias sensoriales y utilizando fracciones simples aisladas e inodoras con propiedades de gusto y táctiles consistentes. Estas fracciones simples se obtuvieron mediante un método semipreparativo seguido de SPE, que pudo aislar grupos de compuestos no volátiles que provocaban diferentes propiedades en la boca. El uso

de fracciones definitivamente simplifica el estudio del sensometaboloma del vino. En este primer estudio se observó que las fracciones que contenían oligómeros de flavanoles (desde tetrámeros hasta decámeros) resultaron principalmente descritas por los atributos “rugoso”, “granuloso”, “secante en la lengua” y “secante en el paladar”. Curiosamente, la fracción que contenía pigmentos derivados de antocianos resultó especialmente “seca”, “amarga” y “persistente” como era el vino original. Tentativamente fueron sugeridos una serie de antocianos oligoméricas (trímeros glicosilados y/o acilados) como los inductores de la sensación de sequedad percibida en la fracción antociánica.

La identificación de diferentes dimensiones independientes y no correlacionadas de las sensaciones táctiles en boca, sin duda contribuye a su comprensión, ya que además se ha podido relacionar las diferentes dimensiones sensoriales percibidas con la composición química analizada. Cabe destacar que dimensiones del sabor y las sensaciones táctiles han sido determinadas con éxito a partir de la composición química mediante la generación de modelos matemáticos PLS muy satisfactorios y validados.

Esta metodología sensorial se aplicó a una amplia gama de vinos tintos (42 muestras de vino). Se identificaron cuatro dimensiones independientes y no correlacionadas de las sensaciones táctiles en la boca, que incluyen "secante/sedoso" y "pegajoso", "granuloso", "punzante" y "graso". Con respecto a los resultados de los modelos matemáticos PLS, se demostró que la actividad de los taninos resulta ser un buen predictor de la sequedad del vino percibida en el paladar y la concentración de taninos de los términos "secante" (en general) y "secante en el paladar". Sin embargo, las otras dimensiones identificadas no pudieron vincularse con las otras variables químicas estudiadas, lo que destaca la necesidad de cuantificar otras variables químicas sensorialmente activas. En este sentido, los posibles compuestos químicos involucrados en las otras dimensiones táctiles en boca podrían incluir polisacáridos u otras familias de polifenoles además de los taninos.

Para estudiar más a fondo las propiedades táctiles en boca de las fracciones aisladas, cuarenta fracciones que contenían taninos o antocianos se analizaron tanto química como sensorialmente en términos del gusto y sensaciones táctiles en la boca. El amargor y la sequedad fueron los atributos destacados que diferenciaron los espacios sensoriales de ambas familias de polifenoles del vino. Los resultados confirmaron la actividad sensorial de las fracciones antociánicas estudiadas, lo que demostró de manera inequívoca una implicación relevante de ciertas antocianos en la percepción de sequedad. Cabe señalar que la adición de una fracción antociánica extremadamente amarga a vinos solo aumentó el amargor en algunos de ellos, lo que sugiere que el amargor en los vinos puede resultar de interacciones y que algunos vinos contienen fuertes supresores del amargor.

Además, la aplicación de estrategias senso-químicas a fracciones fenólicas de la uva ha demostrado ser una herramienta interesante para evaluar la calidad de la uva. Se extrajeron los principales compuestos activos sensoriales de las uvas y se caracterizaron sensorialmente. Esta estrategia permite solventar las principales limitaciones que presenta la evaluación clásica de las bayas (número reducido de panelistas involucrados en la evaluación sensorial y falta de representatividad de la uva). Los resultados, permitieron identificar tres dimensiones sensoriales distintas, independientes y no correlacionadas para el conjunto de la muestra: 1) "secante en la lengua/acuoso", 2) "pegajoso/amargo" y 3) "secante/rugoso". La actividad y la concentración de taninos junto con el grado medio de polimerización (mDP) de los taninos resulttaron ser buenos predictores de la sequedad percibida. Los flavonoles presentaron un alto coeficiente de participación en el modelo de predicción tanto para el atributo “amargo” como para la dimensión “pegajoso/amargo”. Además, los antocianos de bajo peso molecular parecen estar involucrados en la formación del atributo “secante”, mientras que los pigmentos poliméricos en el atributo “pegajoso” y la dimensión “pegajoso/amargo”. A diferencia, la dimensión “rugoso” no pudo ser modelada, lo que sugiere que hay otras (macro)moléculas involucradas en la formación de esta percepción.

Además de la regresión PLS, el presente trabajo ha aplicado con éxito, modelos no lineales derivados de enfoques de aprendizaje automático para predecir la astringencia del vino a partir de su composición química. Las principales variables implicadas en el modelo de astringencia fueron el % de procianidinas que constituyen los taninos y el contenido de etanol, seguidas de otras ocho variables relacionadas con la estructura y acidez de los taninos.

Además, en el presente trabajo se han estudiado diferentes conceptos enológicos que incluyen "carácter verde", madurez y crianza oxidativa aplicando las diferentes estrategias quimio-sensoriales.

Del estudio del "carácter verde" se infiere que se trata de término multidimensional asociado a descriptores tanto del aroma como de sensaciones táctiles boca tales como vegetal, astringencia, taninos verdes y secantes de acuerdo con los expertos de la región del Somontano, lo que producía una depreciación en la aceptabilidad del producto por los consumidores. Los resultados demostraron que dos fracciones no volátiles, relacionadas con la astringencia (sequedad y pegajosidad o adherencia) fueron sugeridas como responsables del alto "carácter verde" de sus respectivos vinos originales. De estas dos fracciones, la que contenía taninos con un grado medio de polimerización de diez inducía la percepción de sequedad, mientras que la segunda fracción conteniendo pigmentos derivados de antocianos (< tetrámeros) fue descrita principalmente como “pegajosa”. Aunque no se identificaron compuestos aromáticos específicos, los vinos verdes contenían niveles significativamente más altos de alcoholes de fusel. Del estudio, se deduce que la interacción entre el alcohol isoamílico y la fracción antociánica y/o taninos contribuye al “carácter verde” y lo aumenta en los vinos tintos. Además, se demostró que el “carácter verde” depende del vino y se sugirió que está enmascarado por los aromas de madera, de oxidación y/o de frutas maduras presentes en los vinos tintos envejecidos en roble.

En lo que respecta a la madurez de la uva, la madurez de la uva indujo efectos sensoriales significativos sobre la astringencia del vino y los aromas afrutados que incluyen “pasa”, “fruta negra” y “fruta roja”. Cabe señalar que los vinos elaborados con uvas recolectadas prematuramente presentaron mayores aromas de oxidación. Este atributo se relacionó con mayores concentraciones de acetaldehído libre, metional, fenilacetaldehído e isoaldehídos, así como bajos niveles de ciertos polifenoles capaces de reaccionar con los aldehídos antes mencionados, denominados en trabajos previos ARP (polifenoles reactivos con aldehído). La astringencia se relacionó con el contenido de etanol, la actividad de los taninos (medida como la interacción de los taninos con una superficie hidrofóbica) y el contenido en compuestos derivados de antocianinas.

En cuanto al envejecimiento oxidativo, destaca que el efecto del consumo de oxígeno indujo un aumento significativo de la actividad tánica. Además, las fracciones fenólicas de los vinos que contienen manganeso presentan un consumo de oxígeno significativamente mayor. Curiosamente, este metal ha sido mencionado en varios trabajos como elemento implicado en el proceso de oxidación de los vinos.

Dando un paso adelante, el uso del análisis no dirigido se presenta como una valiosa herramienta para superar las limitaciones de las técnicas instrumentales dirigidas clásicas. Esta metodología permite considerar tanto metabolitos desconocidos como los que se encuentran en bajas concentraciones y que, sin duda, pueden desempeñar un papel determinante en la formación de las propiedades táctiles en boca. Los resultados de este estudio permitieron obtener modelos PLS muy satisfactorios prediciendo variables sensoriales a partir de características químicas. Entre los marcadores encontrados están los derivados de flavanoles sulfonados, los cuales, de acuerdo con los PLS obtenidos, están implicados en la reducción de la sensación táctil de sequedad del vino. Del mismo modo, los resultados sugieren que los aminoácidos o péptidos son posibles candidatos implicados en la modulación de la sequedad y el atributo “graso” de los vinos. Además, se pudo confirmar el papel sensorial de las antocianos y derivados de

antocianos en la percepción del sabor y las propiedades táctiles en boca de los vinos tintos. Son necesarios estudios de reconstitución para confirmar el papel de los diferentes compuestos y sus estructuras en las sensaciones táctiles en boca y del gusto.

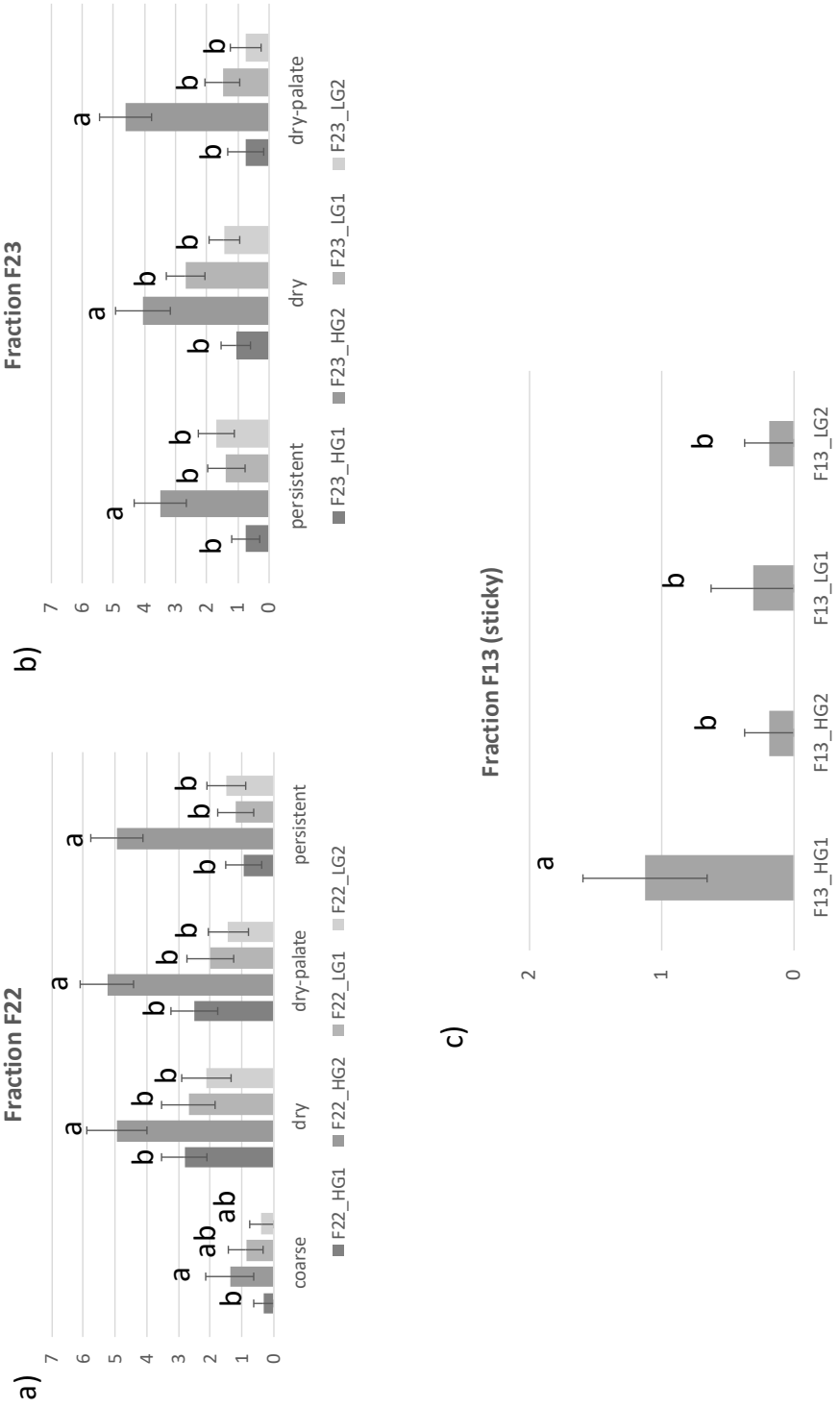
En el presente trabajo las estrategias empleadas siguiendo métodos sensoriales dirigidos y métodos químicos dirigidos y no dirigidos han mostrado resultados muy satisfactorios. No obstante, debe ser reconocido que todavía tienen ciertas limitaciones, puesto que es ampliamente reconocida la dificultad de analizar compuestos polifenólicos polimerizados (>3 unidades), incluidas las proantocianidinas y los pigmentos poliméricos, mediante métodos de HPLC. En este contexto, el desarrollo de nuevas herramientas analíticas es un factor clave para el éxito en la comprensión del gusto y las propiedades táctiles impulsadas por compuestos sensorialmente activos. La determinación de la estructura molecular de los compuestos implicados en el gusto y las sensaciones táctiles se plantea como un gran reto que es fundamental para poder comprender plenamente su papel. En este contexto, técnicas como voltamperometría y espectrofluorometría, podrían aumentar la variedad de atributos sensoriales satisfactoriamente modelados.

ANNEXES

Annexe II-1. 1. List of wines evaluated by the panel of wine experts of Somontano region in the 1st screening task devoted to select wines with different level of green character (ns*: not specified)

code	vintage	country	region	variety	months in oak barrels
1	2011	Spain	DOCa Rioja	Tempranillo, Graciano, Mazuelo	12
2	2013	Spain	DO Somontano	Merlot, Syrah	4
3	2014	Spain	DO Campo de Borja	Grenache, Syrah, Tempranillo	0
4	2014	Spain	DO Somontano	C. Sauvignon	0
5	2011	Spain	DO Cariñena	Grenache	4
6	2011	Spain	DO Somontano	C. Sauvignon, Tempranillo, Merlot	12
7	2014	Spain	DO Valencia	Grenache, Monastrel, Syrah	4
8	2013	France	Pays d´Oc	Grenache	0
9	2013	Italy	IGT-Terre Siciliane	Nero d'Avola	0
10	2011	Portugal	DOC Alentejo	Tricadeira, Touriga nacional, Alicante Bouschet	6
11	2013	Italy	IGP Salento	Primitivo	0
12	2013	Spain	DOCa Rioja	Grenache	9
13	2014	Spain	DO Somontano	Moristel, C. Sauvignon	0
14	2013	Spain	DO Somontano	Moristel, Grenache, Syrah	4
15	2014	Spain	DO Somontano	Merlot	0
16	2012	Spain	DOCa Rioja	Tempranillo	14
17	2014	Spain	DO Navarra	C. Sauvignon	3
18	2014	Spain	DO Somontano	Syrah	0
19	2014	Spain	DOCa Rioja	Grenache	0
20	2014	Spain	DO Somontano	Grenache	0
21	2012	France	Pays d´Oc	Grenache Noir	0
22	2011	Spain	DO Somontano	Tempranillo, C. Sauvignon, Moristel	12
23	2009	Spain	DO Somontano	Tempranillo, C. Sauvignon, Moristel	14
24	2014	Spain	DOCa Rioja	Syrah	3
25	ns*	Spain	Spain	ns*	0
26	2014	Spain	DOCa Rioja	Tempranillo	5
27	2013	Chile	Colchagua	C. Sauvignon	12
28	2012	Spain	DOCa Rioja	Tempranillo, Graciano	14
29	2013	Spain	DO Valencia	Syrah, Marselan, Alicante Bouschet	12
30	2013	Spain	DO Ribera del Duero	Tempranillo	6
31	2011	France	Coteaux du Languedoc AOP	Syrah, Grenache, Mourvèdre, Carignan	12
32	2013	Spain	DOCa Rioja	Grenache, Graciano	9
33	2011	Spain	DOP Jumilla	Monastrell, Tempranillo, Merlot, Syrah	24
34	2010	Spain	DO Ribera del Duero	Tempranillo	20
35	2013	Italy	DOC Primitivo di Mandura	Primitivo	0
36	2013	Spain	DO Somontano	Grenache, C. Sauvignon, Syrah	4
37	2013	Spain	DO Mancha	Bobal	6
38	2014	Spain	DO Mancha	Bobal	0

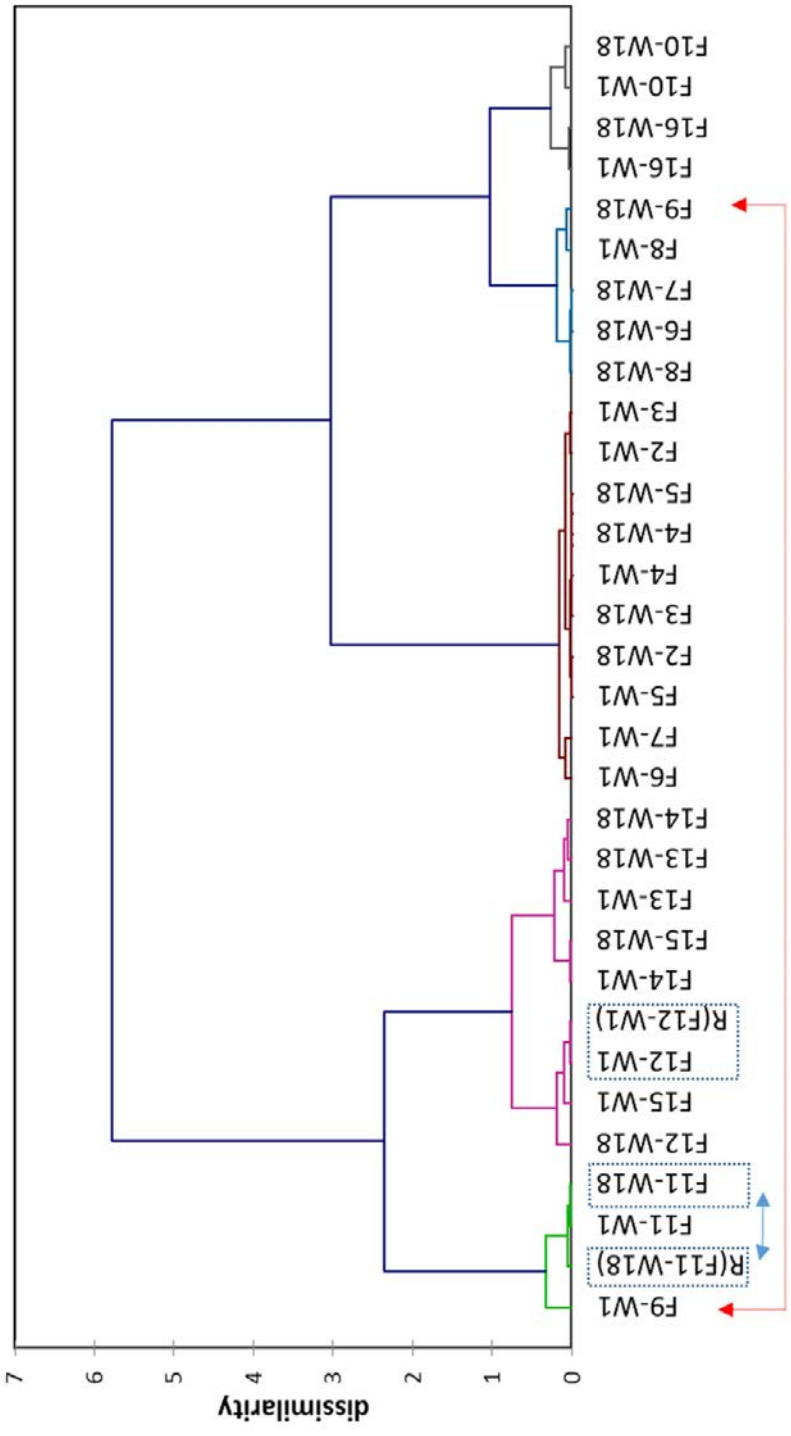
Annexe II-1. 2. Average intensity of in-mouth attributes for fractions F22, F23 and F13. For each fraction, only attributes significantly differing (P<0.05) among wines are displayed. For an attribute and a fraction, different letters means significant (P<0.05) differences among wine fractions.



Annexe II-1. 3. Total polyphenol index (TPI) and colour intensity (CI) in the six fractions of four wines with different level of green character (Low Green: LG and High Green: HG).

	TPI			
	W1_LG1	**W19_LG2**	**W37_HG1**	**W38_HG2**
F11	2.8±0.0	2.8±0.0	2.6±0.0	2.1±0.0
F12	3.4±0.0	3.0±0.0	3.5±0.0	3.3±0.0
F13	**21.3±0.1**	**18.6±0.1**	**24.3±0.0**	**24.5±0.1**
F21	2.4±0.0	0.7±0.0	4.1±0.1	4.5±0.0
F22	2.6±0.0	1.2±0.0	7.5±0.0	11.6±0.0
F23	0.0±0.0	0.6±0.0	0.7±0.0	0.7±0.0

	CI			
	W1_LG1	**W19_LG2**	**W37_HG1**	**W38_HG2**
F11	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0
F12	0.3±0.1	0.4±0.0	0.3±0.0	0.3±0.0
F13	**8.5±0.0**	**6.0±0.0**	**6.1±0.0**	**6.2±0.0**
F21	0.5±0.0	0.1±0.0	1.1±0.0	1.0±0.0
F22	0.5±0.0	0.2±0.0	1.7±0.1	1.8±0.0
F23	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0



Annexe II-1. 4. Tree diagram obtained by Hierarchical Cluster Analysis (HCA) calculated with all the MDS dimensions derived from the sorting task carried out with 32 aroma fractions (F2-F16 for two wines and two replicates: F11-W18 and F12-W1) of wine 18 (high scores for green character and vegetal aroma) and wine 1 (low scores for green character and vegetal aroma). Replicates are marked in blue and the fraction of the two wines belonging to different clusters is marked in red (F9-W1 and F9-W18).

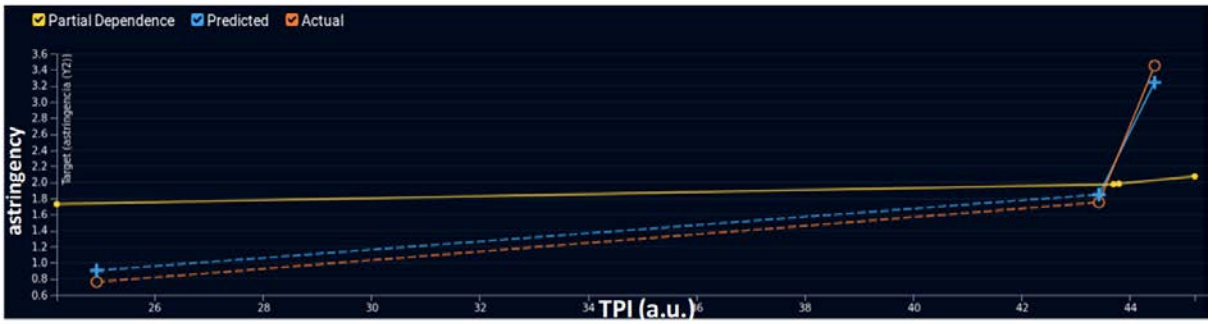
Annexe II-2. 1. **a.1)** and **a.2)** aroma sensory attributes; **b.1)** Taste and mouthfeel sensory attributes (expressed as the average scores of three tanks and 17 panellists in duplicate; error bars are calculated as s/n1/2: (s) standard deviation; (n) number of panellists) for the seven wines elaborated in the present work.

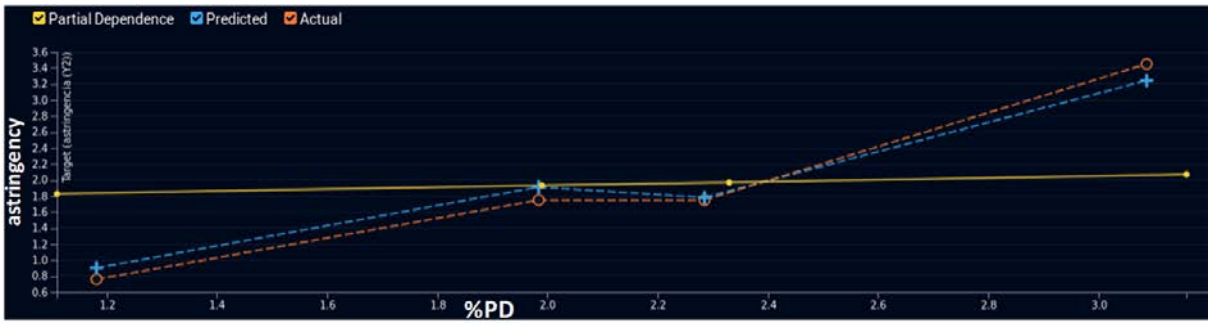
	Raisin	Red fruit	Black fruit	Spicy	Roasted/smoky
BLA_1	0.56±0.22	0.59±0.22	0.47±0.20	1.25±0.29	0.66±0.31
BLA_2	0.81±0.25	0.47±0.20	0.66±0.28	0.88±0.26	0.91±0.32
BLA_3	0.44±0.19	0.66±0.21	1.03±0.30	0.50±0.19	0.63±0.21
BLA_4	1.19±0.29	0.50±0.20	0.59±0.24	0.94±0.30	0.56±0.28
BLB_1	0.94±0.14	0.52±0.11	0.62±0.11	0.48±0.11	0.43±0.09
BLB_2	1.10±0.18	0.43±0.10	0.56±0.13	0.57±0.12	0.32±0.09
BLB_4	0.64±0.12	0.89±0.17	1.01±0.14	0.72±0.12	0.46±0.10

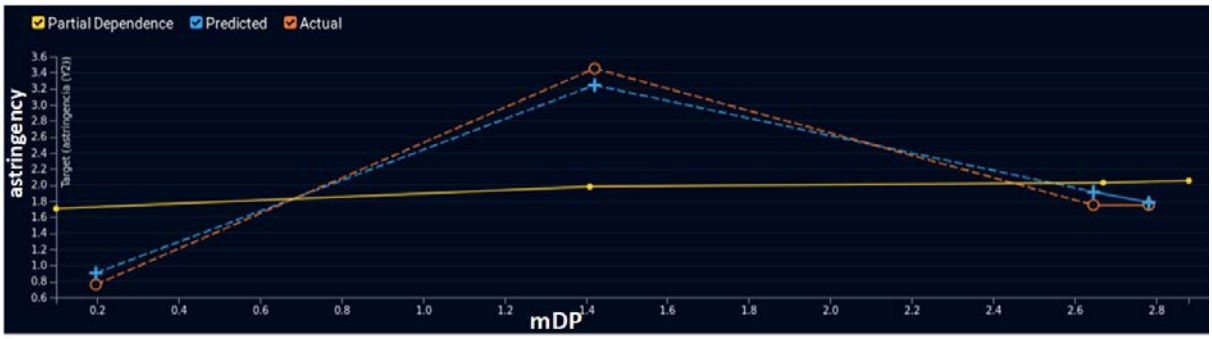
	Alcohol	Fresh vegetal	Fresh grass	Oxidation
BLA_1	0.63±0.15	0.41±0.17	0.78±0.35	1.28±0.53
BLA_2	0.75±0.22	0.63±0.20	0.41±0.19	0.69±0.29
BLA_3	0.78±0.28	0.88±0.29	1.03±0.31	0.47±0.24
BLA_4	1.00±0.30	0.34±0.16	0.47±0.17	0.88±0.27
BLB_1	0.50±0.10	0.59±0.16	0.70±0.12	1.08±0.09
BLB_2	0.75±0.12	0.47±0.12	0.63±0.11	1.72±0.14
BLB_4	0.57±0.10	0.60±0.14	0.62±0.11	0.29±0.06

	Sweet	Sour	Bitter	Alcohol feeling	Astringency
BLA_1	0.13±0.13	2.09±0.39	1.28±0.38	0.16±0.09	3.45±0.34
BLA_2	0.16±0.13	1.56±0.28	2.09±0.36	0.09±0.09	2.90±0.47
BLA_3	0.19±0.09	2.16±0.38	1.38±0.40	0.06±0.06	1.75±0.35
BLA_4	0.19±0.10	1.66±0.33	1.72±0.36	0.28±0.17	2.10±0.44
BLB_1	0.15±0.06	2.53±0.18	1.07±0.17	0.08±0.04	1.14±0.15
BLB_2	0.11±0.04	2.49±0.18	1.06±0.17	0.20±0.06	0.76±0.11
BLB_4	0.13±0.04	2.54±0.19	1.05±0.17	0.18±0.05	1.21±0.15

Annexe III-1. 1. Partial dependence plots of astringency and chemical variables: total polyphenol index (TPI), %prodelphinidins (%PD), mean degree of polymerization (mDP), volatile acidity, tannin activity, total acidity, % tannins linked to malvidin (%T-M) and % galloylated flavanols in tannins (%G).







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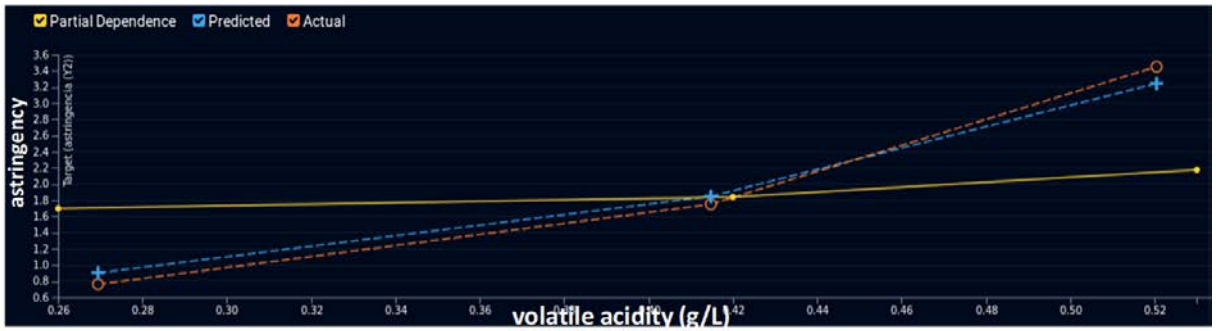

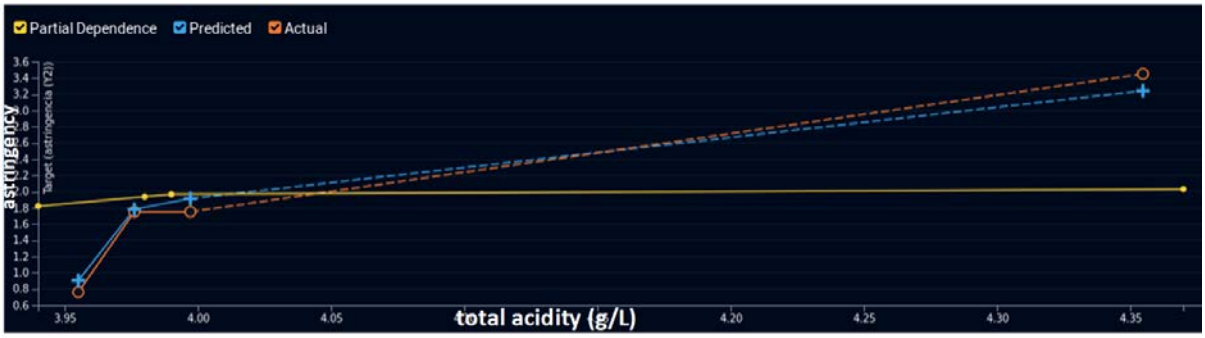

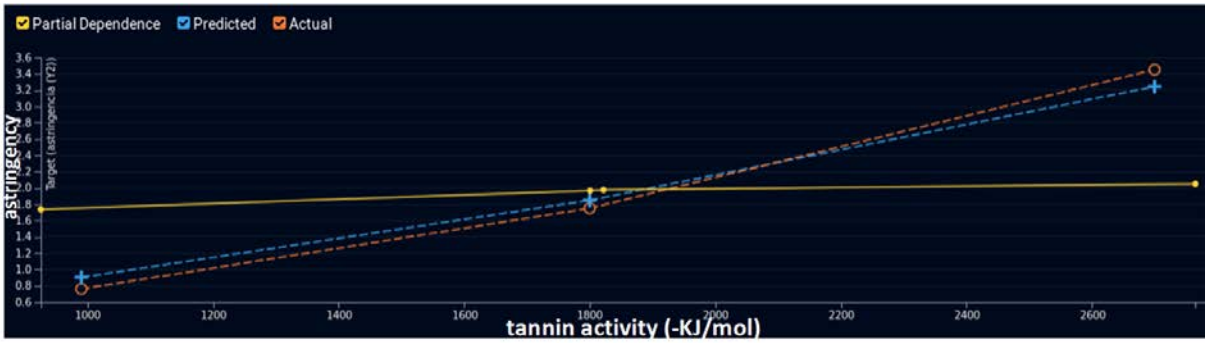

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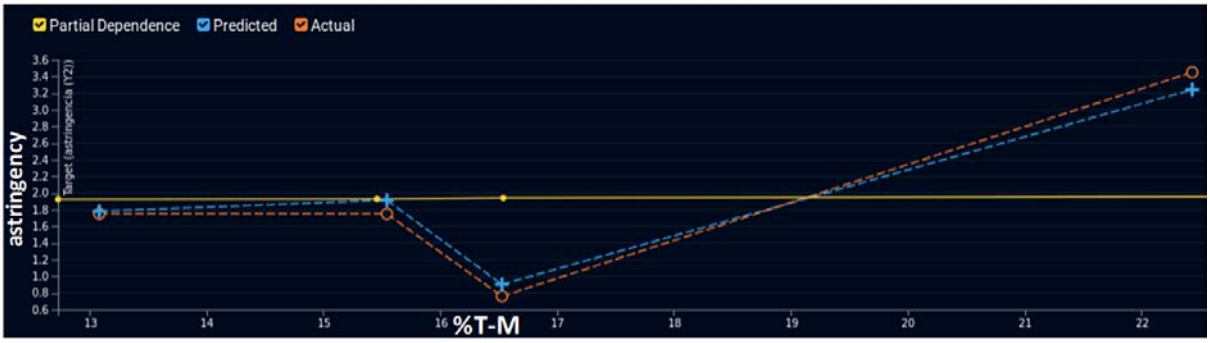
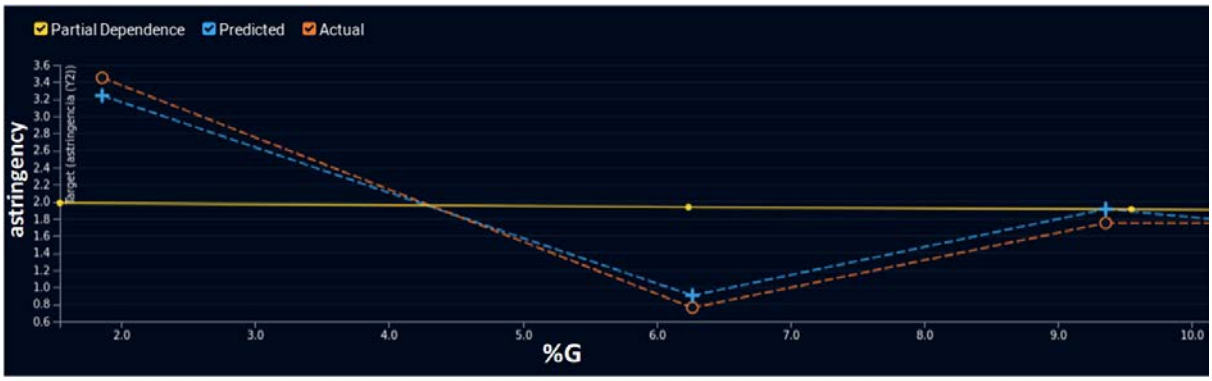




Annexe III-2. 1. Detailed list of wines employed in the study. Chemical data are the average of duplicate analyses (± standard error).

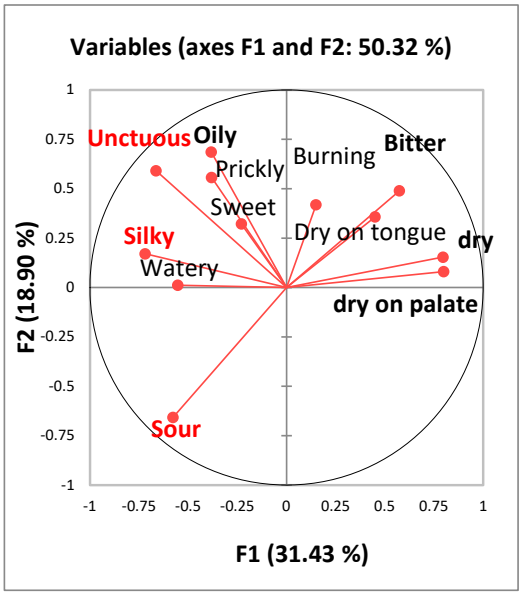
Code	Vintage	Variety	Origin	Ethanol (% v/v)	pH	VA (g L^{-1})	TA (g L^{-1})	RS (g L^{-1})	MA (g L^{-1})	LA (g L^{-1})	TPI
SM1*	2017	Gamay	Côte du Puy, France	13.5±0.0	3.32±0.01	0.3±0.0	5.7±0.0	2.9±0.1	0.0±0.1	0.9±0.0	65±0
SM2	2017	Bonarda	Valle de Uco, Argentina	13.3±0.0	3.80±0.00	0.4±0.0	4.5±0.0	2.6±0.2	0.0±0.0	0.9±0.0	67±1
SM3	2018	Mencía	Bierzo, Spain	12.2±0.0	3.58±0.01	0.3±0.0	5.0±0.0	1.5±0.0	0.2±0.0	1.2±0.0	54±2
SM4*	2018	Mencía	Bierzo, Spain	13.8±0.0	3.73±0.00	0.4±0.0	4.2±0.0	2.1±0.0	0.0±0.1	1.3±0.0	59±1
SM5*	2017	Malbec	Valle de Uco, Argentina	13.4±0.0	3.62±0.00	0.3±0.0	5.0±0.0	2.5±0.1	0.0±0.1	1.0±0.0	46±2
SM6*	2018	Bonarda	Mendoza, Argentina	12.9±0.0	3.76±0.00	0.5±0.0	4.8±0.0	1.8±0.1	0.0±0.0	1.4±0.0	57±2
SM7*	2017	Mencía	Bierzo, Spain	12.7±0.0	3.76±0.00	0.9±0.0	5.4±0.0	2.3±0.1	0.0±0.0	1.9±0.0	57±1
SM8*	2015	Gamay	Beaujolais, France	12.7±0.0	3.48±0.01	0.3±0.0	5.0±0.0	1.6±0.0	0.0±0.0	1.0±0.0	59±5
SM9	2017	Mencía	Bierzo, Spain	13.6±0.0	3.70±0.00	0.6±0.0	4.9±0.0	2.9±0.0	0.0±0.1	1.4±0.0	66±2
SM10*	2017	Mencía	Bierzo, Spain	14.0±0.0	3.75±0.00	0.4±0.0	4.1±0.0	2.5±0.1	0.0±0.0	1.2±0.0	61±1
SM11	2014	Pint Noir	Mercurey, France	12.3±0.0	3.33±0.00	0.4±0.0	5.5±0.0	1.9±0.1	0.0±0.0	1.2±0.0	35±1
SM12*	2016	Pint Noir	Bourgogne, France	12.8±0.0	3.50±0.00	0.3±0.0	4.4±0.0	2.0±0.1	0.0±0.0	1.0±0.0	54±0
SM13	2017	Carignan	Cariñena, Spain	13.3±0.0	3.30±0.01	0.2±0.0	6.3±0.0	4.8±0.1	0.2±0.0	0.7±0.0	53±0
SM14	2016	Prieto Picudo	León, Spain	13.0±0.0	3.32±0.01	0.3±0.0	5.2±0.0	2.2±0.0	0.2±0.0	0.9±0.0	35±1
SM15*	2017	Carignan	Montsant, Spain	12.5±0.0	3.32±0.00	0.2±0.0	5.7±0.0	2.5±0.0	0.1±0.0	1.0±0.0	26±0
SM16*	2017	Prieto Picudo	Castilla, Spain	12.9±0.0	3.51±0.00	0.3±0.0	5.7±0.0	2.4±0.0	0.0±0.0	0.8±0.0	48±1
SM17	2016	Cabernet Sauvignon	Penedés, Spain	12.5±0.0	3.38±0.01	0.2±0.0	5.2±0.0	2.3±0.1	0.1±0.0	0.8±0.0	48±0
SM18	2017	Cabernet Sauvignon	Cariñena, Spain	13.2±0.0	3.62±0.01	0.2±0.0	5.0±0.0	4.5±0.1	0.1±0.0	0.8±0.0	65±1
SM19	2018	Grenache	Cariñena, Spain	14.3±0.0	3.64±0.00	0.4±0.0	4.9±0.0	1.7±0.0	0.0±0.0	0.8±0.0	43±0
SM20	2018	Bobal	Utiel-Requena, Spain	12.7±0.0	3.60±0.00	0.3±0.0	4.8±0.0	2.3±0.0	0.0±0.0	0.9±0.0	49±0

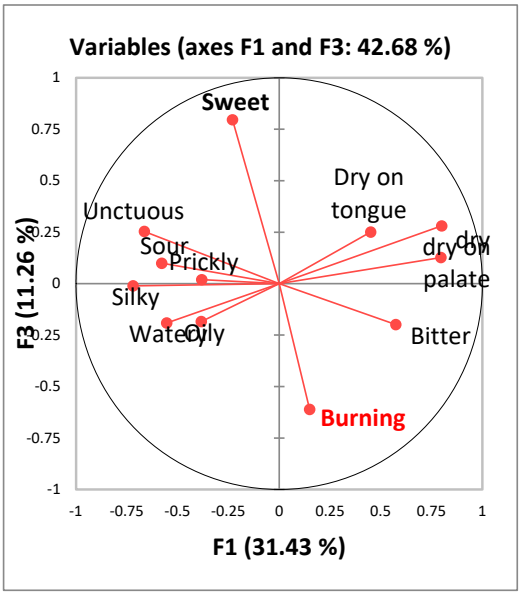
VA: volatile acidity (expressed in g L-1 of sulfuric acid); TA: titratable acidity (expressed in g L-1 of tartaric acid); RS: reducing sugars; MA: malic acid; LA: lactic acid; TPI: total polyphenol index calculated as absorbance at 280 nm

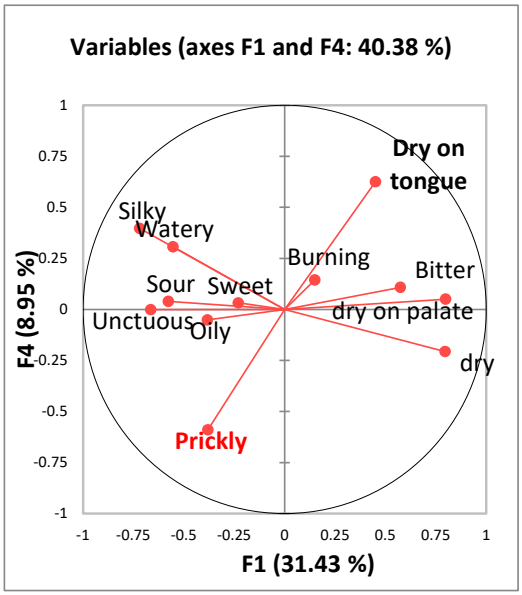
Annexe III-2.1. Continued

Code	Vintage	Variety	Origin	Ethanol (% v/v)	pH	VA (g L^{-1})	TA (g L^{-1})	RS (g L^{-1})	MA (g L^{-1})	LA (g L^{-1})	TPI
SM20	2018	Bobal	Utiel-Requena, Spain	12.7±0.0	3.60±0.00	0.3±0.0	4.8±0.0	2.3±0.0	0.0±0.0	0.9±0.0	49±0
SM21	2018	Merlot	Somontano, Spain	14.5±0.0	3.69±0.00	0.3±0.0	5.8±0.0	2.3±0.0	0.4±0.0	0.9±0.0	93±2
SM22	2018	Syrah	Somontano, Spain	15.5±0.0	3.46±0.01	0.2±0.0	6.3±0.0	2.8±0.0	0.4±0.0	0.6±0.0	71±1
SM23*	2018	Merlot	Somontano, Spain	14.8±0.0	3.37±0.01	0.3±0.0	6.5±0.0	1.7±0.0	0.5±0.0	0.6±0.0	60±1
SM24	2018	Cabernet Sauvignon	Somontano, Spain	15.3±0.0	3.56±0.00	0.3±0.0	6.3±0.0	2.3±0.1	0.6±0.0	0.5±0.0	59±0
SM25*	2018	Merlot	Somontano, Spain	14.8±0.0	3.60±0.01	0.4±0.0	6.2±0.0	2.6±0.1	0.4±0.0	0.9±0.0	111±3
SM26*	2018	Cabernet Sauvignon	Somontano, Spain	15.2±0.0	3.63±0.00	0.2±0.0	6.1±0.0	3.9±0.0	0.6±0.0	0.7±0.0	82±2
SM27	2018	Cabernet Sauvignon	Borja, Spain	13.7±0.0	3.52±0.00	0.2±0.0	5.9±0.0	1.2±0.0	0.5±0.0	0.9±0.0	82±0
SM28	2018	Tempranillo	Borja, Spain	14.2±0.0	3.70±0.01	0.3±0.0	4.5±0.0	1.7±0.0	0.0±0.0	0.9±0.0	59±1
SM29*	2018	Grenache	Borja, Spain	13.8±0.0	3.37±0.00	0.2±0.0	6.3±0.0	1.9±0.0	0.5±0.1	0.3±0.0	47±0
SM30*	2018	Syrah	Borja, Spain	14.3±0.0	3.49±0.00	0.1±0.0	5.6±0.0	1.7±0.1	0.4±0.0	0.7±0.0	61±6
SM31*	2018	Merlot	Borja, Spain	14.9±0.0	3.62±0.01	0.3±0.0	5.1±0.0	1.7±0.0	0.1±0.0	0.6±0.0	58±1
SM32	2018	Grenache	Borja, Spain	14.6±0.0	3.43±0.00	0.2±0.0	5.5±0.0	2.2±0.0	0.1±0.0	0.6±0.0	56±0
SM33	2017	Tempranillo	Rioja, Spain	13.4±0.0	3.56±0.01	0.2±0.0	4.9±0.0	1.7±0.0	0.0±0.0	1.1±0.0	55±1
SM34	2017	Tempranillo	Rioja, Spain	12.5±0.0	3.65±0.00	0.3±0.0	4.5±0.0	1.3±0.1	0.0±0.0	1.4±0.0	55±1
SM35*	2018	Tempranillo	Rioja, Spain	14.3±0.0	3.67±0.00	0.1±0.0	3.9±0.0	2.1±0.0	0.0±0.0	0.6±0.0	40±1
SM36	2018	Tempranillo	Rioja, Spain	13.6±0.0	3.62±0.00	0.3±0.0	4.9±0.0	1.1±0.1	0.1±0.1	1.1±0.0	43±0
SM37*	2017	Tempranillo	Rioja, Spain	11.8±0.0	3.40±0.01	0.3±0.0	5.5±0.0	2.3±0.0	0.2±0.0	1.0±0.0	43±0
SM38	2018	Grenache	Calatayud, Spain	15.1±0.0	3.63±0.00	0.3±0.0	4.4±0.0	2.1±0.0	0.0±0.0	0.6±0.0	45±0
SM39	2018	Grenache	Rioja, Spain	13.4±0.0	3.46±0.00	0.4±0.0	5.2±0.0	1.7±0.1	0.0±0.1	0.9±0.0	28±6
SM40	2017	Moristel	Somontano, Spain	12.6±0.0	3.23±0.00	0.4±0.0	6.0±0.0	1.8±0.1	0.4±0.1	0.4±0.0	19±2
SM41*	2018	Bobal	La Mancha, Spain	12.4±0.0	3.59±0.01	0.3±0.0	5.1±0.0	2.6±0.3	0.0±0.0	1.0±0.0	60±3
SM42*	2018	Bobal	La Mancha, Spain	12.1±0.0	3.55±0.01	0.3±0.0	5.3±0.0	2.7±0.0	0.0±0.0	0.9±0.0	55±1

Annexe III-3. 1. PCA plots with the projection of dimensions F1 – F4 performed for the significant terms of the 42 samples.







Annexe III-3. 2. Annotated metabolite, for ESI – and ESI +.

ESI -	m/z	RT	MS/MS fragments	Annotation	m/z[Theoretical]	ppm	notes	Annotation level*	References	Anova (p)	q Value	Max Fold Change
1-NEG	369.029	7.07	369,03 - 287,1 - 269,1 - 161,0 - 125,0 - 80,96	procyanidin type B-4β-sulfonate fragment	369.029	-0.17	[M-Flavanol-H]-1	2		7.58E-14	2.01E-10	Infinity
2-NEG	369.028	4.70	287,1 - 269,1 - 161,0 - 125,0 - 80,96	epicatechin sulfonate	369.029	1.06	[M-H]-1	1	1, 6, 8	1.28E-10	2.27E-07	25.191
3-NEG	385.023	3.81	385,09 - 177,05 - 125,05 - 80,97	epigallocatechin-4β-sulfonates	385.023	1.61	[M-H]-1	2		4.44E-09	0.00000295	23327.788
4-NEG	221.067	3.64	108,93 - 85,05 - 71,02	ethyl glucuronide	221.067	-1.13		2		6.2E-09	0.00000366	6.311
5-NEG	300.022	4.97	251, 06 - 155,99 - 80,96 - 57,03	Unknown				4		2.57E-08	0.00000852	Infinity
6-NEG	657.092	5.69	369,03 - 287,1 - 269,1 - 161,0 - 125,0 - 80,96	procyanidin type B-4β-sulfonate	657.092	-0.01	[M-H]-1	1		4.83E-08	0.0000142	57.293
7-NEG	487.089	13.23	487,17 - 307,10 - 289,0 - 235, 08 - 163,05 - 145, 04 - 119,05 - 89,03 - 71,02 - 59,01	Unknown				4		0.0000000201	0.0000453	Infinity
8-NEG	515.119	17.71	515,21 - 353,08 - 163,05 -145,04	B-type vitisin (correlation with 353, 0661)	515.120	0.39		1		0.0000000409	0.0000868	58.507
9-NEG	258.992	8.50	215,05 - 135,07 - 79.97	caffeic acid 3-O-sulfate or (3,5-Dihydroxycinnamic acid sulfate)	258.992	-1.11	[M-H]-1	3		0.000001065	0.00020184	11.729
10-NEG	953.256	20.39	663,26 - 645,25 - 355, 1350 - 329,11 - 289,11	malvidin 3-(6"-p-coumaroyl)-glucoside-ethyl-epicatechin (or catechin)	953.250	-5.54	[M-H]-1	2	1, 2, 3, 4, 8	0.000000617	0.00081978	Infinity
11-NEG	357.080	6.98	277,15 - 217,14 - 146,09 -128,03 - 80,97	pantetheine-SO3H	357.079	-2.03	[M-H]-1	3		0.00000216	0.00191271	6.894
12-NEG	805.193	19.77		Unknown (Pigment derivate)				4		0.0000275	0.00211339	Infinity
13-NEG	501.104	15.84	403,12 - 241,06 - 96,97 - 78,96	Unknown (pyrano petunidin 3-glucoside)				4		0.0000844	0.0040257	Infinity
14-NEG	743.128	8.49	743,26 - 661,28 - 509,21 - 491,17 - 447,17 - 289,12 - 165, 08	Unknown				4		0.000557789	0.02071715	Infinity
15-NEG	807.214	19.34	807,22 - 645,2 - 517,1 - 491.2 - 355,1 - 329,1 - 315,1 - 289,1	malvidin 3-glucoside-ethyl-epicatechin (or catechin)	807.214	-0.69	[M-H]-1	2	1, 4, 8	0.0010061 63	0.03166182	Infinity
16-NEG	743.130	7.66	743,26 - 661,28 - 509,21 - 447,17 - 491,17 - 289,12 - 165, 08	Unknown				4		0.0020878 03	0.05175959	Infinity
17-NEG	577.136	10.76	425,09 - 289.07 - 125,039	procyanidin B2	577.135	-2.81	[M-H]-1	1	1, 2, 7, 8	0.002791892	0.06356928	2.052
18-NEG	645.162	19.77	645,2 - 517,1 - 355,1 - 329,1 - 315,1 - 289,1	malvidin 3-glucoside-ethyl-epicatechin (or catechin) fragment (correlation with BD7.2151434)			[M-H]-1	3	1, 4, 8	0.003433349	0.07098398	Infinity
19-NEG	865.200	9.67	865,28 - 713,21 - 575,17 - 407,11 - 289,10 - 125,039	trimer procyanidin type B (catechin-catechin-catechin type)	865.199	-1.26	[M-H]-1	2	1, 8	0.003619085	0.07280998	18.379
20-NEG	849.226	20.51	645,25 - 559,20 - 533,17 - 355, 12 - 329,10 - 315,12 - 289,11 - 245,12	malvidin 3-(6"-acetyl)-glucoside-ethyl-epicatechin (or catechin)	849.224	-2.62	[M-H]-1	2	1, 2, 3, 4, 8	0.004756287	0.08411265	Infinity
21-NEG	495.111	5.83	495,20 - 315,11 - 300,07 - 283,07 - 191,0 - 169,03 - 125,05 - 59,02	carbinol pseudobase petunidin 3-glucoside derivative	495.114	-4.97	[M]-1	3	10	0.007226968	0.10545103	Infinity
22-NEG	447.094	15.16	301.02	Unknown - Flanonol derivate- Quercetin 3-O-rhamnoside- (Quercitrin) Or (-)-Epicatechin 7-O-glucuronide (Fragment)	447.093	-3.41		4	1, 2, 3, 4, 7, 8	0.007848564	0.11057193	Infinity
23-NEG	425.088	12.60	347,11 - 261,1 - 165,03- 138,05 - 121,04	procyanidin type B (PBS) fragment	425.087	-1.22		2	1, 2, 3, 7, 8	0.009081823	0.12187088	7.175

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Annexe III-3.2. Continued

ESI +	m/z	RT	MS/MS fragments and Annotation comments	Annotation	Theoretical m/z	ppm	notes	Data Base	Formula	Annotation level*	References	Anova (p)	q Value	Max Fold Change
1-POS	503.119	15.8	503,18 - 341,12 - 326,12 - 298,1 - 269,1 - 241,11 - 227,09	Pyrano petunidin-3-glucoside	503.1184	-1.92	[M + H]+			1	1, 4, 8, 10, 11	0.000000	0.000004	22.399
2-POS	517.138	17.7	355,16 - 339,13 - 323,13 - 311,13 - 294,10 - 266,13 - 202,07	pyrano malvidin -3- glucoside	517.1345	-6.58	[M + H]+			1	1, 4, 8, 10, 11	0.000000	0.000072	15.104
3-POS	487.124	17.0	325.07	pyrano peonidin 3-glucoside	487.1235	-1.71	[M + H]+			1	1, 4, 8, 10, 11	0.000001	0.000199	52.974
4-POS	207.065	10.4	good correlation with the m/z: 333,0969	Unknown (posible anthocyanin)						4		0.000008	0.001765	8.390
5-POS	248.947	7.5	good correlation with the m/z: 106,98 - 150.97	Unknown						4		0.000048	0.005100	120.147
6-POS	492.279	20.3	492,33 - 331,12 - 315,09 - 287,09 - 283,05 - 269,08 - 242,09 - 181,0 - 123,06 659,21 - 425,10 - 289,08 - 127,04	Anthocyanin - Malvidin 3-glucoside (fragment)						3		0.000213	0.012509	Infinity
7-POS	659.108	5.6	good correlation with the m/z: 577,1353	procyanidin type B-4β-sulfonate	659.1071	-0.92				1	1, 4, 8	0.000232	0.013229	Infinity
8-POS	635.143	18.3		Unknown						4		0.000260	0.014083	Infinity
9-POS	181.097	6.2	166,08 - 120,08 - 103,05	L-Amino--phenylalanine	181.0977	3.12		CSID 130094		3		0.000391	0.018382	Infinity
10-POS				malvidin 3-(6"-p-coumaroyl)-β-glucoside - ethyl - catechin										
11-POS	851.243	20.5	851,42 - 561,28 - 357,17 - 331,16		851.2399	-3.36				2	1,4,8,,10,11	0.000660	0.025056	Infinity
	430.230	19.9	199,18 - 136,93 - 86,096 - 69,069	Leu-Leu-Tyr (Leucyl-leucyl-tyrosine)	430.2312	1.89	M+Na	FDB098391 HMDB0003337	C21H33N3O5	3		0.001211	0.036950	85.316
12-POS	613.161	4.3	695,29 - 484,22 - 355,15 - 231,09 - 177,07 - 130,08	Glutathione disulphide (Oxidised glutathione)	613.1592	-2.39		CSID588335 CAS-27025-41-8 FDB023147	C20H32N6O12S2	3		0.001239	0.037384	Infinity
13-POS	781.200	16.1	781,34 - 619,26 - 491,20 - 329,12 - 287,11	malvidin 3-glucoside-epicatechin (or catechin)	781.1974	-3.63	[M + H]+			2	1, 4, 8, 10, 11	0.001334	0.038579	Infinity
14-POS	273.054	7.3		Unknown						4		0.001450	0.040745	5.028
15-POS	867.215	9.2	867,38 - 715,32 - 577,25 - 453,08 - 425,18 - 189,13 - 247,11 - 127,06 good correlation with the m/z: 453.085178	trimer procyanidin type B (catechin-catechin-catechin type)	867.2136	-2.07		HMDB0038370CSID935 7147 CSID1485540 CSID10673607	C45H39O18	2		0.001651	0.043665	18.645

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Annexe III-3.2. Continued

ESI +	m/z	RT	MS/MS fragments and Annotation comments	Annotation	Theoretical m/z	ppm	notes	Data Base	Formula	Annotation level*	References	Anova (p)	q Value	Max Fold Change
16-POS	441.091	17.7		Unknown - epicatechin (or catechin) gallate derivate fragment						4		0.001937	0.047828	Infinity
17-POS	439.157	12.4	441,25 - 309,1234 - 201,05 - 189,06 - 98,99	Unknown						4		0.002368	0.054410	4985.446
18-POS	224.080	18.8	good correlation with the m/z: 227.089 - 289.06 - 294.98 - 341.03 - 355.07	pyrano malvidin glucoside fragment						3		0.002793	0.059552	6.774
19-POS	563.316	20.5	401,13 - 357,09 - 181,06	proanthocyanin -ethyl- anthocyanidin derivate fragment						3		0.004315	0.074533	Infinity
20-POS	299.056	12.4	good correlation with the m/z: 517.079	Unknown - Carboxy cyanidin 3-glucoside fragment						4		0.009865	0.115300	3.123
21-POS	579.153	10.7	579,14 - 484,31 - 427,10 - 291,08 - 139,04 - 127,04	procyanidin B2	579.1497	-6.08				2		0.010275	0.117480	2.106
22-POS	816.638	4.9	493,13 - 331,10 - 181,06	Unknown						4		0.011316	0.121600	Infinity
23-POS	269.011	10.1	Unknown - Posible Anthocyanidin fragment							4		0.011593	0.123295	Infinity
24-POS	239.026	20.4	good correlation with the m/z: 73.02 - 87.99 - 97.00 - 111.01 - 115.03 - 121.01 -129.0 - 185.01 - 197.07 - 231,0522 - 243.02 - 275.02 - 285,013 - 287.01 - 485.10 -	Posible por correlacion de fragmentos Kaempferol 3-glucuronide sodiate	485.1054	-7.42	[M + Na]+		[M + Na]+	4	8	0.011676	0.123575	2.208
25-POS	163.075	7.6	136,93 - 118,92 - 86,09 - 70,06	Unknown						4		0.012414	0.126744	4608.392
26-POS	563.093	7.9		Unknown						4		0.012780	0.128576	Infinity
27-POS	795.216	17.4	795,36 - 633,25 - 505,22 - 343,14	petunidin 3-glucoside-ethyl-epicatechin (or catechin)						3	1, 4, 8, 10, 11	0.014161	0.135188	Infinity
28-POS	285.076	17.8	good correlation with the m/z: 552,18 - 311,08	unknown						4		0.015246	0.139838	10.719
29-POS	729.242	6.8	729,24 - 621,15 - 595,14 - 497,07 - 289,08 -	Epicatechin (or Catechin)-(4alpha->8)-epigallocatechin (or gallocatechin) 3'-gallate [746.1483] (adduct) Or Epigallocatechin (or gallocatechin)-(4beta->8)-epicatechin (or Catechin) 3'-gallate (adduct)	729.1456	-132.63	M+H-H2O	FDB018852 FDB016766 FDB004461	C37H30O17	3	11	0.015913	0.141514	Infinity
30-POS	867.216	12.2	good correlation with the m/z: 577.1346209	trimer procyanidin type B (catechin-catechin-catechin type)	867.2136	-2.93		HMDB0038370CSID83 57147 CSID148540 CSID10673607	C45H38O18	2		0.017019	0.144883	7.562
31-POS	453.085	9.2	good correlation with the m/z: 867.2154	procyanidin type B (Trimer) fragment						3		0.019686	0.155199	3.050
32-POS	289.070	12.6	good correlation with the m/z: 579,1514 - 453,1091 - 427,1024	procyanidin type B (Dimer) (PB5) fragment						2	1, 4, 8, 10, 11	0.028452	0.184491	3.868
33-POS	883.217	8.2	883,40 - 715,35 - 593,26 -579,27 - 425,19 - 287,12 - 127,07	trimer procyanidin type B Catechin-(4alpha->8)-gallocatechin-(4alpha->8)-catechin Or Gallocatechin-(4alpha->8)-catechin-(4alpha->8)-catechin	883.2086	-9.37	[M + H]+	HMDB0038375 HMDB0038374	C45H38O19	3	11	0.028496	0.184570	Infinity
34-POS	491.320	20.7	491,31 - 357,12 - 331,12 - 283,06 - 269,09 - 181,07 - 120,09 - 86,11	Malvidin 3-glucoside (fragment)						3	10, 11	0.034527	0.200405	Infinity
35-POS	577.135	12.2	good correlation with the m/z: 867.2154	procyanidin type B (Trimer) fragment						3		0.034538	0.200405	8.646
36-POS	385.256	5.8	385,27 - 183,14 - 158,11 - 129,12 - 116,08 - 110,08 - 84,09 - 70,07	Unknown						4		0.035564	0.202966	Infinity
37-POS	391.140	14.6	107,02 - 118,92 - 229,08 - 391,14	Stilbene derivate						3		0.039919	0.212393	Infinity
38-POS	517.104	15.8	355,11 - 341,13 - 181,08 - 151,07 - 86,11	Unknown (posible carboxypyrano cyanidin 3-glucoside tr=19.80)	517.0982	-11.88	[M + H]+			4		0.043962	0.220197	Infinity

Annexe III-3. 3. List of 108 targeted compounds.

	Compound	rt (min)	Chemical formula	Theoretical m/z	Measured m/z	error (ppm)	HMDB ID	CHEBI ID	ChemSpider ID
	Flavanols								
1	Gallocatechin	6.60	C15H14O7	305.0667	305.0658	2.89	HMDB0038365	CHEBI:31018	58594
2	Epigallocatechin	9.70	C15H14O7	305.0667	305.0662	1.58		CHEBI:42255	65231
3	Catechin	9.92	C15H14O6	289.0718	289.0692	8.88	HMDB0002780	CHEBI:15600	8711
4	Epicatechin	13.02	C15H14O6	289.0718	289.0714	1.27	HMDB0001871	CHEBI:90	65230
5	Procyanidin B3 dimer	8.27	C30H26O12	577.1352	577.1350	0.27	HMDB0033974	CHEBI:75630	129476
6	Procyanidin B1 dimer	8.50	C30H26O12	577.1352	577.1318	5.81	HMDB0029754	CHEBI:75633	9425166
7	Procyanidin B4 dimer	9.57	C30H26O12	577.1352	577.1359	-1.29	HMDB0013690	CHEBI:27589	129882
8	Procyanidin B2 dimer	10.76	C30H26O12	577.1352	577.1315	6.33	HMDB0033973	CHEBI:75632	109417
9	Procyanidin B5 dimer	12.60	C30H26O12	577.1352	577.1350	0.27			110533
10	Epicatechin-Gallate	15.29	C22H18O10	441.0827	441.0820	1.65	HMDB0037944	CHEBI:70255	97034
11	Procyanidin type B trimer	5.30	C45H38O18	865.1985	865.1951	3.98			10673607
12	Procyanidin type B trimer	8.70	C45H38O18	865.1985	865.1924	7.10			10673607
13	Procyanidin type B trimer	9.30	C45H38O18	865.1985	865.2002	-1.91			10673607
14	Procyanidin type B trimer	12.30	C45H38O18	865.1985	865.1979	0.74			10673607

Annexe III-3.3. Continued

	Compound	rt (min)	Chemical formula	Theoretical m/z	Measured m/z	error (ppm)	HMDB ID	CHEBI ID	ChemSpider ID
	Flavonols								
15	Myricetin	20.28	C15H10O8	317.0303	317.0317	-4.43			4444991
16	Myricetin-3-Galactoside	17.24	C21H20O13	479.0831	479.0842	-2.25			10188643
17	Myricetin-3-Glucuronide	16.97	C21H18O14	493.0624	493.0637	-2.67		CHEBI:75807	4589353
18	Myricetin-3-Glucoside	17.38	C21H20O13	479.0831	479.0823	1.71		CHEBI:75813	10188643
19	Myricetin-3-Rhamnoside	18.80	C21H20O12	463.0882	463.0873	1.94			4444992
20	Quercetin	20.90	C15H10O7	301.0354	301.0349	1.60	HMDB0005794	CHEBI:16243	4444051
21	Quercetin-3-Galactoside	19.45	C21H20O12	463.0882	463.0876	1.30		CHEBI:64621	4444962
22	Quercetin-3-O-Glucuronide	19.41	C21H18O13	477.0675	477.0643	6.71		CHEBI:131700	4438874
23	Quercetin-3-Glucoside	19.68	C21H20O12	463.0882	463.0843	8.42	HMDB0037362	CHEBI:68352	4444361
24	Taxifolin	16.18	C15H12O7	303.0510	303.0505	1.65			621332
25	Kaempferol	21.27	C15H10O6	285.0405	285.0397	2.69	HMDB0005801	CHEBI:28499	4444395
26	Kempferol-3-Galactoside	20.24	C21H20O11	447.0933	447.0971	-8.52			4445311
27	Kaempferol-3-Glucuronide	20.34	C21H18O12	461.0726	461.0760	-7.47		CHEBI:75721	4477252
28	Kaempferol-3-Glucoside	20.37	C21H20O11	447.0933	447.0923	2.22			4445311
29	Syringetina	21.37	C17H14O8	345.0616	345.0604	3.48		CHEBI:18215	4445230
30	Syringetin-3-Glucoside	20.46	C23H24O13	507.1144	507.1125	3.75			16736532
31	Isorhamnetin	21.35	C16H12O7	315.0510	315.0507	1.06	HMDB0002655	CHEBI:6052	4444973
32	Isorhamnetin-3-Glucoside	20.44	C22H22O12	477.1039	477.1031	1.58		CHEBI:75750	4444973
33	Laricitrin	21.00	C16H12O8	331.0459	331.0458	0.44	HMDB0126497	CHEBI:31763	4445351
34	Laricitrin-3-Glucoside	19.90	C22H22O13	493.0988	493.0953	7.04			

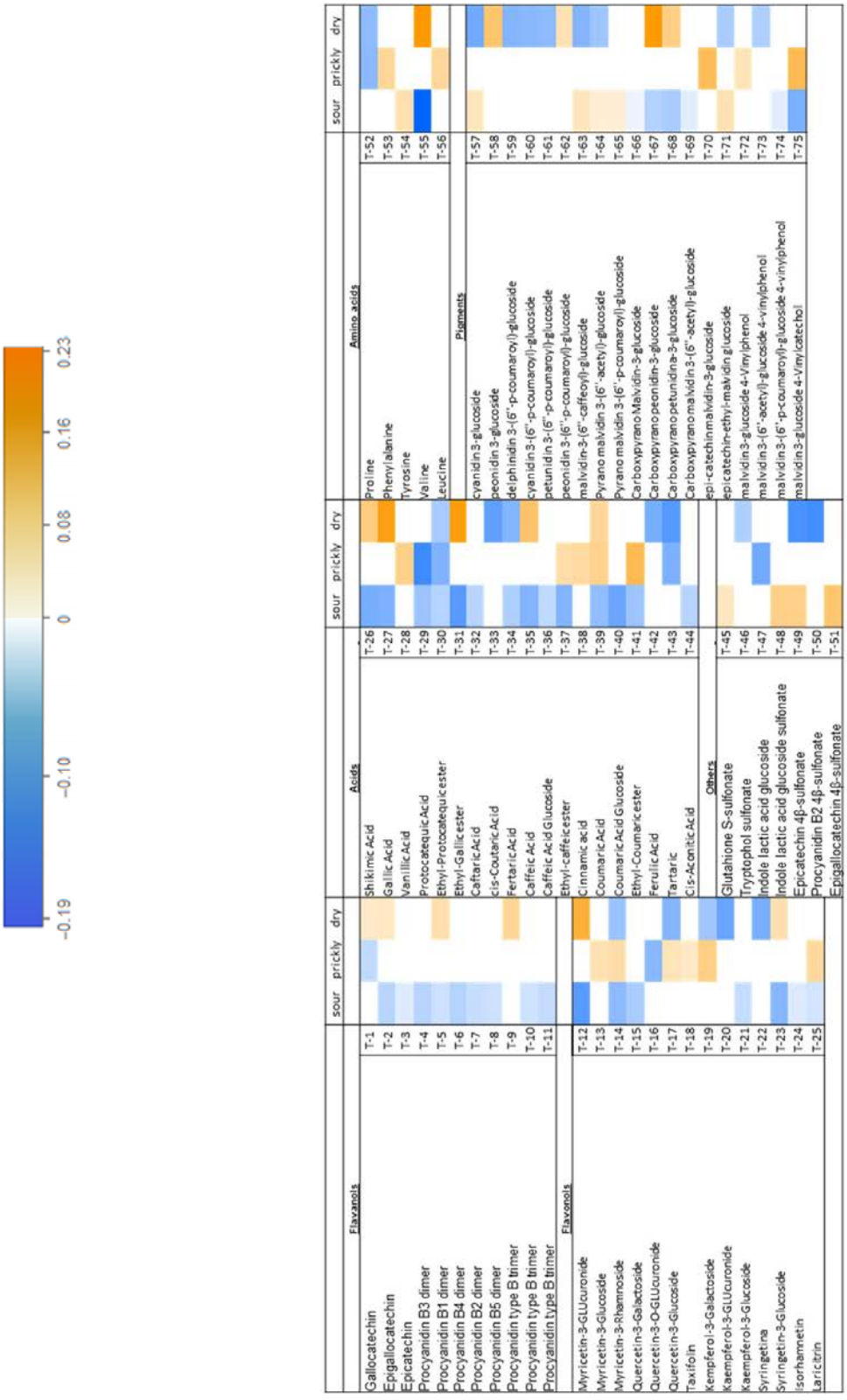
	Compound	rt (min)	Chemical formula	Theoretical m/z	Measured m/z	error (ppm)	HMDB ID	CHEBI ID	ChemSpider ID
	Acids								
	Phenolic Acids								
35	Shikimic acid	2.15	C7H10O5	173.0455	173.0467	-6.93	HMDB0003070		8412
36	Gallic acid	5.01	C7H6O5	169.0137	169.0122	8.88	HMDB0005807	CHEBI:30778	361
37	Vanillic acid	16.62	C8H8O4	167.0350	167.0349	0.60	HMDB0000484		8155
38	Protocatequic acid	7.32	C7H6O4	153.0193	153.0200	-4.57	HMDB0001856		
39	Ethyl-Protocatequic ester	19.36	C9H10O4	181.0506	181.0507	-0.55		CHEBI:132364	69954
40	Ethyl gallic acid	14.72	C9H10O5	197.0453	197.0427	13.19	HMDB0033836	CHEBI:87247	12693
41	Ellagic acid	20.08	C14H608	300.9990	300.9973	5.64	HMDB0002899	CHEBI:4775	4445149
42	Gentisic acid	13.03	C7H6O4	153.0193	153.0197	-2.61	HMDB0000152		3350
	Cinnamics								
43	Caftaric acid	7.99	C13H12O9	311.0409	311.0397	3.74	HMDB0013680	CHEBI:76075	8033613
44	cis-Coutaric acid	9.66	C13H12O8	295.0459	295.0466	-2.21		CHEBI:76096	35013748
45	trans-Coutaric acid	10.01	C13H12O8	295.0459	295.0462	-0.86		CHEBI:76095	4816367
46	Fertaric acid	11.23	C14H14O9	325.0565	325.0547	5.57		CHEBI:91032	20058463
47	Caffeic acid	12.15	C9H8O4	179.0350	179.0332	9.99	HMDB0003501	CHEBI:36281	600426
48	Caffeic acid 3-glucoside	9.89	C15H18O9	341.0870	341.0865	1.47			4445073
49	Ethyl-caffeic ester	20.81	C11H12O4	207.0657	207.0659	-0.97		CHEBI:132714	4476132
50	Cinnamic acid	15.27	C9H8O2	147.0452	147.0449	2.04	HMDB0000567		
51	p-Coumaric acid	15.31	C9H8O3	163.0401	163.0392	5.52	HMDB0041592		553148
52	Coumaric acid 2-glucoside	11.27	C15H18O8	325.0928	325.0933	-1.54			
53	Coumaric acid 3-glucoside	12.06	C15H18O8	325.0928	325.0929	-0.31			
54	Coumaric acid 4-glucoside	13.27	C15H18O8	325.0928	325.0932	-1.23			
55	p-Coumarate ethyl ester	21.27	C11H12O3	191.0708	191.0684	12.66	HMDB0131282		589606
56	Ferulic acid	16.64	C10H10O4	193.0506	193.0510	-2.07	HMDB0000954		393368
57	Ferulic acid glucoside	12.10	C16H20O9	355.1030	355.1063	-9.29			79715434
	Organic acids								
58	Tartaric acid	1.49	C4H6O6	149.0092	149.0068	15.89		CHEBI:26849	852
59	Cis-Aconitic Acid	2.01	C6H6O6	173.0092	173.0095	-1.73	HMDB0000072		558863

Annexe III-3.3. Continued

	Compound	rt (min)	Chemical formula	Theoretical m/z	Measured m/z	error (ppm)	HMDB ID	CHEBI ID	ChemSpider ID
	Others								
60	Glutahione S-sulfonate	1.86	C10H18N3O9S2	386.0328	386.0305	5.96		CHEBI:147425	
61	Pantetheine sulfonate	6.96	C11H22N2O7S2	357.0790	357.0797	-1.96			
62	Tryptophol sulfonate	11.00	C10H11NO4S	240.0336	240.0336	0.00			
63	Indole lactic acid glucoside	14.48	C17H21NO8	366.1196	366.1209	-3.55			
64	Indole lactic acid glucoside sulfonate	10.42	C17H21NO11S	446.0763	446.078	-3.91			
65	Epicatechin 4β-sulfonate	4.70	C15H14O9S	369.0286	369.0258	7.59			
66	Procyanidin B2 4β-sulfonate	5.68	C30H26O15S	657.0920	657.0923	-0.51			
67	Epigallocatechin 4β-sulfonate	3.81	C15H14O10S	385.0234	385.0234	0.26			
	Amino acids								
68	Arginine	1.22	C9H14N4O2	175.1189	175.1165	13.97	HMDB0003416	CHEBI:29016	227
69	Proline	1.58	C5H9NO2	116.0706	116.0692	12.05	HMDB0000162	CHEBI:26271	594
70	Phenylalanine	6.20	C9H11NO2	166.0862	166.0862	0.30	HMDB0000159	CHEBI:28044	14847048
71	Tyrosine	4.18	C9H11NO3	182.0812	182.0814	-1.30	HMDB0000158	CHEBI:18186	1121
72	Valine	1.39	C5H11NO2	118.0862	118.0853	8.04	HMDB0000883	CHEBI:27266	1148
73	Leucine	4.40	C6H13NO2	132.1019	132.1021	-1.52	HMDB0000687	CHEBI:25017	14833106

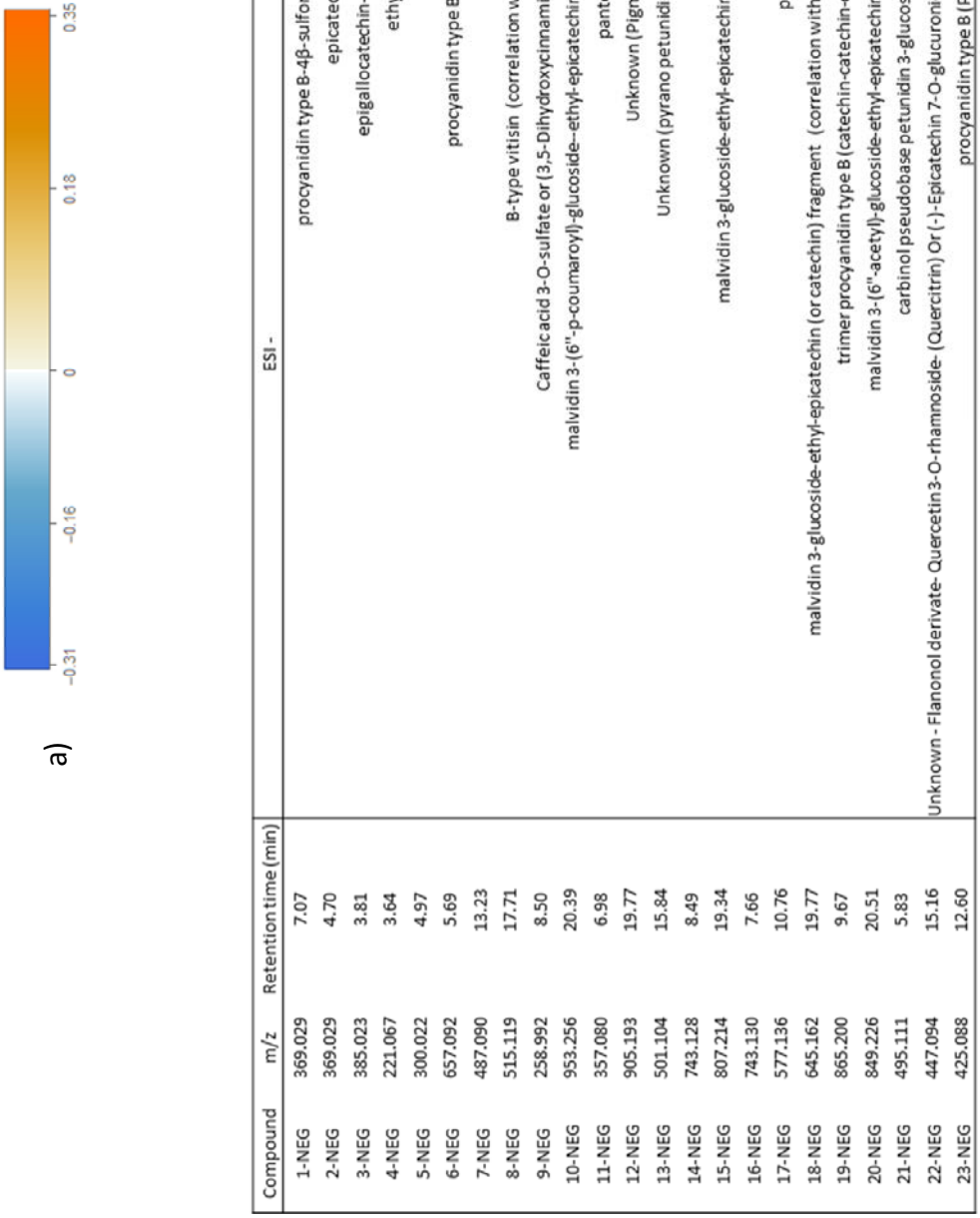
	Compound	rt (min)	Chemical formula	Theoretical m/z	Measured m/z	error (ppm)	HMDB ID	CHEBI ID	ChemSpider ID
	Pigments								
74	delphinidin 3-glucoside	11.50	C21H21O12	465.1028	465.1028	-0.10	HMDB37997	CHEBI:31463	391783
75	cyanidin 3-glucoside	12.85	C21H21O11	449.1078	449.1078	0.08	HMDB0030684	CHEBI:28426	390284
76	petunidin 3-glucoside	13.42	C22H23O12	479.1184	479.1169	3.14	HMDB38097	CHEBI:31985	391784
77	peonidin 3-glucoside	14.71	C22H23O11	463.1235	463.1225	2.13	HMDB0013689	CHEBI:74793	391786
78	malvidin 3-glucoside	14.98	C23H25O12	493.1341	493.1337	0.72	HMDB30777	CHEBI:31799	391785
79	delphinidin 3-(6''-acetyl)-glucoside	17.00	C23H23O13	507.1133	507.1118	2.99	HMDB38004	CHEBI:75678	30777226
80	petunidin 3-(6''-acetyl)-glucoside	18.53	C24H25O13	521.1290	521.1290	-0.06		CHEBI:147427	30779241
81	peonidin 3-(6''-acetyl)-glucoside	19.70	C24H25O12	505.1341	505.1303	7.43		CHEBI:75697	30779239
82	malvidin 3-(6''-acetyl)-glucoside	19.67	C25H27O13	535.1446	535.1427	3.58	HMDB38008	CHEBI:75689	30779236
83	delphinidin 3-(6''-p-coumaroyl)-glucoside	20.04	C30H27O14	611.1395	611.1403	-1.31	HMDB30099	CHEBI:75677	26559505
84	cyanidin 3-(6''-p-coumaroyl)-glucoside	20.24	C30H27O13	595.1446	595.1478	-5.38	HMDB37982	CHEBI:29560	4445294
85	petunidin 3-(6''-p-coumaroyl)-glucoside	20.24	C31H29O14	625.1552	625.1564	-1.95		CHEBI:75709	4445294
86	peonidin 3-(6''-p-coumaroyl)-glucoside	20.40	C31H29O13	609.1603	609.1609	-1.04		CHEBI:75707	30779238
87	malvidin 3-(6''-p-coumaroyl)-glucoside	20.37	C32H31O14	639.1708	639.1726	-2.82	HMDB0038012	CHEBI:75693	30779237
88	malvidin-3-(6''-caffeoyl)-glucoside	20.08	C32H31O15	655.1657	655.1680	-3.51		CHEBI:131453	
89	Pyrano malvidin-3-glucoside (Vitisin B)	17.82	C25H25O12	517.1341	517.1355	-2.80	HMDB0036825		58191428
90	Pyrano peonidin-3-glucoside (Vitisin B)	17.07	C24H23O11	487.1235	487.1234	0.21			
91	Pyrano petunidin-3-glucoside (Vitisin B)	15.92	C24H23O12	503.1184	503.1170	2.78			
92	Pyrano malvidin 3-(6''-acetyl)-glucoside	18.56	C27H27O13	559.1446	559.1429	3.04			
93	Pyrano peonidin 3-(6''-acetyl)-glucoside	18.28	C26H25O12	529.1341	529.1333	1.51			
94	Pyrano malvidin 3-(6''-p-coumaroyl)-glucoside	20.18	C34H31O14	663.1708	663.1680	4.22			
95	Carboxypyrano malvidin-3-glucoside (Vitisin A)	18.90	C26H25O14	561.1239	561.1250	-1.99	HMDB0036348		24842477
96	Carboxypyrano peonidin-3-glucoside (Vitisin A)	18.29	C25H23O13	531.1133	531.1133	0.00			
97	Carboxypyrano petunidina-3-glucoside (Vitisin A)	16.79	C25H23O14	547.1082	547.1101	-3.47			
98	Carboxypyrano malvidin 3-(6''-acetyl)-glucoside	19.47	C28H27O15	603.1344	603.1356	-1.99			
99	Malvidin 3-glucoside-8-ethyl-proanthocyanidin	19.08	C55H53O24	1097.2922	1097.2954	-2.92			
100	epi/catechin malvidin-3-glucoside	9.58	C38H37O18	781.1974	781.1959	1.97			9449309
101	malvidin 3-glucoside ethyl-epi/catechin	19.40	C40H41O18	809.2287	809.2272	1.90			13170147
102	malvidin 3-glucoside ethyl-epi/catechin	19.79	C40H41O18	809.2287	809.2294	-0.81			13170147
103	malvidin 3-glucoside ethyl-epi/catechin	19.92	C40H41O18	809.2287	809.2294	-0.81			13170147
104	epi/catcehin-ethyl-malvidin 3-(6''-p-coumaroyl)-glucoside	20.43	C49H47O20	955.2655	955.2704	-5.11			
105	malvidin 3-glucoside 4Vinylphenol	20.72	C31H29O13	609.1603	609.1629	-4.32	HMDB0031968		30776925
106	malvidin 3-(6''-acetyl)-glucoside 4-vinylphenol	20.76	C33H31O14	651.1708	651.1733	-3.84			
107	malvidin 3-(6''-p-coumaroyl)-glucoside 4-vinylphenol	20.84	C40H35O15	755.1974	755.1974	0.00			
108	malvidin 3-glucoside 4-Vinylcatechol(Pinotin A)	20.60	C31H29O14	625.1552	625.1545	1.09	HMDB0029240		10286568

Annexe III-3.4. Map with regression coefficients of variables included in validated PLS-model predicting some flavours from target chemical variables.



Annexe III-3.5. Map with regression coefficients of variables included in validated PLS-model predicting some flavours from untargeted chemical variables a) variables identified in ESI- b) variables identified in ESI+.

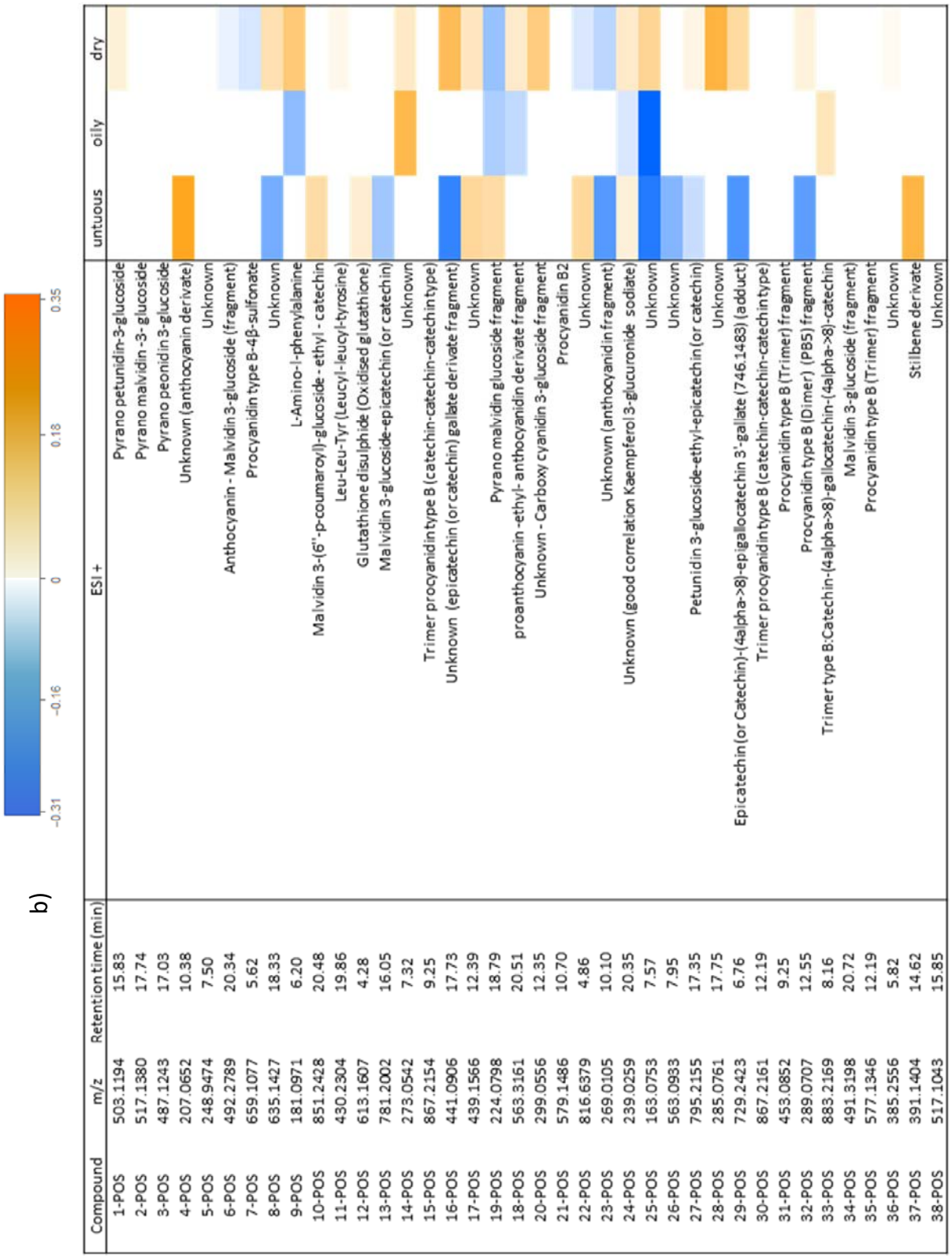
a)



Compound	m/z	Retention time (min)	ESI -
1-NEG	369.029	7.07	procyanidin type B-4β-sulfonate fragment
2-NEG	369.029	4.70	epicatechin sulfonate
3-NEG	385.023	3.81	epigallocatechin-4β-sulfonates
4-NEG	221.067	3.64	ethyl glucuronide
5-NEG	300.022	4.97	Unknown
6-NEG	657.092	5.69	procyanidin type B-4β-sulfonate
7-NEG	487.090	13.23	Unknown
8-NEG	515.119	17.71	B-type vitisin (correlation with 353, 0661)
9-NEG	258.992	8.50	Caffeic acid 3-O-sulfate or (3,5-Dihydroxycinnamic acid sulfate)
10-NEG	953.256	20.39	malvidin 3-(6"-p-coumaroyl)-glucoside--ethyl-epicatechin (or catechin)
11-NEG	357.080	6.98	pantetheine-SO3H
12-NEG	905.193	19.77	Unknown (Pigment derivate)
13-NEG	501.104	15.84	Unknown (pyrano petunidin 3-glucoside)
14-NEG	743.128	8.49	Unknown
15-NEG	807.214	19.34	malvidin 3-glucoside-ethyl-epicatechin (or catechin)
16-NEG	743.130	7.66	Unknown
17-NEG	577.136	10.76	procyanidin B2
18-NEG	645.162	19.77	malvidin 3-glucoside-ethyl-epicatechin (or catechin) fragment (correlation with 807.2151434)
19-NEG	865.200	9.67	trimer procyanidin type B (catechin-catechin-catechin type)
20-NEG	849.226	20.51	malvidin 3-(6"-acetyl)-glucoside-ethyl-epicatechin (or catechin)
21-NEG	495.111	5.83	carbinol pseudobase petunidin 3-glucoside derivative
22-NEG	447.094	15.16	Unknown - Flanonol derivate- Quercetin 3-O-rhamnoside- (Quercitrin) Or (-)-Epicatechin 7-O-glucuronide (Fragment)
23-NEG	425.088	12.60	procyanidin type B (PB5) fragment

Annexe III-3.5. Continued

b)



Compound	m/z	Retention time (min)	ESI +
1-POS	503.1194	15.83	Pyrano petunidin-3-glucoside
2-POS	517.1380	17.74	Pyrano malvidin -3- glucoside
3-POS	487.1243	17.03	Pyrano peonidin 3-glucoside
4-POS	207.0652	10.38	Unknown (anthocyanin derivate)
5-POS	248.9474	7.50	Unknown
6-POS	492.2789	20.34	Anthocyanin - Malvidin 3-glucoside (fragment)
7-POS	659.1077	5.62	Procyanidin type B-4β-sulfonate
8-POS	635.1427	18.33	Unknown
9-POS	181.0971	6.20	L-Amino-l-phenylalanine
10-POS	851.2428	20.48	Malvidin 3-(6''-p-coumaroyl)-glucoside - ethyl - catechin
11-POS	430.2304	19.86	Leu-Leu-Tyr (Leucyl-leucyl-tyrosine)
12-POS	613.1607	4.28	Glutathione disulphide (Oxidised glutathione)
13-POS	781.2002	16.05	Malvidin 3-glucoside-epicatechin (or catechin)
14-POS	273.0542	7.32	Unknown
15-POS	857.2154	9.25	Trimer procyanidin type B (catechin-catechin-catechin type)
16-POS	441.0906	17.73	Unknown (epicatechin (or catechin) gallate derivate fragment)
17-POS	439.1566	12.39	Unknown
19-POS	224.0798	18.79	Pyrano malvidin glucoside fragment
18-POS	563.3161	20.51	proanthocyanin -ethyl- anthocyanidin derivate fragment
20-POS	299.0556	12.35	Unknown - Carboxy cyanidin 3-glucoside fragment
21-POS	579.1486	10.70	Procyanidin B2
22-POS	816.6379	4.86	Unknown
23-POS	269.0105	10.10	Unknown (anthocyanidin fragment)
24-POS	239.0259	20.35	Unknown (good correlation Kaempferol 3-glucuronide sodiate)
25-POS	163.0753	7.57	Unknown
26-POS	563.0933	7.95	Unknown
27-POS	795.2155	17.35	Petunidin 3-glucoside-ethyl-epicatechin (or catechin)
28-POS	285.0761	17.75	Unknown
29-POS	729.2423	6.76	Epicatechin (or Catechin)-(4alpha->8)-epigallocatechin 3'-gallate (746.1483) (adduct)
30-POS	867.2161	12.19	Trimer procyanidin type B (catechin-catechin-catechin type)
31-POS	453.0852	9.25	Procyanidin type B (Trimer) fragment
32-POS	289.0707	12.55	Procyanidin type B (Dimer) (PB5) fragment
33-POS	883.2169	8.16	Trimer type B:Catechin-(4alpha->8)-gallocatechin-(4alpha->8)-catechin
34-POS	491.3198	20.72	Malvidin 3-glucoside (fragment)
35-POS	577.1346	12.19	Procyanidin type B (Trimer) fragment
36-POS	385.2556	5.82	Unknown
37-POS	391.1404	14.62	Stilbene derivate
38-POS	517.1043	15.85	Unknown

Annexe IV-2. 1. Detailed list of the 20 wines employed in the study.

Code	Vintage	Variety	Origin	% etanol (v/v)	pH	VA (g L^{-1})	TA (g L^{-1})	RS (g L^{-1})	MA (g L^{-1})	LA (g L^{-1})	TPI
SM1	2017	Gamay	Côte du Puy, France	13.5	3.32	0.3	5.7	2.9	0.0	0.9	65
SM4	2018	Mencía	Bierzo, Spain	13.8	3.73	0.4	4.2	2.1	0.0	1.3	59
SM5	2017	Malbec	Valle de Uco, Argentina	13.4	3.62	0.3	5.0	2.5	0.0	1.0	46
SM6	2018	Bonarda	Mendoza, Argentina	12.9	3.76	0.5	4.8	1.8	0.0	1.4	57
SM7	2017	Mencía	Bierzo, Spain	12.7	3.76	0.9	5.4	2.3	0.0	1.9	57
SM8	2015	Gamay	Beaujolais, France	12.7	3.48	0.3	5.0	1.6	0.0	1.0	59
SM10	2017	Mencía	Bierzo, Spain	14.0	3.75	0.4	4.1	2.5	0.0	1.2	61
SM12	2016	Pint Noir	Bourgogne, France	12.8	3.50	0.3	4.4	2.0	0.0	1.0	54
SM15	2017	Carignan	Montsant, Spain	12.5	3.32	0.2	5.7	2.5	0.1	1.0	26
SM16	2017	Prieto Picudo	Castilla, Spain	12.9	3.51	0.3	5.7	2.4	0.0	0.8	48
SM23	2018	Merlot	Somontano, Spain	14.8	3.37	0.3	6.5	1.7	0.5	0.6	60
SM25	2018	Merlot	Somontano, Spain	14.8	3.60	0.4	6.2	2.6	0.4	0.9	111
SM26	2018	Cabernet Sauvignon	Somontano, Spain	15.2	3.63	0.2	6.1	3.9	0.6	0.7	82
SM29	2018	Grenache	Borja, Spain	13.8	3.37	0.2	6.3	1.9	0.5	0.3	47
SM30	2018	Syrah	Borja, Spain	14.3	3.49	0.1	5.6	1.7	0.4	0.7	61
SM31	2018	Merlot	Borja, Spain	14.9	3.62	0.3	5.1	1.7	0.1	0.6	58
SM35	2018	Tempranillo	Rioja, Spain	14.3	3.67	0.1	3.9	2.1	0.0	0.6	40
SM37	2017	Tempranillo	Rioja, Spain	11.8	3.40	0.3	5.5	2.3	0.2	1.0	43
SM41	2018	Bobal	La Mancha, Spain	12.4	3.59	0.3	5.1	2.6	0.0	1.0	60
SM42	2018	Bobal	La Mancha, Spain	12.1	3.55	0.3	5.3	2.7	0.0	0.9	55

VA: Volatile acidity (expressed in g L-1 of acetic acid); TA: Titratable acidity (expressed in g L-1 of tartaric acid): RS: reducing sugars; MA: malic acid; LA: Lactic acid; TPI: total polyphenol index calculated as absorbance at 280 nm.

Annexe IV-2. 2. Average scores of the 18 participants (± error calculated as $s/n^{1/2}$: (s) standard deviation; (n) number of participants) for significantly (P<0.1) sensory attributes calculated for the 20 selected wines evaluated with nose clips following rate-K attributes methodology.

codes	bitter	sweet	sour	dry	dry on tongue side	dry on palate	unctuous	oily	silky	watery	burning	puckering
SM1	1.9±0.5	1.4±0.5*	1.2±0.4	2.8±0.5	1.5±0.5*	2.1±0.6	0.8±0.4	0.2±0.1	0.1±0.1	1.1±0.5	0.6±0.3	0.4±0.3
SM4	2.1±0.6	0.5±0.2	0.6±0.2†	1.5±0.5	0.2±0.2	1.9±0.6	0.5±0.3	1.0±0.5	0.4±0.2	0.8±0.5	0.7±0.4	0.6±0.3
SM5	0.9±0.4	0.7±0.3	1.2±0.4	2.6±0.7	0.9±0.4	0.6±0.4	1.1±0.4	0.5±0.3	0.3±0.2	0.8±0.5	1.4±0.5	0.8±0.4
SM6	1.8±0.5	0.4±0.2	1.2±0.4	1.8±0.5	0.7±0.4	1.0±0.4	0.8±0.4	0.7±0.4	0.8±0.4	1.7±0.5*	0.8±0.4	0.4±0.3
SM7	1.4±0.4	1.1±0.4	2.0±0.5	2.1±0.5	1.2±0.5	0.5±0.4	1.4±0.5*	0.4±0.3	0.8±0.4	0.8±0.4	0.6±0.3	1.0±0.4*
SM8	1.7±0.5	1.3±0.5	1.0±0.4	1.1±0.4	1.2±0.5	2.1±0.6	1.3±0.5	1.1±0.5*	0.7±0.4	0.4±0.2	1.3±0.5	0.7±0.3
SM10	1.9±0.6	0.5±0.3	1.1±0.4	2.8±0.6	1.2±0.5	2.5±0.7	0.5±0.3	0.4±0.2	0.1±0.1	0.6±0.4	1.4±0.5*	0.6±0.4
SM12	1.6±0.5	0.6±0.3	1.8±0.5	0.9±0.4†	0.8±0.4	1.3±0.5	0.2±0.2	0.3±0.2	0.4±0.2	1.2±0.5	0.7±0.3	0.2±0.2
SM15	0.4±0.4	0.7±0.3	2.2±0.5	3.2±0.6	0.7±0.4	0.3±0.2†	1.1±0.4	0.8±0.4	0.9±0.4*	1.0±0.5	0.6±0.4	0.1±0.1
SM16	0.8±0.3	0.3±0.2	1.2±0.4	2.5±0.6	0.4±0.3	1.1±0.5	0.2±0.2	0.9±0.4	0.3±0.2	0.9±0.5	0.9±0.4	0.3±0.3
SM23	0.7±0.3	0.7±0.3	1.7±0.4	3.3±0.5	1.1±0.5	2.5±0.7	0.3±0.2	0.1±0.1	0.2±0.2	0.3±0.2	0.7±0.4	0.3±0.2
SM25	1.7±0.5	0.0±0.0†	0.9±0.4	3.3±0.5	1.3±0.6	3.3±0.7*	0.0±0.0†	0.0±0.0	0.0±0.0†	0.0±0.0†	1.1±0.5	0.2±0.2
SM26	2.1±0.5*	0.3±0.2	1.3±0.4	3.4±0.5	1.1±0.5	2.3±0.6	0.1±0.1	0.2±0.2	0.0±0.0†	0.6±0.3	1.1±0.4	0.0±0.0
SM29	0.4±0.3	0.5±0.2	1.9±0.4	2.3±0.6	0.2±0.2	0.7±0.4	0.2±0.2	0.2±0.2	0.4±0.3	0.8±0.3	0.9±0.4	0.6±0.3
SM30	0.8±0.4	0.6±0.3	1.4±0.5	3.4±0.6*	0.2±0.2	0.8±0.5	0.8±0.4	0.0±0.0	0.7±0.4	0.5±0.3	1.1±0.4	0.4±0.3
SM31	1.8±0.5	0.2±0.1	1.7±0.5	2.2±0.5	1.0±0.5	1.2±0.5	0.5±0.3	0.2±0.2	0.1±0.1	0.2±0.2	0.8±0.4	0.1±0.1
SM35	1.9±0.5	0.7±0.3	1.4±0.5	2.3±0.5	1.5±0.5	1.2±0.5	0.4±0.3	0.3±0.3	0.4±0.2	0.1±0.1	1.1±0.4	0.2±0.2
SM37	0.3±0.2†	0.5±0.3	2.5±0.5*	1.6±0.5	0.1±0.1†	1.1±0.5	0.2±0.2	0.0±0.0	0.4±0.3	1.2±0.5	0.4±0.2	0.2±0.2
SM41	1.4±0.5	0.7±0.4	1.3±0.5	1.3±0.5	1.2±0.4	1.9±0.6	0.4±0.3	0.3±0.2	0.3±0.1	0.5±0.1	0.2±0.4†	0.0±0.1†
SM42	0.6±0.3	1.2±0.5	1.4±0.4	1.9±0.5	0.4±0.4	2.5±0.6	0.1±0.1	0.0±0.0	0.2±0.1	0.2±0.2	0.2±0.1†	0.0±0.0†
max*	2.1	1.4	2.5	3.4	1.5	3.3	1.4	1.1	0.9	1.7	1.4	1.0
min†	0.3	0.0	0.6	0.9	0.1	0.3	0.0	0.0	0.0	0.0	0.2	0.0
max-min	1.8	1.4	1.9	2.6	1.4	3.0	1.4	1.1	0.9	1.7	1.3	1.0
average	1.3	0.6	1.4	2.3	0.8	1.5	0.6	0.4	0.4	0.7	0.8	0.3

Annexe IV-2. 3. Average scores of the 17 participants (± error calculated as $s/n^{1/2}$: (s) standard deviation; (n) number of participants) for significantly (P<0.1) sensory attributes calculated for the 20 F_{antho} fractions evaluated following rate-K attributes methodology.

codes	bitter	sweet	sour	dry	unctuous	grainy	silky	watery	mouthcoating	persistent
F_{antho}_SM1	1.7±0.5	0.2±0.2	0.8±0.4	0.4±0.4	0.8±0.4	0.0±0.0†	0.9±0.5	0.8±0.4	0.3±0.2	0.4±0.3
F_{antho}_SM4	1.6±0.4	0.4±0.2	0.4±0.2	1.4±0.6	0.4±0.2	0.0±0.0†	0.6±0.3	0.9±0.4	0.4±0.2	0.4±0.2
F_{antho}_SM5	6.1±0.5*	0.0±0.0†	0.5±0.4	1.5±0.7	0.0±0.0†	0.0±0.0†	0.2±0.2†	0.0±0.0†	0.0±0.0†	4.8±0.7*
F_{antho}_SM6	2.1±0.5	0.1±0.1	0.3±0.2	1.4±0.6	0.0±0.0†	0.4±0.4	0.6±0.3	0.7±0.4	0.2±0.2	0.9±0.4
F_{antho}_SM7	1.0±0.4	0.4±0.2	0.5±0.3	0.5±0.5	0.4±0.3	0.0±0.0†	0.9±0.5	0.9±0.4	0.3±0.2	0.3±†0.2
F_{antho}_SM8	1.0±0.3	0.8±0.3	0.3±0.2	0.5±0.4	0.5±0.3	0.0±0.0†	1.2±0.5	1.6±0.5*	0.4±0.4	0.4±0.3
F_{antho}_SM10	2.5±0.5	0.5±0.2	0.9±0.4	1.2±0.6	0.5±0.3	0.0±0.0†	1.1±0.4	0.2±0.2	0.3±0.2	0.5±0.3
F_{antho}_SM12	1.9±0.4	0.2±0.1	0.7±0.3	1.7±0.6	0.6±0.3	0.1±0.1	0.9±0.5	1.1±0.4	0.2±0.2	0.4±0.2
F_{antho}_SM15	0.5±0.2†	0.6±0.3	0.2±0.1	0.4±0.3†	0.2±0.2	0.0±0.0†	1.3±0.5	1.4±0.5	0.3±0.2	0.4±0.3
F_{antho}_SM16	0.8±0.4	0.5±0.3	0.2±0.2	0.5±0.4	0.5±0.3	0.1±0.1	1.1±0.4	1.1±0.4	0.3±0.2	0.5±0.3
F_{antho}_SM23	3.1±0.5	0.1±0.1	0.2±0.2	1.3±0.5	0.0±0.0†	0.0±0.0†	0.6±0.3	1.1±0.4	0.3±0.3	0.9±0.4
F_{antho}_SM25	4.6±0.5	0.2±0.2	0.2±0.1	2.2±0.7	0.3±0.2	0.2±0.2	0.2±0.2	0.4±0.2	0.1±0.1	1.1±0.4
F_{antho}_SM26	3.2±0.6	0.6±0.2	0.3±0.3	2.7±0.7*	0.0±0.0†	0.1±0.1	0.8±0.4	0.0±0.0†	0.0±0.0†	1.2±0.4
F_{antho}_SM29	1.4±0.4	0.0±0.0†	0.1±0.1†	1.8±0.7	0.4±0.3	0.4±0.4	0.2±0.2	0.5±0.3	0.9±0.4*	0.4±0.3
F_{antho}_SM30	1.9±0.6	0.2±0.2	0.1±0.1†	1.4±0.7	0.0±0.0†	0.9±0.5*	0.3±0.3	0.2±0.2	0.6±0.3	0.4±0.3
F_{antho}_SM31	2.1±0.5	0.2±0.1	0.1±0.1†	1.3±0.6	0.4±0.2	0.0±0.0†	0.8±0.3	0.3±0.2	0.2±0.2	1.0±0.4
F_{antho}_SM35	0.6±0.3	1.1±0.4*	0.3±0.2	0.5±0.2	0.5±0.3	0.1±0.1	1.5±0.5*	1.6±0.5*	0.7±0.3	0.6±0.3
F_{antho}_SM37	2.2±0.5	0.4±0.2	1.2±0.4*	2.0±0.7	0.0±0.0†	0.0±0.0†	0.4±0.2	0.4±0.2	0.2±0.2	0.5±0.3
F_{antho}_SM41	1.9±0.5	0.5±0.3	0.5±0.2	0.4±0.3†	0.2±0.2	0.0±0.0†	0.5±0.4	1.1±0.5	0.8±0.4	0.6±0.4
F_{antho}_SM42	0.9±0.4	0.3±0.2	0.1±0.1†	0.9±0.4	0.9±0.5*	0.4±0.2	0.6±0.4	0.9±0.4	0.7±0.4	0.4±0.3
F_{antho} max*	6.1	1.1	1.2	2.7	0.9	0.9	1.5	1.6	0.9	4.8
F_{antho} min†	0.5	0.0	0.1	0.4	0.0	0.0	0.2	0.0	0.0	0.3
F_{antho} max-min	5.6	1.1	1.1	2.4	0.9	0.9	1.3	1.6	0.9	4.5
F_{antho} average	2.1	0.4	0.4	1.2	0.3	0.1	0.7	0.8	0.4	0.8

Annexe IV-2. 4. Average scores of the 17 participants (± error calculated as $s/n^{1/2}$: (s) standard deviation; (n) number of participants) for significantly (P<0.1) sensory attributes calculated for the 20 F_{tannin} fractions evaluated following rate-K attributes methodology.

codes	bitter	sweet	dry	dry on tongue side	dry on palate	sticky	oily	silky	gummy	watery	persistent
F_{tannin}_SM1	2.9±0.6	1.1±0.5	2.2±0.6	1.3±0.5	1.3±0.5	0.9±0.5	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.1±0.1	1.6±0.6
F_{tannin}_SM4	2.5±0.5	0.4±0.2	2.2±0.6	1.4±0.6	1.1±0.4	0.9±0.5	0.4±0.3	0.1±0.1	0.0±0.0†	0.1±0.1	1.7±0.5
F_{tannin}_SM5	1.2±0.5	1.3±0.4*	1.5±0.5	0.1±0.1	0.4±0.3	0.3±0.2	0.4±0.3	1.1±0.5	0.0±0.0†	1.1±0.5	0.6±0.4
F_{tannin}_SM6	3.6±0.5	0.8±0.4	2.6±0.6	1.5±.5	1.6±0.6	0.8±0.5	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.9±0.4
F_{tannin}_SM7	1.5±0.5	0.4±0.2	1.4±0.5	0.5±0.3	0.5±0.3	0.6±0.4	0.4±0.2	1.1±.4	0.0±0.0†	1.2±0.4	0.6±0.3
F_{tannin}_SM8	0.8±0.3	1.1±0.4	1.2±0.4	0.9±0.4	0.5±0.3	0.5±0.3	0.3±0.3	0.9±0.4	0.6±0.4*	1.0±0.4	0.9±0.4
F_{tannin}_SM10	1.7±0.5	0.6±0.3	2.4±0.6	1.1±0.5	0.8±0.4	1.1±0.5	0.10.1±	0.3±0.2	0.20.2±	0.2±0.1	0.5±0.3†
F_{tannin}_SM12	1.8±0.5	0.4±0.2	1.4±0.4	0.6±0.4	1.1±0.4	0.4±0.2	0.30.3±	0.3±0.3	0.2±0.1	0.4±0.3	0.6±0.3
F_{tannin}_SM15	0.3±0.1†	1.2±0.5	0.8±0.4†	0.0±0.0†	0.0±0.0†	0.4±0.2	0.9±0.5*	1.2±0.5*	0.1±0.1	1.6±0.5*	0.8±0.4
F_{tannin}_SM16	1.5±0.5	0.4±0.2	1.5±0.5	0.6±0.5	1.1±0.5	1.4±0.5*	0.1±0.1	0.2±0.2	0.6±0.5*	0.2±0.2	1.2±0.5
F_{tannin}_SM23	2.5±0.5	0.2±0.2	2.2±0.6	1.3±0.5	1.5±0.6	0.7±0.4	0.3±0.3	0.5±0.4	0.0±0.0†	0.3±0.2	0.9±0.5
F_{tannin}_SM25	4.5±0.7*	0.1±0.1†	3.1±0.7	1.8±0.6*	2.2±0.7*	1.2±0.6	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.0±0.0†	2.2±0.7
F_{tannin}_SM26	3.9±0.6	0.1±0.1†	3.8±0.7*	1.6±0.6	2.2±0.7*	1.1±0.5	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.3±0.3	2.3±0.7*
F_{tannin}_SM29	0.4±0.2	0.6±0.3	1.5±0.4	0.5±0.4	0.2±0.2	0.6±0.3	0.5±0.3	0.7±0.4	0.2±0.2	1.1±0.5	0.5±0.3†
F_{tannin}_SM30	2.4±0.5	0.4±0.2	1.5±0.5	1.2±0.5	0.8±0.4	0.6±0.3	0.1±0.1	0.1±0.1	0.5±0.3	0.2±0.1	0.8±0.4
F_{tannin}_SM31	2.9±0.6	0.4±0.2	2.5±0.6	1.6±0.7	2.1±0.6	0.9±0.4	0.0±0.0†	0.6±0.4	0.2±0.2	0.4±0.3	0.8±0.4
F_{tannin}_SM35	0.9±0.4	0.9±0.4	2.5±0.5	0.2±0.2	0.4±0.3	0.2±0.2†	0.7±0.4	0.6±0.3	0.2±0.2	0.6±0.3	0.9±0.4
F_{tannin}_SM37	0.8±0.4	0.8±0.4	0.9±0.4	0.0±0.0†	0.0±0.0†	0.2±0.1†	0.5±0.2	0.8±0.4	0.6±0.4*	1.4±0.4	0.6±0.3
F_{tannin}_SM41	2.5±0.6	0.5±0.3	2.6±0.6	0.5±0.3	1.5±0.5	1.1±0.5	0.0±0.0†	0.4±0.3	0.0±0.0†	0.0±0.0†	0.8±0.4
F_{tannin}_SM42	3.2±0.6	0.3±0.2	2.6±0.7	1.1±0.5	2.0±0.6	1.4±0.6*	0.0±0.0†	0.0±0.0†	0.0±0.0†	0.0±0.0†	1.6±0.5
F_{tannin} max*	4.5	1.3	3.8	1.8	2.2	1.4	0.9	1.2	0.6	1.6	2.3
F_{tannin} min†	0.3	0.1	0.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.5
F_{tannin} max-min	4.2	1.2	2.9	1.8	2.2	1.2	0.9	1.2	0.6	1.6	1.8
F_{tannin} average	2.1	0.6	2.0	0.9	1.1	0.8	0.2	0.5	0.2	0.5	1.1

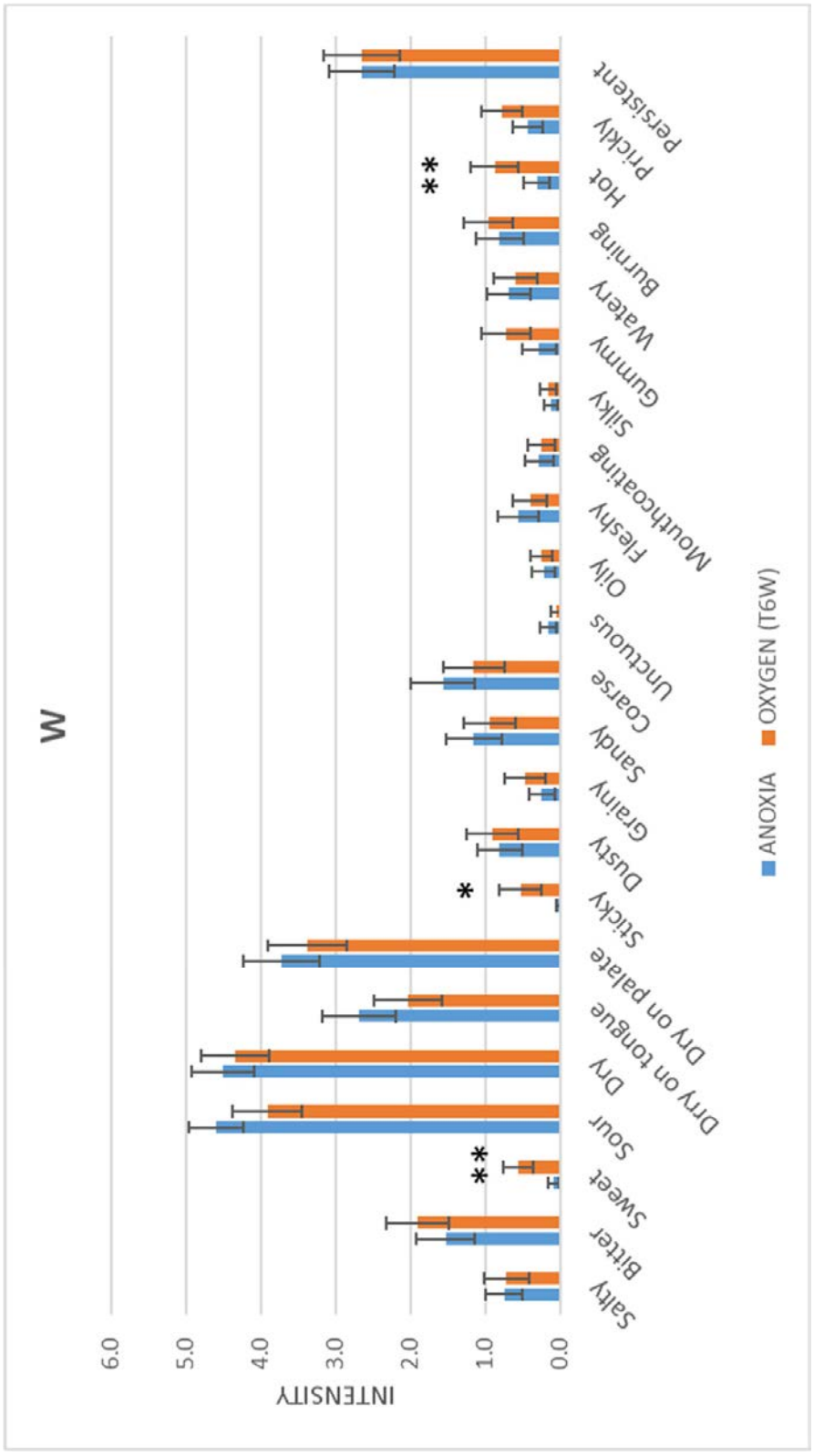
Annexe IV-3. 1. Original wine parameters.

Parameter	Description	Units	Value
pH	Potential of hydrogen	-	3.66 ± 0.00
TPI	Total polyphenol index	(a.u.)	64.550 ± 0.003
CI	Colour intensity	(a.u.)	15.29 ± 0.33
VA	Volatile acidity	g L^{-1} of acetic acid	0.19 ± 0.00
TA	Total acidity	g L^{-1} of tartaric acid	2.99 ± 0.00
RS	Reducing sugars	g L^{-1}	2.31 ± 0,03
ETOH	Ethanol content	%v/v	14.30 ± 0.042
MA	Malic acid	g L^{-1}	-
LA	Lactic acid	g L^{-1}	1.025 ± 0.007
CH_3CHO	Acetaldehyde	mg L^{-1}	6.4 ± 0,33
Fe	Iron	µg L^{-1}	1661.6 ± 18.3
Cu	Copper	µg L^{-1}	<LOQ
Mn	Manganese	µg L^{-1}	1094.0 ± 6.5
Zn	Zinc	µg L^{-1}	948.6 ± 25.6

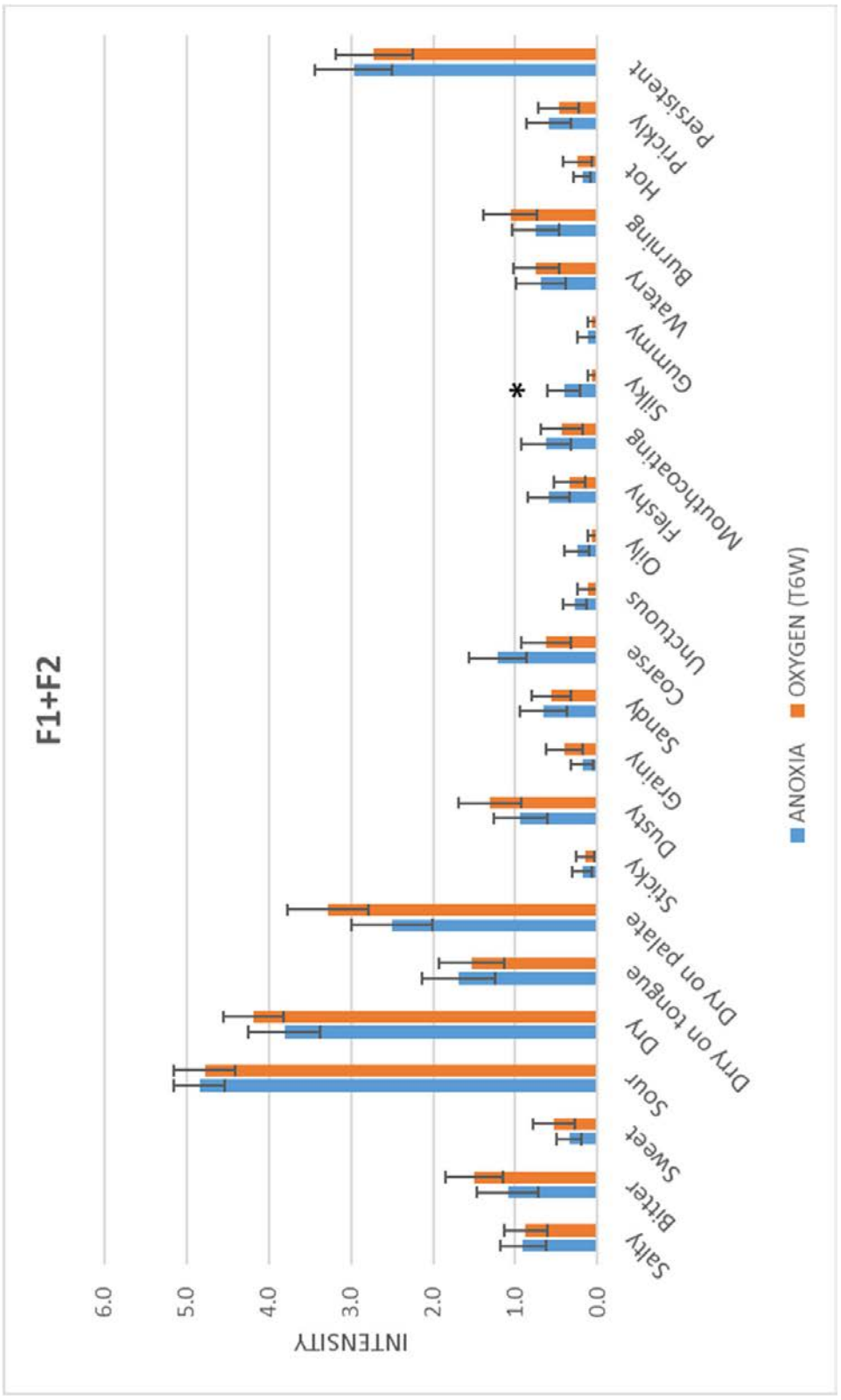
Annexe IV-3.2. Table with the list of taste and mouthfeel attributes evaluated. Two ANOVA (participants as random factor, wine as fixed factors) calculated to evaluate the effect of oxygen on taste and mouthfeel sensory properties. Significance values are shown; P-significance: *P<0.1, **P<0.05, ***p<0.01, -: not significant).

	W		F1+F2		F1L		F2		F11+F12		F13		F13+F2	
	T6W	T24W	T6W	T24W	T6W	T24W	T6W	T24W	T6W	T24W	T6W	T24W	T6W	T24W
Salty	-	-	-	-	-	**	-	-	-	**	**	-	-	-
Bitter	-	-	-	**	-	**	-	-	-	**	-	-	**	-
Sweet	**	-	-	-	-	-	-	-	-	-	-	-	-	**
Sour	-	-	-	-	-	-	**	-	-	-	-	-	-	**
Dry	-	-	-	-	-	-	*	-	-	-	-	-	-	-
Dry on tongue	-	-	-	-	*	-	***	-	-	-	-	-	-	-
Dry on palate	-	-	-	-	-	-	-	-	*	-	-	-	-	**
Sticky	*	-	-	-	-	-	-	-	-	-	-	-	-	-
Dusty	-	-	-	-	-	-	-	-	-	**	-	-	-	-
Grainy	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sandy	-	-	-	-	-	-	-	**	-	**	-	-	-	-
Coarse	-	**	-	**	-	-	*	*	-	-	-	-	-	-
Unctuous	-	-	-	-	-	-	-	*	-	-	*	-	-	-
Oily	-	-	-	-	-	**	-	-	-	-	-	-	-	-
Fleshy	-	-	-	-	-	-	-	*	-	-	-	-	-	-
Mouthcoating	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Silky	-	-	*	-	-	-	-	-	-	-	-	-	-	-
Gummy	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Watery	-	-	-	-	-	-	-	-	-	**	-	-	-	-
Burning	-	-	-	-	-	-	-	***	-	*	-	-	-	-
Hot	**	-	-	-	-	-	-	-	-	-	-	-	-	-
Prickly	-	-	-	-	-	-	-	-	-	-	-	-	-	**
Persistent	-	-	-	-	-	-	-	-	-	-	-	-	-	-

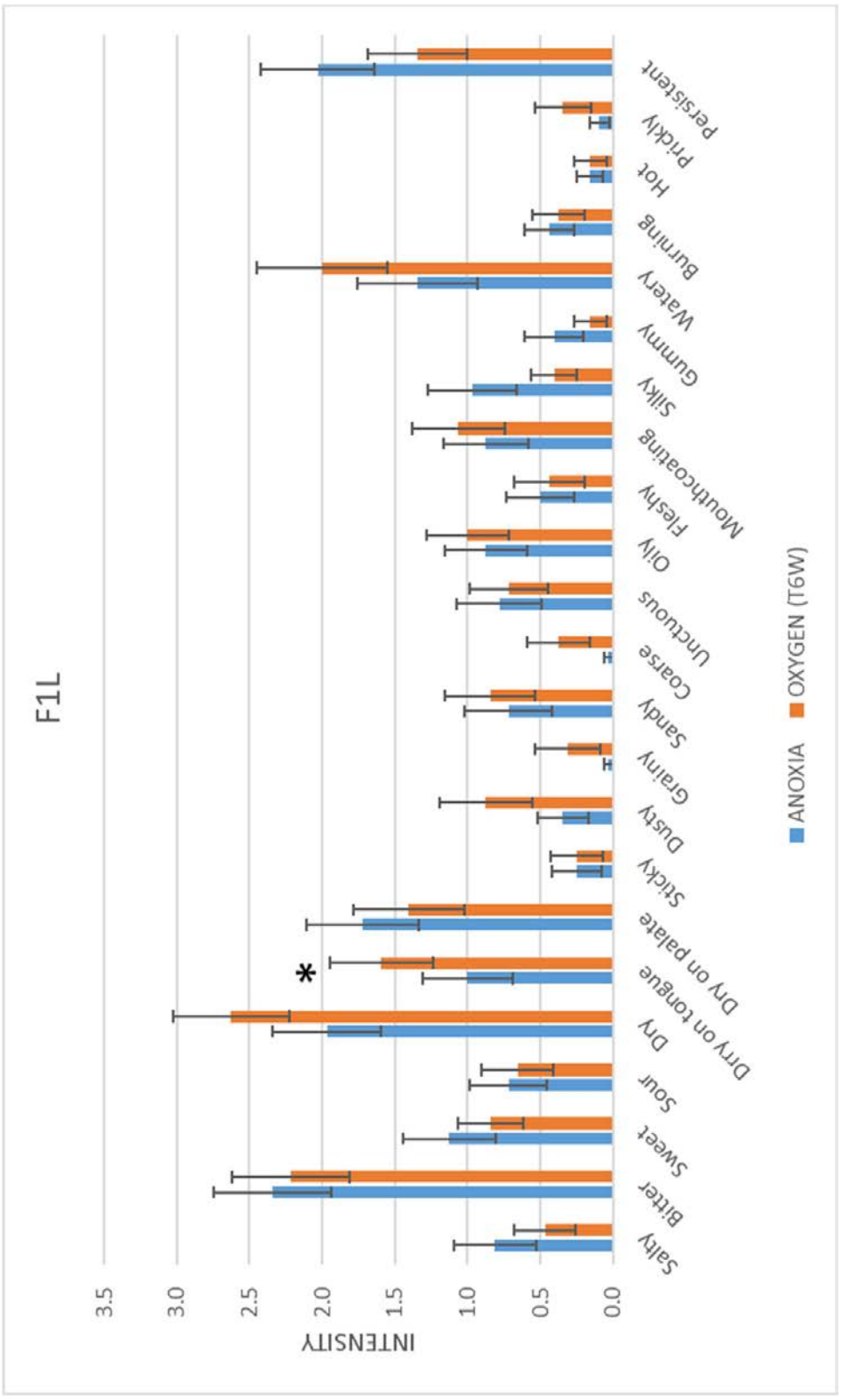
Annexe IV-3.3. Graphic representation of oxygen effect at T6W on taste and mouthfeel sensory properties, in each of the samples. Significance values are shown; P-significance: *P<0.1, **P<0.05, ***p<0.01).



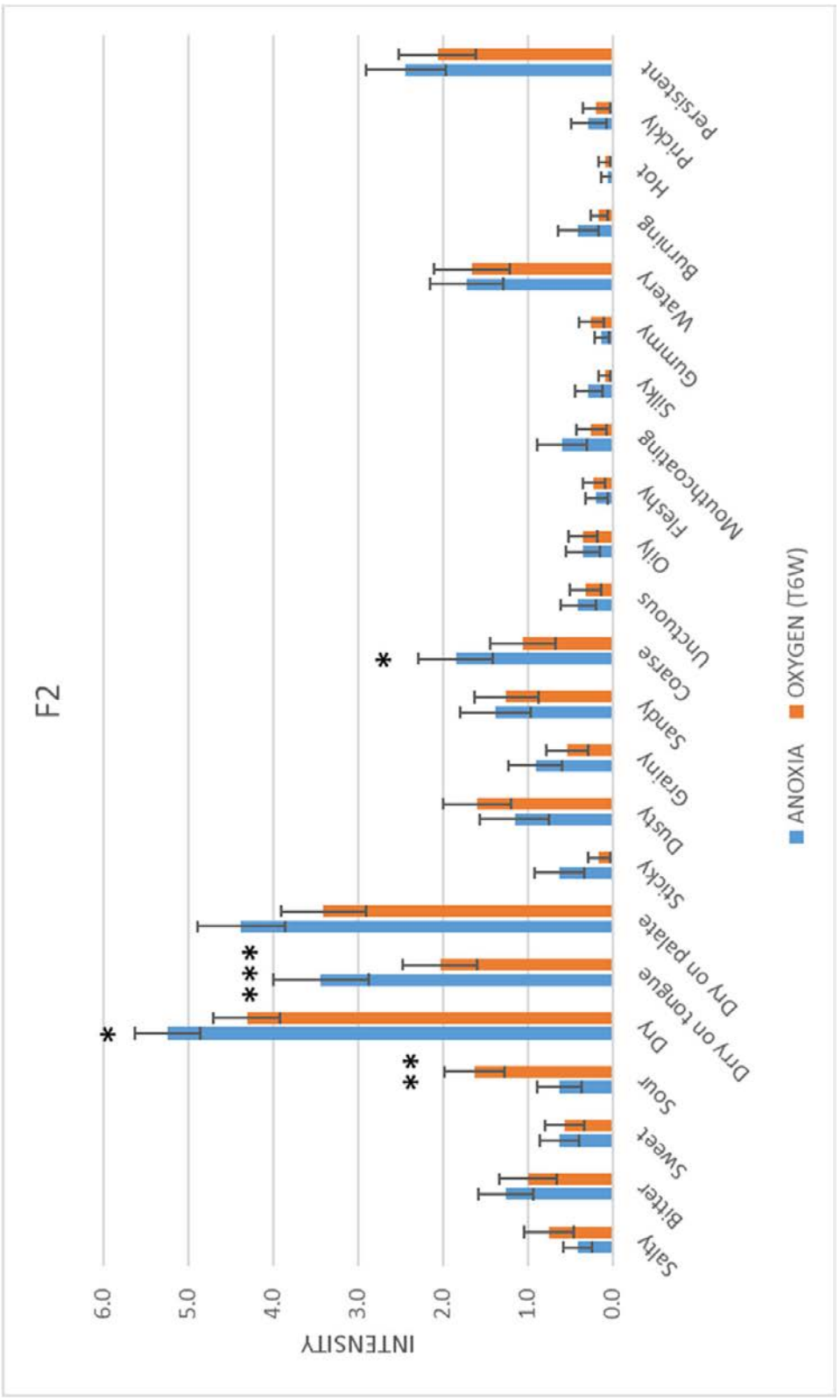
Annexe IV-3.3b. Continued



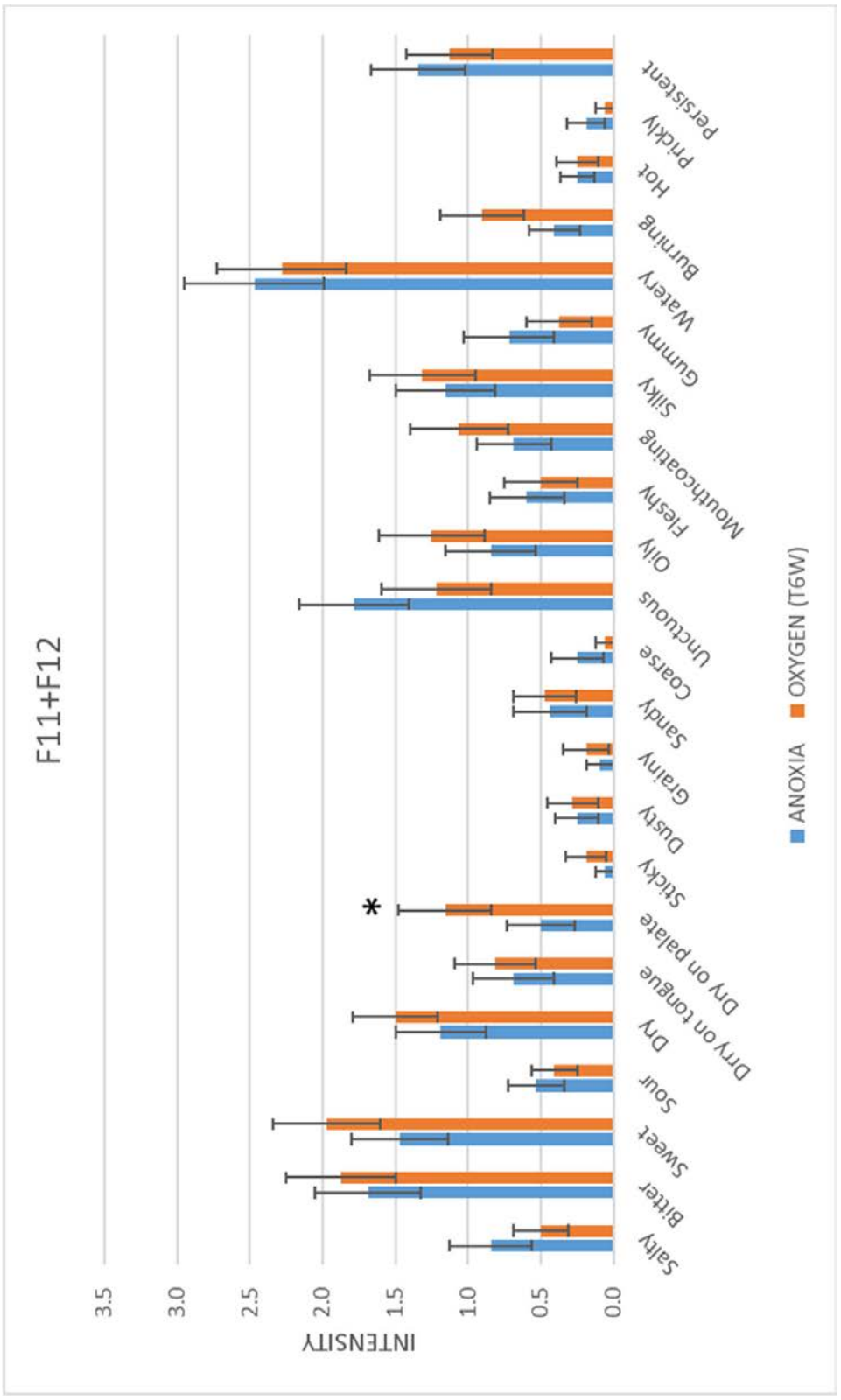
Annexe IV-3.3c. Continued



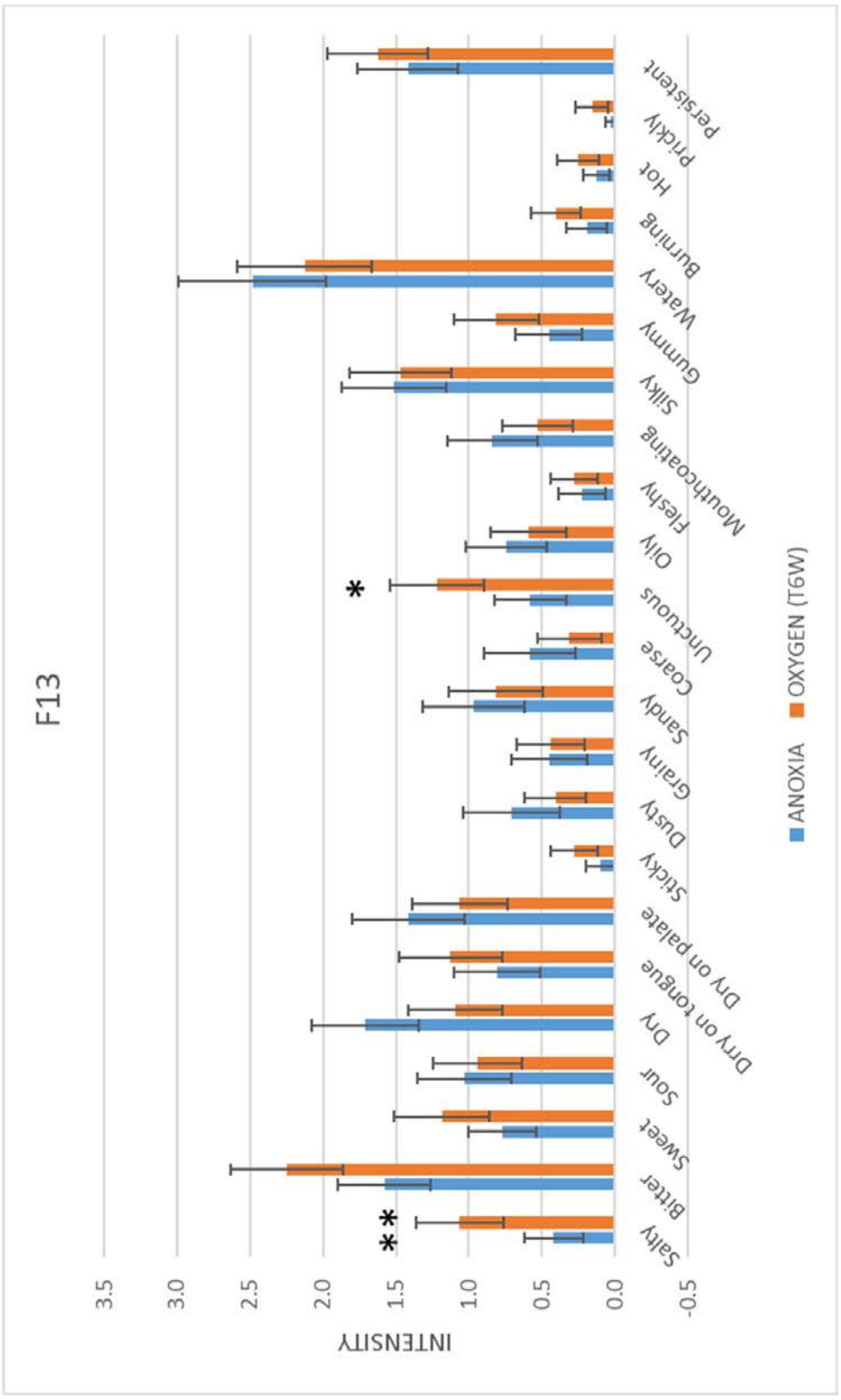
Annexe IV-3.3d. Continued



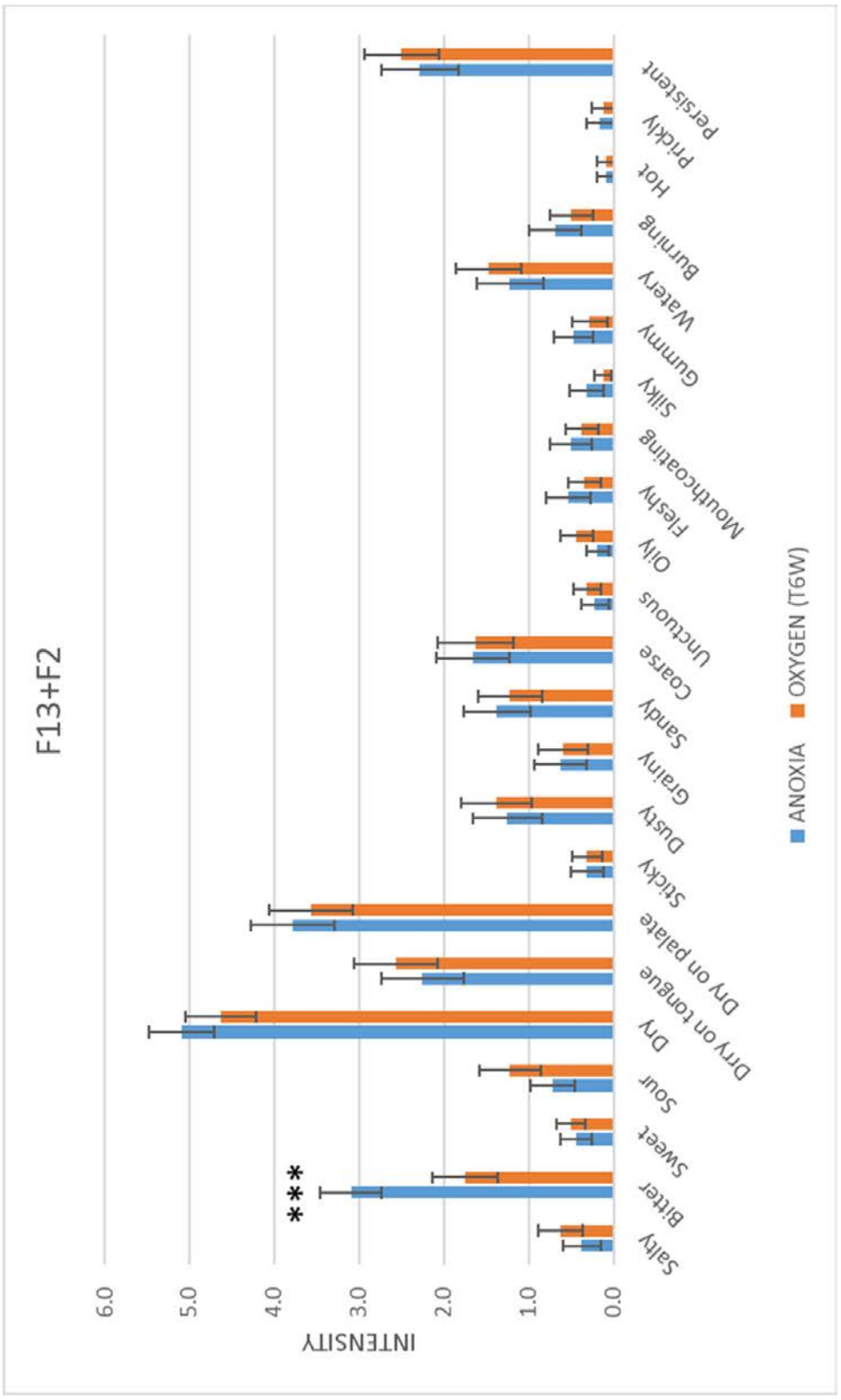
Annexe IV-3.3e. Continued



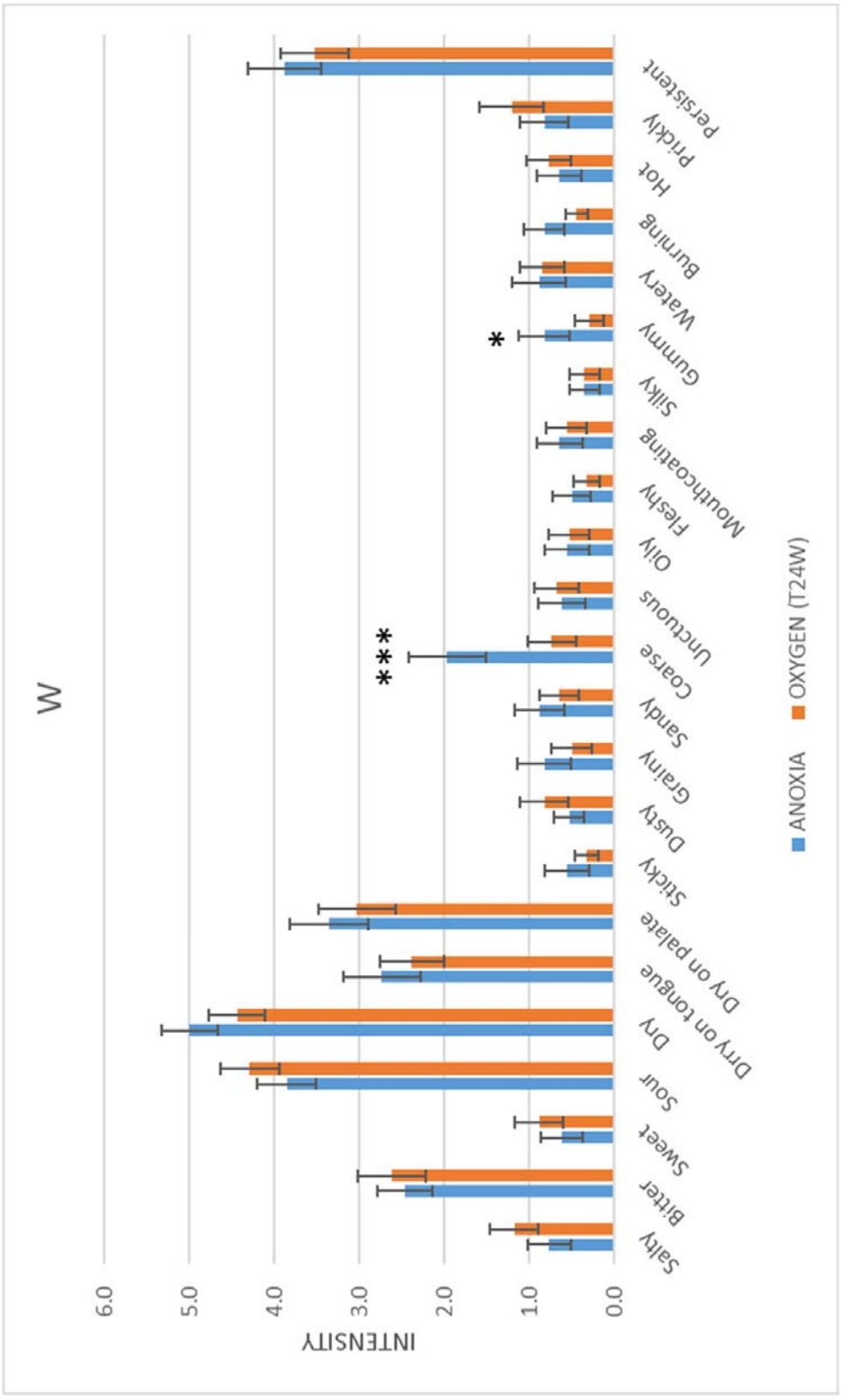
Annexe IV-3.3f. Continued



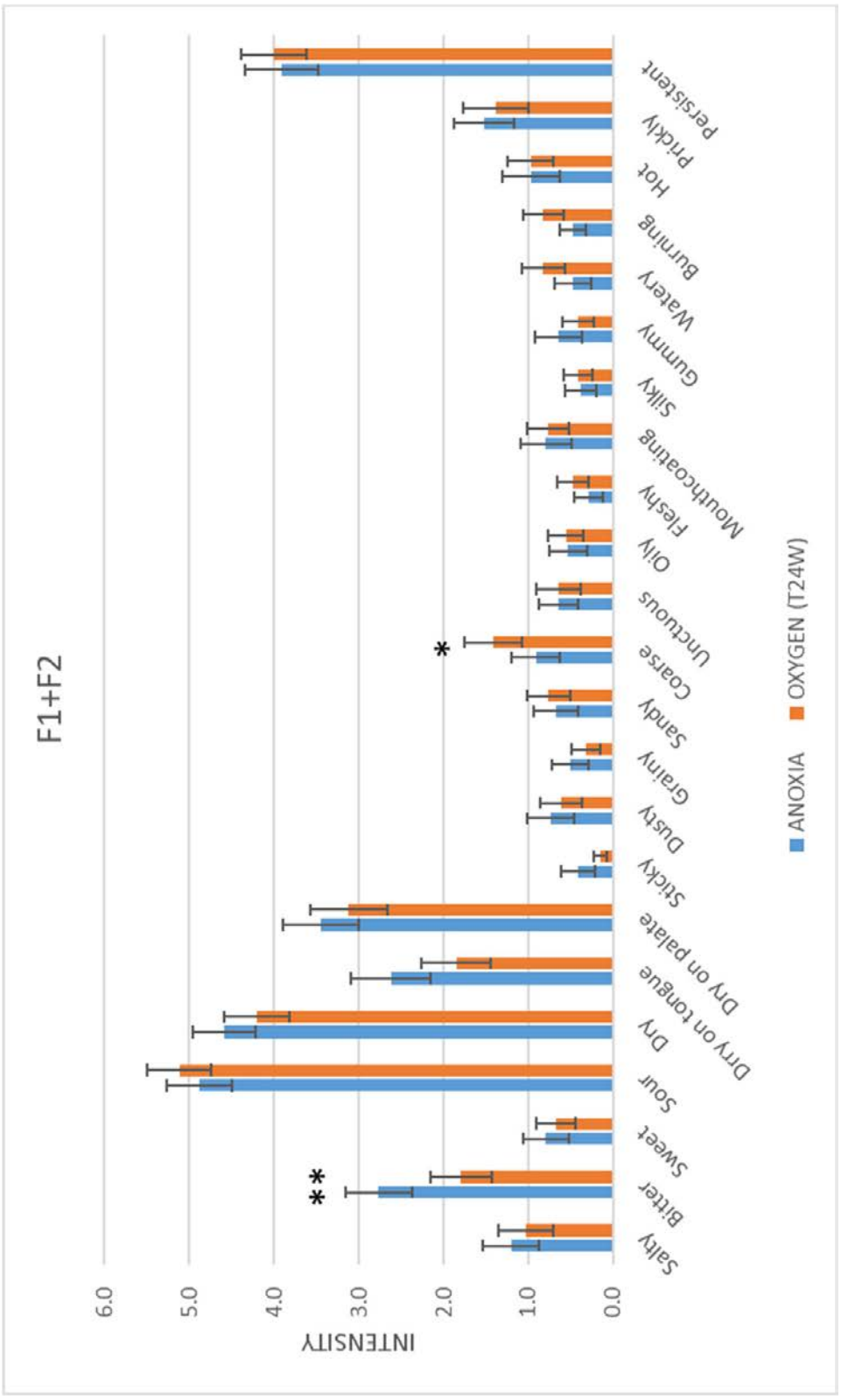
Annexe IV-3.3g. Continued



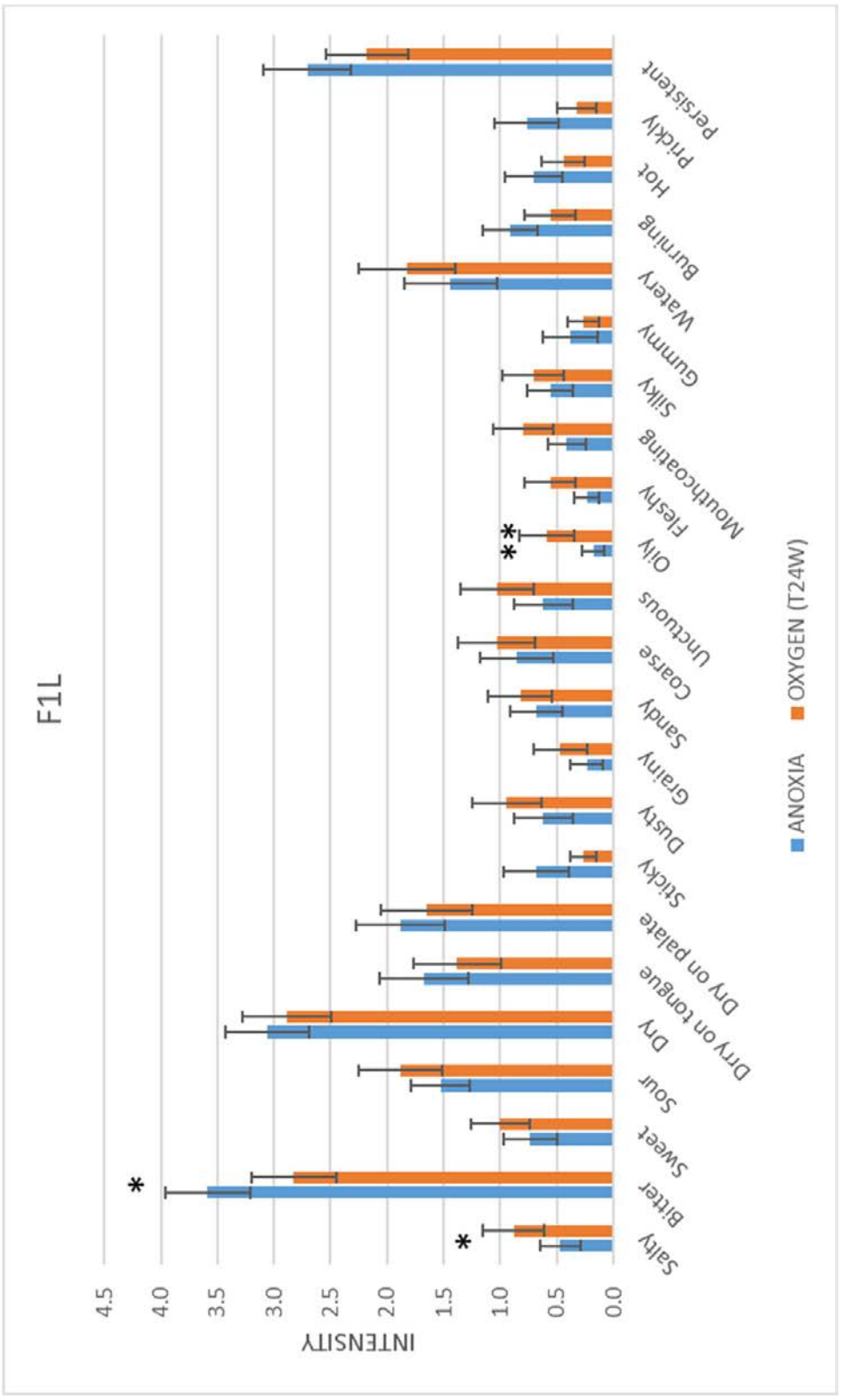
Annexe IV-3.4. Graphic representation of oxygen effect at T24W on taste and mouthfeel sensory properties, in each of the samples. Significance values are shown; P-significance: *P<0.1, **P<0.05, ***p<0.01).



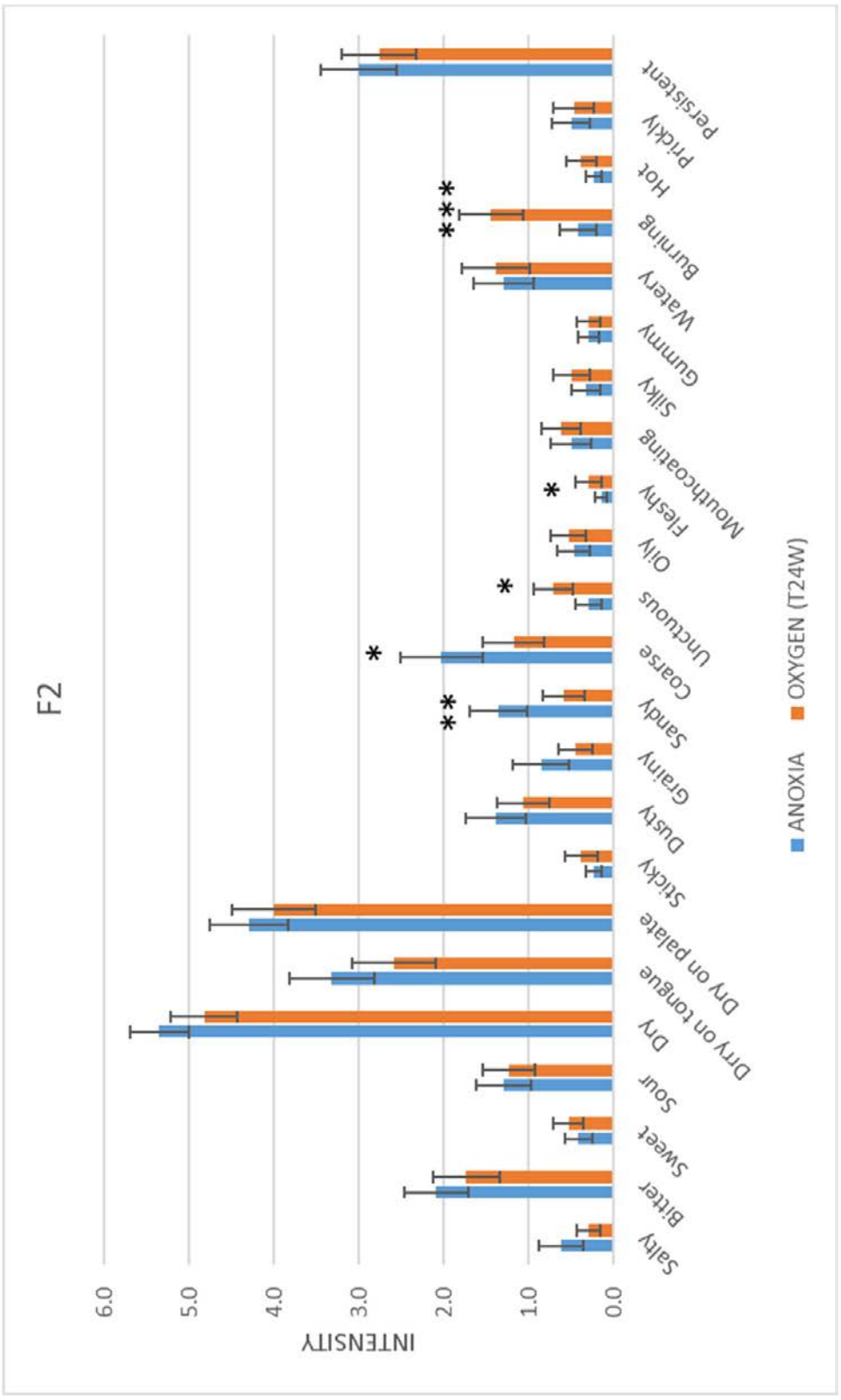
Annexe IV-3.4b. Continued



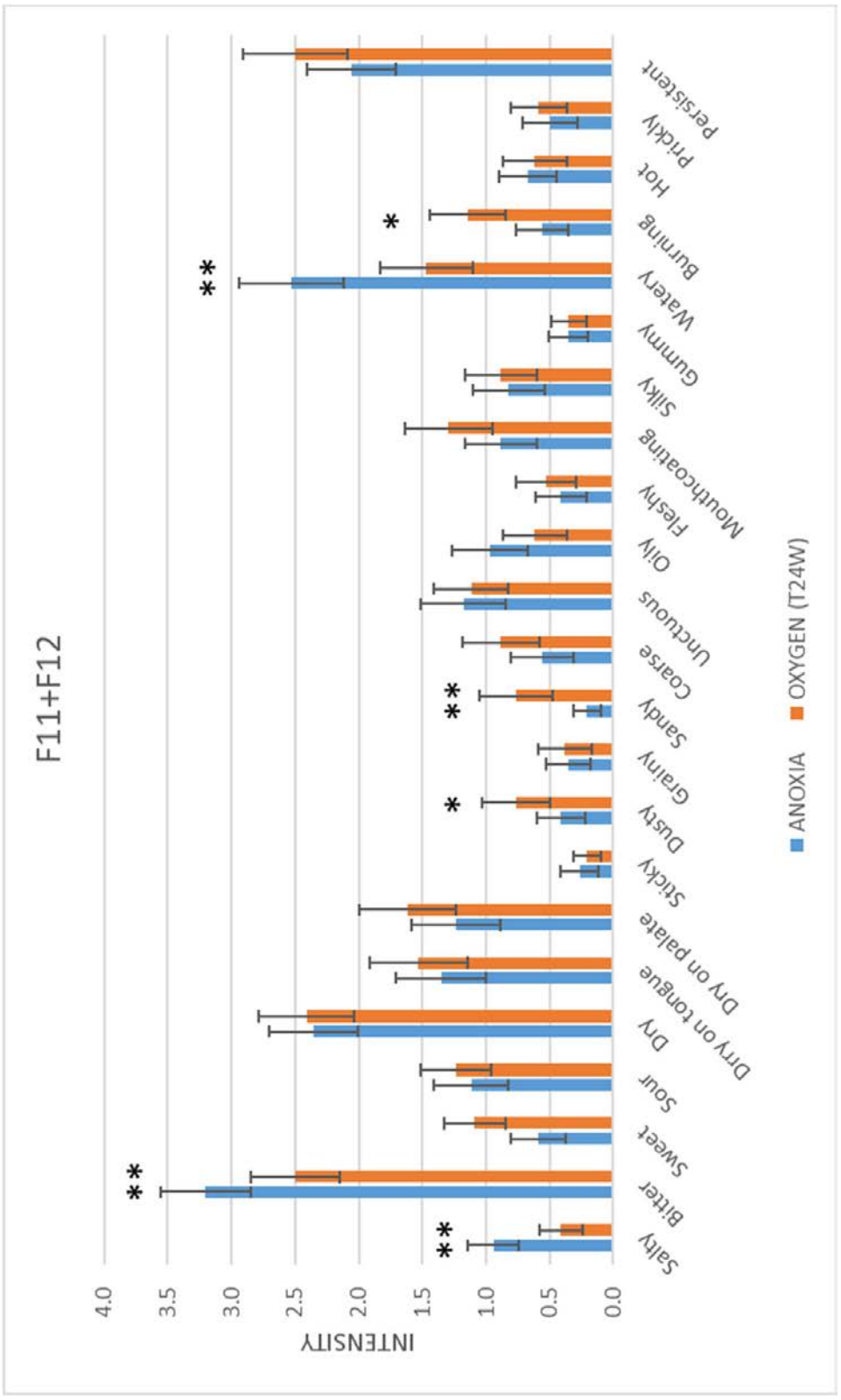
Annexe IV-3.4c. Continued



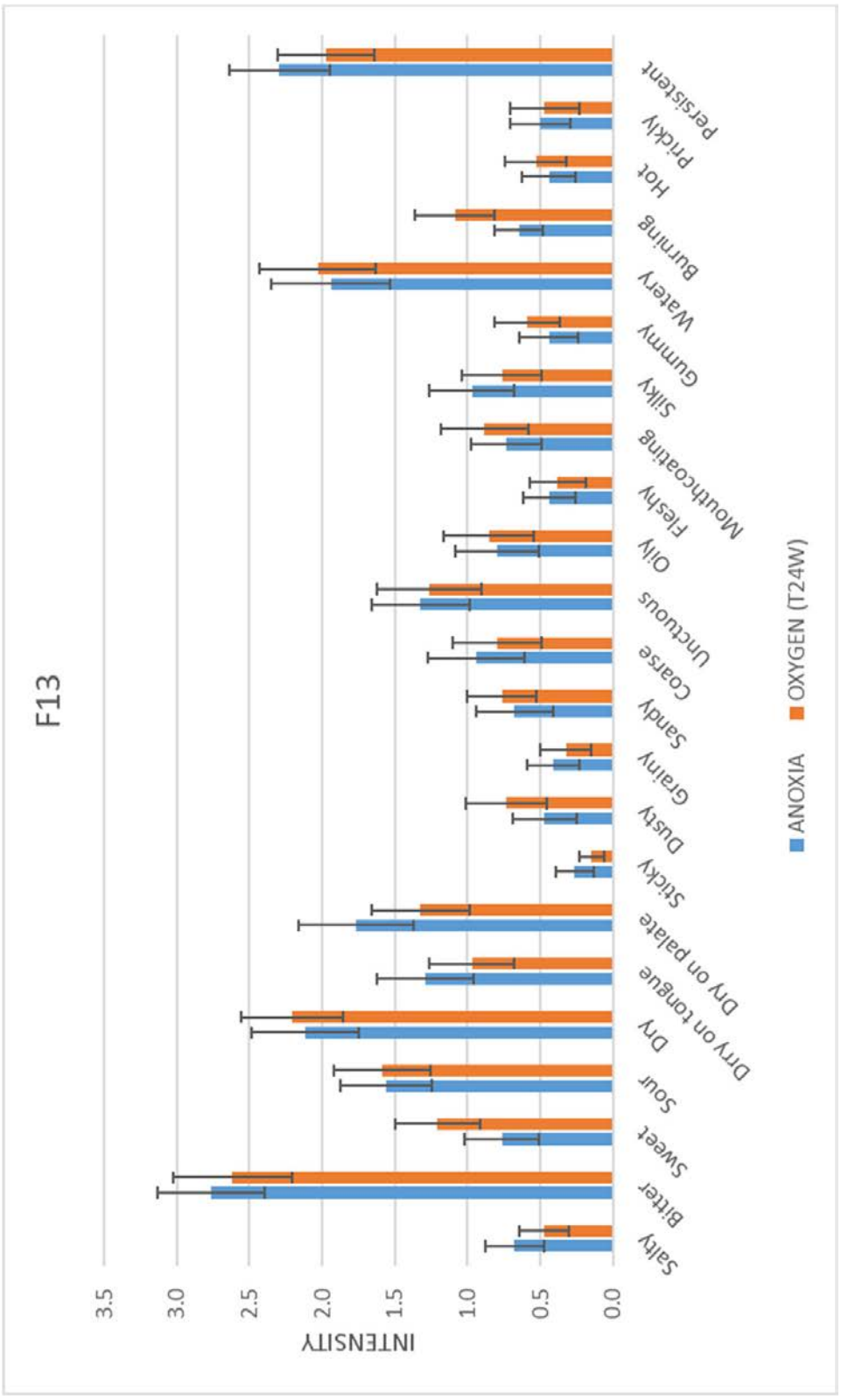
Annexe IV-3.4d. Continued



Annexe IV-3.4e. Continued



Annexe IV-3.4f. Continued



Annexe IV-3.4g. Continued