Cátia Andreia Leal da Costa

**Assessing the role of climate variability
on grape berry quality parameters
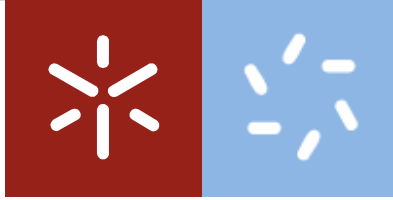in Portugal**

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Universidade do Minho
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Assessing the role of climate variability on grape berry quality parameters in Portugal

Dissertação de Mestrado
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Trabalho efetuado sob a orientação do
Doutor António Graça
e do
Prof. Doutor Hernâni Gerós

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A vida é feita de nadas:
De grandes serras paradas
À espera de movimento;
De searas onduladas
Pelo vento.
De casas de moradia
Caídas e com sinais
De ninhos que outrora havia
Nos beirais;
De poeira;
De sombra de uma figueira;
De ver esta maravilha:
Meu Pai a erguer uma videira
Como uma Mãe que faz a trança à filha.

Miguel Torga

ABSTRACT

Climate is a strong modulator of grape berry composition. Over the last decades, model projections have shown that ongoing climate changes are to continue in the future, but the direction and degree of climate change impact on fruit composition of winegrape cultivars is puzzling. Thus, the study how climatic variables, namely temperature and precipitation, have impacted berry composition during the last decades is of utmost importance to understand future scenarios.

This was the main idea of the present study that was carried out in three Portuguese wine regions – Douro, Dão and Alentejo – and targeted two varieties with a strong national importance (Touriga Nacional and Aragonez). Berry weight, titratable acidity (TA), pH, probable alcohol (PA), anthocyanins and total phenols index (TPI) data, which were collected two to three weeks after the end of the *véraison* until technological maturity for > 10 years, since 1991 in Douro, 2004 in Alentejo and 2008 in Dão, were collected and compiled into a database. Climatic data were obtained from automatic weather stations (owned by Sogrape Vinhos S.A.) and from an estimated climatic database (provided by UTAD) at a very-high resolution grid (<1 Km). The influence of climate variables - monthly average (TG), maximum (TX) and minimum (TN) air temperature (November-October) and precipitation totals (April to June and July to September) – on the above-mentioned berry quality parameters was explored through correlation analyses. This approach was a starting point to identify the months/periods more influential in grape berry composition, as well as the most affect vineyards. Next, different statistical approaches were carried out to explore in detail the influence of climate variables in the vineyards identified as most affected previously.

Results showed that, at technological maturity, temperature was negatively correlated to berry weight, TA, anthocyanins and TPI, but was positively correlated to pH and PA. Moreover, in warmer regions the berry weight was lower, and TA, anthocyanins and TPI followed the same trend, while in colder regions, pH and PA were lower than in warmer regions. Besides, a clustering analysis showed significant temperature differences between years where the quality parameter was higher (cluster 1) and years where it was lower (cluster 2). Additionally, results showed that the temperature differences between clusters resulted from differences between warmer and colder years. Moreover, we showed that temperatures at *véraison* and maturation periods (namely June to August) are more influential in determining grape berry composition at maturity. Regarding precipitation, its influence was dependent on vineyard and variety. Results also suggested that the berry composition from Alentejo vines is more affected by inter-annual climate variability than Douro vines. Furthermore, Aragonez variety seems more resistant to climatic variations than Touriga Nacional.

The obtained results about the role of climate variability on grape berry quality parameters presented here provided important knowledge for the development of forecasting models. These models can predict changes in berry composition in response to changing climate.

Keywords: climate, variability, maturation, grape berry, quality, Touriga Nacional, Aragonez.

Resumo

O clima é um forte modulador da composição do bago de uva. Nas últimas décadas, projeções modelo mostraram que as mudanças climáticas presentes devem continuar no futuro, mas a direção e a extensão do impacto das alterações climáticas na composição do bago da uva não são totalmente conhecidas. Assim, a compreensão de como as variáveis climáticas, nomeadamente a temperatura e a precipitação, influenciaram a composição do bago nas últimas décadas é de extrema importância para a compreensão de cenários futuros.

Esta foi a ideia principal do presente estudo que foi realizado em três regiões vitivinícolas portuguesas – Douro, Dão e Alentejo – e em duas variedades de forte importância nacional (Touriga Nacional e Aragonez). Dados de peso do bago, acidez titulável (AT), pH, álcool provável (AP), antocianinas e índice de fenóis totais (IFT) - medidos duas a três semanas após o fim do *véraison* até à fase de maturação tecnológica durante > 10 anos, desde 1991 no Douro, 2004 no Alentejo e 2008 no Dão - foram recolhidos e compilados numa base de dados. Os dados climáticos foram obtidos a partir de estações meteorológicas automáticas (pertencentes à Sogrape Vinhos S.A.) e a partir de uma base de dados climática estimada (fornecida pela UTAD) numa grelha de resolução muito elevada (<1 Km). A influência das variáveis climáticas - temperatura média (TG), máxima (TX) e mínima (TN) mensal (novembro-outubro) e totais de precipitação (abril a junho e julho a setembro) - nos parâmetros de qualidade do bago foi explorada através de análises de correlação. Esta abordagem constituiu um ponto de partida para identificar os meses/períodos mais influentes na composição do bago, bem como as vinhas mais afetadas. Depois seguiram-se diferentes abordagens estatísticas para explorar em detalhe a influência das variáveis climáticas nas vinhas anteriormente identificadas como as mais afetadas.

Os resultados mostraram que, à maturação tecnológica, a temperatura foi negativamente correlacionada com o peso do bago, AT, antocianinas e IFT, mas positivamente com o pH e o AP. Além disso, nas regiões mais quentes, o peso do bago foi menor, e a AT, antocianinas e IFT seguiram a mesma tendência, enquanto nas regiões mais frias o pH e o AP foram menores do que nas regiões mais quentes. Uma análise de agrupamento mostrou diferenças significativas de temperatura entre os anos em que o parâmetro de qualidade foi maior (grupo 1) e os anos em que foi menor (grupo 2). Adicionalmente, provou-se que as diferenças de temperatura entre grupos resultaram de diferenças entre os anos mais quentes e os mais frios. Mostrou-se ainda que as temperaturas na fase de pintor e na maturação (de junho a agosto) são mais determinantes na composição do bago à maturidade. Relativamente à variável climática precipitação, os resultados mostraram que o seu efeito foi dependente da vinha e da variedade. Os resultados mostraram ainda que a composição do bago das vinhas do Alentejo é mais afetada pela variabilidade climática inter-anual do que as vinhas do Douro. Além disso, a variedade Aragonez parece ser mais resistente às variações climáticas do que a Touriga Nacional.

Os resultados obtidos sobre o papel da variabilidade climática nos parâmetros de qualidade do bago da uva obtidos no presente trabalho constituem uma ferramenta importante para o desenvolvimento de modelos de previsão. Esses modelos podem prever mudanças na composição do bago em resposta às alterações climáticas.

Palavras-chave: clima, variabilidade, maturação, bago da uva, qualidade, Touriga Nacional, Aragonez.

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ABBREVIATIONS AND ACRONYMS

ARA   Aragonez

AWS   Automatic Weather Station

GST   Growing season temperature

IPCC   Intergovernmental Panel on Climate Change

PA   Probable alcohol

SV   Sogrape Vinhos S.A.

TA   Titratable acidity

TG   Average temperature

TN   Minimum temperature

TON   Touriga Nacional

TPI   Total phenols index

TSS   Total soluble solids

TX   Maximum temperature

UTAD   Universidade de Trás os Montes e Alto Douro

1. Introduction

1. INTRODUCTION

Grapevines are one of the most economically important fruit species worldwide. In 2017, world's vineyard area was of 7.5 million ha and Portugal ranked in fifth and eleventh places as wine producer in Europe and worldwide, respectively (OIV, 2017). Almost all world wine is produced from *Vitis vinifera* L. native to the area south of the Caucasus Mountains and the Caspian Sea. Nowadays, wine is an integral component of the culture of many countries, a form of entertainment in others, and its health benefits, when consumed moderately, have been recognized (Conde et al., 2007). Wine's attraction relies not only on strong consistent flavours, but upon a delicate array of shifting sensations that make its charm difficult to define (Bisson et al., 2002).

The wine quality is strongly influenced by berry chemical components and their concentration at harvest (Fontoin et al., 2008). Berry composition is influenced by factors such as genotype, agricultural practices, soil type and climate, with the latter being the most dominant factor (Gladstones, 1992). Therefore, it is of great interest for the wine industry to understand the role of climate variability on grape berry composition to develop and implement optimized agronomic/oenological strategies.

In this context, this master's thesis was developed in the company Sogrape Vinhos S.A. (SV) taking advantage from the access to unique data about the Portuguese vineyards and climates. Grape berry composition data are available since 1991 in some vineyards and climatic data, provided from a network of automatic weather stations (AWS), from 2011 to 2015. We hope this work will be of interest to SV because the scientific knowledge generated will facilitate the implementation of stress mitigation agronomic practices while reducing water, fertilizers and pesticides usage. Also, a better knowledge of the impact of the environment on grape berry quality attributes may potentiate the development of decision support tools for assessing climate change impacts on future climates.

1.1 Importance of climate change upon the global production of winegrapes and wine

Climate is a key factor in the success of all agricultural systems. In viticulture, climate impacts crop production and grape quality, as well as the suitability of a given region for grapevine culture. Thus, the average climatic conditions of wine regions determine the grape cultivars that can be grown there, while wine production and quality are mainly influenced by site-specific factors, management decisions and short-term climate variability (Jones, 2012). Generally, the suitability of a region for grapevine culture is controlled by temperature that should range between 12 and 22 °C for the growing

season (Gladstones, 1992). Large areas of eastern Europe, western Asia, China, the mid-western and eastern United States, south-eastern Argentina, south-eastern South Africa and Southern Australia fall within this temperature range. However, other limiting factors, such as winter minimum temperatures, spring and fall frosts, short growing seasons, and water availability would limit much of these areas mapped (figure 1) (Jones et al., 2012).
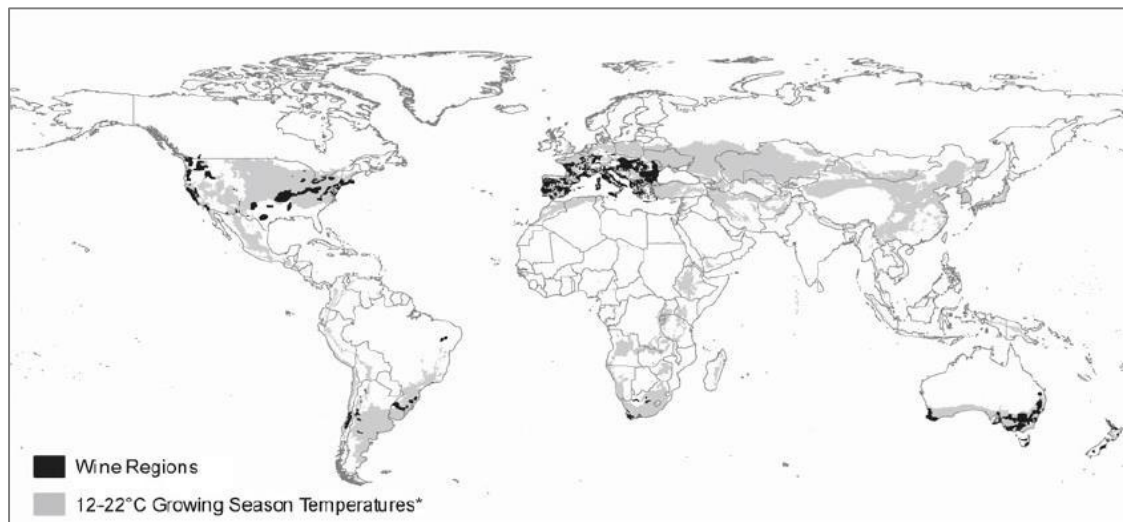
Figure 1. Global wine regions and 12-22 °C growing season temperature zones (Apr-Oct in the Northern Hemisphere and Oct-Apr in the Southern Hemisphere (Jones et al., 2012).

Wine can be defined by its character, quality and personality. Character comes from the *terroir* – all natural conditions such as the climate, the soil and the landscape, which influence the vine biology and thus the composition of the grape itself, – while the wine quality is largely determined by the winemaker. Wine personality is highly dependent on the weather (Halliday and Johnson, 1994). Therefore, although viticulture/oenological practices have a strong influence on wine production and quality (Santos et al., 2013), climatic conditions, namely air temperature and precipitation, are the main factors influencing grape berry and wine quality (van Leeuwen et al., 2004).

Air temperature plays a central role in determining grapevine phenology (Bonada and Sadras, 2014), influencing the onset of phenological stages like bud break, flowering and *véraison* and the phenophase intervals (Fraga et al., 2016). Earlier phenological timings and shorter intervals under future warmer climates are expected (Webb et al., 2007). Other studies have shown that temperature also affects grapevine yield (Bock et al., 2013), wine production (Santos et al., 2013) and quality (De Orduna, 2010).

The main measurable parameter of climate change is a steady global increase in temperature. According to Intergovernmental Panel on Climate Change (IPCC), global temperature is predicted to

increase by from 1 °C to 3.7 °C until the end of the century, compared to the reference period 1985-2005. The total annual amount of precipitation and its distribution are much more uncertain than temperature predictions. IPCC expects increasing water deficiencies in most grape growing regions. Moreover, it is likely that modifications in rainfall will differ from one region to another (IPCC, 2014). However, a warmer climate is also a dryer climate, even when rainfall does not decrease (van Leeuwen and Darriet, 2016). Model projections show that the warming trend in wine regions worldwide is expected to continue in the future (IPCC, 2014), so it is expected that the ripening phase will occur at higher temperatures with increasing frequency, which may impair grape berry and wine quality. In addition, spatial modelling research has also demonstrated potential shifts in the geography of viticulture regions. It is predicted that parts of southern Europe will become too warm to produce high-quality wines, while northern regions will become more stable in terms of consistent ripening climates (Kenny and Harrison, 1992; Jones, 2012).

1.2   Climate variability

While the average climate structure in a region determines the broad suitability of winegrape cultivars as already mentioned, climate variability influences production and quality risk associated with how equitable the climate is year in and year out. Cold temperature extremes during the winter, frost frequency and severity during the spring and fall, high-temperature events during the summer, extreme rain events, and broad spatial and temporal drought conditions are events resulting from climate variability and they influence grape and wine production (Jones et al., 2012).

The inter-annual and inter-seasons temperature variability determines the quality of the wine from a vintage. In cool climates, the warm years are the best, resulting in above-average vintage, because the faster the rate of ripening of grapes between the *véraison* and harvest, the better will be grape berry quality parameters; accordingly, warm years decrease the number of good vintages in hot climates (Gladstones, 1992). Besides that, short-term temperature variability may significantly affect viticulture. The short-term temperature variability includes diurnal fluctuations (the range between day maxima and night minima) and day-to-day fluctuations within a given season (Ashenfelter and Storchmann, 2014).

Relatively constant and intermediate temperatures during ripening favour the biochemical processes of colour, flavour and aroma development in the grape berries. Provided that the substrate sugar is available, and temperatures are favourable for enzyme activity, these processes will occur continuously. However, excessive day and low night temperatures can negatively influence these

processes. In fact, physiological ripening should be rapid and complete for any given mean temperature, so that the time for environmental insults that impair normal berry development is reduced (Gladstones, 1992).

Portuguese wine production is characterized by a strong inter-annual variability, with adverse consequences for wine business (Cunha et al., 2003). Therefore, understanding the impact of the environment on grape berry quality attributes is of utmost practical importance as it may assist better agricultural management and oenological practices, with efficiency gains for the winemaking sector. Furthermore, a better knowledge of these processes may potentiate the development of an operational forecast system to assess climate change impacts in future climates, thereby reducing instability in production.

1.3 Grape berry development and key solutes accumulation

In order to understand the possible impact of climate variability and change on grape berry quality attributes, it is important to understand how and when specific metabolites accumulate and how the metabolism responds to the environment. Moreover, the relative concentration of many compounds, which determine aroma and flavour along maturation is crucial for producing a specific wine.
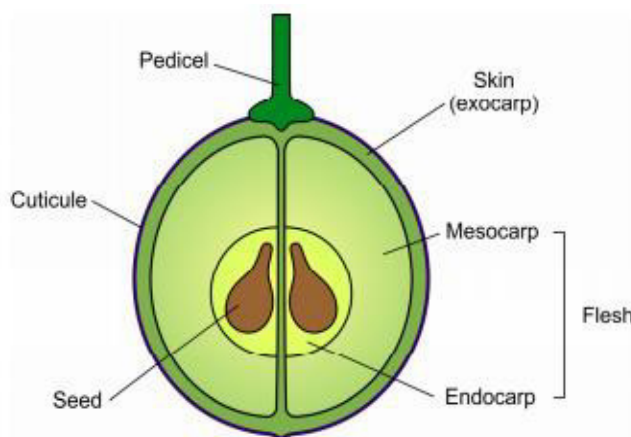
Figure 2. Structure of a ripe grape berry (Conde et. al, 2007).

Grape berries contain three major types of tissue: skin, flesh, and seeds (figure 2). Modifications in size, composition, colour, texture, flavour and in pathogen susceptibility occur with grape berries development (Conde et al., 2007).

The development of grape berry follows a sigmoid pattern (figure 3) with the first growth phase occurring from flowering to about 60 days later. At this stage, several solutes, namely organic acids, are

accumulated in the green berry. Tartaric acid is accumulated during the initial stages of berry development and malic acid is accumulated at the end of the first growth phase. Wine acidity is mainly conferred by tartaric and malic acid, which together may account for over 90% of titratable acidity of the berry (Ford, 2012). The balanced acid properties help the wine maintain freshness and determine their sensory quality. Moreover, grape juice with high pH often results in unstable musts and wines that are more susceptible to oxidative and microbiological spoilage (Conde et al., 2007).

The first growth phase of the berry is followed by a latency phase in which there is little or no growth.
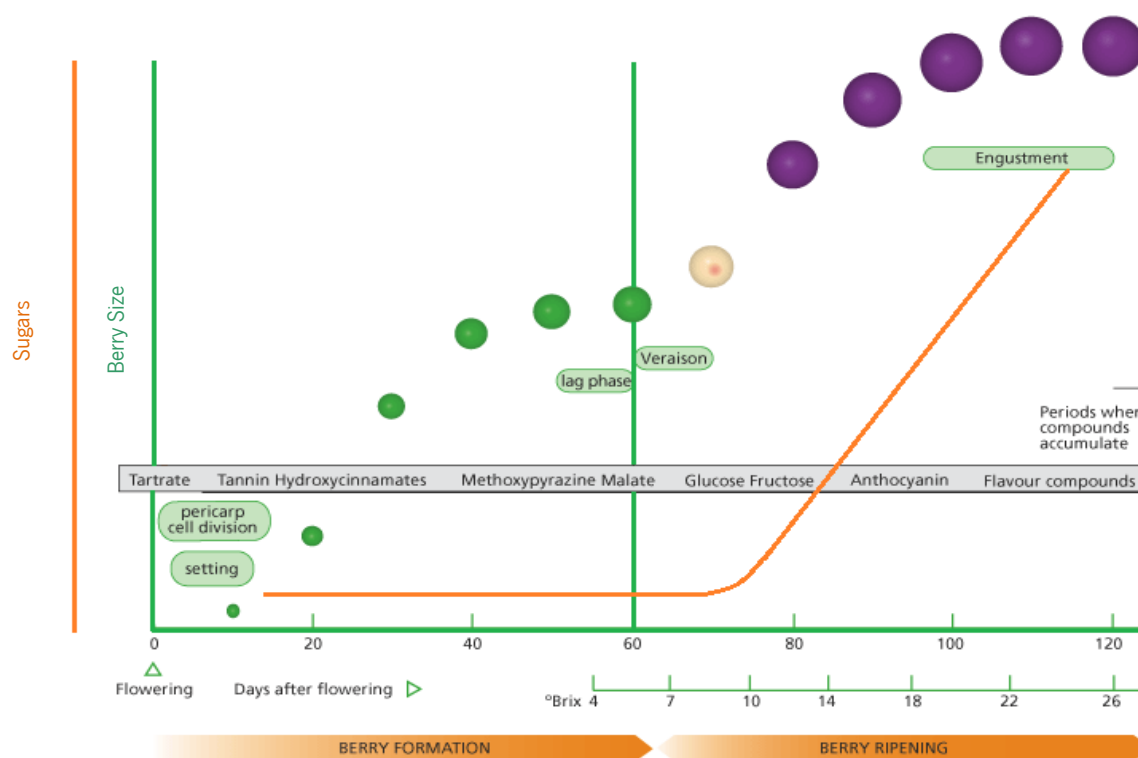
Figure 3. Grape berry development and ripening. Adapted from Kennedy (2002).

During the second growth phase, there is a massive accumulation of key quality solutes, namely sugars (glucose and fructose) and anthocyanins. The sugar content is one of the main indicators to be controlled during this phase. Its concentration increases during maturation until it reaches a maximum. After a plateau, sugars are then concentrated by dehydration of the berry. Thus, the berries switch from a status where they are acidic and with little sugar to a status where they are sweeter and less acidic (Conde et al., 2007; Teixeira et al., 2013). The acid/sugar balance is fundamental to build grape flavour, which determines the quality of final wine. Berry sugar concentration is the major contributor for the final alcohol content of the wine (Davies et al., 2012) and one of the most accessible indicators

of grape and wine quality. Moreover, the sugar status is also important to other grape and wine attributes like colour, flavour and texture, because sugars are precursors of anthocyanins and other phenolic compounds (Pirie and Mullins, 1977; Larronde et al., 1998).

Phenolics are a diverse group of secondary products involved in the protection against biotic and abiotic stresses, and in the response to diverse environmental cues (Castellarin et al., 2012). Berry phenolics contribute to wine quality given its prominent role in determining the colour, flavour, texture and astringency of wine and its antioxidant properties (Teixeira et al., 2013). The phenolic composition depends on the variety and environmental conditions. Three main classes of phenolic compounds are synthesised in grape berries: phenolic acids, stilbenes, and flavonoids. The proanthocyanidins (condensed tannins) and anthocyanins belong to the class of flavonoids - C6-C3-C6 polyphenolic compounds in which two hydroxylated benzene rings (A and B) are joined by a chain of three carbon that is part of a heterocyclic ring (C). The oxidation state of the C ring determines the structural classes of flavonoids (Castellarin et al., 2012).

Proanthocyanidins are the most abundant class of phenolics in grape berries. Synthesis of flavon-3-ols (monomer) and proanthocyanidins begins in flowering phase and proceeds during the fruit growth initial phase. Thus, the content of these phenolics remains stable or decreases a little during fruit ripening (Castellarin et al., 2012). Flavon-3-ols and proanthocyanidins contribute to mouth-feel and smooth sensory perceptions, thereby having a significant impact on wine quality (Cheynier et al., 1997). Moreover, during maturation, proanthocyanidins evolve to softer and more pleasant tastes (Blouin and Peynaud, 2001).

Anthocyanins determine the red, purple and blue pigmentation of grape berries and are accumulated in berry skins of red varieties from the onset of *véraison* until full maturity. Anthocyanins composition is particular to each variety and relatively stable under different growing conditions, however, anthocyanin content is sensitive to environmental effects (Castellarin et al., 2012).

Grape quality is also highly dependent on berry size because changes in size may concentrate (if the size is reduced) or dilute (if the size is increased) berry constituents (Antolín et al., 2003). Changes in the cell number and/or cell volume, which are determined by cell division and cell expansion, respectively, lead to variations in berry weight (Fernandez et al., 2006). Factors such as genotype (Fernandez et al., 2006) and environmental parameters (Ojeda et al., 2001) affect berry weight through modifying cell division and/or cell expansion.

The above mentioned grape berry quality parameters – including total soluble solids (TSS) (namely sugars), anthocyanins, condensed tannins, total phenolics, total acidity, pH and berry weight -

are essential for the production of premium table wines (Francis and Newton, 2005). However, as already mentioned, grapevine growth and development are largely controlled by atmospheric and microclimatic conditions, so berry quality attributes described suffer undesirable irregularity and inter-annual variability.

1.4 Impact of environment on grape berry quality parameters

Grapevines are characterized by phenological growth stages, which are determined by growing season temperatures. In the Northern hemisphere, temperatures should be below 10 °C during the dormancy stage. Winter dormancy affects the vine´s vegetative and berry development and helps synchronizing their growth stages. Temperatures above 10 °C in spring, end the dormancy of the vine and initiate bud break. Accumulation of grape berry compounds is more sensitive to temperature in certain phenological stages. For instance, during *véraison* and ripening, temperature has the strongest impact (Gladstones, 1992). Also, accumulation of TSS is more sensitive to temperature during the onset of accumulation, being less responsive during most of the season (Dai et al., 2011). In contrast, malate accumulation responds to temperature during most of the season (Bonada and Sadras, 2014).

Vine water availability is closely related to the temperature, although other factors are involved. The water balance of vine is mainly determined by the local precipitation, atmospheric humidity and soil water holding capacity (Gladstones, 1992). As few vines in Europe are irrigated, the overall quantity of annual rainfall and its distribution over the growing and non-growing season is crucial. Generally, abundant rainfall during flowering and ripening phases are considered harmful, but during winter and the early vegetative stage, are beneficial. Moreover, under low relative humidity or dry atmospheric conditions, stomata can close in order to conserve moisture, and photosynthesis ceases (Ashenfelter and Storchmann, 2014).

Some reported effects of water stress on grape berry quality attributes are contradictory and/or are strongly dependent on the degree of water stress and on the period in which it is applied. Temperature and water availability effects on the accumulation of grape berry compounds is explored below. Table 1 summarizes these effects.

Phenolic content

Although the mechanisms of phenolic biosynthesis at high temperatures are still poorly understood, high temperature limit for metabolic processes in grapevines is thought to be around 30°C

(Coombe, 1987). It was also reported (Poudel et al., 2009) that optimum temperature for phenolic accumulation is 25 °C; above or below this level, accumulation falls significantly. Therefore, phenolic compounds accumulation decreases in very low and very high temperatures. Nevertheless, further studies are in need to support this thesis.

Regarding the anthocyanins, these are accumulated in berry skins of red varieties from the onset of *véraison* until full maturity and the accumulation process is influenced by environmental effects (Downey et al., 2006; Castellarin et al., 2012). The effect of temperature on anthocyanin content of grape berry has been a target of study over the years in different varieties and regions (Cacho et al., 1992; Ubalde et al., 2010; Lorrain et al., 2011; Barnuud et al., 2013).

Mature berries from Cabernet Sauvignon, Shiraz and Chardonnay varieties cultivated in colder sites accumulate higher levels of anthocyanins than those from warmer climates (Barnuud et al., 2013). High temperatures can reduce anthocyanin accumulation through the impairment of its synthesis (Mori et al., 2005) and/or through the acceleration of its degradation (Mori et al., 2007). In the berry skin of Darkridge (*Vitis vinifera* L. x *Vitis labrusca* L.), the PAL and UFGT activities, two enzymes of anthocyanin biosynthetic pathway, were lower in plants cultivated at high night temperatures (30 °C) than at low night temperatures (15 °C) (Mori et al., 2005). Additionally, the expression level of anthocyanins biosynthetic pathway genes at *véraison* also decreased, although gene expression was not affected in the later stage of ripening.

Under water stress, phenolic compounds, including anthocyanins, can be concentrated because berry size is reduced. On the other hand, depending on the type of phenolic compound, the severity of water deficit and the period when water deficit occurs, the biosynthesis of phenolic compounds can be either positive or negative (Ojeda et al., 2002). A strong water deficit between *véraison* and maturation increases anthocyanins (Matthews and Anderson, 1988; Ojeda et al., 2002; Castellarin et al., 2007), proanthocyanins and flavonols biosynthesis without affecting total flavan-3-ols biosynthesis (Ojeda et al., 2002). On the other hand, enhanced water availability early in the growing season is beneficial for anthocyanins content because it promotes vegetative growth and, consequently, berry development and its composition at later stages (Keller et al., 2010). However, improved moisture availability late in the growing season is often described to reduce anthocyanins content (Jackson and Lombard, 1993).

Total soluble solids content

As referred elsewhere, berry sugars concentration is the major determinant of final alcohol content in wine (Davies et al., 2012). High temperatures promote sugar accumulation and thus

potential alcohol level (Coombe, 1987; Keller et al., 2010). During growing season, the highest sugar content is associated with drier years, while a lowest is related to rainier years (Ubalde et al., 2010). This can be explained by the effect of water limitation on plant hormonal equilibrium and berry weight - responsible for directing sugar accumulation in grape berries. On the other hand, it was shown that TSS accumulation was greater at higher temperatures (30 °C) than lower temperatures (20 °C) at *véraison* and a few days after *véraison* (Poudel et al., 2009)*,* in agreement with previous results (Mori et al., 2005).

In irrigated conditions, TSS concentration can be decreased due to the dilution caused by greater berry size (López et al., 2007). In contrast, irrigated vines are associated with higher photosynthetic activity when compared with water-deficit vines, and consequently, the former have improved sugar accumulation (Bartolomé, 1993).

Titratable acidity

Titratable acidity levels are lower in warmer years than in colder years (Ubalde et al., 2010), and it was shown that some of the strongest and most consistent negative associations of TA with temperature regarded maximum temperatures (Barnuud et al., 2013). In general, respiration of tartaric and malic acids, particularly malic acid, increases with increasing temperatures leading to a drop in titratable acidity and increased pH (Buttrose et al., 1971; Ruffner et al., 1976).

Total acidity is normally reduced by the end of *véraison* due to malic acid respiration and dilution of acids caused by increased berry size, but total acidity values are significantly higher under irrigation than in non-irrigated conditions (López et al., 2007). Moreover, higher levels of malic acid measured under irrigation may be associated with higher malic acid concentrations at the start of ripening, due to greater synthesis during the growth period (López et al., 2007). TA increases with rainfall at maturity (Barnuud et al., 2013). This can be explained by indirect influences via the lowering of air temperature with an increase in rainfall and increased vegetative growth with greater moisture availability, both of which would favour TA levels. It was found that partial irrigation (simulating predicted climate changes) decreased malic acid concentration (Parra et al., 2010), which agrees with the studies reported above.

Table 1. Effect of temperature and precipitation/irrigation on grape berry quality parameters (sugars, anthocyanins, phenolics and acidity) in different phenological stages.

C. V.	Condition	Berry trait	Phenological stage	Response	Reference
Temperature	Driest years	Sugars	During growing season	A	(Ubalde et al., 2010)
	30 °C	Sugars	At *véraison*	A	(Poudel et al., 2009)
	Warmer sites	Anthocyanins	At maturity	↙	(Barnuud et al., 2013)
	30 °C	Anthocyanins	During ripening	↙	(Mori et al., 2005)
	35 °C	Anthocyanins	During ripening	↙	(Mori et al., 2007)
	> 25 °C	Phenolics	During ripening	↙	(Poudel et al., 2009)
	Warmer years	Acidity	During ripening	↙	(Ubalde et al., 2010)
	Warmer sites	Acidity	At maturity	↙	(Barnuud et al., 2013)
Precipitation/ Irrigation	Irrigation	Sugars	During maturation	↙	(López et al. 2007)
	Irrigation	Sugars		↗	(Bartolomé, 1993)
	Water deficit	Phenolics	Between flowering and *véraison*	↗	(Ojeda et al., 2002)
	Water deficit	Phenolics	Between *véraison* and maturity	↗	(Ojeda et al., 2002)
	High P	Acidity	At maturity	↗	(Barnuud et al., 2013)
	Water deficit	Acidity	At maturity	↙	(Parra et al., 2010)
	Irrigation	Acidity	During maturation	↗	(López et al., 2007)
	High P	pH	At maturity	↙	(Barnuud et al., 2013)

↙ - the quality parameter is reduced; ↗ - the quality parameter is increased; A – the quality parameter is accumulated; C. V. – Climate variable; P – precipitation.

1.5 Economic and social impacts of climate changes on vineyards and wine industry

As reported before, grapevine is very sensitive to fluctuations in weather, impacts of global warming on plant physiology and berries' chemical composition are reflected on yield and on grape berry quality and, consequently, wine quality. Changes in yield, quality, prices, revenues and profits can be seen in the short term. Other impacts will materialize in the long term such as scarcity of resources, changes in wine geography and changes in land values, which leads to the need for new policies and adaptation measures (Pomarici and Seccia, 2016).

The equilibrium between climate, soil and grape variety influences the production of high-quality wines as well as grapes yield. In fact, a suitable climate is crucial to characterize the region´s wine styles, so increased warming with a change in rainfall regime can affect wine quality and can cause loss of wines specificity (Moriondo et al., 2013). Thus, wines price also suffer from climate variability, so any variation in weather results in price fluctuation (Chevet et al., 2011). Revenues and profits of grape growers and winemakers will differ among regions because yield, quality, prices, and costs to bear for the adaptation strategies are dependent of climate variability in different regions (Pomarici and Seccia, 2016).

Climate change and variability can raise important long-term issues for the wine industry, particularly in relation to the scarcity of resources. Water management in semiarid areas will probably

be a problem, so new vineyard irrigation techniques will be necessary in order to respond to grapevine water demand caused by high temperatures. Other adaptation strategies will be in order to reduce climate change effects, e.g., changing varieties to adapt to warming temperatures, combined with a need for more information among winegrowers (Pomarici and Seccia, 2016). Changes in the geography of viticulture will occur, some regions will suffer from further warming while others will benefit. Consequently, some areas may be abandoned because they are no longer considered viable for the growth of vineyards. On the other hand, this can lead to an expansion of other suitable areas that could influence global supply and change trade patterns (Ashenfelter and Storchmann, 2014).

The ability of producers to adapt to a changing climate in order to maintain profitability is strongly influenced by social, economic, and political circumstances, including economic capital, education, institutional arrangements and organizational abilities (Hadarits et al., 2010). However, a deep knowledge of future impacts and their main causes can minimize these impacts through better wine management.

1.6   Objectives

Grapevine growth and development are largely controlled by atmospheric and microclimatic conditions, leading to undesirable irregularity and inter-annual variability in berry composition. Thus, the aim of this work was to determine the responses of some key berry attributes – berry weight, titratable acidity, pH, probable alcohol, anthocyanins and total phenols – that are influential for wine quality to changing levels of climate variables.

For this purpose, the study was carried out in vineyards of Douro, Dão and Alentejo for two native varieties with a strong national importance – Touriga Nacional (TON) and Aragonez (ARA). Firstly, a maturation database was created, including quality parameters of grape berry used to assess berry maturation. The data were collected from different vineyards belonging to SV and from farmers associated with SV, located in the main Portuguese wine regions – Alentejo, Bairrada, Douro, Dão and Vinhos Verdes. Next, collection and analysis of climatic data were carried out. These data are available throughout Portugal (provided by UTAD), on a very-high resolution grid (< 1 km), and from a network of automatic weather stations, covering in a total around 750 ha of vineyard's area over the main Portuguese wine growing regions (provided by Sogrape Vinhos S.A.). Lastly, different statistical approaches were tested and applied to correlate berry quality attributes and atmospheric variables (temperature and precipitation).

Therefore, understanding the impact of environment on berry quality parameters is of utmost practical importance as it may assist better agricultural management and oenological practices, with efficiency gains for the winemaking sector. Thus, in the present study, we aimed to obtain a better knowledge of these processes that may leverage the development of decision support tools for assessing climate change impacts in future climates.

2. Material and Methods

2. MATERIAL AND METHODS

2.1 Maturation database

Grape quality data were collected annually, at different time intervals, in five different winemaking regions: Alentejo, Bairrada, Douro, Dão and Vinhos Verdes (figure 4). The data were collected from vineyards of Sogrape Vinhos and from private farmers associated with this company. In each year, most analyses were performed weekly, two to three weeks after the end of the *véraison* until harvest. All the data were compiled into an EXCEL database (Microsoft Corporation, Redmond, WA, EUA) and validation of wet chemical data was performed. The database was then validated and consolidated. The number of vineyards, vine blocks, varieties and the analysed years for the different regions are indicated in table 2. The method of bunch sampling and analysis of berry composition used to obtain these data is presented below.
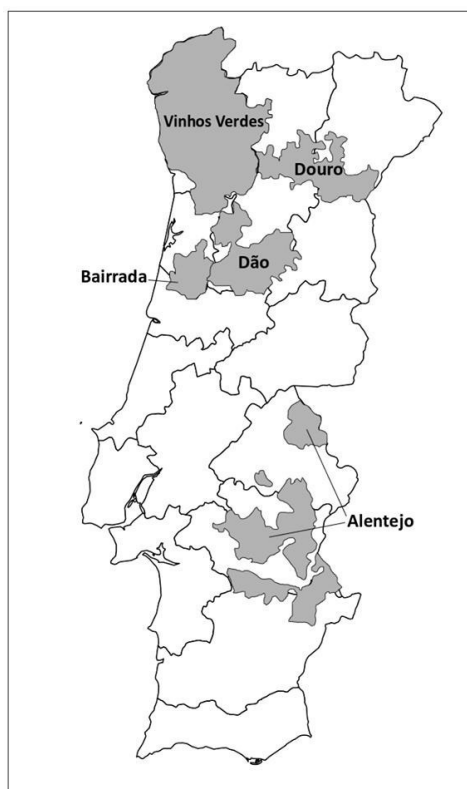
Figure 4. Location of winemaking regions where data were collected. The borders of the winemaking regions in Portugal are delimited as defined by the Portuguese '*Instituto da Vinha e do Vinho (IVV)*'.

Bunch sampling

The sampling method was adapted from previous reports (Blouin and Peynaud, 2001). Bunches were sampled manually, switching randomly between the side exposed to the sun and the shaded side of canopies. Figure 5 shows a sampling site (vine marked in red) in Douro-A vineyard. Berries were sampled randomly from the top to the tip of each bunch and placed in closed plastic bags and stored in refrigerated and insulated boxes during transport to the laboratory. Analyses of grape berry quality parameters commonly selected for maturation control were performed up to 24 h after collection.

Figure 5. Douro-A vineyard. Sampling sites are marked in red. Photo taken on 24/04/18.

Berry composition analysis

Collected berries were divided into two sets of 200 berries each: one for analysis of berry weight, sugars and acids and another for phenolic compounds analysis. Two hundred berries were weighed and then crushed and decanted. The obtained grape must was used to evaluate sugars, acidity and pH. For sugar analysis, results were expressed in terms of probable alcohol (PA) percentage, considering 1% of probable alcohol as equivalent to 17 g/L of total sugars. Titratable acidity was analysed by titration of the sample with 0.1 M NaOH solution and the results were expressed as g of tartaric acid equivalents per L of juice (g/L). pH was analysed with a digital potentiometer. pH is an important indicator of microbiological stability of musts: grape juice with a high pH often results in biologically unstable musts and in wines that are more susceptible to oxidation and microbiological spoilage (Conde et al., 2007).

Phenolic compounds analysis was carried out with the second sample of 200 grape berries and the results were expressed in terms of extractable anthocyanins (mg/L) (a measure of easily extractable

anthocyanins at a pH value similar to the pH of the must) and total phenols index (TPI). TPI is an indirect index of the total polyphenolic abundance in grapes and correlates with other phenolic compounds such as phenolic acids and flavonoids, in addition to anthocyanins. In some cases, other parameters were evaluated: assimilable nitrogen (mg/L), copper (mg/L) and malic acid (g/L).

This work was performed along the years before the present project was set. In the present study, we collected and treated the results obtained over the years indicated in table 2.

Table 2. Regions and subregions, number of vineyards, vine blocks and varieties in each subregion in which annual maturation analysis was performed. The seasons from which maturation data were obtained are also indicated.

Region	Subregion	Vineyards	Vine blocks	Varieties	Years
Alentejo	Vidigueira	7	33	15	2002-2017
Bairrada	Bairrada	18	48	14	2011-2017
Dão	Besteiros	2	2	5	2013-2014
	Castendo	1	1	4	2013-2014
	Serra da Estrela	3	2	5	2013-2014
	Silgueiros	4	3	6	2013-2014
	Terras da Azurara	2	25	8	2008-2017
	Terras de Senhorim	2	2	4	2013-2014
Douro	Baixo Corgo	12	26	9	1991-2017
	Cima Corgo	29	151	26	1991-2017
	Douro Superior	21	80	8	1991-2017
Vinhos Verdes	Ave	1	1	3	2012-2016
	Cávado	22	93	4	2007-2017*
	Lima	5	15	1	2007-2017*

(*) Missing the year 2010.

2.2 Selection of study regions

After assembling the maturation database, a restricted subset of maturation data was selected for correlation with climatic data. Vine blocks of two varieties – Touriga Nacional (TON) and Aragonez (ARA) - were carefully chosen in Douro, Dão and Alentejo regions to study the dynamics of berry weight, titratable acidity, pH, probable alcohol, total phenols index and anthocyanins at maturity. Touriga Nacional produces dark, full-bodied, powerful and aromatic wines with floral notes similar to the scent of purple violet blossoms. It frequently shows blackberry, blueberry, rock rose, and rosemary notes. Aragonez produces full-bodied, inky and highly aromatic wines. The grape has fine and delicate aromas of pepper and berries (Ali et al., 2011).

For a better correlation between climate variables and berry composition, vine blocks were selected based on two criteria: 1) vine blocks should be as close as possible to an automatic weather station and 2) maturity data sets should refer to ten or more years (except for the Alentejo region,

where information about Touriga Nacional variety was only available for six years) (table 3). A geographic information system (Quantum GIS) was used to visualize vine blocks of different regions, allowing the selection of those closer to an automatic weather station. This information had already been inserted previously in the software and was kindly provided by Sogrape Vinhos S.A. For reasons of confidentiality, the names of vineyards and vine blocks were anonymised.

As can be seen in figure 6, in a vineyard of Alentejo, the vine block 18 (Touriga Nacional) is close to AWS 2 and vine block 6 (Aragonez) is close to AWS 3. In these vine blocks a maturation database was previously built over a period > 10 years. The same criteria were used for Douro (vineyard A, B, C and D) and Dão regions and the selected AWS are indicated in table 3.
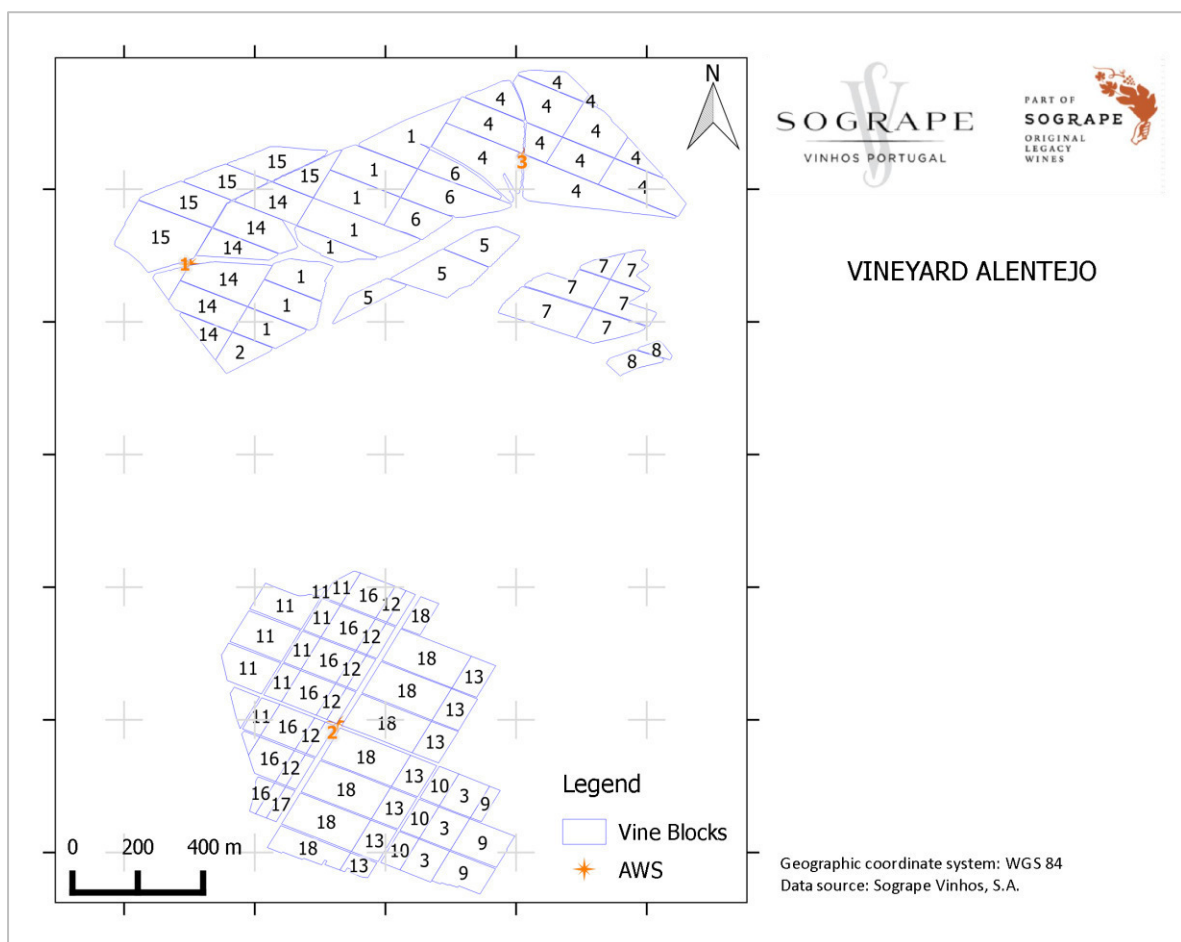
Figure 6. Identification of vine blocks in a vineyard of Alentejo, as well as AWS (1, 2 and 3). Obtained in QGIS.

The selected vine blocks in vineyard Douro-A were vine block 9 (TON) and vine block 7 (ARA) (figure 7).
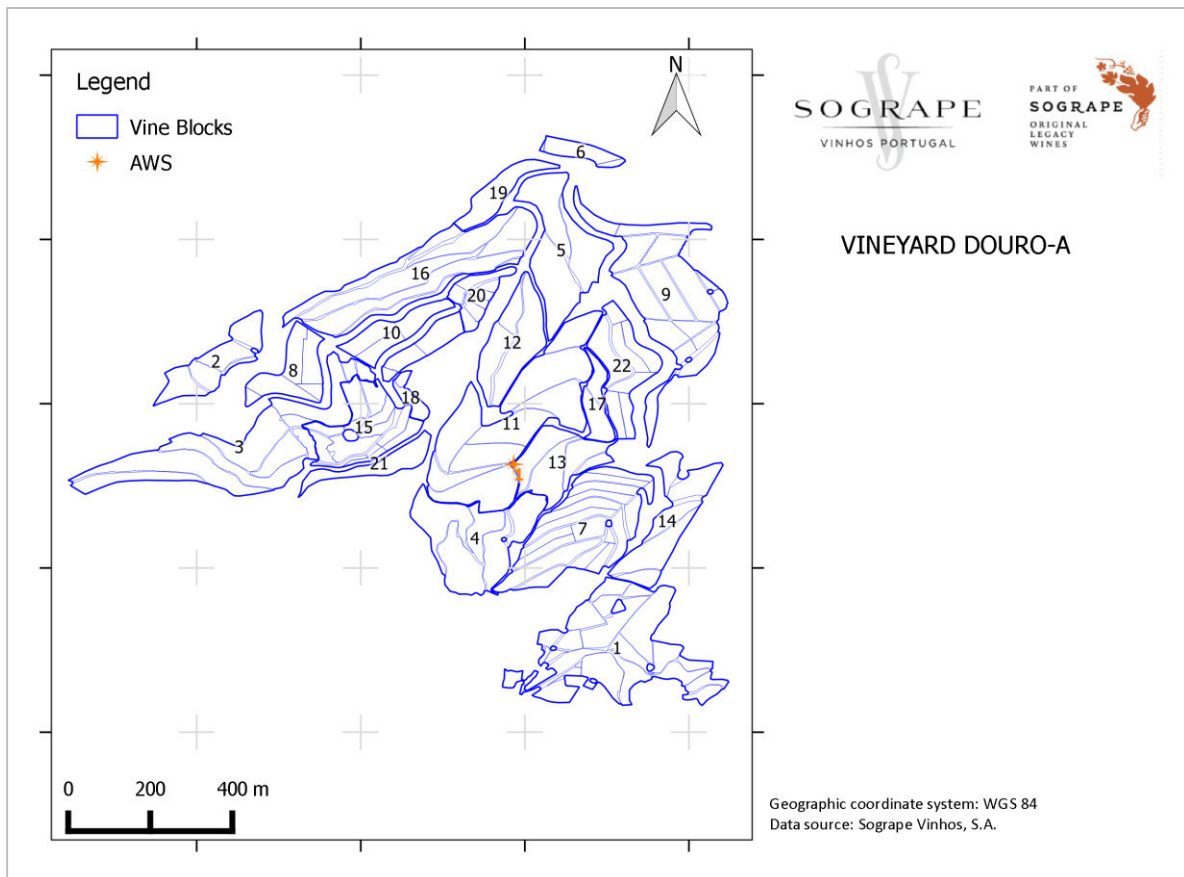
Figure 7. Identification of vine blocks in vineyard Douro-A, as well as AWS (1). Obtained in QGIS.

The selected vine blocks in vineyard Douro-B were vine block 1 (TON) and vine block 5 (ARA) (figure 8). For vineyard Douro-C (figure 9) the vine blocks 4 (TON) and 8 (ARA) were selected. Vine block 8 is closer to AWS 2 and vine block 4 is closer to AWS 1.
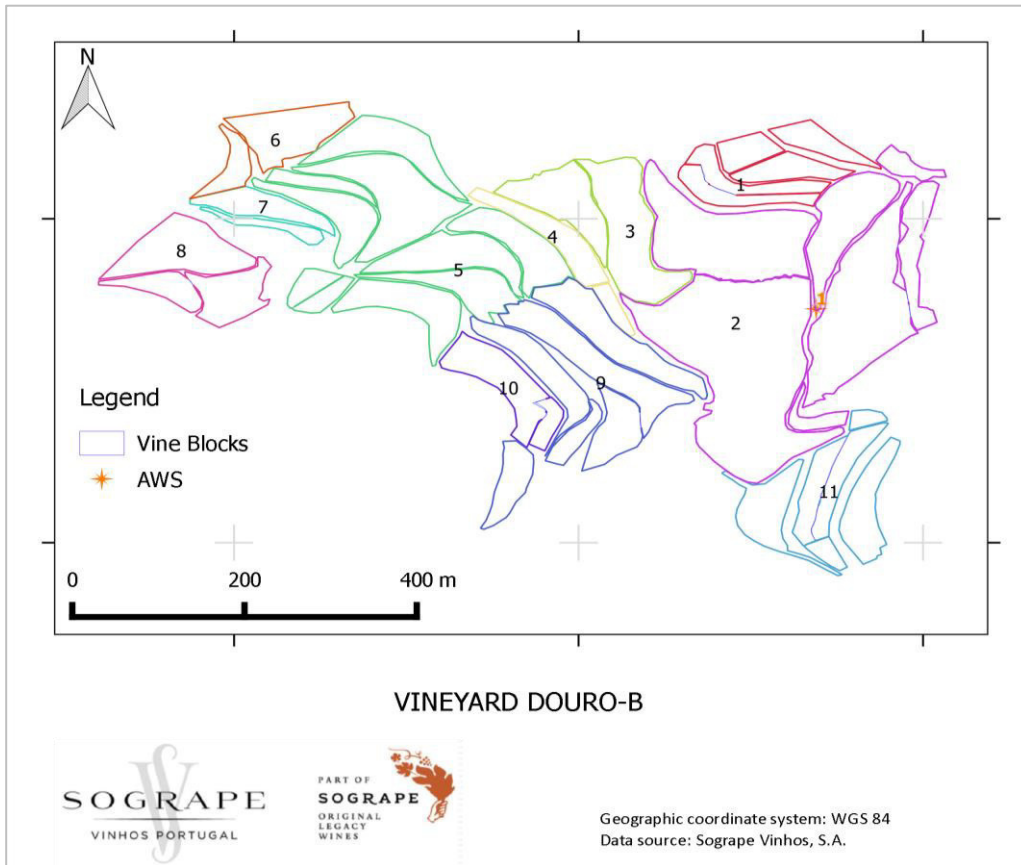
Figure 8. Identification of vine blocks in vineyard Douro-B as well as AWS (1). Obtained in QGIS.
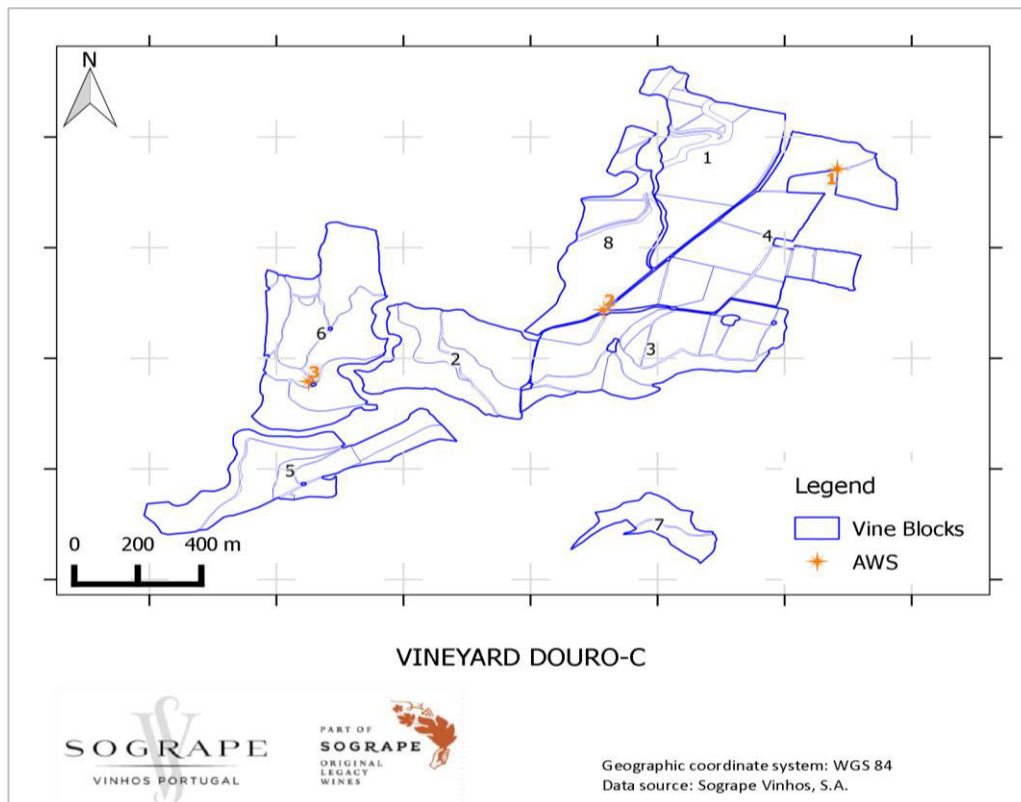
Figure 9. Identification of vine blocks in vineyard Douro-C, as well as AWS (1, 2 and 3). Obtained in QGIS.

In vineyard Douro-D (figure 10), the vine blocks chosen were 8 (TON) and 19 (ARA). Vine block 8 is closer to AWS 2 and vine block 19 to AWS 1.
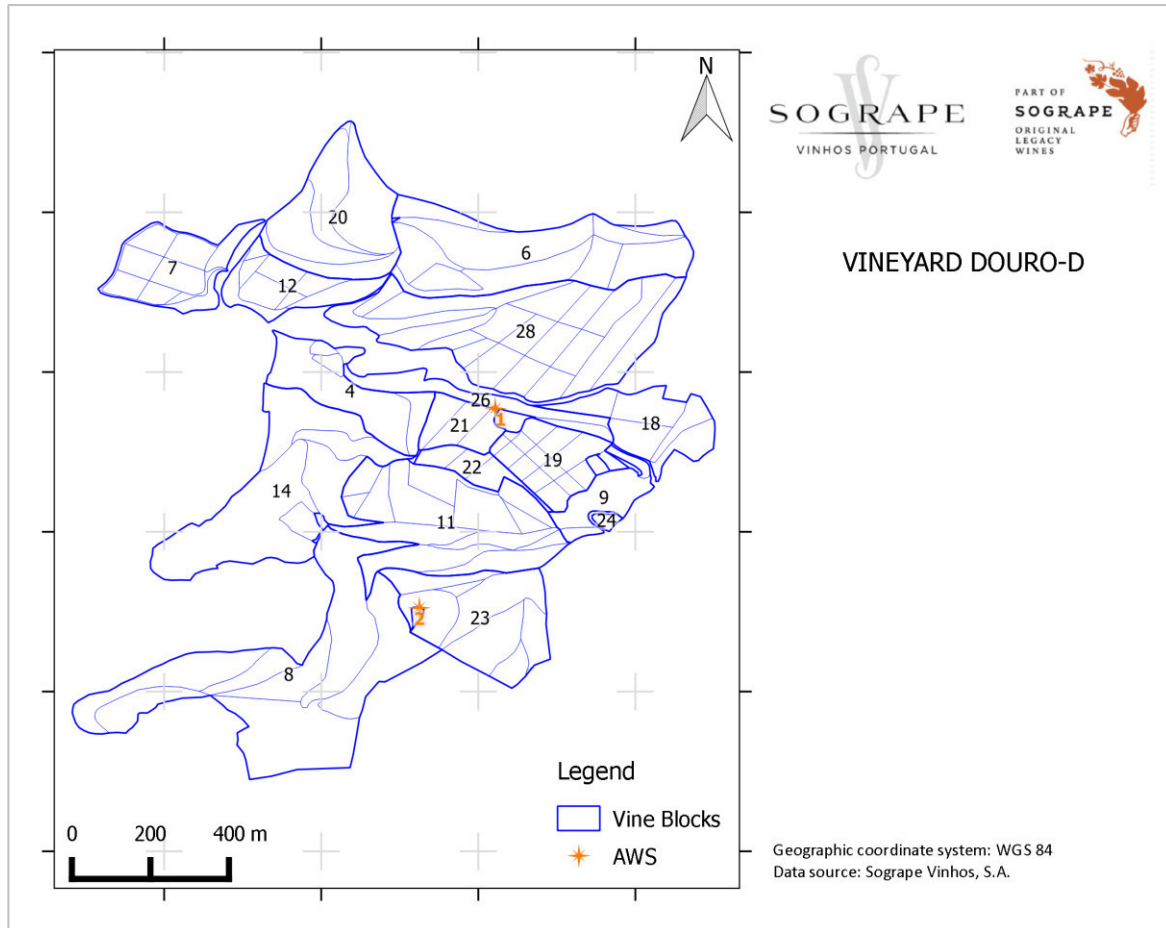
Figure 10. Identification of vine blocks in vineyard Douro-D, as well as AWS (1 and 2). Obtained in QGIS.

In a vineyard of Dão, the vine blocks chosen were 8 (TON) and 7 (ARA) which are close to AWS 2. Maturation data from these two vine blocks were recorded for 10 years (figure 11).
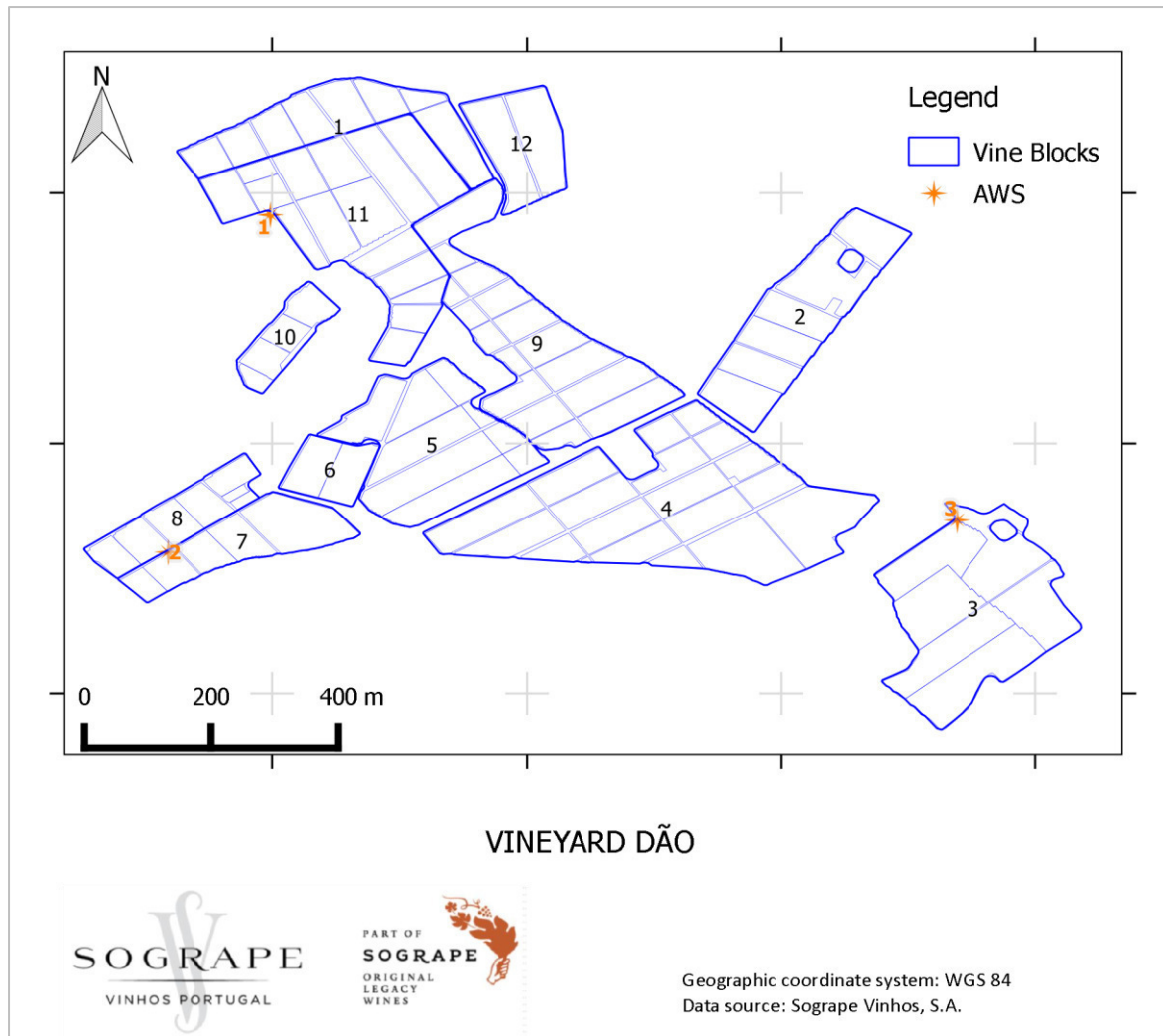
Figure 11. Identification of vine blocks in a vineyard of Dão, as well as AWS (1, 2 and 3). Obtained in QGIS.

Table 3. Maturation data selected for correlation with climatic variables in the present study. The regions, subregions, vineyards and vine blocks, as well as the range of years recorded, and the nearest AWS are presented.

Region	Subregion	Vineyards	Variety	Vine block	Years	Nearest AWS
Alentejo	Vidigueira	Vineyard Alentejo	TON	18	2012-2017	Alentejo 2
Alentejo	Vidigueira	Vineyard Alentejo	ARA	6	2004-2017	Alentejo 3
Dão	Terras da Azurara	Vineyard Dão	TON	8	2008-2017	Dão 2
Dão	Terras da Azurara	Vineyard Dão	ARA	7	2008-2017	Dão 2
Douro	Cima Corgo	Douro-A	TON	9	1991-2017	Douro-A 1
Douro	Cima Corgo	Douro-A	ARA	7	1991-2017	Douro-A 1
Douro	Cima Corgo	Douro-B	TON	1	1993-2017	Douro-B 1
Douro	Cima Corgo	Douro-B	ARA	5	1994-2017	Douro-B 1
Douro	Cima Corgo	Douro-C	TON	4	2007-2017	Douro-C 1
Douro	Cima Corgo	Douro-C	ARA	8	2007-2017	Douro-C 2
Douro	Douro Superior	Douro-D	TON	8	1999-2017	Douro-D 2
Douro	Douro Superior	Douro-D	ARA	19	1999-2017	Douro-D 1

TON – Touriga Nacional; ARA – Aragonez.

2.3 Climate data

Automatic weather stations close to the vine blocks previously selected in Alentejo, Dão and Douro (table 3) provided daily average (TG), minimum (TN) and maximum (TX) air temperatures, as well as daily precipitation totals for the period of 2011-2017. Climatic data prior to 2011 are not available since AWS´s were installed in 2010, so these data were obtained from an estimated climatic database by a research group of UTAD (PTHRES), available throughout Portugal on a very-high resolution grid (< 1 km).

For this purpose, statistical relations between observed daily average, minimum and maximum air temperatures and daily precipitation and the same estimated data (PTHRES) was established (in 2011-2015 period). The first ones were used as dependent variables in the linear regression models. As the determination coefficient was high in the linear regression models performed between estimated and observed temperatures, linear equations were used to estimate the TG, TN and TX prior to 2011 (table 4). The determination coefficient was lower in the linear regressions performed between estimated and observed precipitation totals, therefore, this method cannot be used for estimating daily precipitation totals before 2011 (table 5). The multiplicative method was used for this purpose. First, the average of observed and estimated monthly precipitation totals was calculated for each month of the 2011-2015 period. Then, the quotient between the observed data average and the estimated data average was calculated for each month and the daily precipitation totals data prior to 2011 were obtained by multiplying this quotient by the daily estimated data.

Table 4. Linear equations used to estimate TG, TN and TX prior to 2011. Determination coefficients of linear regression models are indicated.

AWS	Linear equation (TG)	R^2	Linear equation (TX)	R^2	Linear equation (TN)	R^2
Alentejo 2	y = 1.0001x + 0.1459	0.987	y = 1.0866x - 1.5017	0.994	y = 1.016x - 0.1484	0.990
Alentejo 3	y = 0.9929x - 0.4101	0.971	y = 1.0602x - 0.2323	0.992	y = 1.0098x - 1.7713	0.907
Dão 2	y = 1.0276x - 0.0435	0.955	y = 1.0833x - 0.6819	0.981	y = 0.9247x + 0.4509	0.902
Douro-B 1	y = 1.0204x + 0.5626	0.980	y = 1.0526x - 0.5688	0.974	y = 0.9597x + 0.3643	0.955
Douro-C 1	y = 0.9861x - 0.8406	0.980	y = 0.9954x - 1.8099	0.976	y = 0.9147x - 0.7594	0.937
Douro-C 2	y = 0.9698x - 0.1636	0.987	y = 0.9955x - 1.3195	0.979	y = 0.8953x - 0.1773	0.968
Douro-A 1	y = 0.9686x - 0.3989	0.977	y = 0.9686x - 1.4239	0.974	y = 0.9489x - 0.269	0.940
Douro-D 1	y = 1.0844x - 1.4776	0.979	y = 1.0841x - 0.5099	0.974	y = 1.0695x - 2.9495	0.954
Douro-D 2	y = 1.0772x - 1.4794	0.983	y = 1.0712x - 1.1463	0.975	y = 1.0418x - 1.9773	0.971

Table 5. Determination coefficient obtained from linear regression models between the estimated and observed total precipitation data.

AWS	R^2
Alentejo 2	0.917
Alentejo 3	0.574
Dão 2	0.810
Douro-B 1	0.728
Douro-C 1	0.716
Douro-C 2	0.767
Douro-A 1	0.773
Douro-D 1	0.506
Douro-D 2	0.615

2.4 Relationships of grape berry components with climate variables

In this study, influences of climate variables on grape berry composition were examined at maturity (last analysis carried out before harvest, up to two weeks before). Thus, the technological maturity level was established based on the winemaker's decision to harvest. The influence of climate variables (average, maximum and minimum air temperature and total precipitation) on berry weight, titratable acidity, pH, probable alcohol, total phenols index and anthocyanins at maturity during vine vegetative cycle (November-October) was explored through correlation analyses. This exploratory correlation analysis aimed to identify the climatic variables and critical periods that affect berry composition. Thus, correlation analyses were performed with monthly averages of TG, TX and TN and monthly precipitation totals between November and October. However, this approach was ineffective for precipitation, because rainfall days were not uniform over each month and in some months did not rain at all. For this reason, precipitation totals were used from April to June (flowering/fruiting phase) and from July to September (maturation phase) to study their influence on grape berry composition. Moreover, this preliminary analysis allowed to identify the regions more affected by inter-annual climate variability, and thus then study these regions in detail.

2.5 Maturation data clustering

Exploratory correlation analyses of climate variables and grape berry composition at maturity allowed to identify the average, maximum and minimum air temperatures of the months of June, July and August as determinants of grape berry quality. The results obtained for precipitation varied significantly between regions and varieties. In addition, this analysis showed that Douro (C and D), Dão

and Alentejo were the regions more affected by climate. Therefore, the subsequent analysis focused only on these regions and on the average, maximum and minimum air temperatures.

Each grape berry quality parameter was divided into two clusters by the median: years where the maturation parameter was higher (cluster 1) and years where it was lower (cluster 2) (annex I). The average of the two clusters for each grape quality parameter, region and variety studied, as well as the difference between the averages was calculated (annex II).

2.6   Temperatures analysis between maturation clusters

The difference between the monthly averages of TG, TX and TN for June, July and August between maturation clusters was calculated for Aragonez and Touriga Nacional varieties in Douro-C, Dão and Alentejo. This analysis allowed the identification of the temperature variation that may explain the average difference in grape quality attributes between clusters. For the largest monthly temperature differences found, daily temperature differences between clusters were calculated from June 1 to August 31. This analysis allowed for the identification of critical days/periods that may have contributed to the inter-annual variability of maturation parameters. Furthermore, the evolution of daily temperatures for each year of the two clusters was plotted from June 1 to August 31 to determine if differences between monthly temperatures found were the result of the difference between warmer and colder years.

2.7   Statistical analysis

The statistical significance of the difference between averages of the two maturation clusters for each parameter and monthly temperature differences between clusters was obtained through the Student's t-test in software R 3.4.3 (R core team 2017). For each grape quality parameter, cluster 1 averages of each region were compared to each other through by one-way ANOVA analysis in Prism® 6 software (GraphPad Software, Inc.).

3. Results

3. RESULTS

3.1 Relationships of grape berry quality parameters with climate variables

Exploratory bivariate correlation analyses between climate variables (precipitation and mean, maximum and minimum air temperature) during the vine vegetative cycle (November to October of the following season) and grape berry attributes at maturity allowed the identification of climate variables and critical periods that were influential on berry composition of Touriga Nacional and Aragonez varieties. This analysis was a starting point to identify inter-annual climatic variability influences on grape berry quality parameters, including berry weight, titratable acidity, pH, probable alcohol, total phenols and anthocyanins in the previously selected regions (figure 12).
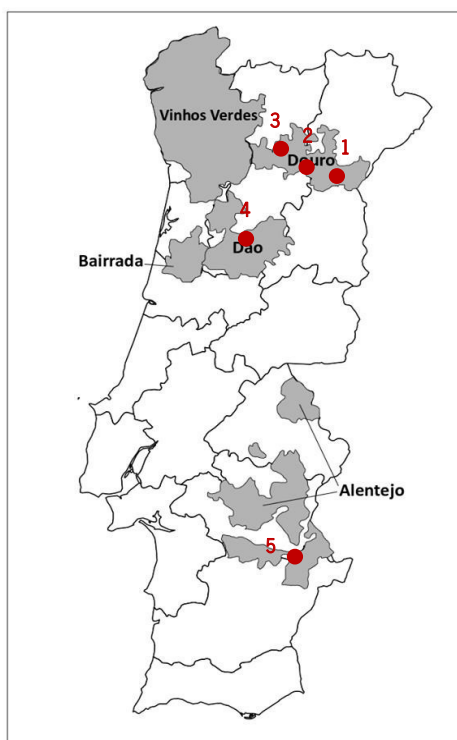
Figure 12. Wine regions selected to study the influence of climate variables in grape berry quality parameters. 1- Douro-D vineyard, 2- Douro-C vineyard, 3- Douro-A and Douro-B vineyards, 4- Dão vineyard, 5- Alentejo vineyard.

Correlation analyses showed that the analysed grape berry quality parameters were mostly influenced by the temperatures of June, July and August. Thus, in figure 13 and 14 only correlations between the temperatures of these months and grape berry quality parameters are presented. The correlations between monthly precipitation totals (November to October) and the grape berry quality parameters were not conclusive because precipitation days are not uniform over each month and in

some months does not rain at all. Thus, precipitation totals were used from April to June and from July to September to study the effect of precipitation on berry composition.

The correlations between the climatic variables and the grape quality parameters in Douro-A and Douro-B vineyards (Cima Corgo subregion) were very weak (data not shown). Therefore, in the following analyses, these two vineyards were not considered.

Berry weight

Touriga Nacional berry weight at maturity was negatively correlated with temperature in the four vineyards – Dão, Alentejo, Douro-C and Douro-D. Particularly, moderate to strong negative correlations were observed with average (-0.50 to -0.83) and maximum temperature (-0.56 to -0.81) in Alentejo, with August temperatures showing more influence in berry weight. Moreover, in Dão and Douro-C, moderate negative correlations were also found with average and maximum July temperatures. In Douro-D, correlations were weak for all temperatures and months analysed. In general, in all regions, the minimum temperature little influenced berry weight, compared with the average and maximum temperature (figure 13). Figure 13 also shows that precipitation during the maturation period (July to September) increased berry weight of Touriga Nacional, except in the Dão region. A strong positive correlation was observed in Alentejo (0.81) (figure 13).

The weight of the Aragonez berries was influenced by temperature much like the berries of Touriga Nacional but, in general, correlations were weak to moderate. Berry weight in Alentejo and Douro-C was mainly affected by the temperature of June, while in Dão, July temperature was more influential. In Douro-D very weak correlations were observed between temperature and berry weight. A strong positive correlation between berry weight and precipitation during the maturation period (July to September) was only observed in Douro-C (0.79) (figure 14).

As a whole, results suggested that high temperatures are associated with a reduction in the weight of the berries of both varieties but Touriga Nacional berries were much more sensitive.

Titratable acidity

Temperature increase negatively impacted titratable acidity (TA) of Touriga Nacional berries at maturity, but in Douro-D vineyard the correlation was weak. In Dão, the strength of correlations increased steadily as the season progressed, becoming very strong in August. TA in Alentejo was mainly

affected by the temperatures of July. In Douro-C, correlations were moderate for all temperatures and months analysed (figure 13).

Temperature negatively impacted TA levels of Aragonez berries at maturity but mainly in Dão region, with a growing trend throughout maturation (figure 14).

Generally, correlations between TA and precipitation variables are positive but weak (figure 13 and 14). Negative correlations with precipitation were found in Alentejo and Douro-D, but in these two vineyards grapevines are irrigated. Results suggested that higher temperatures are associated with low levels of TA, but correlations between precipitation and TA at maturity were not evident.

pH

Generally, the correlations between the berry pH and temperature were opposite to TA and temperature because the pH tends to decrease with the increase of titratable acidity (figure 13 and 14). Correlations of pH with temperatures during all months studied were weak and varied in Douro-D vineyard. In Aragonez berries, temperature had significant positive impacts on pH levels only in Dão vineyard, with stronger correlations in August for all temperature variables.

Negative correlations between berry pH and precipitation were found in Dão and Douro-C for Touriga Nacional and Aragonez varieties. An unexpected positive correlation between pH of Touriga Nacional berries and precipitation in April to June period was observed in Alentejo (figure 13). Therefore, precipitation effect in berry pH is not consistent in all regions.

Probable alcohol

In Alentejo and Douro-C, probable alcohol levels of Touriga Nacional berries were positively correlated with temperature, and the effect of July average and maximum temperatures were more evident. Weak to moderate positive correlations were found between minimum temperature and PA in these regions. In Dão and Douro-D, minimum temperature did not impact probable alcohol levels because correlations were very weak (figure 13). Only the temperature in June consistently affected positively alcohol levels in Aragonez grapes of Dão region (figure 14).

In Dão region, a strong negative correlation between precipitation and PA was observed in Touriga Nacional and Aragonez berries (figure 13 and 14). In the remaining regions, precipitation did not affect PA levels, or the effects were very weak.

Total phenols index

Results showed that temperature negatively impacted total phenols index at maturity in Touriga Nacional berries from Alentejo. However, an opposite effect of temperature on TPI was observed in Douro-D for Touriga Nacional berries (figure 13). Correlations between temperature and TPI in Aragonez grapes were weak to moderate and dependent on the region (figure 14). Precipitation can also increase or reduce phenols level, depending on the region (figure 13 and 14). Analysis of the impact of climatic variables on TPI is ambiguous since the impact on different phenols may be different.

Anthocyanins

Results presented in figure 13 and 14 show that the concentration of anthocyanins in Touriga Nacional and Aragonez berries were negatively associated with all temperature variables. In particular, the correlations were strong in Alentejo where the climatic conditions of July (particularly average and maximum temperatures) showed a great impact on the concentration of anthocyanins at maturity. Moderate correlations between minimum temperatures and anthocyanins at maturity were found for all regions. Correlations between precipitation and anthocyanins in Touriga Nacional berries were weak and depended on the region (figure 13). However, moderate positive and negative correlations were found between precipitation (from April to June) and anthocyanins in Aragonez grapes (figure 14).
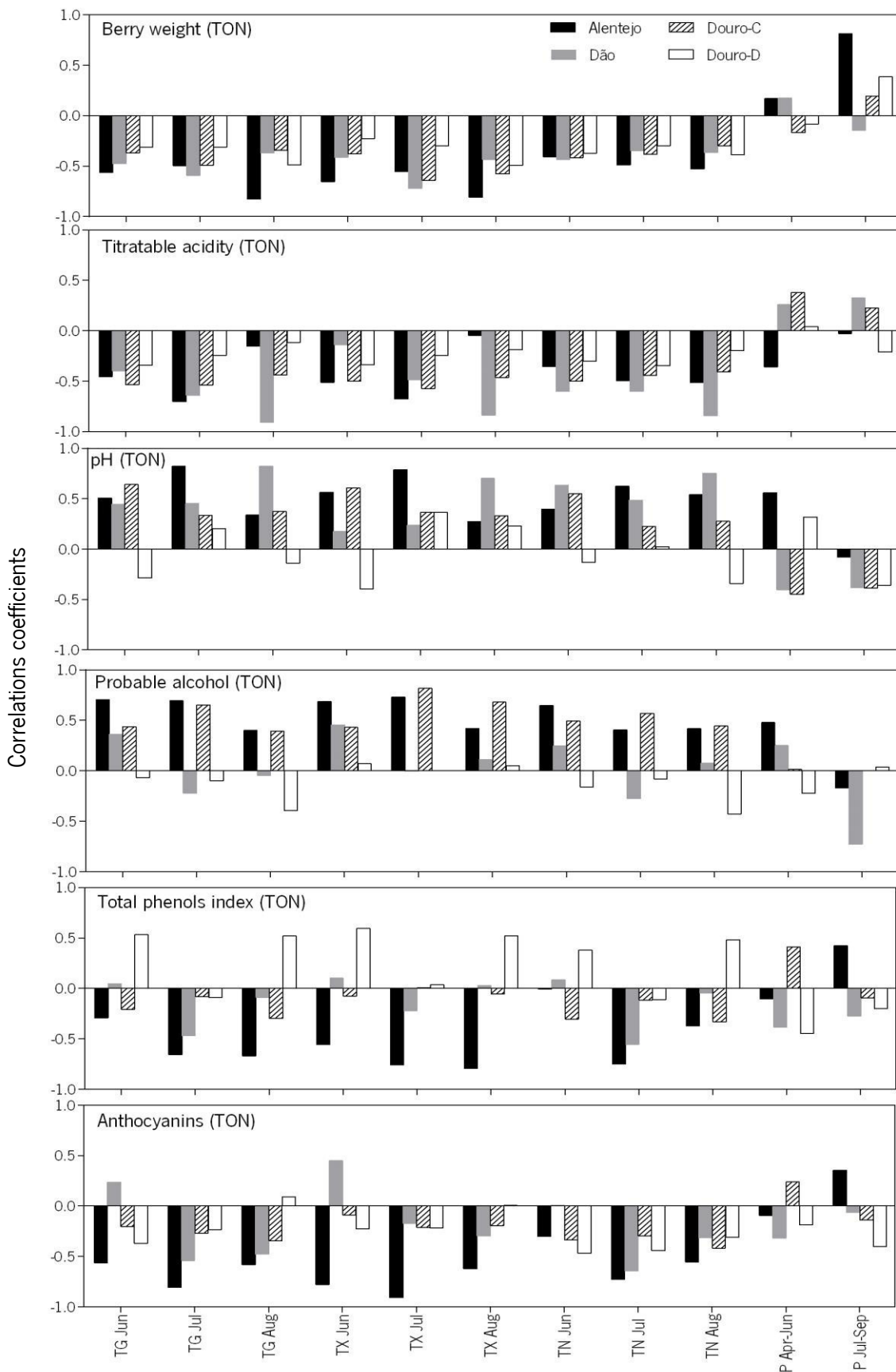
Figure 13. Correlations between grape berry quality parameters (berry weight, TA, pH, PA, TPI and anthocyanins) at maturity and climate variables for Touriga Nacional (TON) variety. Months are described by their initial letters. TG - average temperature; TX – maximum temperature; TN – minimum temperature; P – precipitation.
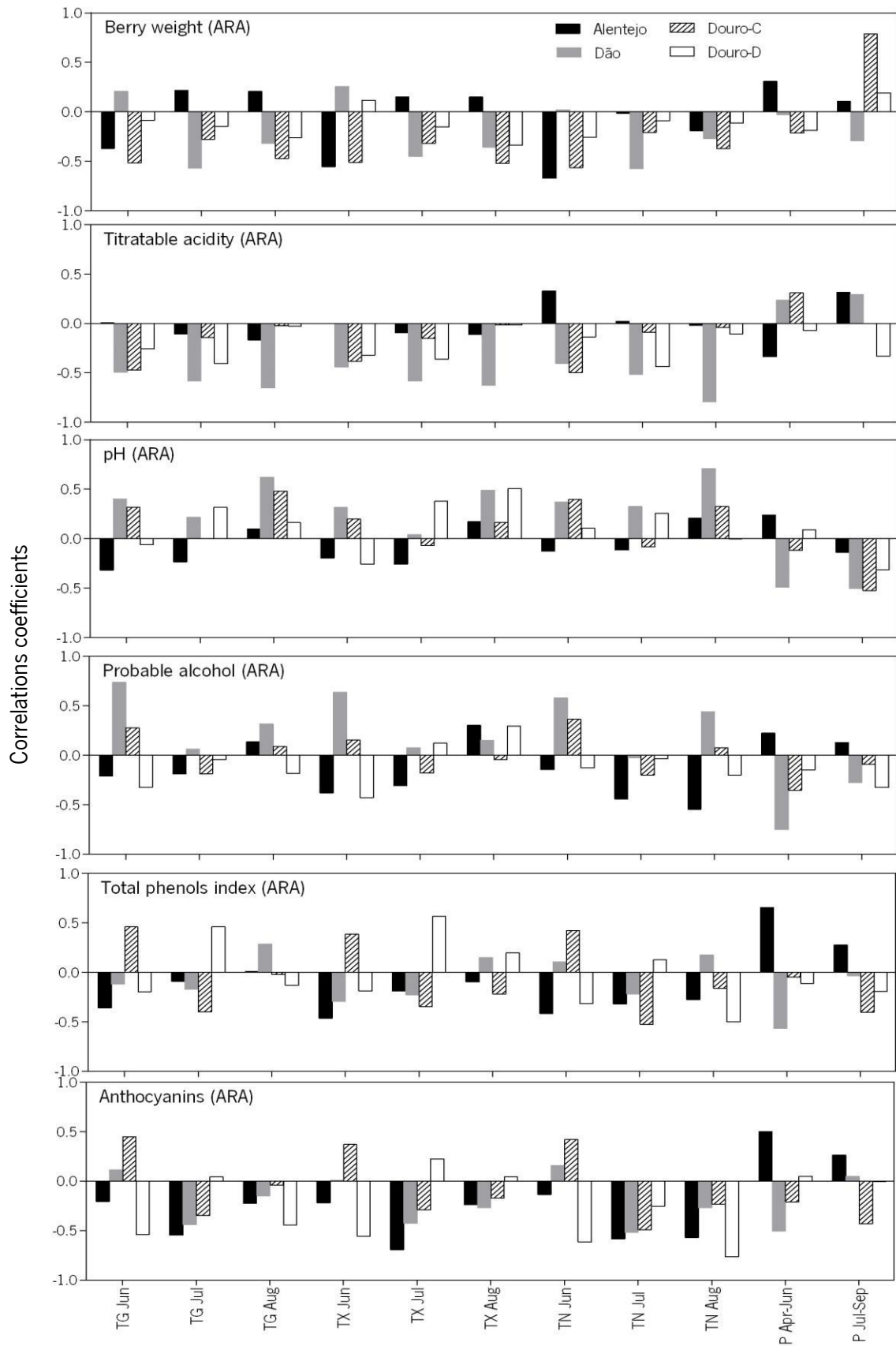
Figure 14. Correlations between grape berry quality parameters (berry weight, TA, pH, PA, TPI and anthocyanins) at maturity and climate variables for Aragonez (ARA) variety. Months are described by their initial letters. TG - average temperature; TX – maximum temperature; TN – minimum temperature; P – precipitation.

3.2   Climatic characterization of the studied regions

The exploratory bivariate correlation analysis allowed to identify Alentejo, Dão, Douro-C and Douro-D vineyards as those most affected by temperature and precipitation throughout years. Thus, a climatic characterization of these regions regarding temperature and precipitation is crucial to understand many of the results obtained below. Average (1991-2017) growing season temperature (GST) (April-October) was calculated, which ranged from 18.1 °C in Dão to 21.1 °C in Alentejo. Average, maximum and minimum air temperatures from June to August (1991-2017) were also greater in Alentejo and Douro-D than in Douro-C and Dão. The average annual precipitation (1991-2017) was also calculated, and the results obtained allowed to identify Dão and Douro-C regions as the rainiest (table 6) when compared with Alentejo and Douro-D. Figure 15 shows the geographic localization of these regions and an automatic weather station in Douro-C from which climatic data were collected.
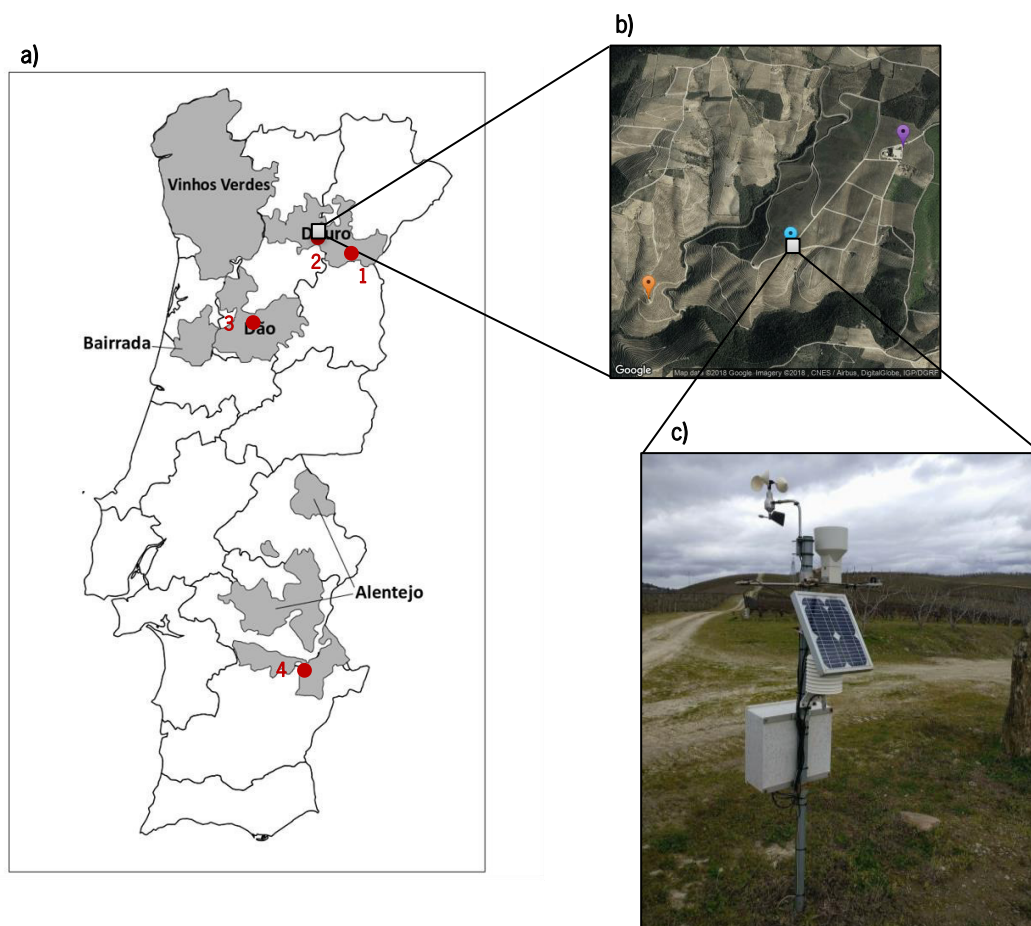
Figure 15. (a) Study areas in Portugal. (b) Douro-C. (c) Douro-C 2 AWS (March 2018 photo).

Table 6. Climatic characterization of the studied regions during 1991-2017 period.

Vineyard	Annual precipitation (mm)	Growing season TG (°C)	Jun - Aug TG (°C)	Jun – Aug TX (°C)	Jun - Aug TN (°C)
1. Douro-D	563	20.5	24.4	31.8	16.21
2. Douro-C	681	18.2	21.7	29.0	14.0
3. Dão	992	18.1	21.3	29.5	12.9
4. Alentejo	637	21.1	24.2	33.1	15.5

3.3   Patterns in grape berry composition at maturity between regions

The following analyses will help to understand the effects of climate variables on grape berry quality parameters in the regions previously identified as the most affected by temperature and precipitation throughout years – Alentejo, Dão and Douro (C and D). For this purpose, the maturation parameters of these regions were grouped into 2 clusters: cluster 1 (years where maturation parameter was higher) and 2 (years where maturation parameter was lower) – annex I. Differences between averages of cluster 1 and 2 were significant ($p \leq 0.05$) for all studied berry parameters and regions, except for titratable acidity in Alentejo and total phenols index in Alentejo and Douro-D (Touriga Nacional) ($p > 0.05$) – annex II and figure 16.

Figure 16 also shows differences in berry composition between colder and warmer regions. Generally, in warmer regions and with lower annual precipitation (Alentejo and Douro-D), berry weight and titratable acidity were lower than in colder regions and with higher annual precipitation (Dão and Douro-C). Conversely, the pH of the berries was higher in warmer than in colder regions. Probable alcohol at maturity did not show a clear trend across regions. Even so, the highest probable alcohol level in Aragonez berries was observed at the warmer region (Alentejo) and is significantly different from the lowest level observed in the colder region (Dão). Total phenols index levels were very similar in all regions for both clusters, although a slight decrease (from the warmer region to the colder region) was observed for the Aragonez variety. Anthocyanins at maturity did not show a clear trend across regions. Moreover, the highest levels of anthocyanins were unexpectedly observed in Alentejo. In fact, the unclear pattern observed between regions for anthocyanins, TPI and probable alcohol can be due to temperature extremes. Temperature extremes are potentially determinant for quality and were not considered (because the identification of warmer/colder regions was made by GST).
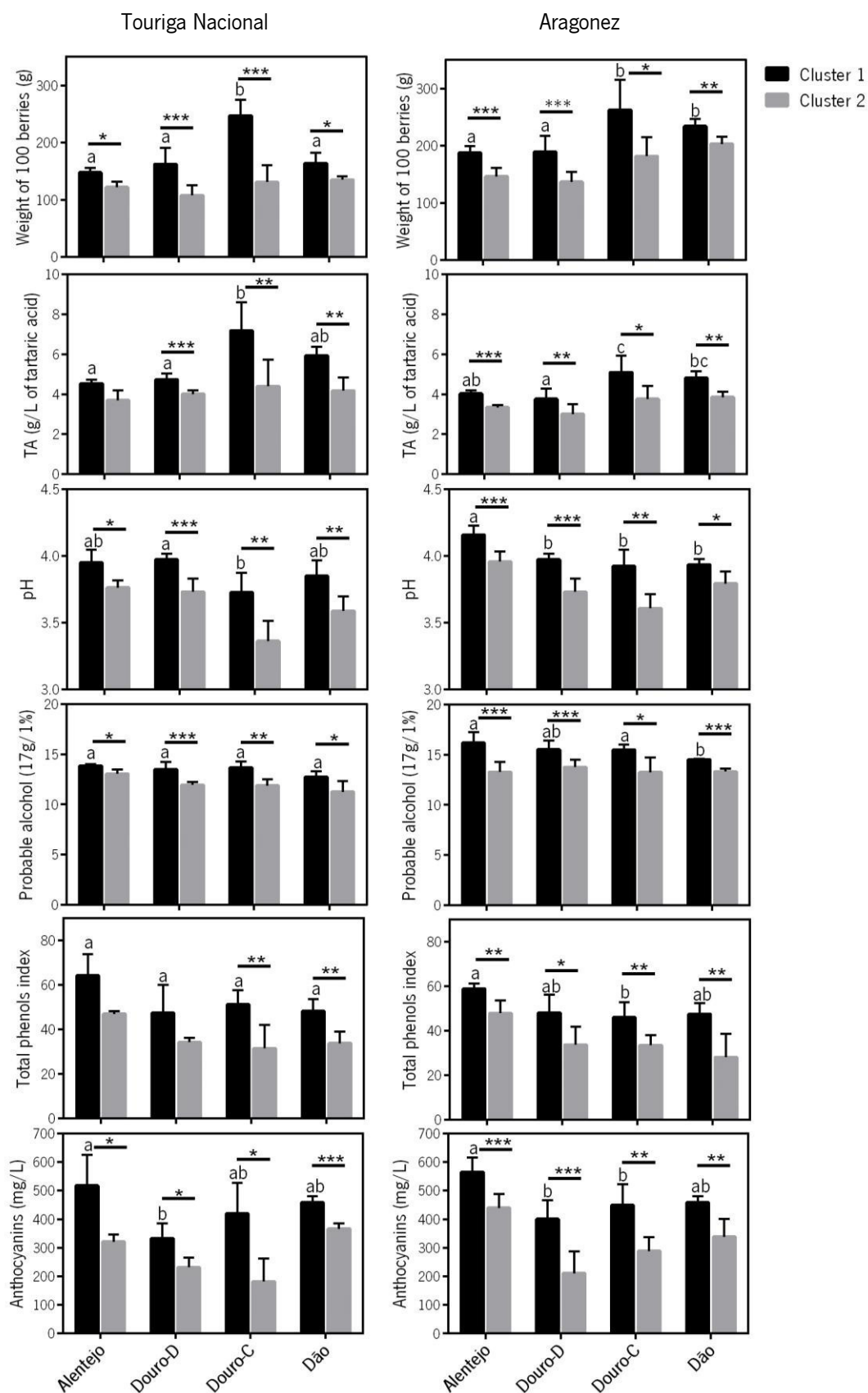
Figure 16. Levels of grape berry quality parameters at maturity. Regions are listed from left to right according to average GST (April to October) in decreasing order. Results represent the mean ± SD; the statistically significant differences between clusters are indicated by asterisks (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$). Different letters indicate statistically significant differences between regions (for cluster 1).

3.4   Temperature differences between maturation clusters

In general, exploratory correlation analyses between climate variables and grape berry quality parameters at maturity allowed for identifying TG, TX and TN of June, July and August as determinants of grape composition. In addition, this analysis showed that grape quality parameters are less affected by climatic conditions in Douro-D, compared to the other studied regions. For precipitation, the results obtained by the exploratory correlation analysis varied significantly between regions and varieties (figure 13 and 14).

Therefore, grape berries composition of Alentejo, Dão and Douro-C are the most affected by climatic conditions. Difference between monthly averages of TG, TX and TN for June, July and August between maturation clusters was calculated in these regions for Touriga Nacional and Aragonez (table 7 and 8) (only statistically significant temperature differences are shown). This analysis allowed the identification of temperature differences that may explain the average difference in grape quality attributes between clusters. The temperature differences presented are associated with a decrease in the grape quality parameter (Cluster 2 – Cluster 1).

Table 7. Difference between the maturation clusters for average monthly TX, TG and TN in June, July and August for Touriga Nacional variety. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

Quality parameter	Temp.	Alentejo			Dão			Douro-C		
		Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
Berry weight	ΔTX	-	1.95**	3.01***	-	1.53**	-	1.83**	1.84***	1.50***
	ΔTG	-	1.01*	1.26***	-	1.26*	-	1.22**	1.00*	-
	ΔTN	-	1.02**	-	0.82*	1.00**	-	1.03**	-	-
Titratable acidity	ΔTX	-	1.42*	-	-	2.07***	2.00***	-	1.54***	
	ΔTG	-	1.32***	-	-	1.89***	1.79***	0.88*	1.83***	0.92**
	ΔTN	-	0.77*	1.00**	-	1.54***	1.36***	0.95**	1.91***	0.94**
pH	ΔTX	-2.72***	-3.51***	-1.95***	-	-1.62***	-2.62***	-1.16*	-	-1.23***
	ΔTG	-1.71**	-1.84***	-0.84*	-	-1.43***	-1.86***	-1.24**	-1.16**	-1.06***
	ΔTN	-1.11*	-0.99**	-0.75*	-0.86*	-1.55***	-1.69***	-1.23***	-1.10**	-
Probable alcohol	ΔTX	-2.72***	-3.51***	-1.95***	-2.06***	-	-	-1.12**	-3.28***	-2.13***
	ΔTG	-1.71**	-1.84***	-0.84*	-	-	-	-0.96*	-2.66***	-1.28***
	ΔTN	-1.11*	-0.99**	-0.75*	-	-	-	-1.25***	-2.93***	-1.75***
Anthocyanins	ΔTX	2.72***	3.51***	1.95***	-1.28*	-	0.98*	-1.44**	-	-
	ΔTG	1.71**	1.84***	0.84*	-	1.42***	0.93**	-	-	-
	ΔTN	1.11*	0.99**	0.75*	-	1.84***	0.60*	-	0.81*	0.96**
TPI	ΔTX	-	1.95**	3.01***	-	2.01***	1.15*	-1.44***	-	-
	ΔTG	-	1.01*	1.26***	-	2.08***	0.97**	-	-	-
	ΔTN	-	1.02**	-	-	2.22***	0.83**	-	-0.81*	-0.96**

Table 8. Difference between the maturation clusters for average monthly TX, TG and TN in June, July and August for Aragonez variety. *$P \leq 0.05$. **$P \leq 0.01$. ***$P \leq 0.001$.

Quality parameters	Temp.	Alentejo			Dão			Douro-C		
		Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
Berry weight	ΔTX	1.06*	-	-	-1.24*	1.35**	1.09*	1.94***	1.60***	-
	ΔTG	-	-	-	-	1.43***	0.93**	1.52***	1.32***	-
	ΔTN	1.01***	-	-	-	1.24***	-	1.33***	1.09**	-
Titratable acidity	ΔTX	-	-	-	2.13***	1.27*	1.22**	-	-	-
	ΔTG	-	-	-	1.32**	-	1.00**	-	-	-
	ΔTN	-	-	-	-	-	1.33***	-	-	-
pH	ΔTX	-	-	-	-	-	-1.52***	-	-	-
	ΔTG	-	-	-	-	-	-1.23***	-	-	-
	ΔTN	-	-	-	-	-	-1.52***	-	-	-
Probable alcohol	ΔTX	-	-	-	-1.97**	-	-	-	-	-
	ΔTG	-	-	-	-1.48***	-	-	-	-	-
	ΔTN	-	-	-	-0.86*	-	-	-	-	-
Anthocyanins	ΔTX	-	1.14**	-	-	-	-	-	-	-
	ΔTG	-	0.67**	-	-	-	-	-	-	-
	ΔTN	-	-	0.80**	-	-	-	-	-	-
TPI	ΔTX	-	-	-	-	-	-	-	-	-
	ΔTG	-	-	-	-	-	-	-	-	-
	ΔTN	-	-	-	-	-	-	-	-	-

These results again confirm that an increase in temperature induces a reduction on berry weight, titratable acidity, anthocyanins and total phenols index. Conversely, a decrease in temperature induces a reduction in pH and probable alcohol.

In general, significant temperature differences between clusters were lower for Aragonez variety. At large, higher temperature differences between clusters were found in July and for maximum temperature in Touriga Nacional variety. In all studied regions, larger temperature differences between clusters were observed in Touriga Nacional from Alentejo for all parameters, except titratable acidity. Temperature differences for titratable acidity clusters were higher in Dão region. In Douro-C, the greater differences were found between probable alcohol clusters. However, the conclusion that these temperatures differences are due to differences between warmer and colder years still remains to be proved.

3.5 Daily temperature analysis between maturation clusters

For the largest monthly temperature differences found, daily temperature differences between clusters were calculated from June 1 to August 31. Therefore, daily maximum temperature differences between clusters were carried out for Alentejo (berry weight, pH, probable alcohol, TPI and anthocyanins), Dão (titratable acidity) and Douro-C (probable alcohol) samples of Touriga Nacional

variety. This analysis allowed for identifying critical days/periods that may have contributed to inter-annual variability of maturation parameters.

In addition, evolution of daily temperatures of each year allowed for proving that differences in monthly temperatures resulted from differences between warmer and colder years.

Berry weight and total phenols index in Alentejo
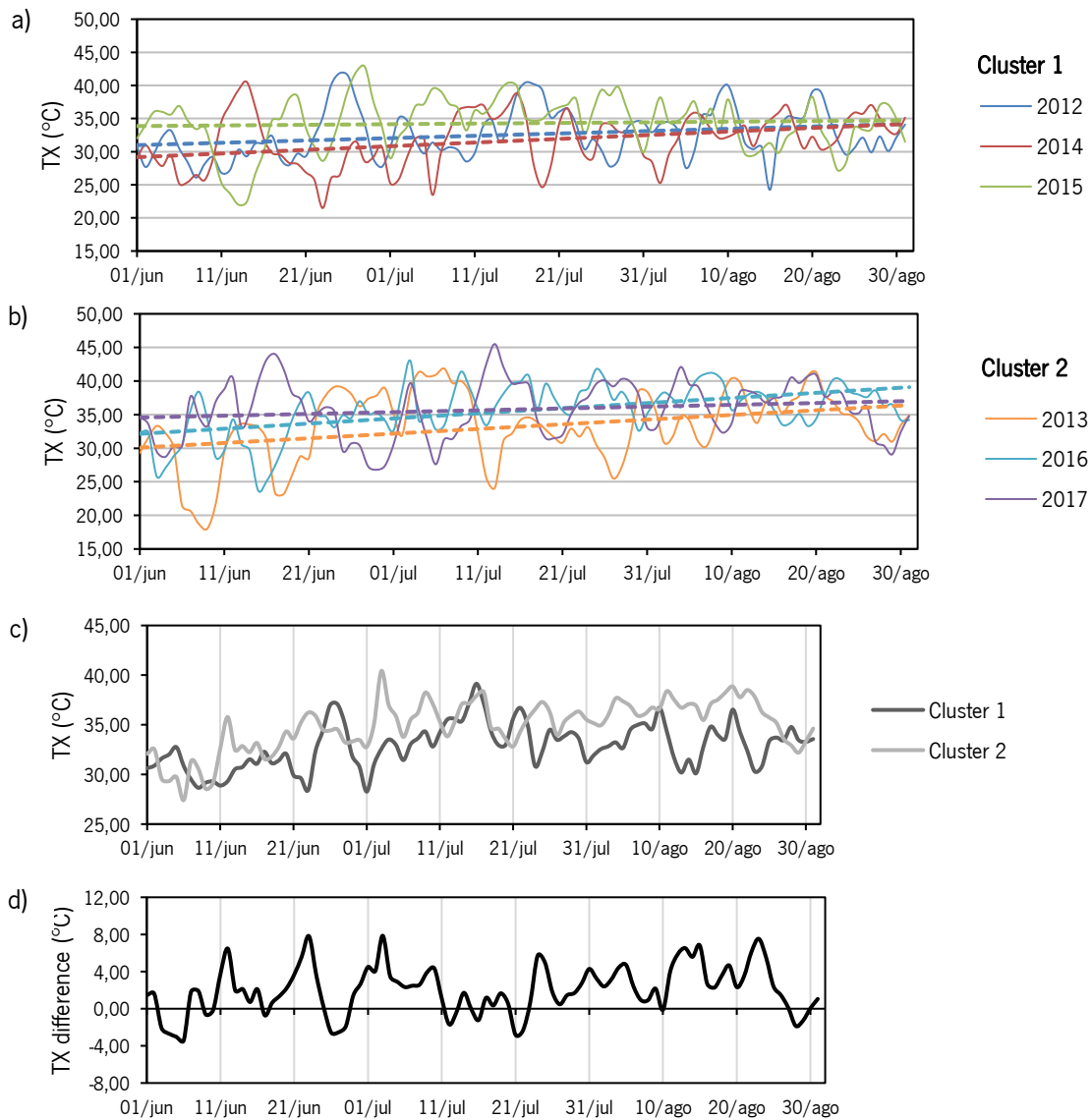
Figure 17. Daily temperatures evolution from June 1 to August 31 for cluster 1 (a) and cluster 2 (b) of berry weight and total phenols index in Alentejo. (c) shows average of maximum temperatures for cluster 1 and 2 in the indicated period, with their difference represented in (d).

Daily maximum temperature evolution from June 1 to August 31 of years in the two clusters showed that daily TX of cluster 1 years was generally lower (essentially between 30 and 35°C) than those of cluster 2 (essentially between 30 and 40 °C) (figure 17 a, b). Therefore, the observed

differences in monthly temperatures result from the difference between warmer and colder years, showing that cluster 1 years (higher berry weight and TPI) are coolest and those of cluster 2 (lower berry weight and TPI) are warmest. Likewise, average maximum temperature of cluster 1 years is lower than that of cluster 2, reaching differences of almost 8 °C at the end of June, beginning of July and mid and late August (figure 17 c, d). Indeed, in correlation analyses, correlation was higher between TX and berry weight (-0.81) and TPI (-0.79) in August compared to June and July. Therefore, inter-annual TX variability of months between June and August was determinant for berry weight and TPI in Alentejo region, with August TX showing more influence in these grape berry attributes.

Anthocyanins, pH and probable alcohol in Alentejo

Figure 18. Daily temperatures evolution from June 1 to August 31 for cluster 1 (a) and cluster 2 (b) of anthocyanins in Alentejo. (c) shows average of maximum temperatures for cluster 1 and 2 in the indicated period, with their difference represented in (d).
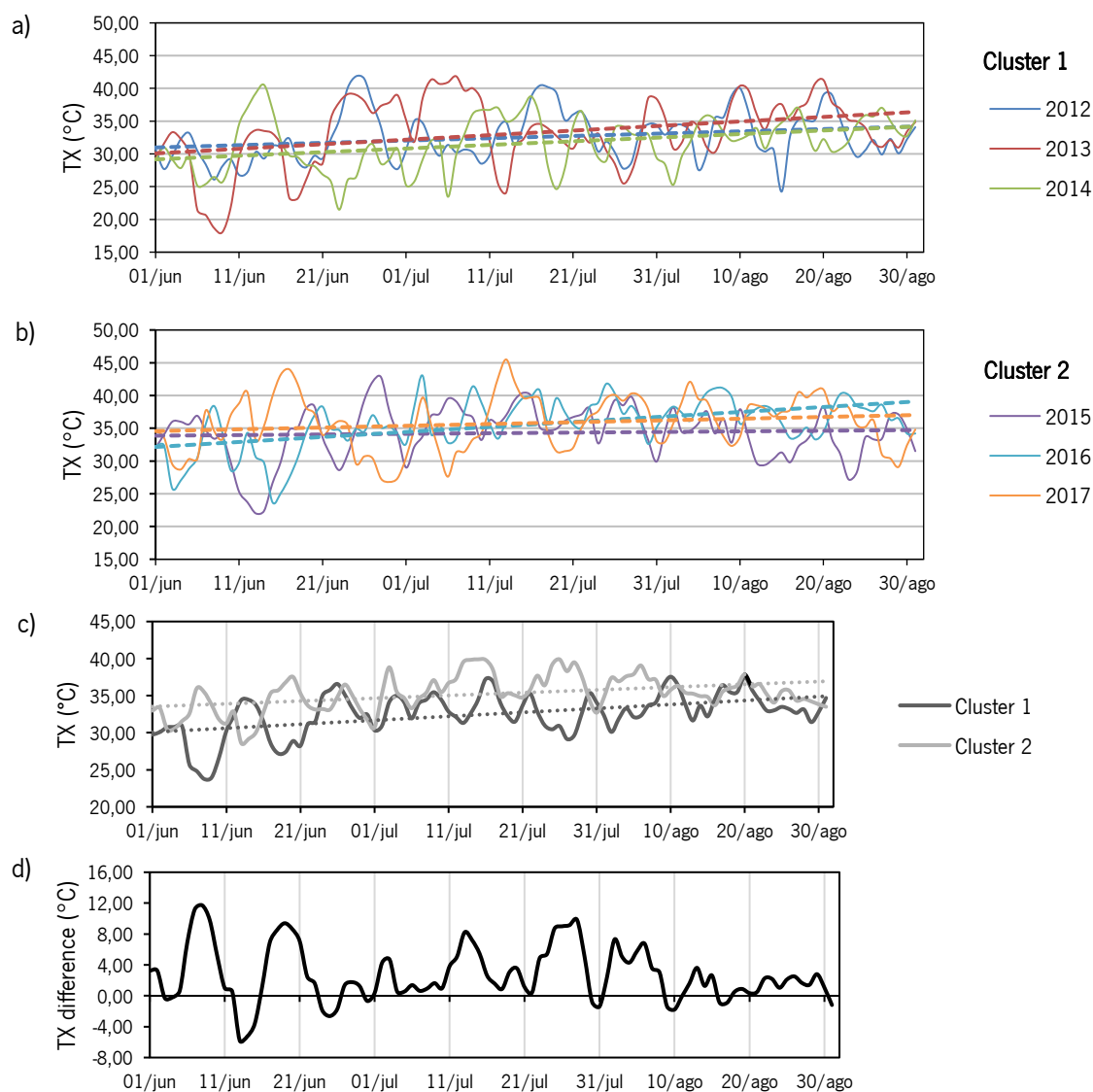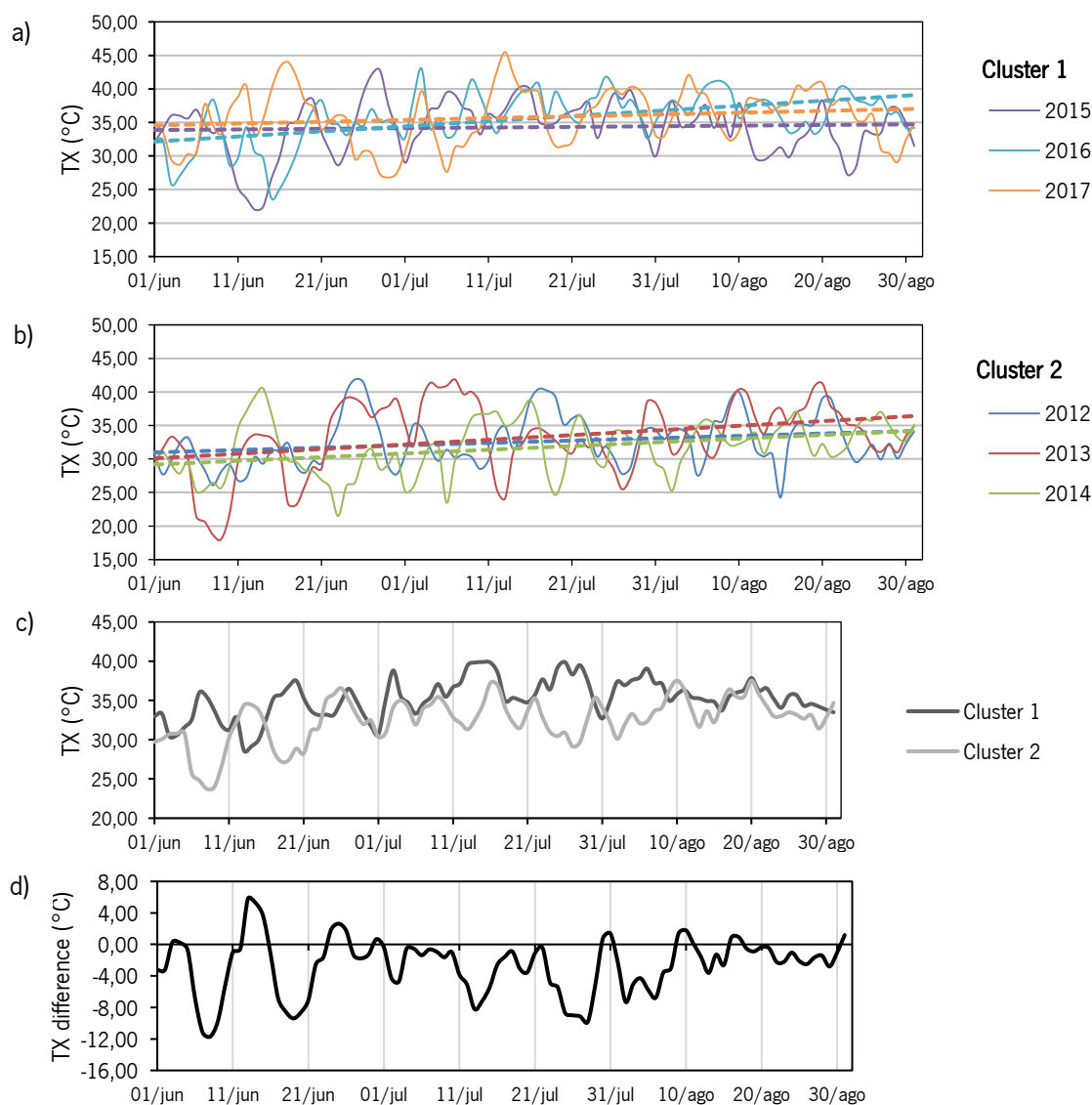
Figure 19. Daily temperatures evolution from June 1 to August 31 for cluster 1 (a) and cluster 2 (b) of pH and probable alcohol in Alentejo. (c) shows average of maximum temperatures for cluster 1 and 2 in the indicated period, with their difference represented in (d).

Figure 18 a, b and 19 a, b show that daily maximum temperatures of 2012, 2013 and 2014 were generally lower (essentially between 30 and 36 °C) than those of 2015, 2016 and 2017 (essentially between 32 and 40 °C). Thus, evolution of daily TX of each year allowed for proving that differences in monthly temperatures found were a result of the difference between warmer and colder years. Colder years (2012, 2013, and 2014) were associated with higher anthocyanin levels (figure 18 a) and lower pH and probable alcohol levels (figure 19 b). In addition, the difference between average maximum temperatures of clusters was substantial in July, namely in the middle and end of July (figure 18 d and 19 d). In June, two peaks of TX difference between clusters are also clear (11.7 °C; 8.7 °C). In fact, in correlation analyses between pH, probable alcohol and anthocyanins and maximum

temperatures, correlations were higher in June and July compared to August. Therefore, inter-annual variability of June and July maximum temperature was determinant for pH, probable alcohol and anthocyanins levels of grape berries in Alentejo region.

Titratable acidity in Dão
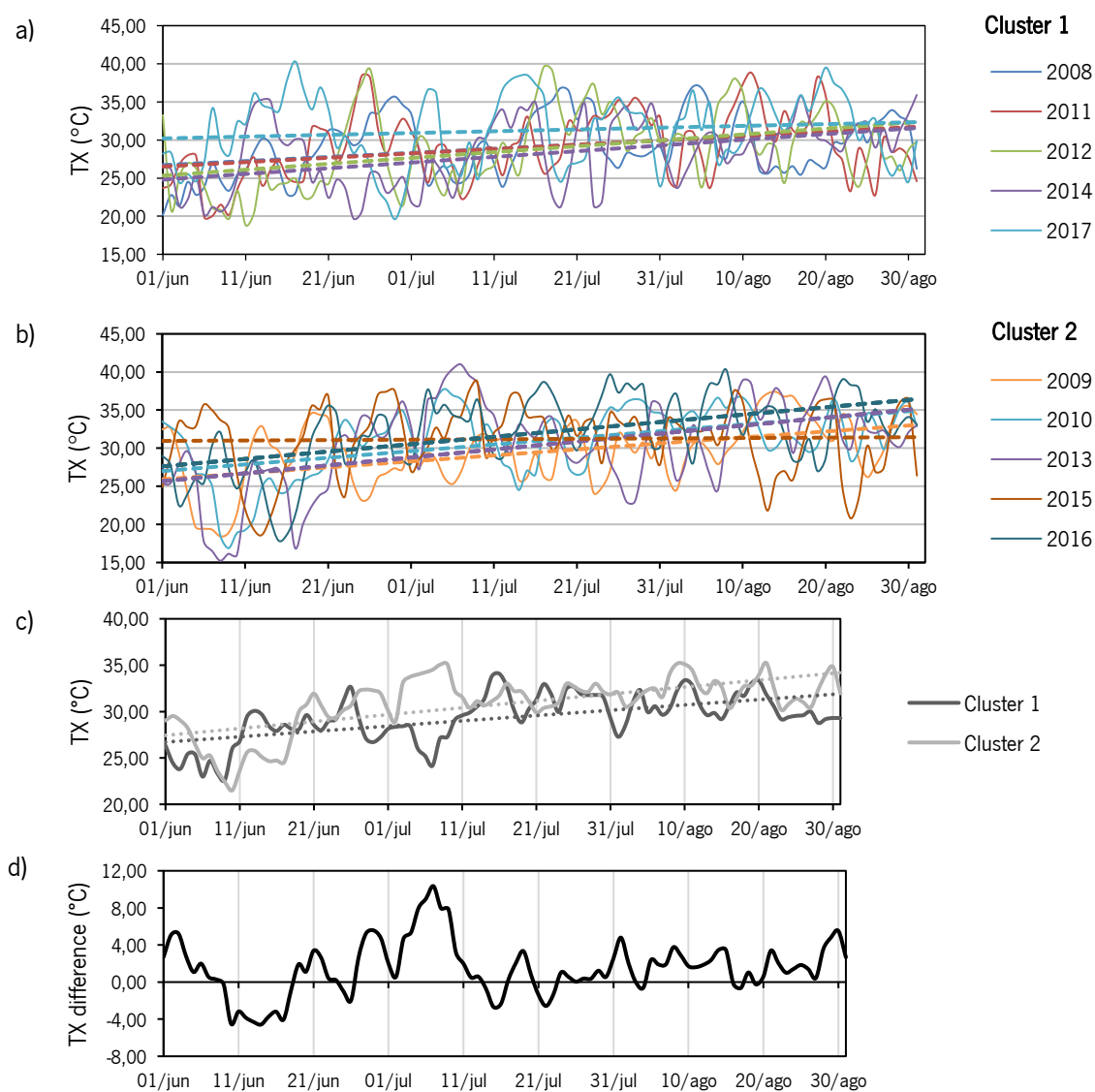
Figure 20. Daily temperatures evolution from June 1 to August 31 for cluster 1 (a) and cluster 2 (b) of titratable acidity in Dão. (c) shows average of maximum temperatures for cluster 1 and 2 in the indicated period, with their difference represented in (d).

Figure 20 a, b show that the years of the cluster 1 have generally lower values of maximum temperatures than those of cluster 2. Therefore, differences in monthly temperatures found were a result of the difference between warmer years and colder years. These latter (cluster 1) are associated with higher titratable acidity levels (figure 20 a). Conversely, warmer years are associated with lower titratable acidity levels (figure 20 b).

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Furthermore, the difference between average maximum temperatures of clusters was almost always positive in August, showing that in general maximum temperatures of cluster 2 years were higher than those of cluster 1 (figure 20 c, d). Although a difference of about 10 °C is visible in early July between clusters, in some days the average temperatures of cluster 1 are higher than cluster 2. In fact, in exploratory correlation analysis, August seems the more influential month for titratable acidity in Dão. Thus, inter-annual variability of August maximum temperature was determinant for titratable acidity of grape berries in Dão region.

Probable alcohol in Douro-C
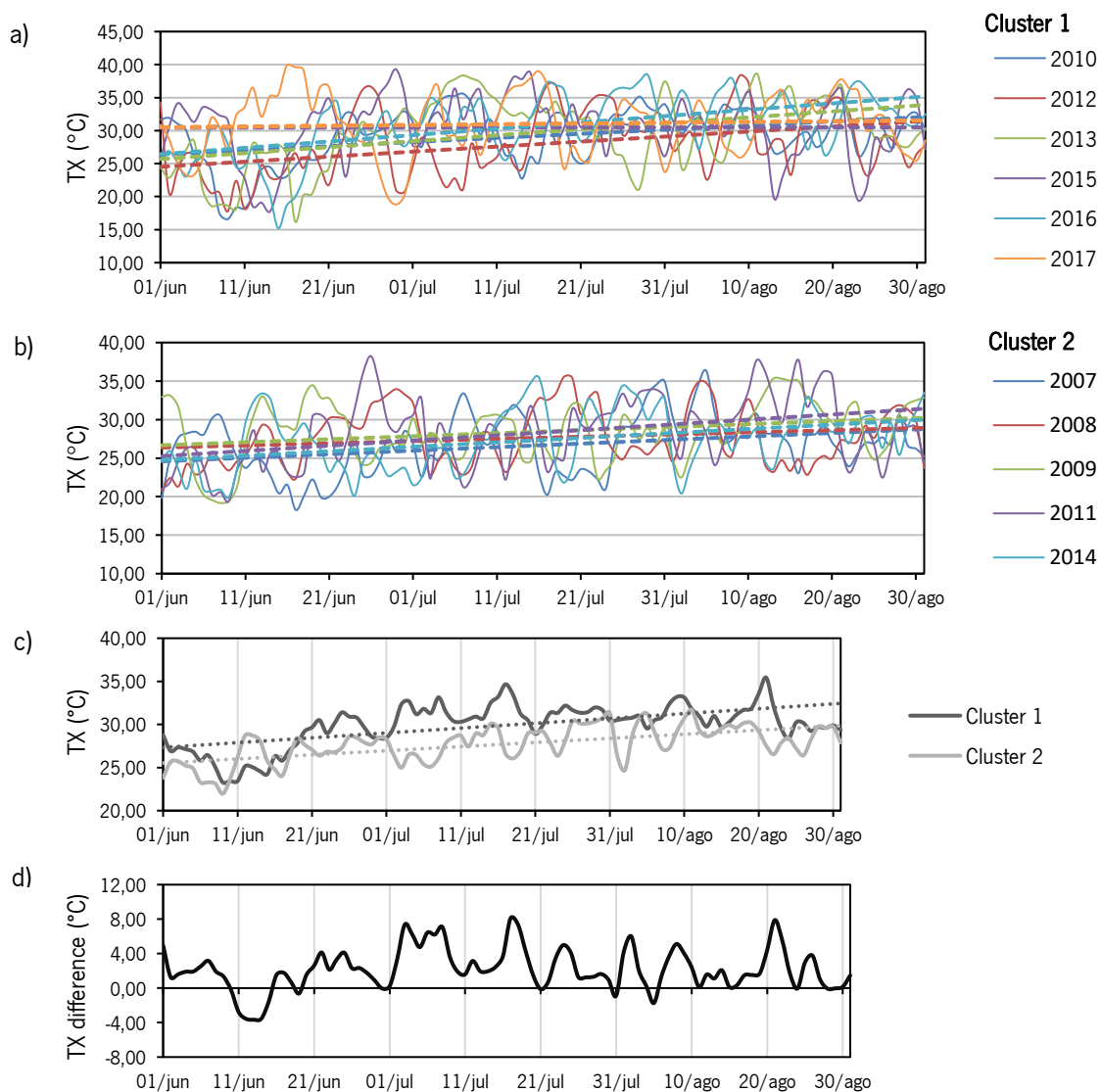
Figure 21. Daily temperatures evolution from June 1 to August 31 for cluster 1 (a) and cluster 2 (b) of probable alcohol in Douro-C. (c) shows average of maximum temperatures for cluster 1 and 2 in the indicated period, with their difference represented in (d).

Figure 21 a, b show that daily TX of cluster 1 years was generally higher than those of cluster 2. Thus, differences in monthly temperatures result from the difference between warmer and colder years, showing that cluster 1 years (higher probable alcohol) are the warmest years and those of cluster 2 (lower probable alcohol) are the coolest. Moreover, differences of average maximum temperatures between clusters are noticeable in July and August (figure 21 d). Indeed, in correlation analyses between probable alcohol and maximum temperatures, correlation was higher in July (0.82) and August (0.68) compared to June (0.43). Therefore, it seems that inter-annual variability of July and August TX was determinant for probable alcohol levels in grape berries of Douro-C, with July TX showing more influence.

4. Discussion

4. DISCUSSION

This work aimed to assess the role of climate variables, in particular, temperature and precipitation, on grape berry quality parameters of two varieties (Touriga Nacional and Aragonez) cultivated in six vineyards from Alentejo, Dão and Douro (A, B, C and D). In some of these regions, berry composition data are available since 1991 at weekly intervals from two to three weeks after the end of *véraison* until harvest. In the context of the present study, this dispersed information was organized and compiled into a database.

Climatic data after 2011 were obtained from automatic weather stations owned by Sogrape Vinhos S.A. and those before 2011 from the estimated climatic database (PTHRES). An exploratory correlation analysis showed that climatic variables were weakly related to grape berry quality parameters in the Douro-A and Douro-B vineyards and therefore these regions were not considered in the subsequent work. Results showed that the strength of correlations was higher in June, July and August, showing that the climate at *véraison* and ripening periods are more influential in determining grape berry composition at maturity, as previously reported (Gladstones, 1992; De Orduna, 2010).

Berry composition data were examined at same maturity level based on winemaker's decision (last analysis before harvest), and results showed clear differences across regions differing in growing season average temperature, particularly in berry weight, titratable acidity and pH. This indicates that macroclimate can influence regional patterns of grape berry composition (Smart, 1985; Barnuud et al., 2013). How inter-annual climate variability affect each grape berry quality parameter was an important question of the present study.

4.1 Climate variables affect berry weight, titratable acidity and pH, probable alcohol, total phenols index and anthocyanins

For all four vineyards from Alentejo, Dão, Douro-C and Douro-D berry weight of Touriga Nacional and Aragonez at maturity was negatively influenced by temperature while strong positive correlations with precipitation (July to September) were found only in Alentejo (TON) and Douro-C (ARA). As vine water availability is closely related to temperature (Gladstones, 1992), an increase of temperature can lead to water deficit. Some studies have shown that when the vine is exposed to water deficit stress, berry weight decreases (Castellarin et al., 2007; van Leeuwen et al., 2009; Arrizabalaga et al., 2018)

which is in agreement with obtained results. Water deficit generally leads to smaller berries since it inhibits both cell division and/or cell expansion (Conde et al., 2007; Dai et al., 2011).

For both studied varieties from Alentejo, Dão and Douro (C and D), exploratory correlation analysis of titratable acidity at maturity with climate variables showed that titratable acidity is negatively correlated with all monthly temperatures (TX, TG and TN) between June to August while correlations with precipitation variables were generally positive but weak. Temperature increases the respiration of tartaric and malic acids leading to a drop in titratable acidity and increased pH (Buttrose et al., 1971; Ruffner et al., 1976). Similar results were obtained by Barnuud et al. (2013) and Ubalde et al. (2010). The observed positive influence of precipitation on titratable acidity levels at maturity is supported by previous studies (López et al., 2007; Parra et al., 2010; Barnuud et al., 2013). The observed negative correlations with precipitation found in Alentejo and Douro-D can be explained by the fact that these two vineyards are the only ones that are irrigated (Alentejo since always and Douro-D since 2016).

The decrease of the titratable acidity with increasing precipitation found in Douro-D and Alentejo is puzzling, however, it would be necessary to consider other factors than precipitation, such as atmospheric humidity and soil water holding capacity, since they determine the water balance of the vine (Gladstones, 1992). They were not studied in this work but may account for this result.

The correlations between pH and monthly TX, TG and TN were generally positive, in accordance with previous studies (Barnuud et al., 2013) while the associations between pH and precipitation variables were negative, except for Alentejo and Douro-D (April to June period), as found in correlations with titratable acidity. The observation that the strength of correlations between climate variables and pH is lower than between climate variables and TA (more evident in Aragonez variety) is possibly explained by previous results showing that pH is more affected by microclimate, i.e. climatic conditions within a vine canopy, than TA (Smart, 1985). Future studies with microclimate data could further clarify these correlations.

Correlation analysis between climate variables and probable alcohol was dependent on the regions and varieties. In Alentejo and Douro-C (TON) and in Dão (ARA), temperature exerts a strong positive influence on probable alcohol levels, but weak negative influences were found in other regions. Moreover, the strongest correlations were found between PA and monthly temperatures of June and July (fruit set/*véraison* period). These results go in agreement with previous reports showing that TSS accumulation was stimulated at higher temperatures at *véraison* and few days after (Mori et al., 2005; Poudel et al., 2009).

A strong negative correlation between PA and precipitation was only found in Dão for both varieties studied while a moderate positive correlation was found in Alentejo (TON). As previously described, under irrigated conditions, TSS concentration can be decreased due to the dilution effect caused by greater berry size (López et al., 2007). But other reports have shown that in irrigated vines the accumulation of sugar is improved due to a stimulation of the photosynthetic activity (Bartolomé, 1993). Thus, both positive and negative correlations found with precipitation can be explained.

Correlations between TPI and climate variables depended on the regions and varieties. The optimum temperature for phenolics accumulation is 25 °C and above or below this value, accumulation decrease significantly (Poudel et al., 2009). Therefore, the positive and negative correlations can be explained by the temperature differences between regions. In fact, Alentejo is characterized by GST higher than other regions, which may explain the observed decrease of total phenols levels. However, analysis of impact of climatic variables on TPI is ambiguous since the impact of an environmental factor on each phenolic compound may be different. So, further studies are needed to understand phenolic biosynthesis under different temperatures.

Our results showed that TPI can be reduced or increased depending on the region and precipitation period. It has been shown that phenolic compound biosynthesis can be stimulated or inhibited depending on the type of phenolic compound, severity of water deficit and the period when water deficit occurs (Ojeda et al., 2002). On the other hand, the positive effect of water stress on phenolics concentration can be explained by the reduced berry size under water limitation (Ojeda et al., 2002). Surface area/volume ratio of the berries increases with the decrease of berry size. Then, since phenolic compounds accumulate in the berry skin, smaller berries have a relatively greater solute to solvent ratio than larger berries (Conde et al., 2007).

Results showed that anthocyanins are negatively correlated with all monthly temperatures (TX, TG and TN) from June to August. Previous reports have shown that high temperatures negatively affect the content of anthocyanin at harvest due to an impairment of biosynthesis (Mori et al., 2005) and/or accelerated degradation (Mori et al., 2007). For both varieties, average minimum temperature in July and August was the most influential variable in all regions. In agreement a previous report (Jackson and Lombard, 1993), above and below a night temperature of 15 °C, grape berry anthocyanins are reduced and enhanced, respectively. For the majority of studied regions, the minimum temperature average (1991-2017) was higher than 15 °C from June to August. Therefore, minimum temperature may have exerted more influence on anthocyanins content than other climate variables. Similar results were already reported (Barnuud et al., 2013).

Correlations between precipitation variables and anthocyanins at maturity were weak for Touriga Nacional variety but strong for Aragonez variety. For both varieties, the correlations were different from one region to another. Specifically, a moderate positive correlation was found for Alentejo and a moderate negative correlation for Dão in April-June period (Aragonez). In fact, enhanced water availability early in the growing season is beneficial because it promotes vegetative growth and, consequently, berry development and its composition at later stages (Keller et al., 2010). Thus, the positive correlation found in Alentejo region can be explained (besides that, the vineyard of the Alentejo is irrigated). On the other hand, improved moisture late in the growing season has been described to reduce anthocyanins content (Jackson and Lombard, 1993), although this effect is not clear in our results. However, it is necessary to take into account that only Alentejo and Douro-D vineyards are irrigated and that other parameters such as air relative humidity and water holding capacity of soils should be assessed for a correct evaluation.

4.2 Grape berry composition varies across regions and years

Analysis of berry quality parameters at maturity across regions (figure 16) showed that lowest levels of berry weight and TA occurred more frequently in warmer regions with lowest annual precipitation (Alentejo and Douro-D). Accordingly, in colder regions with highest annual precipitation (Douro-C and Dão), berry weight and TA were higher (opposite effect observed with pH). The difference between warmer and colder regions relative to berry weight, TA and pH was particularly well established. Moreover, berry weight and TA levels are more negatively affected in warmer than in the colder years (figure 17 and 20). Conversely, pH is more negatively affected in colder than in warmer years.

Anthocyanins at maturity did not show a clear trend across regions when only average temperature of the growing season was considered. However, precipitation levels influenced berry anthocyanins. In particular, low levels of precipitation in Alentejo may have accounted for high anthocyanin content when compared to other regions (even with higher temperatures). Besides that, in Alentejo, the highest anthocyanin content was in colder years and the lowest in warmer years (figure 18). Higher minimum temperatures recorded in Douro-D vineyard can explain lower anthocyanins levels. In fact, the positive effect of low minimum temperatures during ripening on anthocyanin accumulation was already reported (Jackson and Lombard, 1993).

Highest sugar content was associated with warmer years, and an opposite effect was observed in colder years (figure 19 and 21), which goes in agreement with previous reports (Ubalde et al., 2010). Water limitation, often associated with heat stress, may affect plant hormonal equilibrium and berry weight, and compromise sugar accumulation in grape berries (Ubalde et al., 2010).

4.3 Touriga Nacional and Aragonez show different susceptibility to climate

In general, the results showed that grape berry quality parameters of the Aragonez variety are less affected by inter-annual climatic variability than Touriga Nacional variety. Thus, Aragonez variety seems to be more resistant to climatic variations, which is clearly reflected in their grape berry composition, namely in studied grape berry quality parameters.

Physiological response of grapevines to environmental stress is complex and not yet completely understood. It depends on genotype and is largely influenced by both root (rootstock) and scion traits. Concerning scion traits, leaf stomatal regulation is a key parameter influencing plant water relations (Zarrouk et al., 2016). Thus, one explanation for the obtained results may arise from the fact that Aragonez variety is considered isohydric while Touriga Nacional variety is anisohydric (Carvalho et al., 2016). These terms are related to stomatal regulation, i.e., some genotypes display better stomatal regulation under drought conditions/water stress than others and they were classified as isohydric (drought avoiders or 'pessimistic'). In turn, anisohydric ('optimistic') genotypes have less control of stomatal aperture under water stress (Zarrouk et al., 2016).

In Semillon variety (anisohydric), high stomatal conductance during the day results in an incomplete rehydration before dawn, especially during warm and windy nights, which could affect grape berry composition (Rogiers et al., 2009). A similar behaviour could account for the results obtained in the present study for Touriga Nacional. Besides, Touriga Nacional displays high capacity of heat dissipation via evaporative cooling, which can facilitate adaptation to warmer climates under plentiful water supply (i.e. irrigated vines) (Costa et al., 2012; Carvalho et al., 2016). However, the majority of Touriga Nacional vineyards under study were not irrigated, so that adaptation to warmer climates by evaporative cooling is limited. Moreover, Touriga Nacional may face early senescence of basal leaves and further leaf drop with negative consequences for berry quality (Zarrouk et al., 2016). Therefore, isohydric varieties are more tolerant to drought/water stress than the anisohydric ones.

However, whether or not isohydric some genotypes have different behaviour according to growing conditions (e.g. field versus greenhouse) and the degree of stress imposed on vines (Chaves et al.,

2010). But in this study, comparison between varieties was carried out in the same regions and over the same growing conditions. So, the different behaviour of Touriga Nacional and Aragonez regarding stomatal regulation is likely an important physiological condition that accounts for the observed results.

5. Conclusion

5. CONCLUSION

Grapevine growth and development are largely controlled by climatic conditions and have already suffered from significant impacts of global climate change over the last years (Santos et al., 2013; Mozell and Thach, 2014). In this context, the study of the influence of climate variability on grape berry quality parameters is of utmost importance because a better knowledge of these processes may potentiate the development of decision support tools and forecasting models for assessing climate change impacts in future climates. Agricultural and oenological practices can then be adjusted in order to keep good levels of productivity and maximizing grape berry and wine quality, even under adverse climatic scenarios. This topic was the main target of this study.

For six vineyards located in Douro, Dão and Alentejo regions, the composition of grape berries from vines cultivated in Alentejo appears as the more affected by inter-annual climate variability while some vineyards from Douro seem little or not affected at all (Douro-A and Douro-B). The results also showed that climate at *véraison* and maturation periods (namely June to August) is more influential in determining grape berry composition at maturity and TX, TG and TN have an important influence. The influence of precipitation on berry composition at maturity varied between regions and varieties. However, some results have shown a role of precipitation during flowering and maturation period.

Results also demonstrated that grape berry composition of the Aragonez variety is less affected by temperature and precipitation, or the response to climate is not so clear when compared to Touriga Nacional variety. Thus, Aragonez variety seems to be more resistant to climatic variations.

Moreover, the characterization of daily TX difference between clusters in Alentejo (berry weight, pH, probable alcohol, TPI and anthocyanins), Dão (titratable acidity) and Douro-C (probable alcohol) of Touriga Nacional variety allowed for identifying critical days/periods that may have contributed to inter-annual variability of maturation parameters. Furthermore, it was possible to associate warmer/colder years with higher/lower levels of berry quality parameters.

Previous studies had already demonstrated the role of some climatic variables in grape berry quality parameters (Ubalde et al., 2010; Barnuud et al., 2013). However, to our knowledge, this is the only study that uses a long-time series of biochemical (in field conditions) and climatic data: maximum of 27 years for Douro region (1991-2017) and a minimum of 6 years for Alentejo region (2012-2017). This confers robustness to obtained results.

5.1   Work weaknesses and constraints

In this study, influences of climate variables on grape berry composition were examined at maturity (last analysis carried out before harvest, up to two weeks before), so the established common maturity level is influenced by the winemakers' decision to harvest, which can have introduced a variation between samples. This additional source of variation could be minimised by fixing a common maturity date when the crop first reaches 22° Brix TSS (Barnuud et al., 2013), but this type of experimental methodology would be almost impossible to introduce in a study like the one performed here which was based in maturity data from different vineyards and regions in a very large time-scale.

The influence of precipitation on berry composition at maturity varied from one region to another and between varieties. Although some results have shown a role of precipitation during flowering and maturation period, other variables such as soil water holding capacity and air relative humidity should be considered in order to guarantee that the variations found due to precipitation are not being influenced by other important factors in the plant water status. On the other hand, some vineyards are irrigated (Alentejo and Douro-D), which limits the study of precipitation influence on berry composition.

5.2   Future perspectives

After confirming the influence of climatic variability on grape berry composition, by identifying the most affected regions and the most important climatic variables, in the future, we should invest in the development of a model to be used for both operational short-to-medium range forecasting and climate change impact assessments. Strong trends in berry composition – berry weight, titratable acidity, pH, probable alcohol, anthocyanins and total phenols index – were detected which demonstrates that, although other factors could potentially affect the grape berry biochemistry, climate is dominant in shaping regional patterns of grape berry composition. This provides support for the use of studied climate variables in evaluating potential impacts of climate changes on some aspects of viticulture in future scenarios. These models can be run at local/regional scales, thus providing forecast information for the sector, namely for varieties across the wine regions of Portugal.

The grape maturation database that was prepared in the context of the present study still contains crucial information that can be further exploited in order to increase the level of knowledge about the influence of climatic variability on grape composition. In particular, it would be interesting: i) to search correlations between climatic variables and grape quality parameters not studied in this work, such as malic acid, and ii) to extend the study to other regions and varieties.

The enrichment of the study with more information will increase its robustness and will allow the improvement of the forecast model to be developed.

6. REFERENCES

Ali, K., Maltese, F., Fortes, A. M., Pais, M. S., Choi, Y. H., Verpoorte, R. (2011). Monitoring biochemical changes during grape berry development in Portuguese cultivars by NMR spectroscopy. *Food Chemistry, 124*, 1760-1769.

Antolín, M., Baigorri, H., De Luis, I., Aguirrezabal, F., Geny, L., Broquedis, M., Sánchez-Díaz, M. (2003). ABA during reproductive development in non-irrigated grapevines (Vitis vinifera L. cv. Tempranillo). *Australian Journal of Grape and Wine Research, 9*, 169-176.

Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J. J., Hilbert, G., Pascual, I. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Science, 267*, 74-83.

Ashenfelter, O., Storchmann, K. (2014). Wine and Climate Change. *American Association of Wine Economists*.

Barnuud, N. N., Zerihun, A., Gibberd , M., Bates, B. (2013). Berry composition and climate: responses and empirical models. *International Journal of Biometeorology, 58*, 1207-1223.

Bartolomé, M. (1993). Respuestas de la vid (Vitis vinifera L.) a condiciones de estrés hídrico: efectos sobre las relaciones agua-planta, el crecimiento, la producción y la calidad (cv. Tempranillo). Doctoral Thesis. Spain: Universidad Politécnica de Madrid.

Bisson, L. F., Waterhouse, A. L., Ebeler, S. E., Walker, M. A., Lapsley, J. T. (2002). The present and future of the international wine industry. *Nature, 418*, 696-699.

Blouin, J., Peynaud, É. (2001). *Connaissance et travail du vin* (3ª ed.). Paris: Dunod.

Bock, A., Sparks, T., Estrella, N., Menzel, A. (2013). Climate-induced changes in grapevine yield and must sugar content in Franconia (Germany) between 1805 and 2010. *Plos ONE, 8*, 1-10.

Bonada, M., Sadras, V. (2014). Review: critical appraisal of methods to investigate the effect of temperature on grapevine composition. *Australian Journal of Grape and Wine Research, 21*, 1-17.

Buttrose, M., Hale, C., Kliewer, W. (1971). Effect of temperature on the composition of Cabernet Sauvignon berries. *American Journal of Enology and Viticulture, 22*, 71-75.

Cacho, J., Fernández, P., Ferreira, V., Castells, J. (1992). Evolution of five anthocyanidin-3-glucosides in the skin of the Tempranillo, Moristel, and Garnacha grape varieties and influence of climatological variables. *American Journal of Enology and Viticulture, 43*, 244-248.

Carvalho, L. C., Coito, J. L., Gonçalves, E. F., Chaves, M. M., Amâncio, S. (2016). Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. *Plant Biology, 18*, 101-111.

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Perterlunger, E., Di Gaspero, G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant, Cell and Environment, 30*, 1381-1399.

Castellarin, S., Bavaresco L., Falginella, L., Gonçalves, M., Di Gaspero, G. (2012). Phenolics in grape berry and key antioxidants. In H. Gerós, M. Chaves, & S. Delrot (Eds.), *The Biochemistry of the Grape Berry* (pp. 89-100). Bentham Science Publishers.

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., Rodrigues M. L., Lopes, C. M. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany, 105*, 661-676.

Chevet, J.-M., Lecocq, S., Visser, M. (2011). Climate, grapevine phenology, wine production, and prices: Pauillac (1800-2009). *American Economic Review, 101*, 142-146.

Cheynier, V., Prieur, C., Guyot, S., Riguad, J., Moutounet, M. (1997). The structures of tannins in grapes and wines and their interactions with proteins. In T. R. Watkins (Ed.), *Wine: Nutritional and Therapeutic Benefits* (Vol. 661, pp. 81-93). American Chemical Society.

Conde, C., Silva, P., Fontes, N., Dias, A., Tavares, R., Sousa, M. J., Agasse, A., Delrot, S., Gerós, H. (2007). Biochemical changes throughout grape berry development and fruit and wine Quality. *Food, 1*, 1-22.

Coombe, B. (1987). Influence of temperature on composition and quality of grapes. *Acta Horticulturae, 206*, 23-35.

Costa, J. M., Ortuño, M. F., Lopes, C. M., Chaves, M. M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. *Functional Plant Biology, 39*, 179-189.

Cunha, M., Abreu, I., Pinto, P., Castro, R. (2003). Airbone pollen samples for early-season estimates of wine production in a mediterranean climate of northern Portugal. *American Journal of Enology and Viticulture, 54*, 189-184.

Dai, Z. W., Ollat, N., Gòmes, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C., Vivin, P., Delrot, S. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *American Journal of Enology and Viticulture, 62*, 413-425.

Davies C., Boss, P., Gerós, H., Lecourieux, F., Delrot, S. (2012). Source/sink relationships and molecular biology of sugar accumulation in grape berries. In H. Gerós, M. Chaves, & S. Delrot (Eds.), *The Biochemistry of the Grape Berry* (pp. 44-66). Bentham Science Publishers.

De Orduna, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International, 43*, 1844-1855.

Downey, M. O., Dokoozlian, N. K., Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture, 57*, 257-268.

Fernandez, L., Pradal, M., Lopez, G., Berud, F., Romieu, C., Torregrosa, L. (2006). Berry size variability in Vitis vinifera L. *Vitis, 2*, 53-55.

Fontoin, H., Saucier, C., Teissedre, P.-L., Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference, 19*, 286-291.

Ford, C. M. (2012). The Biochemistry of Organic Acids in the Grape. In H. Gerós, M. Chaves, & S. Delrot (Eds.), *The Biochemistry of the Grape Berry* (pp. 67-88). Bentham Science Publishers.

Fraga, H., Santos, J. A., Moutinho-Pereira, J., Carlos, C., Silvestre, J., Eiras-Dias, J., Mota, T., Malheiro, A. C. (2016). Statistical modelling of grapevine phenology in Portuguese wine regions: observed trends and climate change projections. *Journal of Agricultural Science, 154*, 795-811.

Francis, I., Newton, J. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research, 11*, 114-126.

Gladstones, J. (1992). *Viticulture and Environment.* Adelaide, South Australia: Winetitles.

Hadarits, M., Smit, B., Diaz, H. (2010). Adaptation in viticulture: a case study of producers in the Maule region of Chile. *Journal of Wine Research, 21*, 167-168.

Halliday, J., Johnson, H. (1994). *The art and science of wine.* (M. Beazley, Ed.) London: Mitchell Beazley.

IPCC. (2014). *Climate Change 2014: Impacts, Adaptation, and Vulnerability.* Retrieved October 16, 2017, from http://www.ipcc.ch/report/ar5/wg2/.

Jackson, D. I., Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality - a review. *American Journal of Enology and Viticulture, 44*, 409-430.

Jones, G. (2012). *A climate assessment for the Douro wine region: an examination of the past, present, and future climate conditions for wine production.* Oregan, USA: ADVID.

Jones, G. V., Ried, R., Vilks, A. (2012). Climate, grapes and wine: structure and suitability in a variable and changing climate. In *The Geography of Wine: Regions, Terroir and Techniques* (pp. 109-133). Springer Press.

Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research, 16*, 56-69.

Keller, M., Tarara, J. M., Mills, L. J. (2010). Spring temperatures alter reproductive development in grapevines. *Australian Journal of Grape and Wine Research, 16*, 445-454.

Kennedy, J. (2002). Understanding grape berry development. *Practical Winery & Vineyard*.

Kenny, G., Harrison, P. (1992). The effects of climate variability and change on grape suitability in Europe. *Journal of Wine Research, 3*, 163-183.

Larronde, F., Krisa, S., Decendit, A., Chèze, C., Deffieux, G., Mérillon, J. (1998). Regulation of polyphenols production in Vitis vinifera cell suspension cultures by sugars. *Plant Cell Reports, 17*, 946-950.

López, M.-I., Sánchez, M.-T., Diaz, A., Ramirez, P., Morales, J. (2007). Influence of a deficit irrigation regime during ripening on berry composition in grapevines (Vitis vinifera L.) grown in semi-arid areas. *International Journal of Food Sciences and Nutrition, 58*, 491-507.

Lorrain, B., Chira, K., Teissedre, P. (2011). Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: comparison to 2006, 2007 and 2008 vintages. *Food Chemistry, 126*, 1991-1999.

Matthews, M. A., & Anderson, M. M. (1988). Fruit ripening in Vitis vinifera L.: responses to seasonal water deficits. *American Journal of Enology and Viticulture, 39*, 313-320.

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany, 58*, 1935–1945.

Mori, K., Sugaya, S., Gemma, H. (2005). Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae, 105*, 319-330.

Moriondo, M., Jones, G. V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., Bindi, M. (2013). Projected shifts of wine regions in response to climate change. *Climatic Change, 119*, 825-839.

Mozell, M. R., Thach, L. (2014). The impact of climate change on the global wine industry: Challenges & solutions. *Wine Economics and Policy, 3*, 81-89.

OIV. (2017). *World Vitiviniculture Situation.* Paris: OIV Statistical Report on World Vitiviniculture.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A. (2002). Influence of pre- and postversaison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of Vitis vinifera cv. Shiraz. *American Journal of Enology and Viticulture, 53*, 261-267.

Ojeda, H., Deloire, A., & Carbonneau, A. (2001). Influence of water deficits on grape berry growth. *Vitis, 40*, 141-145.

Parra, C. S., Aguirreolea , J., Sánchez-Díaz , M., Irigoyen , J. J., Morales, F. (2010). Effects of climate change scenarios on Tempranillo grapevine (Vitis vinifera L.) ripening: response to a combination of elevated CO_2 and temperature, and moderate drought. *Plant Soil, 337*, 179-191.

Pirie, A., Mullins, M. (1977). Interrelationships of sugars, anthocyanins, total phenols, and dry weight in the skin of grape berries during ripening. *American Journal of Enology and Viticulture, 28*, 204-209.

Pomarici, E., Seccia, A. (2016). Economic and social impacts of climate change on wine production. *Reference Module in Food Science*.

Poudel, P. R., Mochioka, R., Beppu, K., Kataoka, I. (2009). Influence of temperature on berry composition of interspecific hybrid wine grape 'Kadainou R-1' (Vitis ficifolia var. ganebu × V.vinifera 'Muscat of Alexandria'). *Journal of the Japanese Society for Horticultural Science, 78*, 169-174.

Rogiers, S. Y., Greer, D. H., Hutton, R. J., Landsberg, J. J. (2009). Does night-time transpiration contribute to anisohydric behaviour in a Vitis vinifera cultivar? *Journal of Experimental Botany, 60*, 3751–3763.

Ruffner, H. P., Hawker, J. S., Hale, C. R. (1976). Temperature and enzymic control of malate metabolism in berries of Vitis vinifera. *Phytochemistry, 15*, 1877-1880.

Santos, J. A., Gratsch, S., Karremann, M., Jones, G., Pinto, J. (2013). Ensemble projections for wine production in the Douro Valley of Portugal. *Climatic Change, 117*, 211-225.

Smart, E. R. (1985). Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. *American Journal of Enology and Viticulture, 36*, 230-239.

Teixeira, A., Eiras-Dias, J., Castellarin, S., Gerós, H. (2013). Berry phenolics of grapevine under challenging environments. *International Journal of Molecular Sciences, 14*, 18711-18739.

Ubalde, J. M., Sort, X., Zayas, A., Poch, R. M. (2010). Effects of soil and climatic conditions on grape ripening and wine quality of Cabernet Sauvignon. *Journal of Wine Research, 21*, 1-17.

van Leeuwen, C., Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *Journal of Wine Economics, 11*, 150-167.

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *American Journal of Enology and Viticulture, 55*, 207-217.

van Leeuwen, C., Tregoat, O., Choné, X., Bois, B., Pernet, D., Gaudillère, J.-P. (2009). Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *Journal International des Sciences de la Vigne et du Vin, 43*, 121-134.

Webb, L., Whetton, P., Barlow, E. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Australian Journal of Grape and Wine Research, 13*, 165-175.

Zarrouk, O., Costa, J. M., Francisco, R., Lopes, C., Chaves, M. M. (2016). Drought and water management in Mediterranean vineyards. In H. Gerós, M. M. Chaves, H. M. Gil, & S. Delrot (Eds.), *Grapevine in a changing environment* (pp. 38-59). John Wiley & Sons.
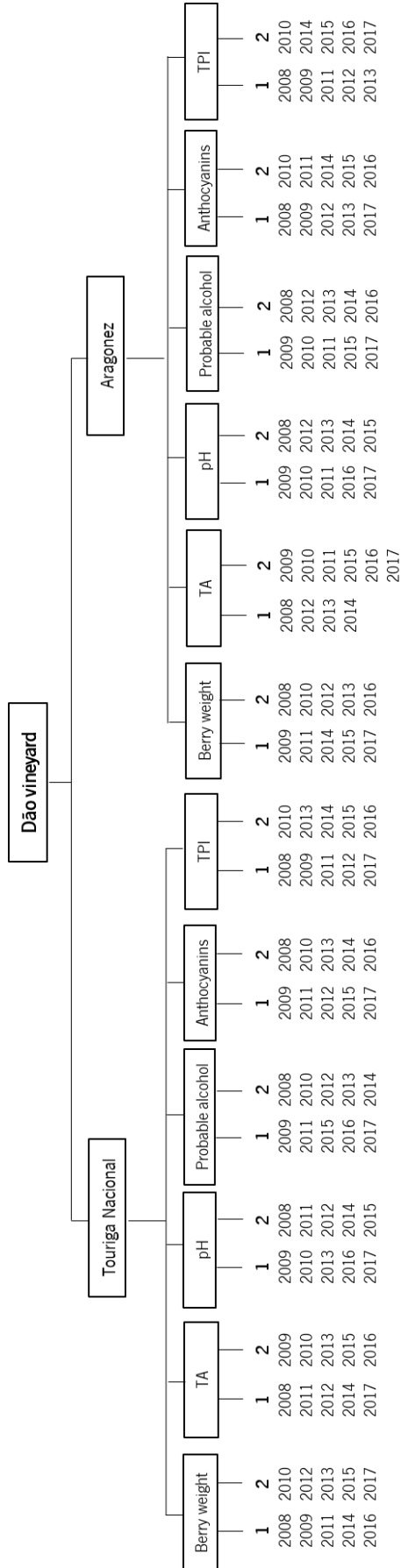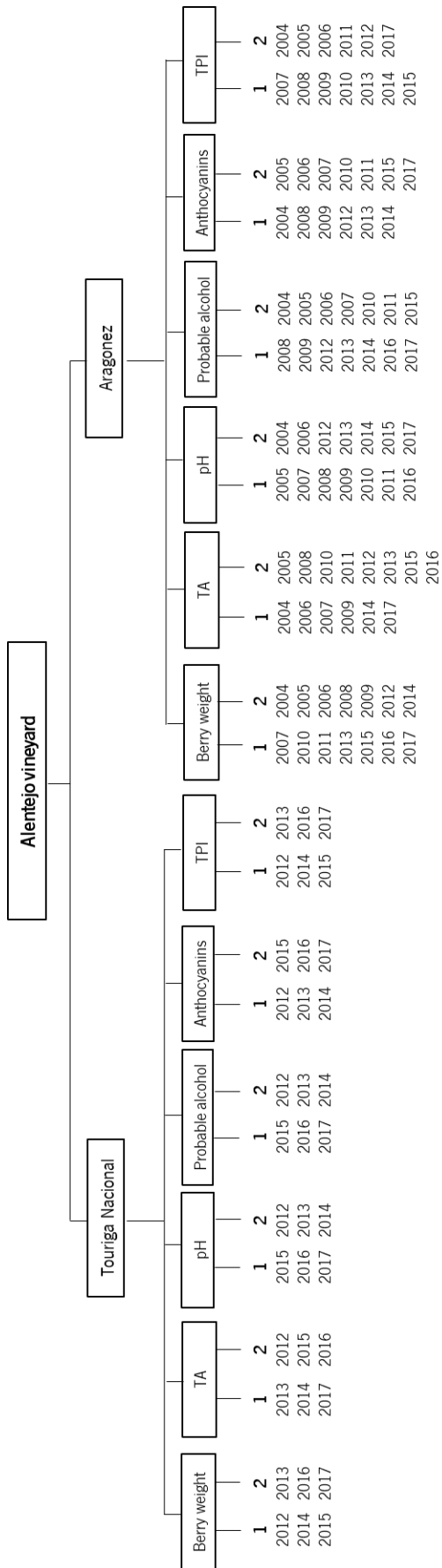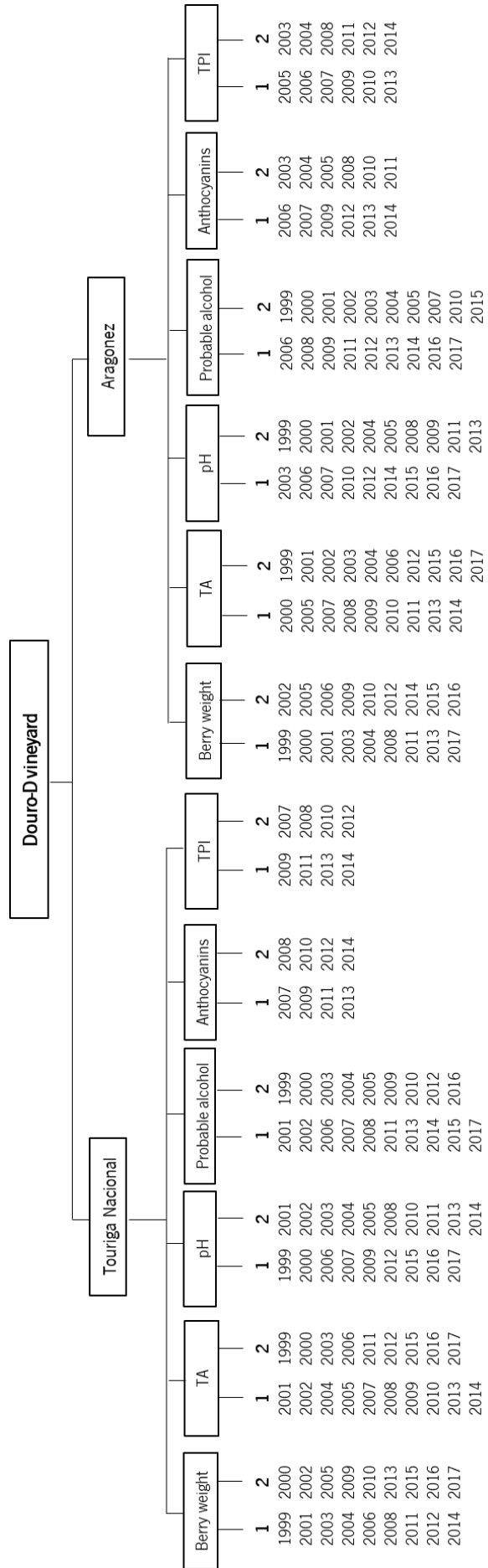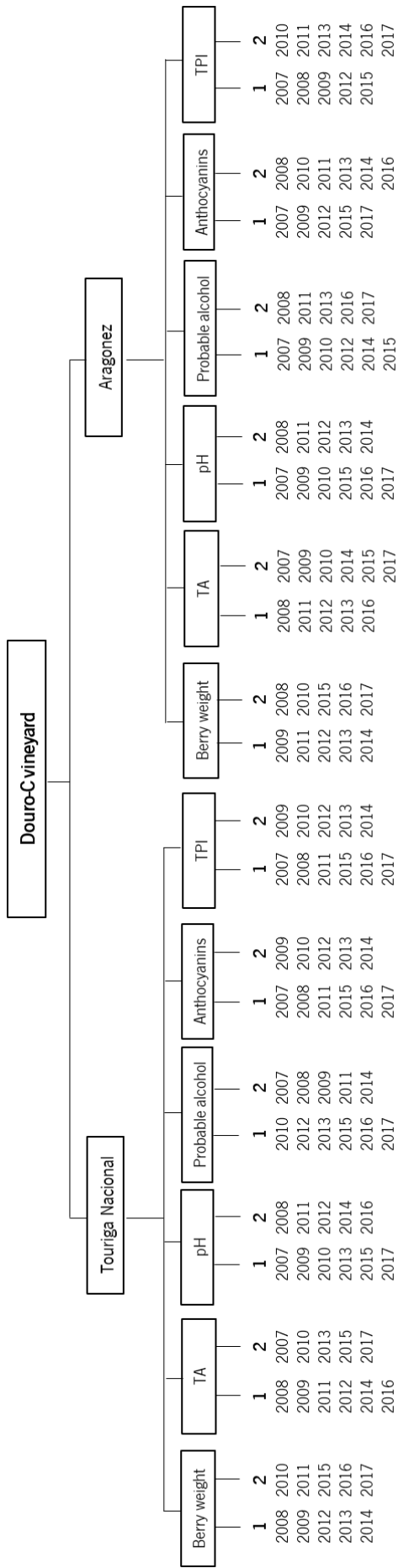
ANNEX II – ANALYSIS OF MATURATION CLUSTERS

Region	Quality parameter	Touriga Nacional			Aragonez		
		Cl1 average	Cl2 average	Difference between averages	Cl1 average	Cl2 average	Difference between averages
Alentejo	Weight of 100b (g)	148	122	26*	188	146	42***
	Titratable acidity (g/L T.A.)	4.5	3.7	0.8	4.0	3.4	0.6***
	pH	3.95	3.76	0.19*	4.16	3.96	0.20***
	Probable alcohol (17g/1%)	13.9	13.1	0.80*	16.2	13.3	2.9***
	TPI	64.1	46.9	17.2	58.9	47.7	11.2**
	Anthocyanins (mg/L)	517	320	197*	565	440	125***
Dão	Weight of 100b (g)	164	135	29*	234	203	31**
	Titratable acidity (g/L T.A.)	5.9	4.2	1.7**	4.8	3.9	0.9**
	pH	3.85	3.59	0.26**	3.93	3.79	0.14*
	Probable alcohol (17g/1%)	12.7	11.3	1.4*	14.5	13.3	1.2***
	TPI	48.2	33.7	14.5**	47.5	28.1	19.4**
	Anthocyanins (mg/L)	485	366	119***	458	339	119**
Douro-C	Weight of 100b (g)	247	131	116***	263	181	81*
	Titratable acidity (g/L T.A.)	7.2	4.4	2.8**	5.1	3.7	1.3*
	pH	3.73	3.36	0.37**	3.92	3.61	0.31**
	Probable alcohol (17g/1%)	13.7	11.9	1.8**	15.5	13.3	2.2*
	TPI	51.1	31.4	19.7**	46.1	33.5	12.6**
	Anthocyanins (mg/L)	419	182	237*	449	288	161**
Douro-D	Weight of 100b (g)	162	108	54***	189	137	52***
	Titratable acidity (g/L T.A.)	4.7	4.0	0.7***	3.8	3.0	0.8**
	pH	3.97	3.73	0.24***	4.03	3.84	0.19***
	Probable alcohol (17g/1%)	13.5	11.9	1.6***	15.5	13.8	1.8***
	TPI	47.4	34.2	13.2	48.1	33.7	14.4*
	Anthocyanins (mg/L)	332	232	100*	401	211	190***