

## **Impact of vine age on grape composition: a review**

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*To my family*

## ACKNOWLEDGMENT

*Most of all, I want to thank my family and relatives for the support and understanding they have shown me. It has been a tough time and they were always there for me.*

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## Abstract

*Vitis vinifera* L. is a perennial cultivation plant capable of showing remarkable longevity. Some of its characteristics evolve as they get older. Thus, vine age and its relation with grape and wine quality are subjects of recurring interest. This topic remains discussed and the understanding of the mechanisms through which vines of different ages would produce wines of different quality is still poorly studied.

This bibliographic work aims to evaluate previous studies on the effect of vine age on some of the main grape descriptors. Vine age impacts reproductive performance: older vines generate higher yields, but all vines, regardless of age, are affected by seasonal variability as expected.

Wine quality is mainly influenced by the characteristics of the grapevine used for production. *Vitis* genus contains some plasticity inside, that is the amount by which the individual expression of a genotype can be regulated by the environment where it is immersed. This research analyzed key metabolites to elucidate qualitative traits that contribute to the dynamics of maturation, describing biosynthesis, evolution, the main factors that influence grape maturation, and their relationship to vine age.

Analyzing the various published works on the topic of vine age, it is possible to notice quite contradictory results. This is due to the difficulties in obtaining appropriate plant material and organizing field tests with equivalent conditions based on vine age. Furthermore, it may be difficult to distinguish vine age-related impacts related to vine age due to seasonal and environmental variability.

## Keywords

Old vines – grape composition– wine quality

## Resumo

*Vitis vinifera* L. é uma planta de cultivo perene capaz de mostrar uma longevidade notável. Algumas das suas características evoluem à medida que envelhecem. Assim, a idade da videira e a sua relação com a qualidade da uva e do vinho são temas de interesse recorrente. Este tema continua a ser discutido e a compreensão dos mecanismos através dos quais as vinhas de diferentes idades produziram vinhos de qualidade potencialmente diferente ainda é pouco estudado.

Este trabalho de pesquisa bibliográfica visa fazer uma revisão sobre os trabalhos publicados sobre o efeito da idade da videira em alguns dos principais componentes das uvas. A idade da videira teve impacto no desempenho reprodutivo: as vinhas mais velhas geraram maiores rendimentos, mas todas as vinhas, independentemente da idade, foram afectadas pela variabilidade sazonal.

A qualidade do vinho é principalmente influenciada pelas características das uvas utilizadas na produção. O género *Vitis* contém alguma plasticidade no interior, ou seja, a quantidade pela qual a expressão individual de um genótipo pode ser regulado pelo ambiente onde está imerso. Esta investigação analisou os metabolitos chave para elucidar traços qualitativos que contribuem para a dinâmica da maturação, descrevendo a biossíntese, a evolução, e os principais factores que influenciam a maturação da uva e a sua relação com a idade da videira.

Pela análise dos vários trabalhos publicados referentes ao tópico da idade da videira, verifica-se a existência de resultados bastante contraditórios. Este facto deve-se às dificuldades em obter material vegetal apropriado e em organizar testes de campo com condições equivalentes com base na idade da videira. Além disso, pode ser difícil distinguir os impactos relacionados com a idade da videira, devido à variabilidade sazonal e local.

## Palavras - chave

Vinhas velhas – composição da uva – qualidade do vinho

## Resumo alargado

Nas paisagens agrícolas mediterrânicas, um elemento-chave reconhecível é a vinha. O seu significado ambiental e cultural é amplamente reconhecido, com particular impacto em Portugal. O património vitícola e o valor dos vinhos e das paisagens das regiões vinícolas são dimensões importantes para o país. A riqueza do material vegetal, a história e a qualidade são também relevantes à escala mundial. Actualmente, Portugal beneficia de projectos notáveis na viticultura: adegas e empresas promoveram a caracterização e selecção de castas autóctones de vinha. De facto, tal como descrito por da Silva *et al.* (2018), estas contribuições técnicas e científicas proporcionaram a qualificação e conservação do património varietal português. Também em Itália, através do Centro de Viticultura e Enologia, são realizados planos de melhoramento genético e selecção varietal de vinhas; além disso, são realizados estudos sobre as técnicas de conservação, propagação, cultivo e protecção fitossanitária da vinha, com atenção às interacções entre os métodos de produção e a qualidade da uva e do vinho (CREA, 2021).

A vinha produz vinho desde antes do registo histórico: o vinho é um produto tão único (Bisson *et al.*, 2002; McGovern, 2013). As vinhas são plantas que podem persistir durante séculos; de facto, há exemplos de plantas cultivadas e que ainda dão frutos após mais de 400 anos (Vršič *et al.*, 2011; Grigg *et al.*, 2018). O ciclo biológico de uma vinha é caracterizado por uma fase inicial de crescimento improdutivo, que dura cerca de 3 anos. Depois, a produtividade aumenta ao longo do tempo (Eynard e Dalmasso, 1990; Fregoni, 2013). Mais tarde, a vinha atinge a idade adulta, caracterizada por uma produtividade que se mantém constante durante 20 ou 30 anos. Finalmente, segue-se uma fase de diminuição do rendimento, que corresponde à velhice da videira. Nesta última fase do ciclo de vida, as vinhas produzem frutos mais pequenos e os rendimentos diminuem, o que resulta em vinhos mais concentrados e complexos (Robinson e Harding, 2006).

Estudos sobre a análise do ciclo biológico das vinhas criam precedentes na literatura. Em particular, esta dissertação visa investigar a resposta fisiológica à dinâmica da maturação, verificar o impacto potencial da idade, e comparar a reacção das vinhas mais novas às mais velhas. Estas revisões podem ser utilizadas para a "restauração" da vinha: estas operações não requerem trabalho indiferente e recursos económicos; portanto, estimar o comportamento da planta durante a maturação pode ajudar a ter imediatamente limpo as práticas culturais a serem implementadas. Isto permite a aquisição de uma resposta vegetativa desejada na vinha, permitindo a expressão de características específicas das uvas e, conseqüentemente, do vinho obtido a partir delas. As vinhas mais jovens produzem vinhos com poucas características ou estruturas particulares como vinhos obtidos a partir de vinhas mais velhas

(Reynolds *et al.*, 2008). Como relatado por Zufferey e Maigre (2008), um dos factores mais importantes que explica a qualidade é o equilíbrio vegetativo e reprodutivo observado nas vinhas velhas. Os seus rendimentos mais baixos permitem o armazenamento de uma maior quantidade de metabolitos nas uvas, tais como antocianinas, flavonas e flavonóides: o vinho adquire um perfil organoléptico e nutricional muito interessante.

No entanto, é um desafio extrapolar exclusivamente o impacto da idade da videira até à maturidade. De facto, muitas variáveis podem afectar o estudo de forma cruzada, o que pode complicar a separação dos efeitos relacionados com a idade. De acordo com muitos autores, a composição química dos cachos é dependente da cultivar, mas também depende das condições climáticas e das mudanças climáticas sazonais (De Pascali *et al.*, 2014; Fraga *et al.*, 2017a), localização, e características do solo em que as uvas são cultivadas (Cheng *et al.*, 2015), práticas ambientais e de gestão da vinha (Romeyer *et al.*, 1983; Jackson e Lombard, 1993; Deloire *et al.*, 2005).

O recurso único também precisa de ser monitorizado e preservado das futuras alterações climáticas, apesar das incertezas sobre a sua verdadeira extensão. As tendências de aquecimento e seca podem levar a novos desafios para a produção de videiras em todo o país (Santos *et al.*, 2011). Por conseguinte, são necessários estudos anuais que monitorizem a resposta fisiológica à dinâmica de maturação e o ambiente climático registado. Assim, a indústria vinícola pode implementar medidas apropriadas para lidar com os impactos das alterações climáticas através do desenvolvimento de estratégias à escala regional (Metzger *et al.*, 2008). Entretanto, os viticultores podem implementar um planeamento estratégico e atempado para beneficiar também.

Este trabalho de investigação introduziu o processo de desenvolvimento da vinha, descrevendo a dinâmica da maturação dos compostos químicos do fruto e como estes podem ser afectados pelas variáveis. Depois, foram pormenorizados projectos de investigação em curso no Novo Mundo, que visam criar uma consciência sobre o valor das vinhas velhas. Em seguida, analisando os trabalhos de investigação presentes na literatura, o impacto da idade da vinha foi colocado em relação aos aspectos vitivinícolas, processo de maturação e composição da uva, bem como a composição do vinho e o perfil sensorial. É possível notar imediatamente resultados bastante contraditórios, tanto devido à dificuldade em obter material vegetal apropriado e organizar ensaios de campo com condições equivalentes, como porque é difícil distinguir impactos relacionados com a idade da vinha devido à variabilidade sazonal e ambiental.

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## 1. INTRODUCTION

In Mediterranean agricultural landscapes, a key recognizable element is vineyard. Its environmental and cultural significance is widely acknowledged, with particular impact in Portugal. Viticultural heritage and the value of wines and landscapes of wine regions are important dimensions for the country. The richness of plant material, history, and quality are also relevant on a worldwide scale. Currently, Portugal benefits from remarkable projects in viticulture: wineries and enterprises have promoted the characterization and selection of autochthonous vine varieties. Indeed, as described by da Silva *et al.* (2018), these technical and scientific contributions have provided the qualification and conservation of the Portuguese varietal heritage. Also in Italy, through the Center of Viticulture and Enology, plans for genetic improvement and varietal selection of vines are realized; in addition to this, studies are conducted on the techniques of conservation, propagation, cultivation, and phytosanitary protection of vines, with attention to the interactions between production methods and grape and wine quality (CREA, 2021).

Vines have been producing wine since before history was recorded: wine is such a unique product (Bisson *et al.*, 2002; McGovern, 2013). Vines are plants that can persist for centuries; in fact, there are examples of grown and still bearing fruit after more than 400 years (Vršič *et al.*, 2011; Grigg *et al.*, 2018). The biological cycle of a vineyard is characterized by an initial phase of unproductive growth, which lasts about 3 years. Then, productivity increases over time (Eynard and Dalmasso, 1990; Fregoni, 2013). Later the vine reaches adulthood, characterized by productivity that remains constant for 20 or 30 years. Finally, a phase of decreasing performance follows, which corresponds to the old age of the vines. In this last phase of the lifecycle, vines produce smaller fruit and yields decrease, which results in more concentrated and complex wines (Robinson and Harding, 2006).

Studies on the analysis of the biological cycle of vines create precedents in the literature. In particular, this dissertation aims at (i) investigating the physiological response to the ripening dynamics, (ii) verifying the potential impact of age, and (iii) comparing the response of younger to older vines. This enables the acquisition of a desired vegetative response in the vineyard, allowing the expression of specific characteristics of grapes and, consequently, of the wine obtained from them. Younger vines produce wines with not many particular characteristics or structures as wines obtained from older vines (Reynolds *et al.*, 2008). As reported by Zufferey and Maigre (2008), one of the most important factors explaining quality is the vegetative and reproductive balance observed in old vines: their lower yields allow the storage of a higher quantity of metabolites in the grapes, such as anthocyanins, flavones, and flavonoids: the wine acquires a very interesting organoleptic and nutritional profile.

However, it is challenging to exclusively extrapolate the impact of vine age to maturity. Indeed, many variables can affect the study crosswise, which may complicate the separation of effects related to age. According to many authors, the chemical bunch composition is cultivar dependent but also rely on climatic conditions and seasonal changing in the weather (De Pascali *et al.*, 2014; Fraga *et al.*, 2017a), location, and soil characteristics in which the grapes are grown (Cheng *et al.*, 2015), environmental and management vineyard practices (Romeyer *et al.*, 1983; Jackson and Lombard, 1993; Deloire *et al.*, 2005).

The unique resource of old vines also needs to be monitored and preserved from future climate change, despite uncertainties about its true extent. The warming and drought trends could lead to new challenges for grapevine production all over Portugal (Santos *et al.*, 2011). Therefore, annual studies that monitor the physiological response to ripening dynamics and the recorded climatic environment are needed. Thus, the wine industry can implement appropriate measures to deal with the impacts of climate change through the development of regional-scale strategies (Metzger *et al.*, 2008). Meanwhile, winemakers can implement strategic and timely planning to benefit as well.

This research work introduced the process of vine development, describing the dynamics of the maturation of the chemical compounds of the fruit and how they may be affected by variables. Then, it was detailed ongoing research projects in the New World, that aim to create awareness about the value of old vines. Then analyzing the research works present in the literature, the impact of vine age was placed in relation to viticultural aspects, ripening process, and grape composition, as well as wine composition and sensory profile. It is possible to notice immediately quite contradictory results, both because of the difficulty to obtain appropriate plant material and organize field trials with equivalent conditions, and because it is difficult to distinguish impacts related to vine age because of seasonal and environmental variability.

## 2. LITERATURE REVIEW

### 2.1 The Portuguese vine industry

In Portugal, viticulture is acquiring an increasingly important role from a socio-economic point of view: the sector has recorded a continuous average annual growth until 2019, then recording a contraction of 0.2% according to the latest available data for 2020. From a worldwide vision, the total area planted in Portugal with vines covers about 194.000 hectares, equivalent to 2.7% of the world surface under vines (OIV, 2021). Nowadays Portugal still has a great diversity of autochthonous grapevine cultivars: 343 cultivars are officially authorized for wine production in Portugal; however, only 34 vines are most used for the wine sector (IVV, 2018). In 2020, wine production amounted to  $6.4 \times 10^6$  hL (-2% / 2019), which places Portugal in 11<sup>th</sup> position among the main wine-producing countries in the world (OIV, 2021).



Figure 1 – Wine regions in Portugal, both mainland and islands. Source: Cunha et al., 2013.

The country is divided into 14 viticultural regions (Figure 1), or Protected Geographical Indications (PGIs), 12 on the mainland and 2 in the islands, including 31 Protected Denominations of Origin (PDOs). Douro/Porto, Lisboa, Alentejo, and Minho are the main in terms of production declared by region. Even though the Alentejo has the highest productivity of PDO wine (Fraga and Santos, 2018), the Douro/Porto region has the largest vineyards area and accounts for approximately 21% of total wine production in Portugal during the 2018/2019 vintage (IVV, 2018).

### 2.1.1 Douro Wine Region

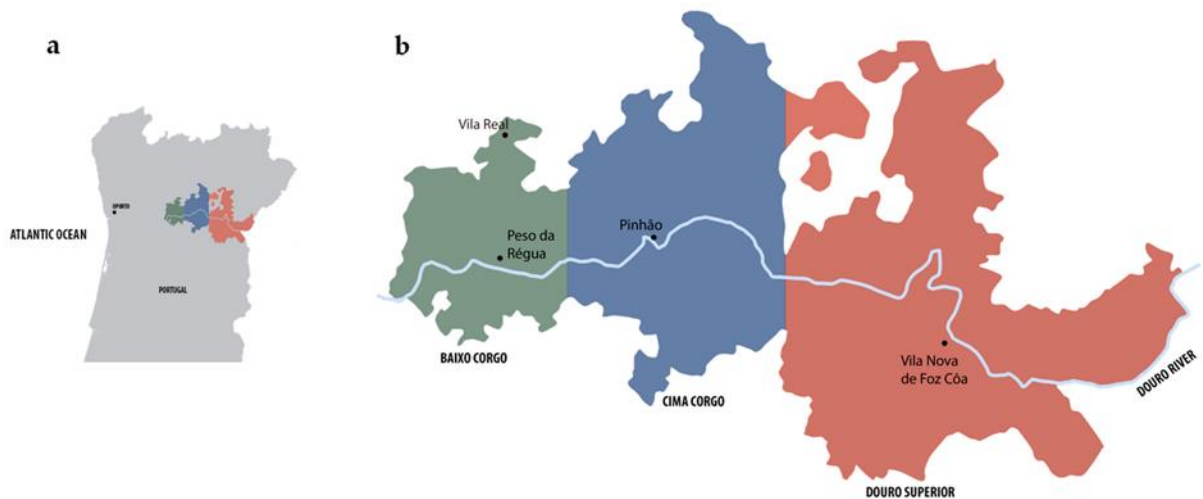


Figure 2 – Map of Douro Wine Region: (a) Illustration of the position of the Douro Wine Region within Portugal. (b) Each coloured zone represents an individual subregion: Baixo Corgo (BC); Cima Corgo (CC); Douro Superior (DS). Source: Barbosa et al., 2018.

The Douro Wine Region is the one of the oldest delimited and demarcated region in the world, dated 1756 (Pereira, 2003). A part of it, the Alto Douro Vinhateiro, obtained UNESCO World Heritage status in 2001, thanks to its "heroic, living and evolving" viticulture (Bianchi-de-Aguiar, 2002). The first vineyards of which there are traces date back to Roman times (Almeida and Almeida, 2004); in the Middle Ages, several Cistercian monasteries promoted viticulture, expanding the area of vineyards (Sebastian, 2018).

Located within the Douro basin in northeastern Portugal, the region is known for its deep valleys and mountain terrain; its altitude can vary between 40 and 1400 m (Jones, 2013). It is divided into three subregions: Baixo Corgo, Cima Corgo, and Douro Superior. The positioning of the region and the location of the subregions are shown in Figure 2. The total area of the region covers 250.000 hectares, and vines take up approximately 18% of the total (IVDP, 2021). These vineyards are relatively small in size, often bordering non-cultivated habitats (forests, grassy slopes, terraces with natural vegetation) and soils covered with wild vegetation (Andresen et al., 2004). The complex landscape characteristics produce a wide range of terroirs, allowing for the growth of a large number of autochthonous grape varieties (Fraga et al., 2017b). The most prevalent grape varieties are: Touriga Nacional, Touriga Franca, Tinta Roriz, Tinta Barroca, Tinta Amarela, Sousão for red grapes; Códèga, Malvasia Fina, Rabigato, Moscatel Galego for white ones. Considering the amount of total wine produced in the Douro Valley, Port Wine represents as much as 50% (IVV, 2018), being the leading product of Portugal.

## 2.2 The Italian vine industry

Viticulture is a relevant aspect of the Italian economy. Since viticulture was already performed long before Roman times, wine production is strongly rooted in the Italian heritage. Nowadays, Italy is among the main actors of the wine world, and indeed, wine is the real food emblem of Made in Italy: the Italian wine sector leads the national agri-food industry, being its main contributor thanks to exports. The sector is continuously growing, with the latest available data for 2020 indicating an increase in the area planted with vines in Italy of +0.8% compared to 2019. The total area under vine covers about 719.000 ha, equivalent to 9.8% of the world's area under vine (OIV, 2021). Italy has an enormous diversity of cultivars and clones included in the register of varieties: 798 cultivars are officially recognized for the production of wine and table grapes, and, of these, 526 varieties are used for the production of PDOs and PGIs (MIPAAF, 2022). In 2020, wine production amounted to  $49.1 \times 10^6$  hL (+3% / 2019), which places Italy in 1st position among the main wine-producing countries in the world (OIV, 2021).



Figure 3 – Wine regions in Italy, both mainland and islands. Source: vineyards.com.

In Italy, grapevines are grown in a range of ecosystems, including coastal regions, mountains, hills, and alluvial plains, as well as dry and marshy locations, with a huge range of substrates of all sorts (Figure 3). As a result, the Italian wines are divided into 92 "macro-areas" based on geological, soil, topographical, and climatic characteristics (Pollini *et al.*, 2014). Veneto, Apulia, Emilia Romagna, and Sicily are the main regions in terms of declared production per region, while Veneto and Piedmont have the highest production of PDOs wine (Federvini, 2021).

Sicily has the largest area of vineyards (MIPAAF, 2018), but the Puglia region led total wine production in Italy during the year 2020/2021 (Federvini, 2021). The average land value of Italian vineyards increased by +9.3% in Piedmont compared to 2015; indeed, in the first place are Barolo vineyards that can reach quotations of 1.5 million euros per hectare (Barbaresco *et al.*, 2021).

### 2.2.1 Piedmont Wine Region

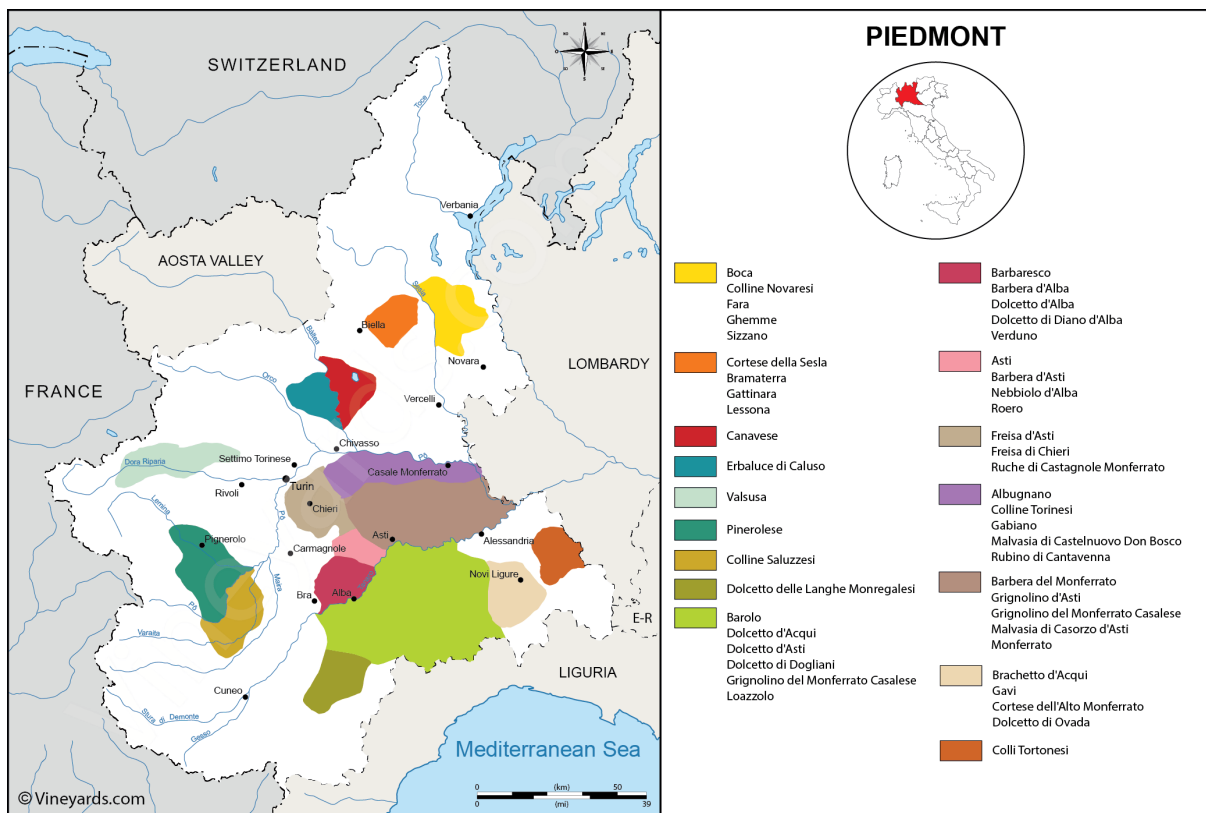


Figure 4 – Map of Piedmont Wine Region. Subregion's location (left) and positioning in the Italian territory (right). Source: vineyards.com.

Piedmont is one of the most important Italian wine regions and produces prestigious wines such as Barolo and Barbaresco. In 2014, The Viticultural Landscape of Piedmont: Langhe, Roero, and Monferrato were recognized as a UNESCO World Heritage Site for their exceptional landscapes and the importance of viticulture and winemaking in the Region (UNESCO, 2014). Viticulture has been practiced in the area since Pre-Roman and Roman times, however, large-scale production of grapes did not begin until the 17th century. Phylloxera (acquired from the Americas) devastated the vineyards in the second half of the 19th century. Several vineyards were completely destroyed and had to be replaced (Eynard and Dalmasso, 1990).

The Piedmont region extends in the northwest of the Italian territory for a total area of 2.538.700 hectares (ISTAT, 2021), corresponding to 24% of the Italian territory and 21% of its agricultural area (Sapino *et al.*, 2020). The territory can be divided into six macro-areas (Figure 4): the area between Saluzzo and Turin; Monferrato Astigiano; Upper Monferrato; Roero; Langhe; and Upper Piedmont (Assovini, 2021). The central areas, namely Langhe, Monferrato, and Roero, are now world-renowned as territories of high-quality wines, and place names such as Barbaresco and Barolo have become trademarks of top wines (Corinto and Pioletti, 2019). Producing some of Italy's best-known and premium wines, it has 60 PDOs, divided into 42 DOCs, and 18 DOCGs (Piedmont Region data, 2021a). Indeed, this characteristic situates the region on top, being the one that manages the largest number of wine appellations of origin (Cerrato *et al.*, 2014). Vineyards cover 45.979 hectares, or about 6,7% of the Italian wine area (MIPAAF, 2018; OIV, 2021). According to the Piedmont Regional Administration, nearly 90% of the vineyard surface is on hilly land, with only 2% on mountain ones, and vineyards are concentrated in the southern part of the region, in the provinces of Asti, Cuneo, and Alessandria (Piedmont Region data, 2021b). Barbera is the most common vine in the region: in 2010, the year of the last agricultural census, ISTAT reported more than 13.900 hectares, representing 30% of total regional vineyards. These areas ensure leadership at the national level: in Piedmont is located 68% of Barbera vineyards are planted in Italy. The second most common cultivar is Moscato Bianco, with 9.750 hectares and a 21% share. Dolcetto is also relevant with 5.990 hectares, as well as Nebbiolo and Cortese, with 4.480 and 2.630 hectares respectively. Piedmont has the largest share of these grapes, between 80 and 98% of the corresponding national surface. Lastly, Nebbiolo is the base of DOCG Barolo and Barbaresco, as well as DOC Nebbiolo d'Alba (UIV, 2021).

I had the opportunity to get involved with the reality of the Douro and Piedmont Region during my university career, which allowed me to examine the two *terroirs* in depth. I deepened the enological relevance of these two regions to highlight how, even in prestigious contexts, the bibliographic research revealed a lack of research studies which analyzed the impact of vine age as a variable in itself. However, I was able to find that the problem is mainly Old World, with the exception of very few cases, also because, from what emerges in subitem 2.7.2, the New World is organizing registries and Charters in which they can keep track of this world heritage.

### 2.3 Grapevine variability system

Variability is a fundamental characteristic of all biological systems, including the grapevine. Given the vast diversity within the *Vitis* genus, the genotype is a significant source of variability (This *et al.*, 2007). Variability is the outcome of plasticity, which is the quantity by which a genotype's individual expression may be adjusted by its environment within a particular genotype, cultivar or clone (Bradshaw, 1965). Variability has been shown to occur within berries, between berries within a cluster, between clusters on a vine, and between vines within a vineyard due to environmental conditions and viticultural operations (Gray, 2002; Keller, 2010a). Existing variability may or may not be an advantage (Tijsskens *et al.*, 2003). On the one hand, genetic variability and plasticity allow cultivars to be fitted to a specific growing zone, to offer a wide range of wines from the same variety, or to raise new ones that are well-adapted to a different growing area. On the other hand, in response to climatic circumstances and viticulture methods, the plasticity of genotype might result in uneven maturity (Selvaraj *et al.*, 1995) and interseasonal oscillations (Clingeffer, 2010). Additionally, wine quality and complexity are determined by the range of diversity within clusters and berries composition (Singleton *et al.*, 1966; Kontoudakis *et al.*, 2011). Also under appropriate ripeness, heterogeneity in berry maturity could lead to green (from immature berries) or jam (from overripe berries) flavors in the finished wine (Long, 1987). Identifying the underlying reasons for variation in grape berries and main metabolites may help decrease heterogeneity of a cultivar within a vineyard and across vintages, and also select the best cultivar for a specific growing zone. The study of grape variability is becoming increasingly essential due to global warming: in viticulture, one of the most significant impacts of climate change is the alteration of phenological stages of grape growth and ripening, with the period between flowering and harvest becoming both earlier and shorter (Duchêne and Schneider, 2005). In the event that the vineyard approaches the final ripening period under increasingly warmer and drier conditions, certain physiological processes necessary for the production of high-quality grapes are going to be inhibited (Deloire *et al.* 2004). The alteration of vine physiology results in significant changes in wine quality and typicality (Schultz, 2000; Jones *et al.*, 2005). Indicators that attest to climate change are growing global temperatures, warming and rising oceans, diminishing ice sheets, decreasing Arctic Sea, glacier retreat, and an increase in the frequency of catastrophic events (NASA, 2020). Climate change and variability play a significant effect on a cultivar's adaptation to a growing season (Jones, 2006; Webb *et al.*, 2007; Duchêne *et al.*, 2010), along with changing the average grape yield and variability for a cultivar (Bindi *et al.*, 1996). These scenarios are promoting the anticipation of vine phenological events also in the interest area of the Douro Valley (Jones and Alves, 2012; Jones, 2013; Real *et al.*, 2015). According to Santos *et al.*, 2011, future climate change scenarios seem to favor an increase

in wine production in the Douro region. Due to climatic and production variations in this area, future production cannot be expected to be uniform across the region. In the eastern zone (Douro Superior), for example, further decreases in rainfall will make it extremely impossible to continue producing, but the western parts (Baixo Corgo and Cima Corgo) may respond slowly to these shifts (Gouveia *et al.*, 2011). Furthermore, according to an interview with eight Piedmonts winemakers conducted by Monzini (2020), was found that unusual temperatures are associated with unusual grape ripening, a phenomenon also reported in California and Australia (Poni, 2011). Another effect of climate change is excessive exposure to radiation, which can cause sunburn, thus damaging vine ripening. Most winemakers associate extreme weather elements and drought problems with a new "comeback" of certain diseases that affect grapevines, such as esca disease and powdery mildew, as also stated by other authors (Caffarra *et al.*, 2012; Merloni, 2017). Santos *et al.* (2020) specified that the spread of these diseases is the result of increased globalization, while their survival and continued spread to new areas are attributed to more mild winters (Langille, *et al.*, 2017) Climate change will have a considerable impact on future grape and wine production (Bisson *et al.*, 2002), which could result in benefits or disadvantages depending on location (Jones *et al.* 2005).

## 2.4 Grape berry development and ripening

Grape development stages undergo molecular, genetic, and biochemical processes that will affect grape composition (Palliotti *et al.*, 2018) and ultimately the quality of the resulting wine. Berry growth is defined by a double sigmoid model with three distinct growth phases (Figure 5), namely the first rapid growth phase, the lag phase, and the second rapid growth phase (Coombe, 1973, 1976, 1992; Coombe and McCarthy, 2000).

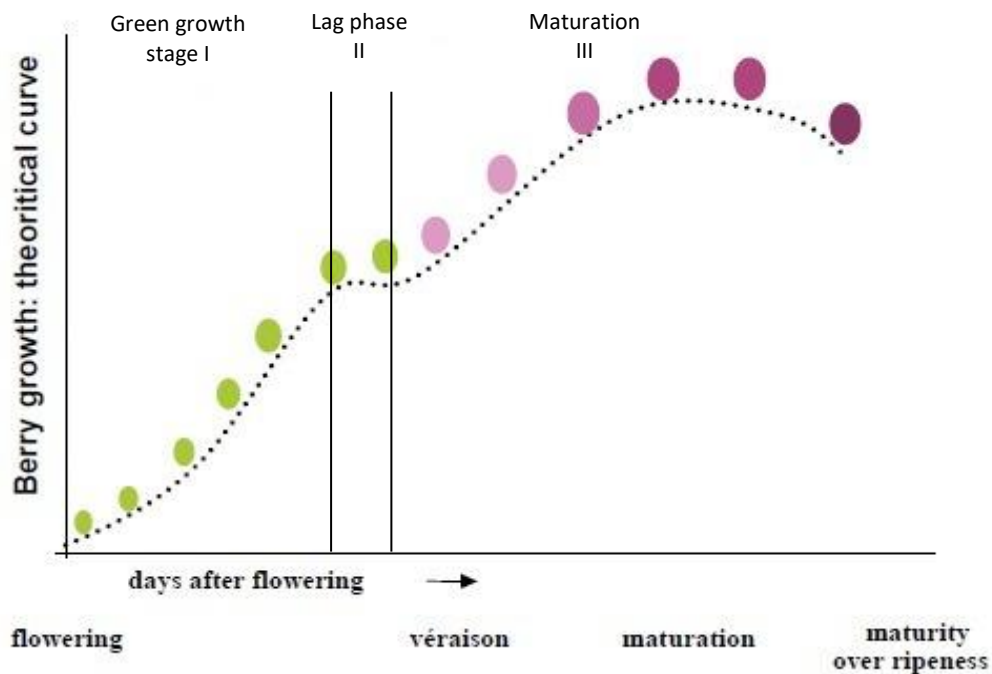


Figure 5 – Double sigmoid growth curve of berry development showing different phases of growth. Adapted source: Deloire, 2010.

**Phase I.** The fruit set starts with flowering and ends after about 60 days: it is a period of intense growth of the berry and seed embryos (Kennedy, 2002; Conde *et al.*, 2007), promoted by processes of cell division and distension. In the end, the fruit will reach 50-60% of its final weight (Palliotti *et al.*, 2018). Chlorophyll is the dominant pigment, the grapes show strong metabolic activity, characterized by intense levels of respiration and a rapid accumulation of acids (Ribéreau-Gayon *et al.*, 1997). Indeed, the most abundant solutes are tartaric and malic acids: the former is stored in the outer berry layers and accumulated in the initial growth steps; the latter is in the pulp just before *véraison*.

Hydroxycinnamic acids accumulates during this phase, important as precursors of volatile phenols (Romeyer *et al.*, 1983). Tannins (including monomeric catechins) increase as well, located more in skin and seed tissues and almost absent in the pulp. These compounds will

provide astringency, bitterness, and colour stability (Kennedy, 2002). Lastly, even amino acids are deposited during this first evolution phase (Stines *et al.*, 2000).

**Phase II.** Named 'lag phase', its length is strictly cultivar-dependent and there is little or no increase in the berry dimension, which only begins to soften (Palliotti *et al.*, 2018), changes colour due to the release of chlorophyll and accumulates high levels of sugars (Robinson and Davies, 2000).

**Phase III.** Corresponding to the proper ripening, it is a complex phase that ends when the compositional framework is ideal for the expected use (Table 1). It begins at the time of the *véraison* and lasts about 45 days. An additional cellular growth enables a double of the size of the berry, as well as a softening of the berry itself. Whereas, malic acid is used as a source of energy, that is why its amount decreases more than tartaric acid, whose portion remains constant throughout ripening typically (Conde *et al.*, 2007). The seed tannins decrease due to oxidation since the tannins become part of the seed membranes (Kennedy *et al.*, 2000). Instead, the skin tannins decrease or change, increasing their polymerization degree (Kennedy, 2002). The most important biochemical phenomenon of ripening is the increase in sugars that takes place after *véraison*: the sucrose synthesized by photosynthesis is introduced into the berry and is hydrolyzed into its simple sugars, glucose, and fructose (Robinson and Davies, 2000). Other important aspects are the synthesis of anthocyanin pigments in red grapes, and the accumulation of volatile aromatic components (Kennedy, 2002). The major components of the cluster and their biochemical composition at maturity, are shown in Table 1.

Table 1 – Main components of the cluster at maturity (stem, skin, pulp, and seeds) and their biochemical composition. Adapted source: Palliotti et al., 2018; Blackford et al., 2021.

Stems 2–6% FW	Skin 6–12% FW	Seeds 2–6% FW	Pulp 75–90% FW
<b>Average cluster components</b>			
Stems		4% FW	
Skins and seeds		14% FW	
Pulp		82% FW	
<b>Stem components</b>			
Water		60–80% DW	
Cellulose		15–40% DW	
Tannins		1–3.5% DW	
Ashes		2–2.5% DW	
Nitrogen substances		1–1.5% DW	
Acids		1–1.5% DW	
Sugars		<1% DW	
<b>Skin components</b>			
Water		60–80% v/v	
Cellulose		18–20% v/v	
Polyphenols		0.5–3.5 v/v	
Ashes		1.5–2% v/v	
Nitrogen substances		1.5–2% v/v	
Acids		0.8–1% v/v	
<b>Seeds components</b>			
Water		30–40% DW	
Cellulose		30–40% DW	
Emicellulose		10–15% DW	
Lignin		13–47% DW	
Fat		6–12% DW	
Tannins		3–6% DW	
Proteins		5–6% DW	
Ashes		1–3% DW	

Pulp components	
Sugars	150–250 g/L
Acids	8–10 g/L
Gum	0.1–6 g/L
Ashes	2–5.5 g/L
Phenolic compounds	0.2–2 g/L
Nitrogen substances	0.1–1 g/L
Pectic substances	0.1–1 g/L

## 2.5 Berry weight

Berries develop in size according to a double sigmoid growth pattern. Growth is a multi-step process in which both dry (cell wall, membrane, solutes, etc.) and fresh weight grow. The cell sap collects solutes, generating a water potential gradient down which water flows, allowing it to enter the cells. Solute are either translocated into the fruit or generated from translocated solutes in berry cells. The overall quantity of solute in the berry is dynamic, rising in general as the fruit grows and develops. Water inflow must surpass solute influx in order for the latter to be diluted. Plant tissues, particularly fleshy fruit (as berries) more than others (as woody stems), clearly operate as water capacitors on a daily basis. But after *véraison*, the grape berry's ability to retain solvent water is drastically diminished (Greenspan *et al.*, 1996; Matthews and Nuzzo, 2007).

Within the *Vitis* genus, berry weight reveals a lot of genetic variabilities (Boursiquot *et al.*, 1995; Shellie, 2007; Houel *et al.*, 2010). Berry weight varies both across different cultivars and clones of the same cultivar. Grenache, Mourvèdre, Ugni Blanc, and their reference wildtype clones, for example, have been found to have a difference in berry weight of 49 to 90%. Cell division and cell expansion, which affect cell number and/or cell volume, respectively, can induce variations in berry weight (Fernandez *et al.*, 2006). Intrinsic and extrinsic factors, that affect berry weight through cell division and/or expansion, include genotype (Boursiquot *et al.*, 1995; Fernandez *et al.*, 2006; Houel *et al.*, 2010; Mejia *et al.*, 2011), seed number within a berry (Cawthon and Morris, 1982; Walker *et al.*, 2005), source-sink ratio, and environmental parameters such as water supply (Ojeda *et al.*, 2001; Van Leeuwen *et al.*, 2009; Shellie, 2010).

The seed number is a significant factor of berry weight, especially inside a grape cluster (Cawthon and Morris, 1982; May, 2000; Walker *et al.*, 2005). Following many authors, result in a connection between berry and seed weight (Ebadi *et al.*, 1996; Friend *et al.*, 2009; Gray and Coombe, 2009). Thompson seedless berries had a variation of 25 to 30% of berry weight

(Kasimatis *et al.*, 1975), which was equivalent to Merlot (Shellie, 2010); while Syrah's weight variation is between 41 and 53% during its berry development (Gray and Coombe, 2009).

The skin/pulp ratio and the number of solutes extracted from skins may be affected by differences in berry size (Roby *et al.*, 2004; Roby and Matthews, 2004; Walker *et al.*, 2005; Matthews and Kriedemann, 2006; Matthews and Nuzzo, 2007). However, in well-watered grapevines, the fresh weight skin/pulp ratio was independent of berry size when comparing different irrigation regimes (Roby and Matthews, 2004). Only when berry flesh weight was impacted in vines treated to water stress during *véraison* did the skin/pulp ratio change (Barbagallo *et al.*, 2011). According to a study on cv. Merlot, water stress limited berry growth in a cluster uniformly, ruling out, or at least reducing, its function in determining intracluster variation in berry weight (Shellie, 2010).

Berry weight fluctuation may be caused by the length of the flowering period and the size of the inflorescence, both within and between clusters (Poni and Libelli, 2008). Furthermore, cultural practices and environmental conditions that occur during floral differentiation, as well as during and after blooming, can increase or alleviate berry size variations (Gray and Coombe, 2009).

Finally, the fresh weight of berries within a cluster is determined by their location. Berries near the distal end are substantially thinner than those in the proximal part (Tarter and Keuter, 2005; Pagay and Cheng, 2010). This aspect may be impacted by sink competition, also because the influence of source-sink connections on berry weight is well established (Kliewer, 1970; Ollat and Gaudillere, 1998). During berry growth, supply restriction usually results in a drop in individual berry weight. However, its impact on the amount of intracluster variability requires more research. In addition, cluster variability is generally higher within than between clusters or vines at maturity (Sato *et al.*, 2000; Pagay and Cheng, 2010). Factors causing within-cluster variation are discussed in the following sections.

## 2.6 Evolution of main grape metabolites during maturation

### 2.6.1 Sugars

The sugars composition of berries has a role in wine quality, since fermenting into ethanol during winemaking, its measurement allows the determination of the potential alcohol content in wines (Conde *et al.*, 2007). During alcoholic fermentation, an average of 17g/L sugar is required to obtain 1° of alcohol (Deloire, 2010). However, it should be considered that sugars accumulation is not strictly correlated to grape maturity (Rolle *et al.* 2022).

The sugar is derived from leaf photosynthesis and is transported into the berry via phloem via sugar-transporting proteins. In the initial phase of the ripening process, most of the sugar is metabolized, thus its content will be relatively low (Figure 6). At *véraison*, the sugars are hydrolyzed into glucose and fructose, accumulate in the vacuoles of flesh cells, and continue in this trend until the end of ripening (Conde *et al.*, 2007).

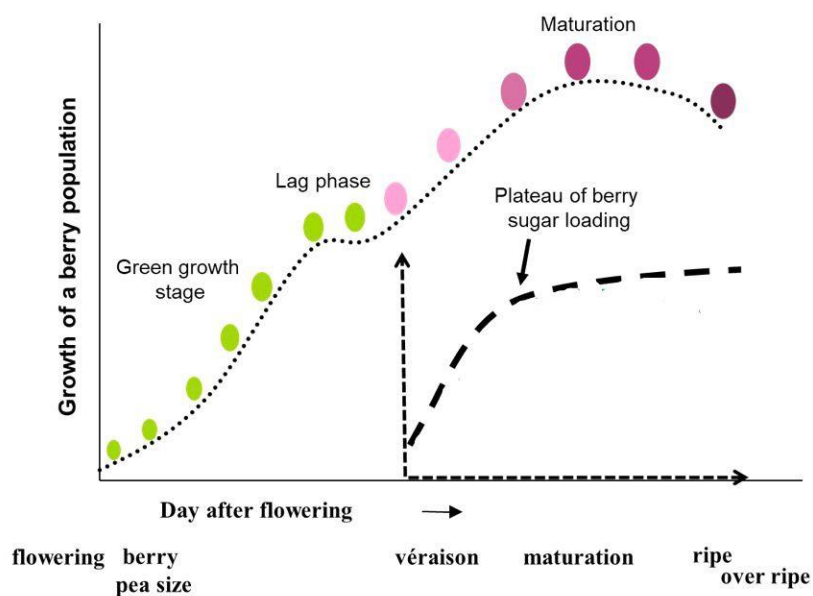


Figure 6 – Evolution of the sugar accumulation (theoretical quantity) during maturation. Adapted source: Deloire *et al.*, 2020.

Its total concentration ranged from 18.7 to 27 Brix at maturity among 78 cultivars, including table grapes and red and white wine grape cultivars (Kliewer, 1967b). Among 26 North American and Middle Eastern species, Kliewer (1967a) reported a wider variation at maturity, ranging from 13.7 Brix (*V. champinii*) to 31.5 Brix (*V. riparia* of Wyoming). A total of 98 grape cultivars were studied for two consecutive years, glucose and fructose were the predominant sugars and scaled from 45.86 to 122.89 mg/mL, and 47.64 to 131.04 mg/mL, respectively (Liu *et al.*, 2006). The glucose/fructose ratio is reported to fluctuate from 0.47 to 1.12, with only two

species containing more glucose than fructose (*V. champinii* and *V. doaniana*). Most *Vitis vinifera* vines have a glucose/fructose ratio of 1 at maturity (Kliewer, 1967b). Grape berry sugar composition and concentration change during ripening: although sugar composition is mainly determined by genotype (Shiraishi, 2000; Liu *et al.*, 2006, 2007; Shiraishi *et al.*, 2010), sugar concentration varies with berry development (Coombe, 1992), environment, and cultural management (Nuzzo and Matthews, 2006; Sadras *et al.*, 2007; Keller *et al.*, 2008; Cosme *et al.*, 2016).

An important environmental factor that affects grape sugar accumulation is the temperature (Hawker, 1982; De Orduna, 2010). For temperatures above 25°C, net photosynthesis decreases even at constant sun exposure (Huglin, 1986). In addition, for temperatures above 30°C, several authors have reported a reduction of berry size and weight, and metabolic processes and sugar accumulation may completely stop (Kriedemann and Smart, 1971; Coombe, 1986). Higher temperatures (30°C) may lead to higher suspended solid concentrations, but Brix levels higher are likely due to concentration by evaporation (Keller, 2010a, b). Also, in the last years, the alcohol content of wines tended to increase, due to climate change (García-Martín *et al.*, 2010).

Irrigation is one of the viticultural practices that influence vine and grape metabolism: the response of berry sugar concentration to water supply depends on the intensity and timing of water stress applied to vines (Roby *et al.*, 2004; Sadras and McCarthy, 2007), exhibiting increases (Van Leeuwen and Seguin, 1994), decreases (Esteban *et al.*, 1999; Santesteban and Royo, 2006), or no change (Sivilotti *et al.*, 2005). Water stress can affect sugar outflow (Wang *et al.*, 2003), sugar metabolism (Kobashi *et al.*, 2000), or berry water requirements (Van Leeuwen and Seguin, 1994; Keller *et al.*, 2006), with a resulting change in sugar concentration. Water stress during the period from fruit set to *véraison* reduces fruit size (McCarthy, 1997). On the other hand, late-season water restriction may reduce fruit cell enlargement and water accumulation (Smart and Coombe, 1983), and in general, has a less deleterious impact on final berry size than early season water stress (McCarthy, 1997). Irrigation might also indirectly affect berry quality because of increased and prolonged vegetative growth. Increased vegetative growth could damage cluster microclimate as well, particularly fruit light exposure (Smart *et al.*, 1985). On the opposite side, excess irrigation has led to a delay in obtaining the desirable sugar levels (Bravdo *et al.*, 1984) due to dilution. Irrigation allows increasing yields (Williams and Matthews, 1990), though a moderate water deficit is used to improve wine composition (Jackson and Lombard, 1993), so as to reduce canopy vigor, increase fruit exposure to light, and reduce berry growth to avoid dilution (McCarthy *et al.*, 2002).

Various research studies analyzed the water deficit effects toward sugar concentration in grape berries. For example, no significant alterations were observed in the sugar concentration of Merlot grapes under a water deficit when compared to the irrigated control (Shellie, 2014). As reported by Alves *et al.* (2012), the study results showed that non-irrigated grapevines experienced severe water stress and consequently lower photosynthetic rates. By contrast, moderate levels of irrigation had a positive impact on yield and berry quality. However, the metabolic responses of grapes to water deficit varied with the cultivar and fruit pigmentation. Water deficit increased sugar in Cabernet Sauvignon, but not Chardonnay berries (Deluc *et al.*, 2009). The concentration of glucose and fructose in Tempranillo grapes was significantly higher in the irrigated vines than in the non-irrigated, especially at the ripening end (Esteban *et al.*, 1999).

### 2.6.2 Organic acids

Organic acids do not exceed more than 1% of the total juice weight (Muñoz-Robredo *et al.*, 2011), tartaric and malic acid are usually the most relevant acids, accounting for 70-90 % of total acids (Peynaud and Ribéreau-Gayon, 1971; Winkler *et al.*, 1974). Other organic acids found in a variable, but always low concentrations are citric, acetic, lactic, succinic acids (Fuleki *et al.*, 1993). Tartaric acid is responsible for wine biological stability, while malic acid confers the typical “green tones” to wines (Bakker and Clarke, 2011). The quantitative ratio between malate and tartrate is depending on the grape variety. Cultivars such as Carignane, Malbec, and Pinot Noir have a relatively high malate content at maturity, but the majority of varieties, among them Chasselas, Semillon, and Thompson Seedless, predominantly store tartrate (Amerine, 1956; Winkler *et al.*, 1974). Specifically, it was determined that Thompson Seedless shows the lowest tartaric/malic acid ratio of 1:19 (Muñoz-Robredo *et al.*, 2011).

When analyzed in detail, both acids are synthesized and degraded due to different chemical pathways. The synthesis of tartaric acid is limited from flowering to *véraison* (Saito and Kasai, 1982; Coombe and McCarthy, 2000; DeBolt *et al.*, 2006); during maturation, the tartaric acid changes from acid to salt form and, simultaneously, dilute at a rate depending on variety and berry weight increment (Zoecklein B., 1988; García-Escudero *et al.*, 1995). The malic acid content varies considerably between berry growth and ripening. Six to nine weeks after flowering, the level of malic acid goes down and its degradation is related to its use as an energy source for respiration (Conde *et al.*, 2007).

In a nutshell, these organic acids are synthesized in the grape berry, their accumulation culminates at *véraison* and decreases during ripening (Eskin *et al.*, 1971) – Figure 7.

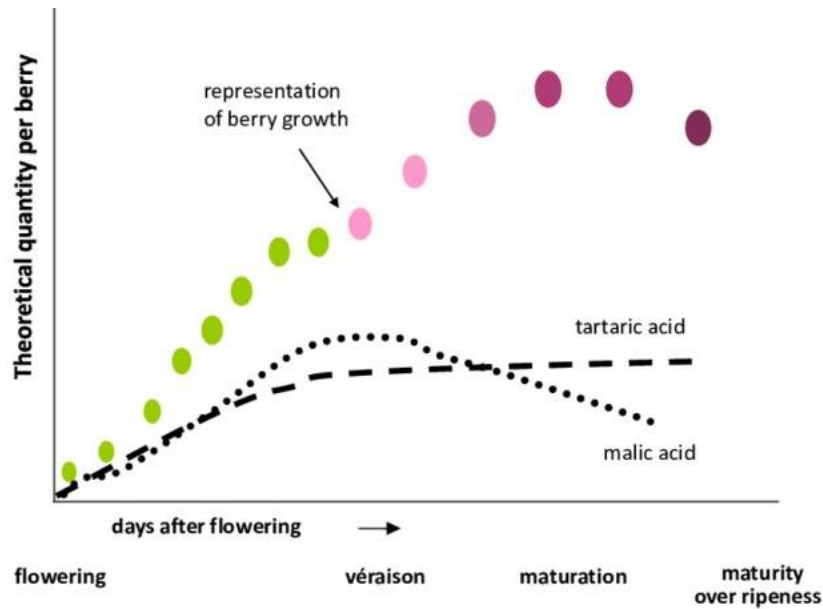


Figure 7 – Evolution of the organic acids (theoretical quantity) during maturation. Source: Deloire, 2010.

There is genetic diversity for organic acid concentration in grape berries (Kliewer, 1965, 1967a; Liu *et al.*, 2006; Shiraishi *et al.*, 2010). In the ripe berry, tartaric acid usually accounts for 5 to 40% of total tartrate and malic for 30 to 70% of total malate (Kliewer *et al.*, 1967). For 78 table and wine cultivars, a concentration range at maturity of 4 to 9.4 g/L for tartrate and 1.5 to 6.8 g/L for malate has been reported (Kliewer *et al.*, 1967). On a set of 98 varieties, including interspecific hybrids, an interval of 1.6 to 9.1 g/L for tartrate and 0.36 to 7.06 g/L for malate was recorded (Liu *et al.*, 2006). Wine grapes Cabernet Sauvignon and table grapes Gora Chirine differed in the acidity of their berry juice: at harvest, Gora Chirine berry juice had a pH of 5.2 and a titratable acidity (TA) of 32 meq/L, whereas Cabernet Sauvignon berry juice had a pH of 3.2 and a TA of 150 meq/L (Diakou *et al.*, 1997). The tartrate/malate ratio also shows wide genetic variability (Shiraishi, 1995): this ratio varied from 0.64 (Pinot St. George) to 3.41 (Palomino) (Kliewer, 1967a).

Deficit irrigation reduces acidity (Freeman *et al.*, 1980; Ruhl and Alleweldt, 1983; Climaco and Chaves, 1987), this phenomenon is intensified in unirrigated vines, as malic acid loss increases with water deficit and warm temperatures (Hepner and Bravdo, 1985). Contrary, in over-watered vines, the higher organic acid content of the fruit may be caused either by a delay in fruit ripening or by excessive growth of leaves shading the berries (Smart and Coombe, 1983; Jackson and Lombard, 1993). In grapes, especially if grown in dry climatic regions, irrigation can increase titratable acidity and organic acid content (Esteban *et al.*, 1999; des Gachons *et al.*, 2005; De La Hera Orts *et al.*, 2005). Another important factor is temperature: continuous heat exposure leads to a decrease in acidity, mainly due to accelerated oxidative

degradation of malic acid (Kanellis and Roubelakis-Angelakis, 1993). Instead, tartaric acid is resistant to combustion at high temperatures (Calo *et al.*, 1995).

Small differences in pH reflect large changes in titratable acidity (Iland, 1987). Esteban *et al.* (2002) observed that pH increased linearly with berry ripening while titratable acidity decreased. In the beginning, when titratable acidity is high, its decrease did not result in a consistent change in pH; as maturation progressed, the change in pH became greater (De La Hera Orts *et al.*, 2005). The decrease in titratable acidity during ripening is normally attributed to falling concentrations of malic acid (Calo *et al.*, 1995), due to its loss through respiration (Hepner e Bravdo, 1985), while the decrease in tartaric acid was not as intense, as also observed by Iacono *et al.* (1995). Titratable acidity and pH were only slightly affected by irrigation (Nadal and Arola, 1995; De La Hera Orts *et al.*, 2005).

### 2.6.3 Phenolic compounds

Colour, astringency, bitterness, and aroma are all grape sensorial qualities where phenolic compounds play an essential influence. Figure 8 schematizes the localization of the main polyphenols in the bunch.

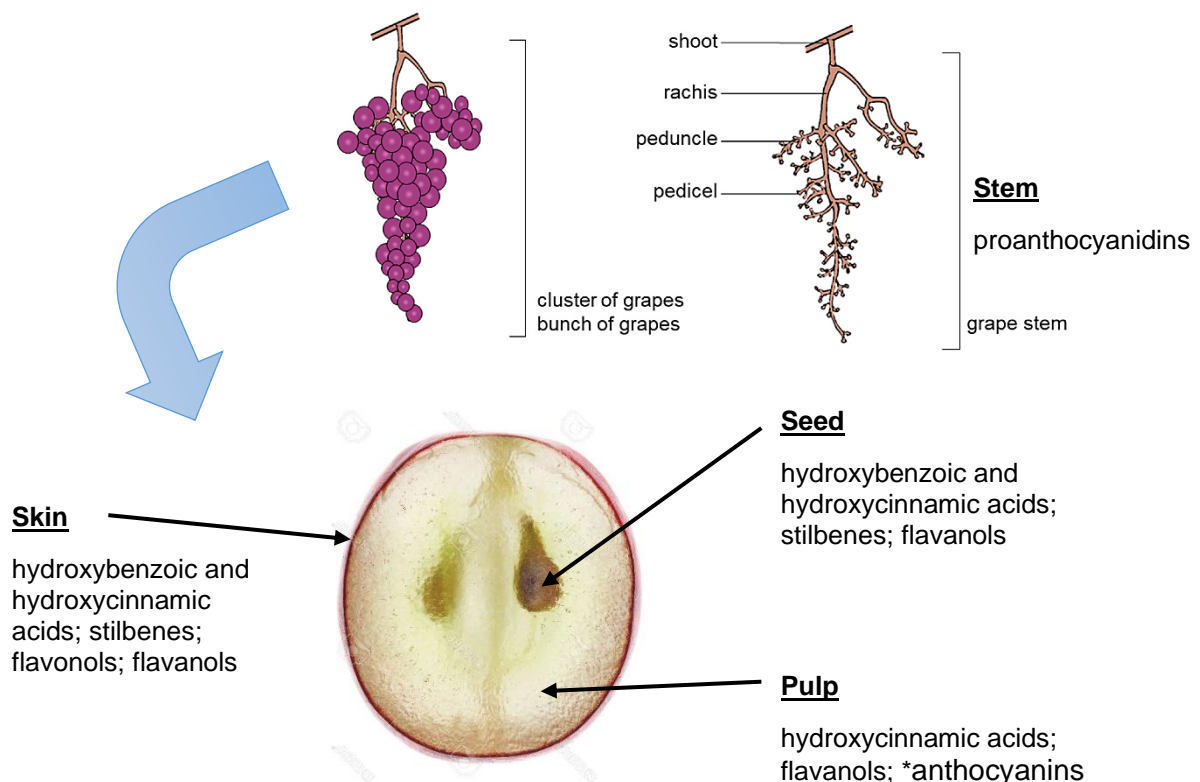


Figure 8 – Distribution of the main phenolics compounds in a cluster and ripe grape berry. \*Anthocyanins are synthesized in the so-called 'teinturier' varieties. Adapted source: Blackford *et al.*, 2021.

Total extractable phenols are approximately 60-70% in the seeds, 30-35% in the skin, and 10% or less in the pulp (Guerrero *et al.*, 2013; Sirohi *et al.*, 2020). Due to the presence of anthocyanins in the skins, red grapes have a higher total phenolic concentration than white grapes (Waterhouse *et al.*, 2016). Arapitsas *et al.* (2015) first reported anthocyanins in the skins of international white grape varieties (Chardonnay, Sauvignon Blanc, and Riesling). Polyphenols are mainly found in the skin and seeds of the berries, while their concentration in juice and pulp is lesser (Waterhouse *et al.*, 2016). Exceptions are reported in 'teinturier' varieties, which accumulate anthocyanins in both skin and pulp (Cabezas *et al.*, 2003; Santiago *et al.*, 2008). Indeed, the pink-colored wine from the white grape Síria was due to the presence of anthocyanins (mainly malvidin-3-O-glucoside) in the skin and pulp of the grape (Andrea-Silva *et al.*, 2014). These compounds can be classified into two groups: flavonoids and non-flavonoids. The flavonoids include flavonols, flavanols, anthocyanins, and proanthocyanidins – or condensed tannins (Schwinn and Davies, 2004; Kitamura, 2006). The non-flavonoids incorporate hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes (Jordão and Correia, 2012; Costa *et al.*, 2015a).

The biosynthesis of phenolic compounds begins with the aromatic amino acid phenylalanine, a product of the shikimate pathway. The initial precursors are erythrose-phosphate and phosphoenol-pyruvate (Conde *et al.*, 2007). This pathway is responsible for the production of phenylalanine, which is subsequently converted to cinnamic acid (Teixeira *et al.*, 2013). This acid undergoes several transformations, leading to the formation of precursors for more complex phenolic compounds.

The quantity of total phenols increases in association with the berry development: hydroxycinnamates and proanthocyanidins are already synthesized in the 'green growth' phase, while anthocyanins are formed during the ripening phase (Figure 9).

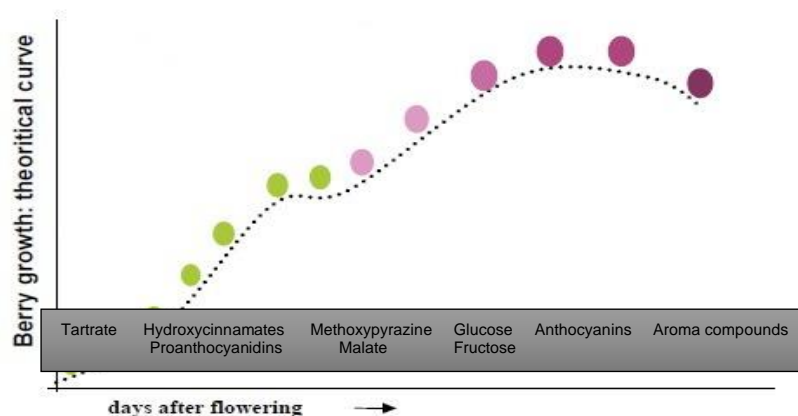


Figure 9 – Representation of the main compounds in berry development during ripening. Adapted source: Deloire, 2010.

Table 2 – Phenolic compounds produced and accumulated in the grape berry. Source: Teixeira et al., 2013.

Compound	Level of synthesis			Location	Berry phenological scale			
	Skin	Pulp	Seed		Blooming	Green stage	Véraison	Ripening
<i>Flavonoids</i>								
Flavonols	++	-	-	Dermal cell vacuoles of the skin tissue and cell wall of skin and seeds.	++	+	+++	++
Flavan-3-ols	++	+	+++	Specific vacuoles of hypodermal skin cells and seed coat soft prequima.	+	++	+++	++
Anthocyanins	+++	- *	-	Cell layers below the epidermis; storage confined to the vacuoles and cytoplasmic vesicles named anthocyanoplasts.	-	-	+	+++
<i>Non-flavonoids</i>								
Hydroxycinnamic acids	++	+++	++	Hypodermal cells and placental cells of the pulp; primarily in the vacuoles of mesocarp cells.	+++	+++	+	+
Hydroxybenzoic acids	+	-	++	Berry skin and seeds.				
Stilbenes	+++	+	++	Berry skin and seeds	-	+	++	+++

(+++) Very abundant compound to (-) absent; \* 'Teinturier' contains anthocyanins also in mesocarp cells.

Table 2 details where the flavonoids and non-flavonoids are found in the berry tissues, and how they accumulate during physiological stages (blooming, green stage, *véraison*, and ripening). The evolution of each phenolic compound will be described in the following paragraph.

In the study conducted by Sabir *et al.* (2010) on 25-year-old red and white cultivars, the total phenol content accelerated from *véraison* to reach the range of 2253 (Alphonse Lavallée) and 2847 (Muscat of Alexandria) mg/L at the ripe stage. The average concentration of total phenolic compounds in wine grapes is about 2178.8 mg/g gallic acid equivalent in seeds, 374.6 mg/g gallic acid equivalent in skins, and 23.8 mg/g gallic acid equivalent in pulps (Pastrana-Bonilla *et al.*, 2003). In addition, some researchers have identified significant levels of total and individual phenolic compounds in table grapes as well (Eyduran *et al.*, 2015).

As recorded by several works, phenolic content relies on the grape cultivar (Costa *et al.*, 2015a, 2015b), but also on geological and soil conditions (Ubalde *et al.*, 2010), vineyard altitude (Mateus *et al.*, 2002), sunlight exposition (Bergqvist *et al.*, 2001), climate (Bergqvist *et al.*, 2001; Downey *et al.*, 2006), and solar radiation (Madeira, 2011) of a region. Lastly, additional factors like cultivation techniques (Downey *et al.*, 2006), disease exposure (Bruno and Sparapano, 2007), and grape maturity (Jordão *et al.*, 2001; Obreque-Slier *et al.*, 2012) can influence the phenolic content of grapes, either directly or indirectly.

#### 2.6.3.1 Flavonoids

The most relevant class of flavonoids are anthocyanins, responsible for grapes colour (Teixeira *et al.*, 2013) and, consequently, influence the colour intensity and tone of red wines (Boss *et al.*, 1996). These pigments are mainly distributed in the skin and, as mentioned before, in the pulp itself regarding the varieties 'teinturier'. The basic structure of anthocyanidin is the flavylium cation, which includes two benzene rings linked by a cationic oxygenated heterocycle, derived from the 2-phenyl-benzopyrylium nucleus (Jordão *et al.*, 2012). *Vitis vinifera* anthocyanins are composed of five aglycones / anthocyanidins: malvidin, cyanidin, delphinidin, peonidin, and petunidin, which vary according to the number and position of hydroxyl groups and degree of methylation (Goufo *et al.*, 2020).

The accumulation of anthocyanin components begins from *véraison* (Table 2), or even two/three weeks before the colour change (Darné, 1988). Some authors (Pirie and Mullins, 1977; Budin, 1983; Darné, 1988; González-SanJosé *et al.*, 1990) have previously documented that during berry ripening, anthocyanins evolve in a three-step process: at first showing a slow increase, followed by a rapid accumulation, then a stabilizing (Table 2) before a decrease just before harvest and during over-ripening stage (Ryan and Revilla, 2003; Fournand *et al.*, 2006).

Figure 10 provides an example of the evolution of skin anthocyanin glucosides during the maturity stages of two Portuguese red grapes.

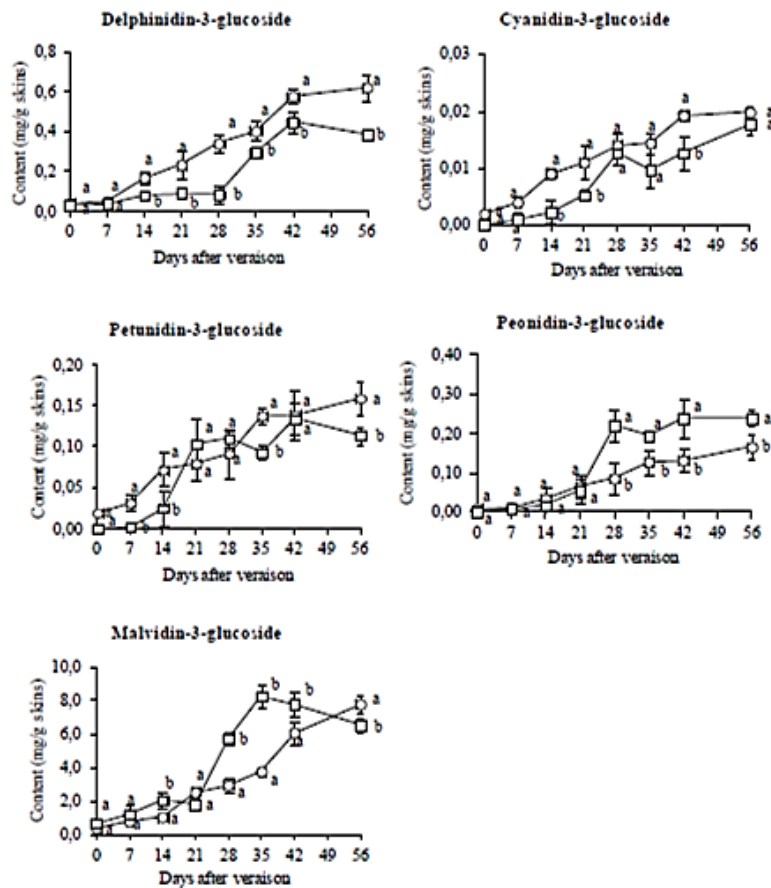


Figure 10 – Evolution of skin anthocyanin glucoside derivatives during the maturation of two red grapes (-□-Touriga Nacional and -o-Tinta Roriz). Source: Jordão and Correia, 2012.

Several aspects impact the anthocyanins amount in grapes, such as cultivars (Mazza and Francis, 1995), stage of ripeness (Fournand *et al.*, 2006; Holt *et al.*, 2010), environmental and viticultural practices as light exposure, temperature, availability of water and nitrogen (Downey *et al.*, 2006; He *et al.*, 2010; Kappel, 2010; Ollé *et al.* 2011).

The most plentiful class in the grape berry is the flavanols (Singleton, 1992). Located essentially in the seeds and skins (Sun *et al.*, 2001; Table 2), they are particularly influential in red wines (Kennedy *et al.*, 2006; Conde *et al.*, 2007). This phenolic group includes both monomers and polymers. The monomers are (+) catechin, (-) epicatechin, (+) galocatechin, (-) epigallocatechin, and catechin-3-O-gallate. The polymers are usually known as proanthocyanidins or condensed tannins (Teixeira *et al.*, 2013), and it has been confirmed that proanthocyanidins in grapes and wine can have a positive effect on human health, due to their oxygen radical-scavenging ability (Ricardo-da-Silva *et al.*, 1991; Frankel *et al.*, 1992). Climatic

and geographical factors, cultural practices, ripening stage, and, consequently, vine cultivars can affect the flavonols and proanthocyanidins quantity (Pérez-Magariño and González-SanJosé, 2002).

The maximum expression of the genes involved in flavanol synthesis is around flowering (Bogs *et al.*, 2006), and proanthocyanidins content reaches its maximum just before véraison (Kennedy and Jones, 2001; Downey *et al.*, 2003; Table 2). From véraison until harvest, there was a decline in its concentration, as specified by several research works (Downey *et al.*, 2003; del Rio and Kennedy, 2006; Verries *et al.*, 2008). Anyway, the decreased pattern of proanthocyanidins might be influenced by the polymerization (Downey *et al.*, 2003) and/or hydrogen bonding to cell walls (Hanlin *et al.*, 2010), which can lead to reduced extraction rates (Rustioni *et al.*, 2014).

Figure 11 provides an example of the development of the various seed and skin proanthocyanidin fractions throughout the ripening of grapes.

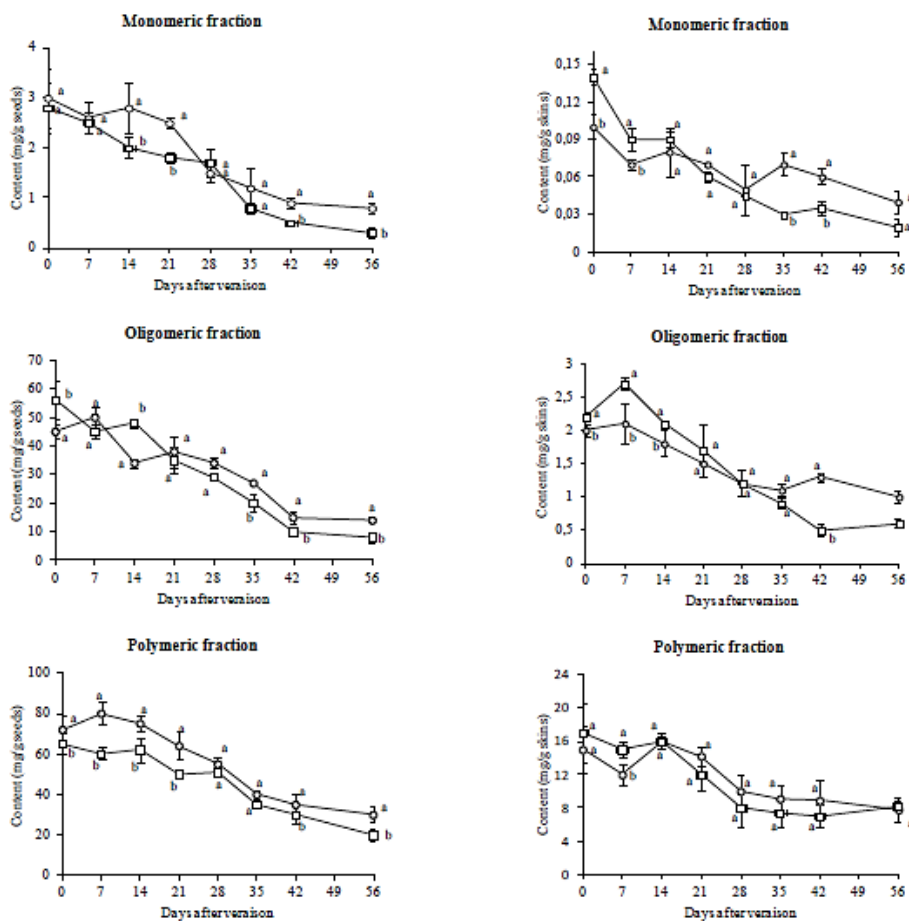


Figure 11 – Evolution of different seed and skin proanthocyanidin fractions during the ripening of two red grape varieties (-□-Touriga Nacional and -o-Tinta Roriz) from véraison to harvest. Source: Jordão and Correia, 2012.

Flavonols are mainly found in the skins of both red and white grapes (Table 2). The main reported in grape berries are kaempferol, quercetin, and myricetin (Makris *et al.*, 2006). Through copigmentation, flavonols help to stabilize the flavylium form of anthocyanins in young red wines (Boulton, 2001). Its biosynthetic pathway is the same that also produces anthocyanins and condensed tannins (Stafford, 1990; Darné, 1993). High concentrations of flavonols in grapes were found at blooming, followed by a decrease with the grape size growth. Subsequently, a significant level of flavonols biosynthesis was observed during *véraison* (Downey *et al.*, 2003; Table 2).

Localized mainly in the skin, flavonols have a photo-protective function against UV radiation (Haselgrove *et al.*, 2000) and their concentration is directly correlated with sunlight (Downey *et al.*, 2004). Indeed, wines from grapes cultivated in sunny areas have greater flavonol contents: in Pinot Noir wines made from sun-exposed clusters, the amount of quercetin glycosides is 10 times higher than those in wines made from shaded ones (Price *et al.*, 1995). Moreover, wines with higher concentrations of flavonols are made with thick skinned grapes such as Cabernet Sauvignon (high skin/volume ratio), rather than thin skinned grapes, such as Grenache (low skin/volume ratio) (McDonald *et al.*, 1998).

#### 2.6.3.2 Non-flavonoids

In grapevines, it is possible to find non-flavonoid compounds as well, such as hydroxycinnamic acids. They have a C<sub>6</sub>-C<sub>3</sub> structure and can be detected in all berry tissues (Easterling *et al.*, 2000; Braidot *et al.*, 2008), but they accumulate largely in pulp (Singleton *et al.* 1986; Table 2). The predominant acids are caffeic acid, ferulic acid, and *p*-coumaric acid. They are usually found esterified with tartaric acid or in heterosidic form (Ribéreau-Gayon *et al.*, 2006). In the green stage, the synthesis and accumulation of hydroxycinnamates begin, their concentration increases until *véraison*, and then their content per berry remains almost constant (Table 2). A number of papers have studied the changes in hydroxycinnamic acids in relation to grape variety and maturity degree (Ribéreau-Gayon, 1972; Nagel *et al.*, 1979).

Hydroxybenzoic acids are commonly found in seeds (Table 2), although to a much lesser extent than other phenolic compounds. They are found in the C<sub>6</sub>-C<sub>1</sub> structure and include gallic acid, ellagic acid, gentisic acid, and salicylic acid (Vanhoenacker *et al.*, 2001; Pozo-Bayón *et al.*, 2003; Ali *et al.*, 2010). Riesling wine from Germany contains other benzoic acids such as protocatechuic, vanillic, and syringic (Baderschneider and Winterhalter, 2001).

Finally, stilbenes are a non-flavonoid chemical class that has garnered much attention, despite being found in small amounts in grape and wine (Conde *et al.*, 2007). The synthesis of these compounds occurs primarily in the skin and its concentration increases throughout the grape

berry development (Table 2). Trans-resveratrol has the simplest molecular structure and is utilized as a precursor for other phenolic compounds (Teixeira *et al.*, 2013). Considering its potential therapeutic benefits, especially against heart disease and cancer, resveratrol is the most studied stilbene-type compound (Wolter and Stein, 2002; Borra *et al.*, 2005). Several resveratrol derivatives have been implicated in plant defense systems against biotic and abiotic stress (Jeandet *et al.*, 2002; Richter *et al.*, 2006). In particular, fungal infection – principally *Botrytis cinerea* (Ebel, 1986; Adrian *et al.*, 1997) – and UV irradiation (Douillet-Breuil *et al.*, 1999; Adrian *et al.*, 2000) rapidly induce stilbenes synthesis in *Vitis vinifera*.

#### 2.6.4 Nitrogen, minerals and other minor compounds

In grape and must, nitrogen is found in inorganic (ammonium salt) and organic (amino acids, peptides, proteins) forms. Total nitrogen in the must can vary from 100 to 1200 mg/L, (Conde *et al.*, 2007); its content is lower for red grape wines than white grape wine (Verdenal *et al.*, 2021). Table 3 provides a list of amino acids commonly found in the grape berries and juice at harvest: the most abundant amino acids are arginine and proline (Garde-Cerdán *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2018); glutamine, glutamic acid, serine, threonine, and alanine are found in considerable quantities.

*Table 3 – The identity and concentration of amino acids found in the whole grape and/or juice at harvest. Source: Bell and Henschke, 2005.*

Amino acid	Concentration range reported in the literature (mg/L)
Alanine	10 – 227
Arginine	20 – 2322
Asparagine	1 – 171
Aspartic acid	10 – 138
Citrulline	0.1 – 83
Cysteine	1 – 8.2
Glutamine	9 – 4499
Glutamic acid	27 – 454
Glycine	1 – 20
Histidine	5 – 197
Isoleucine	1 – 117
Leucine	2 – 160
Lysine	0.7 – 45
Methionine	1 – 33
Ornithine	0.1 – 27.2
Phenylalanine	2.8 – 138

Proline	9 – 2257
Serine	13 – 330
Threonine	9 – 284
Tryptophan	0.2 – 11
Tyrosine	2 – 33
Valine	7 – 116

Amino acids and ammonium ions are key growth factors for yeast and bacteria during alcoholic fermentation and malolactic fermentation (Batch *et al.*, 2011). Low nitrogen levels in vines can result in lower Yeast Available Nitrogen (YAN) levels, slowing fermentation and increasing the risk of formation of hydrogen sulfide and thiol-containing chemicals. (Malherbe *et al.*, 2007).

The concentration and total nitrogen content of grapes and juice increase during ripening (Figure 12a). Specifically, the concentration of total amino acids in grapes and juice increases from *véraison* to harvest (Figure 12b). Instead, the concentration of ammonium in grape berries decreases during ripening (Figure 12c). Relevant proline accumulation in the berry begins after *véraison*, and arginine accumulation begins before *véraison* (Bell and Henschke, 2005; reference there in; Figure 12d). Cabernet Sauvignon has been demonstrated to be high proline-accumulating, while cultivars with high arginine-accumulating are Gewürtztraminer and Muscat Gordo (Stines *et al.*, 2000).

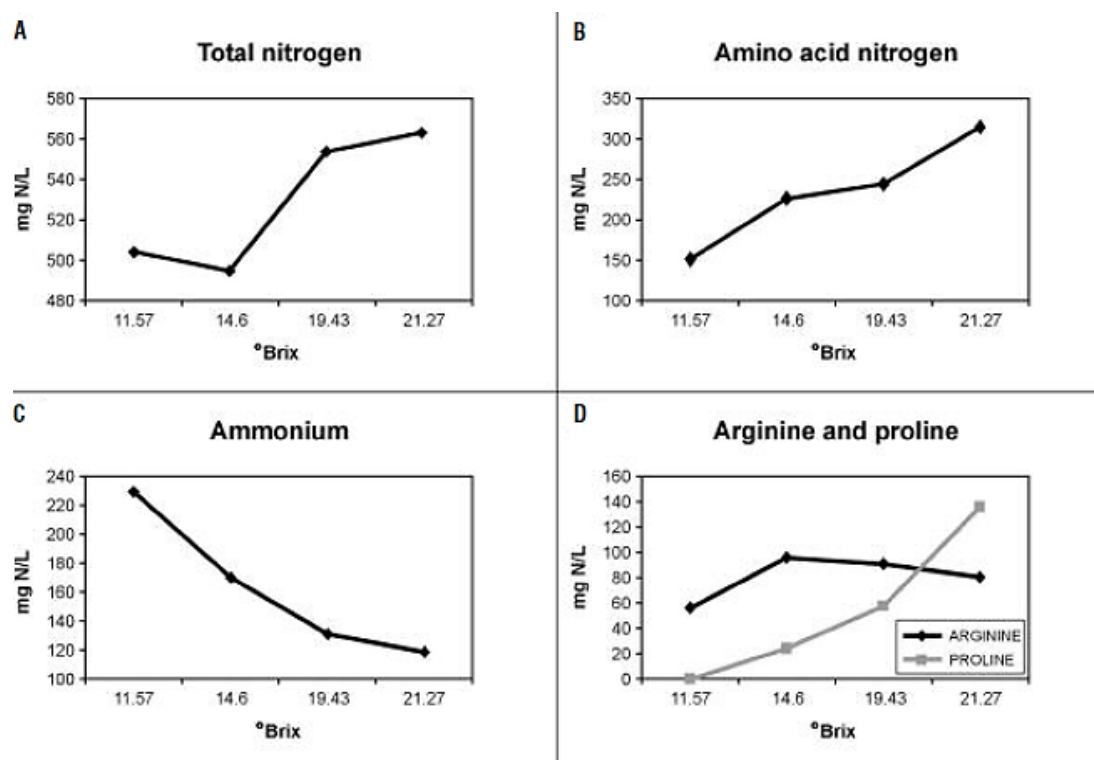


Figure 12 – Nitrogen compounds changes in Cabernet Sauvignon from *véraison* to harvest. Source: Bell and Henschke, 2005.

Nitrogen content is affected by numerous viticultural and oenological factors. The amounts of free amino acids in grapes, as well as their profiles, may varied due to cultivar, rootstock, vine care, vineyard location, and growing season (Huang and Ough, 1991; Spayd and Andersen-Bagge, 1996; Treeby *et al.*, 1998; Gump *et al.*, 2002; Rodriguez-Lovelle and Gaudillere, 2002; Bell and Henschke, 2005).

The storage of mineral compounds in the grape berry occurs at two different time intervals. The first elements (potassium, phosphorus, sulfur, magnesium, boron, iron, and copper) accumulate during berry development and ripening via the phloem. The second elements (calcium, manganese, and zinc) accumulated largely prior to *véraison* via the xylem (Rogiers *et al.*, 2006a). Potassium is the most abundant mineral cation within a grape berry and it is an essential macronutrient for a number of physiological and biochemical processes in plants. It plays an important function in controlling the cell membrane potential, making it essential for the absorption of other ions and sugars. Plant signaling, osmoregulation, cation-anion balance, cytoplasmic pH regulation, enzyme activation, and protein and starch production are all dependent on it (Wang and Wu, 2013; Ahmad and Maathuis, 2014; Shabala and Pottosin, 2014). At the vine level, this cation is involved in photosynthesis and a variety of functions connected to plant water relations, such as growth, turgor maintenance, and phloem transport (Hsiao and Läuchli, 1986; Liesche, 2016). The mineral is available over 5 mg by harvest and its accumulation into the berry seeds, pulp, and skin is constant from flowering through maturity, but most quick following the beginning of ripening (Hale, 1977; Possner and Kliewer, 1985; Williams and Biscay, 1991; Rogiers *et al.*, 2000, 2006b; Ramos and Romero, 2017). Within the fruit, the pulp has the highest concentration. In Grenache Noir, this berry layer stored 60% of the total potassium, while the skin received 37% and the seeds only 3% (Etchebarne *et al.*, 2009). In Shiraz, the pulp and skin were responsible for 59% and 32% of the total potassium, respectively, with the seeds accounting for 6%. (Rogiers *et al.*, 2006a). Due to this cation has such a large impact on juice pH, it's also crucial for berry, juice, and wine acidity and color (Mattick *et al.*, 1972; Somers, 1977; Boulton, 1980; Walker and Blackmore, 2012). Nevertheless, high potassium levels can also affect microbiological stability and fermentation processes (Walker *et al.*, 1998). Its levels in grape berries can be influenced by a range of factors, including soil potassium levels, grape type, and viticultural methods (Mpelasoka *et al.*, 2003; Davies *et al.*, 2006).

Regarding calcium concentration, it is highest at *véraison*, and remains stable or decreases during ripening; due to its low mobility in the phloem (Rogiers *et al.*, 2000, 2006a; Knipfer *et al.*, 2015). Calcium plays a significant role in resistance to *Botrytis cinerea* infection (Martins *et al.*, 2021). Magnesium, a component of chlorophyll, is present in grapes and must be at

levels similar to calcium (Conde *et al.*, 2007); its high levels may limit potassium absorption in the grapevine (Ashley, 2011). Copper can be transported into grapes through root adsorption; the maximum tolerance limits for copper residue are 20 mg/L and 1 mg/L in grape must and in wines, respectively (García-Esparza *et al.*, 2006; Ferreira *et al.*, 2006). However, vineyard management and winemaking practices might cause an uncommon rise in this copper in must, influencing wine flavor by oxidizing thiol-containing compounds (Blouin and Cruège, 2003). As example, the final concentrations of mineral compounds in the three main tissues (seeds, pulp, and skin) are shown in the Table 4.

*Table 4 – Mineral element concentrations ( $\mu\text{g/g}$ ) in the seeds, pulp and skin of Shiraz berries 90 day after flowering. Source: Rogiers *et al.*, 2006a.*

	<b>Seed</b>	<b>Pulp</b>	<b>Skin</b>
Calcium	4960 $\pm$ 191	112 $\pm$ 9	252 $\pm$ 11
Potassium	3660 $\pm$ 98	3360 $\pm$ 93	4380 $\pm$ 185
Phosphorus	2720 $\pm$ 86	211 $\pm$ 16	320 $\pm$ 23
Magnesium	822 $\pm$ 29	78 $\pm$ 3	130 $\pm$ 5
Sulfur	700 $\pm$ 22	64 $\pm$ 4	132 $\pm$ 4
Manganese	26 $\pm$ 3	0.96 $\pm$ 0.08	2.3 $\pm$ 0.2
Iron	19.6 $\pm$ 0.8	3.5 $\pm$ 0.2	8 $\pm$ 0.6
Boron	14.4 $\pm$ 0.2	7.4 $\pm$ 0.1	13.5 $\pm$ 0.2
Copper	8.7 $\pm$ 0.3	1.23 $\pm$ 0.04	2.83 $\pm$ 0.08
Zinc	10 $\pm$ 0.6	0.52 $\pm$ 0.04	1.6 $\pm$ 0.1

Glutathione is a tripeptide composed by glutamic acid, cysteine, and glycine. It is a non-protein thiol compound and can exist in a reduced (GSH) or oxidized form (Li *et al.*, 2004; Šuklje *et al.*, 2012). White and, to a lesser extent, red wines are susceptible to oxidative spoilage, which can result in a loss of aroma, the development of unwanted aging traits, and undesired colour changes. GSH has a key role because it reduces *o*-quinones generated during the oxidation process of musts, hindering the formation of browning pigments (Singleton *et al.*, 1985; Singleton and Cilliers, 1995; Du Toit *et al.*, 2006). In addition, glutathione reduces oxidized caftaric acid to 2-S-glutathionylcaftaric acid, also known as grape reaction product (GRP) (Singleton *et al.* 1985). Furthermore, it has a protective effect on several aroma compounds in wine (Papadopoulou and Roussis, 2008; Ugliano *et al.*, 2011).

The GSH in grape juice ranged from 53 up to 95 mg/L, at the first sampling and harvest point respectively (Figure 13); its concentration increased with berries development (Šuklje *et al.*, 2013).

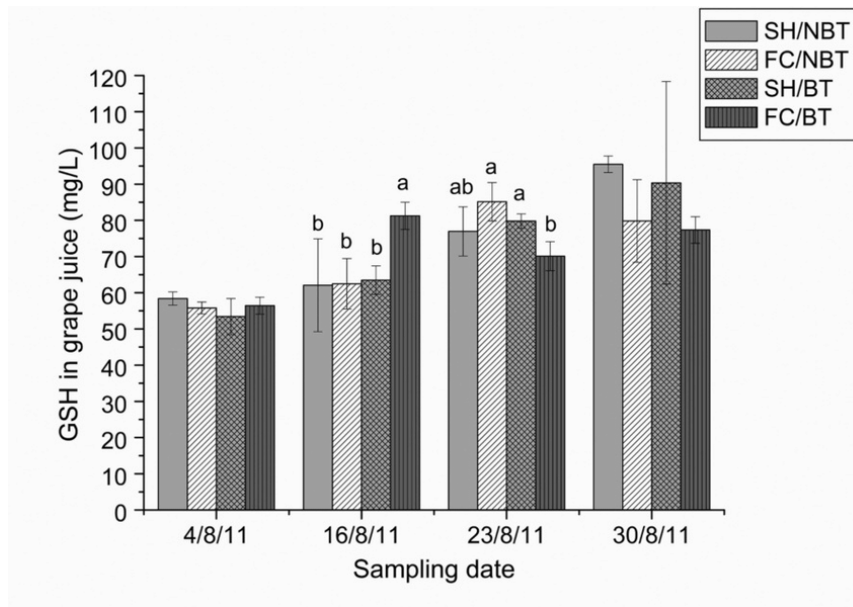


Figure 13 – Glutathione (GSH) concentration in grape juice during grape maturation from véraison to harvest, in response to different canopy management treatments. Source: Šuklje *et al.*, 2013.

GSH levels increased with greater sugar levels, while berry diameter or bunch exposure had no effect on GSH levels (Šuklje *et al.*, 2012). Furthermore, glutathione in berries during ripening is in the reduced form (Okuda and Yokotsuka, 1999). The increased input of phloem components to the berry from sources such as mature leaves may account for the rise in GSH during the onset of maturation. Another research specified that a rise in berry GSH is accompanied by a reduction in leaf GSH content, which supports this theory (Liyanage and Adams, 1992). The amount of GSH in grapes is directly connected to the vine nitrogen status, which is defined as the amount of yeast assimilable nitrogen in the grape juice (Choné *et al.*, 2006). The Australian Wine Research Institute (2020) reported that the minimum YAN requirement for low-risk fermentation is approximate 150 mg/l for white and 100 mg/L for red grapes/musts/juices. In grapevine, GSH depends on the vintage, location, and technological practices, which affect the content of readily assimilable nitrogen in the soil and, consequently, the final GSH content (Cheynier *et al.*, 1989; Lavigne and Dubourdieu, 2004).

Following the progressive ripening of grape berries, the soluble protein content in grape juice increased positively (Murphey *et al.*, 1989). Their concentration in finished wine typically ranges from 15 to 300 mg/L (Ferreira *et al.*, 2002; Waters *et al.*, 2005). The production of these proteins can be induced as a consequence of environmental or stress factors (Jacobs *et al.*, 1999; Robinson and Davies, 2000; Monteiro *et al.*, 2003a, 2003b).

## 2.7 Old vines

### 2.7.1 General aspects

*Vitis vinifera* L. is a perennial cultivation plant capable of showing remarkable longevity (Grigg et al., 2017; Robinson et al., 2013; Vršič et al., 2015). The world's oldest live and fruiting cultivated grapevine is called the 'Žametovka' grapevine from Lent in Maribor, also known as the 'Old Vine' and documented as more than 400 years old (Vršič et al., 2011). Many European nations have a considerably older but not comparable history of vine production since grape phylloxera destroyed most of these vines in the 19th century (Wapshere and Helm, 1987; Gale, 2011). This pest attacked the roots (Benheim et al., 2012) or leaves of the vines (Goussard, 2015), reducing their capacity to produce quality grapes (Teubes, 2014) and making them vulnerable to viral and fungal diseases (Granett et al., 2001), which often resulted in the vines mortality. After trying several control methods, the main successful management technique was grafting the vines onto phylloxera-resistant rootstocks imported from America (Granett et al., 2001). This allowed the progressive rebuilding of European vineyards during the next decades: in Italy and Portugal, as well as in other European viticultural countries, the introduction of *Vitis vinifera* varieties to American strengthen rootstocks gave rise to a real revolution in viticulture (Wapshere and Helm, 1987). First of all, it totally changed the location of vineyards which moved to plains with more fertile soils. Secondly, new management techniques were introduced, such as the planting of more spaced vines and more intense cultivation (Morrow, 1973). Currently, in the world, 80% of planted vines are phylloxera-susceptible cultivars grafted onto resistant hybrid rootstocks of American species (Whiting, 2004). Few vineyards date back to the pre-phylloxera period, located where soil conditions have been unfavorable for aphid development or in areas where natural barriers prevented spread (Ray, 1988; Robinson and Harding, 2015).

Despite these backgrounds, old vines continue to divide the viticulture world, both in terms of cost-effectiveness and recent New World efforts to regulate this informal section of the industry. As stated by Easton (2015), science has still to prove on measurable parameters that old vines wines are superior. Indeed, there is still no agreed-upon and regulated definition of 'old vine' that is valid worldwide. The life expectancy of vineyards before being economically unsustainable is different according to the continent: in Australia is 40 years, whereas in Europe and in South Africa, the expectancy is lower and around 25 years. Nowadays, there is no experimental evidence that a 30, 50, or 70-years-old vine, produces better wines than a balanced vine, well trained, 12-years-old. Indeed, with a suitable site (soil and climate) even 10 or 12-year-old vine is capable to produce high-quality wines when a proper balance between cultivation load and vegetative growth is achieved. There is a gradual accumulation

of more mature varietal taste and structural traits between the ages of 20 and 30 years, while tannic and structural maturity is clearer in vines over 30-years-old. Old vines have better root systems that are deeper and developed over a significant area; they have more wood and reserves, which provides better adaptability to climate change and more regular water nutrition than young vines. Old vines require less irrigation because their root systems are precisely deeper and well expanded in the soil. Since they are less vigorous, canopy management is limited as well. Disease management is similar to that of younger vines, except for the presence of wood diseases that force the replacement of dead vines. Young vines' lack of reserves makes them more susceptible to diseases and pests such as powdery mildew and spider mites, both can have extremely detrimental effects on both fruit quality and the long-term longevity of the vine. Older vines have faced more viruses than young ones and these can sometimes affect the quality and complexity of resulting wines.

### 2.7.2 The definitions of old vine

In recent years, the term 'old vine' has appeared on several wine bottle labels, with a new meaning: it no longer refers to vines dating back to the pre-phylloxera period or bred according to the traditional farming system, but simply that the wine was made from vines of a certain age. However, there is not still official age to define the term 'old vine' which is why, over the past few years, projects have been spreading with the purpose of conserving ancient vineyards and assisting in their good management. In accordance with Easton (2015), these projects are taking place primarily in the New World, which is searching for appropriate standards to describe wines created from 'aged' grapes. Exception for the Portuguese case, according to which vines must be more than 40 years old in order to use the designation 'vinhas velhas' (IVDP, 2020), in Europe, the appellations (vieilles vignes, viñas viejas, alte reben, and so on) are used exclusively in relation to the producer's quality or implied marketing aim. The New World is adopting a different method, as demonstrated by the Barossa Valley in Australia, California in the United States, Maule in Chile, and South Africa.

Phylloxera has been introduced in Australia in 1877, but it has stayed isolated due to stringent quarantine and has not been detected in South Australia so far (Gale, 2011). In South Australia, and particularly in the Barossa Valley, there are huge sections of phylloxera-free vineyards surviving, some of which have been produced continuously since the 1840s and 1850s (Ioannou, 2018; Barossa Wine, 2017). In the Barossa Valley, the discussion had been debated for years, and then a 'Charter' was elaborated to help categorize historic vineyards and increase the value of their older vines. The Charter was designed by Robert Hill-Smith of Yalumba Winery in 2007 and evolved into an industry-recognized Charter with the support of the Barossa Grape and Wine Association in 2009 (Barossa Wine, 2017; Yalumba Wine, 2020).

The four categories, each having a distinct definition of 'old', are Old, Survivor, Centenarian, and Ancestor. The Charter's age classification is reported in Table 5. Hill-Smith claimed that the Old Vine Charter is dedicated to the recognition, preservation, and promotion of old vineyards (Barossa Wine, 2017). Although this categorization is not official, it is designed to raise awareness of the status and significance of the region's ancient grape varieties.

*Table 5 – Barossa Old Vine Charter and age classifications. Source: Barossa Wine, 2017.*

<b>Term</b>	<b>Vine age</b>	<b>Details</b>
Old	≥ 35 years	These vines are fully matured. With a solid trunk, their structure allows the harvesting of grapes with different organoleptic characteristics.
Survivor	≥ 70 years	These very old vines are a milestone that pays tribute to the commitment of growers and winegrowers.
Centenarian	≥ 100 years	These pre-phylloxera vines have been allowed to mature and protected in their thick and gnarly trunks. They are known for their low yield and intensity of flavor.
Ancestor	≥ 125 years	These are vines that have been growing strong and have helped sustain the wine-growing tradition. Dry-grown, low-yielding vines produce grapes of great flavor and intensity.

In California, a non-profit and official organization, The Historic Vineyard Society, was founded in an effort to raise awareness and protect the oldest vineyards. It was established in 2011 and is dedicated to the preservation of California's historic vineyards. The purpose is to develop a register of California's oldest vineyards, which includes all vineyards with one-third or more of their original vines planted at least 50 years ago (Easton, 2015). Before being included in the registry, all vineyards must satisfy specific criteria and undergo a screening procedure. To accomplish their aim, the founders wrote a resolution stating the organization's mission, which was approved by the California Assembly Agriculture Committee (Historic Vineyard Society, 2011).

In Chile, Vigno is an abbreviation for Vignadores de Carignan, a producer group whose purpose is to develop an appellation of origin for wines made from ancient Carignan vines planted dry and bush-trained in the Maule Secano region (Conway, 2018). The old Carignan vine was a forgotten heritage of Chile's wine industry. It was originally planted on a wide scale in Maule in the early 1940s when the Ministry of Agriculture advocated encouraging the cultivation of Carignan to promote the region's wines following a massive earthquake in 1939. The Vigno project has 12 founding wineries (Goode, 2020), and wines must be made from at

least 65% old Carignan vines and aged for at least two years to be labeled as Vigno wines (Conway, 2018).

In South Africa, vines have a life expectancy of about 25 years, assuming they reach this age and are not removed sooner owing to viral illnesses (Lloyd, 2013). Rosa Kruger is one of South Africa's foremost experts on ancient vines (Mkhwanazi, 2016). Because there is no legal age to define the term 'old vine', she determined that 35 years and older is an appropriate age to characterize an 'old vine' based on her knowledge and experience dealing with ancient vines in South Africa and throughout the world. Kruger started an informal initiative to develop a blog that is a database of all ancient vines over 35 years old located in South Africa to raise awareness of old vines in the country. The database includes information about the age of vineyards, cultivars grown on the farms, the number of covered hectares, the district of vineyards, and the name of farmers' owners. The blog also acts as a communication platform for farmers to include their historic vineyards or contribute extra information to assist complete the database (Old Vine Project, 2020).

Regarding Old World, France, in particular, is one of the world's oldest wine-producing countries, having produced wine since very ancient times. The country does not have a legally or informally recognized age that characterizes an old vine, despite having a highly sophisticated and accurate classification system, Appellation d'Origine Protégée. It is widely agreed that the term 'Vieilles Vignes' refers to an old vineyard (Meuse, 2015). It has also been suggested that the term 'old' might vary according to the variety and soil in which the vine is cultivated. Some types are more susceptible to illness than others, and some are better adapted to extreme environmental difficulties. Thus, in certain harsher environments, even 20 years may be considered 'old' (Old Vine Project, 2020). As a result, it appears that the expression 'old vine' refers to recalling specific traits rather than the vineyard being of a given age.

While the term is also becoming more popular due to research projects initiated in the New World, the legislation governing its application is still inadequate and unclear. The critical point is the lack of agreement on how long it takes for a vine to be considered 'old enough' to have any effect on wine quality (Robinson and Harding, 2015). Therefore, it is necessary to start studying a precise definition and quantification of the term 'old vine' in years, following the steps of the New World. This will make it easier to organize future experimental designs and subsequent comparisons between studies.

## 2.8 The effect of vine age on grape and wine

The thesis according to older vines produce better and more structured wines is well-rooted in the wine industry and in all the people who revolve around it. As previously described, there are several projects in progress, especially in the New World, to give more visibility to the vine age. This idea has been spread by specialized magazines, books, and wine critics (Koblet and Perret, 1980; Smart, 1993; Goode, 2005; Caputo, 2008; Dry, 2008), where the relevance of this aspect from different points of view has been investigated. However, the literature supporting this contention remains sparse, with the most relevant scientific articles having been published in the last twenty years. Hence, this question still remains unanswered, both officially and scientifically (Mkhwanazi, 2016).

The next two subitems have reported the studies where the impact of vine age has been examined in relation to viticultural aspects, maturation process, and grape composition, as well as wine composition and sensory profile.

### 2.8.1 Impact of vine age on viticultural aspects, maturation process and grape quality

Multiple factors influence vine performance and fruit quality, including environmental elements (soil, temperature, solar radiation) and those related to management, such as site selection, variety, rootstock choice, planting density, irrigation, and canopy management (Ibacache et al., 2020); as well wood diseases such as esca (Williams and Matthews, 1990; Bertsch et al., 2013; Tyminski, 2013) and viral diseases may reduce vineyard longevity (Credi and Babini, 1997; Walker et al., 2004). In some circumstances, inadequate management procedures may result in dense canopies and compact soils, decreasing crop value while increasing production costs (Smart and Robinson, 1991; Morlat and Jacquet, 2003), which are reflected in vine productivity and grape and wine quality (Reynolds and Wardle, 1989; Smart and Robinson, 1991; Dry et al., 2005).

Focusing on vegetative capabilities, in a study conducted in the Barossa Valley, Grigg *et al.* (2017) tested the influence of age on Syrah vines' performance ranging in age from 6 to 168 years. Vine age had an effect on reproductive performance: older vines produced higher yields than younger vines. Older vines had a higher mass of fruit per meter because of the higher number of berries per bunch, due to the ability to store more perennial wood. Regardless, it is specified that even after 160 years of existence, vegetative and reproductive growth can be affected by vineyard management and season variations. In this study, it was found that age alone is not a preponderant factor in the balanced production and consequent quality of wines produced: in fact, the effects associated with the site were greater than the effect of age.

The phenological aspect has been mostly examined in several works. Ezzili (1992) compared 13 and 50-year-old vines of Alicante (Grenache Noir), stating that the older vines had lower vigor and reduced fruit set. Considine (2004) presented a case study in which the Zante cultivar was analyzed for five vineyards of different ages (the vines were between 6 and 50 years old) and management. Likewise, it was found that vigor began to decline after the vines reached peak productivity at 25-30 years. The decline was accompanied by a reduction in the number of berries per cluster. Obviously, external factors such as water deficit could alter the reading of the data, so age was not correlated with total yield, number of clusters, or berry volume. When confronted with the quality topic of old vines, one of the major explanations of wine experts, producers, and viticulturists is that these old vines have formed a large and deep root system (Ross, 1999; Easton, 2015). The study conducted by Zufferey and Maigre (2007), was intended to understand the influence of grapevine age on its physiological behavior. Six vines (both red and white), planted between 1971 and 2000, were compared. It was found that the old vines presented less water scarcity due to the well-developed root system, which moderated climatic demand and increased photosynthetic activity of the foliage in case of climatic stress. Moreover, in rainy weather, the vineyard did not consume all of the water on the soil surface, resulting in watery grapes, as a young vine may. An old vine with a deeper root system may also be able to reach new minerals, adding dimensions to the vine's resources and allowing it to produce more complex and concentrated fruit. In addition, the more concentrated vegetative expression of the old vines meant high nitrogen content due to the larger leaf areas and higher weight of green and winter pruning wood. Vineyards get increasingly acclimated to their surroundings as vines age; and are reported to have formed a 'memory' of their surroundings, resulting in better-balanced yields and crops. (Crouse, 2016). Nader (2018) took a multidisciplinary approach to the Riesling white variety, by following three vineyards planted in 1971, 1995, and 2012 for a four-year time period (from 2014 to 2017). The 2-year-old vines initially had significantly lower vegetative productivity than 19 and 43-year-old vines. Their lower capacity and lack of canopy foliage led to greater cluster exposure to light and increased nitrogen accumulation, which resulted in increased amino acids, monoterpenes, norisoprenoids, and flavonols in 2014 and 2015. In the following years 2016 and 2017, yield and pruning weight, as well as berry composition, were comparable to older vines. Technological maturity parameters were not affected by vine age. The 19 and 43-year-old vines had similar physiological characteristics throughout the study, but the older group demonstrated a higher incidence of esca malady. Individual yields remained equivalent for both groups, nonetheless. Another aspect that has been tested on the previously defined criteria is the influence of vine age on water status and productivity. Subsequently, Nader *et al.* (2019) concluded that photosynthesis, stomatal conductance, and transpiration were lower for younger vines, although the response was dependent on seasonal factors. They also

exhibited smaller canopy size and lower productivity than adult vines, although by the fourth year after planting they achieved similar levels of bud fruiting, yield, and pruning weight. The adult vines, planted in 1995 and 1971, did not differ in water status, canopy conformation, bunch parameters, and technological maturity. However, the older vines had a higher incidence of trunk diseases, which as a consequence decreased their final yield. From this study, it appears that the physiological behavior, productivity, and berry composition of 20 and 40-year-old vines can be compared as long as the vineyards are managed likewise. Lastly, Riffle *et al.* (2021) studied the effect of vine age on phenological parameters and gas exchange for the Zinfandel cultivar. To minimize seasonal variability and any other disturbing factors, the study was performed in a single block of vineyards interplanted with young (5 to 12 years old) and old (40 to 60 years old) vines, under uniform management practices, for two consecutive vintages. Young vines progressed more slowly during berry formation and more rapidly during berry ripening, resulting in young vines being harvested earlier than old vines due to variation in the timing of sugar accumulation. No differences were found in the leaf water potential of the vines. Young vines tended to have higher stomatal conductance, and thus photosynthesis rates, at mid-day. The results of this study suggest that vine age is a factor in the phenological timing and length of the growing season.

Fruit from older vines was typically found to have lower pH and greater total acidity than fruit from younger vines, while the sugar content of grapes is comparable regardless of age. In terms of quality results, the evidence that acid balance or a favorable pH to total acidity ratio may be related to storage capacity is critical to comprehend (Zufferey and Maigre, 2007). Concerning berry, must, and wine composition, Reynolds *et al.* (2008) conducted a study of five cultivars ranging in age from 4 to 14 years and determined that vine age had an effect on the primary structure of the red varieties. The impact was greater on must and wine composition than berry composition. Anyway, musts and wines from young vines had more concentrated phenolics and anthocyanins than old vines therefore, from a sensory point of view, wines from old vines were not necessarily of higher quality. Moreover, Grigg (2017) published a manuscript in which he evaluated the influence of vine age on grape and wine production. The areas selected for investigation were several: vine performance, fruit, and wine composition and sensory analysis, wine metabolomic analysis, and molecular analysis (genome and epigenome). Five Syrah vineyards with 'young' and 'old' plantations were selected, with an average age difference of more than 97 years and studied for a period of time of three years. Vine age was found to impact reproductive capacity; older vines registered higher yields, but all were affected by seasonal variations, whatever their age. Differences in vine age did not produce differences in basic grape composition; in fact, tannins and phenols showed greater differentiation by growing region. Furthermore, differences associated with

vine age were found in both grape and wine samples: grapes and wine from older vines showed consistent sensory characteristics across seasons. In conclusion, despite the large differences in vine age, both site and season were influential in the quality assessments conducted.

An overview of what was described in the current paragraph is defined in Table 6. The age-related studies are listed in chronological order.

*Table 6 – Relevant studies in the literature that studied the impact of vine age on viticultural aspects, vine cycle, maturation process, and grape composition. Adapted source from Grigg et al., 2017; Nader, 2018.*

Measures	Vine age (years)	Location	Cultivars	Findings	Reference
-Fruitset kinetics	13 / 50	El Khanguet, Tunisia	Alicante Grenache Noir	Older grapevines had lower vigor and reduced fruit set.	Ezzili (1992)
-Vegetative and fruit	6 / 50	Western Australia	Zante Currant	Older grapevines had lower vigor and berry number per bunch. Grapevine age was not related to total yield, bunch number or berry volume.	Considine (2004)
-Vegetative, fruit	5 / 34	Wädenswil, Switzerland	Chasselas, Arvine, Pinot Blanc, Gamay, Syrah and Humagne Rouge	Older grapevines had higher total acidity, yeast available nitrogen and pruning mass. Age had no impact on sugar concentration.	Zufferey and Maigre (2007)
-Vegetative, fruit and wine	4 / 14	Ontario, Canada	Cabernet Sauvignon, Cabernet Franc, Pinot Noir and Riesling	Old grapevines had higher yield, bunch number, bunch mass and berry mass and lower total soluble solids in one season only. Age had little impact in second season. Wine pH and total acidity were contrasting in each season, and wines from old grapevines were more vegetal in 2002 but not in 2003.	Reynolds <i>et al.</i> (2008)
-Vegetative and fruit	6 / 168	South Australia	Syrah	Older grapevines had a higher yield, which may be due to their increased size. The effects associated with planting site were more important than the effect of grapevine age.	Grigg <i>et al.</i> (2017)
-Vegetative, fruit and wine	6 / 168	South Australia	Syrah	Older vines produced higher yields, but all were affected by seasonal variations. Grapes and wine from older vines showed consistent sensory characteristics across all seasons. Both site and season were found to be influential in the quality evaluations conducted.	Grigg (2017)

-Vegetative, fruit	7 /24 /48	Geisenheim, Germany	Riesling	Young and old vines exhibited similar physiological characteristics at the individual vine level, including yield, pruning weight, berry structure and composition, and ultimately, wine profile. Age appears to be less influential on wine profile than the growing season, region, or management practices.	Nader (2018)
-Vegetative and fruit	7 /24 /48	Geisenheim, Germany	Riesling	Photosynthesis, stomatal conductance, and transpiration were lower for younger vines, although the response depended on seasonal factors. Adult vines did not differ in water status, canopy conformation, bunch parameters, and technological maturity. Older vines had a higher incidence of trunk disease, which decreased their final yields.	Nader <i>et al.</i> (2019)
-Fruitset kinetics and vegetative	5 / 60	California, USA	Zinfandel	Young vines progressed slower during berry formation and faster during berry ripening, so were harvested earlier. Young vines tended to have higher stomatal conductance, and thus photosynthesis rates, at mid-day.	Riffle <i>et al.</i> (2021)

In conclusion, these studies showed that the strength of old vines is the very deep root system, which allows them to moderate climatic demand, increase photosynthetic activity in case of water stress, and acclimatize to the surrounding environment. Also, due to the deep roots in the soil, they are able to reach new minerals, allowing them to produce more complex and concentrated fruits. Moreover, vine age had an effect on reproductive performance: older vines produced higher yields than younger vines. However, through these articles, it was found that age alone is not a preponderant factor in the productive balance and in the consequent quality of wines produced. In fact, even after 160 years of existence, vegetative and reproductive growth can be influenced by vineyard management and by seasonal variations. Some evidence reported that old and young vines had physiological characteristics; despite the older group showing a higher incidence of esca disease, individual yields were still equivalent.

### 2.8.2 Wines produced from old vines: compositional and sensory profile

Several factors influence a wine's aroma character, including grape variety, climatic conditions, soil, region, cultivation practices, yeast, enological procedures, and aging (Jackson and Lombard, 1993; Moret *et al.*, 1994; Dirninger *et al.*, 1998; Fischer *et al.*, 1999; Jones and Davis 2000; Falqué *et al.*, 2001; Gómez-Míguez *et al.*, 2007). However, studies that investigated the effect of vine age on aroma compounds in wine are detailed below.

In terms of vegetative-productive balance, Zufferey and Maigre (2008) analyzed the influence of age on grape and wine quality. The vegetative-productive balance (modest yields) of old vines is the main factor defining the best quality of wines obtained from them. As a matter of fact, from the tasting of red wines, results that the ones coming from old vines were judged more stable and structured; in the same way, from the tasting of wines made from white and old vines, in particular from Pinot Blanc, wines were judged better for typicality and finesse of the bouquet. It has been determined that the aging potential of wines produced from old vineyards, over 30 years, is superior to those made from vines aged 4-8 years for both red and white wines. Vineyards lose vigor as they age and produce fewer berries with smaller berries and thicker skins. This has also been recognized as a contributing factor to the high quality of old vine grapes. The titratable acidity, phenolic, and flavonol content of smaller berries differed considerably from that of medium and smaller berries, according to a study on the impact of fruit size on red wine color and chemical composition (Gil *et al.*, 2015). From the analysis of wines aged for more than 40 years of Chenin Blanc (Crouse, 2016), it was found they were not characterized by distinct aromatic attributes although presenting a subtle range of aromas. Their aromatic profile was complex, in fact, wines had prominent in-mouth properties but were different within a set. From the sensorial data collected, body, balance, concentration, and complexity were all positively correlated, and acidity was negatively correlated. In addition, the body had a strong positive correlation with ethanol, dry extract, and reducing sugars. There were distinctive phenolic differences between older Chenin Blanc wines and Chenin Blanc wines produced from 30-year-old vineyards, however, this could depend on winemaking practices. The composition of these aged wines had a great impact on the perception of in-mouth sensations. Lastly, in the multidisciplinary study conducted by Nader (2018), it was found that wines from younger vines were associated with ripe fruit aromas and the kerosene aroma typical of Riesling. In addition, concentrations of potential monoterpenes, norisoprenoids, and volatile compounds were elevated in 2014 and 2015. Sensory and chemical profiles of wines from older vines were dependent on vintage, not their age. When 2-year-old vines had reached their fruiting potential, productivity, berry composition, and resulting wine quality converged with that of the other groups. It turned out that 19 and 43-year-old vines

behaved similarly throughout the study and produced comparable wines in terms of sensory analysis, which goes against the notion that older vines produce wines with different profiles. Similarly, vines planted in 1995 and 1971 exhibited similar physiological characteristics at the individual vine level, including yield, pruning weight, berry structure and composition, and ultimately wine profile. It follows that even if vine age did indeed have an impact on wine quality, the present work suggests that its effect would be less influential on wine profile than the growing season, region, or management practices.

The sensorial aspect has been specified by Heymann and Noble (1987), Cabernet Sauvignon wines from 5 and 20-years-old vines were compared, showing a correlation between the age of the vine and the aroma of the finished wines. The correlation is positive between vine age and intensity of berry aroma, and negative between age and vegetal aromas. Whereas, Du *et al.* (2012) investigated the effect of vine age (3, 6, and 12 years) on volatile compounds in 'Beihong' wine. Thirty-three volatile components were discovered and measured, and a descriptive examination of its flavor profile revealed that the fruity and floral series contributed the most impact. The analysis of volatile compounds on this wine showed that as vine age increased, total volatile compound concentrations and odorant activity values increased significantly.

An element that contributes to the value of these old vines is the perception that they are a real reflection of their terroir. As a result, they have the potential to produce wines that are distinct because of the land and environment in which they are cultivated. Thus, wines might be created that are unique to a certain place and could not be replicated anywhere else in the world. This would also elevate the perceived quality of old vine wines since research has suggested that wine origin is highly valued because it is related to tradition and the uniqueness of the wines (Sáenz-Navajas *et al.*, 2013).

An overview of what was described in the current paragraph is defined in Table 7. The age-related studies are listed in chronological order.

Table 7 – Relevant studies in the literature that studied the impact of vine age on the compositional and sensorial profile of resulting wines. Adapted source from Grigg *et al.*, 2017; Nader, 2018.

Measures	Vine age (years)	Location	Cultivars	Findings	Reference
-Wine	5 / 20	California, USA	Cabernet Sauvignon	Vine age was correlated with berry aroma and fruit flavor in finished wines. Wines of old grapevines had higher ratings. Negative correlation between grapevine age and green bean and vegetative flavor in wines. Younger grapevines from cooler areas produced more vegetative wines.	Heymann and Noble (1987)
-Wine	5 / 34	Wädenswil, Switzerland	Chasselas, Arvine, Pinot Blanc, Gamay, Syrah and Humagne Rouge	Wines of old grapevines were more preferred early and after 4 years of aging.	Zufferey and Maigre (2008)
-Wine	3 / 6 / 12	Beijing, China	Beihong	As grapevine age increased, the concentration of total volatiles and the odor activity values of the wines increased.	Du <i>et al.</i> (2012)
-Wine	≥ 40	South Africa	Chenin Blanc	Wines aged for more than 40 years of Chenin Blanc were not characterized by distinct aromatic attributes although presenting a subtle range of aromas. Their aromatic profile was complex, and wines had prominent in-mouth properties. However, this could depend on winemaking practices.	Crouse (2016)
-Wine	7 / 24 / 48	Geisenheim, Germany	Riesling	Young and old vines exhibited similar physiological characteristics at the individual vine level, including yield, pruning weight, berry structure and composition, and ultimately, wine profile. Age appears to be less influential on wine profile than the growing season, region, or management practices.	Nader (2018)

In conclusion, the vegetative-productive balance (modest yields) of the old vines is the main factor that defines the best quality of the wines obtained from them: these wines are judged better for the typicality and finesse of the bouquet. From the analysis of volatile compounds in old wines, it is shown that with the progression of vine age, total concentrations of volatile compounds increase significantly. In fact, the correlation appears to be positive between the age of the vine and the intensity of berry aroma, and negative between age and vegetal aromas. However, analysis of wines aged for more than 40 years found that they were not characterized by distinct aromatic attributes and exhibited a subtle range of aromas. It happens that when young vines reached their fruiting potential, productivity, berry composition, and resulting wine quality converged with the older ones. In fact, one study showed that 19 and 43-years-old vines behaved similarly and produced comparable wines in terms of sensorial analysis. It follows that even though vine age has an impact on wine quality, its effect may be less influential on the wine profile than the growing season, region, or management practices.

## 2.9 Wine quality

Evaluating the quality of any food or beverage product has proven to be challenging, and this is even more true for a multidimensional product such as wine (Parr *et al.*, 2011). Quality cannot be attributed to a specific aspect of a product; rather, it is a combination of all of the aspects that constitute the final product (Peri, 2006). In order to analyze its quality, the product is usually divided into its many features and traits, and the quality of each is studied separately. In general, the first quality evaluation consisted of monitoring a set of objective criteria that simply had to satisfy the requirements of the product's producers and scientists, such as the chemical, microbiological, and even compositional quality of the food (Crosby, 1979; Cardello, 1995). If the product satisfied all of the criteria, it was declared to be of excellent quality. Anyway, there was no proof that the product would respond adequately to customer expectations. In 1870, Clarke wrote the first essay pointing out this issue, as maintained by Cardello (1995), but the first article to properly address this topic for the first time was published in 1995, and it proposed that quality rating should be determined by consumer satisfaction. Since then, there has been a significant change in attention toward customers' expectations and perceptions of quality. Grunert *et al.* (1995, 1997) developed the Total Food Quality Model, which was the first model to integrate the quality factors that customers perceive when purchasing food. This model (Figure 14) was created based on the multidimensional aspects of customer quality standards (Peri, 2006), commonly known as 'perceived quality' (Olshavsky, 1985; Ophuis and Van Trijp, 1995). It demonstrates what customers expect before making a purchase choice (*homo oeconomicus*) as well as when using the product after purchase (*homo edens*).

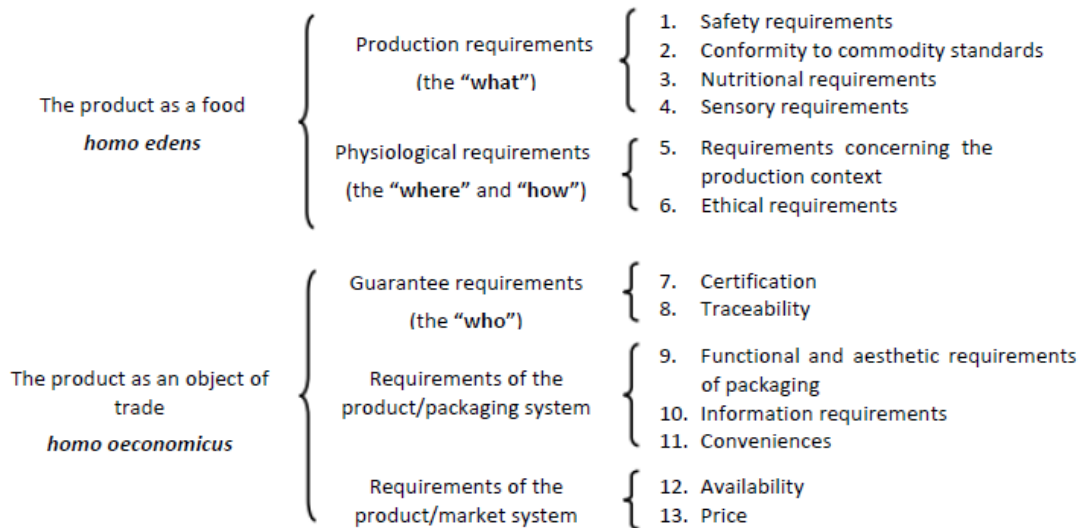


Figure 14 – An analytical model of food product quality factors, in relation to customer requirements. Source: Peri, 2006.

Figure 14 illustrates how a product's perceived quality manifests itself in several dimensions. The approach would also be relevant to wine, as wine has a multidimensional structure. Nevertheless, following a study on red wine, wine quality is not simply related to customer expectation and experience (Jover *et al.*, 2004). Wine production is certainly customer-driven, but due to the impact of the winemakers, it is also driven by the producers' requirements, which influences the consumer's impression (Stuen *et al.*, 2015). Due to the complex nature of wine, the quality of wine is the overall judgment that results from the interaction of intrinsic and extrinsic factors (Holbrook and Corfman, 1985; Jover *et al.*, 2004). Intrinsic factors are described as the chemical and physical aspects of wine that are experienced only after consumption (Jover *et al.*, 2004; Veale and Quester, 2009) and, consequently, contribute to the direct consumption experience, such as taste, sight, and smell (Charters and Pettigrew, 2007; Hopfer and Heymann, 2014). Extrinsic factors have been defined as product-related attributes that are not part of the actual product that is consumed (Olson and Jacoby, 1972), such as region of origin (Lockshin and Corsi, 2012), price (Curzi and Pacca, 2015), and packaging (Piqueras-Fiszman and Spence, 2012). Likewise, the relationship between vine age and wine quality creates an extrinsic aspect for perceived quality that is unrelated to quantifiable intrinsic ones. In Figure 14, while wine experts (*homo edens*) can recognize the intrinsic qualities of a wine, consumers (*homo oeconomicus*) rely more on extrinsic factors (Sáenz-Navajas *et al.*, 2015). Hence, given the effect of both consumers and experts on perceived wine quality, as well as the fact that the categories of factors associated with wine

quality may differ from those associated with other products, the model for wine quality may differ slightly from Figure 14.

When assessing the perceived quality of wine, researchers have frequently focused on extrinsic factors. According to Jover *et al.* (2004), this is due to the inability of the intrinsic factors to be examined prior to consumption. This is intricate even by the fact that previous consumption experience may not be a prediction of future purchase quality owing to differences in manufacturing processes and vintages, among other reasons. The study of wine complexity discovered that consumers prefer to focus on the sensory aspects of wine and associated 'complexity' more with the intrinsic qualitative features of wine (Parr *et al.*, 2011). However, wine experts identified the term 'complexity' in wine with extrinsic traits such as vine, soil, and production processes, but they also valued relevant organoleptic attributes and vine age (Ross, 1999; Veale and Quester, 2009; Sáenz-Navajas *et al.*, 2013). According to these researches, both consumers and experts agree that quality is a multidimensional phenomenon and that the in-mouth aspects play a key role in their evaluation of wine quality. However, little study has been conducted on evaluating key intrinsic qualities linked with quality. The sensory evaluation of wine has mostly concentrated on wine aroma and basic tastes but has not considered other aspects regarded to be essential intrinsic quality criteria (Charters and Pettigrew, 2007; Parr *et al.*, 2011) as balance, weight, length, complexity, and concentration. Mueller *et al.* (2010) reported that the two factors (extrinsic and intrinsic) cannot be separated since product consumption can only occur once a product has been purchased, and consumption experience influences future purchases. According to them, because there isn't much investigation that unifies the two dimensions of extrinsic and intrinsic, most research on this topic is treated unrealistically. Thus, while studying a product's intrinsic features, it is critical to comprehend and have information on the product's properties.

### 3. CONCLUSION AND FUTURE PERSPECTIVES

Investigation of the vegetative performance of significantly different ages was performed by comparing vines of different cultivars, located at different sites. Despite large age discrepancies, measurements of vine performance were shown to be unaffected by plant age on a regular basis. In isolation, age-based comparisons revealed considerable disparities, leading to the conclusion that vine age had a greater impact on reproductive than on vegetative performance. In general, older vines produced greater yields, which was attributed to a rise in the yield components berry number and bunch mass. Although increased vine age can affect reproductive capacity, it was not consistently observed in this work. Anyway, higher yields and variances in reproduction might still be attributed to vine reserves or overall capacity. It has previously been found that vines with a higher amount of old wood generate better yields. In addition, their root system is more developed, since they have formed a strong grounding in the soil over time. This shows that as age progresses, their seasonal fluctuation smoothing out capability may increase. Consequently, in more difficult seasons, such as those marked by drought or severe temperatures, older vines are likely to outperform younger vines, ensuring long-term stability. Further studies will be needed to detect this aspect.

Climate and season have an impact on vine development. In every field experiment, seasonal variance is a difficulty: if the research works were conducted over a longer period of time, more relevant tendencies could be discovered. This would also increase the chances of recording seasonal extremes. The analysis of grape composition found few clear age-specific associations. Compositional examination found that the growing season and location had a bigger impact on several of the characteristics than the vine age. Because acid balance is a fundamental quality factor in winemaking, it should be determined if vine age, resulting in a balance in acid composition, is connected to vine age or size, or canopy structure. The main compositional parameters used to estimate harvest time and assess quality were found to have no significant association with vine age in this study. The composition of grape berries is complicated, with different chemical components, that affect wine quality, and are present in different amounts at each grape berry tissue and stage. The composition and development of various grape compounds are influenced by the biosynthetic processes that take place during the grape maturity process, as well as by cultivar-dependent, environmental and management vineyard practices, climatic conditions, and seasonal changing in the weather. Therefore, it is very difficult to evaluate the extent of influence of vine age, as the variables affecting the ripening process are really different.

The discrepancies noted in the various studies presented to this point may result from several factors. The first is the diversity of climatic regions in which the studies were conducted. Then,

the ages of vines considered 'young' and 'old' vary widely among the articles (Tables 6 and 7). This stems from both the difficulty of finding suitable plant material and of performing experiments regarding vine age under comparable conditions. Indeed, clonal material from very old vineyards may be difficult to find after decades. For grafted vines, the nature of the scion and rootstock combination may also influence vine longevity (Lider et al., 1978; Bauerle et al., 2008; Keller et al., 2012), and this adds another level of complexity.

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