



Sensory and phenolic characterization of red wine according to the vintage year

A case study related to the red wines from the North of Italy

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Al mio angelo custode,

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Abstract.

Phenols are essential compounds that characterized the composition of red wines. The evolution of these compounds during the aging in bottle contributes to the perception of the quality of the product.

The aim of this work was to study the evolution of polyphenolic compounds according to the vintage from the sensory and chemical point of view, trying to find indices that could be useful for the prediction of the evolution of wine in bottle. Four Italian producers agreed to participate in this project. The work was developed in three phases: sensory analysis, chemical analysis and subsequent data analysis.

The results showed that the experience of the judges did not influence the evaluation of wine quality. Moreover, quality over time did not follow the model proposed in literature, moreover was independent according to the type of wine. For the panel it was easier to evaluate the evolution stage and to place the samples in chronological order. The evaluation of astringency followed the expected trend: a progressive decrease over time. From the correlation between the sensorial attributes was created an equation that help to predict the quality of the wine. Color was a determining factor in understanding the age and evolution of wine in bottle: the judges predicted with higher precision the age of the product when it was possible to see its color.

Among the chemical analyses, the study of astringency through Bovine Serum Albumin (BSA) reported expected trends but a low correlation with the results of sensory analysis. Among the different chemical indices studied those that can be considered most relevant for the evaluation of bottle evolution are Monomeric Anthocyanins (%), Long Polymeric Pigments (%), Absorbance₅₂₀, Absorbance₄₂₀ and Hue. Finally, through a multifactorial analysis it was possible to divide wines according to the results obtained from sensorial and chemical analysis, in four distinct groups according to the attribute "Quality".

Further investigations are necessary to confirm the goodness of the results obtained.

5 key words: Bottle Aging, Overall Quality, Phenols Evolution, Sensory Analysis, Color.

Resumo.

Os fenóis são compostos essenciais que caracterizam a composição dos vinhos tintos. A evolução destes compostos durante o envelhecimento em garrafa contribui para a percepção da qualidade do produto.

O objetivo deste trabalho foi estudar a evolução dos compostos polifenólicos em função do ano de colheita do ponto de vista sensorial e químico, tentando encontrar índices que possam ser úteis para a previsão da evolução do vinho em garrafa. Quatro produtores italianos aceitaram participar neste projeto. O trabalho foi desenvolvido em três fases: análise sensorial, análise química e posterior análise de dados.

Os resultados mostraram que a experiência dos juízes não influenciou a avaliação da qualidade do vinho. Além disso, a qualidade ao longo do tempo não seguiu o modelo proposto na literatura, além disso, foi independente de acordo com o tipo de vinho. Para o júri, foi mais fácil avaliar o estado de evolução e colocar as amostras em ordem cronológica. A avaliação da adstringência seguiu a tendência esperada: uma diminuição progressiva ao longo do tempo. A partir da correlação entre os atributos sensoriais foi criada uma equação que ajuda a prever a qualidade do vinho. A cor foi um fator determinante para compreender a idade e a evolução do vinho em garrafa: os juízes previram com maior precisão a idade do produto quando era possível ver a sua cor.

Entre as análises químicas, o estudo da adstringência através da Serioalbumina Bovina (BSA) registou tendências esperadas, mas uma baixa correlação com os resultados da análise sensorial. Entre os diferentes índices químicos estudados, os que podem ser considerados mais relevantes para a avaliação da evolução da garrafa são as Antocianinas Monoméricas (%), os Pigmentos Poliméricos Longos (%), a Absorvância₅₂₀, a Absorvância₄₂₀ e a Tonalidade. Finalmente, através de uma análise multifatorial, foi possível dividir os vinhos de acordo com os resultados obtidos na análise sensorial e química, em quatro grupos distintos de acordo com o atributo "Qualidade".

São necessárias mais investigações para confirmar a bondade dos resultados obtidos.

5 palavras-chave: Envelhecimento em Garrafa, Qualidade Geral, Evolução de Fenóis, Análise Sensorial, Cor.

Resumo Alargado.

Os fenóis são compostos essenciais para caracterizar a composição dos vinhos tintos. A evolução destes compostos durante o envelhecimento em garrafa contribui para a percepção da qualidade do produto. De acordo com a literatura, espera-se um "amolecimento" geral do vinho, atingindo uma menor adstringência e a perda de nuances vermelho-púrpura para atingir uma cor mais vermelha granada. É importante compreender quais são os efeitos reais do envelhecimento que ocorre durante os anos em garrafa e perceber quais são os índices que podem dar a ideia da perspectiva de envelhecimento do produto.

O objetivo deste estudo foi estudar a evolução dos compostos polifenólicos durante o período de envelhecimento em garrafa, do ponto de vista sensorial e químico, tentando encontrar índices que possam ser úteis para compreender a evolução do vinho em garrafa durante os anos passados em garrafa. Quatro produtores italianos do Norte de Itália aceitaram participar neste projeto, disponibilizando 21 vinhos diferentes, desde a colheita de 2021 até 1995. O trabalho foi dividido em três fases: a primeira foi uma análise sensorial para 30 juízes, divididos em 15 estudantes e 15 especialistas na área, em que cada juiz teve de preencher um formulário para cada amostra proposta. A segunda fase foi a análise química, na qual foram efectuadas as análises básicas para cada amostra e foram estudados diferentes índices de polifenóis para investigar a cor e o teor de polifenóis. Finalmente, foram efectuadas análises estatísticas para perceber se havia relações entre os resultados sensoriais e químicos.

Os resultados sensoriais foram submetidos a testes estatísticos não paramétricos porque os pressupostos dos testes paramétricos não foram cumpridos. Os resultados mostraram que a experiência dos juízes não foi um fator fundamental na avaliação da qualidade e evolução dos vinhos durante o estágio em garrafa. Assim, decidiu-se utilizar as respostas conjuntas dos estudantes e dos peritos, sem ir investigar os diferentes comportamentos baseados na experiência no mundo do vinho. As avaliações para o atributo "qualidade" não seguiram o modelo proposto na literatura, mas sim tenderam para um valor central da escala e muito relacionado com o tipo de vinho. Para o júri, foi mais fácil avaliar a evolução do produto e colocar as amostras por ordem cronológica. No entanto, houve uma tendência para subestimar a idade real da amostra provada. Por conseguinte, mesmo que os provadores tenham compreendido a evolução diferente das amostras, a qualidade não diminuiu durante mais de 20 anos de envelhecimento. Foi notável constatar que os vinhos puderam ser apreciados neste período. Quanto aos atributos que envolvem polifenóis durante a análise sensorial, entre eles, a avaliação do "corpo" e do "amargo" não mostrou diferenças

significativas. Além disso, avaliando o desempenho de cada vinho, não foi possível encontrar uma tendência linear semelhante entre eles. Mais surpreendentes foram os resultados relativos à adstringência: para todos os vinhos, parece haver uma diminuição gradual da percepção ao longo do tempo. Este facto foi interessante porque os juizes não sabiam a priori o tipo de vinho, mas avaliaram uma diminuição deste atributo ao longo do tempo. Foi também criada uma equação que poderia ajudar a prever a qualidade global do vinho com base nos atributos sensoriais avaliados. Os resultados mostraram que a qualidade poderia ser prevista principalmente pela complexidade e pelo equilíbrio, com uma contribuição menos positiva do corpo e uma contribuição negativa do defeito. Como última secção da análise sensorial, foi pedido aos juizes que avaliassem a cor e a idade prevista com base na cor. No que diz respeito à avaliação visual da cor, verificou-se que, ao longo dos anos, os juizes foram capazes de discriminar os vinhos mais jovens mais próximos do vermelho-violeta e os vinhos mais velhos mais próximos da cor tijolo. A cor parece, portanto, ser um elemento fundamental para a avaliação da idade do vinho: a componente cromática dos vinhos influencia fortemente a avaliação do vinho e a sua percepção. No entanto, estes resultados indicam que os provadores ficaram surpreendidos com a qualidade dos vinhos envelhecidos.

Quanto aos resultados da análise físico-química. Foram considerados diferentes parâmetros de base e de impacto para estudar os perfis polifenólicos. Entre os parâmetros de base analisados, o teor de dióxido de enxofre total foi o único considerado como tendo um interesse direto nas reacções que envolvem os polifenóis durante o período de envelhecimento. Como previsto na literatura, o teor total de SO₂ diminuiu ao longo do tempo devido às múltiplas reacções a que está sujeito. Os índices Cielab foram utilizados para interpretar a cor do vinho e para indicar os índices fiáveis que permitem prever o envelhecimento do vinho. Entre estes, certamente h^* (que representa a tonalidade) aumentou e a^* (correspondência cromática vermelho/verde) diminuiu com o tempo, e foram os que mais claramente tiveram a mesma tendência para os quatro vinhos. Os índices espectrofotométricos foram dos mais imediatos e fáceis de interpretar. As medidas de absorvância a 420 nm e 520 nm (A_{420} , A_{520}) e de tonalidade (A_{420}/A_{520}) podem ser bons indicadores da evolução dos vinhos tintos em garrafa. Além disso, a excelente correlação entre A_{520} e a^* e entre as tonalidades e h^* , mostrou como estas medidas podem ser consideradas para a previsão da evolução do vinho em garrafa, mesmo quando os vinhos são provados em copos escuros. O estudo do grau de polimerização dos pigmentos corantes confirmou a literatura: uma diminuição das antocianinas monoméricas (MP) e dos polímeros de cadeia curta (SPP) associada a um aumento dos pigmentos LPP de cadeia longa. O índice de polifenóis totais (IPT) e o teor de proantocianidinas seguiram a tendência esperada: registou-se uma diminuição gradual ao longo do tempo. A partir da relação tanino/antocianina (T/A), verificou-se que a diminuição foi

maior para as antocianinas e que a sua relação aumentou com o tempo. Como referido por Ribèreau-Gayon et al. (2006), este índice é importante para avaliar a formação de pigmentos poliméricos durante o período de conservação do vinho. Um dos resultados mais importantes foi observado pela avaliação do "poder tânico" ao longo do tempo: este pode ser considerado um índice químico para avaliar a adstringência do vinho. De acordo com o método de Boulet et al. (2016), para todos os vinhos, registou-se um aumento da adstringência até cerca de 15 anos de idade e depois uma diminuição gradual. O resultado obtido está de acordo com a polimerização dos taninos e com a sensação relativa de adstringência. No entanto, a correlação entre este índice e os resultados obtidos nas avaliações de adstringência da análise sensorial foi baixa ($R^2=0,46$), indicando que são necessárias mais investigações para encontrar testes químicos fiáveis para a avaliação desta sensação sensorial aquando da avaliação de vinhos de longa duração.

Por fim, procurou-se estabelecer ligações entre as diferentes análises físico-químicas, o que permitiu tirar conclusões importantes para a previsão da evolução da fração polifenólica durante o envelhecimento em garrafa. Uma matriz de correlação mostrou correlações muito significativas, sendo A520, A420 e tonalidades entre os índices com maior número de correlações e entre os mais fáceis e rápidos de estimar. Além disso, com a ajuda do mapa de calor e da análise de componentes principais (ACP), foi possível diferenciar os vinhos em função de vários parâmetros: os vinhos mais velhos foram mais bem descritos pelo LPP (%) e pelas tonalidades. Por outro lado, os vinhos jovens foram melhor distinguidos pela percentagem de antocianinas monoméricas (MP %), pela concentração de antocianinas e pelo dióxido de enxofre total.

Uma análise multifatorial evidenciou as relações entre os resultados sensoriais e químicos. Com base nos resultados, foi possível agrupar os vinhos em 4 grupos, de acordo com a idade média do grupo e as avaliações do atributo "Qualidade global". De acordo com os grupos, verificou-se que a qualidade e a "evolução" seguiram tendências opostas: com o aumento da evolução, a qualidade percebida diminuiu e vice-versa, os vinhos jovens foram considerados de nível de qualidade superior. Isto pode ter sido influenciado pelo facto de os juízes estarem mais facilmente habituados a provar vinhos jovens. A partir daí, verificou-se que os índices mais simples e imediatos de compreender e calcular para avaliar o estado evolutivo do vinho são os seguintes MP (%), LPP (%), A520, A420 e tonalidade.

São necessárias mais investigações para confirmar a bondade dos resultados obtidos.

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List of Abbreviations:

- *Amarone* = Amarone della Valpolicella DOCG, Jago, Cantina Sociale di Negrar
- *Luce* = Luce della Vite IGT, Frescobaldi
- *Teroldego* = Teroldego Rotaliano DOC, NOS, Mezzacorona
- *Valpolicella* = Valpolicella Classico Superiore DOC, Monte Sant'Urbano, Speri
- A15 = Amarone della Valpolicella DOCG vintage 2015
- A10 = Amarone della Valpolicella DOCG vintage 2010
- A04 = Amarone della Valpolicella DOCG vintage 2004
- A00 = Amarone della Valpolicella DOCG vintage 2000
- A95 = Amarone della Valpolicella DOCG vintage 1995
- L16 = Luce della Vite vintage 2016
- L09 = Luce della Vite vintage 2009
- L07 = Luce della Vite vintage 2007
- L03 = Luce della Vite vintage 2003
- L98 = Luce della Vite vintage 1998
- T21 = Teroldego Rotaliano DOC vintage 2021
- T16 = Teroldego Rotaliano DOC vintage 2016
- T12 = Teroldego Rotaliano DOC vintage 2012
- T06 = Teroldego Rotaliano DOC vintage 2006
- T01 = Teroldego Rotaliano DOC vintage 2001
- V19 = Valpolicella Classico Superiore DOC vintage 2019
- V16 = Valpolicella Classico Superiore DOC vintage 2016
- V12 = Valpolicella Classico Superiore DOC vintage 2012
- V09 = Valpolicella Classico Superiore DOC vintage 2009
- V05 = Valpolicella Classico Superiore DOC vintage 2005
- V00 = Valpolicella Classico Superiore DOC vintage 2000
- MP (%) = Monomeric Pigments percentage (Adams-Harbertson assay)
- LPP (%) = Long Polymeric Pigments percentage (Adams-Harbertson assay)
- SPP (%) = Short Polymeric Pigments percentage (Adams-Harbertson assay)
- IPT/ TPI= Total Polyphenols Index

- PACs = Proanthocyanidins
- H.A. = Hydroxycinnamic Acids mg/L
- Ant. = Anthocyanins mg/L
- C.I. = Color Intensity
- D.I. = Dialysis Index

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1. Introduction

The aging of fermented beverages is a practice that is carried out for millennia. Among these products, wine is considered the most valuable (Echave et al., 2021). The interest in understanding what happens during the period of rest in bottle is a subject of high interest in the wine sector. During the years all these alcoholic beverages change their composition: as a result of the chemical transformations, the flavor and the taste of the wine look different during the ages (Ribèreau-Gayon et al., 2006). Moreover, it is important to figure out when the wine could be considered “aged” and when “oxidized” or “defective” since the shelf-life of every wine depends on several factors and, on the other side, not all the products could be aged for several years.

Phenols are essential compounds that characterized the quality of the red wines. They are responsible for the main differences between white and reds (color, bitterness, and astringency) and they play a fundamental role in the stabilization of wines. They are also compounds with an important nutraceutical effect on human health. Their origin can be different: endogenous (from the parts of the bunch) or exogenous (ex. wood). Their structure and content change according to the storage and bottle aging conditions of the wines (Gutiérrez-Escobar et al., 2021).

The main goals of this dissertation work are:

- To study the sensory and phenolic characteristics of red wines with different bottling ages.
- To find out possible correlation between the sensory and the chemical analysis that could explain the evolution of the wine in bottle through the years.

Moreover, the analysis could give important information to find out compounds that can be used as index of aging and try to create an evolution curve connected to factors related to the phenols' sensory attributes, as shown by Pereira et al. (2011).

Several Italian wineries took part in this thesis project supplying products that are representative of the most important wine regions in the North of Italy. Having different wines for variety, nature and origin is very useful to look for general perspectives and try to discover correlations that are not only related to the variety or style of the winery. The wines were: *Luce della Vite* by Frescobaldi; *Teroldego Rotaliano* by Mezzocorona; *Amarone della Valpolicella Classico* by Cantina Sociale di Negrar and *Valpolicella Classico Superiore* by Speri.

The work is organized in three steps: firstly, the sensory analysis of the wines with different judges: experts and students. The goal of this first experience is to evaluate the different

perception of the overall quality, the evolution state, and the polyphenols attributes from the point of view of many connoisseurs of the world of wine.

The second step is represented by the chemical analysis. Several analyses will be made on polyphenols to understand how the different fractions evolve during aging: Polymerization of Anthocyanin, Tannin Power, Total Polyphenol Index, Total PACs, Total Anthocyanins, Total Tannins, Hydroxycinnamic Acids, Dyalis Index, Hue, Color Intensity and CIELab*. Moreover, the basic chemical analysis (alcohol, pH, sulphur dioxide (free and total), titratable acidity, residual sugar and volatile acidity) are made for understanding how these values may affect the sensorial perception of the wine.

To conclude, to find out a possible correlation between the tasting notes and the analytical result a statistical analysis is made.

2. Literature review

2.1 The aging process

The sensory evolution of red wine, depending on the type of wine and the variety. The aging process is characterized by a complex development of the three main components of tasting: color, aroma, and flavor.

During this evolution, the color evolves towards the brick. Purple hues, typical of young wines, give way to yellowish-orange hues (Ribèreau-Gayon et al., 2006). Losses of color compounds during the aging period result mainly from: enzymatic oxidation, action of sulfur dioxide, presence of metal ions, formation of long-chain polymeric pigments that decay, precipitation with bitartrate salts, formation of colorless or neutral pigments, micro-oxidation, and linkages with other phenolics derived from wood aging (Tirelli, 2021).

The taste also evolves, becoming softer, with less bitterness and different astringency according to the initial characteristics of the product. Too long aging can lead to the risk of wine thinning and flatting in the mouth as it matures.

It should also be considered that the rate of variation is different for each wine, depending on the intrinsic composition of the wine, the external conditions and the reactions occurring during the aging in bottle. A wine is often considered prestigious when it has the ability to age for a long time, unlike the slenderer wines that develop their full potential in the short term (Ribèreau-Gayon et al., 2006).

According to Dubourdieu et al., (1992) the ageing process comprises three distinct phase (Fig.2.1). In the first period of aging, the wines ripen and there may be some fluctuations in quality based on endogenous and exogenous conditions. During the second step, wines reach their best expression and are considered in the best period to be drunk. The third and final phase brings wine to its natural decline, when wines no longer express themselves for what

they were to be and to become flats. This “qualitative parabola” takes place at variable speeds and the compositional changes are accompanied by the gradual loss of clarity of the wine, which can cause precipitation in the bottle.

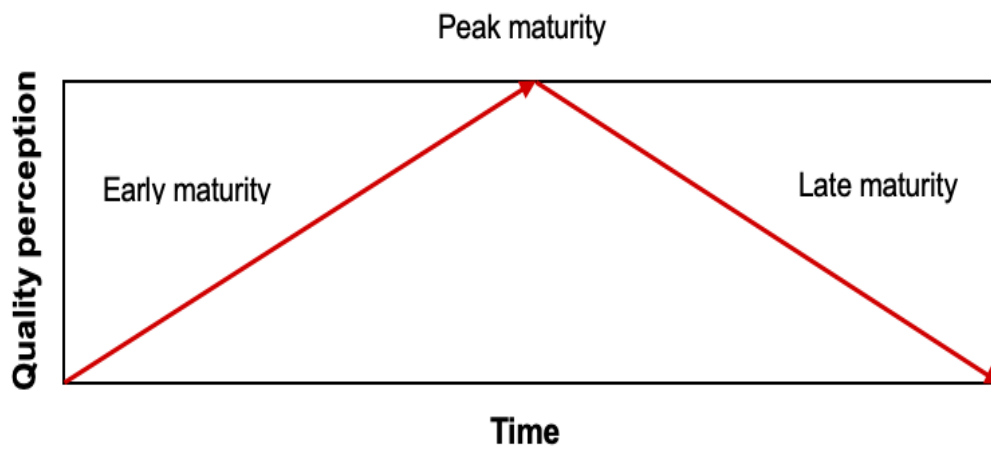


Figure 2.1 Evolution of the wine during aging according to Dubourdieu et al., (1992).

Aging has been noted as one of the factors that most affect the concentration of phenolic compounds, with a significant transformation/ evolution of these elements as a function of the time of storage in the bottle (Gutiérrez-Escobar et al., 2021). In a recent study of Aleixandre-Tudo & du Toit (2020), evaluated the effect of the change in the phenolic fraction of wine first in cask and then in bottle. During the bottle aging, it seems that there was an intense formation of polymeric pigments, which influenced the chromatic properties of wines. The phenolic chemistry that occurs during ageing therefore seems to be based on a complex balance that involves further pigment formation and the decomposition and reorganization of existing chemical structures (Aleixandre-Tudo, 2020).

2.2 Phenolic composition of the wine

Phenols are secondary metabolites of the berry; their purpose is to protect the seed from UV radiation. In addition, they attract predators and aid in seed shedding when the cluster reaches maturity. Tannins, on the other hand, protect against predators in the early stages, as the degree of polymerization is lower and therefore more bitter, which is why it has a low palatability for animals. Flavanols have a maximum synthesis at flowering, which is the most delicate time to protect the cluster and are then synthesized and metabolized differently depending on light (Mattivi, 2019).

The phenols are highly correlated both to the sensory quality of the wine (color, smell and taste) and to the beneficial and nutraceutical properties for health (antioxidants and cardioprotective among others). This class of compounds can be grouped into two broad categories: flavonoids (anthocyanidins, flavanols, flavanols, hydrolysable and condensed

tannins, flavanones, flavones, and chinks) and non-flavonoids comprising hydroxycinnamic acids, hydroxybenzoic acids, stilbenes, tyrosol and hydroxytyrosol (Fig. 2.2) (Mattivi, 2019).

Anthocyanins (Fig. 2.2A) are compounds that have as their basic structure: a phloroglucinol year together with an acyl ester on the C6 carbon of the 3-O-glucoside moiety. They are all named after flowers since there are species of flowers in which almost only one type of anthocyanin is synthesized. All B-rings of the structures are oxidizable depending on the substituents present. The difference between anthocyanin and anthocyanidin is that the former molecule is not bound to a sugar unlike the latter. Anthocyanins have a profile that distinguishes one cultivar from another (Jackson, 2020). The absorption spectrum of these compounds varies between 420 and 620 nm, where tri-substituted molecules are more purplish (absorbance 620 nm) and di-substituted ones are redder (absorbance 520 nm). Anthocyanins can be defined as polyprotic acids: the composition of the wine greatly influences their dissociation. The most obvious effect occurs with a change in pH. Anthocyanins are found in the colored form when they are at the "flavylium" stage, that is, when they lose the electron and remain positively charged. At this (electrophilic) stage and lower pH, however, they are more reactive with nucleophilic compounds (ex. Sulfur) (Tirelli, 2021). However, the color attributed to wine also depends on other factors: terroir, yeast strain, aging conditions, execution of a blend, maceration conditions, grape health, seasonality, and others.

Flavanols (flavan-3-ols) are compounds that actively influence the taste-visual characteristics of wine (Fig. 2.2A). They can be found in monomeric form (catechin and Epicatechin) and in their polymer form (proanthocyanidins, also called condensed or non-hydrolysable year) (Jackson, 2020). Flavanols, commonly called tannins, are responsible for the sensorial characteristics (mainly astringency and bitterness) of wines and contribute to the stabilization of wine's color. The concentration range of 4 to 120 mg/L in young red wine (Gutiérrez-Escobar et al., 2021).

Hydroxycinnamic Acids (Fig.2.2 B) are present in a small percentage as free, most are esterified with tartaric acid. An alcoholic function of phenolic acid esterifies an alcoholic function of tartaric acid. The three hydroxycinnamic acids found in the notes and then in wines are: coumaric (-OH group), caffeic (2 -OH) and ferulic (-OH + CH₃O). Concentration varies according to variety and the aging vats. They are very oxidizable compounds, especially in free form, by polyphenol-oxidase (PFO) enzymes in function of the number of -OH groups (Tirelli, 2021).

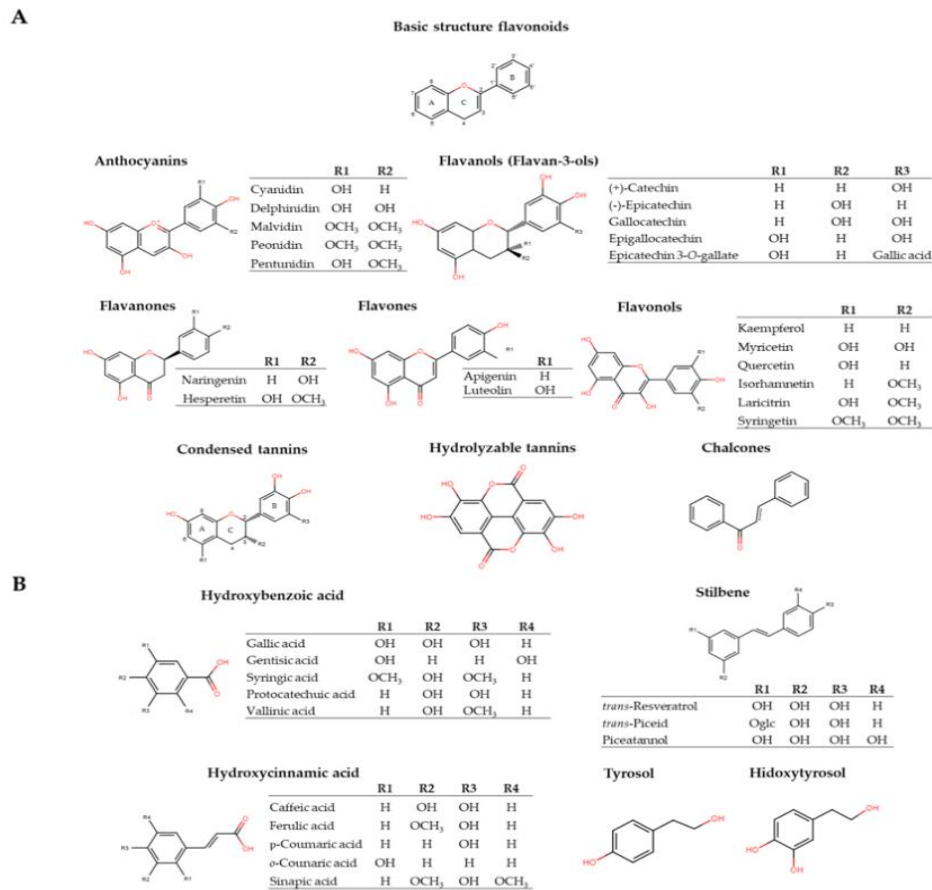


Figure 2.2 Example of **A** Flavonoid and **B** non-Flavonoid compounds that are present in wine (Gutiérrez-Escobar et al., 2021).

The origin of the compounds and their mouthfeel perception depending on the part of the grapes from where they are extracted and the starting variety (Fig. 2.3A). Grape seed tannins are astringent, as opposed to catechin and gallic acid, which are more bitter. The structure of the molecules (Fig. 2.3B) affects the associated taste sensations: low polymeric PACs are more acidic and have low persistence; oligomeric PACs are bitter and very persistent; polymeric PACs: sour-bitter sensation with gradual persistence; stalk tannins are very astringent (Mattivi, 2019).

The levels of phenols obtained prior to bottling will have an impact on the aging of the bottle, as well as on the overall oxidative stability of the bottled wine (Echave et al., 2021). For these reasons, the phenolic fraction can be managed to obtain identity products with unique and desirable characteristics.

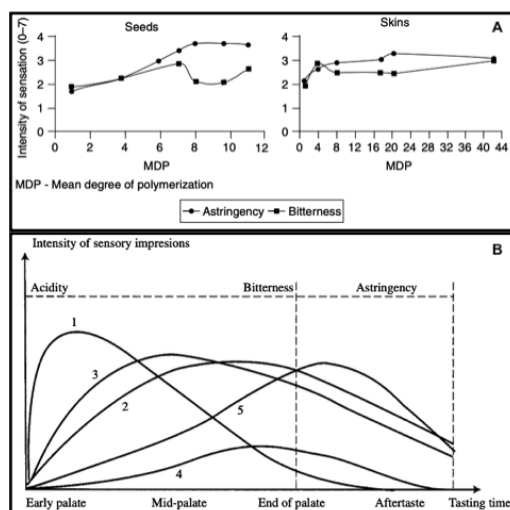


Figure 2.3 **A** Changing in sensorial attributes (astringency and bitterness) in function of the MDP (Mean degree of polymerization) of seeds and tannins (Mirabel, 2000) **B** Influence of the chemical structure of polyphenols on the different mouthfeel associated perceptions: 1, procyanidins little-polymerized procyanidins; 2, oligomeric procyanidins; 3, polymerized procyanidins; 4, anthocyanins; 5, tannins from stalks (Glories, 1994, unpublished)

2.3.1 Chemical reactions involving phenols during aging.

During the aging process, phenolic compounds undergo numerous chemical reactions (Fig. 2.4). Some of these reactions involve anthocyanins, which are transformed into more stable oligomeric and polymeric pigments with important consequences for the color and astringency properties. The role of anthocyanins from the gustatory point of view is linked to the formation of tannin-anthocyanin structures that cause the decrease in astringency (Ribèreau-Gayon et al., 2006). Homogeneous polymerization of tannins takes place, condensation of anthocyanins and tannins (involving the carbocations of procyanidins). Flavanols also participate in condensation reactions. For all these reasons, the aging process is required in some cases to achieve optimal quality. However, longer periods of aging could cause unpleasant consequences.

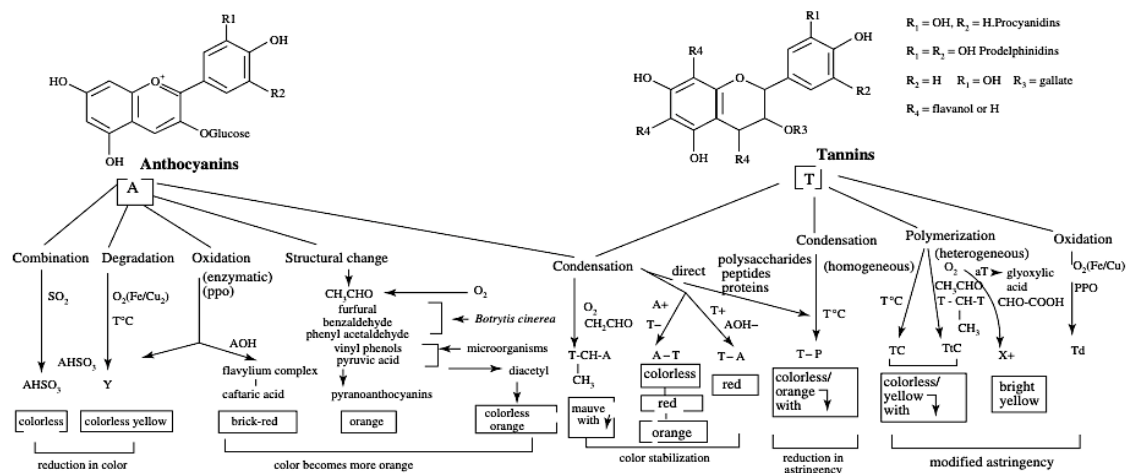


Figure 2.4 Changes in phenols (A, anthocyanins; T, tannins) in red wine during aging. Impact of these reactions on organoleptic characteristics. (Glories 2003, unpublished, P. Ribèreau-Gayon et al., 2006)

In the following bulled list, the main reactions that occur during aging and their effects are summarized (Ribèreau-Gayon et al., 2006):

- During the period of aging in bottle, it can be observed a "smudging" of the wine. These reactions are conditioned by the form of the tannins evolved both during this period and in the previous sentence of fermentation. If, on the one side, it is true that wines with a higher content of phenols usually have a higher potential of expression during time, the opposite is not always true. If a wine will be extremely astringent when it is bottled, it is not said it will lose this characteristic during the time.
- Through a homogeneous polymerization of PACs phenols mutate, together with the condensation of anthocyanins and tannins, which also involves the carbocations that form after the protonation of procyanidins. The formation of carbocations is necessary for polymerization: this leads to breakage reactions of the compounds after bottling. All these reactions take place (condensation and polymerization) occur depending on the type of red wine, which involves a change in intensity and speed of reaction. The resultant compounds react less rapidly, resected to those from which they derive (oligomeric).
- The increase in colour intensity during ageing is caused by a decrease in the anthocyanin content (which can reach a minimum of close to 50 mg/L). The wine pass from violet-reddish complexes in the first years after bottling, to orange-brick shades. The gradual decrease of these compounds is caused by the progressive disappearance of free fraction. The reactivity of the new formed complexes is very variable depending on the type of compound. Wines with high content of anthocyanins combined evolve less quickly than wines with low concentration of anthocyanins combined; but more rapidly with anthocyanins combined with a process of direct oxidative reaction.

In the Table 2.1 are indicated the most important markers for the evolution of the phenols, according to the articles already present in literature.

Typically, older wines have higher hue values and higher concentrations of polymeric pigments, while younger wines have higher concentrations of anthocyanins and other simple phenolic compounds. These chemical reactions depend on certain internal factors (initial concentration of phenolic and volatile compounds, additives, oxygen, pH, acidity, minerals, microorganism, etc.) and external factors (temperature, barrels, closure, humidity, luminosity) (Gutiérrez-Escobar et al., 2021).

The reactions that occurred during aging was the main goal of the work of Monagas et al. (2006). They measure different index connected to the polyphenols content during bottle aging. The results show that the decrease in total anthocyanins (due to the disappearance of monomeric anthocyanins), connected to an increase registered in proanthocyanidins and catechins (due to the cleavage of proanthocyanidins and their structural transformations), was in accordance with the cut in low-polymerized polyphenols, suggesting the occurrence of condensation reactions during ageing in bottle.

Table 2.2 Main phenols markers of bottle aging according to literature.

Markers	Varieties	Trend	Reference
Hidrocinnamic Acid	Tempranillo, Grenache, Cab.Sav; Shiraz	Increase ↑	Garrido-Banuelos et al. (2020) ; Monagas et al. (2006)17/01/2024 19:51:00
Flavonols	Tempranillo, Grenache, Cab.Sav; Shiraz	Decrease ↓	Burin et al. (2011); Monagas et al. (2006); Garrido-Banuelos et al. (2020).
Astringency	Shiraz; Cabernet Sauvignon	Decrease ↓	Garrido-Banuelos et al. (2020); Chira et al. (2011).
Tannins	Malbec, Merlot	Decrease ↓	Deshaies et al. (2022); Casassa et al. (2019); Chira et al. (2011); Gómez Gallego et al. (2013).
Catechins	Tempranillo, Grenache, Cab.Sav;	Decrease ↓	Burin et al. (2011); Monagas et al. (2006).
Epicatechin	Tempranillo, Grenache, Cab.Sav;	Decrease ↓	de Oliveira et al. (2019); Monagas et al. (2006).
Total Anthocyanins	Tempranillo, Grenache, Cab.Sav; Aglianico; Malbec, Merlot	Decrease ↓	Gambutti et al. (2020); Casassa et al. (2019); Chira et al. (2011); Monagas et al. (2006); Gómez Gallego et al. (2013).17/01/2024 19:51:00
Monomeric Anthocyanins	Shiraz; Sangiovese; Aglianico; Cabernet Sauvignon; Malbec, Merlot	Decrease ↓	Maioli et al. (2022); Garrido-Banuelos et al. (2020); Gambuti et al. (2020); Casassa et al. (2019); Burin et al. (2011).
Tonality (Hue)	Sixteen different Spanish commercial red wines; Shiraz; Sangiovese; Aglianico	Increase ↑	de Oliveira et al. (2019); Maioli et al. (2022); Garrido-Banuelos et al. (2020); Gambuti et al. (2020); Chira et al. (2011); Gómez Gallego et al. (2013).

Color Intensity	Sangiovese; Cabernet Sauvignon	Decrease ↓	de Oliveira et al. (2019); Maioli et al. (2022); Chira et al. (2011); Gómez Gallego et al. (2013).
Polimeric Pigments	Sixteen different Spanish commercial red wines; Shiraz; Sangiovese; Cabernet Sauvignon	Increase ↑	Avizcuri et al. (2016); Maioli et al. (2022); Garrido-Banuelos et al. (2020); Chira et al. (2011); Burin et al. (2011).
Malvidin-3-O-glucoside Peonidin	Aglianico; Cabernet Sauvignon	Decrease ↓	Gambutti et al. (2020); Burin et al. (2011).
	Aglianico; Cabernet Sauvignon	Decrease ↓	Gambutti et al. (2020); Burin et al. (2011).

2.3.2 Modification of Astringency

During the period of aging of red wines in bottle, and even before, tannins undergo changes that have an important mouthfeel effect such as the softening of the taste, accompanied by a reduction in astringency. Gómez Gallego et al. (2013) suggested that after the first year of bottle aging there is a “turning-point” from the mouthfeel point of view.

The mechanism of astringency, follows three steps (Mattivi, 2019):

1. Combination between tannins and mucosal glycoproteins and salivary proteins resulting in precipitation.
2. Reduced lubrication of saliva and increased friction between surfaces.
3. Tactile sensation of the tongue associated with astringency.

The intensity of perceived astringency is additive if the ingestion time between one sip and the next is too short. The intensity of the sensation of bitterness and astringency depends on: alcohol content, pH, and tannin type. In wines with the highest alcohol content, it will result higher. At pH values > 3.6, tannin persistence increases, especially when associated with high ethanol content. The size and degree of polymerization of the molecule cause different taste sensations within the mouth. Sensations of "bitterness" are associated with small monomers or polymers. Astringency, on the other hand, increases with a higher number of gallic esters, with the degree of polymerization, with a higher concentration at a higher alcoholic degree, and with a low pH (Gawel et al., 2001). A decrease in the sensation of astringency occurs in wines with a high concentration of anthocyanins that bind to PACs creating large molecules that can precipitate, in the mixture of high degree of polymerization molecules that precipitate without binding to salivary proteins or the presence of soluble polysaccharides that can react with tannins not making them more reactive. Reason why, from very ripe grapes is made a wine with a lower perception of astringency due to the higher concentration of soluble polysaccharides and higher polymerization. More astringent tannins are extracted from grape seeds as they are larger in size. The large molecules are not very soluble, if they were extracted, they would not be retained (Tirelli, 2021).

Different reactions are responsible for these changes, including several oxidative phenomena (Ribèreau-Gayon et al., 2006)

- Acetaldehyde: It is therefore evident how a wine is kept, both in hermetic vats and in the presence of oxygen, has a significant effect on the development of its organoleptic characteristics. It has also been observed that aging in bottle is not able to soften wine with high phenolic content: an initially “aggressive” wine will always keep that character. There is also a risk of thinning due to precipitation.
- Tannins may react with voluminous polymers (polysaccharides) by forming colloidal precipitates. Tannins may be implicated in other reactions involving plant polymers, and proteins. They can precipitate during aging when Brownian motion decreases due to low temperatures. This is the origin of the colloidal coloring substance in wine. These combined molecules improve the taste of a wine, thanks to the fact that tannins become less active than proteins and make the wine more full-bodied: these are considered higher quality wines (Jackson, 2020). The stability of the combinations still depends on temperature.
- In an acid medium, the components of the procyanidins of tannins produce carbocations. This changes both the structure and the organoleptic characteristics of the tannin molecules. These modifications lead to a certain softening of the wine.

During the resting time in bottle, the effect may be different, depending on the variety, the ripening process, and the harvest. The main risk associated with these phenomena is that the set of these processes can start from the precipitation of tannins, which could lead to a non-positive perception of quality. This danger is greater in case of aging with bottles exposed to not controlled conditions.

2.3.3 Effect on Color

Color is probably the characteristic of the wine most subject to aging. Anthocyanins are initially found in monomeric form; however, due to their reactive nature, many reactions and interactions involving anthocyanins occur during vinification and aging (Jackson, 2020). These molecules collapse during aging, on average in 5 years the concentration reaches values close to zero (Mattivi, 2019). The percentage of those bound to sulfur increases in the early stages and then persists and remains stable over time. The polymeric tannin-anthocyanin compounds still decolorized by the action of SO₂ gradually decrease over time (Mattivi, 2019). When the wine exceeded its ideal aging time: anthocyanins disappeared, remaining only yellow-orange complexes (Jackson, 2020).

In red wines, the initial purple hue is associated with copigmentation. It decreases due to the dissociation of these complexes and release of oxidizable anthocyanins, changing their color (Jackson, 2020; Peng, 2002). At the same time, the polymerization of anthocyanins with tannins takes place. The changes historically have been associated with a decrease in the

optical density causing a shift in the absorbance of wine (Peng, 2002). The degree of polymerization was typically measured with the evaluation of Color Intensity and Hue (Somers et al., 1977). Low 420/520 nm ratios indicate a bright red color, while high values indicate a shift towards brick shades. The amount of initial free and combined sulfur dioxide, as well as that of anthocyanins and tannins, are key factors in driving polymerization reactions and the aging of red wines (Gambutì et al., 2020; Ribèreau-Gayon et al., 2006)

Different polymeric pigments are formed and different reactions take place (Fig. 2.5) (Jackson, 2020; Ribèreau-Gayon et al., 2006):

- The first change to mention is the direct combination of anthocyanins in flavylum form (positive charge and red color), with flavanol molecules (procyanidins, catechins, etc...). The result of this reaction is the formation of a colorless flaven, which if oxidized becomes red. An acidic pH of less than 3.5 and a molar ratio of T/A <5 promotes the formation of these compounds. This process causes the wine to deepen in colour after discharge.
- The pigments that are formed by anthocyanin-tannin reaction, depend on the structure of the tannin that reacts. In the case of simple condensation reactions, oxygen is not required: these reactions are favored at high temperatures that lead to the formation of carbocations. However, this type of reactions occurs slowly and during aging in hermetic tanks (stainless steel) or in bottles.
- In addition, a loss of color may occur accompanied by a change towards yellow-orange shades, caused by the degradation of anthocyanins and tannins. The breakdown of anthocyanins causes a loss of molecular structure in the red dye, probably accompanied by the appearance of a yellowish hue. Among the few reactions of which we are certain to date and whose contribution is strongly impacting there is the ethanal. Direct polymerization may be more significant in the long term than the polymerization associated with acetaldehyde that causes the emergence of tannin-pyroanthocyanin complexes orange (more marked at the beginning of maturation/ aging) (Jackson, 2020).
- Another source of phenolic polymerization is the oxidation of tartaric acid to glyoxylic acid. The reaction can generate both colorless and yellow polyphenols. This transformation takes place in function of the acetaldehyde count that is formed inside the wine, the temperature and the oxygen content dispersed in the wine (Tirelli, 2021).

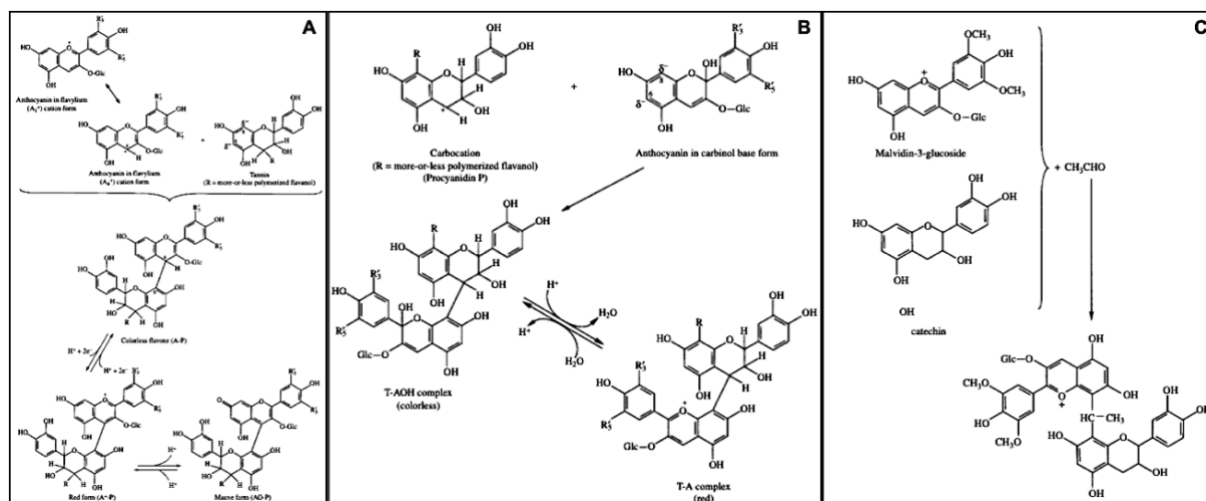


Figure 2.5. Example of reactions that occur between anthocyanins and tannins (Ribèreau-Gayon et al., 2006) : **A** Direct A-T type condensation of anthocyanins and tannins (Galvin C., 1993) **B** Direct T-A type condensation of procyanidins and anthocyanins (Galvin C., 1993) **C** Reaction between catechin and malvidin-3-glucoside in an acid medium, in the presence of ethanal (Timberlake & Bridle, 1976).

2.4 Factors that affect aging process.

The evolution of the wine inside the bottle it is not only connected to its composition, moreover to other factors that are responsible for its changing and that can affect the speed of the wine's evolution. These elements could be exogenous and endogenous. Evolutionary phenomena occur slowly in a reductive environment (such as that of the bottle), but the kinetics of reactions can be directly affected by temperature changes. If the volume inside the bottle decrease, due to different causes, air can be sucked into the bottle causing spoilage. However, the minimum quantities of oxygen play an impact role, also depending on the position of the bottle and the type/quality of the closure.

The effect of pH effects on the color and stability of red wine and phenolic susceptibility to oxidation. The redox potential decreases progressively to a minimum value. Anthocyanins are polyprotic acids and their chemical behavior in wine is strongly influenced by their form of dissociation. The most noticeable effect that occurs with the pH change is the color change. They are colored until the continuous sequence of double bonds conjugated from the phloroglucinol ring to the catechol/pyrrolyl ring is preserved. The hemiketal shape is highly unstable in acidic solution and the central ring easily opens to form a calcon. pH influences the ionic/neutral state of anthocyanins and, consequently, their electrophilic/nucleophilic behavior. The form of flavylium (positive ion) is low in electrons so it is a poor electron donor (high redox potential) and behaves like an electrophile, being able to react with other compounds and lead to a decrease in dye intensity (Janeiro & Oliveira-Brett, 2007).

The aging in bottle takes place practically in the absence of oxygen. From the chemical point of view, with a lower oxidation-reduction potential compared to the processing conditions in the cellar. The glass, being airtight, does not allow the passage of air. Theoretically, the cap should be impermeable to liquids and air, as it lies tight in the neck of the bottle and wet by the wine in the other end (Margalit, 2016). For the evolution and aging of white wines, oxygen is generally considered as harmful. On the contrary,

the minimum quantities of air absorbed through closures (different types of closures involve different quantities of oxygen dissolved inside the bottle) are associated with beneficial effects for red wines, especially "premium" wines (Gutiérrez-Escobar et al., 2021). These products benefit from the effects of oxygen that leads to greater complexity and greater polymerization between the various phenolic components. This also causes the slow and positive softening of the bitterness/ astringency of quality red wines (Jackson, 2020).

In a relatively controllable manner, oxygen is dissolved within the wine at bottling, causing rapid oxidation of all highly reactive compounds until the oxygen is depleted. In this way, the wine should continue to further be aging theoretically in the absence of air. In this first month after bottling, these rapid changes occur: the main consequences are formation of acetaldehyde, consumption of free sulphur dioxide and phenolic oxidation. Following this rapid phase, the wine in bottle is considered ready for the real phase of aging in bottle. All types of wine, in the absence of contact with oxygen, lead to the deterioration of wine through different chemical reactions until the end of its "shelf-life" (Margalit, 2016). The work of Gambuti et al. (2013), is focused to understand the effect of the oxygen before and after the bottling. Oxygen ingress through the closure post bottling was positively correlated with the decrease of astringency. Controlling the oxygen rate that come inside the bottle after the bottling, the producers can modulate the mouthfeel of the polyphenols the evolve during aging. Obviously, the modification of the astringency is a result of aging time, wine initial composition (pH and phenolic composition), and oxygen level. Moreover, for these reasons the selection of the right closure for the wine and the oxygen present at bottling are two fundamental aspects that will influence the evolution of the product (Gambuti et al., 2013). To evaluate the effect of different doses of oxygen during the bottle aging period, Avizcuri et al. (2016) studied this phenomenon related to the changes in color and anthocyanin content. The presence of oxygen seems to slightly accelerate the phenomena that occur during aging. The results confirmed an approximate model of general evolution of color indices and anthocyanin composition of red wines. The author agrees with the fact that the coloring substance of aged wines is more stable to changes produced by time, temperature, or oxygen available during aging (McRae et al. 2012).

The previous storage period and the type of tank that is used for the preliminary aging period in the cellar, influence the wine composition and its reactivity to the oxygen during the period in the bottle. A large part of the red wines follows a period in wood before the final operations. There are significant differences in phenolic concentrations depending on the type of wood used, which change the reactivity and the type of oxidizable compounds. In addition to the type of wood, also the intensity of toasting has a significant effect on the subsequent composition of the wine in bottle. Usually, a greater loss of anthocyanins occurs in wood obtained with a higher roasting temperature, then with a lower ellagitannins content that do not have a strong limited power against oxidation. For these reasons, the types of wood used for casks have a direct influence on the polyphenolic development also later in bottle. However, the chemical nature of polyphenols is more impactful than the type of wood used. For instance, chestnut woods is richer in oxidizable compounds compare to classic oak casks. By re-using barrels several times, a decrease in the extraction of polyphenols is caused (Tirelli, 2021).

To evaluate the effect of the oxygen, in the study of Sánchez-Gómez et al. (2020) the researchers compared the effect of the wood and the bottle aging to the final color of the red wines. Likewise, for the

simulated bottle conditions studied, the simulation of short bottle aging and the simulation of long bottle aging, the results depended on the aging time in cask and on the level of dissolved oxygen during this first period of aging. Although wines coming from casks with different percentages of oxygenation tend to be similar after a first short period in bottle, it was observed that with the progress of time they differentiated again. Wines with a longer period of aging in bottle have been characterized by a greater evolution, linked to the increase of yellow and the loss of purple tones, more evident in wines made from casks that showed the highest greed for oxygen.

Temperature is probably the factor that most affects the quality of the aging process in bottle during storage period. It has been proven, more than once, to be directly related to the kinetics of reactions that occur during aging. Results in literature show that wines that have undergone tests of aging at higher temperatures (20+°C), show a relative age between 1 and 18 months older than the wines with conventional storage at 13° C (Catarino, 2022). This reveals the importance of temperature on the aging process and, therefore, the degradation of phenolic compounds. This has been associated with the reaction of anthocyanins with H₂O₂ generated during the activation of molecular oxygen catalyzed by metal ions, which is favored by higher temperatures (Gutiérrez-Escobar et al., 2021). Free anthocyanins are easily degraded by light and high temperatures. Ageing at temperatures close to 40 °C is about 10 times faster than ageing at low storage temperatures (near to 12 °C) (Margalit, 2016). In order not to compromise the tightness and stability of the closure, the storage temperatures must remain constant and in the best range for the wine during the storage period. Avoid temperature fluctuations between different seasons is the only way to prevent the rapid maturation of the wine (Catarino, 2022). Rapid temperature changes can generate fluctuations in the volume of wine, making pressure on the seal of the cap/ neck. If the break of the stopper is sufficiently marked or repeated, it can facilitate the entry of oxygen. If the wine freezes, the volume increase may be enough to force the cork (Jackson, 2020).

For many reasons, the selection of the stopper is a crucial aspect that impact on the aging process. The cork is basically the only membrane permeable, or not, between the wine and the environment. In function of the type of closure, the amount of oxygen entering the bottle and the migration of some volatile compounds is different. The paradox for the keeping of red wines is that corks with higher permeability, on one hand would help the stabilization of color but would increase the oxidation of phenolic and aromatic compounds. As already mentioned above, the loss of free sulfur and flavan-3-ol monomers, and the change of anthocyanins in other colored compounds increased with the oxygen rate. For these reasons, the choice of the type of closure becomes decisive for the type of wine that will be released on the market. (Gutiérrez-Escobar et al., 2021). Humidity inside cellars must ensure that the cap does not dry out (Catarino, 2022). Red wines designed to be aged for a long time require a higher rate of oxygen transfer to ensure the evolution of the product in bottle; therefore, the corks chosen are made with natural cork or filled. Synthetic caps are usually used for "low" quality products as they tend to have a lower waterproofing to oxygen (except screw caps) and cause accelerated/premature oxidation (Echave et al., 2021). In the article published by Gao et al. (2015) they investigate the effect of the stopper and the time of aging on the phenolic compounds.

The duration of the aging period is another crucial aspect that influence the sensory composition of the wine at the tasting. The authors suggested that the 9-month period could be the key turning point for

the modification of the phenolic fraction during the bottle aging (Jackson, 2020). Combining the data of the oxygen dissolved inside the bottle during the period in bottle, the hypothesis of the researchers was that after 9 months the effect of closure on phenolic compounds was perceived both at the analytical level and through tastings.

There are a lot of other aspects that could influence the evolution of the wine during bottle aging. Like the color of the bottle: it should be avoided to bottle with transparent glass, in case it is better to keep the wine in question in dark rooms. The light would accelerate and catalyze the reactions of oxidation in wine.

2.5 Sensory analysis and overall quality of red wines

The first experimental part of the thesis was devoted to sensory analysis. This type of analysis is performed with verified and standardized procedures such as evaluation forms, control of the execution environment and Panel control. Personal evaluation is "limited" so that only the sensory characteristics of the wine are emphasized (no hedonic judgments, coded language). It generates essential, relevant, and comprehensive data, the statistical treatment of which cancels out any "subjectivity." It differs from simple tasting, which verbally translates the taster's perceptions by communicating his mental representation of the wine. The latter is strongly related to the individual's attitude for tasting and the individual's ability to communicate. Tasting provides us with opinions, unlike sensory analysis, which provides information (Ubigli, 2020).

From a scientific point of view, the objectives of sensory analysis are: to determine of the different impressions that the wine tasted causes (consumer); to evaluate in itinere of the suitability of the commercial (marketing) production route and to determine the timeliness of any alterations (professional / winemaker), to search for any defects (professional / winemaker) and to evaluate the overall quality of the product at any point in the production process. This thesis focused more in this latter application of sensory analysis (Ubigli, 2020).

Several mechanisms are triggered during this type of evaluation. Initially, the taster is subjected to a "stimulus," that is, physical or chemical agent that causes stimulation by interacting with specific sensory receptors. That differs from "sensation" in that it is more commonly defined as a subjective (reflex/unconscious) phenomenon that results from the stimulation of a sensory apparatus. For the final evaluation according to what is required by the tasting card, the judge has to extricate himself from his own subjectivity to evaluate a "perception", thus sensory awareness and interpretation of sensation based on prior experience (Ubigli, 2020). This type of technical tasting is divided into several stages:

- Observation using the senses.
- Comparison relative to known norms and (sight, smell, taste) expression of sensations with reference to an attribute.

- Expression of a reasoned judgment including by common vocabulary the assignment of a grade.

The tasting sheets proposed in sensory analysis can be of any type and arise from the combination of different types of questions. The goal is always to gather useful information according to the focus of the sensory analysis. In the sheet proposed in this thesis, we chose to divide the sheet into three parts: a first part with unstructured 10-cm scale questions, the second part with two CATA and finally a fill-in question. In this case, the proposed sheet is unstructured. According to Kobler (2008), this graphic type is applied in the detailed description of wines for viticultural and oenological research purposes. They are used to characterize wines from different production areas or help define characters of typicality of wines with controlled designation of origin. While numerical scales offer the taster a limited number of graduations, the chosen continuous scales are characterized by only two reference points placed at the ends. As there are several variations within the category of unstructured scales. This allows the taster to evaluate the wine under consideration as freely as possible, enabling the judge to make highly differentiated judgments. It is also not bound to a small number of pre-constituted subdivisions and does not come to be conditioned by the semantic meanings of the numbers at their disposal. The work Parr et al. (2020) was taken as inspiration for the formulation of the tasting sheet. The researcher uses eight continuous scales (100 mm linear scale) anchored with pictures, designed to clarify the concept being evaluated. The structure associated with the image plays a key role in simplifying the voting associated with the attribute by the judges. The use of a 10-cm unstructured line scale to assess the magnitude of attributes has also been used in many other works such as Cliff et al. (2007) and Gawel et al. (2000). Each judge had a different order of presentation of samples, chosen at random and presented with a random three-digit number. In this way, each judge most likely has a unique selection and sequence of wines that no other judge has. Each taster can thus be considered as a committee in its own right, thus maximizing the number of possible combinations (Kobler, 2008). Furthermore, in order not to influence the perception of attributes, judges were not told the variety or vintage of the wine (Cliff et al., 2007).

The use of ISO glasses for sensory analysis tests is a very important precondition to standardize in principle the instrument for the acquisition of sensory perception (Ubigli, 2020). In the first part of the test, it was decided to use an ISO glass with dark glass for the evaluation of only the taste characteristics of the product. The goal was to limit the influence of color on the perception of age and all the attributes asked about the wine within the tasting sheet. Confirming, Parr et al. (2020)'s publication, viewing wine color was a determining factor in judgments of attributes associated with quality by wine professionals that have been demonstrated to have colour bias.

One of the aims of the sensory analysis was to understand the evaluation of quality and its relationship to other attributes as a function of the age associated with wines during tasting. Therefore, to understand the relationship between quality, wine aging and the evaluation of the various sensory attributes of wine. Overall quality, often avoided in scientific articles because it is difficult to define, it is described as a multidimensional concept or a complicated construct. Regarding wine, the three main components for assessing its nature are color, taste and mouthfeel, and aroma (or flavor) (Brand et al., 2020). As (Parr et al., 2020) said:

“Wine is a complex beverage, both psychologically and in terms of its chemical composition. Perceiving the intrinsic attributes of a wine involves multisensory perception with visual, olfactory, gustatory, and tactile phenomena that integrate to guide the taster's judgments.”

Quality is classified primarily through two classes of product properties: intrinsic and extrinsic. The focus of this paper was only on intrinsic attributes, as tasters were not aware of extrinsic attributes such as: origin, year, label, price, marketing, etc... Intrinsic attributes are related to the actual composition of the products.

They cannot be altered in any way without changing the product at origin and are unique to each unit (Verdú Jover et al., 2004). The results of Charters (2007), showed that intrinsic dimensions of wine quality received more weight than extrinsic ones. The panel proposed many intrinsic characteristics. However, closer analysis indicated the existence of five major intrinsic dimensions (Fig. 2.6).

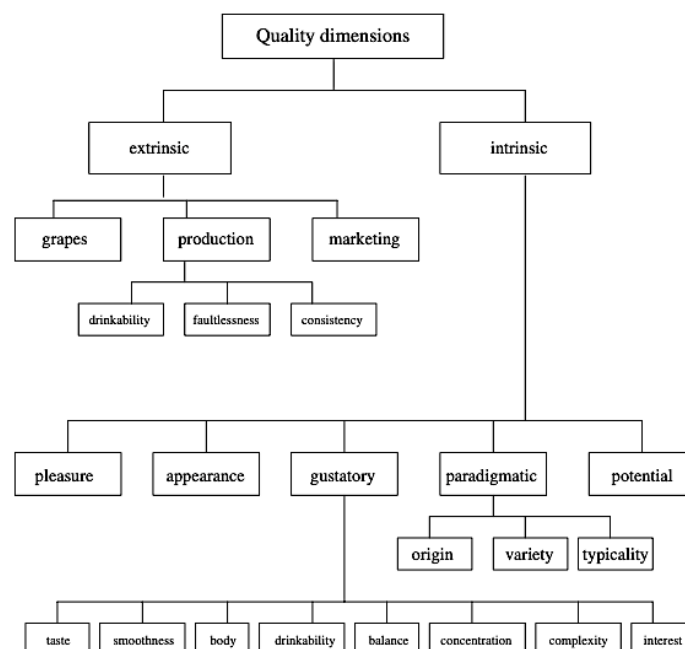


Figure 2.6. The dimensions of wine quality (Charters, 2007).

In the following thesis, it was chosen that the participants of the tasting were students and experts in the field to understand whether there was a real different response to quality according to degree of experience. Since, quality assessment can be carried out by judges

with different degrees of knowledge in the wine industry (experts, trained workers, consumers...), but it has been shown that these types of people differ in their perception of quality and its dimensions (Brand et al., 2020). From a personal point of view, wine can be faced with two possible ways of expressing quality, whose perceptions are discriminated against based on many factors. The first is composed by experts, the second is that of consumers (Verdú Jover et al., 2004). In according to Brand et al. (2020), for the evaluation of wine quality experts are preferred. This could be linked to the perception of "objectivity of experts". It is believed expert judges have a more objective (and systematic) approach to wine tasting and a better overall technique, developed over time through experience and exposure. However, their emotional response cannot be ignored. As suggested by Malfeito-Ferreira (2021), judgments about the quality of experienced tasters can be strongly influenced by cognitive factors and personal taste. In order to be as objective as possible in the evaluation of the product, it is necessary to be able to identify at the best the synthetic substances of the wine product (e.g. body, balance, complexity...) and be able to associate them to the wine's input elements (e.g. variety, production area, type of vinification) in order to evaluate the wine independently from the personal taste.

For Sáenz-Navajas et al. (2013), which aimed to assess the effect of knowledge and the level of competence on the perception of the intrinsic quality of wine. The results show no meaningful correlation between the quality scores provided by experts and regular consumers. While French and Spanish experts' judgements are highly correlated, as are consumer quality judgements. This shows that perceived intrinsic quality is dependent on the skill level of the consumer.

Focusing on the relationship between wine quality and aging, as suggested by Pereira et al. (2011) the study of aged wines considers more dimensions of quality, where intrinsic attributes, such as complexity, balance, and positive aging evolution, play an important role. In the course of the study of Charters (2007), some tasters suggested that the ability of a wine to improve with age belongs to the quality sphere of wine. This shows how the potential of aging is intrinsic to wine and it is gustatory because it is necessary to understand how the gustatory dimension evolves over time and how to discern from the immediate pleasure. If aging capacity is a quality dimension, then wine excellence is evident now, but its enjoyment is through deferred gratification. However, this was not recognized as the main dimension of quality, but many considered it an integral part of the concept along with others.

Moreover, due to the fact that this work is focused on polyphenols, it was asked to the judges to evaluate the taste sensations connected to these compounds in red wines, like bitterness and astringency. Among all sensations, astringency is considered the most valuable sensation of table red wines. Astringency is a multi-phase perceptive phenomenon. In order, several

stages have been defined, from the combination of which astringency is given (Gawel et al., 2001):

1. Drying, the lack of lubrication or humidity resulting in friction between oral surfaces.
2. Roughing, the non-smooth consistency in the oral cavity marked by perceived inequalities, ridges and/or protrusions when the oral surfaces meet each other.
3. Puckery, the feeling of drawing or tightening felt in the mouth, lips and/or cheeks.

Over the years, many have worked with the aim of characterizing the various sub-qualities related to astringency reaching many and detailed definitions based on the tactile perception left in the mouth by the tasting of red wines (Gawel et al., 2000; Gawel et al., 2001; Rinaldi & Moio, 2018).

The connection between the phenolic composition and the overall quality of wine, is the purpose of the work of Sáenz-Navajas et al. (2015). Wines with high content of astringent related molecules such as PACs, cis-aconitic acid, some flavonols and oxycinnamic acid derivatives have been classified by testers as lower quality samples. On the other hand, panelists assessed those wines with lower concentrations of lactones of whisky and volatile phenols, with higher levels of norisoprenoids were of higher quality. Moreover, there is an important role of the matrix and origin of wine, in the gustatory sensation of astringency. The variety undoubtedly plays a very important role on astringency and phenolic characteristics of wines. Therefore, the initial phenolic composition of wine seems to be the main factor influencing the evolution of wine during aging in bottle (Rinaldi & Moio, 2018).

The starting matrix is therefore undoubtedly the most impacted element in the perception of the sensorial characteristics related to polyphenols in wine. Therefore, not only the initial phenolic composition of the grape, but also characteristics such as acidity, pH, sugar and alcohol heavily influence global gustatory perceptions (Gawel et al., 2001). Moreover, it is fundamental to keep in mind that there are great inter-individual differences, also linked to human physiological factors, in the perception of these attributes linked to the phenols of wine (Parr et al., 2020).

3. Materials and Methods

3.1. Wine Samples

The wine used for the analysis are from several wineries in the North of Italy. For each wine, there were present at least 5 vintage and for each vintage a minimum of 3 bottles. The wines and the companies that take part to the study are described in the following table (Tab. 3.1).

Table 3.1 Samples used in the following experimental thesis.

Wine	Company	Origin	Varieties	Vintage	Aging Process	DOC
Luce della Vite	Frescobaldi	Tuscany; Montalcino (SI)	50% Merlot; 50% Sangiovese	2016, 2009, 2007, 2003, 1998	This wine is aged for 24 months in barrique before the bottling. After the fermentation the wine stays for 2 years in barriques and another year in bigger oak casks, before 12 months of bottle aging before the commercialization.	IGT (Typical Geographical Indication)
Teroldego Rotalaino DOC Riserva NOS	Mezzacorona	Trentino Alto- Adige; Piana Rotaliana (TN)	100% Teroldego	2021, 2016, 2012, 2006, 2001.	Once fermentation is complete, the wine is aged in Slavonian oak barrels for 24 months and then later in the bottle for 6-8 months. After fermentation, the wine stays for 24 months in 500 L French oak barrels and another 6 months in the bottle	Teroldego Rotalaino DOC
Amarone della Valpolicella DOCG Classico Jago	Cantina Sociale di Negrar (Domini Veneti)	Veneto, VR (Valpolicella)	Corvina, Corvinone, Rondinella and other indigenous varieties from Valpolicella	2015, 2010, 2004, 2000, 1995	After fermentation, the wine stays for 24 months in 500 L French oak barrels and another 6 months in the bottle	Amarone della Valpolicella DOCG
Valpolicella DOC Classico Superiore Sant'Urbano	Speri	Veneto, VR (Valpolicella)	75% Corvina + Corvinone, 20% Rondinella, 5%Molinara	2019, 2016, 2012, 2009, 2005, 2000		Valpolicella DOC Classico Superiore

3.2 Methods

3.2.1 Sensorial Analysis

The sensory evaluation was carried out over the past few months at the Microbiology laboratory of *Istituto Superiore di Agronomia* from 20th of April to 15th of May in different sessions. The tasting analysis was divided into several days according to the people who will participate in the panel. During one whole day, students, or judges with little experience in the world of wine tasted. During the following weeks, several more experienced judges (such as oenologists, wine professionals, sommeliers...) took part in the tasting. In Figure 3.1 is summarized the master data of the 30 tasters who took part in the thesis test. The aim of having people with different experience in the world of wine was to understand the different perception of aged wines from different points of view and experiences.

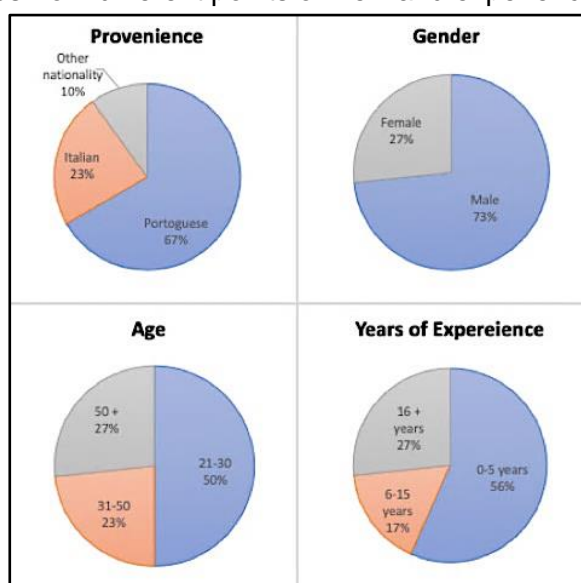


Figure 3.1 Analytical data of the 30 judges who took part the tasting.

The wines were presented in a random order for each judge. Each wine was associated with a random three-digit code to make it unrecognizable and unrelated to any order to the judges. There were 21 red wines for tasting, fully described in the chapter 3.1. They were divided into 4 sessions: in 3 of these 5 samples were presented to the judges and in one 6. The order of presentation was randomized and different for each judge. Between each session there was always a minimum break of 2-3 minutes to avoid sensory fatigue. At the end of the second session there was a 10-minute break for the same reason. The wines were presented in dark glasses so that the colour of the wine could not affect the evaluation of taste and aroma. Each sample was covered with a plate to avoid contamination of the wine with external smells and loss of aromas. The serving temperature was controlled in the way that it remained constant between 16 °C and 18 °C. Each judge in his post found in front of him: the tasting card, a pen, glasses marked with tails and lids, a spittoon, a glass for water, a bottle of water and crackers.

It was suggested to the judges to clean the palate with a sip of water and a piece of cracker after each taste, to limit sensory fatigue.

During the test, each panelist received 3 tasting forms to fill out. The first concerned filling in personal data (age, gender, education, years of experience in the wine sector) and compliance with privacy regulations. The second sheet concerned the evaluation of flavor and aroma. The panelists received a copy of the tasting sheet for each sample. The questions are presented in a different, randomized order for each judge.

The tasting sheet is divided into three parts (Appendix N° 1).

In the first, the judges were asked 8 different questions about wine attributes. Each judge had to draw a mark on a 10 cm continuous scale according to his or her personal idea. For each question, the scale ranged from low to high value of each attribute. The choice of using a continuous scale was because later in the statistical analysis the data to be worked on were continuous and not discrete. The model was based on the work of Parr et al. (2020). In the questions in this section, one was asked to rate: intrinsic quality, rate of evolution, complexity, balance, body, defect, bitterness, astringency.

In the second section, they were asked to fill in 2 CATAs for the evaluation of taste and aroma attributes. The judges could select a maximum of 5 descriptors for each wine. Judges have to choose between these descriptors for taste: dryness, sourness, minerality, saltiness, smoothness, viscosity, vegetal, umami, sweetness, acidity and others. Judges have to choose between these descriptors for aroma: balsamic, tobacco, chocolate, earthy, dried fruit, mature fruit, fresh fruit vegetal, floral, spicy, oak, kerosene, honey, wet stone, straw and off flavor.

In the last section, the judges had to indicate, based on the previous questions and their idea of the wine, the number of years after the vintage of the wine in their opinion.

Throughout this first evaluation of aroma and taste, the wines were presented in black glasses so that the colour could not influence this perception.

After the evaluation of the mouthfeel, the judges assessed the colour of the wines with the third and final tasting sheet (Appendix N° 2). There were 8 samples to be evaluated, respectively the youngest and the oldest of the 4 different wines. The samples are presented in transparent glasses all together in one session. Each glass was marked with the random three-digit wine code. On this sheet, each judge had to draw a mark on a continuous 10 cm scale to assess the colour from deep purple to brick orange. After that, they had to indicate the predicted age of each wine based solely on colour. This part was done with the aim of understanding how colour influences the perception of ageing.

3.2.2 Chemical Analysis

The determination of the main chemical parameters are made by W.Tejo enological lab and by the University of Porto.

Total Alcohol Content (% vol.), Reductive Sugars (g/L), Volumetric Mass (M.Vol. g/mL), Volatile Acidity (g/L acetic acid), Total Acidity (g/L tartaric acid), Malic Acid (Malic g/L), Lactic Acid (Lactic g/L), Malolactic fermentation (FML) and Carbon Dioxide are identified by F.T.I.R. The tool used for the analysis is BACCHUS 3 MultiSpec, FTIR - Bacchus Thermo Scientific. The Thermo Scientific Bacchus is an IRTF and UV-VIS infrared analyzer. Wine samples are inserted into tubes and placed in the needle. In the FTIR analysis the actual alcohol content (AT), density, volatile acidity (VA), total acidity (TA), pH values, reducing sugars (RA), malic and lactic acid content are obtained.

For the calculation of the Reductive Sugars, it is created a calibrated straight line by the method of reducing sugars of the official journal of the European communities' regulation (CEE) N.o 2676/90 of the commission, page - 52. We consider dry the wines below 3 g/L of reducing sugars. The malolactic fermentation is considered over when the concentration of the Malic Acid is under 0.2 g/L.

The pH is calculated by potentiometric method. The difference in potential between two electrodes immersed in the liquid under test is measured. One of these two electrodes has a potential that is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode. The calibration of the pH meter is made at 20°C using buffer solutions connected to the SI. The pH values selected must encompass the range of values that may be encountered in musts and wines. Usually, for wines is made by three buffer-solution at pH at 3.0, 7.0 and 9.0 For the determination, to dip the electrode into the sample to be analyzed, the temperature of which should be between 20 and 25 °C and as close as possible to 20°C. Read the pH value directly off the scale. The pH is reported to two decimal places.

The Free sulphur dioxide is calculated following the Ripper method (OIV, 2023). It is determined by direct iodometric titration in acid medium. The materials used are: 250 mL Erlenmeyer flasks, 20 mL volumetric pipette; 5 mL pipettes, burette. The reagents needed are starch weld 5 g/L, sulphuric acid (H_2SO_4) 1/3, Indine N/50. The procedure is to introduce in the 250 mL Erlenmeyer flasks (wide neck) 20 mL of the wine, 5 mL starch weld 5 g/L and 5 mL of sulphuric acid (H_2SO_4) 1/3. After, to titrate with Iodine N/50 until the color turns purplish. "n" is the volume of iodine spent in the titration. The result of Free SO_2 is "n" x 32, expressed in mg/L.

The Total sulphur dioxide is calculated following the Ripper method (OIV, 2023). It is determined by direct iodometric titration in acid medium. The materials used are: 250 mL Erlenmeyer flasks, 20 mL volumetric pipette; 5 mL pipettes, 20 mL pipette, burette. The reagents needed are starch weld 5 g/L, Sodium hydroxide 1N, Sulphuric acid (H_2SO_4) 1/3, Indine N/50. The procedure is to introduce in the 250 mL Erlenmeyer flasks (wide neck) 20 mL of the wine and 20 mL Sodium hydroxide 1N and to wait for 15 minutes. After, to add 5 mL of

5 mL starch weld 5 g/L and 10 mL of 1/3 Sulphuric acid (H₂SO₄). After, to titrate immediately with Iodine N/50 until the color turns. “n” is the volume of iodine spent in the titration. The result of Total SO₂ is “n” x 32, expressed in mg/L.

3.2.3 Polyphenols Analysis

3.2.3.1 Total Polyphenols Index (IPT), Anthocyanins and Tannins

The reagent needed is a solution at 0,1N of HCL. The procedure is: clarify the wine sample by centrifugation or filtration. Dilute the sample 1/100 in HCl solution. Measure absorbances at: 280 nm in a 10mm optical path; 320 nm in a 10mm optical path; 520 nm in a 10mm optical path. Calculations: IPT = 100 X D280; Anthocyanins (mg/l) = 100 (22.76 x D520); Tannins (mg/l) = 100 x 76 (D280 - 0.6 x D520 - 0.4 (D320 - 0.2 x D520)).

The measurement of anthocyanins is an estimation based on the same principle as the PUISSANT-LÉON method (same coefficient). The measurement of tannins is also an estimate based on the IPT by deducting the contribution of the absorbances of anthocyanins and phenolic acids (considering that these two families of compounds are the ones that contribute most to the TPI of red wines together with tannins).

3.2.3.2 Analysis of polymerization of pigments

According to Harbertson et al. (2002), Simultaneous analysis of the polymeric pigments and tannin in wines required two 1.5 mL microfuge tubes for each sample. The first tube was made up by dispensing 1 mL of the acetic acid/ NaCl buffer (containing 200 mM acetic acid and 170 mM NaCl adjusted to pH 4.9 with NaOH) into the tube and then adding 500 µL of the diluted wine (with a buffer of 12% aqueous ethanol (v/v), containing 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl). 1 mL of the mixture was transferred to a cuvette and the absorbance at 520 nm was determined (reading A). Then 80 µL of 0.36 M potassium metabisulphite was added and mixed, and the absorbance at 520 nm was redetermined after a 10 min incubation (reading B). For the second tube, 1 mL of the acetic acid/NaCl buffer containing bovine serum albumin (BSA) at 1 mg/mL was put into a 1.5 mL microfuge tube, and 500 µL of the diluted wine was added. The mixture was allowed to incubate at room temperature for 15 min with slow agitation. After incubation, the sample was centrifuged for 5 min at 13 500 g to pellet the tannin-protein precipitate. One milliliter of the supernatant was put into a cuvette, 80 µL of 0.36 M potassium metabisulphite was added and, after a 10 min incubation, the absorbance was determined at 520 nm (reading C). The absorbance due to MP, SPP and LPP is given as: Total polymeric pigments (SPP + LPP) = B x 1.08; Short-chain polymeric pigments (SPP) = C

$\times 1.08$; Long-chain polymeric pigments (LPP) = $(B \times 1.08) - (C \times 1.08)$; Anthocyanin monomers (MP) = $A - (B \times 1.08)$. (Versari et al., 2008)

3.2.3.3 Tannin Power-Precipitation of tannin with BSA

The relative concentration of procyanidins is measured by the ability of proteins present in BSA (bovine serum albumin) to bind with procyanidins and precipitate them. The method proposed by Boulet et al. (2016) is based on the potential of proteins to complex and precipitate tannins in solution. It is a subtractive approach: the selective determination of tannins is done by reading the absorbance at 280 nm before and after precipitation with bovine serum albumin (BSA) protein.

An initial dilution of 1 mL of wine and 3 mL of pH 3.3 buffer solution is made because it must be like the pH of the wine. Into one Eppendorf is placed: 1 mL of the solution, 500 μ L of pH 4.9 buffer solution. In the other Eppendorf it is placed: 1 mL of the solution, 500 μ L of pH 4.9 buffer solution with 1 mg/mL BSA. For both we vortex, wait 15 min for precipitation to occur and centrifuge at 13500 g for 5 min. The supernatant is removed and diluted with 2% HCl in a 5 ml flask. At a more acidic pH, the tannins bound to the anthocyanins are detached, so this contribution is also eliminated. Afterwards, we wait 30 min and record the spectrum between 230 and 400 nm with a 1 cm quartz cuvette. Tannins were estimated as the difference between the 280 nm absorbance measured on the control sample and that measured on the test sample (Boulet et al., 2016). The spectrophotometer used for the analysis is a Shimadzu UV-2100, UV-Visible Recording Spectrophotometer. The software used for the lecture of the absorbances is UV-2101PC. Take the spectrophotometric measurements using distilled water as the reference liquid, in a cell of the same optical path (1 cm), to set the zero on the absorbance scale of the apparatus at the wavelengths between 230-400 nm.

3.2.3.4 Dialysis index

The procedure (Ribèreau-Gayon et al., 2006) consists of putting 10 ml of wine into a cellophane tube. It is dialyzed with a 100 ml model wine solution (5 g/l tartaric acid, pH 3.2, 10% EtOH) for three days and agitated manually twice a day. After dilution to 1/10 with water, the optical density (d_1) of the dialysate is measured at 280 nm on a 1 cm optical path. The control is measured in the same way (d_0). $I(\text{dialysis}) = (d_0 - d_1) \times 100 d_0$.

3.2.3.5 Total Proanthocyanidins.

Total proanthocyanidins (condensed tannins) was determined based on the Bate-Smith reaction (Gonçalves et al., 2011). This method consisted of the measurements of sample absorbance (at 520 nm) of anthocyanidins resulting from the acidic decomposition of condensed tannins, at 100° C for 30 minutes, in strongly acid conditions (Ribèreau-Gayon et

al., 2006). Unknown concentrations of condensed tannins in wines were determined using a calibration curve ($R^2 = 0.9911$) obtained using different concentrations (ranging from 0.264 to 5.92 mg/mL) of a procyanidin fraction composed of dimeric and trimeric procyanidins purified from grape seeds (Gonçalves et al., 2011). Each sample was prepared in duplicate and injected in triplicate and results were expressed as mean \pm standard deviation and presented as g/L equivalent of total procyanidins.

3.2.3.6 Chromatic characteristics

3.2.3.6.1 Hue and Intensity through absorbance detection at $\lambda = 420$, $\lambda = 520$, $\lambda = 620$

Young red wines exhibit an absorbance maximum at 520 nm, which is responsible for the frankly red colour. As the wine ages, the absorbance maximum at 520 nm tends to decrease or even disappear, which corresponds to an increase in the yellow colour (absorbance maximum at 420 nm). This explains the evolution of the frank red colour into an orangey red or even an onion-skin colour. The absorbance at 520 nm provides an arbitrary estimate of the concentration of red pigments present in wine under natural conditions. The absorbance measurement at 420 nm provides an estimate of the concentration of yellow-brown pigments present under natural wine conditions.

This is spectrophotometric method. Conventionally, the chromatic characteristics of red and rosé wines are described by the intensity of color and shade. The intensity of color is given by the sum of absorbances (or optical densities) in reference to 1 cm optical path and wavelengths of wave lights 420, 520 and 620 nm. The shade is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm. Before to start the analysis, if the wine is cloudy, clarify it by centrifugation. Ideally, the optical path of the glass cell must be chosen so that A falls between 0.3 and 0.7. Take the spectrophotometric measurements using distilled water as the reference liquid, in a cell of the same optical path b , to set the zero on the absorbance scale of the apparatus at the wavelengths of 420, 520 and 620 nm. Using the appropriate optical path b , read off the absorbencies at each of these three wavelengths for the wine. The color intensity (CI) is conventionally given by: $CI = A_{420} + A_{520} + A_{620}$. (It is expressed to three decimal places) The shade (N) is conventionally given by: $N = A_{420} / A_{520}$. (It is expressed to three decimal places) (OIV, 2023)

3.2.3.6.2 CIEL*a*b* coordinates

The purpose of this spectrophotometric method is to define the process of measuring and calculating the chromatic characteristics of wines and other beverages derived from trichromatic components: X, Y and Z, according to the *Commission Internationale de l'Eclairage* (CIE, 1976), by attempting to imitate real observers regarding their sensations of

colour. The technique is fully described in the OIV correlated publication (OIV, 2023). The three main CIE Lab coordinates were:

- x: a^* , when greater than zero means proximity to red, when less than zero means proximity to green
- y: b^* , when greater than zero means proximity to yellow, when less than zero means proximity to blue
- z: L, or the brightness: 0 corresponds to black or the absence of light, 100 corresponds to white.

C^* indicates Chroma/Saturation; H^* indicates the hue or angle of the Chroma.

3.2.4 Statistical Analysis

The data collected were subsequently subjected to statistical analysis. As for the results obtained from the sensory analysis, after the appropriate checks, it was understood that parametric statistical tests (ANOVA) could not be used, as the data did not meet the assumptions for these tests. Then, using the R studio and Jamovi software, non-parametric tests, specifically the Kruskal-Wallis's test, were applied for the statistical analysis of this data. For the results collected from the various chemical tests, no statistical analysis could be applied because the samples available to us were insufficient to perform an adequate number of repetitions necessary for the statistics. Then, it was decided to perform the "Pooled Sample" technique, that is to insert the same 50 ml tube, the same wine but coming from 3 different bottles (those supplied by the cellars) so that the variability existing in the three bottles was collected in a single sample.

4. Results and Discussion

4.1 Sensory evaluation of synthetic parameters

4.1.1 Taster characterization

The tasting panel was composed by individuals with different backgrounds because the division based on experience and knowledge has been largely described in the world of wine (Verdú-Jover et al., 2004, Sáenz-Navajas et al., 2013, Brand et al., 2020). In this study, the cluster analysis of the responses to overall quality was shown in Figure 4.1. These results evidenced that each cluster was composed by students and professionals (Fig. 3.1).

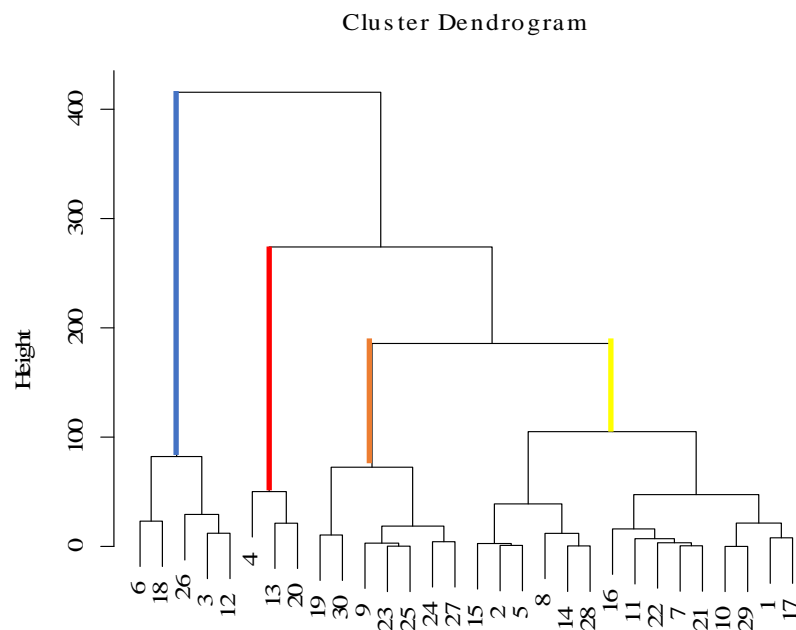


Figure 4.1 Clustering of the judges based on their answer for "Overall Quality" of the wines.

Figure 4.1 illustrates the division of the tasters in four clusters. The two groups on the left were the ones who gave the answers to the extremes: the judges of the blue cluster were the ones who gave the highest scores, the judges 4, 13 and 20 (red cluster) are the ones who gave the lowest votes. All the other judges were in the middle: the two successive clusters that were on the right put together the judges who gave medium-high (orange) and medium-low (yellow) grades. Since there was no clear separation between the evaluation of judges from 1 to 15 (students) and 16 to 30 (experts), it can be said that these are distributed all over the clusters. The only difference that can be noticed was in the orange cluster (Figure 4.1), where there were more "expert" judges, unlike the yellow cluster where there are many novice judges. This highlights how experienced tasters tend to evaluate wines aged in bottle with a higher rating. This probably derived from the greater familiarity in the tasting of these wines and a greater frequency in wine tasting. For example, a judge not accustomed to tasting aged wines could

easily define as 'off-flavour' an aroma that, on the contrary, an expert judge associates to the evolution of wine.

To check if the high overall quality scores were related to the utilization of higher scores across all synthetic parameters, another cluster analysis was performed using all of these scores. Comparing the judges' dendrogram based on grades on the quality attribute (Figure 4.1), with that based on the division of judges by grades on all attributes (Figure 4.2), there were some differences. The blue cluster included the judges who gave the highest score for all categories, in which there were again judge 12 and 26, as previously. In the successive clusters there were a homogeneous division between the expert and student judges.

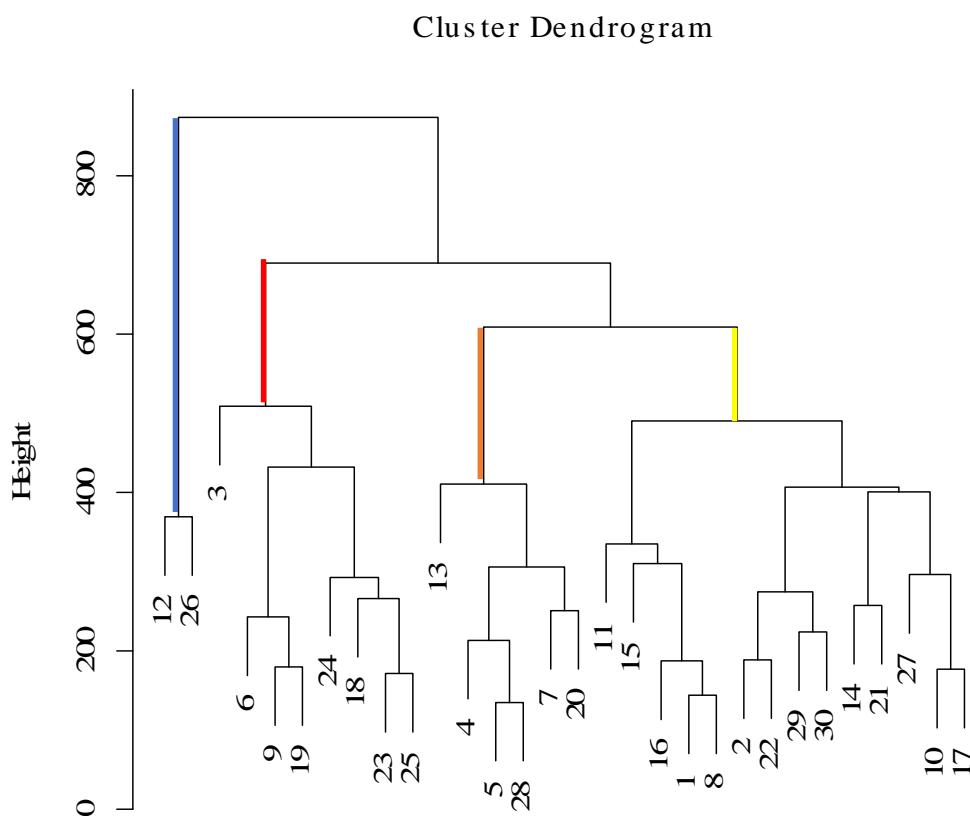


Figure 4.2 Clustering of the judges based on the answers for all the attributes of the wines.

The previous observations are clarified in table 4.1. The order of the judges was given according to the column "sum of all the scores" which should be in accordance with the cluster of Figure 4.2. As it is possible to see from the sums of votes, the order of judges did not follow a criterium of experience but rather the order was highly heterogeneous, confirming that the experience did not allow to separate the tasters according to experience.

As a results, , and taking in consideration the low number of respondents in two of the clusters, it was decided to merge all the responses irrespective of taster background.

Table 4.1 Sum of the votes of each individual judge for the Quality attribute and sum of all the votes for the synthetic attributes given by the judges. These two values are associated with the reference cluster. Judges are sorted by the sum of the total votes.

<i>Judge</i>	Years of Experience	Quality	Quality Cluster	Sum of all the scores	Cluster of all attributes
26	38	541	1	4.047	1
12	5	524	1	3.132	1
6	5	482	1	3.055	2
3	4	512	1	2.843	2
18	15	459	1	2.685	2
4	5	176	2	2.507	3
13	5	147	2	1.974	3
20	17	125	2	2.153	3
19	3	389	3	2.842	2
30	50	399	3	2.481	2
9	2	342	3	2.698	2
23	2	345	3	2.251	2
25	35	346	3	2.573	2
24	2	327	3	2.546	2
27	35	331	3	2.129	4
15	10	214	4	2.746	4
5	2	215	4	2.354	3
2	2	216	4	2.054	4
8	1	240	4	2.240	4
14	2	252	4	2.130	4
28	2	253	4	2.483	3
16	10	271	4	2.601	4
11	5	287	4	2.832	4
22	19	280	4	2.101	4
7	8	283	4	2.719	3
21	25	284	4	2.440	4
10	4	297	4	2.060	4
29	20	298	4	2.493	4
17	10	319	4	1.998	4
1	3	311	4	2.550	4

4.1.2 Synthetic evaluation scores

The average scores given by the panel of 30 judges is shown in Table 4.2. The statistical test used to highlight significant differences was a non-parametric test because the statistical population did not meet the requirements of parametric tests (normal distribution). Therefore, it was necessary to use the Kruskal-Wallis test, which is not based on the use of variances and averages of statistical data but on the relative position of the sum of the judges' votes for each wine in the evaluation of each attribute. For each wine was reported the average for each attribute, the standard error, and the significance according to the Kruskal-Wallis test. The use of the standard error and not of the standard deviation was used as this error index considers the number of the population, quantifying the change in the sample average from sample to sample.

Table 4.2 Results of the average value of the synthetic attributes. The error is related to the standard error for each attribute. Different letters refer to the post-hoc nonparametric test of Kruskal-Wallis, when the p-value indicated a significative difference.

Sample	Quality	Evolution	Body	Bitterness	Astringency	Predicted Age
A15	5.62 ± 1.75	4.32 ± 2.04 efg	5.91 ± 1.92 abcd	4.28 ± 2.48	5.72 ± 1.96 abc	6.00 ± 4.73 de
A10	5.62 ± 2.28	4.81 ± 2.08 cdefg	6.61 ± 1.92 a	4.89 ± 2.65	6.65 ± 1.52 a	6.90 ± 5.78 cde
A04	5.88 ± 2.07	5.56 ± 1.77 abcd	6.42 ± 1.91 ab	3.95 ± 2.41	5.66 ± 2.22 abcd	9.47 ± 7.25 abc
A00	5.94 ± 2.30	6.14 ± 1.65 ab	5.73 ± 1.74 abcde	3.74 ± 2.52	4.13 ± 2.43 fg	10.83 ± 8.39 a
A95	5.47 ± 2.31	6.17 ± 1.67 ab	5.88 ± 2.19 abc	3.62 ± 2.36	4.17 ± 2.04 g	9.73 ± 5.70 a
L16	5.57 ± 2.13	5.36 ± 1.89 bcde	5.96 ± 2.05 abc	4.94 ± 2.50	6.78 ± 2.07 a	7.17 ± 7.59 cde
L09	6.20 ± 1.70	4.84 ± 1.61 cdefg	5.87 ± 1.80 abcde	3.91 ± 2.33	5.57 ± 1.83 bcde	6.77 ± 4.72 bcd
L07	5.96 ± 2.20	4.83 ± 1.65 cdefg	5.60 ± 1.85 bcde	3.99 ± 2.25	6.32 ± 1.74 ab	6.87 ± 5.01 cd
L03	5.57 ± 2.62	5.60 ± 2.41 bcd	6.20 ± 2.48 abcd	4.14 ± 2.54	5.53 ± 2.79 cdef	7.90 ± 6.20 abcd
L98	5.73 ± 1.76	4.92 ± 2.24 cdefg	5.68 ± 1.66 abcde	5.00 ± 2.35	5.57 ± 2.12 bcde	7.30 ± 5.13 abcd
T21	5.75 ± 1.90	4.14 ± 1.70 g	5.37 ± 1.90 cde	3.19 ± 2.27	5.44 ± 2.68 cdef	5.67 ± 3.97 de
T16	5.57 ± 1.73	5.05 ± 1.59 cdefg	4.73 ± 2.20 e	4.21 ± 2.42	4.69 ± 1.99 defg	6.47 ± 5.82 cde
T12	5.05 ± 1.92	4.42 ± 2.27 efg	4.73 ± 2.39 de	3.49 ± 2.23	4.87 ± 2.29 cdefg	4.83 ± 5.57 e
T06	5.87 ± 2.24	4.40 ± 1.89 efg	5.70 ± 2.25 cde	3.69 ± 2.13	5.58 ± 2.08 bcd	5.67 ± 5.65 de
T01	4.64 ± 2.37	6.42 ± 2.21 a	4.71 ± 2.20 e	3.74 ± 2.17	4.69 ± 2.09 defg	9.30 ± 7.21 abc
V19	5.70 ± 2.15	4.24 ± 1.77 fg	5.13 ± 2.22 cde	3.97 ± 2.38	4.56 ± 2.39 cdefg	5.30 ± 4.47 de
V16	5.77 ± 1.81	4.83 ± 1.77 defg	5.10 ± 1.86 cde	4.23 ± 2.17	4.43 ± 2.52 fg	5.80 ± 4.63 de
V12	5.21 ± 1.99	5.25 ± 1.87 bcde	5.36 ± 1.88 cde	4.59 ± 2.32	4.80 ± 2.20 cdefg	6.23 ± 5.09 cde
V09	5.02 ± 2.37	5.02 ± 2.43 cdef	5.27 ± 2.44 cde	4.66 ± 2.14	4.50 ± 2.33 fg	6.70 ± 6.60 de
V05	4.67 ± 2.31	5.80 ± 2.16 abc	4.82 ± 2.38 e	3.81 ± 2.28	4.61 ± 2.36 efg	6.57 ± 4.76 cde
V00	5.16 ± 2.43	6.49 ± 2.35 a	4.68 ± 2.49 e	3.81 ± 2.40	3.83 ± 2.42 g	10.07 ± 7.11 ab
p.value	0.4387239	5.46E-09 ***	0.001628 **	0.0785762	1.72E-09 ***	5.24875e-05***

4.1.3 Overall Quality

By means of statistical analysis, it has been found that for the intrinsic quality of wine there was no difference according to the samples. Therefore, the results given by the judges showed that the perceived quality of wines, during the aging in bottle, did not change the perception of quality itself. This can lead to several conclusions:

- The age of wine did not influence the perception of its quality.
- The taster adapted his quality evaluation to the evolutionary state of each wine.
- Experience was not a determining factor influencing the different evaluation of the quality of wine as previously demonstrated by the clustering of judges.

Through the Figure 4.3, it is possible to understand the general trend based on the curves on the evaluation of the quality of the different wines. In the literature, the evolution of a wine during the aging in bottle is described by Dubourdieu (1992), who stated that the "quality" describes a parable in time reaching a peak (10) and then decaying until the "death" of the wine itself (0). By graphing the results for each wine in the range from 0 to 10 (Fig. 4.3A), it can be noticed how the real behavior was very different from the expected one. Pereira et al. (2011) and Charters (2007), suggested that the potential of aging and the "positive" evolution were elements to be attributed to a higher intrinsic quality of wine.

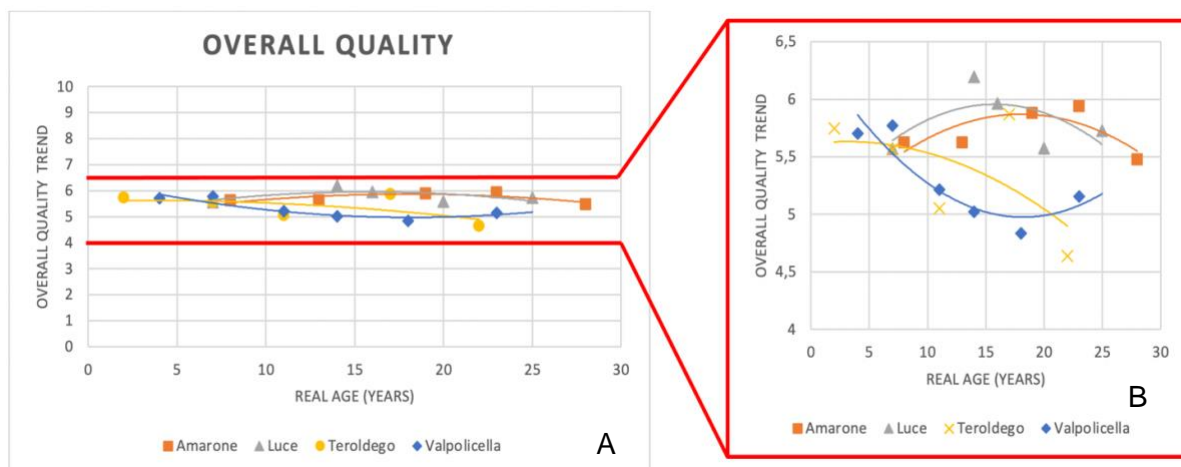


Figure 4.3 Curves describing the development of the average quality ratings of the 4 wines according to the judges' scores. **A**: Evaluation curves for the 4 wines in the entire mark's scale. **B**: detail of the judge's votes in the main range (from 4 to 6,5) of the evaluation scale.

Overall, the wines considered in this work showed different tendencies (Fig. 4.3):

- By observing the position of the averages of the different wines in the chart, young wines were appreciated in the same way as old wines.

- The averages of evaluations tended to be always close to the central values of the scale (Fig. 9A). This confirms how judges on a scale tend to exclude extremes in favor of central values, although some did not follow this observation (see Table 3.1).
- The vintage factor and the tasting sample seemed to affect the perceived quality itself, rather than the real evolution of the wine in bottle.
- The fact that there was different behavior in different wines confirms the high variability in the judgment of intrinsic quality.
- Only in two wines, *Amarone della Valpolicella* (orange curve) and *Luce* (grey curve) (Fig. 4.3B), there was a tendency to have an evolution of quality as expected.
- For the yellow curve (Fig. 4.3B), related to *Teroldego* wine, it can be noticed the quality tended to be higher for younger wines and the evaluation then gradually decreased over time (except for vintage 2006).
- For the blue curve (Fig. 4.3B), relative to the *Valpolicella* wine, there was an opposite behavior to the expected one: peaks occur at extremes, that was with very young or very old wines. In the middle there was a "loss" of quality.

Through the box-plot graph (Fig. 4.4), it is possible to observe the high variability in the evaluation of this attribute. Therefore it is possible to hypothesize that the results showed a different evolution according to the type of wine taking in account, and that the model proposed by Dubourdieu (1992) had given an excessive simplification of what can be the real evaluation of each wine.

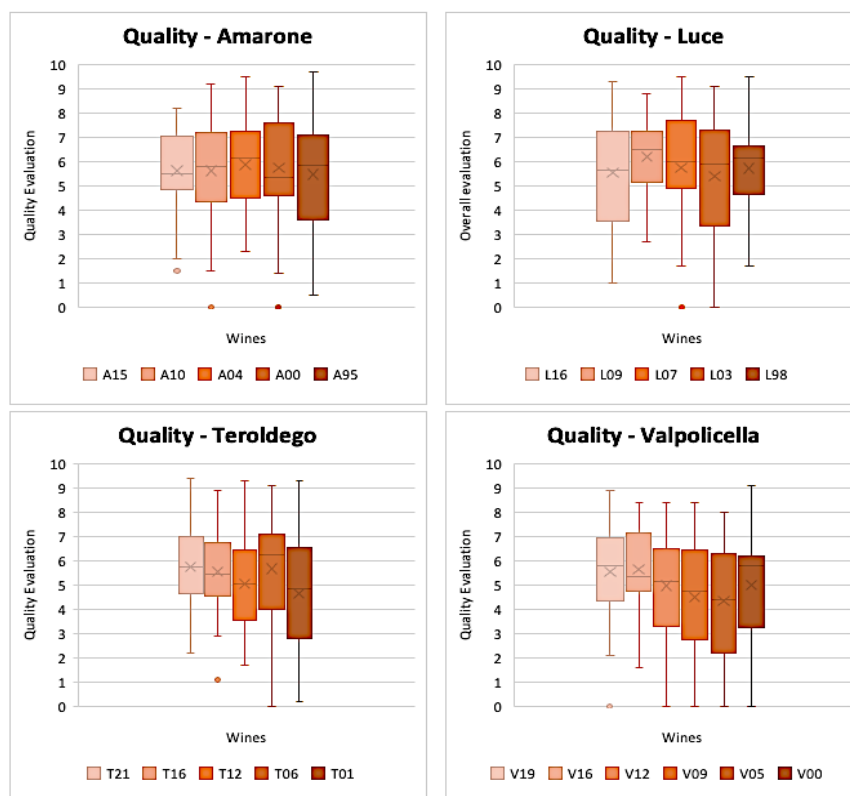


Figure 4.4 Boxplots describing the ratings of each wine according to the attribute Quality.

4.1.2 Wine evolution state

In the tasting sheet, it was asked to evaluate the evolution of wine. The judges indicated whether the wine was in its initial phase (0), its maximum expression (5) or its terminal phase (10). The graphs in Figure 4.5 were obtained by modifying the results obtained. The results awarded by the judges were inserted in the following equation: $(\text{Score Given} - 5)^2$. The formula was applied to obtain model close to a parabola, as reported in literature by Dubourdieu (1992). The obtained curves could be read in the following way: the more the average of the sample for this attribute is close to zero, the more that wine has been evaluated as at its maximum expression. The averages farthest from zero indicated wines too young or too old according to the panel. The non-parametric Kruskal-Wallis test showed that there was a highly significant difference between samples. Since graphic corrections were made in the chart representation, the post-hoc test letters were reported in Table 4.2.

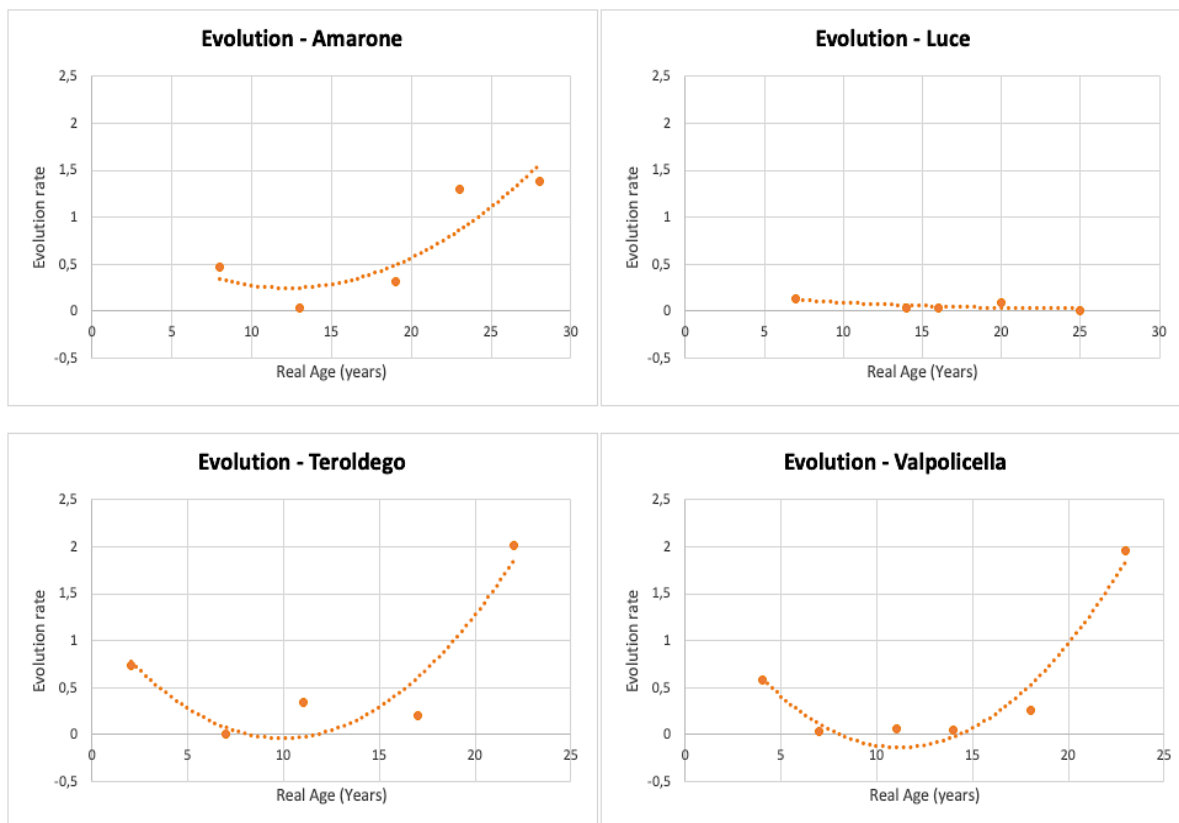


Figure 4.5 Curves describing the "Evolution score" of the wines

The curve of the evolution of *Amarone* wine showed the expected trend. The *Amarone* wine with 13 years was the less developed. On the left there was the *Amarone* 2015 wine which was quite close to the next sample. All wines after A10 (see table 4.2) were evaluated towards the terminal stage of their expression. The post-hoc test (Tab. 4.2) showed that there was a significant difference between the younger wine (A15) and the three older *Amarone* wines (A04, A00 and A95).

The *Luce* wine showed different results compared to other wines: all the samples were evaluated at zero, therefore there were no differences in their development from 7 to 25 years of age. This was probably due to the production technology, the varieties used and the effect of the different vintages considered.

The curve of the *Teroldego* wine was consistent with what was expected: the younger (T21) and older (T01) wines are at the extremes, the other samples were evaluated close to zero. For the non-parametric test, only the wine of vintage 2001 was different from the others.

Finally, the chart for *Valpolicella* wine agreed with that of *Amarone* and *Teroldego* wine. The wines of the vintages 2009, 2012 and 2016 were close to zero, therefore at the top of their expression considering the equation applied to the equation. The wine of the 2019 vintage, had been recognized as the youngest. The 2005 and 2000 wines were far from the axis of the abscisse, therefore in their decreasing or terminal phase, as reported also by the singular difference in the post-hoc test (Tab. 4.2).

As it is possible to see from Figure 4.6, it did not exist a good correlation between these two synthetic attributes. There was an inverse relationship but the correlation index (R^2) was rather low (0,1403), indicating that the perceived evolution of the different wines was not reflected in lower quality scores, as shown in table 4.2.

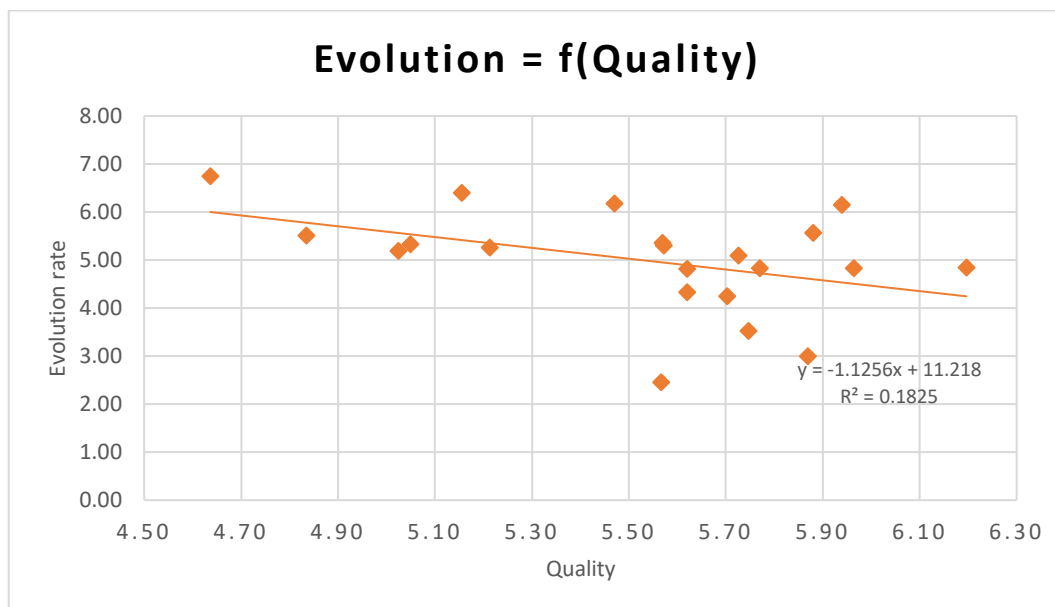


Figure 4.6 Correlation between the averages values of the quality and evolution marks according to the judges.

4.1.3 Body

Body was another attribute that caused a difference between the different samples (Table 4.2). But what is meant by full-bodied wine? The body, in the case of sweet wines, is correlated to the sugar content, whereas, in the case of dry wines, it is more associated to the alcohol content. It is also argued that glycerol can increase body perception and that acidity can,

instead, decrease this perception. Tannins also seem to be involved in the definition of full-bodied wine (Jackson, 2009). Regarding this attribute it is in fact important to compare these results with those of the basic analysis and those on astringency and tannins (Fig 4.9).

Statistical analysis shows that there was a significant difference in the evaluation of this attribute between the different wines (Table 4.2). The result was statistically significant (< 0.01). Through the box-plot (Fig. 4.7) It showed that there was a high variability in the votes associated with the body for the wines in question. The values tended to stay higher than the 5 rating. It can be noticed that there was a common trend of decrease of the body during the aging period in the bottle. Chart boxes tended to be very large, indicating that the range in which 50% of the values could be found was very wide. Moreover, the margins of the first and last quartile were also very wide, covering the entire scale (from 0 to 10) in many cases, confirming the high variability of the results. Many box-plots described a hyponormal distribution. About all wines had a positive or negative misalignment with respect to the average.

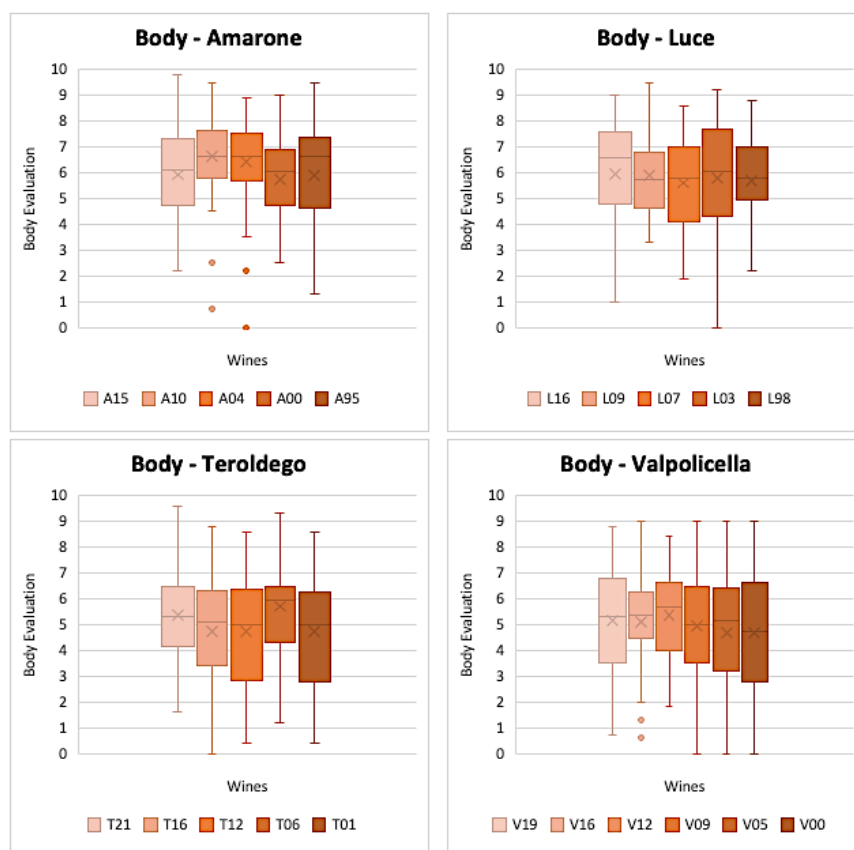


Figure 4.7 Boxplots describing the ratings of each wine according to the attribute Body.

Considering individually the evaluation of each of the 4 wines, several considerations could be done (Fig. 4.8). It should be pointed out that in the evaluation of this activity it was clear there was a difference between the different wines, but there were only small differences between vintages of the same product. Regarding *Amarone della Valpolicella* wine, it can be noticed

that with the advance of the vintages there was a slow tendency to decrease of body evaluation. According to the Kurkall-Wallis test, there were no statistically high differences between the different vintages, the only one to report is for A10 wine which had been attributed a higher value than the other samples. In general these wines were considered the ones with the highest value in this attribute. This depend on the nature of the matrix.

For the wine "*Luce della Vite*", it can be noticed a tendency similar to that of Amarone wine, even if less accentuated. There were no significant differences between the different vintages. This could be influenced by a probable effect of vintage and the varieties on the composition of wine.

In the graph for the "*Teroldego Rotaliano*", it was possible to notice a soft decrease in the evaluation of this attribute, despite the linear tendency was quite flat. There were no significant differences between the different vintages on the basis of the post-hoc test. The wine Teroldego in the vintage 2006 received the higher values. The values of "Body" were lower than the previous wines, this could be in accordance with the type of the wine itself.

About *Valpolicella Classico Superiore* wine, it can be noticed a tendency to decrease in the evaluation of the body more accentuated than other wines, as the curve had a higher slope. There were no statistically large differences between the vintages according to the Kruskal-Wallis test. Valpolicella wines in 2005 and 2000 vintages were considered among those with the lowest body by the judges in the tasting. The tendency to an increase in the body in years with a medium aging was in agreement with the increase in the polymerization of the tannic component and with a consequent increase in the perceived astringency (Ribèreau-Gayon et al., 2006).

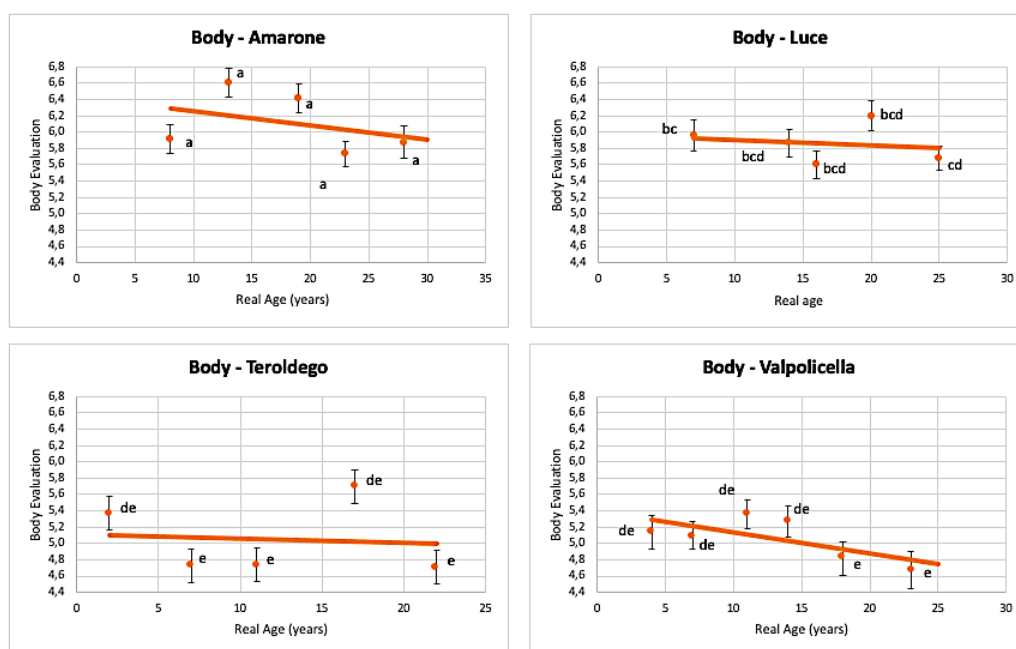


Figure 4.8 Curves describing the "Body Evaluation" of the judges for each of the 4 wines.

It was also tried to confirm what was described by Jackson (2009) about the possible correlation between sugar content, alcohol and body (Appendix N°3). As it is possible to see from Figure 4.9A, there was a good, but not very strong, correlation between the Body evaluation and the alcohol content of the wine ($R^2 = 0,63$). By the contrary, it was not exactly the same between Body and sugar content (Fig. 4.9B): the correlation index was 0,28. The wines with higher ethanol have also higher sugar, case of the Amarone.

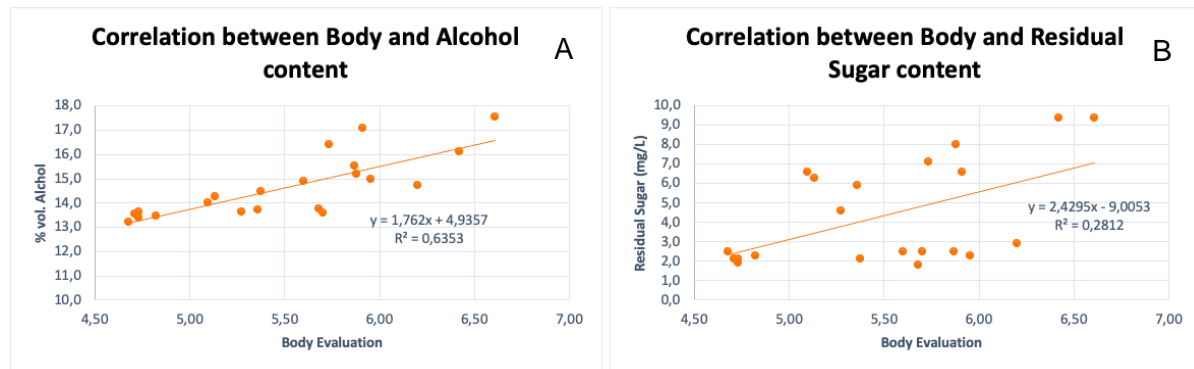


Figure 4.9 **A** Correlation between Body evaluation made by the judges during the tasting and Alcohol content from the chemical analysis. **B** Correlation between Body evaluation made by the judges during the tasting and Residual Sugar Content from the chemical analysis.

4.1.4 Astringency

Astringency is the sensory feeling is related to the reaction that occurs between Salivary proteins and flavan-3-ols that cause precipitation of the tannin-protein complex and lack of lubrication (Mattivi, 2019). This attribute of wine should tend to increase during aging due to the growing of the average degree of polymerization of wine tannins. To this phenomenon, should follow a relative "softening" of this sensation due to the continuous increase in the degree of polymeric pigments polymerization up to their precipitation and also to the formation of high more complex compounds always destined to precipitate, causing the decrease in the binding tannin-salivary protein (Jackson, 2020).

Statistical analysis showed that there is a significant difference in the evaluation of astringency for the different samples (Tab. 4.2). Samples are significantly different ($p.value < 0.001$ ***). Through the box plot in Fig. 4.10, it can be noticed that there is a high variability in wines and between samples. For some wines there seemed to be a gradual tendency to decrease over time, for others the evaluation remained constant over time. Obviously, astringency was a value very linked to the organoleptic composition of grapes and also to the effect of the vintage on the accumulation of polyphenolic compounds. There are varieties that had very high values and that, despite the evolution in time in bottle, in very old vintages, however, a higher

astringency than younger wines made with other varieties. For every wine, the youngest vintage had always been voted the most astringent. It is possible to observe the great variability in the results according to the size of the box-plot, that was quite in accordance with all the other results of the sensory analysis shown by box-plot.

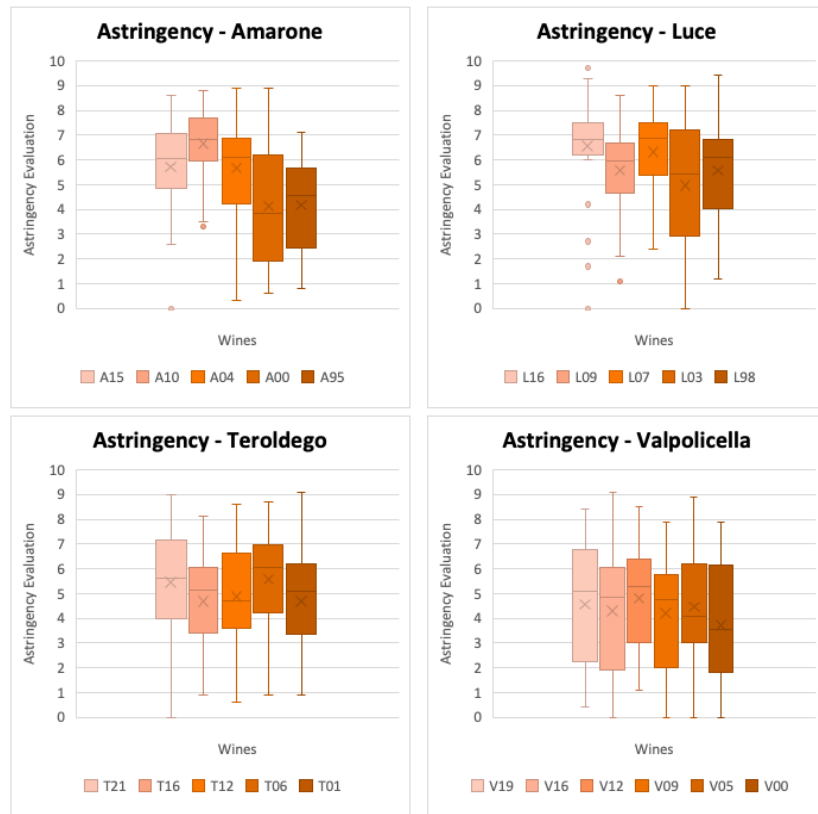


Figure 4.10 Boxplots describing the ratings of each wine according to the attribute Astringency.

Considering individually the evaluation of each of the 4 wines, several considerations can be made (Fig. 4.11). About the graph of *Amarone Della Valpolicella* wine, it can be noticed a decreasing tendency in the evaluation of astringency. This result was strengthened by the statistic that confirms that there was a strong difference between Amarone wine in vintage 2010, the one with the highest value, and wine in vintages 2000 and 1995 with the lowest values. This trend has been confirmed by the chemical tests on astringency (Fig 4.30-4.31). As for *Luce della Vite* wine, it can be noticed a decreasing tendency in the evaluation of astringency, although less pronounced than Amarone. There were significant differences in sample evaluation, according to the Kruskal-Wallis Parametric Test. The Luce 2016 wine was evaluated as more astringent than the 2009, 2003 and 1998 vintages. The decrease in the evaluation of astringency is constant and with a lower tendency than the previous wine but like subsequent wines. The use of two varieties, such as Sangiovese and Merlot, very rich in the polyphenolic component has had an impact on the evaluation of this attribute with medium-high values (Rinaldi et al., 2020; Mattivi, 1997).

For the graph of *Teroldego* wine, it can be noticed a slight decrease in the slope of the curve associated with the linear tendency describing the evaluation of astringency. There was no significant difference in the evaluation of samples, according to the Kruskal-Wallis Test. The value of *Teroldego* wine 2006 received the highest ratings. This was in accordance with the marks assigned to the attribute "Body" (Fig 4.7): astringency is a component that contributes to the perception of the body of a wine (Jackson, 2009).

For *Valpolicella Classico Superiore* wines, it can be noticed a decreasing tendency in the evaluation of astringency. There was no significant difference in the evaluation of samples, according to the post-hoc test. In general, this wine recorded the lowest marks in the evaluation of this attribute among all the ones proposed by the tasting. This agrees with the work of Giacosa et al. (2021), as the varieties that constitute it were poor in polyphenolic component, especially flavan-3-oils. Despite *Amarone* was produced with the same varieties, the production technology influences the content of these compounds as through drying there was a concentration of these molecules.

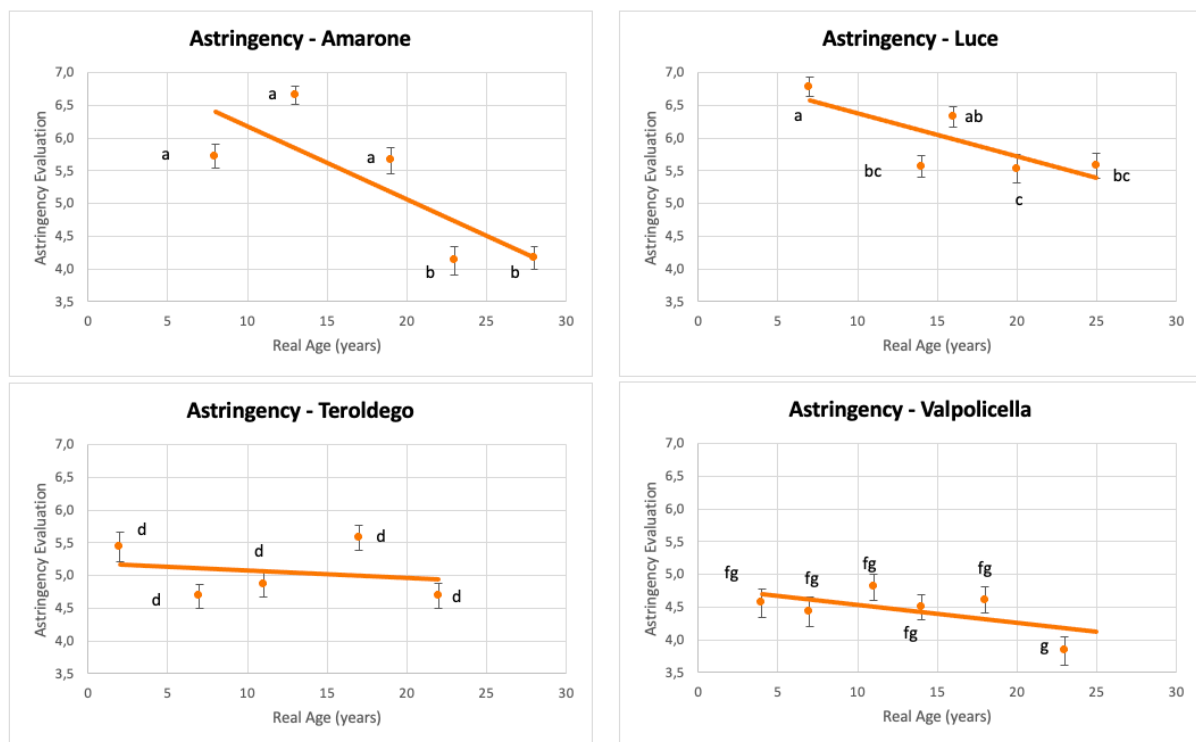


Figure 4.11 Curves describing the "Astringency Evaluation" of the judges for each of the 4 wines.

4.1.5 Bitterness

The bitterness sensation in red wines is connected to flavan-3-OIs of small dimensions. According to the literature (Ribèreau-Gayon et al., 2006), it is expected that during the evolution of wine the sensation of bitterness will decrease, along with an increase in perceived astringency and over time leading to the softening of tannic perception. Higher polymers, that

usually come from the skin (especially those between 2-5 monomer units) (Jackson, 2009), will tend to be more astringent than bitter in solution (Ribèreau-Gayon et al., 2006).

From the results of the tasting, the statistical tests showed no significant differences regarding this attribute. In the boxplot chart below (Fig. 4.12) there had been a high variability in bitterness evaluation. The values of the averages had tended to be low, as they were lower than 5 as an estimate. For wines it was difficult to discriminate a trend during the time as it did not correspond to the expected one that can be found in literature. The chart boxes tended to be very large, indicating that the range in which can found 50% of the values is very wide. In addition, the margins of the first and last quartile were also very wide, covering the entire scale (from 0 to 10) in many cases, confirming the high variability of the results. Almost all boxplots described a hyponormal distribution. About all wines had a positive or negative misalignment with respect to the average.

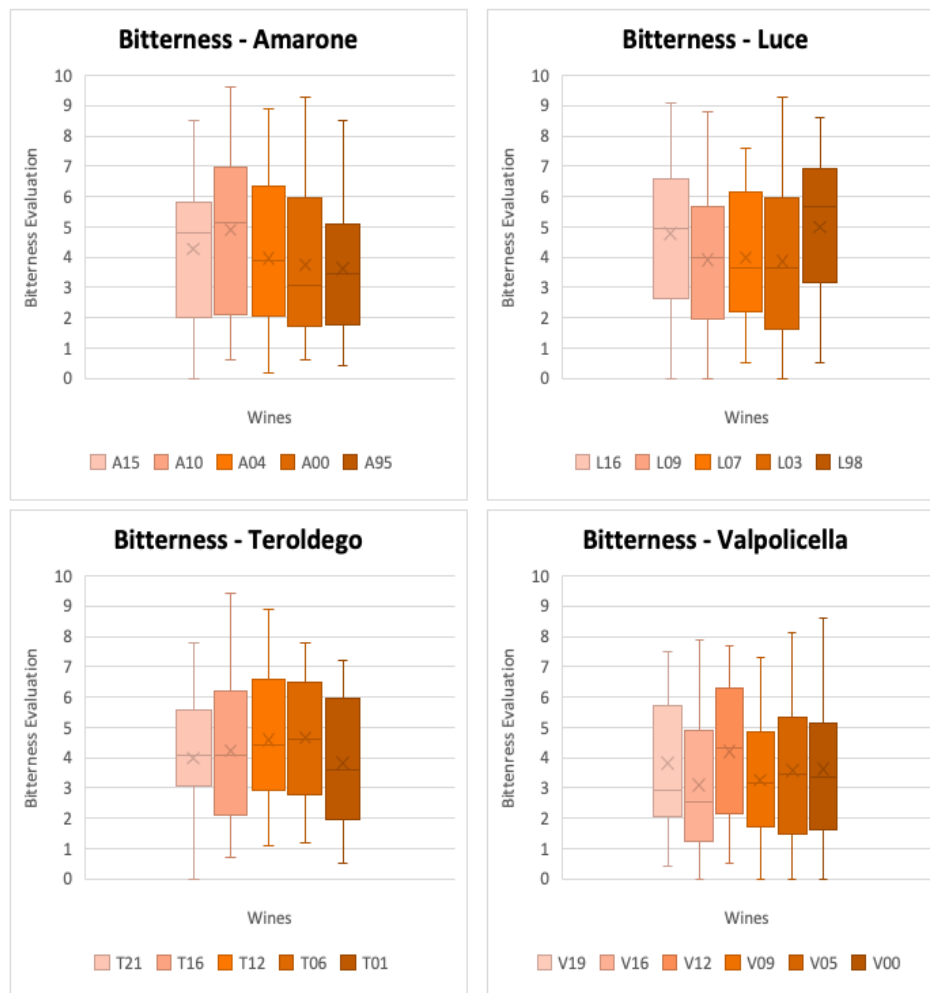


Figure 4.12 Boxplots describing the ratings of each wine according to the attribute Bitterness.

In Fig. 4.13 it is shown the trend of the average votes for each of the 4 wines for the bitterness attribute. In the graph (Fig. 4.13A), it can be noticed that all the wines are close to the central part of the scale. This highlights how it was difficult to statistically discriminate wines in this

case. Moreover, it emphasized how it is difficult for an untrained panel to evaluate the bitterness of wine, as it was certainly an attribute of wine difficult to evaluate without a proper experience. Looking at the course of bitterness in the different wines (Fig.4.13B), it can be noticed how each wine had about a different evolutionary curve. The only two samples that could be compared are *Teroldego* wine (yellow) and *Amarone* wine (orange): the trend grew and then drastically decreased. Nevertheless, the *Teroldego* 2021 was evaluated as slightly bitter. In contrast, *Luce della Vite* had the youngest wine and the oldest vintage with the two highest ratings and the vintages in the middle received very low ratings. For Valpolicella wine the trend went to describe a curve that remained almost constant as the associated average values were very variable during the years.

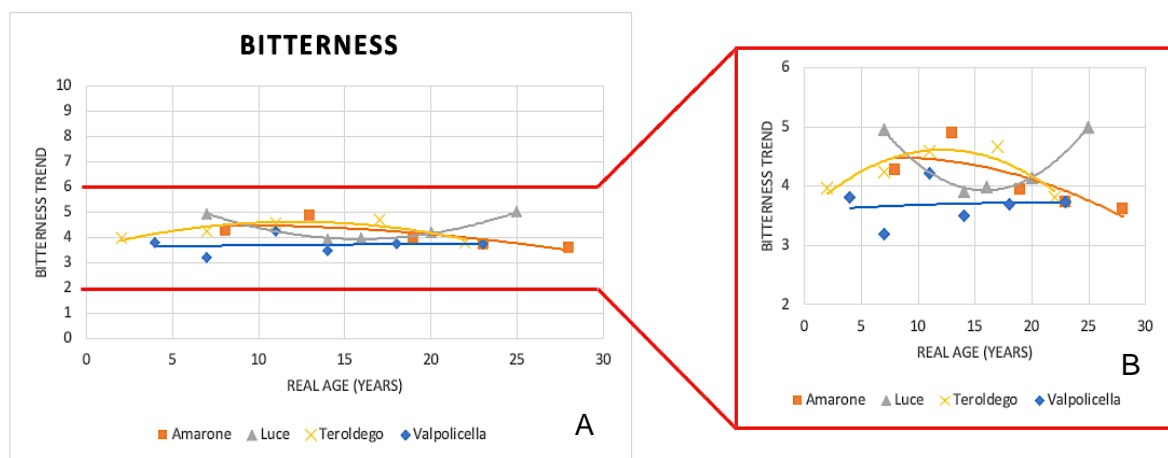


Figure 4.13 Development of the average for bitterness ratings of the 4 wines according to the judges' scores. **A:** Evaluation curves for the 4 wines in the entire mark's scale. **B:** detail of the judge's votes in the main range (from 2 to 6) of the evaluation scale.

According to the production and vinification technology, and to the evaluations for the synthetic attributes of these wines, some interesting considerations could be made.

For the wine "Luce della Vite", the long aging process in barrique, the use of international varieties and the style of the product that could be grouped into the "super Tuscan" category, may have had an influence on the evaluation of the product by greatly improving the taste of the product and losing the "vintage factor" that was easier to find in other wines.

In the other products, larger woods were used, and the starting varieties were less identifiable with the tastes of international varieties already known. Amarone and Valpolicella, although it was important to emphasize that they were produced in two different cellars, started from the same reference varieties but the drying process, to which the grapes destined for the production of Amarone were subjected, gave totally different characteristics (Jackson, 2020). The main effect of the drying brought a concentration of all the main constituents of the starting varieties, being poor in polyphenolic content (Giacosa et al., 2021); this technology contributed to the achievement of more intense tannins and stronger colors. This was in agreement with

what was reported by the evaluation of astringency, which was more marked in Amarone than in Valpolicella. Moreover, Valpolicella was considered as a "younger and fresher" wine with a lower aging potential than its dried version. This was confirmed by the results for the attribute "Evolution", where older vintages were considered on average closer to the end of the curve for Valpolicella than for Amarone. To conclude, Teroldego was produced with a variety that was very rich in anthocyanins and not as rich in tannins (Giacosa et al., 2021). The production technology that involved the use of wood of different sizes, aimed to extract tannins in order to stabilize the color and contributed to a greater astringent sensation of the product (Mattivi, 2019). The use of wood in the aging phase before the storage in bottle, had a strong influence in the subsequent evaluation and evolution of the product (Jackson, 2020).

4.1.6 Quality Prediction

By comparing the grades of the different attributes with the overall quality grades through a correlation analysis, it is possible to predict quality. The results made possible to predict that higher quality values were associated to wines considered by the judges more complex, balanced, and full-bodied. Conversely, wines with a lower quality had been considered as the most "defective" (Fig. 4.15).

In the following CorPlot graph (Fig. 4.14), the correlation ratio between the different attributes was described according to the results of the tasting. The graph is read based on the color and size of the circle that is intersected by the attribute that is at the top and the one that is in the scale on the left. If the circle intersecting the two attributes is white it means that there is a positive correlation, if black the correlation is negative. This means that if white as one attribute grows, the other also grows, if the circle is black as one attribute grows the other decreases. The intensity of the correlation is explained by the size of the circle: the greater the relationship between the two attributes. Obviously, the circles at the beginning of each line where the attribute intersects with itself in the vertical axis, are white and with the maximum size, indicating a total correlation.

These reports are translated numerically in the table below (Tab. 4.3).

The ratios described above by the equations describing quality (Fig. 4.15) as a function of quality confirmed the circles present in the CorPlot. Interestingly, the Faulty had a negative correlation with all attributes excepted for the Bitterness. One hypothesis may be that tasters had associated faulty wines as well as more bitter ones. The Evolution attributed also had a negative correlation with all other attributes, outside of Real Age and Faulty. This was interesting because despite this relationship was not very strong from a statistical point of view, it proved that judges had sometimes evaluated faulty wines more evolved and as evolved wines that had more years. Complexity had a very strong relationship with quality, Balanced

and Body. This confirms how frequently a complex wine was evaluated as more qualitative, balanced, and full bodied.

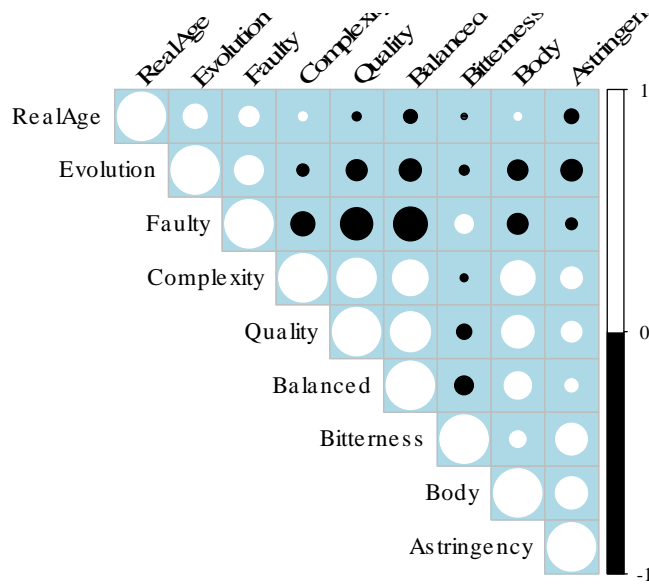


Figure 4.14 CorPlot describing the correlation between each attribute of the tasting.

Table 4.3 Table describing the correlation (R^2) between each attribute of the tasting. In bold are indicated the higher correlation and * indicated the significance.

	Real age	Quality	Evolution	Complexity	Balanced	Body	Faulty	Bitterness	Astringency
Real age	1,000								
Quality	-0,037	1,000							
Evolution	0,249	-0,193	1,000						
Complexity	0,034	0,661*	-0,066	1,000					
Balanced	-0,083	0,691*	-0,215	0,542	1,000				
Body	0,026	0,452*	-0,179	0,508	0,320	1,000			
Faulty	0,176	-0,450*	0,357	-0,251	-0,492	-0,187	1,000		
Bitterness	-0,018	-0,101	-0,045	-0,027	-0,153	0,119	0,150	1,000	
Astringency	-0,092	0,183	-0,198	0,204	0,077	0,447	-0,061	0,432	1,000

The following conclusions were reached:

- There was no relationship between Quality and Evolution, Bitterness and Astringency
- There was a relationship between Quality and Complexity, Balanced, Body and Faulty
- There was a positive correlation between Quality and the attributes Complexity, Balanced, Body, that was, if one increased, so did the other.

- There was a negative correlation between Quality and Faulty, that was, as the one increased the other factor decreased.
- The equation that could described the following model for higher statistically significant correlations was $Quality = 1,12205 + 0,38153 Complexity + 0,36652 Balanced + 0,12065 Body -0,12 Faulty$.

In addition to the equation that encompasses all the assets that had influenced the evaluation of quality, it can be seen from the equations below (Fig. 4.15) that even taking only some of the following attributes can explain the model, with a similar level of correlation. In fact, the value of R² oscillated in a very limited range: between 0.5942 and 0.6228.

Figure 4.15 Correlation between overall quality and other sensorial attributes with statistical correlation.

Quality = 1.12205 + 0.38153 Complexity + 0.36652 Balance + 0.12065 Body -0.12 Faulty	R ² = 0.6228
Quality = 1.42769 + 0.44371 Complexity + 0.37098 Balance – 0.12483 Faulty	R ² = 0.6120
Quality = 0.48370 + 0.37403 Complexity + 0.43608 Balance + 0.12801 Body	R ² = 0.6064
Quality = 0.78147 + 0.43984 Complexity + 0.44380 Balance	R ² = 0.5942

4.1.7 Age Prediction

As the last question of the tasting sheet, the judges had to indicate the age of the wine according to the olfactory tasting of the wine, the scores assigned for the previous applications and the overall idea of what represented the predicted age according to their opinion. The averages of the results of the predicted age of the wines were compared with the actual age of the samples. In Fig. 4.16 the relations between real age and foretold through the trend lines have been represented. As it can be noticed, for all the wines it was a growing and positive trend. Nevertheless, for all the samples it can be noticed how the inclination of the curve did not indicate a linear relationship but rather underlines how the tendency of the judges was to underestimate the age of the wines. The inability to estimate the real age was probably caused by the fact judges were not used to tasting very old wines and therefore they were inclined to underestimate the actual age of wines. The non-parametric Kruskal-Wallis test showed significant differences in samples based on predicted age. Surely, several factor influence wine profile.

For *Amarone della Valpolicella wine*, there was a growing relationship between the real age and the average of the predicted age of each sample under examination. The judges accurately arranged the samples in chronological sequence despite being unable to determine the true ages of the wines. Judges tend to underestimate the age of wines. The Kruskal-Walli's test shows that there was a significant difference for the term 'predicted age' between younger and older wines.

The graph concerning the wine *Luce della Vite*, showed differences from that of the other wines taken into consideration. The judges only managed to place in order the younger wine (L16) but were not able to correctly position the older wines. Many judges rated the L03 wine as defective, and therefore it may have been rated as older for this reason. The Kruskal-Wallis test confirms the linear trend: there are no significant differences between the various vintages. For Teroldego wine, according to the non-parametric test for this attribute, there was a significant difference between the younger wine, T21 and all the others. The two older wines, respectively 17 and 22 years old, were correctly placed as the older ones, even if the judges also in this case underestimated the real age.

The graph of *Valpolicella* wine, through post-hoc testing, showed that there were significant differences only between the oldest wine and all the others. Also in this case, the judges correctly identified the progressive age of the wines but nevertheless underestimated the values.

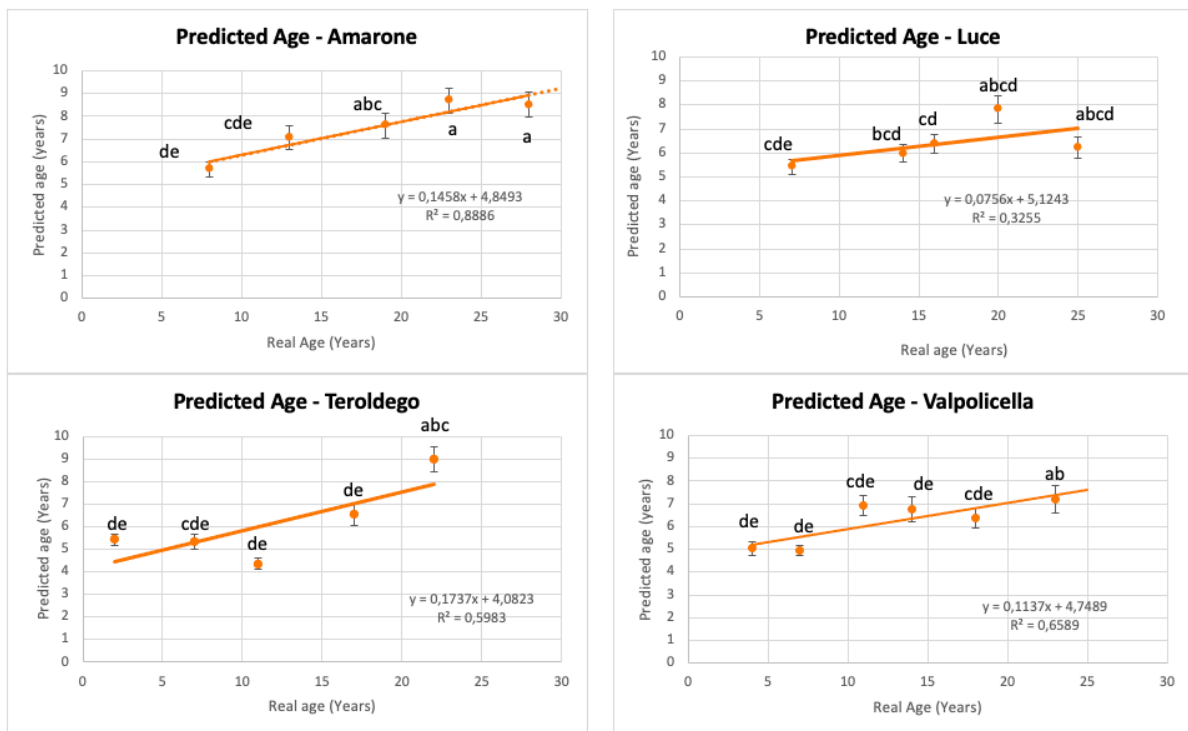


Figure 4.16 Average values of Evaluation of the Predicted Age made by judge during the tasting.

In Figure 4.17, it is shown the relationship between the real age and the age predicted by the judges based on the tasting of the wine alone (without the vision) had given a good but not high correlation ($R^2 = 0,6107$). It is also important to note that the slope of the line was very low (0.1336), which indicated that the judges underestimated the age of a factor close to 1/10 compared to the true age of the products tasted.

Although with only two wines, the younger wines with less than 5 years, were predicted as older. This indicated that the winemaking process tends to make younger wines look like older, probably by the use of barrique.

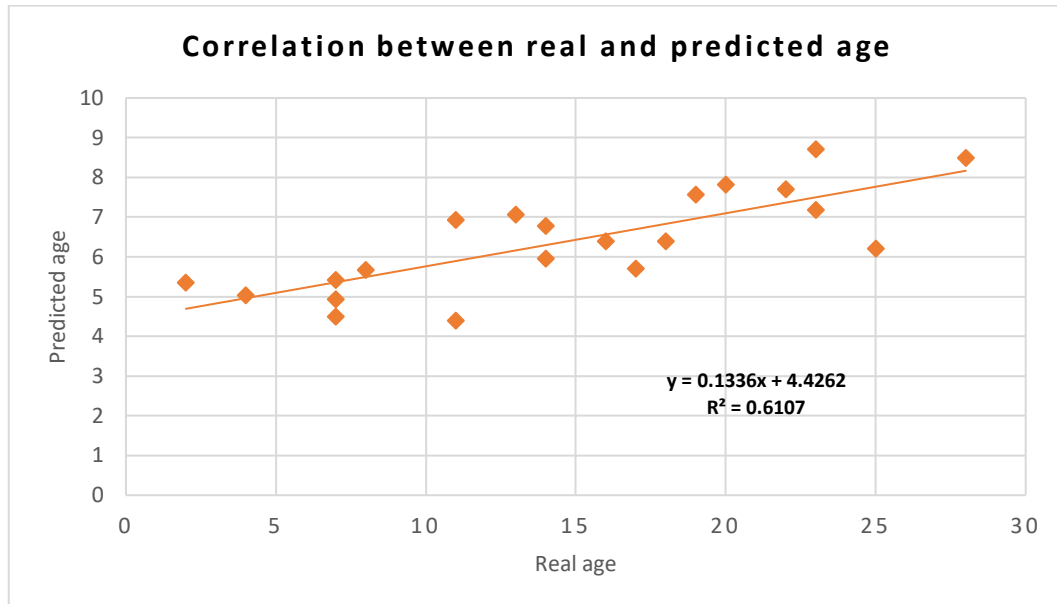


Figure 4.17 Correlation between real age and the average of the predicted age based on the taste of the wine given by the panel.

4.1.8 Color Evaluation

During the experience of sensorial analysis, the judges, after having evaluated the olfactory taste attributes of wines, had to evaluate the color of wines. The two experiences were separated because the goal was to understand how much color could really affect the evaluation of the age of wine. In fact, during the gustatory analysis the wines were presented in dark glasses, and then they were then moved to transparent glasses for the analysis of color. It was chosen to carry out this evaluation only for the older and younger vintages of each wine to understand how the evaluation changed in case of samples that were visibly different to the naked eye (Parr et al., 2020). This difference was later confirmed by chemical analysis. Each judge for the following samples had to give a color rating in a scale ranging from "deep Purple" to "light brick, on a continuous scale of 10 cm. Also, based on the color alone they had to give their own assessment of the age of the sample.

In the figure (Fig. 4.18) were represented the judges' evaluations of the color of the wines during the sensorial analysis. The judges were asked for each wine proposed to evaluate the color from 0 equivalent to "deep Purple" to 10 equivalents to "Orange brick". The results are presented with the corresponding image related to the evaluation of the color through the chemical examination of CieLab (Tab. 4.5). It can be noticed that there had been an increase

in the evaluation of color with the increase in the age of the wine: in the graph the order of wines had been placed from left to right from the younger to the older one. This underlines how judges were able to discriminate the visual differences of wines. The average evaluations of the panel were visually in accordance with the CieLab test: it is possible to see how moving from the wine that received the lowest ratings, Teroldego 2021 (T21), to wines with the highest average ratings, Luce 1998 (L98) and Amarone 1995 (A95), the color went from a red with purple shades to a soft orange. Color therefore seemed to be a fundamental element for the evaluation of the age of wine.

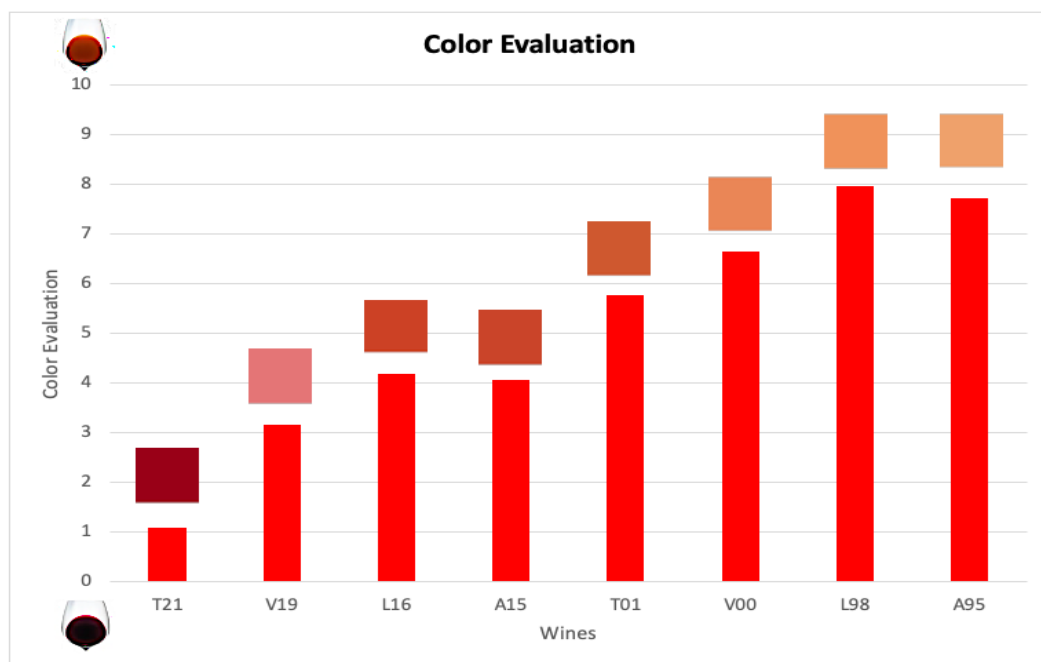


Figure 4.18 Average scores of the evaluation of the color made by the judges during the tasting. The scale goes from 0 (deep Purple) to 10 (orange brick). The colored squared on top of each column are the equivalent evaluation of the color made by the CieLab analysis.

4.1.9 Age prediction based on Color Evaluation

Regarding the predicted age based only on the visual evaluation, it was found that color plays a crucial role in the evaluation of wine's age. Comparing the predicted age that had been given to wines through the taste-olfactory evaluation and that given only according to color, it was noted that (Tab. 4.4):

- For all wines, the evaluation of the predicted age by colour gave an average value closer to the actual age of the samples.

- It was easier for all judges to judge young wines than older wines; perhaps because it was more common to taste recent wines rather than products that had undergone a long period of aging in bottle. This confirmed how in the evaluation and identification of age in older wines the color had not been so bad as in the evaluation of young wines. The chromatic component of wines greatly affected the evaluation of the wine itself, but it did not depend only on the age of the sample but also on the influence of the starting variety and the production technology of the product. For example, the varieties used to produce Luce wine were richer in anthocyanins than the one used for Amarone and Valpolicella, so the color would be always deeper than the wines from Valpolicella valley.
- According to Appendix N°5, in younger samples the percentage difference between the predicted age and the actual age was less. In older specimens this difference increased, and the gap between age predicted by color and taste-olfactory attributes increased.
- The wines in the first years after the harvest presented colors that were more familiar to tasters. This factor made it easier to assign the age for younger samples, independent of the nature of the wine itself.

In Figure 4.19, the differences in the age estimation of the different wines have been graphically represented. For all 4 younger samples the differences were limited, and, in some cases, the predicted age coincided. For older wines, the differential increased greatly. Moreover, it was interesting to observe how the difference between age prediction according to taste and sight had grown but also how with color the judges were closer to the real age. Nevertheless, the only visual evaluation was not enough to be able to approach the true age of the wines. The factors that could help in the exact estimation of this value are of two types:

1. Intrinsic to the tasting: that is to make the tasters evaluate the wines with clear glasses and giving the possibility to taste.
2. Outside the tasting: the frequency of tasting and the experience, not so much personal, as in the drinking of very old wines, seem to be essential to be able to really approach the real age of wines.

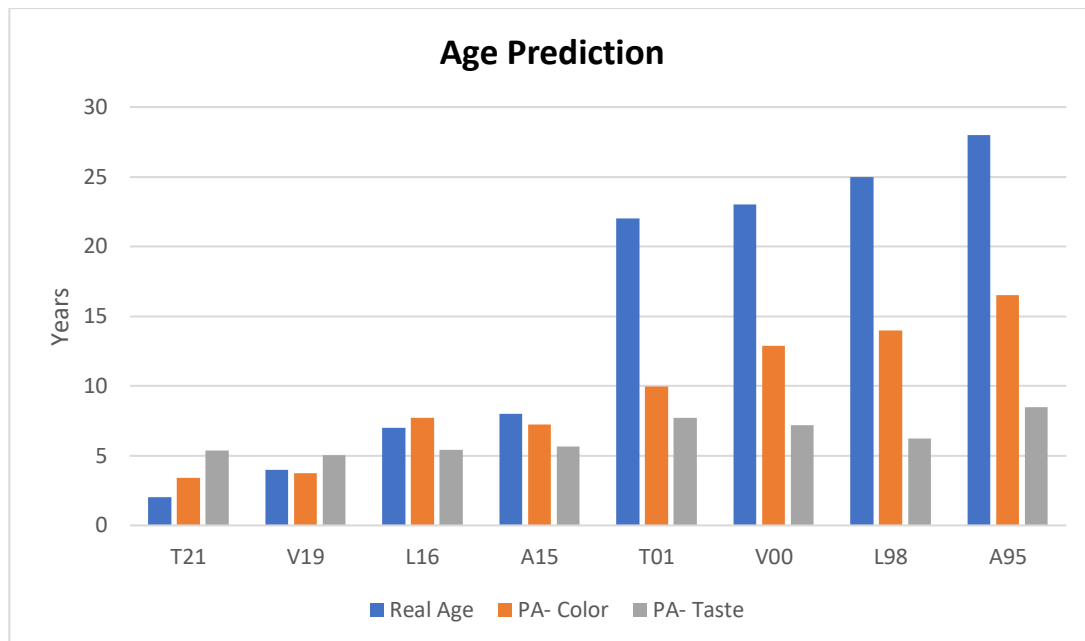


Figure 4.19 Comparison between the real age of the wine and the Predicted age based on the color (orange column) and the taste (grey column).

In Figure 4.20, the lines resulting from the judges' assessment of predicted age based on colour and taste were compared. It is necessary to emphasize, however, that a limiting factor of this diagram was the constitution of straight lines formed only by two points, that cannot explain in exhaustive way the true course of the appraisal. Looking at the angular coefficients of the lines in blue, that was those based on the age prediction based on color, it was possible to see that they were higher and therefore closer to a real perception of real age, compared to the evaluations based only on the tasting of wines. They were close to 0.5, meaning that on average the judges halved the real age of the wines tasted. The angular coefficients in orange had values close to 0.1, which therefore indicate that the age was underestimated by a factor of 1/10 compared to the real age.

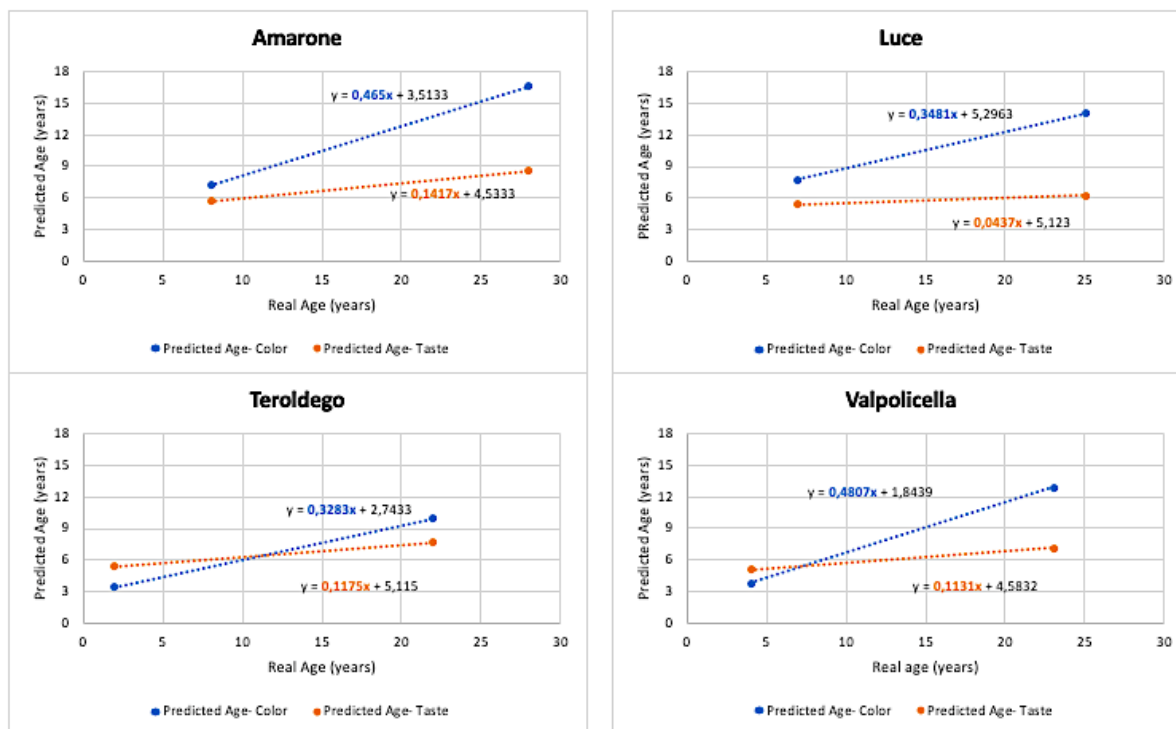


Figure 4.20 Lines describing the evaluation of the predicted age based on the color (blue) and taste (orange).

4.2 Chemical results of basic analysis and polyphenols

The numeric results of the chemical analysis are given in the appendix. The results of the basic analyses have been reported in Appendix N° 3 and the results of the polyphenolic analyses in Appendix N° 4 A-B.

4.2.1 Sulphur Dioxide evolution

To evaluate the aging of the phenolic fraction of red wines, an important parameter to monitor is the sulfur dioxide content. SO₂ binds with the phenolic compounds of the wine during the aging period in bottle, causing a progressive disappearance of red-violet nuances and a progressive softening of astringency for the bonds respectively with anthocyanins and tannins (Jackson, 2020; Ribèreau-Gayon et al., 2006). As reported by Avizcuri et al. (2016), the concentration of this index is expected to decrease. As shown in Figure 4.21, the content gradually tended to decrease for all wines. Wines starting from a lower content, e.g. Amarone and Luce, had a smaller decrease than others. In the case of Teroldego, the first vintage, 2021, had a very low value as it came from a cask sample still to be bottled. In any case, this index was confirmed as a good indicator of the state of evolution of wine in bottle; however, it is crucial to know the initial sulfur content at the time of bottling in order to evaluate the course of the process.

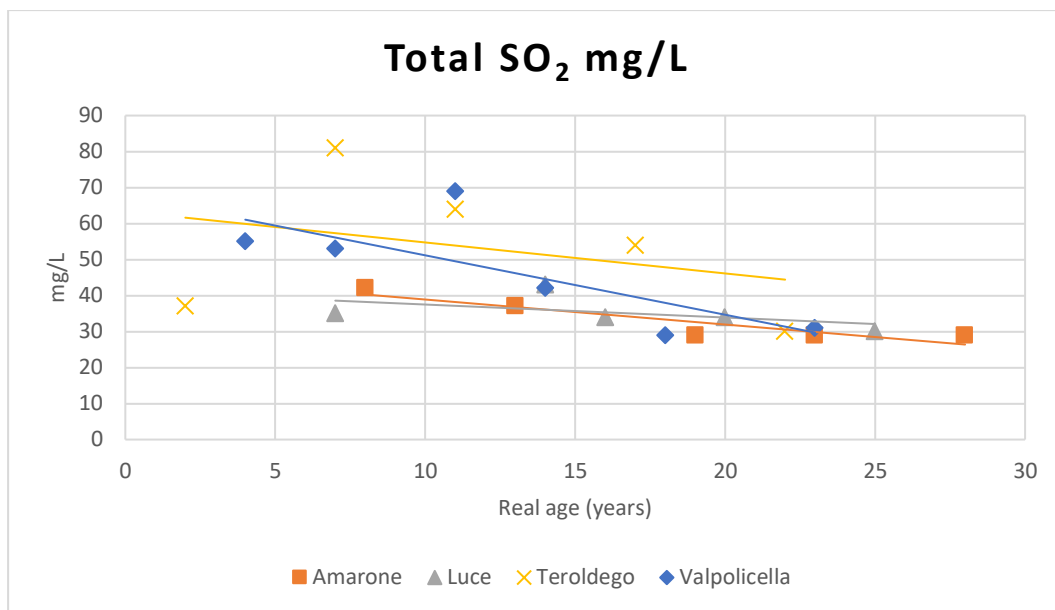


Figure 4.21 Evolution of Total SO₂ (mg/L) during bottle aging.

4.2.2 Color Indexes.















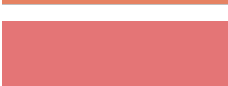

4.2.2.1 CieLab* Results.

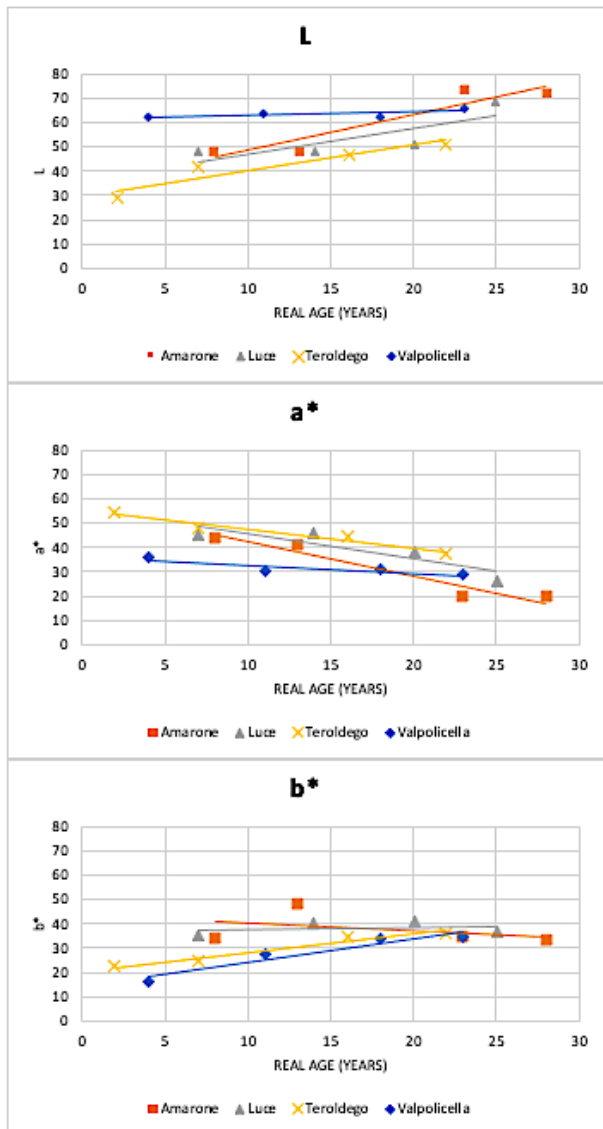
Among the chemical analyses, the evaluation of color was carried out through the CieLab test (OIV, 2023). The purpose of this measure was the definition of a process of measuring the chromatic characteristics of wine using a three-dimensional model and trying to imitate real observers (Rio-Segade, 2022). The detected attributes were: tonality, that was the color itself (red, yellow, green, or blue); brightness, that was the attribute for a +/- luminous wine; chromaticism, which measures the intensity of color.

In the sphere CieLab there are all the colors, after calculating all the coordinates it is possible to see the wine's color spectrum (Rio-Segade, 2022).

The table 4.5 shows the results obtained for the wines considered for the following thesis. In this case only the results of 4 vintages per wine were reported, together with the relative standard deviation. Through the translation of the CieLab coordinates obtained through a software, it was possible to notice the "translation" of the color of the wine, according to the mathematical model proposed by the colorimeter. In general, it can be seen how for all wines there a progressive abandonment was of the red and rose nuances present in younger vintages, for less intense colors and with clear and orange nuances. This was confirmed by the fact that during the aging period in bottle tends to decrease the coordinate a*, which at higher values indicated a more red color and increased the coordinate b*, which indicated a greater presence of yellow color, as indicated by the work of McRae et al. (2012). These results were compared with the ones obtained by the visual evaluation of the wine from the sensorial analysis (Fig. 4.18).

Table 4.5 Results of CieLab analysis. For each coordinate is reported the average and the standard deviation of the measurements.

Color Translation	Sample	L*	a*	b*	C*	h
	A95	72,4745 ± 0,4197	19,5800 ± 0,4629	33,0814 ± 0,2547	38,4440 ± 0,0417	59,3801 ± 0,7848
	A00	73,1353 ± 0,0144	19,3048 ± 0,0155	34,8079 ± 0,0237	39,8028 ± 0,0280	60,9868 ± 0,0067
	A10	48,7750 ± 0,1196	41,1735 ± 0,0670	48,0012 ± 0,0336	63,2406 ± 0,0691	49,3784 ± 0,0263
	A15	47,7957 ± 0,1056	43,8397 ± 0,0690	34,2189 ± 0,2704	55,6138 ± 0,1504	37,9733 ± 0,2421
	L98	68,7934 ± 0,0642	25,8468 ± 0,0613	36,5386 ± 0,0734	44,7563 ± 0,0944	54,7251 ± 0,0185
	L03	51,7187 ± 1,1429	38,6066 ± 0,6554	40,6276 ± 0,1017	55,9812 ± 0,4911	46,4003 ± 0,04492
	L09	47,9814 ± 0,0599	46,1095 ± 0,1435	40,2863 ± 0,0095	61,2297 ± 0,1123	41,1442 ± 0,0842
	L16	47,6912 ± 0,0802	45,4905 ± 0,0292	35,3337 ± 0,0744	57,6008 ± 0,0634	37,8374 ± 0,0484
	T01	52,0548 ± 0,1377	37,3800 ± 0,1408	36,0729 ± 0,0632	51,9473 ± 0,1439	43,9807 ± 0,0614
	T06	46,5734 ± 0,1733	44,5792 ± 0,0967	34,4233 ± 0,0580	56,3230 ± 0,0479	37,6747 ± 0,1039
	T16	42,3328 ± 0,1282	48,3564 ± 0,0590	24,5875 ± 0,0883	54,2484 ± 0,0898	26,9517 ± 0,0598
	T21	28,3029 ± 0,0236	54,3187 ± 0,0411	22,4325 ± 0,0107	58,7685 ± 0,0339	22,4396 ± 0,0250
	V00	65,4998 ± 0,1332	28,5946 ± 0,0644	34,3756 ± 0,0329	44,7139 ± 0,0645	50,2454 ± 0,0417
	V05	62,8712 ± 0,1149	30,9114 ± 0,0504	34,1825 ± 0,0226	46,0865 ± 0,0235	47,8768 ± 0,0622
	V12	64,3217 ± 0,1732	30,2437 ± 0,0746	28,0156 ± 0,1353	41,2257 ± 0,1349	42,8097 ± 0,1049
	V19	62,1370 ± 0,6235	35,6874 ± 0,1865	15,7482 ± 0,2881	39,0081 ± 0,2375	23,8102 ± 0,3643



As shown in the figure 4.22, this tendency is followed by all wines, except for Amarone in which the b^* coordinate did not decrease significantly over time. In addition, Valpolicella wine, except for vintage 2019, remains quite stable over time as confirmed by the representation of color made by the software. Teroldego wine remained in time the one with the matching corresponding to the red color (a^*) greater; this confirmed the high content of monomeric anthocyanins, which contribute to the red-purple color (Giacosa et al., 2021). The wine Luce della Vite, has always been kept in a range of intermediate values compared to the other samples. As for coordinate L, relative to brightness, there is a progressive increase during the years of rest in the bottle. This indicates a progressive "loss of color" of wine as over time wines are brighter and therefore less "dense" in color.

Figure 4.22 Evolution of the coordinates a^* , b^* and L during the years of bottle aging.

The additional information that can be calculated from the coordinates a^* , b^* and L are C^* and h^* (Fig. 4.23). C^* indicates Chroma/Saturation, during the aging period in the bottle there seems to be a general decrease of this value, except for Valpolicella wine that, As with other indices, it tends to remain unchanged. h^* indicates the hue or angle of the Chroma, in the course of time for all wines with approximately the same linear slope. indicating that the further away from the 0 the greater the red and the greater the yellow tint approaching 90 the greater. This index seems to give a robust indication of the variation of color over time of red wines.

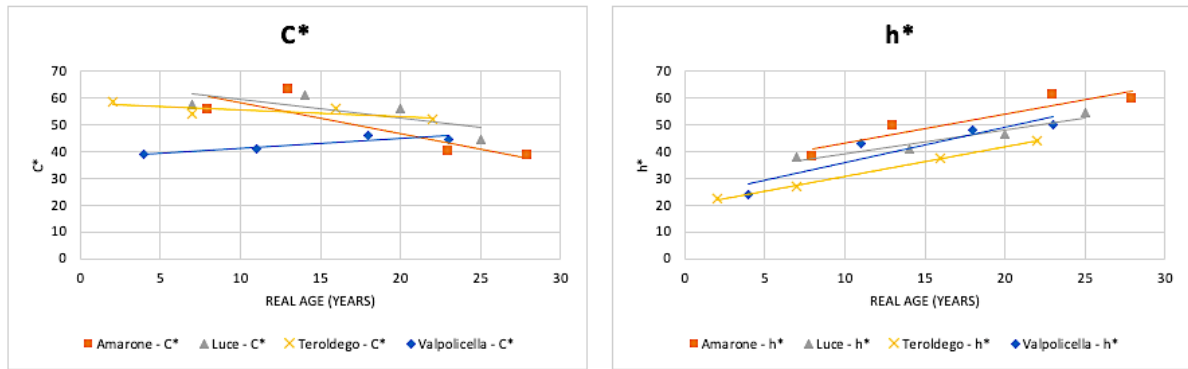


Figure 4.23 Evolution of Chroma (C^*) and Tonality (h) during bottle aging, according to CieLab coordinates.

4.2.2.2. Spectrophotometric results.

As reported in the literature by de Oliveira et al. (2019); Maioli et al. (2022); Garrido-Banuelos et al. (2020); Gambuti et al. (2020); Chira et al. (2011), the values of coloring intensity and hues (Hue) are two of the most used chemical indicators to evaluate the evolution of the phenolic component (anthocyanin) of red wines in bottle. The colour intensity corresponded to the sum of the absorbances at 420 nm, 520 nm and 620 nm, corresponding respectively to the absorption peaks of yellow, red and blue/violet. Over time it is expected to decrease due to the progressive decrease in pigments that occurs during time (Gutiérrez-Escobar et al., 2021).

In this case (Fig. 4.24A), for all wines, outside Valpolicella, the trend was the one expected: the coloring intensity was progressively decreasing. In a wine like Amarone, which started with a content of coloring matter higher than others, as Teroldego, it seemed the collapse was even more evident, this was evidenced by a higher slope of the curve. However, this was not the case for Valpolicella wine: the trend curve underlined an almost constant trend or even a slight increase. Also, in this case the starting varieties were very poor in coloring pigments, nevertheless, the content remained constant. This value was underlined by the fact that the value of A_{520} remains constant even though it was expected to decrease in time (Aleixandre-Tudo, 2020). The value of A_{620} decreased as expected and there was a marked increase in the absorbance at 620 nm that causes this slight increase. Moreover, the 2009 vintage of Amarone wine had values outside the scale of other wines, as confirmed by other chemical analyses.

Hue was a spectrophotometric index indicating the ratio of the absorbances at 420 nm and 520 nm. These two peaks indicated the absorbances of yellow pigments and red pigments of wines, respectively. During the aging in bottle, it was expected that the A_{520} decreased and that A_{420} increased as the compounds that attribute a red color to wine, distinctive of young wines, should decrease, and increased those that lead to more yellow/orange/brick colors that

are detectable at 420nm. During the ageing period this value is expected to increase (Ribèreau-Gayon et al., 2006). In all four wines this value increased (Fig. 4.24B), with a trend line almost with the same slope. Wines with a higher initial A420/A520 ratio, such as Amarone, kept this record over time. Probably the growth was proportional to the increase of the absorbance at 420 nm, which corresponded to the yellow/orange/brick pigments. This index was a good indicator to evaluate the state of evolution of the aging of wine in bottle.

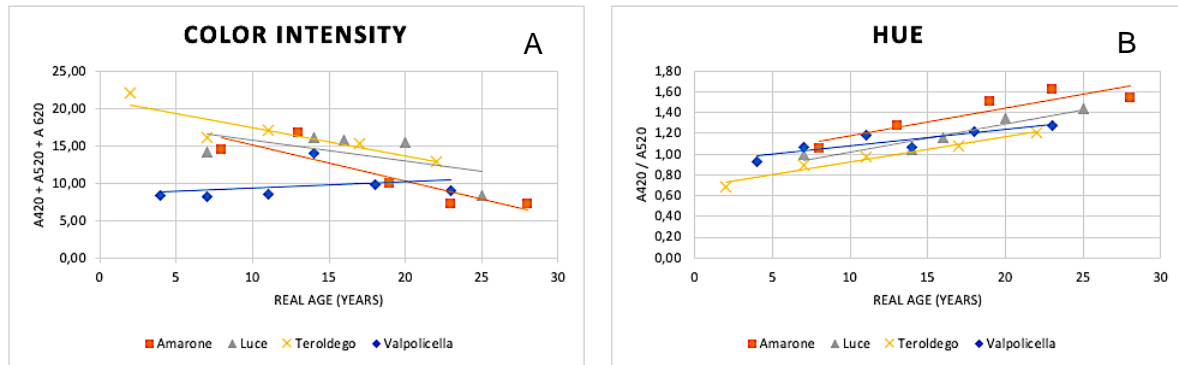


Figure 4.24 Evolution of Color Intensity (A) and Hue (B) during bottle aging.

The absorbance values at 520 nm and 420 nm are index used in red wines that correspond to the absorption spectrum of the red and yellow shades (Ribèreau-Gayon et al., 2006). During ageing, the Abs520nm was expected to decrease, because of the decrease in colored pigments, mainly monomeric, which reflect the color and red shades. These compounds during the aging period will tend to bind with other molecules, increase in size and precipitate, favoring the decrease of the red fraction that corresponds to this absorbance peak. All these reactions carry out the increasing of the yellow-orange compounds, that are identified by Abs420nm (Aleixandre-Tudo, 2020).

According to Fig. 4.25A, the average values of young wines agreed for what was reported in literature about the absorption spectrum according to the content and the type of anthocyanins present in the starting varieties (Giacosa et al., 2021). For A520, Amarone, Teroldego and Luce wines, it gradually decreased during the aging period, with trend lines that describe the same trend. On the other hand, Valpolicella wine tended to keep the absorbance peak constant during time; the only difference, as for other analyses, was the anomalous value of the wine of vintage 2009 compared to the others. For the absorbance value at 420 nm (Fig. 4.25B), it can be noticed that there was the same trend: all wines, outside the growing Valpolicella, had a general decrease in the course of time. It was important to note, however, that the reference scales were different: this value, unlike A520, started from lower absorbance values and a lower decrease in time. This explains why the ratio of A420/A520 (Hue) increased over time.

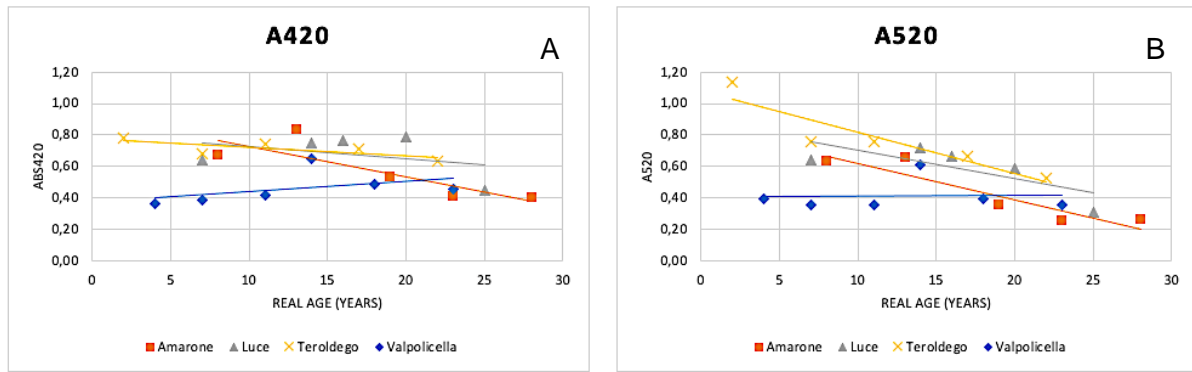


Figure 4.25 Evolution trend of Absorbance 520 nm (A) and 420 nm (B) during bottle aging.

The ratio of the color indices A520/a* and Hue/h* (Fig. 4.26) showed high correlation values. In the evaluation of the age of a wine these relationships can be used as good index to understand the progress of the aging of the product.

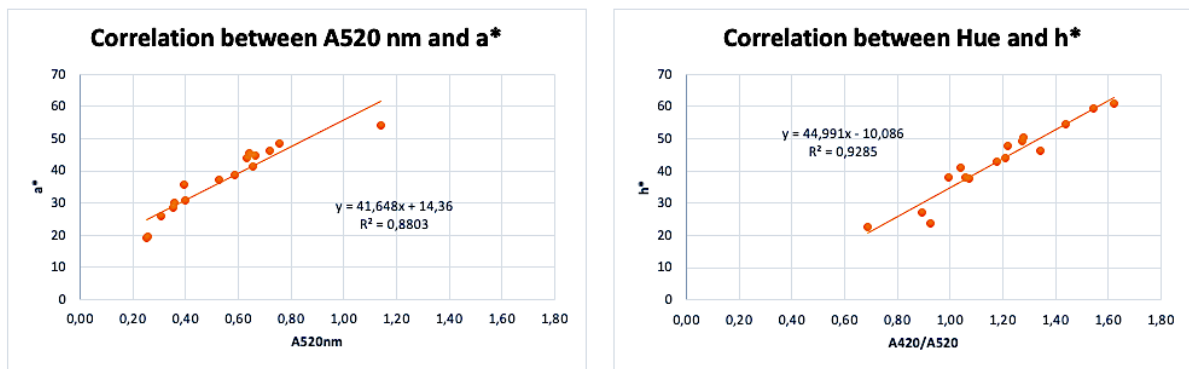


Figure 4.26 Correlation between spectrophotometric and Cielab parameters.

4.2.2.3. Degree of polymerization.

The Adams-Harberston assay (Harbertson et al., 2003) distinguishes short and long chain polymer pigments using a protein that precipitates long chain pigments. The protein used was bovine serum albumin. In addition, it is possible to distinguish monomer anthocyanins from polymeric pigments based on the resistance to discoloration by SO₂.

The results obtained are expressed as a percentage of total polymer pigments (Fig. 4.27). For all wines the trend is the one expected and described in literature by Avizcuri et al. (2016), Maioli et al. (2022), Garrido-Banuelos et al. (2020), Chira et al. (2011) and Burin et al. (2011). Short chain polymer pigments and monomer anthocyanins decreased over time as they reacted with other molecules. The main effect may be due to the formation of long-chain polymeric pigments, the action of sulfur against the monomer anthocyanins and reactions with reactive molecules, such as tannins, over time. In almost all wines, it could be noticed that the slope of the curves was proportional but inverse between the meaning (SPP) and (LPP): negative and positive respectively. Monomeric compounds decreased in proportional to SPP. It was interesting that in a variety known for its high amount of anthocyanin compounds such

as Teroldego (Giacosa et al., 2021), up to about 8 years of age the % of SPP and MP was greater than long chain polymer molecules.

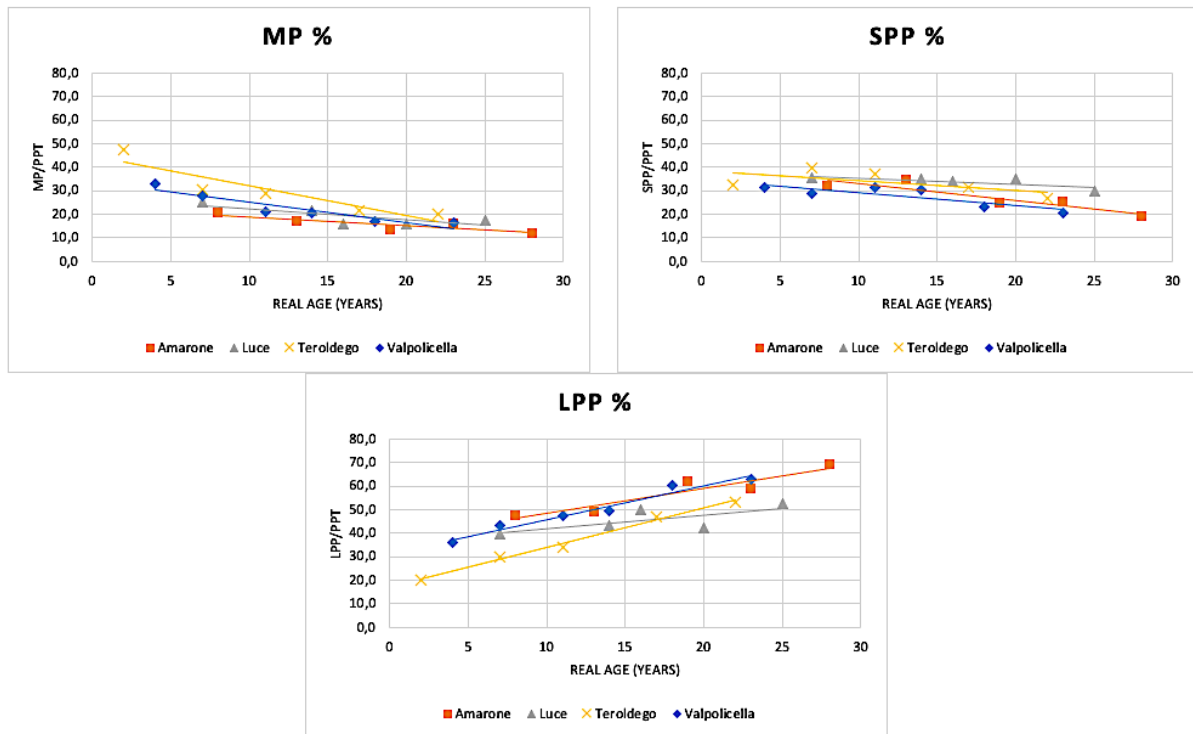


Figure 4.27 Evolution of Monomeric Anthocyanins (MP), short polymeric pigments (SPP) and long polymeric pigments (LPP) during bottle aging. The results are expressed as % of the total polymeric pigments.

4.2.3 Phenolic indexes

4.2.3.1 Total Polyphenol Index

Total polyphenol index is used to have an idea of the overall polyphenol content of wine. It is a simple spectrophotometric method, which quickly and without the use of chemical reagents can be used in the cellar to make decisions (Rio-Segade, 2022).

Due to the different reactions that occur over time, polyphenols were expected to decrease during the ageing period, and consequently also during this index (Casassa et al., 2019).

What it is possible to notice from Fig. 4.28, is that in all wines there was a progressive decrease of this index during the aging in bottle. All wines showed a decreasing trend, with a greater or lesser inclination of the trend line according to the type of wine. Amarone and Valpolicella were the two wines with an inclination of the highest straight line, despite it can be noticed that Amarone wine with 13 years, had a very high value in this index, and then collapsed with older wines. This result agrees with other chemical tests such as the content of PACs and the BSA test for astringency. The same result was quite similar for Valpolicella wine, which, as for the tests, had an increase only for the 2009 vintage.

Teroldego and Luce wines had a lower slope than the regression line and followed roughly the same trend: for L03 and T06 wines there was a peak in this index, and then fell with the two older vintages (L98 and T01). This index seems to be effective and useful for evaluating the stage of aging of wine in bottle.

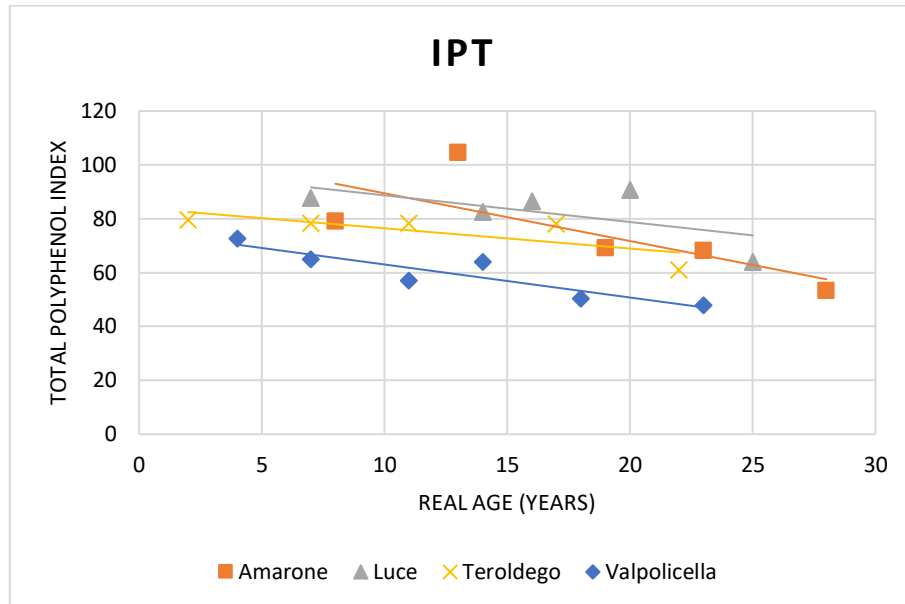


Figure 4.28 Evolution of the Total Polyphenol Index during the bottle aging.

4.2.3.1 Proanthocyanidins concentration.

Proanthocyanidins are molecules that arise from the polymerization of several molecules of flavan-3-ols put together and constitute the compounds of red wines linked to the gustatory sensations of astringency (Jackson, 2020). Over time, it is expected that these compounds may increase in the first years of bottle aging, and then decrease as reported by Monagas et al. (2006). Each wine starts from a starting level that is influenced by variety, origin, type of vinification and aging.

The wines taken into consideration in this work (Fig. 4.29), followed a tendentially decreasing trend as expected by the literature (Monagas et al., 2006). Looking at the single average values, for Amarone, Teroldego, and Luce wines there was an increase, accentuated, between 7 and 15 years after the harvest period, and then progressively decreasing. Valpolicella wine showed a constant and slight decrease over time, its low content agreed with the starting varieties of wine (Giacosa et al., 2021).

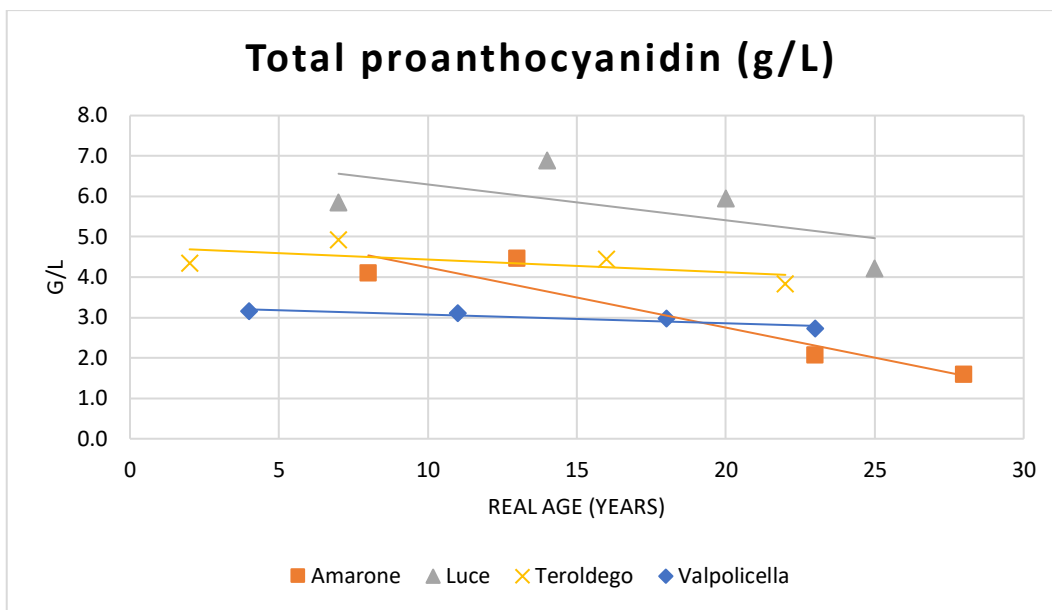


Figure 4.29 Evolution of Total PACs during bottle aging.

4.2.3.1 Anthocyanins and Tannins content

The polyphenolic component of wines is mainly composed by the sum of anthocyanins and tannins. These two classes of compounds undergo important changes during the aging period. According to literature, both classes of compounds are expected to decrease in content over time.

Regarding anthocyanins (Fig. 4.30A), the result agrees with that reported in the literature by Maioli et al. (2022), Garrido-Banuelos et al. (2020), Gambuti et al. (2020), Casassa et al. (2019) and Burin et al. (2011). During ageing there was a progressive decrease in these compounds. It was immediately noticed as the Teroldego, the wine that obtained with a coloring intensity and a color from the most intense CieLab test, was the one that had a greater decrease in time. All the other wines follow the same trend: the decrease was less marked than Teroldego and the trend lines had a very similar inclination.

Regarding tannins (Fig. 4.30B) the result was in agreement with that reported in the literature by Deshaies et al. (2022), Casassa et al. (2019) and Chira et al. (2011). For this class of compounds, the decrease was very similar among the various wines. All the wines started from an initial content in agreement with the reference varieties as reported by Giacosa et al., (2021) e Mattivi et al. (1997). It can be seen how Amarone and Luce had the highest values compared to Teroldego and Valpolicella. Amarone had the greatest decrease; instead, Luce had a constant content over the years and then collapsed after 20 years of age. Valpolicella was the most constant linear regression sample among the wines considered. The Teroldego had a more similar pattern to that of Luce.

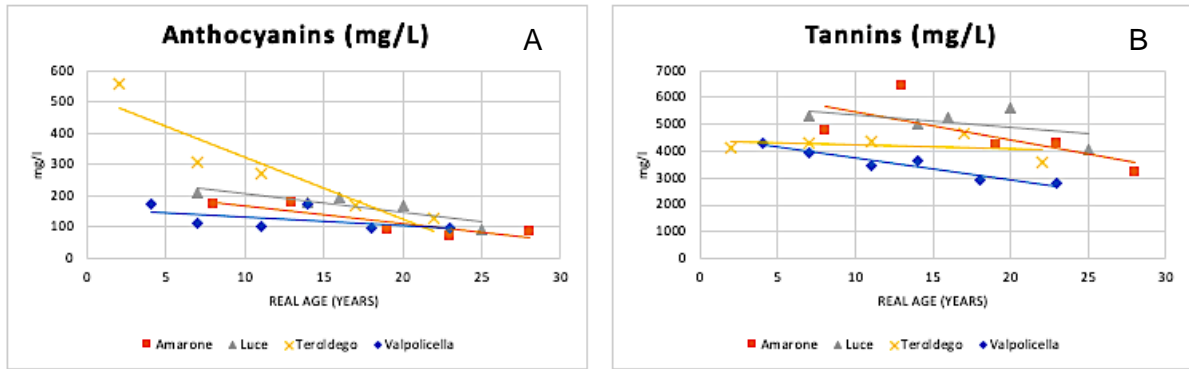


Figure 4.30 Evolution of Anthocyanins (A) and Tannins (B) during bottle aging.

In addition, the T/A ratio has been evaluated over time. The graph (Fig. 4.31) indicated a progressive increase for all wines, outside Valpolicella. Teroldego, Amarone and Luce followed the same trend with a similar angular coefficient. For Valpolicella Classico Superiore wines, the trend was flat: this meant that during the aging in bottle the tannin-anthocyanin ratio remained constant. The reason under this tendency could be related to the starting polyphenol profile of the varieties used for this wine: both anthocyanins and tannins had low concentration and their decrease was proportional. Looking at the previous graphs (4.28), the decrease in tannins also decreased anthocyanins. This was consistent with what Giacosa et al. (2021) reported about these varieties. As reported by Ribèreau-Gayon et al. (2006), this ratio was important for evaluating the formation of polymeric pigments during the shelf-life of wine.

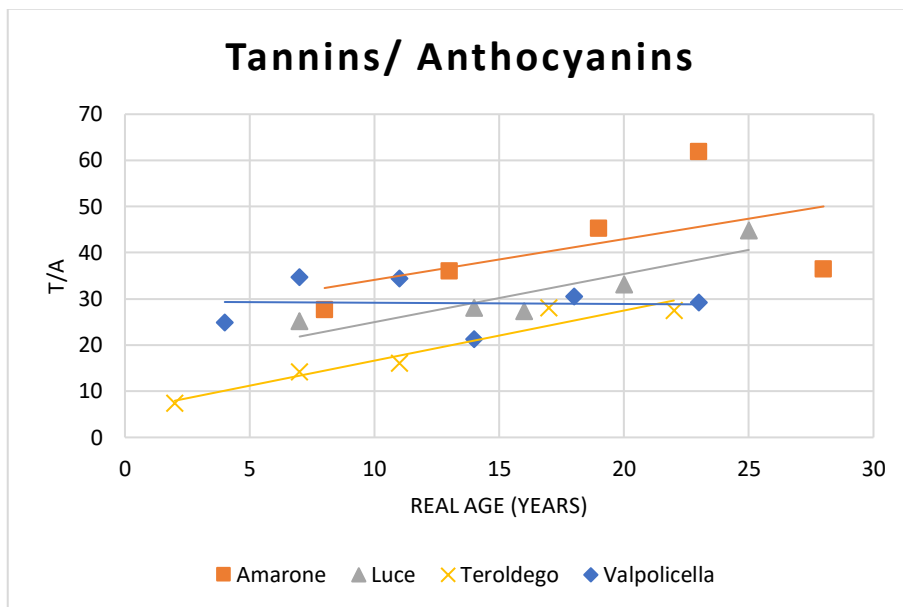


Figure 4.31 Evolution of relationship between Tannins / Anthocyanins during bottle aging.

4.2.3.1 Tannin Power

Through the test described by Boulet et al. (2016), an indicator of the perception of astringency was calculated. This assay-based test is based on the potential of proteins to complex and

precipitate the tannins present in solution. It is a subtractive approach: the selective determination of tannins takes place by reading the absorbance at 280 nm before and after precipitation with the bovine serum albumin protein (BSA) (Rio-Segade, 2022). Moreover, the BSA index allows to evaluate the perceived astringency. The choice of this method and not of other types of existing methods that help to predict astringency has been taken because the method presented by Boulet has the highest correlation index between astringency by sensory analysis and that expressed by the method (Wilhelmy et al., 2021).

From the results (Fig. 4.32), it can be noticed that all wines followed roughly the same trend. The astringency seemed to increase up to an interval going from 13 to 17 years and then decreasing progressively. This was quite in agreement with the results present in the literature, where it was reported that astringency grows during aging and did not have a moment of decrease (Garrido-Banuelos et al. 2020; Chira et al., 2011; Preez et al., 2022). As polymerization, direct or indirect, during the aging period leads to an increase in medium-long chain polymeric compounds. With time, these compounds will tend to precipitate and bind with other molecules not making them more reactive to salivary proteins and causing a partial softening of wine. The effect of the matrix, the type of wine, the starting variety, the vintage, the origin, and the production technology certainly have a fundamental effect on the quantification of this index (Mattivi, 2019; Ribèreau-Gayon et al., 2006).

As reported in the literature, Valpolicella wine, which was produced with grapes with a low phenolic content (Giacosa et al., 2021), despite it had a gradual increase over the first years of aging, was however the least astringent among the wines considered for the thesis. Conversely, Amarone wine, produced from the same varieties, but obtained with a different production technology (drying) that led to the concentration of the compounds present in the grapes, achieved the highest values in the two younger vintages taken into consideration, then collapsed later and reached values close to those of Valpolicella. On the other hand, Teroldego and Luce wines followed the same trend: the index of Tannin Power grows up until 15 years after the harvest, and then decreased gradually.

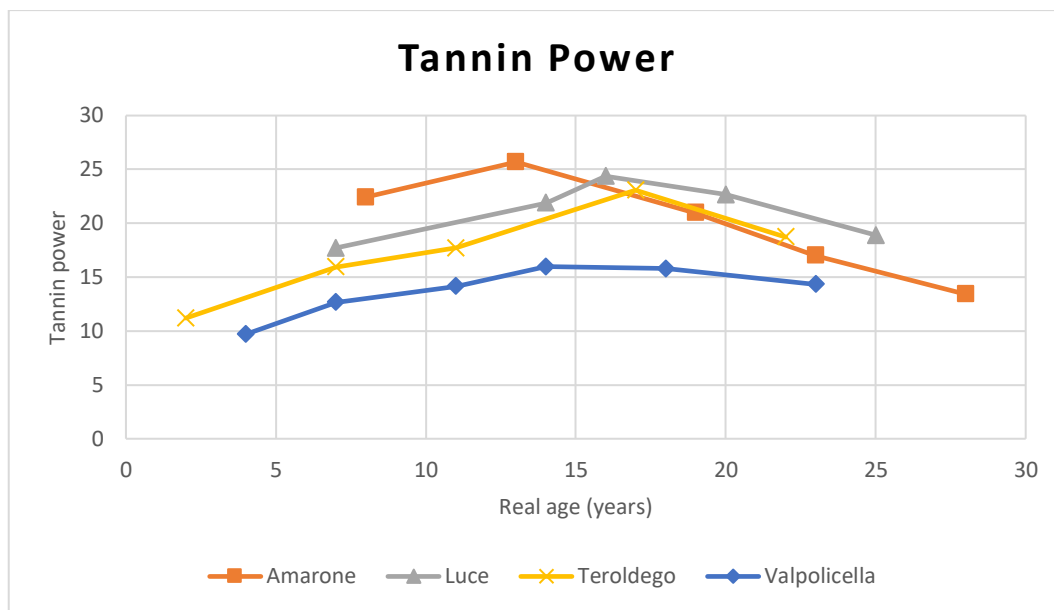


Figure 4.32 Evolution of the astringency by BSA assay during bottle aging.

Comparing the results obtained from the astringency test with the BSA protein and those from the evaluations of the judges by sensory analysis (Fig. 4.33 B), it was important to make several considerations. From the obtained graphs, only Amarone wine showed that the linear trend was like the one obtained by the Power Tannin assay. For other wines, using the full scale of values (from 0 to 10), trend was almost linear. The influence of aging on the tannic composition in young wines could be very impactful at the time of tasting. This was why very often wines from more recent vintages obtained higher ratings. This result helped to understand how the effect of the matrix was fundamental in the perception of the attributes of wine. Moreover, the evaluation carried out by an uninformed panel on the perception and the distinction of astringency can surely had affected the result. Each person had a different ability to perceive astringency which is dictated by personal and external factors during the tasting (Sáenz-Navajas et al. 2015).

By carrying out a regression analysis between the results obtained from sensory analysis and the chemical analysis concerning astringency (Fig. 4.33 A), it can be noted that the value of the correlation coefficient (R^2) was not very significant (0.46). In the perception of astringency, it was also important to consider the effect of the matrix: the pH, the alcohol content and the residual sugar had an effect on the gustatory perception of wine (Jackson, 2009). This result was in contrast with the literature on the correlation between astringency and the Boulet test ($R^2= 0.83$) (Wilhelmy et al., 2021). There was however to be taken into account that the conditions of the experiment were different and the judges had been trained on the evaluation of astringency before sensory analysis. The quantification of the astringency of wine by means of chemical analysis was a subject that very often leads to conflicting results and that is constantly being studied.

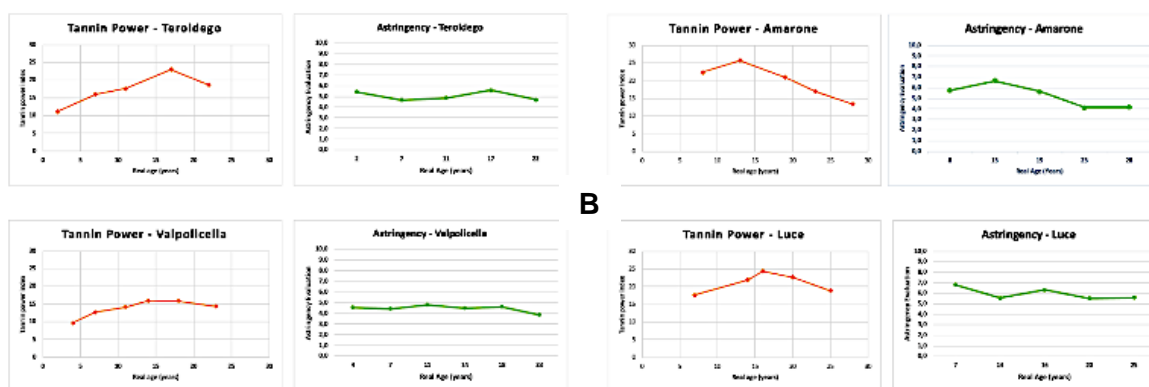
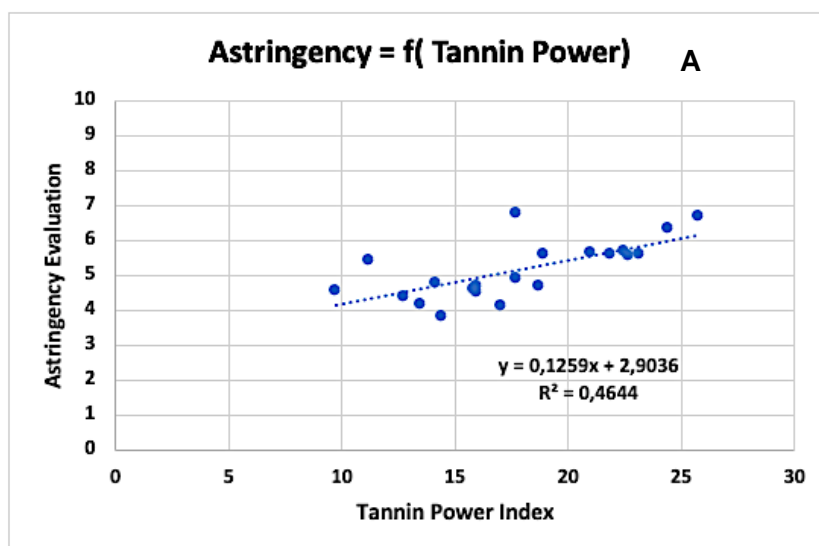


Figure 4.33 **A** Correlation and comparison between astringency evaluation made by sensory analysis and Tannin Power BSA assay. **B** Comparison between the real trend of tannin power and astringence ratings taking into consideration the individual wines.

Another indicator that can give an indication of the size of tannins and the perception of astringency is the dialysis index. This evaluation evaluates the charge and structure of tannins: the average values are usually between 5 and 30. A high dialysis index (25) indicates that there are voluminous pigments, generally polymerized or highly charged (Nioi et al., 2021; Ribèreau-Gayon et al., 2006).

Wines showed a very fluctuating trend for this index (Fig. 4.34). Amarone and Teroldego wines followed a trend that could be expected, as with time tannic molecules could increase their size due to the progressive increasing of polymerization during the period of aging. Luce and Valpolicella wines, however, did not follow the same trend: despite the trend lines indicate a slight decrease, looking at the points the trend was basically constant over time.

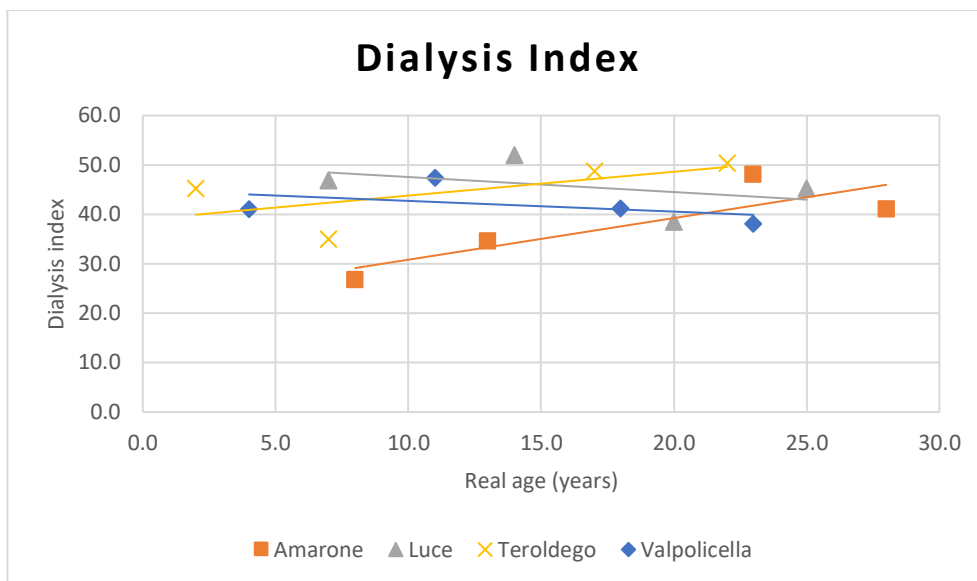


Figure 4.34 Variation of Dialysis Index according to wine age.

Hydroxycinnamic acids are phenolic compounds that are highly influenced by the starting variety and by the type of aging to which wine is subject (Jackson, 2020).

According to Garrido-Banuelos (2020) e Monagas et al. (2006), the content was expected to increase over time due to the release of forms related to other compounds.

However, the content decreased for all wines subject to this thesis (4.35). It was evident that the starting content was different according to the type of wine, however the decrease appeared common for all products.

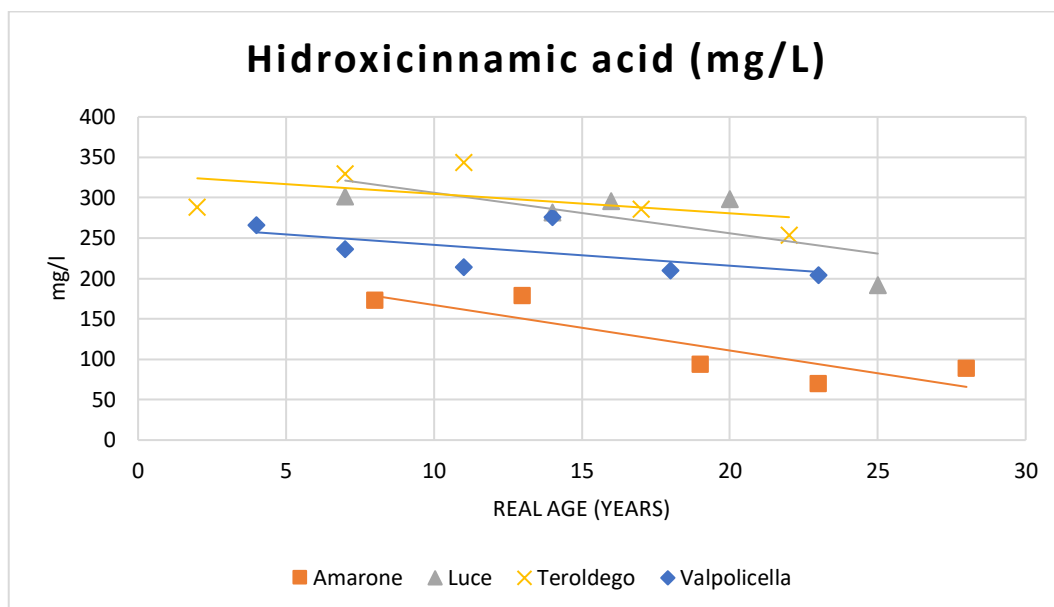


Figure 4.35 Variation of Hidroxicinnamic Acid (mg/L) according to wine age.

4.3 Correlation among chemical parameters

The numerous chemical parameters determined in all red wines were subjected to a correlation analysis. The results are listed in Table 4.6 showing that most determinations were highly

correlated, positive or negative. In bold, highly significant correlations were highlighted. Evaluating this result, it was possible to understand which were the most incisive indices in the evaluation of the evolution of red wines in bottle, and, according to the method of evaluation, to identify which were the simplest and easily replicable for the determination of "ageing potential". Among these, certainly A520 and A420 were among the indices with the highest number of highly significant and, according to the method, the simplest and fastest to estimate. Also, Hue was highly correlated with other indexes and, as would be shown (Fig. 4.38), it was considered a good measure to predict aging potential.

Table 4.6 Correlation matrix between most of the chemical indexes evaluated during the following thesis.

	TPI	Tannins	H.A.	Ant.	A420	A520	A620	C.I.	Hue	MP (%)	SPP (%)	LPP (%)
TPI	—											
Tannins	0.971^{***}	—										
H.A.	0.858^{***}	0.744^{***}	—									
Ant.	0.443*	0.228	0.573**	—								
A420	0.771^{***}	0.666^{***}	0.829^{***}	0.587**	—							
A520	0.613**	0.422	0.749^{***}	0.908^{***}	0.841^{***}	—						
A620	0.600**	0.409	0.743^{***}	0.896^{***}	0.858^{***}	0.970^{***}	—					
C.I.	0.696^{***}	0.531*	0.809^{***}	0.820^{***}	0.938^{***}	0.976^{***}	0.970^{***}	—				
Hue	-0.303	-0.113	-0.530*	-0.769^{***}	-0.408	-0.768^{***}	-0.654^{***}	-0.647^{***}	—			
MP (%)	0.206	0.012	0.355	0.843^{***}	0.191	0.662**	0.597**	0.500*	-0.855^{***}	—		
SPP (%)	0.788^{***}	0.699^{***}	0.813^{***}	0.541*	0.675^{***}	0.676^{***}	0.623**	0.696^{***}	-0.593**	0.446*	—	
LPP (%)	-0.538*	-0.365	-0.640**	-0.838^{***}	-0.474*	-0.780^{***}	-0.717^{***}	-0.686^{***}	0.855^{***}	-0.888^{***}	-0.800^{***}	—

There was a high positive correlation between TPI and Tannins because Tannins represented a main fraction of the Total Polyphenol Index. For all the spectrophotometric indexes (A520, A420, A620; Hue and C.I.) there existed a high correlation due to the fact that they were all connected either by the measurement method, the purpose of the measurement, or by the fact that with one measure you get another. It was important to notice the negative correlation between A520 and Hue because Hue was calculated as A420/A520 and the relationship with A520 was negative. A520, that indicated the spectrum of absorbance of red, was highly correlated with the concentration of anthocyanins. The amount of LPP (%) was negative correlated to the concentration of anthocyanins: since the total anthocyanins decreased during the time, otherwise the degree of polymerization grew up. Therefore, that was the reason why existed a negative correlation between LPP (%) and MP (%): the monomeric pigments diminished in favor of polymeric pigments.

4.4 Comparison of chemical analysis among wines

The comparison of wine chemical analysis was performed through the construction of a Heat Map (Fig.4.36). The wines were clustered according to the best grouping for the results obtained. Older products were located at the bottom of the scheme and had been better characterized by high Hue and LPP (%). In the upper area of the chart, instead, were found wines of medium-old age that were better described by higher values of TPI, Tannin (mg/L), BSA assay and others. In the central area were found the medium-young wines, except for T01 which was clustered together with Valpolicella wines, for the high correlation of the results obtained. These results underlined that most of the time, the vintage was more important than the variety to distinguish the wines.

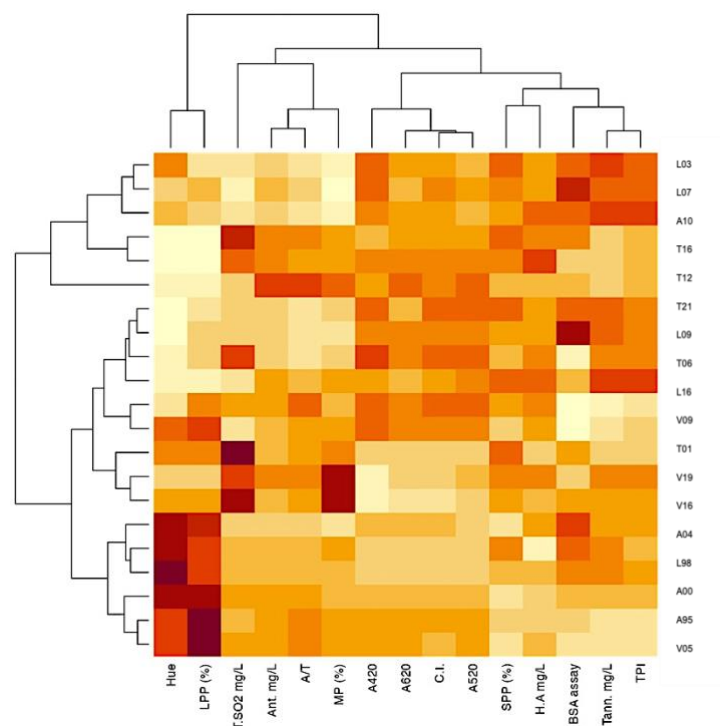


Figure 4.36 Heat Map describing the clustering of all the wines (on the right) according to the chemical results (down part). (-1 close to white; + 1 deep red).

Another Heat Map was produced for another set of chemical parameters determined in a selected group of wines (Fig.4.37). The division became even more evident by including the results of the CieLab* and PACs analyses. CieLab coordinates greatly helped to cluster the wines according to their age. Since, Hue, h^* and L^* during aging (Ribèreau-Gayon et al., 2006) and were major involved in the clustering of old wines. a^* and C^* better described the young wines, because a^* represented the red shades of the wine that were stronger in the first years, as C^* that represented the saturation of the color.

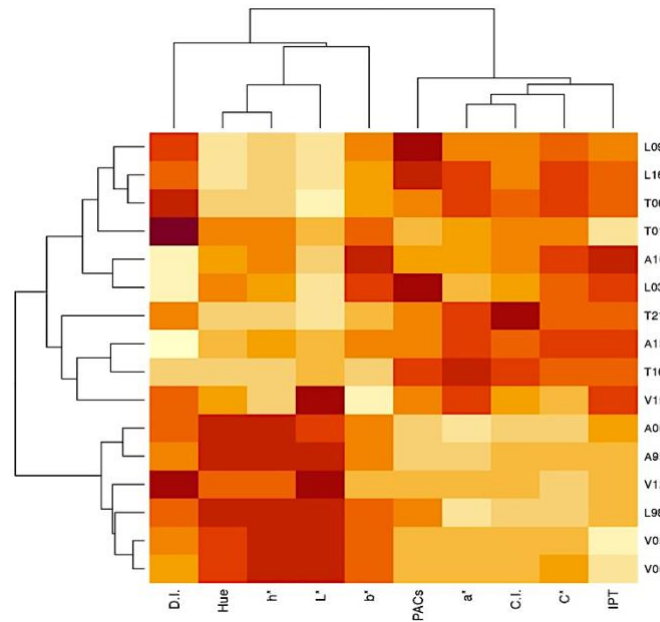


Figure 4.37 Heat Map describing the clustering of some of the wines (right part) according to the chemical results from the University of Porto (down part). (-1 close to white; + 1 deep red).

4.4 PCA of chemical analysis and wines

By carrying out an analysis of the principal components (PCA) (Fig.4.38A), it was further tried to understand how the various measures could better describe young wines or aged wines. The sum of the first and second main components was 82%, indicating the high quality of the model. On the left side, there was a high correlation between Hue and LPP (%), as it would be expected from the previously described correlations. They were totally opposed to MP (%), Total SO₂ mg/L, A/T and Anthocyanins. Moreover, all the other measures were found in the right part of the diagram, indicating a different course.

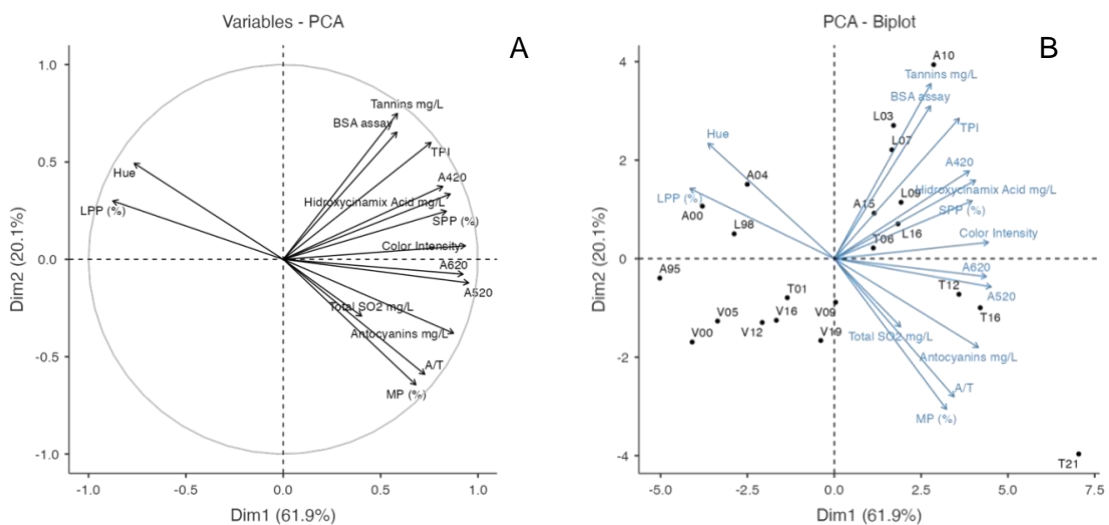


Figure 4.38 Variables-PCA (A) and Biplot-PCA (B) describing the chemical results of the polyphenols.

From the right graph (Fig.4.38B), we can see that older wines were mostly grouped in the left part of the bi-plot graph, therefore closer to Hue and LPP (%). These two indices better explained the characterization of old wines. Seeing the T21 wine, the youngest among all the wines considered, was in the opposite direction compared to the old wines, understanding how the chemical indices MP (%), A520 and Anthocyanins can better describe young wines. This was in accordance with the foregoing and the significance of these measurements.

To confirmation of these results, using other analyses and a shorter number of wines was shown by the Bi-Plot (Fig.4.39). The sum of the first two main components was 82.5%, confirming the goodness of the model. In this case, h^* , L+ and Hue were the analyses that best described the old wines. C^* , PACs and PTI instead the ones better describing the middle-aged wines (in an average of 10-12 years). For younger wines, there were no indices that better described wines at a young age.

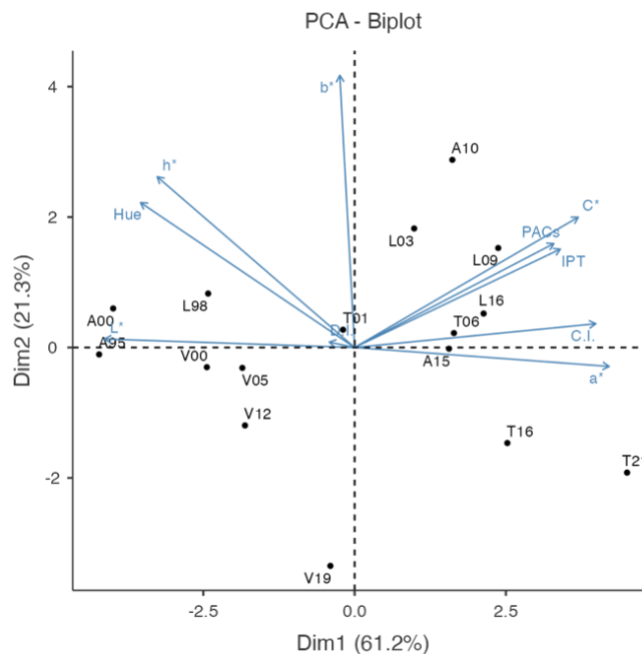


Figure 4.39 PCA-Biplot of different chemical results.

4.5 Multifactorial analysis

By the comparison of chemical results and those obtained from sensory analysis with a Multiple Factor Analysis (MFA), some differences were found (Fig.4.40). The proposed PCA model (Fig.4.40A) had the sum of the two main components 62,1%, indicating that the result gave a good but not excellent interpretation of the model. The "Evolution" attribute was close to Hue and LPP (%) indicating how high ratings were given to older wines which may be considered as remarkable. Indeed, tasters could not be influenced by colour since dark glasses were used. Those ranked with higher "Quality" were in the upper right quadrant, along with the higher results of Tannins and TPI.

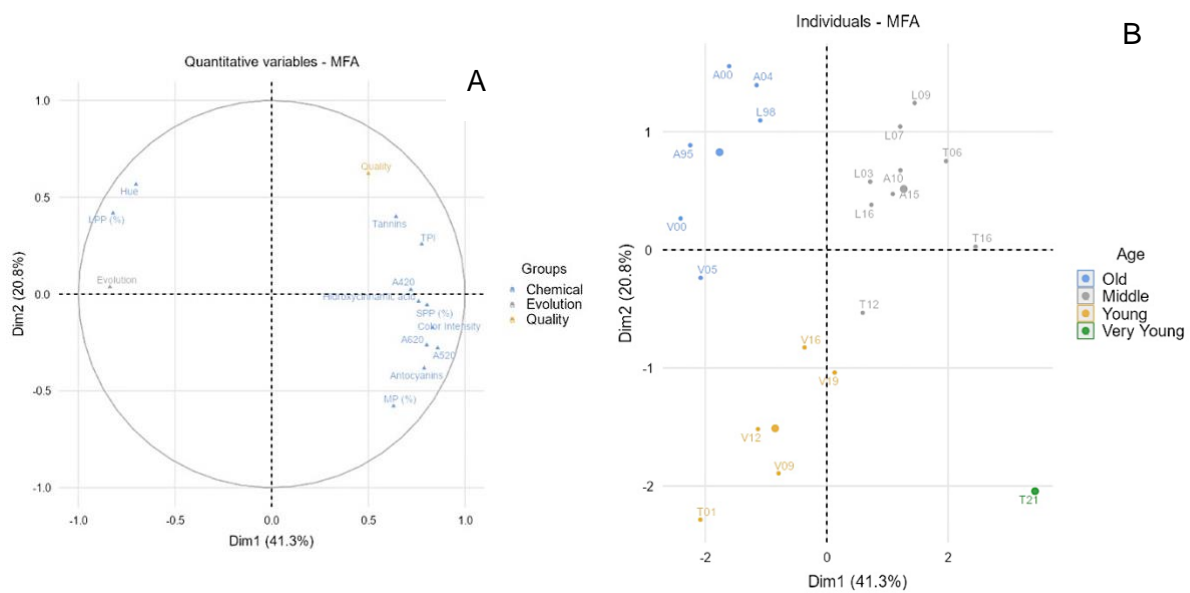


Figure 4.40 Quantitative variables - MFA (A) and Individuals - MFA (B) describing the correlation between sensory and chemical results.

MFA-Individuals (Fig.4.40B) showed how wines were classified in this way: the older wines according to the previous bi-plot (Fig.4.38B-4.39), in middle-aged wines there were all those with an average of about 10-12 years outside the Valpolicella who have all been included in the "Young" category. This could be related to the results of chemical and sensory analysis, Valpolicella, had generally been characterized as younger than the other products. This was in accordance with the style of winemaking and the type of wine produced. As a wine "Very Young" was classified only the T21.

The multifactorial analysis evidenced the separation of wines according to their age, chemical analysis, and sensory evaluation. The parameters that appeared more relevant for the characterization of each group were averaged and listed in Table 4.7. The results should be understood as a preliminary observation and as tendencies, given that Cluster I had only one wine. Cluster IIa and IIb were named according to the quality score. The former was composed by A15, A10, T16, T12, T06, L16, L09, L07 and L03 and the latter by V19, V16, V12, V09 and T01. Interestingly, the lower quality tended to show lower A420, A520, anthocyanins and tannins and higher evolution, MP and LPP than the higher quality. These relations were expected since the correlations already shown in table 4.6 were either highly positive or negative.

Table 4.7 Average chemical and sensory characterization of wines clustered according to the Multiple Factor Analysis.

Cluster	Average Age	A420	A520	Hue	Ant. (mg/L)	Taninns (mg/L)	MP (%)	LPP (%)	Evolutio n	Quality
I - Very Young	2	0.782	1.139	0.69	557	4102	47.5	20.1	3.52	5.75
IIa – Middle, High Quality	13	0.490	0.448	1.09	138	3782.2	24.5	45.9	5.25	5.27
IIb – Middle, Low Quality	12	0.732	0.676	1.09	205	5070.7	21.9	42.5	4.47	5.67
III - Old	23	0.486	0.321	1.43	90	3744.8	15.3	60.9	5.81	5.50

The IIa cluster had values like those of cluster III, indicating that this cluster corresponded to wines that were more evolved for its real age than the cluster IIb. To better distinguish them, it was better to divide them according to the marks received during sensory analysis by the attribute “Quality” (Fig.4.41A). This was the reason why the initial group.

Young, could be considered as “Middle-low” and the initial group Middle became “Middle-high”. However, the amplitude of the scale was low. It was interesting to see how Middle-low quality wines had the same average rate of “quality” of old wines. These groups were respectively evaluated as more and less evolved, based on the average votes received for the attribute “evolution” (fig.4.41B). The results confirmed how higher quality wines were considered at a younger stage of the evolution process.

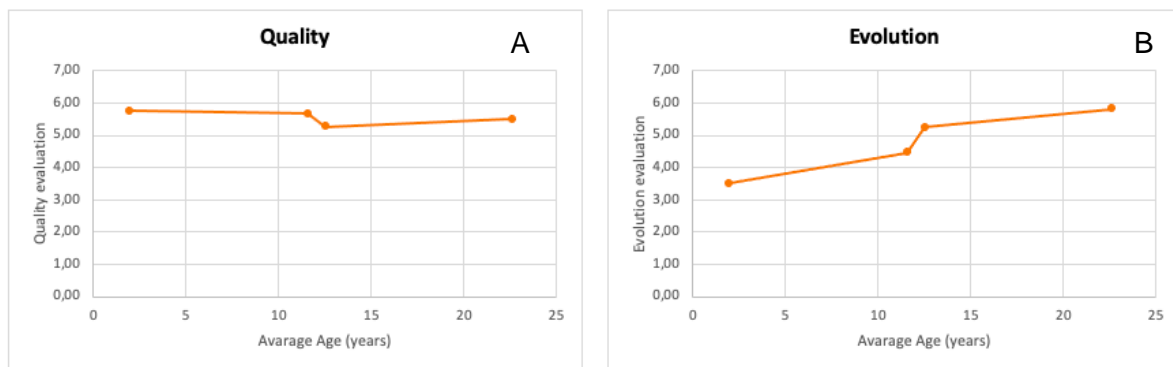


Figure 4.41 Evolution of the attributes "Quality" (A) and "Evolution" (B) according to the 4 clusters born from the Multiple Factor Analysis.

MP (%) and LPP (%) followed opposite trend (Fig. 4.42). There was a perfect evolutionary state depending on the average age of the samples considered. LPP (%) increased progressively over time, as polymerization of pigments increased during the time (Ribèreau-Gayon et al., 2006). Opposed to the Monomeric Pigments MP(%) that decreased.

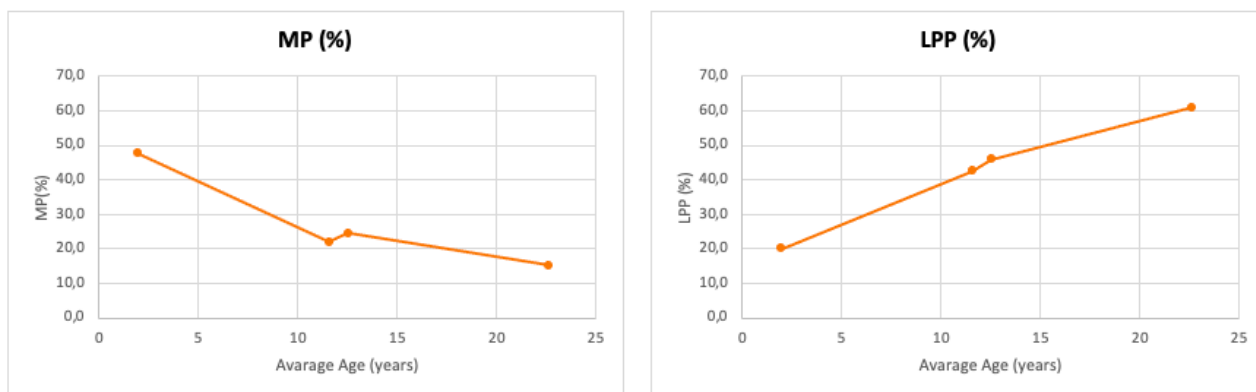


Figure 4.42 Evolution of MP(%) and LPP (%) according to the 4 clusters born from the Multiple Factor Analysis.

Focusing on the spectrophotometric indexes, A420 and A520 progressively decreased over time based on these cluster groups (Fig.4.43). Look at A420, it was interesting to see the similarity to old wines, that was in accordance with “quality”, even if tasters did not see the colour. The decreasing of these two parameters was higher in A520 than in A420, that was the region why the Hue increased over the time. Hue confirmed to be a good indicator to study the evolution of the wine during the years.

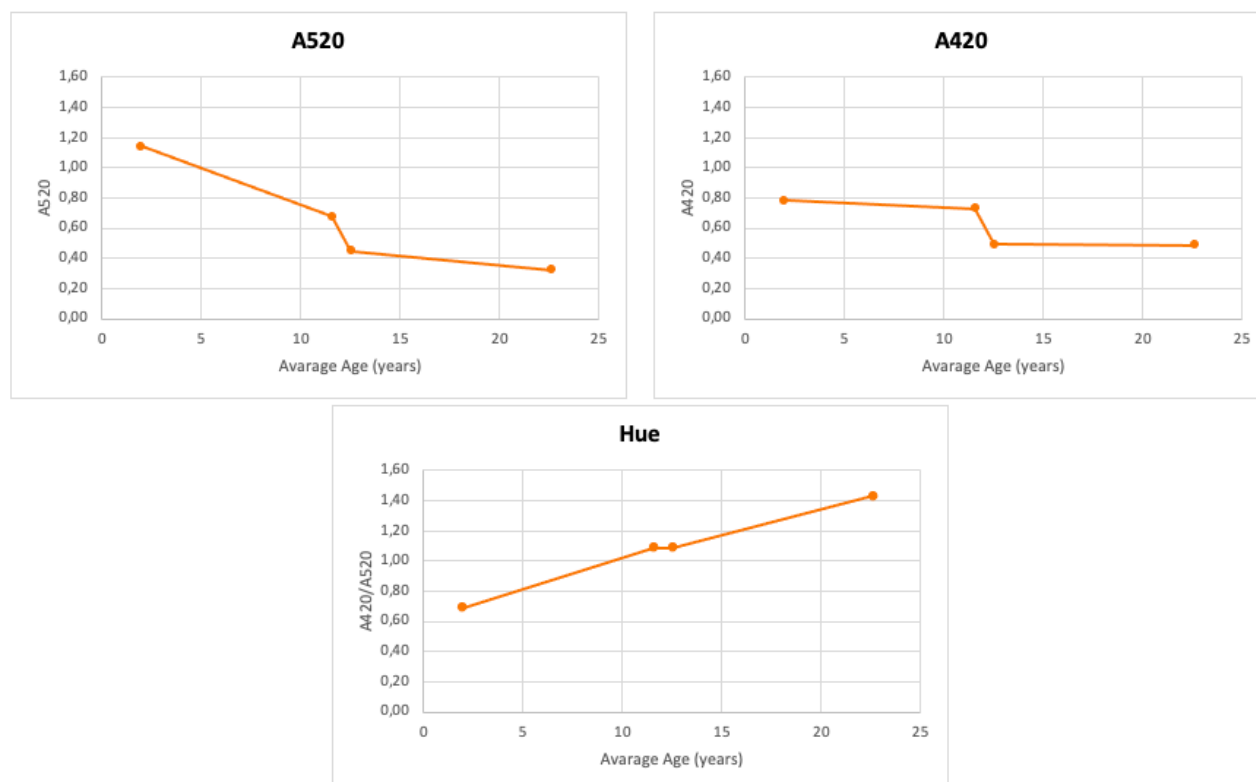


Figure 4.43 Evolution of Anthocyanins (mg/L), MP (%) and A520 according to the 4 clusters born from the Multiple Factor Analysis.

The results emphasized that according to the multifactorial analysis, it was possible to divide wines in a distinct way according to age. The union of the results obtained by sensorial analysis and chemical analysis allowed to distinguish wines and to classify them according to their evolution over time. As it was visible from the groups (Tab.4.7), in the various clusters there were not all wines of the same age, but those that according to the results and the obtained votes could be positioned in the time in function of the own evolutionary stage. For example, the group "Middle Low-Quality wine" was composed mainly by Valpolicella wines. This type of wine had been considered historically as not suitable for aging at the expense of Amarone (Paronetto, 1991), which, depending on the production technology (drying), was more proposed for aging. Since the drying technique was used to concentrate the metabolites of wine (among which tannins and anthocyanins), of which the varieties of Valpolicella were poor. Looking at parameters such as A420 and the votes received in the Evolution category it was possible to understand both from a chemical and sensorial point of view the lesser aging potential of this wine. In conclusion, the easy to determine parameters of A420, A520 or Hue would have sufficed to study the evolution of red wines.

5. Conclusions

Interesting results had been obtained regarding the evolution of red wines during a wide range of vintages.

From the sensorial analysis it emerged that: (a) responses were highly variable; (b) the experience of the judges was not a fundamental factor in the evaluation of the quality and evolution of wines during the aging in bottle. This led to the use of all answers and not to the division of judges into two different panels. The judges' assessments for overall quality showed that the model proposed by literature on the evolution of quality over time was rather simplistic. The statistical analysis did not find differences in quality along the vintages. Moreover, all wines followed different trends and not strictly attributable to a decreasing parabola. Instead, the judges were able to find more or less accurately the evolutionary status of wines according to age. Therefore, although tasters understood the different evolution of the samples, quality was not underscored along more than 20 years of aging. It was remarkable to see that wines could be appreciated over this period.

Regarding the attributes that involved polyphenols during sensorial analysis, among these, the evaluation of "body" and "bitterness" did not show any significant differences, moreover, evaluating the performance of each wine it was not possible to find any similar linear trend among them. More surprising were the results for astringency: for all wines there seemed to be a gradual decrease in perception during time. This was interesting because the judges did not know a priori the type of wine however found an evolution of this tribute during the time. In

general, the results obtained in this first part of the tasting where the judges did not know a priori the characteristics and types of wine they were going to taste, stressed which were the attributes that can be most influenced during a tasting by the knowledge of the extrinsic qualities of wine. Finally, an equation was created that could “predict” quality as a function of other synthetic attributes of wine, as a function of attributes and their correlations with the evaluation of overall quality. The results showed that quality could be mostly predicted by complexity and balance, with a lower positive contribution of body and a negative contribution from faulty.

As the last section of the sensory analysis, the judges were asked to evaluate the color and the predicted age according to the color. Interestingly, in dark glasses predicted wine age was much less than the real age, indicating a high aging potential of the tasted wines. Regarding the visual evaluation of color, it was noticed how with the passing of the years the judges were able to discriminate the younger wines closer to red-violet and the older wines close to the more brick color. Color therefore seemed to be a fundamental element for the evaluation of the age of wine: chromatic component of wines greatly affected the evaluation of the wine itself and its perception. However, these results indicate that tasters were surprised by the quality of the aged wines. Then, it was admissible that they were not familiar with aged red wines, or at least, with aged red wines with high aging potential.

As regards the results of the chemical analysis, it should first be stressed that is no statistical analysis to prove the veracity of the results obtained, since it has not been possible to repeat measurements for all indices due to the lack of sufficient samples. Nevertheless, the obtained correlations provided consistent results. Different indices for the basic and impact analyses of polyphenolic profiles were considered. Regarding the basic analyses, the sulfur content was the only one taken into consideration as of direct interest for the reactions that take place with polyphenols during the aging period. As expected by the literature, the total sulfur content decreased over time due to the multiple reactions to which it is subject, including those with polyphenols. The CieLab indices were used to have an interpretation of the color of the wine and to have an indication of reliable indices to predict the aging of the wine. Among these surely h^* (which represented Hue) and a^* (Red color Shades) decreased during the time, and they were the ones that more clearly had the same trend for all four wines. Spectrophotometric indices were among the most immediate and easy to interpret. The measurements of A420, A520 and Hue, could represent good indicators of the evolution of red wines in bottle. Moreover, the excellent correlation between A520 and a^* and Hue with h^* , highlighted how these measures can be considered for the prediction of the evolution of wine in bottle, even when wines are tasted in dark glasses. The study of the degree of polymerization of dye pigments confirmed the: a decrease in polymeric and short-chain pigments associated with an increase in long-chain pigments. The index of total polyphenols and the content of

proanthocyanidins followed the expected trend: there was a gradual decrease over time. Similarly, anthocyanins and tannins decreased over time but from the T/A ratio it was found that the decrease was greater for anthocyanins and their ratio was increasing over time. This ratio was important for evaluating the formation of polymeric pigments during the shelf-life of wine. One of the most important results was observed by the evaluation of "tannin power" over time: this can be considered a chemical index for evaluating the astringency of wine. According to the method with BSA for all wines there was an increase in astringency up to about 15 years of age and then gradually decreasing. The result obtained agreed about the polymerization of tannins and the associated sensation of astringency. Nevertheless, the correlation between this index and the results obtained from the astringency ratings of sensory analysis was low (0.46), indicating that further investigations are needed to find reliable chemical tests for the evaluation of this sensory feeling when long aged wines are evaluated.

Finally, connections were sought between the various chemical analyses, which highlighted important conclusions for the prediction of the evolution of the polyphenolic fraction during bottle aging. A correlation matrix (see Tab. 4.6) showed highly significant correlations between some of the polyphenols analyses. Among these, certainly A520, A420 and Hue were among the indices with the highest number of highly significant and, according to the method, the simplest and fastest to estimate. Moreover, with the help of heat-map and PCA it was possible to differentiate wines according to analysis: older wines were better described by the engravers LPP (%), Hue, h^* and L^* . On the other hand, young wines were better distinguished by MP (%), Anthocyanins mg/L, Total SO_2 mg/L, PACs, a^* and IPT. Finally, the multifactorial analysis highlighted the connections between sensory and chemical results. Based on the results, it was possible to clustered wines in 4 groups according to the average age of the cluster and the evaluations for the attribute "Overall Quality". According to the groups it was noticed how Quality and "evolution" followed opposite trends: with the increase of the evolution the perceived quality decreased and vice versa, young wines were considered higher in quality level. This may have been influenced by the fact that judges were more easily used to tasting young wines. Moreover, clusters followed the trends highlighted in the study of wines taken individually for the most interesting attributes, those that can be considered the simplest and most immediate to understand and calculate to evaluate the evolutionary status for wine: MP (%), LPP (%), A520, A420 and Hue.

5.1 Limitations and future directions

The outputs of this work must be understood together with its limitations that become apparent after the end of the experiments, encompassing all the performed experiments:

a) Tasting panel

The tasting panel yielded a wide diversity of responses, as measured by the relatively large variation of the scores for each of the synthetic descriptors. We found two main tasting cohorts with similar responses that could be further studied to see if there would be a more consistent set of responses and higher coefficient of correlations regarding quality prediction. We could have found probably different quality predictors according to the taster segment. This may be achieved in the future increasing the number of tasters and proceed to a separate analysis to characterize the responses of each segment.

b) Tasting sheet and fatigue

After the end of tasting, some respondents commented that the sheet was too long and felt fatigued after completing the task. The results showing a high correlation among some of the parameters shows that, in the future, shorter grids may be used. In addition, the first wines appeared to be better evaluated than the last ones, which was counterbalanced by the random presentation of the samples, but this possible bias should have been assessed.

c) Wine choice

The results were representative of the different tested wine styles. It would be interesting to see if other wines would behave in the same way. For instance, using another high colored grape variety from another region/country, and a low colored variety would enable a stronger generalization of the obtained results. Together, a higher number of younger wines should have been tested by asking the companies wines not yet sent to market.

d) The presented chemical analysis should be considered as preliminary because repetitions of different bottles were not done given the little quantity of available wine. Particularly in long aged wines different bottles may have different evolutions due to variability in cork closures.

e) The chemical and sensory characterization of the wines did not allow a distinction according to their origin. Probably, an analysis of the molecular profile of anthocyanins and tannins would provide this distinction. In addition, an elemental analysis should have been performed to check how the style, grape variety or origin could have influenced sample discrimination.

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Appendix

Appendix N° 1 – Example of the mouthfeel tasting sheet.

A) Flavour and Aroma valuation part

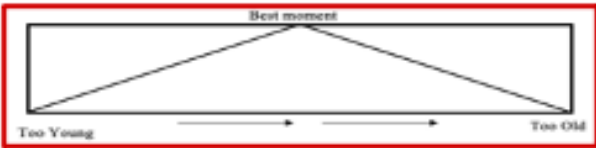
Sample Code: _____

1. Please draw a mark on the scale according to your perception on the **overall quality**:

Very low quality |-----| Very high quality

2. Please draw a mark on the scale to evaluate the **evolution** you perceived according to the age that you attribute to the wine (consider the image as example):

Initial |-----| Best moment |-----| Dead



3. Based on the answers given above, please rate the following attributes, according to your perception.

Evaluate the **bitterness** of the wine.


↓ Low |-----| High ↑

Evaluate the overall **wine body**.

Light |-----| Full









Score the wine **overall complexity**.

Low complexity |-----| High complexity



Appendix N° 2 – Example of Color Tasting Sheet.

Panel Personal Code: _____

Sample Code	How do you describe the colour of this wine?		Predicted age
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____
_____	 Deep purple	_____	_____

Appendix N° 3 – Results of the base chemical analysis.

Sample	Alcohol	M. vol. g/mL	Volatile Acidity g/L	Titrate Acidity	pH	Residual sugar g/L	Malic Acid	Lactic Acid	FML	Free SO2 mg/L	Total SO2 mg/L	CO2 mg/L
A95	15,2	0,9944	0,72	5,6	3,58	8,0	0,0	1,2	Conclosed	2	29	154
A00	16,4	0,9948	1,10	6,2	3,63	7,1	0,0	1,3	Conclosed	2	29	112
A04	16,1	0,9948	0,78	5,7	3,71	9,4	0,0	1,2	Conclosed	2	29	55
A10	17,6	0,9945	0,78	6,5	3,62	9,4	0,0	1,0	Conclosed	2	37	123
A15	17,1	0,9931	0,72	6,7	3,46	6,6	0,0	1,5	Conclosed	2	42	108
L98	13,8	0,9913	0,72	5,2	3,46	1,8	0,0	1,0	Conclosed	2	30	165
L03	14,7	0,9917	0,94	5,7	3,68	2,9	0,0	1,3	Conclosed	2	34	143
L07	14,9	0,9922	0,75	6,5	3,42	2,5	0,1	1,0	Conclosed	2	34	128
L09	15,5	0,9919	0,81	6,1	3,39	2,5	0,0	1,3	Conclosed	3	43	148
L16	15,0	0,9912	0,66	6,2	3,38	2,3	0,1	1,1	Conclosed	2	35	102
T01	13,5	0,9931	0,66	5,9	3,53	2,1	0,0	1,9	Conclosed	2	30	120
T06	13,6	0,9934	0,66	5,9	3,50	2,5	0,0	1,1	Conclosed	2	54	135
T12	13,4	0,9946	0,72	6,9	3,64	1,9	0,0	2,1	Conclosed	3	64	115
T16	13,6	0,9935	0,57	6,1	3,56	2,1	0,0	1,5	Conclosed	15	81	122
T21	14,5	0,9927	0,53	6,1	3,60	2,1	0,0	1,3	Conclosed	8	37	207
V00	13,2	0,9914	0,51	5,4	3,37	2,5	0,0	1,2	Conclosed	2	31	174
V05	13,5	0,9913	0,57	5,5	3,50	2,3	0,0	1,2	Conclosed	2	29	181
V09	13,6	0,9929	0,53	5,4	3,58	4,6	0,1	1,1	Conclosed	2	42	263
V12	13,7	0,9956	0,66	5,7	3,63	5,9	0,0	1,7	Conclosed	2	69	247
V16	14,0	0,9948	0,60	6,1	3,44	6,6	0,0	1,6	Conclosed	3	53	299
V19	14,3	0,9953	0,66	5,6	3,47	6,3	0,1	0,8	Conclosed	10	55	268

Appendix N° 4.1 – Results of the phenols chemical analysis.

Sample	Vintage (Years)	TPI	Tanninos mg/L	Hydroxycinnamic Acid mg/L	Anthocyanins mg/L	AT	A420	A520	A620	Color Intensity	Hue	MP (%)	SPP (%)	LPP (%)
A95	28	53,3	3240	194	89	0,03	0,4	0,259	0,068	0,73	1,54	11,9	19,1	69,0
A00	23	68,3	4325	220	70	0,02	0,41	0,253	0,06	0,72	1,62	15,9	25,4	58,7
A04	19	69,2	4243	252	94	0,02	0,529	0,352	0,112	0,99	1,50	13,3	24,8	61,9
A10	13	104,7	6428	362	179	0,03	0,838	0,657	0,174	1,67	1,28	16,7	34,5	48,8
A15	8	79,0	4769	276	173	0,04	0,669	0,632	0,154	1,46	1,06	20,5	32,1	47,4
L98	25	64,1	4067	192	91	0,02	0,445	0,309	0,084	0,84	1,44	17,4	30,0	52,6
L03	20	90,7	5590	298	169	0,03	0,79	0,589	0,16	1,54	1,34	16,1	36,7	42,2
L07	16	86,6	5242	296	192	0,04	0,764	0,662	0,149	1,58	1,15	16,0	34,2	49,8
L09	14	82,6	5011	282	179	0,04	0,749	0,721	0,145	1,62	1,04	21,7	34,8	43,5
L16	7	87,7	5281	302	210	0,04	0,64	0,643	0,138	1,42	1,00	24,9	35,4	39,6
T01	22	61,1	3561	254	130	0,04	0,636	0,525	0,133	1,29	1,21	20,0	26,7	53,3
T06	17	78,1	4675	286	167	0,04	0,712	0,663	0,153	1,53	1,07	21,8	31,2	46,9
T12	11	78,4	4328	344	270	0,06	0,745	0,761	0,195	1,70	0,98	28,7	37,1	34,2
T16	7	78,4	4312	330	305	0,07	0,677	0,758	0,183	1,62	0,89	30,6	39,6	29,8
T21	2	79,5	4102	288	557	0,14	0,782	1,139	0,283	2,20	0,69	47,5	32,4	20,1
V00	23	48,0	2795	204	96	0,03	0,454	0,355	0,086	0,90	1,28	16,4	20,8	62,9
V05	18	50,4	2950	210	97	0,03	0,484	0,397	0,099	0,98	1,22	16,7	23,2	60,1
V09	14	64,1	3644	276	172	0,05	0,647	0,61	0,143	1,40	1,06	20,4	30,2	49,4
V12	11	57,0	3436	214	100	0,03	0,42	0,356	0,083	0,86	1,18	21,2	31,4	47,5
V16	7	65,0	3945	236	114	0,03	0,382	0,357	0,077	0,82	1,07	28,0	28,6	43,3
V19	4	72,7	4325	266	174	0,04	0,364	0,394	0,077	0,84	0,92	32,8	31,3	35,9

Appendix N° 4.2 – Results of the phenols chemical analysis.

Sample	L*	a*	b*	C*	h	Total Proanthocyanidins (g/L)	Total phenolic compounds	Color intensity	Hue	Dyalisis Index
A00	73,1353 ± 0,0144	19,3048 ± 0,0155	34,8079 ± 0,0237	39,8028 ± 0,0280	60,9868 ± 0,0067	2,06 ± 0,04	64,2 ± 0,02	6,56 ± 0,02	1,48 ± 0,004	48 ± 0,8
A10	48,7750 ± 0,1196	41,1735 ± 0,0670	48,0012 ± 0,0336	63,2406 ± 0,0691	49,3784 ± 0,0263	4,46 ± 0,08	100,7 ± 0,1	14,6 ± 0,19	1,23 ± 0,002	34,6 ± 0,4
A15	47,7957 ± 0,1056	43,8397 ± 0,0690	34,2189 ± 0,2704	55,6138 ± 0,1504	37,9733 ± 0,2421	4,1 ± 0,09	79,9 ± 0,05	13,29 ± 0,06	0,97 ± 0,005	26,7 ± 0,6
A95	72,4745 ± 0,4197	19,5800 ± 0,4629	33,0814 ± 0,2547	38,4440 ± 0,0417	59,3801 ± 0,7848	1,58 ± 0,1	51,4 ± 0,08	6,51 ± 0,06	1,41 ± 0,01	41 ± 1
L03	51,7187 ± 1,1429	38,6066 ± 0,6554	40,6276 ± 0,1017	55,9812 ± 0,4911	46,4003 ± 0,04492	5,94 ± 1,0	84,9 ± 0,05	12,91 ± 0,15	1,24 ± 0,003	38,5 ± 0,07
L09	47,9814 ± 0,0599	46,1095 ± 0,1435	40,2863 ± 0,0095	61,2297 ± 0,1123	41,1442 ± 0,0842	6,89 ± 1,0	82,7 ± 0,03	14,1 ± 0,03	0,99 ± 0,005	51,9 ± 0,6
L16	47,6912 ± 0,0802	45,4905 ± 0,0292	35,3337 ± 0,0744	57,6008 ± 0,0634	37,8374 ± 0,0484	5,83 ± 0,4	83,6 ± 0,03	13,39 ± 0,06	0,94 ± 0,003	46,8 ± 0,3
L98	68,7934 ± 0,0642	25,8468 ± 0,0613	36,5386 ± 0,0734	44,7563 ± 0,0944	54,7251 ± 0,0185	4,21 ± 0,7	62,6 ± 0,5	7,52 ± 0,06	1,34 ± 0,003	45,2 ± 1,0
T01	52,0548 ± 0,1377	37,3800 ± 0,1408	36,0729 ± 0,0632	51,9473 ± 0,1439	43,9807 ± 0,0614	3,82 ± 0,005	62,7 ± 0,06	11,96 ± 0,03	1,16 ± 0,003	50,4 ± 0,5
T06	46,5734 ± 0,1733	44,5792 ± 0,0967	34,4233 ± 0,0580	56,3230 ± 0,0479	37,6747 ± 0,1039	4,44 ± 0,03	81,2 ± 0,09	13,95 ± 0,09	1,01 ± 0,0004	48,6 ± 0,3
T16	42,3328 ± 0,1282	48,3564 ± 0,0590	24,5875 ± 0,0883	54,2484 ± 0,0898	26,9517 ± 0,0598	4,92 ± 0,02	75,9 ± 0,1	14,37 ± 0,05	0,86 ± 0,003	34,9 ± 0,06
T21	28,3029 ± 0,0236	54,3187 ± 0,0411	22,4325 ± 0,0107	58,7685 ± 0,0339	22,4396 ± 0,0250	4,33 ± 0,4	82,9 ± 0,04	21,21 ± 0,09	0,66 ± 0,004	45,2 ± 0,1
V00	65,4998 ± 0,1332	28,5946 ± 0,0644	34,3756 ± 0,0329	44,7139 ± 0,0645	50,2454 ± 0,0417	2,73 ± 0,6	47,6 ± 0,06	8,26 ± 0,11	1,22 ± 0,005	38,1 ± 0,2
V05	62,8712 ± 0,1149	30,9114 ± 0,0504	34,1825 ± 0,0226	46,0865 ± 0,0235	47,8768 ± 0,0622	2,97 ± 0,4	52,8 ± 0,04	8,71 ± 0,07	1,17 ± 0,01	41,1 ± 3
V12	64,3217 ± 0,1732	30,2437 ± 0,0746	28,0156 ± 0,1353	41,2257 ± 0,1349	42,8097 ± 0,1049	3,09 ± 0,04	59,9 ± 0,07	8,01 ± 0,04	1,12 ± 0,001	47,4 ± 0,5
V19	62,1370 ± 0,6235	35,6874 ± 0,1865	15,7482 ± 0,2881	39,0081 ± 0,2375	23,8102 ± 0,3643	3,16 ± 0,07	71,9 ± 0,05	7,54 ± 0,06	0,88 ± 0,02	41 ± 0,2

Appendix N°5 – Results of the evaluation of the predicted age of wines based in the taste, the color and the % difference between the predicted and the real age given by the judges.

Sample	Real Age	Predicted Age- Color	Predicted Age- Taste	% Difference from real Age - Color	% Difference from real Age - Taste
V19	4	3,77	5,04	-5,8	25,9
A15	8	7,23	5,67	-9,6	-29,2
L16	7	7,73	5,43	10,5	-22,4
T21	2	3,40	5,35	70,0	167,5
V00	23	12,90	7,19	-43,9	-68,8
A95	28	16,53	8,50	-41,0	-69,6
L98	25	14,00	6,21	-44,0	-75,1
T01	22	9,97	7,70	-54,7	-65,0

Appendix N° 6 - Photos from the tasting days.





