ALMA MATER STUDIORUM - Università di Bologna

DOTTORATO INDUSTRIALE IN

Scienze e Tecnologie Agrarie, Ambientali e Alimentari

Ciclo XXXIII

Settore Concorsuale: 07-F1

Settore Scientifico Disciplinare: AGR/15

Study of the Chianti and Chianti Classico appellations: Evaluation of enological potential of Sangiovese and complementary varieties by a multiparametric approach

Presentata da: Jelena Jeremić

Coordinatore Dottorato:	**Supervisore tesi:**	**Cosupervisore tesi:**
Prof. Massimiliano Petracci	Prof. Andrea Versari	Prof.ssa. Giuseppina P. Parpinello

Supervisore aziendale (Ruffino s.r.l.):

Dr. Gabriele Tacconi

Esame finale anno 2021

ACKNOWLEDGMENT

To my family: My lovely sister Emina, my mother Vesna and father Radomir, and the other members of family that have always understood my motivation to progress in scientific world.

I would like to express my gratitude to following persons and colleagues:

To my supervisor Professor Andrea Versari and Co-supervisor Professor Giuseppina P. Parpinello and Arianna Ricci from Bologna University for their generous advisements and availability in collaboration.

To my supervisors from Ruffino: Gabriele Tacconi, the chef winemaker and Rita Orrù for their pleasant support and understanding that they showed during all three years of the PhD program.

To Ruffino's human resource office: Emanuele Rossini that recognized the importance to develop the PhD project in collaboration with University of Bologna.

To Ruffino's laboratory personal: Benedetta Bronzi, Sandro Crocchini and Sandra Ciapetti for their kind participation for what concern chemical analysis of wine.

To my Serbian colleagues and friends: Dr. Mirjam Vujadinović Mandić and Dr. Nemanja Teslić for an efective and nice collaboration on climate changing research.

To my dear friends Chiara Carnovale and her altruistic help to get visual design of the PhD thesis . Grazie Mario!; and Milena Đekić for the English language shaping …maco…ti znaš…ova ti je A!

Contents

Table of abbreviations used in this thesis

LIST OF ABREVIATIONS USED IN PHD THESIS	
DO	Dissolved oxygen in wine
HS	Head space oxygen of bottle
TPO	Total packaging oxygen
OTR	Oxygen transmission rate
OCR	Oxygen consumption rate
CI	Color intensity
H	Color hue
ROS	Reactive oxygen species
CIE	International commission on illumination (Commision international eclarage fr.)
IPCC	Intergovernmental Panel on Climate Change
NOAA	National Oceanic and Atmospheric Administration
PDO	Protected Designation of Origin
RMSE	Root mean square error
DOCG	Denominazione di Origine Controllata e Garantita
EasyOx	Easily oxidizable wine compounds
PhenOx	Total oxidizable wine polyphenols
IPT	Total phenolics index
TAN/ACN	Tannin / Anthocyanin ratio

The list of tables

The list of figures

CHAPTER 1

Introduction and Project Aims

1 Introduction and Project aim

1.1 Introduction - Ruffino winery

Two cousins Ilario and Leopoldo Ruffino established the winery in 1877, realizing that the secret to making the finest Tuscan wine was to take care of the vineyards best suited to the purpose. Ruffino takes care about tradition and history, while embracing modern winemaking to achieve the very best from each vintage.

In 2011, Ruffino was purchased by American Constellation Brans Company, a market leader of the beer, wine and spirits sector. The winery employs 300 people whose work contributes to the business's strong continual growing. Ruffino produces and sells more than 30 million bottles across forty labels, most of which come from Tuscany's historic denominations such as Chianti, Chianti Classico and Brunello di Montalcino. On the North of Italy, the winery produces Prosecco and Pinot Grigio in Veneto region. The wines from this company are present in more than 96 countries in the world.

Ruffino grows grapes in over 600 hectares of vineyards across eight estates: six in Tuscany: Poggio Casciano, Montemasso, Santedame, Gretole, La Solatia and Greppone Mazzi; two in Veneto: Cà del Duca and La Duchessa (**Figure 1.1**). In Pontassieve is sited a state-of-the-art aging cellar with bottling facilities.

Figure 1. 1 Ruffino's estates in Tuscany. Poggio Csciano, Santedame, Gretole, La Solatia, Montemasso, Greppone Mazzi.

Poggio Casciano is an estate next to Grassina, only few kilometres from Florence. It produces Super Tuscan full-bodied highly structured wines. Montemasso estate is located in Chianti Classico wine region, south of Florence in Greve municipality. Santedame and Gretole are located in Chianti Classico region near to Castellina in Chianti. There are produced Ruffino's Chianti Classico wines including Gran Selezione, the highest quality of Chianti Classico wines. La Solatia is a winery located next to Monteriggioni, few kilometres from Siena and produces white and rosé wines and red wines including Chianti DOCG and Chianti Superiore DOCG.

When it comes to Chianti family of wines, Ruffino produces all the categories of Chianti and Chianti Classico appellations of origin wines (**Figure 1.2**). Thus, the biggest production in terms of quantity is presented by Chianti DOCG wine. It is followed by Chiati Superiore DOCG wines with labels Chianti Superiore Fiasco and Il Leo. The next category is Chianti Classico DOCG wine represented by Santedame and Aziano labels. It is followed by Chianti Classico Riserva DOCG wine with label Riserva Ducale, and at the and the Ruffino's icon wine Chianti Classico Gran Selezione DOCG: the best quality from Chianti family wines - Riserva Ducale Oro.

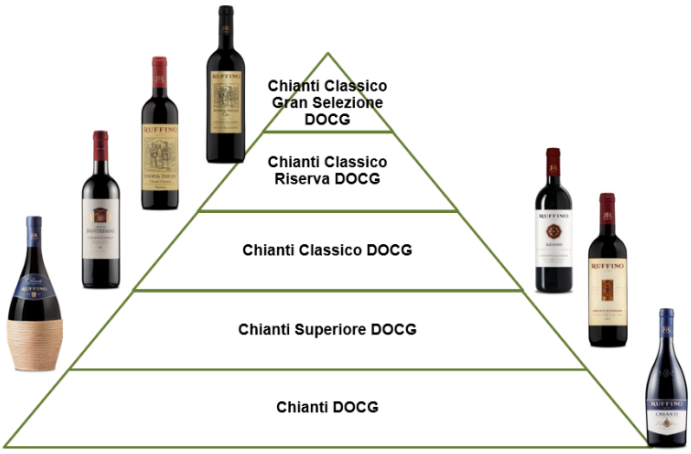


Figure 1. 2 Ruffino's quality piramid of Chianti wines.

Ruffino has always been striving to achieve the best quality of wine and to remain one of the leader of Italian wine sector. Thus, even with this PhD program, Ruffino aims to get in scientific details of wine quality with purpose to improve even more the wine production.

1.2 Project aim

The overall aim of the PhD study is to exploit the enological potential of Sangiovese grape – and additional complementary grape varieties – for the valorization of Chianti red wine. In this view, the following interrelated tasks were planned alongside the entire production chain:

(i) understanding the impact of the climate changes at local level to tailor both the grape growing and winemaking practices to improve the wine quality with respect to the traditional regional winemaking regulations;

(ii) development of the quality control procedures and analytical protocols to improve and ascertain the composition of wine from the grape growing, through the winemaking and aging processes, till transportation and consumption.

The ongoing **climate change** is expected to further impact the European viticulture in the future. The climate variables - like air temperature, rainfall levels and distributions, humidity, winds, light intensity and cloud cover - can affect the grape growing wine industry to large extent (Jackson and Lombard, 1963; Jones, 2007). In particular, the main effects of climate changes on the viticulture are the increasing sugar levels of grapes that leads to wines with high alcohol and low acidity and change of varietal aromas (Jones, 2007; Mira de Orduña, 2010). The mean temperatures have a great impact on the length of the growing season for each variety **(Figure 1.3)**, the grapevine physiology and the metabolism and fruit composition.

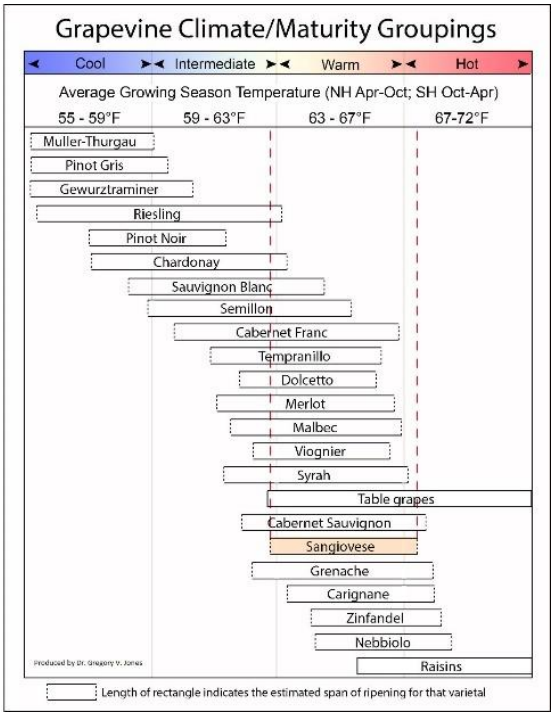


Figure 1. 3 Set of grapevine varieties by maturity groupings based on growing season mean temperatures (modified from Jones, 2007).

Fraga et al. (2012) found that high night temperatures in combination with high diurnal temperatures during the grape maturation/ripening period could lead to low tannins and anthocyanins with reduction in wine color and increased loss of aroma compounds. The annual precipitation is another critical factor of viticulture because the water stress can lead to small shoot growth, poor flower, cluster and berry development causing the decrease of grape yield and increased water demand due to the irrigation. On the other hand, the excessive humidity during the early stages cause the denser canopies that leads to pests and disease problems requiring more intense plant protection and low wine quality (Fraga et al. 2012).

Wine typicity and wine quality parameters

The **typicity** of wine is defined as the physico-chemical and sensory characteristics that are considered representative for the Protected Designation of Origin (PDO) related to a terroir. Three main factors are usually considered to assess the global wine typicity of a PDO wine: the standard profile (i.e., the basic physico-chemical characteristic), the cultivar profile (i.e. the sensory aromatic characteristics coming from the grapes), the style profile (i.e., the characteristics that result from the winemaking methods). The relation between chemical

characteristics of grapes and the chemical and aromatic profile of wine could be revealed using multivariate analysis like Partial Leas Squares (PLS) (Canuti et al., 2017). In the case of Chianti and Chianti Classico DOCG wines, all the wines that obtain these appellations of origin have to acquire the approval by the orgaisations "Consorzio vino Chianti" (Chianti wine Consortium) and "Consorzio di Tutela" (Chinati Classico wine Consortium). These organisations control the wine quality in terms of chemical analysis and sensory characteristisc (See the **Chapter 3**).

The **wine quality** could be described in four notions: (i) the excellence or superiority, (ii) the value, (iii) the conforming to specifications and (iv) meeting or exceeding customer expectations (Canuti et al. 2017). Well run taste-panels relied with the chemical analysis of wine would be an ideal wine quality evaluation model that can be defined by the following selected 'indicators' according to literature (Jackson and Lombard, 1963):

1. *Soluble solids:* presented by sugar levels that indicates the alcohol potential degree after fermentation and the likelihood of residual sugar remaining. It is measured in Brix, Beaumé, Babo, Oechsels or Balling. The limit of 24° Brix is commonly used to indicate the proper maturity of red and white grapes suitable for the quality production of dry wines.

2. *Organic acids* (tartaric, malic, citric, etc.) expressed in total titratable acidity TA: from 6 g/L in warm region where the acidification process should be taken in consideration to high levels of 10 g/L in cool climates where the deacidification may be required.

3. The *pH* amount above the 3.6 may increase risk of microbial stability and may decrease the red wine color intensity and free SO_2 level in wine (Ribéreau-Gayon et al., 2010).

4. *Phenolics and anthocyanin*: phenolic compounds include the tannins that are extracted from the grape skin, seed and rachis during the winemaking and impart structure, astringency and bitterness in wine. Anthocyanins are responsible of red color in wine. Ribéreau-Gayon et al. (2010) describes the sensory properties of phenolic compounds in red wine **(Figure 1.4)** depending on the polymerization level. Thus, low polymerized catechins and procyanidins elicit sour and astringent taste, whereas the oligomers and polymers of procyanidins give the volume in mouth-feel with pronounced bitterness and astringency. The condensate tannins

(procyanidins type) have the astringent characteristic that decrease with degree of polymerization, while the combination of tannins-polysaccharides gives the fat sensation and the pleasant wine body. The anthocyanins and their combined forms with tannins are low astringent, but it gives the pronounced bitterness specially in young wines.

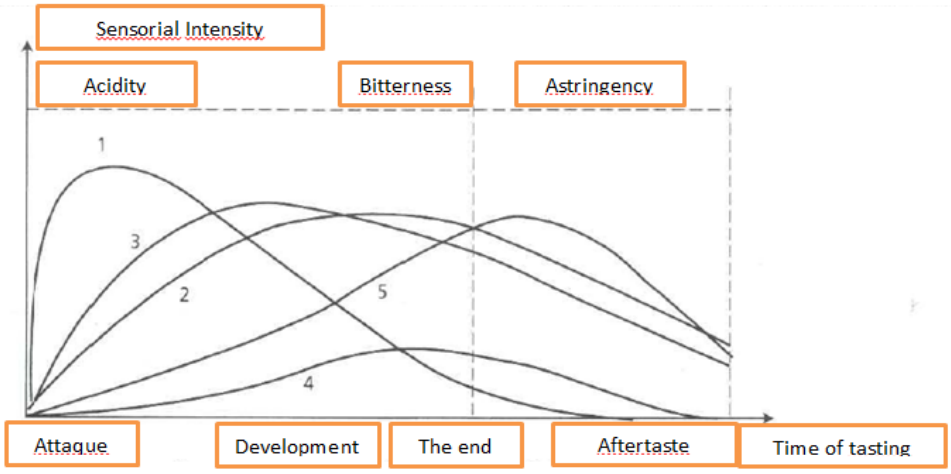


Figure 1. 4 The influence of phenolic compound structure on of their sensory characteristics 1. Procyanidin low degree of polymerization, 2. Procyanidin oligomer, 3. Procyanidin polymer, 4. Anthocyanins, 5. Seed tannins (Adapted from Ribéreau-Gayon et al., 2005).

Scollary et al. (2012) have found the links between sensory descriptors for astringency and analytical measurements of wine. The astringency perception in red wine is a mouthfeel response of a proanthocyanidin assembly with proteins (ethyl-bridged compounds, pyranoanthocyanins) and polysaccharides **(Figure 1.5).** This molecular assembly could be (i) proanthocyanidin-proanthocyanidin, (ii) proanthocyanidin–protein, (iii) proanthocyanidin–polysaccharide and (iv) proanthocyanidin–protein–polysaccharide. The molecular π–π interaction that involve anthocyanins with polyphenols are described as the color enhancement or copigmentation in the red wine (Boulton, 2001).

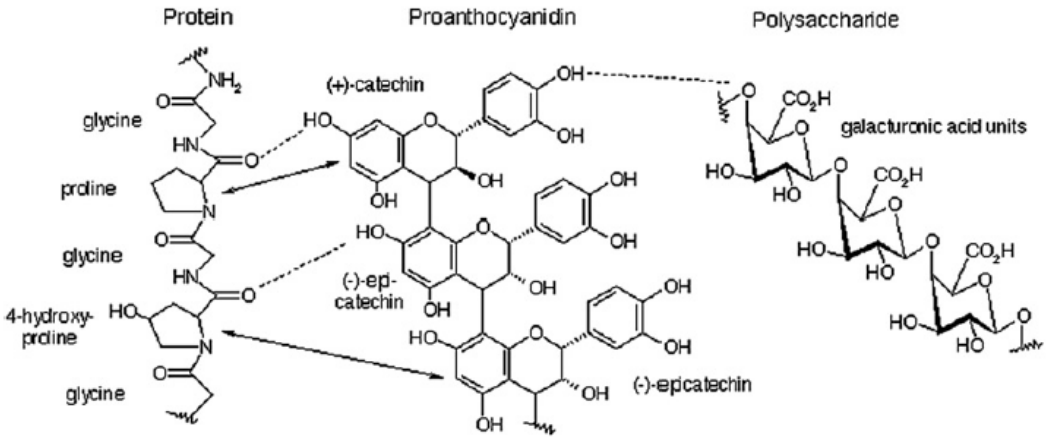


Figure 1. 5 Interaction between protein, proanthocyanidin and polysaccharide fragments (Scollary et al., 2012).

The sub-qualities to astringency as oral sensations elicited in the red wine is described with 33 terms in the "mouth-feel wheel" **(Figure 1.6)**. The polyphenols of molecular weight between 500 and 3000 Da elicit the astringency sensation. The same sensation can give even a smaller molecules such as: 5-O-caffeoylquinic acid, flavan 3–ol monomers, dimers and trimers. Beside the polyphenols, the organic and inorganic acids can also induce the astringency in wine as well as some minerals like zinc (Bajec and Pickering, 2008).

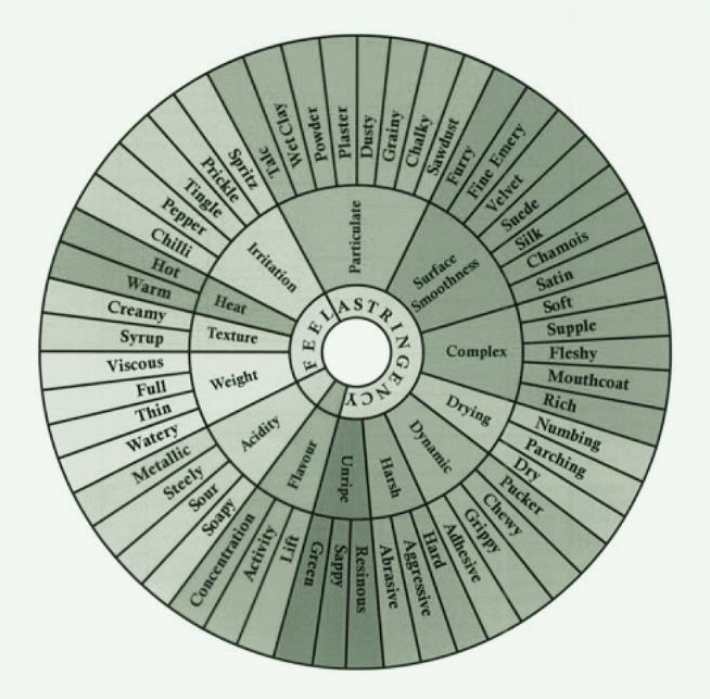


Figure 1. 6 Red wine mouth-feel wheel (Bajec and Pickering, 2008).

5. Other components like *amino acids* could cause unwanted organoleptic characteristics (i.e. reduction) to wine if their amount is not high enough in the must during the fermentation (Jackson and Lombard, 1993).

6. *Wine aromas* could be positive or pleasant characteristics and negative, i.e. wine faults. Ferreira and De la Fuente (2017) recently introduced the concept of "aromatic buffer" mainly driven by approximately 37 chemical compounds common in all wines that seems the main responsible of the "vinous" character of wine. Besides the mentioned molecules, there is a number of volatile compounds that confer typicity to the wine when they occur at supra-threshold level.

7. *Sanitary condition of grape and wine.* If rain occurs during the harvest it is possible to have a grape infection by *Botrytis cinerea* that can lead to the enzymatic wine oxidation by laccase which ruins the wine quality (Thurston, 1994; Belcher and Dienes-Nagy, 2011). To avoid problem of wine filterability the enzymatic treatment may be required (Laffort, 2013).

8. Wine aging, storage and transport conditions can affect the wine composition to the great extent. For example, during the aging of wine, the polyphenols can polymerize in the presence of oxygen leading to the change in wine color as well as the development of tertiary aromas known as wine bouquet **(Figure 1.7)** (Versari, 2012). The long-term wine exposure to the heat could negatively change its olfactory characteristics even its physical and chemical stability (the cloudy or oxidized appearance) during the storage or transportation (Butzke et al., 2012).

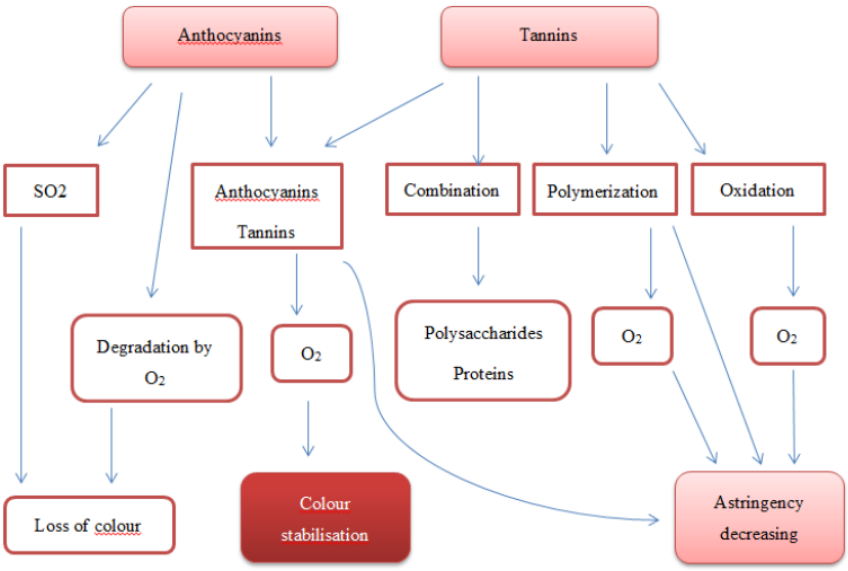


Figure 1. 7 Transformation of phenolic compounds in wine (Adapted from Versari, 2012).

1.3 References

Bajec, M. and Pickering, G. (2008). Astringency: mechanism and perception. Critical reviews. *Food Science and Nutrition*, 48, 1–18.

Belcher, S. and Dienes-Nagy, A. (2011). Indices sanitaires et marqueurs chimique pour évaluer l'état sanitaire du vin. 1. Definicion, principes et tests préliminaires avec Botrytis cinerea. Revue Suisse. *Viticulture, Arboriculture et Horticulture*, 43, 2, 134–137.

Boulton, R.B. (2001). The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *American Journal of Enology and Viticulture*, 52, 2, 67–87.

Butzke, C., Vogt, E. and Chason Rodriguez L. (2012). Effects of heat exposure on wine quality during transport and storage. *Journal of Wine Research*, 23, 1, 15–25.

Canuti, V., Picci, M., Zanoni, B., Fia, G. and Bertuccioli, M. (2017). A multivariate methodological approach to relate wine to characteristics of grape composition: the case of typicality. *American Journal of Enology and Viticulture*, 68, 1, 49–59.

Fraga, H., Malheiro, A.C., Moutinho Pereira, J. and Santos J.A. (2012). An overview of climate change impacts on European viticulture. *Food and Energy Security*, 1, 2, 94–110.

Jackson, D. and Lombard P. (1993). Environmental and management practices affecting grape composition and wine quality – a review. *American Journal of Enology and Viticulture*, 44, 4, 409–430.

Jones, G. (2007). Climate change: observations, projections and general implications for viticulture and wine production. http://www.cnpuv.embrapa.br/publica/anais/cbve12/56-67.pdf.

Laffort (2013). Vinificazione uve alterate da *Botrytis cinerea*. *Laffort-Info*, 89, 1–4.

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, 43, 7, 1844–1855.

Ribéreau-Gayon, P., Glories, Y., Maujean A. and Dubourdieu, D. (2010). Trattato di enologia. Chimica del vino stabilizzazione trattamenti. *Edagricole – Bologna*, ISBN: 8850651961.

Scollary, G., Pasti, G., Kallay, M., Blackman, J. and Clark, A. (2012). Astringency response of red wines: Potential roll of molecular assembly. *Trends in Food Science & Technology*, 27, 25–36.

Thurston, C. (1994). The structure and function of fungal laccase. *Microbiology*, 140, 19–26.

Versari, A., (2012). Grape chemistry & chemical analysis. 8. Phenolic compounds. *Master International Vintage courses*. University of Bologna (Italy).

CHAPTER 2

Climate change in Tuscany wine region

2 Climate change in Tuscany wine region

2.1 Introduction

Climate change is surely one of the most studied scientific topics of the last decade. Despite the numerous recent discussions on the influence of human activity on the climate change and especially on the effect of the anthropogenic greenhouse gas emissions, it is evident that the climate is changing (Barnett et al. 2005; Crowley 2000). According to the Intergovernmental Panel on Climate Change (IPCC), the "climate change" is an alteration in the climate conditions that can be revealed (e.g., applying statistical tests) by shift in the mean and/or the variability of climate patterns that can last for a long period of time usually for 10 years or longer. These alterations of weather conditions may be caused by both natural factors like Earth insolation (Milankovic, 1941), tornado activities, volcano eruption or forest fires, or anthropogenic factors including industrial gas emissions (IPCC, 2007).

2.1.1 Natural factors of climate change

Be that as it may, the changes in the weather patterns were present during the long history of the Earth creation, thus generating the Ice Ages. An early theory that implemented multidisciplinary approach in understanding of the Ice Ages phenomena was introduced by Croll (1889). He suggested that eccentricity of the Earth's orbit creates 'great secular summers and winters', which certainly lead to the contemporary understanding of the climate change. Later on, a Serbian astronomer, climatologist, and geophysicist Milutin Milanković (1941) reported that changes in the Earth insolation were caused by variation of the Earth inclinations in comparison to the Sun, thus creating warm and cold periods throughout history (Berger, 1988). He created the climate modelling for the first time by an accurate calculation of three factors: the astronomical variables, the insolation forcing and the climate response (Knežević, 2010). The summer insolation shows the latitude variations in the long term (**Figure 2.1**). For instance, the same amount of insolation that we find at 65º of latitude nowadays (0 value on the X axis), occurred at 60º of latitude 10,000 years ago (it was warmer). On the other hand, 435,000 years ago, the insolation amount was the same like the one we have today at 72º (it was colder).

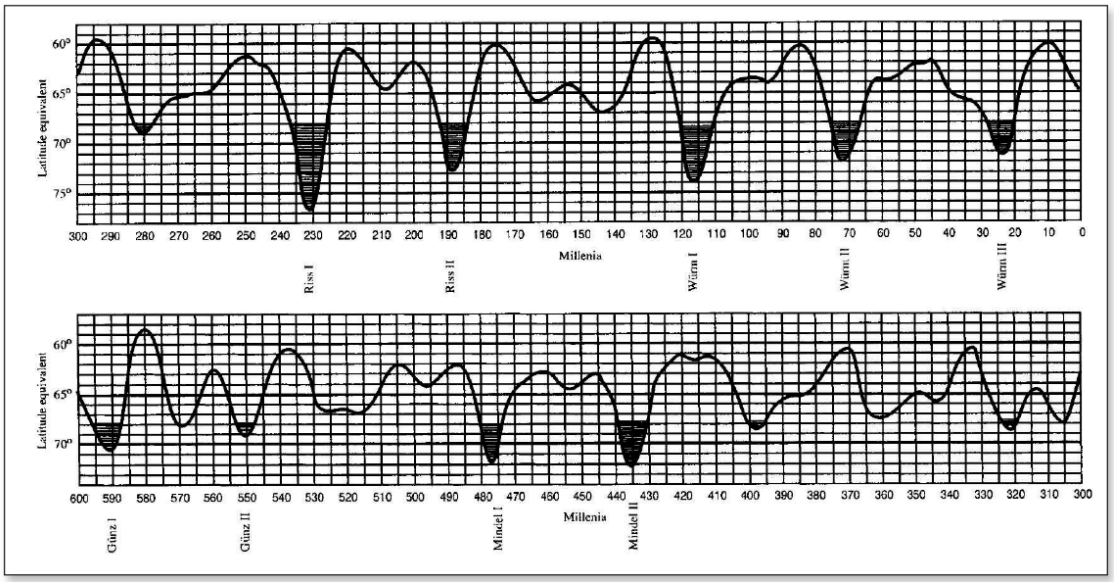


Figure 2. 1 Latitudinal variation of the summer insolation over 600,000 years (Milanković, 1941).

Current research of the atmospheric CO_2 trapped in the ice core from Antarctica confirms the existence of glacial cycles phenomena which date back to 800.000 years ago. The temperature changes are related to the carbon dioxide content as can be seen in **Figure 2.2** (Lithi et al., 2008). The highest CO_2 concentration level throughout the mentioned historical period is about 300 ppm that corresponds to the warmest periods.

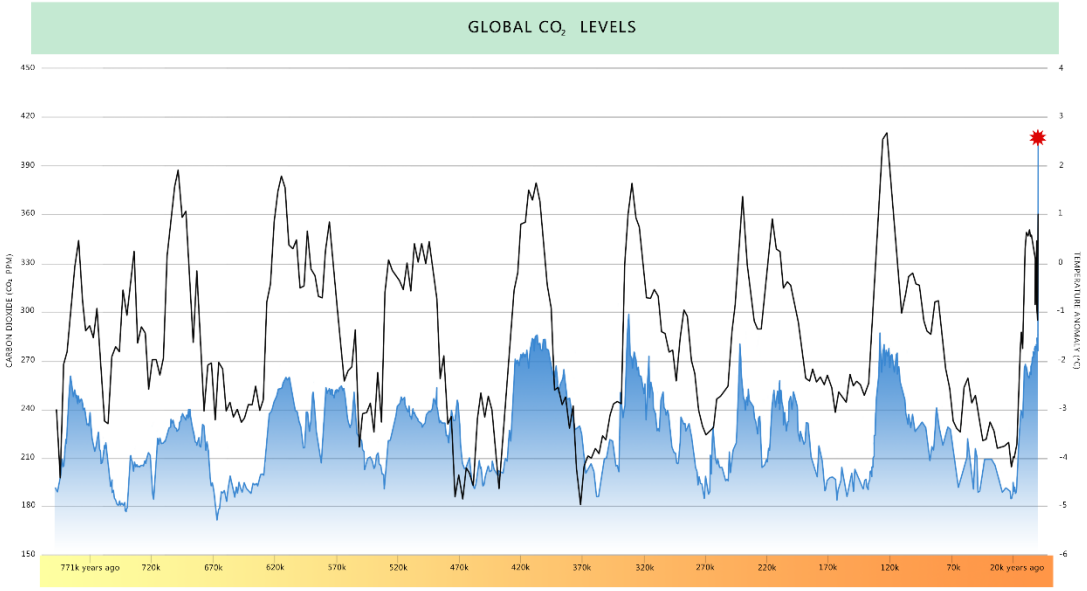


Figure 2. 2 Carbon dioxide level and temperature in Antarctica from 800,000 years ago until 2020. (https://www.co2levels.org).

2.1.2 Anthropogenic factors of climate change

IPCC declared that the human impact on the climate system is evident and that greenhouse gas emissions have been reaching the highest level so far. Climate changes have had an extensive influence on nature and human beings, with numerous changes observed from the 1950's onward that appeared for the first time in history, including the atmosphere and ocean warming, decreasing of snow and ice volume and rising of the sea level. Recent history records the highest amount of carbon dioxide ever released in the atmosphere, especially in the last 100 years, that correspond to the industrial development and emission of greenhouse gasses (e.g., carbon dioxide - CO_2, methane - CH_4 and nitrous oxide - N_2O) into the atmosphere (IPCC, 2014). The trend of global warming at the 20^{th} century was $0.6 \pm 0.2°C$ and the last decade was the warmest of the century (IPCC, 2007).

In November 2020, National Oceanic and Atmospheric Administration reported year-to-date online results of temperature changing, that are recorded as 1ºC higher than the average value of the last century (NOAA, 2020). Furthermore, the atmospheric CO_2 concentration appeared to be 416 parts per million (ppm), which is the highest atmospheric CO_2 content recorded up to this point (Available online: co2levels.org). There is a strong probability (> 99 %) that the next 10 years will be the warmest years in history. So, the overall trend of global warming is constantly on the rise (Agues et al., 2020).

Global warming will cause many risks related to extreme weather conditions, including floods, increased amount of heat waves, occurrence of hurricane strikes typhoons and cyclones in some specific areas, whereas desertification and drought are certain to occur in others (IPCC, 2007). Furthermore, it leads to the accelerated melting of the massive volume of the ground and Antarctic and Arctic glaciers, thus raising the level of the sea (Church, 2001; Huybrechts and Joughin, 2005; Mitrovica et al., 2001). The ecology and human system will be affected by a certain increase in temperatures leading to the asymmetry of ecological responses and the extended risk for flora and fauna (Walther et al., 2002; Thomas et al., 2004), hazard for water resources and reduction of global food production (Arnell et al., 1999; Parry et al., 2004) and threat for the human health (McMichael et al., 2006).

2.1.3 Climate change influence on viticulture and wine quality

The changing in the weather patterns will surely have a large impact on agriculture, and more particularly on viticulture (IPCC, 2014; Rosenzweig et al., 2004; Jones et al., 2005). Grapevine plant *Vitis vinifera* is very delicate when it comes to the weather conditions during the growing season, like precipitation and air temperature. Hence, the climate change can affect the grape and wine structure to significant scale, thus changing their quality (Fraga et al., 2012; Gladstones, 2011; Holland and Smith, 2014; van Leeuwen and Darriet, 2016).

Numerous studies of the weather condition influence on the wine quality have shown that it has an important impact on the wine price and vintage ratings. For instance, Ashenfelter (2010) showed that the price of Bordeaux wine depends on the winter precipitation, growing season temperatures and precipitation during the harvest time. Commonly, low precipitation and warm weather during the summer and autumn time, as well as previously humid winters lead to the high-quality wine production in Bordeaux. Jones and Storchmann (2001) reported that dry and warm growing season is favourable for the high sugar accumulation and low acid content in grapes which defines good quality of the future wine in the same region. Soar et al. (2008) reported that the high-quality wine production in Australia was achieved when the summer temperatures were above 28 to 34 °C depending on the Australian region.

Temperature has a strong impact on vine phenology including bud break, flowering, ripening onset (i.e., véraison) and grape ripening. Temperature can be used for creating models that predict the vine phenology (Parker et al., 2011).

Throughout the fruit set growing stage, the optimum atmosphere temperature ranges from 20 to 26 ºC, while the temperatures <30 ºC may significantly reduce the fruit set (Buttrose and Hale, 1973; Ebadi et al., 1995). During the grape berry growth, the optimum temperature is between 20 and 30 ºC (Kobayashi et al., 1965; Ewart and Kliewer, 1977).

Afterwards, the high temperature that occurs during the grape ripening affects its composition, because it allows sugar compounds to accumulate, while the organic acids and anthocyanins decrease at high temperatures (Coombe, 1987; Kliewer and Torres, 1972). More specifically,

the organic acids content in grape berries decreases at temperatures above 20 ºC (Buttrose et al. 1971; Kliewer, 1971; Kliewer, 1973; Lakso and Kliewer, 1978), whereas the optimum temperatures for the anthocyanins generation is between 17 and 26 ºC (Pirie, 1978). However, during the berry ripening, the night temperature has an impact on the sugar accumulation level. Thus, at lower temperatures (around 10 ºC) the sugar content is higher than the one found in berries ripening at 30 ºC night temperatures (Coombe, 1987; Mori et al., 2005).

In conclusion, temperatures above 40 ºC can inhibit the grape physiology functions like flowering, berry growth, and sugar accumulation, thus reducing the grape quality and quantity (i.e. grape yield) (Greer and Weston, 2010). In the region with high temperatures and high light exposure like Australia, the protection against the sun radiation and high temperature can maintain the grape quality (Abeysinghe et al., 2019).

Light intensity is another climate variable that influences *Vitis vinifera* physiological functions, particularly its photosynthesis and biosynthesis of phenolic substances in the berries (Iland et al., 2011). The intensity of photosynthesis rises up to 1/3 of the maximum level of solar radiation and afterwards it decreases (Kriedeman and Smart, 1971). The level of anthocyanins increases in the grape skin with the light exposure, but it is negatively correlated with the temperature (Spayd et al., 2002).

A photon flux density is strongly connected with temperature in a synergic effect (Antcliff and Webster, 1955; Iland et al., 2011). Moreover, the sunlight plays an important role in heating the vineyard through solar radiation that increases the temperature of the plant surface, and also through warming the surrounding air (Crippen et al., 1986).

Water status of *Vitis vinifera* plant depends on the equilibrium level between water absorption and water release via transpiration. The level of water absorption depends on the root position in the ground and soil moisture related to the mechanical properties of the soil (i.e. percentage of the soil rock, sand, gravel, silt, clay and humus) and rainfall. In addition, the transpiration of the plant depends on the energy necessary to vaporise and move the water in the soils-plant-atmosphere system (van Leeuwen and Darriet, 2016; Smart, 1974). The plant response to a decrease in the midday leaf water potential may lead to reduction of the shoot growth, berry size, cluster weight, yield, trunk growth, cluster number, and berry titratable acidity (Shellie,

2006). The water stress may increase the grape skin and seed tannins and anthocyanins concentration, since the water amount in berries decreases due to the evapotranspiration, while the content of tartaric and malic acid decrease during the grape maturation (Duteau et al.,1981; Matthews and Anderson 1988). On the other hand, some berry compounds like the ºBrix and K element do not change significantly in water stress conditions (Matthews and Anderson 1988). Anyway, a strong water stress may cause leaves injury and problems in the grape ripening (van Leewenand and Darriet, 2016).

2.1.4 Requirements of Vitis vinifera phenological phases regarding weather conditions

The plant *Vitis vinifera* may have different physiological responses to the specific weather conditions during the year, thus influencing the grape compounds accumulation. Numerous studies confirm that the wine quality depends on the climate conditions occurring in each phenological phase. Thus, Jones and collaborators (2005) studied the global wine quality under the climate changing conditions. They reported that an increase of 1°C means the temperature during the growing season may shift up the vintage quality for 13 points on the vintage rating scale. Furthermore, according to van Leeuwen et al. (2009), the vintage ratings in Bordeaux region would never be low if the mild water deficit stress occurred from the véraison to the harvest time. In this water condition, anthocyanin and sugar compounds accumulate in grape berries improving its quality. In contrast, the strong water deficit stress may cause damages to the plant phenology. Additionally, Douro Valley in Portugal that produces high quality Port wine has good vintages in the specific climate conditions like higher temperatures during dormancy and growing season period, compared to the average temperatures of the region for the same period. On the other hand, cooler weather from véraison to ripening enhances the wine quality (Real et al., 2017). As for the Burgundy famous French wine region, Davis et al. (2019) reported that vintages that have growing seasons with warm temperatures (characterised by high diurnal temperature range) and low precipitation were highly rated on the wine quality scale. Moreover, when it comes to Tuscany, Salinger and collaborators (2015) reported in their work on the climate influence on Sangiovese wine quality that the most important factors that distinguish between good and poor wine quality vintages are heat units and precipitation events in summer period of growing season. Thus, the high-quality vintages have low precipitation in véraison phenological stage and high heat accumulation (i.e. average, minimum and maximum temperatures) that is above 35 °C in spring and summer phenological phases.

For what concern duration of Sangiovese phenological phases, Dalla Marta and collaborators (2010) divided Sangiovese grape plant development into two main stages throughout one year:

- Dormancy: form November 1st of previous year until March 15th of the current year;
- Growing season: from April 1st until September 30th (the harvest of the Sangiovese grapes finishes usually before the end of September in Tuscany). Within this stage, there are subsequent phenological phases included: spring growth that contains "bud break" up to appearance of flowers that lasts from April 1st until May 31st, "bloom" from 1st to 15th of June; "summer growth" from 1st to 10th of August; "véraison" from 10th to 20th of August and "ripening" from 21st of August to 30th of September.
-

2.1.5 References

Ashenfelter, O. (2010). Predicting the quality and prices of Bordeaux wine. *Journal of Wine Economics*, 5, 1, 40–52.

Shellie, K.C. (2006). Vine and berry response of merlot (*Vitis vinifera* L.) to differential water stress. *American Journal of Enology and Viticulture*, 57, 4, 514–518.

Dalla Marta, A., Grifoni, D., Mancini, M., Storchi, P., Zipoli, G. and Orlandini, S. (2010). Analysis of the relationships between climate variability and grapevine phenology in the Nobile di Montepulciano wine production area. *The Journal of Agricultural Science*, 6, 148, 657–666.

Davis, R.E., Dimon, R.A., Jones, G.V. and Bois, B. (2019). The effect of climate on Burgundy vintage quality rankings. *International Viticulture and Enology Society – IVES*, 1, 59–73.

Duteau, J., Guilloux, M. and Seguin, G. (1981). Influence des facteurs naturels sur la maturation du raisin, en 1979, à Pomerol et Saint-Emilion. *Connaissances de la Vigne et du Vin*,15, 3 ,1–27.

Jones, G.V. and Storchmann, K.H. (2001). Winemarket prices and investment under uncertainty: an econometric model for Bordeaux Crus Classés. *Agricultural Economics*, 26, 2, 115–133.

Jones, G.V., White, M.A., Cooper, O.R. and Storchmann, K. (2005). Climate change and global wine quality. *Climatic Change*, 73, 3, 319–343.

Matthews M.A. and Anderson M.M. (1988). Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. *American Journal of Enology and Viticulture*, 39, 4, 313–320.

Real, A.C., Borges, J., Cabral, J.S. and Jones, G.V. (2017). A climatology of vintage Port quality. *International Journal of Climatology*, 37, 10, 3798–3809.

Salinger, M.J., Baldi, M., Grifoni, D., Jones, G.V., Bartolini, G., Cecchi, S., Messeri, G., Dalla Marta, A., Orlandini, S., Dalu, G. and Maracchi, G. (2015). Seasonal differences in climate in the Chianti region of Tuscany and the relationship to vintage wine quality. *International Journal of Biometeorology*,59, 12, 1799–1811.

Smart, R.E. (1974). Aspects of water relations of grapevine (*Vitis vinifera). American Journal of Enology and Viticulture*, 25, 2, 84–91.

Soar, C.J., Sadras, V.O. and Petrie, P.R. (2008). Climate drivers of red wine quality in four contrasting Australian wine regions. *Australian Journal of Grape and Wine Research*, 14, 2, 78–90.

van Leeuwen, C., Trégoat, O., Choné, X., Bois, B.,Pernet, D. and Gaudillère, J.P. (2009). Vine water statusis a key factor in grape ripening and vintage qualityfor red Bordeaux wine. How can it be assessed for vineyard management purposes? *Journal International des Sciences de la Vigne et du Vin*, 43, 3, 121–134.

2.2 Prediction of climate change in Tuscany's DOP appellations until the end of 21st century

2.2.1 Introduction

Agriculture and its sectors of grape production and winemaking are strongly dependant on climate since the crop production relies on weather conditions. Wine quality characteristics and typicity are determined by the climate that makes part of a specific wine terroir. It is well known that the climate influences the wine quality and that specific weather conditions during growing seasons may cause quality variations between vintages (see the previous **section 1.1.1**.). Furthermore, climate affects the canopy microclimate, the grape production per hectare (i.e. yield), *Vitis vinifera* physiology, plant growth and berry solutes (Santos et al., 2020). The world area under the grapevine production covers almost all the continents (6 out of 7) and encompasses a broad spectrum of climates including oceanic, warm oceanic, transition temperate, continental, cold continental, Mediterranean, subtropical, attenuated tropical, arid and hyperarid climates that are located between 4th and 51st parallel in the Northern Hemisphere and between 6th and 45th parallel in the Southern Hemisphere. However, the biggest part of the production is located in moderate climate area (Tonietto, 1999). According to the statistical report from 2019 (OIV), nearly one half of the world's vineyards is covered by wine grape production, with the following countries having the largest areas: Spain (13 %), China (12 %), France (11 %), Italy (9 %) and Turkey (6 %). The wine production leading countries in terms of volume produced are listed in a decreasing order: Italy (54,8 million hL), France (48,6), Spain (44,4), USA (23,9).

Based on this information, it can be inferred that the importance of grape production and associated activities is more than evident since it has a strong socioeconomical and cultural impact on numerous countries. ProWien Business Report (2019), which surveyed more than 1700 wine experts (producers, intermediaries and retailers) from 46 countries, stated that climate change presents one of the biggest threats in the grape and wine production. One half of participants considered the climate change as one of the biggest risks for their business. Among the wine experts, 90% of grape and wine producers have already experienced a

negative influence of the CC such as a decrease in yield caused by the extreme climate conditions like hail, heavy rains and late frost; increased inconsistency of the grape yield, resulting from water stress and deficit of the vine plant; shorter timeframe of the harvest due to the simultaneous grape maturity; increased necessity of the phytosanitary protection and other grape cultivars needed. On the other hand, 60 % of retailers stated that they have already had a detrimental influence of CC (Loose and Pabst, 2019). Thus, the climate change (CC) becomes one of the greatest issues for the grape and wine producers worldwide. Furthermore, the study of the future climate conditions draws extraordinary interest (Santos et al., 2020) and adaptation to the new weather conditions gains a sense of urgency. Among the proposals of strategies to adapt to the new climate conditions, many researchers suggest that the grape production should be shifted to the high altitude areas and the grape varieties should be changed to those that are more suitable for warmer weather conditions. (White et al, 2006; Shultz, 2000; Jones et al., 2005).

As described in the section 2.1.2., the human activities are the main cause of the greenhouse gasses (GHG including carbon dioxide, methane, nitrous oxide and halocarbons) release in the atmosphere, which triggers the global warming. Nowadays, the level of the carbon dioxide reaches the highest level recorded in history presented by concentration of 400 ppm (parts per million). In order to predict the future climate conditions, The Intergovernmental Panel of Climate Change (IPCC) created four Representative Concentration Pathways (RCPs) that are used for modelling of the future GHG increase during the 21st century. The first RCP2.6 predicts the lowest GHG emission expressed in CO_2 equivalent concentration (CO_2-eq) and the temperature increase of 2 ºC relative to the pre-industrial temperatures (**Figure 2.3**). Under RCP4.5 (intermediate scenario or "stabilization pathway"), the expected CO_2 equivalent concentration is expected to be between 580 and 720 ppm, which will cause the temperature rising from 3-4°C, where the radiative forcing is predicted to be 4.5 W/m^2 by 2100. Under the RCP6 scenario the CO_2-eq is expected to be from 700 to 1000 ppm and the temperature to increase for about 4°C. The last RCP8.5 scenario represents the highest global warming with CO_2-eq possibly higher than 1000 ppm and the temperature rising more than 6°C, while radiative forcing will probably be 8.5 W/m^2 until 2100, see the **Figure 2.3** (IPCC, 2014).

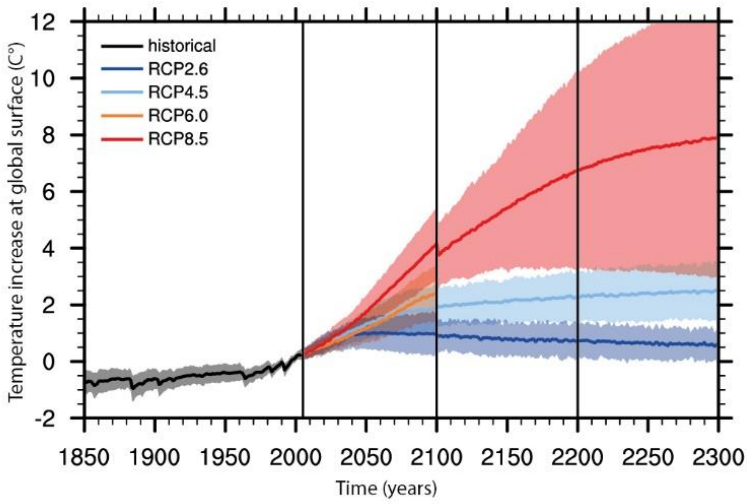


Figure 2. 3 Global warming in the future until 2300 (Modified from: https://open.oregonstate.education/climatechange/chapter/impacts)

Many authors that have studied future climate projections have applied CORDEX (Coordinated Regional Climate Downscaling Experiment), high resolution climate simulations under different RCP scenarios in order to assess the impact of the climate change on the economy and agricultural sectors and to provide information about adaptation strategies that producers may apply in their activities (Koufos et al., 2017; Fraga et al, 2016; Santos et al., 2018).

Numerous studies have shown that the climate change will impact various world's areas, and as far as the European continent is concerned, the strong impact of the climate changes will be experienced in the Mediterranean basin in terms of a decrease of the harvestable yields, increased of the yield variability and a reduction of areas suitable for traditional crops cultivation for traditional crops cultivation (Olsen and Bindi, 2002). In this part of Europe, special attention should be paid to the studies of the climate change impact on the economically most important crops like grape vine considering that the high-quality wine production depends on the fragile equilibrium between climate – soil and grape variety (Mullins et al, 1992; Jones et al., 2005).

Tuscany, the region in the central part of Italy is one of the most famous wine region area in the world and the wine production in this region plays an important socioeconomic and cultural role. The wine production stands for 13% of the total agricultural production. On the other

hand, the vineyard picturesque landscapes of the Tuscany's hills attract many tourists contributing to the development of agritourism (Zhu et al., 2014).

Chianti DOCG wine is one of the most important high quality wine produced in Tuscany. The area encompasses the districts from Siena to Florence near to 43.5°N of latitude to 10-12°E of longitude. Long history of Chianti wine production starts in 1389 when the name Chianti was mentioned for the first time in official documents. Nowadays Chianti wine production is controlled by grower consortium, and it follows the regulations regarding grape and wine production quality parameters. The main grape variety is Sangiovese that must be stand for 80 to 100% of grape varieties in Chianti wine. Therefore, it is important to understand how this grape variety responds to the climate changes in order to understand future conditions and to be able to apply new strategies for the wine production (Ewing-Mulligan and McCarthy, 2001).

Jones (2006) reported that the optimum average growing season temperature (T_{avg}) for Sangiovese is from 17.5 and 19.5 °C in the ripening period, from August to September. Furthermore, Salinger at al. (2015) showed that the highest quality ranged Chianti vintages were produced in the years when the average growing season temperature was from 19.2 to 20.8 °C, while low ranged vintages were produced under T_{avg} condition from 17.3 to 19.8 °C.

Many studies have shown that the global warming started with the industrialisation and that this trend will continue in the future. In the past period, from 1950 to 2009, the rise in temperature especially regarding Southwest Europe was identified by calculation of Winkler index and Huglin index (Santos et al., 2012). More specifically, Bartolini et al. (2008) reported that the maximum summer temperatures in Tuscany have rised for 0.44 °C / decade, while minimum summer temperatures increased for 0.38 °C / decade in the period from 1955 to 2004. The trend of the climate change will continue in the future according to ICCP research and it will be necessary to adapt some measure to mitigate its impact on this agricultural sector, especially in high quality wine production areas such as Chianti in Tuscany. It is therefore necessary to study and create models of the future climate conditions in order to predict the new upcoming weather changes and create new adaptation strategies to soften its negative impact.

Some studies have shown that the crop yields in Mediterranean basin will decrease significantly in the future due to of the lack of water availability (Iglesias et al, 2011). Thus, Alcamo and collaborators (2007) specified that the temperature will continue to rise in the future, increasing for 2.5-5.5 °C until 2070, while the precipitation will decrease for 30-40% in the same period of time.

As far as we know, the models of the future climate conditions in Tuscany have estimated the GCM General Circulation Models for the period 2075-2099 with few or no bioclimatic indices (Moriondo et al., 2011, D'Oria et al., 2017, Zhu et al., 2016). Anyway, the value of these studies is certain, but a study of the more detailed period with a high range of the BIs would provide further information about the grape cultivation and wine production suitability in the future in this region.

2.2.2 Materials and Methods

2.2.2.1 Study region - Tuscany

Tuscany is located in the central part of Italy enclosing about 23,000 km^2 from 44° 28' to 42° 22'N latitude and 10° 19' and 12° 20'E longitude. It is surrounded by other regions Liguria, Emilia Romagna in the north, Marche, Umbria in the east and Lazio in the south. Tuscany is one of the most prominent wine regions of the world the surface of which is covered by hills (67%) and mountains (25%). Mild hills that ensure the great sun exposition of the vine are located between the Apennine mountains in the east, the Tyrrhenian Sea and Ligurian Sea in the west. The climate in Tuscany is characterized by hot and dry summers and mild winters. Tuscany produced 2,652 milion hL of wine on about 55,500 ha of vineyards in 2020 (www.inumeridelvino.it).

Beside Chianti DOCG and Chianti Classico DOCG wines (**see the section 2**), there are other famous DOCG wines produced in Tuscany, like Vernaccia di San Gimignano white wine, and among red wines there are Brunello di Montalcino, Vino Nobile di Montepulciano, Carmignano, Elba AleaticoPassito, Montecucco Sangiovese, Morellino di Scansano, Suvereto, Rosso della Val di Cornia and IGT Bolgheri (**Figure 2.4**).

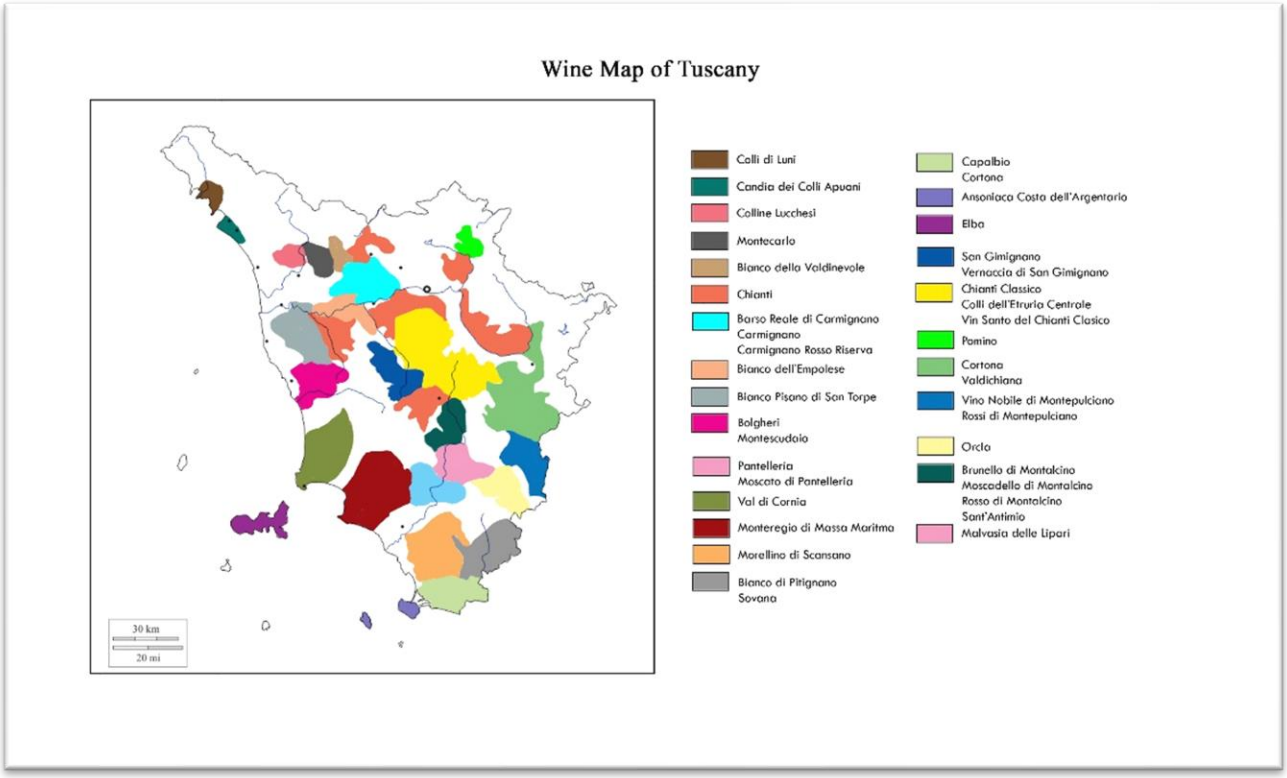


Figure 2. 4 Tuscany wine map: Colli di Luni, Candia dei Colli Apuani, Colline Lucchesi, Montecarlo, Bianco della Valdinevole, Chianti, Barco Reale di Carmignano, Carmignano, Carmignano Rosso Riserva, Bianco dell'Empolese, Bolgheri, Montescudaio, Pantelleria, Moscato di Pantelleria, Val di Cornia, Monteregio di Massa Maritima, Morellino di Scansano, Bianco di Pitignano, Sovana, Capalbio, Cortona, Ansonica Costa dell'Argentario, Elba, San Gimignano, Vernaccia di Sangiminiano, Chianti Classico, Colli dell'Etruria Centrale, Vin Santo di Chianti Classico, Pomino, Cortona, Valdichiana, Vino Nobile di Monteplulciano, Rossi di Montepulciano, Orcio, Brunello di Montalcino, Moscadello di Montalcino, Rosso di Montalcino, Sant'Antonio, Malvasia delle Lipari (Modified from: https://vineyards.com/wine-map/italy/tuscany).

2.2.2.2 Model data and bioclimatic indices

The weather data including daily maximum temperatures (T_{max}), minimum temperatures (T_{min}) and precipitation were downloaded from the website: http://cordex.org for the reference to past period from 1976 – 2005, for the near future from 2031 to 2060 and distant future from 2071 to 2100.

The weather data regarding minimum and maximum temperatures and precipitation are collected from E-OBS observations with 11 x 11 km spatial resolution. For the data elaboration,

the reference period was from 1976 – 2005. The obtained results are divided in three periods: last 30 years from 1990-2019 and the future periods from 2031-2060 and 2071-2100.

CORDEX (Coordinated Regional Climate Downscaling Experiment) high-resolution climate simulations were applied under 4.5 RCP scenario in order to calculate the bioclimatic indices (BIs). Afterwards, the bias correction of nine Regional Climate Model (RCM) data was performed based on http://cordex.org/data-access/bias-adjusted-rcm-data/ (**Table 2.1**)

Table 2. 1 Nine Global Climate Model and Regional Climate Model chains applied in the research (Literature: 1: Soares et al., 2017: 2: Frei et al., 2018)

GCM	RCM	Ensamble	Literature
CNRM-CERFACS-CNRM-CM5	CCLM4-8-17	rlilpl	1
ICHEC-EC-EARTH	CCLM4-8-17	r12ilpl	1
ICHEC-EC-EARTH	HIRHAM5	r3ilpl	1
ICHEC-EC-EARTH	RACMO22E	rlilpl	1
MOHC-HadGEM2-ES	CCLM4-8-17	rlilpl	1
MOHC-HadGEM2-ES	RACMO22E	rlilpl	1
MPI-M-MPI-ESM-LR	CCLM4-8-17	rlilpl	1
MPI-M-MPI-ESM-LR	REMO2009	rlilpl	2
MPI-M-MPI-ESM-LR	REMO2009	r2ilpl	1

Bioclimatic indices based on temperature: temperature is an important climatic factor that has a great impact on grape chemical compounds and its quality. In this view, Winkler index (GDD), Huglin index (HI) and Cool night index (CI) are used in this work.

Growing degree day (GDD) or Winkler index shows the number of degrees above the growing degree base of 10°C, and it is calculated as a sum of daily average temperatures in the growing season from 1stApril until 31stOctober, using the mathematical equation written in **Table 2.2** (Winkler, 1962; Tonietto and Carbonneau, 2004). Winkler and Ameline (1944) created a classification system of grapevine suitability for the quality wine production. Thus, the calculated WI values from 850 to 1667 that are classified as I and II Regions are suitable for the production of the dry table wine with light to medium body. The Region III is good for full-bodied wine production with the index value from 1688-1944. The Region IV (1945 – 2222 value of index) meets the requirements of fortified wine production, whereas the last

Region V (2223-2700 index value) presents the areas that are suitable for the table grape production, and low-quality (table) wine. Another important thermal bioclimatic index is **Heliotermal index** (**Huglin index)** that is more focused on maximum temperatures. It gives further information about heat accumulation over the active vine phase from the beginning of August to the end of September. In both indices calculations, the temperature parameter is presented as average, minimum and maximum temperatures, (T_{avg}, T_{min} and T_{max}, respectively).

The last bioclimatic index, **Cool night index (CI)**, is an average of minimum night temperatures (in C°) that occur during one month of ripening period. September is usually considered as the month of the ripening period in the Northern hemisphere. This assessment of the CI is important to better understand the secondary metabolites accumulation in the grapes (i.e. grape polyphenols and aromas) (Tonietto and Carbonneau, 2004).

When it comes to the **water balance indices**, Tonietto and Carbonneau (2004) created the **Dryness index (DI)** on the basis of Riuo's index that determines the availability of the soil water, i.e. the presence and absence of dryness. It takes into account the soil moisture at the beginning of the growing cycle, then evapotranspiration and precipitation during the growing cycle. It is important for better evaluation of the grape maturation level and wine quality (Jackson and Cherry, 1988; Mérouge et al., 1998).

All of these grape growing indices are important for description of the grape varieties needs, wine quality in terms of soluble solids accumulation (sugar level, polyphenols and wine aroma) and wine typicity (Tonietto and Carbonneau, 2004).

Table 2. 2 : Bioclimatic indices used in this study: Mean temperature in vegetation (VEGTM), Cool night index (CI), Huglin index (HI), Winkler index (WI), Total amount of precipitation during vegetation (VEGRR), Dryness index (DI).

Bioclimatic index	Mathematical equation	Classes	References
Temperature related indices			
Mean temperature in vegetation (VEGTM)	Average temperature during growign season (from 1 April until 31 October)		Fraga et al., 2014
Cool night index (CI)	$CI = \frac{1}{N}\sum_{1.9}^{30.9} \mathrm{Tm}$ Tm- Min.air temperaqture (°C) N-number of days	Warm nights: > 18 Temperate nights: 14-18 Cool nights: 12-14 Very cool nights: < 12	Tonietto, 1999
Huglin index (HI)	$HI = \sum_{1.4}^{30.9} \frac{(Tx - 10°C) + (Tn - 10°C)}{2} * k$ Tx - Max air temperature (°C) Tn - mean air temperature (°C) k - Length of the day correction coefficient	Very warm: > 3000 Warm: 2400-3000 Temperate warm: 2100-2400 Temperate: 1800-2100 Cool: 1500-1800 Very cool: < 1500	Tonietto and Carbonneau, 2004
Growing degree day (WI) Winkler index	$GDD = \sum_{1.4}^{31.10} \frac{(Tx + Tm)}{2} - 10°C$ Tm - Min air temperature (°C) Tx - Max air temperature (°C)	Too hot: > 2700 Region V: 2222 - 2700 Region IV: 1944 -2222 Region III: 1667 - 1944 Region II: 1389 - 1667 Region I: 850 - 1389 Too cool: < 850	Hall and Jones, 2010; Winkler et al., 1974
Temperature, precipitation and evaporation related indices			
Total precipitation amount in vegetation (VEGRR)	Sum of rainfal during growing season (from 1 April to 31 October)		Ruml et al., 2012
Dryness index (DI)	$DI = Wo + \sum_{1.4}^{30.9} [\mathrm{Pm} - (\mathrm{Et} + \mathrm{Es})]$ $\mathrm{Et} = \mathrm{aPET}$ $Es = \frac{\mathrm{PET}(1 - \mathrm{a})\mathrm{Nef\,pec}}{\mathrm{N}}$ Wo - initial soil moisture Pm - monthly precipitation Et - Water loss through transpiration PET - Potential evaporation	Humid: >150 Moderately dry: 50 -150 Sub-humid: 100-50 Very dry: < 100 a- Plant radiation absorption coef (a=0.1, 0.3, 0.5 in April, May, June-September, respectively) Nef pec - Monthly effective soil evaporation N-Number of days in month	Tonietto and Carbonneau, 2004

2.2.2.3 Statistical analysis

Student's t-test was carried out in order to reveal statistical differences in the mean of dependent variables (bioclimatic indices) for each grid cell, with confidence level of 95%. The study covered the period from 1990-2019, and future periods 2031-2060 and 2071-2100. It was applied for one studied model (out of 9) and one period at a time. The statistical differences were designed with one dot in each grid cell of the figure, if it was present in at least 5 models for the same studied period and for one BI. The significance of the data changing is calculated taking into account the reference period. This methodology allows us to better understand if the change comes from the global warming or if it is a natural climate phenomenon (Fraga et al., 2013; Liu et al., 2014; Monjo et al., 2016; Vuković et al., 2018). The bioclimatic indices were calculated using self-created software that is written in Fortran programming language. Maps were created online (http://cola.gmu.edu/grands) using Grid Analysis of Display Sistem (GrADS).

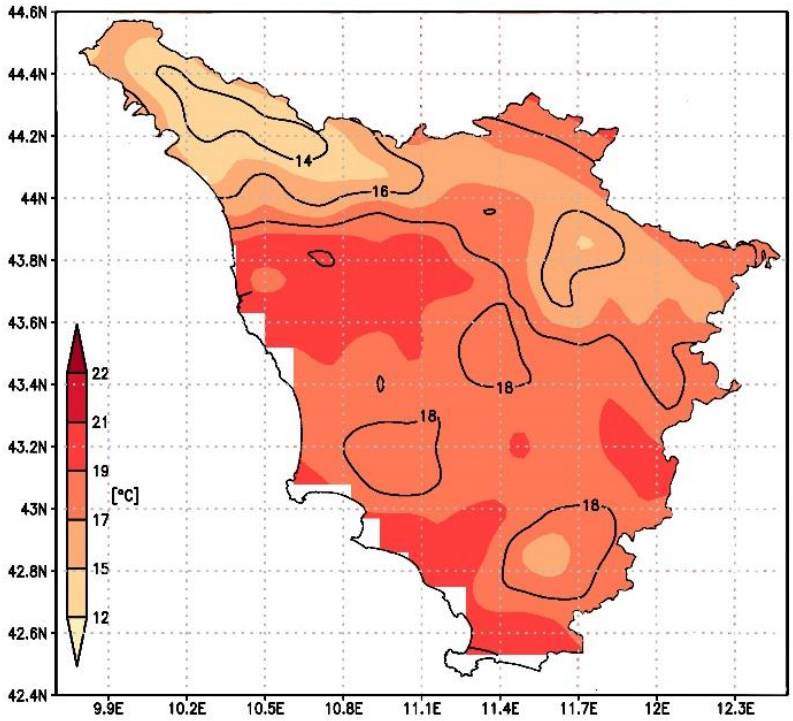
2.2.3 Results and Discussion

2.2.3.1 Temperature corresponding bioclimatic indices

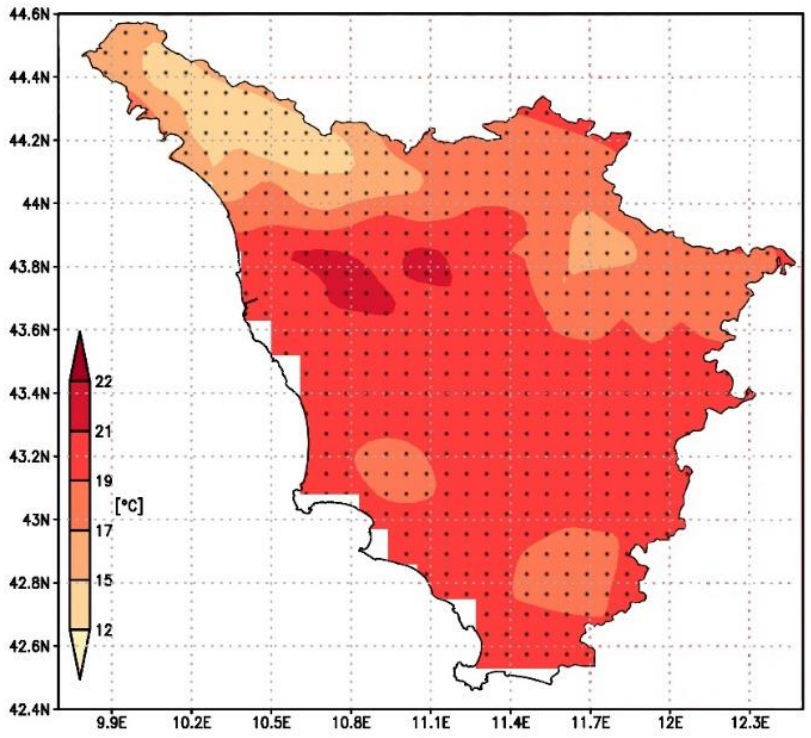
It is well known that temperature is a climate parameter that can strongly impact the grape and wine quality, as well as crop quantity and vine plant phenological phases under the climate change conditions (Coombe, 1987; Kliewer and Torres, 1972;Buttrose etal. 1971, Kliewer, 1971, 1973; Lakso and Kliewer, 1978;Fraga et al., 2016; Abeysinghe et al., 2019).

Mean temperature during the vegetation period (VEGTM) in the central part of Tuscany was ranged from 17 to 19°C in the period from 1990 to 2019. Aferwards, in the period from 2030 to 2060 there will be a 2 degrees increase, with the estimated range of 19-21°C under RCP 4.5 scenario (**Figure 2.5**). The observed trend is consistent with the results of Alcamo et al.(2007) who declared that the temperature rising in the Mediterannean basin will be between 2.5 °C and 5.5°C by 2070. The period from 2070 to 2100 will be warmer in some areas reaching 22°C as the average vegetation period temperature. According to Jones (2006), (see the **Fig. 1.3, section 1)** the optimum growing season temperatures for the production of Sangiovese

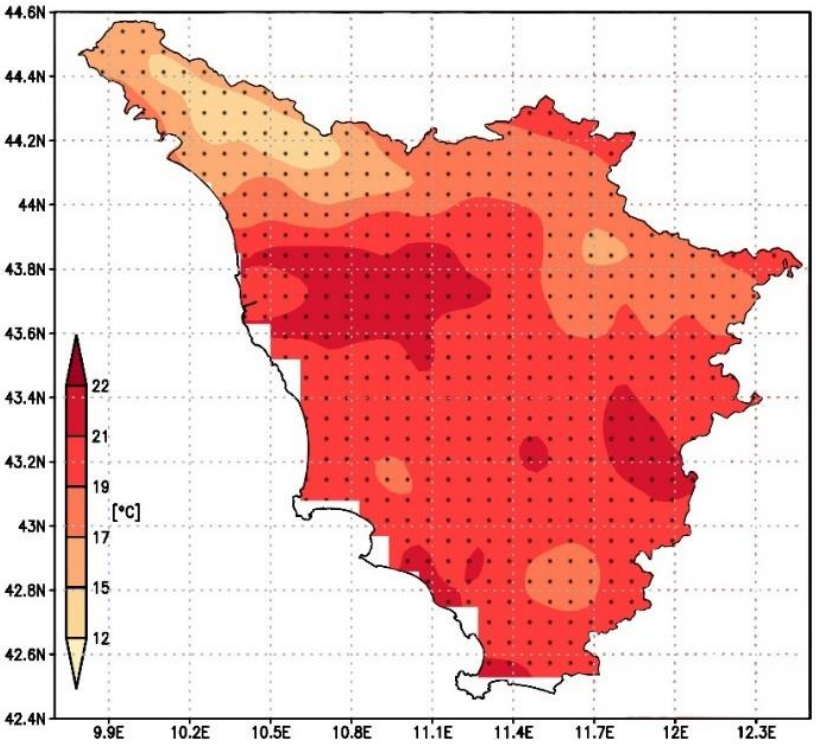
permium quality wines are from 17-19 °C, those being present in the current period (1990 – 2019). Any higher temperature will require switching to other grape varieaties or resin production. Therefore, it is very likely that the future decades in Tuscany will face the mean growing season temperature that is too hot and not suitable for the high quality wine production coming from the tipical Tuscany's Sangiovese grape.



a)



b)

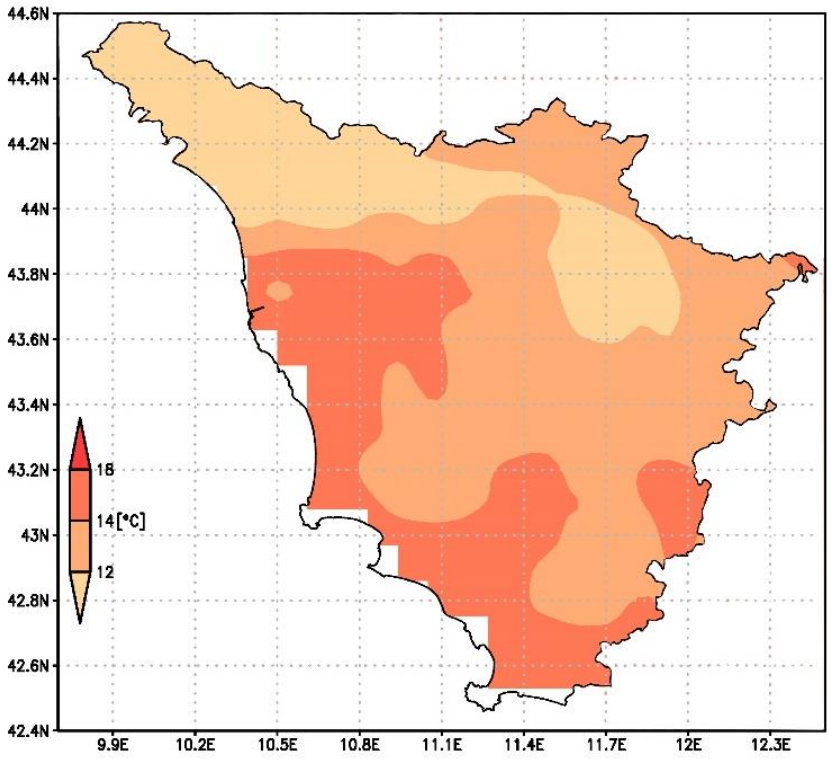


c)

Figure 2. 5 Mean temperature during the vegetation period (VEGTM) in Tuscany region based on 9 model median ensemble projections, calculated for periods: a) 1990-2019 b) 2031-2060 c) 2070-2100 under RCP 4.5 scenario. Statistical significance of changes ($p < 0.05$) is presented by dots and calculated by the Student t-test.

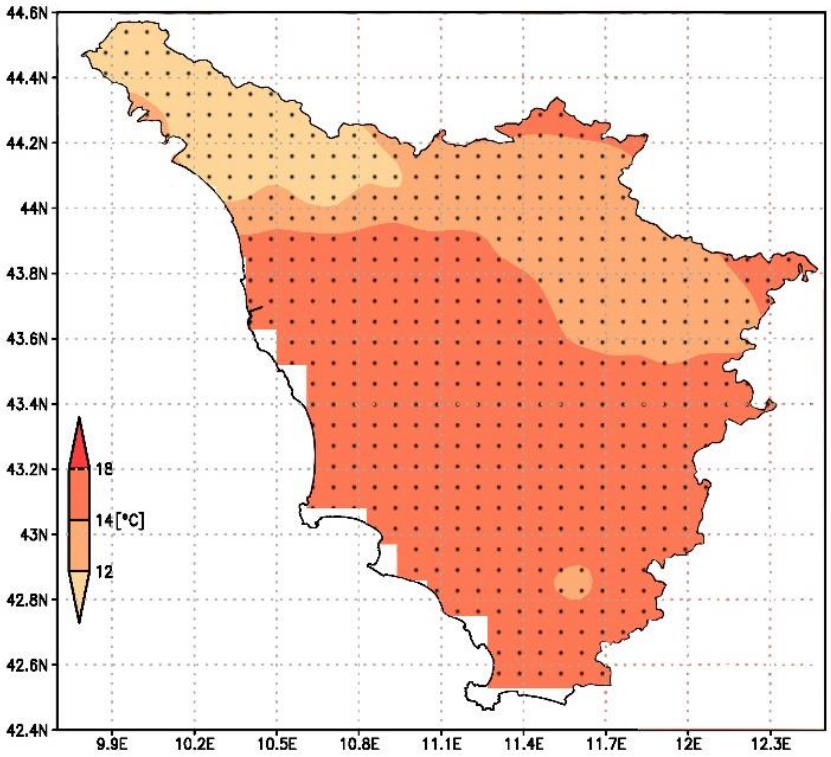
As for the night temperatures, the cool night index (CI) showed that the present period (from 1990 to 2019) has “cool nights” with 12-14 °C in the central part including Chianti Classico area (see the **Table 2.2**). Only the seaside area had the “temperate night” temperatures with 14-18 °C (**Figure 2.6**). The cool night index will significantly increase in all Tuscany grape production areas in the future, and it will be presented by “temperate nights” in both periods from 2031 to 2060 and from 2071 to 2100 under RCP4.5 scenario. All the night temperatures will probably have a statistically significant increase by 2100.

According to Kliewer (1977), the ideal night temperature that allows a good accumulation of anthocyanins and aromas in grapes ranges from 10-15°C. Having this in mind, it can be said that the rise of night temperatures in the future period may have a strong negative impact on the color stability and wine flavour in the future. Moreover, the ripening phenological phase is expected to be shifted to an earlier period of growing season when the temperatures are even higher, and thus, the negative impact on wine quality is expected to be even stronger (Fraga et al., 2016).



a)

b)



c)

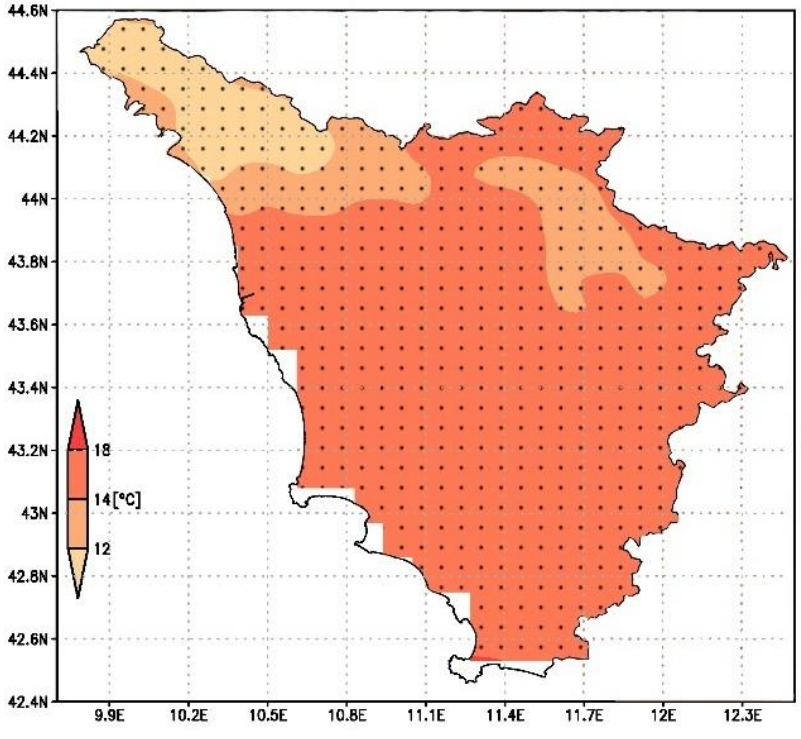
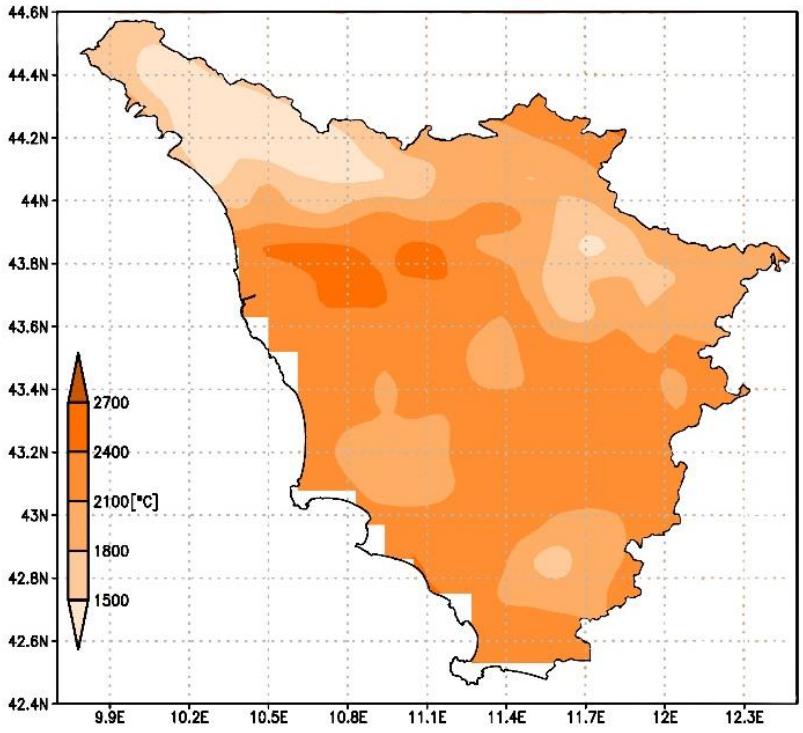
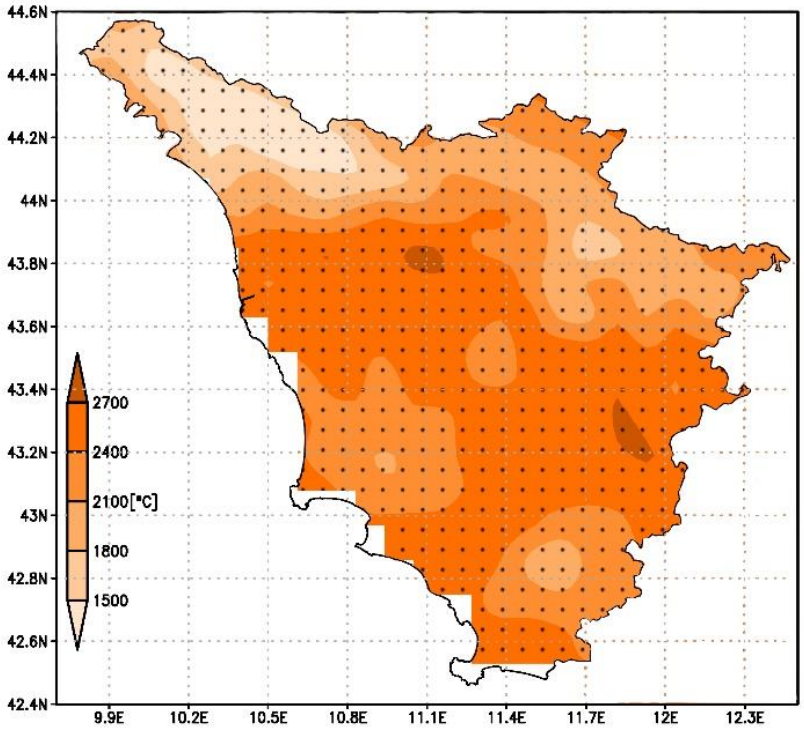


Figure 2. 6 Cool night index (CI) in Tuscany based on 9 model median ensemble projections for the periods: a) 1990-2019 b) 2031-2060 c) 2070-2100 calculated under RCP 4.5 scenario. Statistical significance of changes ($p < 0.05$) is presented by dots and calculated by the Student t-test.

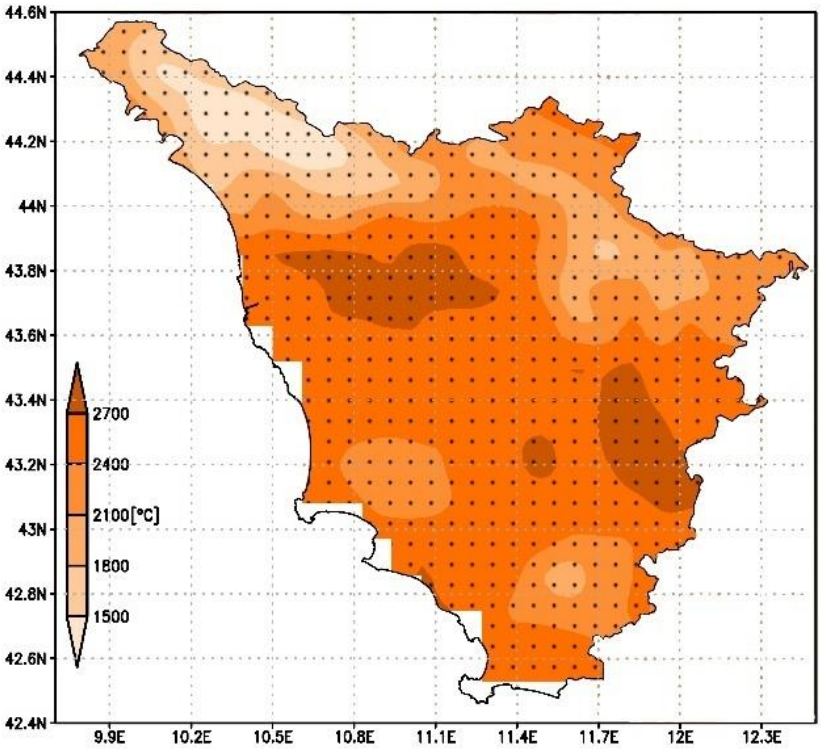
Huglin index (HI) calculated for the present period from 1990 to 2019 classifies the central part of Tuscany as “temperate warm”, having the HI values from 2100-2400 (see the classification according to Huglin in **Table 2.2)**. All the future study periods (2031-2060 and 2071-2100) are supposed to have statistically significant changes of temperature. Thus, the period from 2031 to 2060 will become “warm” under RCP 4.5 scenario fot the biggest part of Tuscany grape production area (**Figure 2.7**). Toward the end of the 21st century, two big spots of “very warm” HI climate appear within the Cortona and Valdichiana areas and in one small part of Chianti DOCG and San Gimignano wine production areas. In the study of the Emilia Romagna region of Italy, Teslić and collaborators (2019) reported similar results for the period from 2071-2100 under RCP4.5 scenario: the climate conditions will probably be “warm” to “very warm”. These findings are in accordance with the study of Fraga et al. (2013) which reported that the climate in these areas will probably be “warm” to “very warm” in the period from 2041 to 2070.



a)



b)

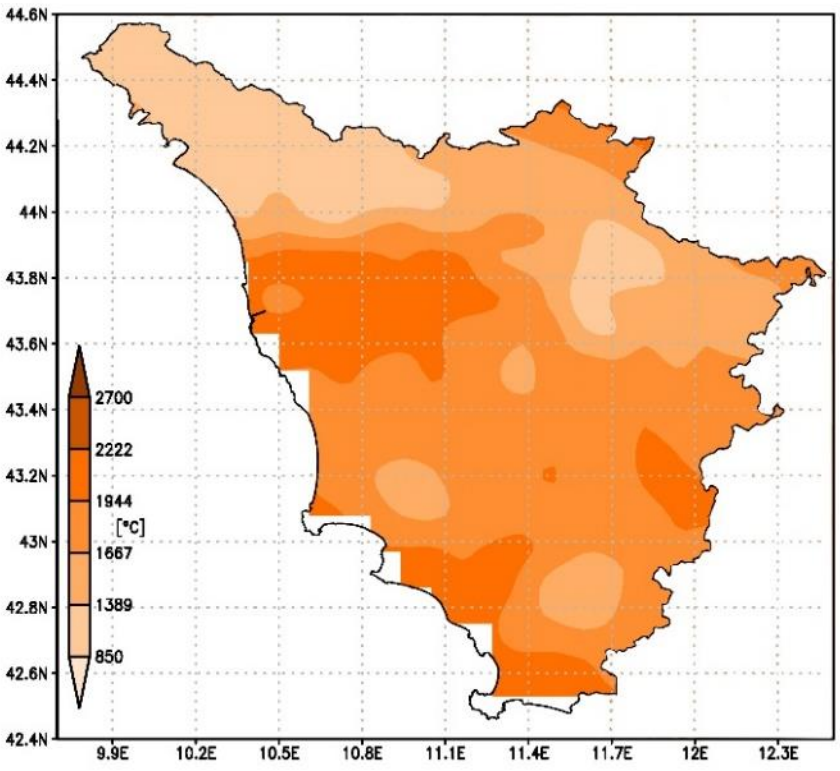


c)

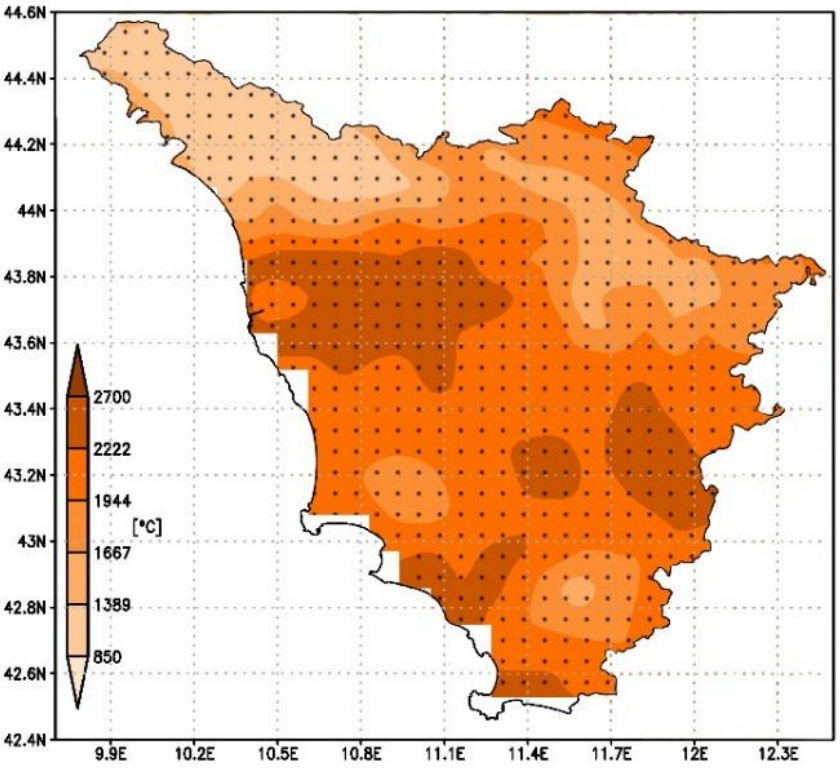
Figure 2. 7 Huglin index (HI) in Tuscany region based on 9 model median ensemble projections, calculated for periods: a) 1990-2019 b) 2031-2060 c) 2071-2100 under RCP 4.5 scenario. Statistical significance of changes ($p < 0.05$) is presented by dots and calculated by the Student t-test.

Winkler index (WI) of the present period (1990 – 2019) shows that the central part of Tuscany, typical for production of Chianti and Chianti classico DOCG wines, is classified as "Region III" according to Winkler classification (see the **Table 2.2**). Yet, there are some spots of the warmer climate classified as "Region IV" next to the Terranean sea close to Livorno and Grosseto districts. As claimed by Winker and Ameline (1944), the "Region iv" climatic conditions are suitable for the fortified wine production. The same seaside areas will become warmer in the period from 2031 to 2060, classified as "Region V" according to Winkler's classification (**Figure 2.8**). The central part of Tuscany will remain in the Region IV in the same period of time. In other words, Tuscany will become the region that is suitable for the fortified wine production and in certain hot areas the quality of produced wines will reach only low-quality level of table wines. Later on, in the period from 2071 to 2100, the biggest part of Tuscany will probably have the "Region V" type of climate, meaning that the region will probably lose the characteristics suitable for the high-quality wine production and switch to the conditions good for the table grape production and low-quality table wine production toward the end of 21st century, if the climate change follows the RCP4.5 scenario. It is worth mentioning that the RCP4.5 is not the "strongest" scenario regarding the GHG emissions, and that the situation may cause even more concern in the future. That is why it is very important to apply a new adaptation measure to the new upcoming climate change conditions. These findings are in accordance with the study of Moriondo and collaborators (2011) who reported that Sangiovese grape variety in Tuscany might be substituted with supplementary grape varieties, which are more suitable for the new warmer climate conditions, like those cultivated in South Italy. Southern grape varieties are suitable for the table grape production and for the higher alcohol wine production as well. On the other hand, the premium wine production made out of Sangiovese grape should be gradually moved from flat to higher locations (> 600 m asl).

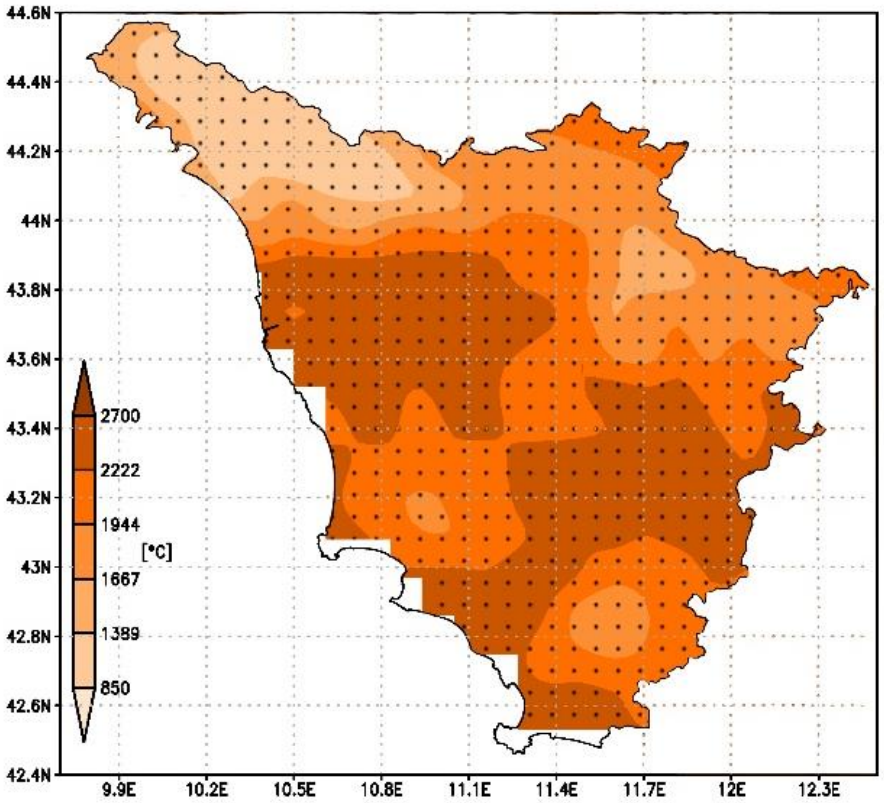
The rising trend of the Huglin and Winkler indices are in agreement with the previous research of the climate conditions in the South Europe from 1950 to 2009 (Santos et al., 2012).


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
2700
2222
1944
[°C]
1667
1389
850

a)


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
2700
2222
1944
[°C]
1667
1389
850

b)

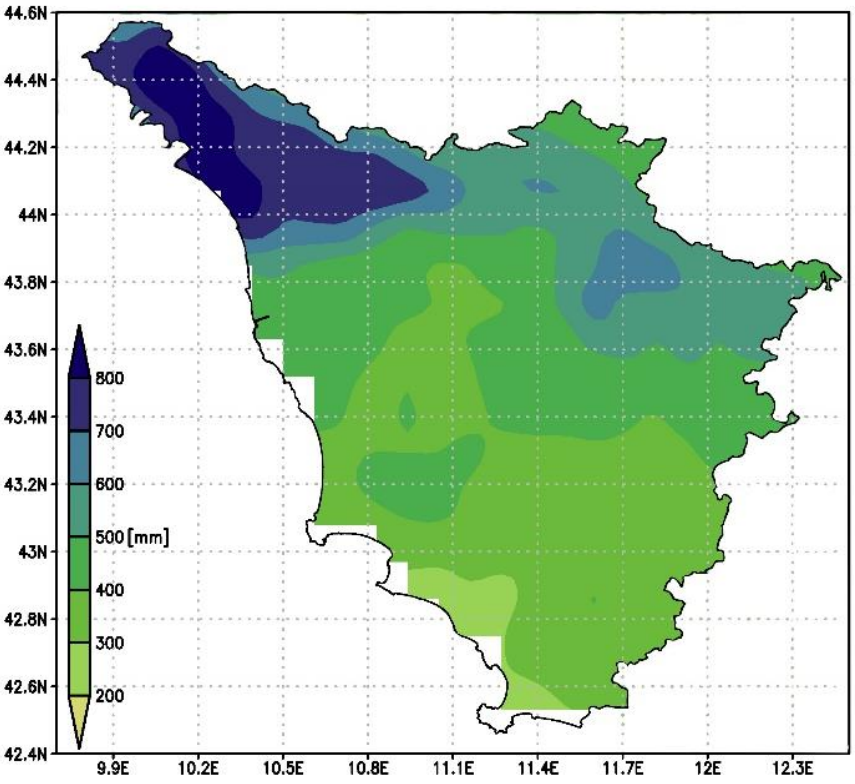


c)

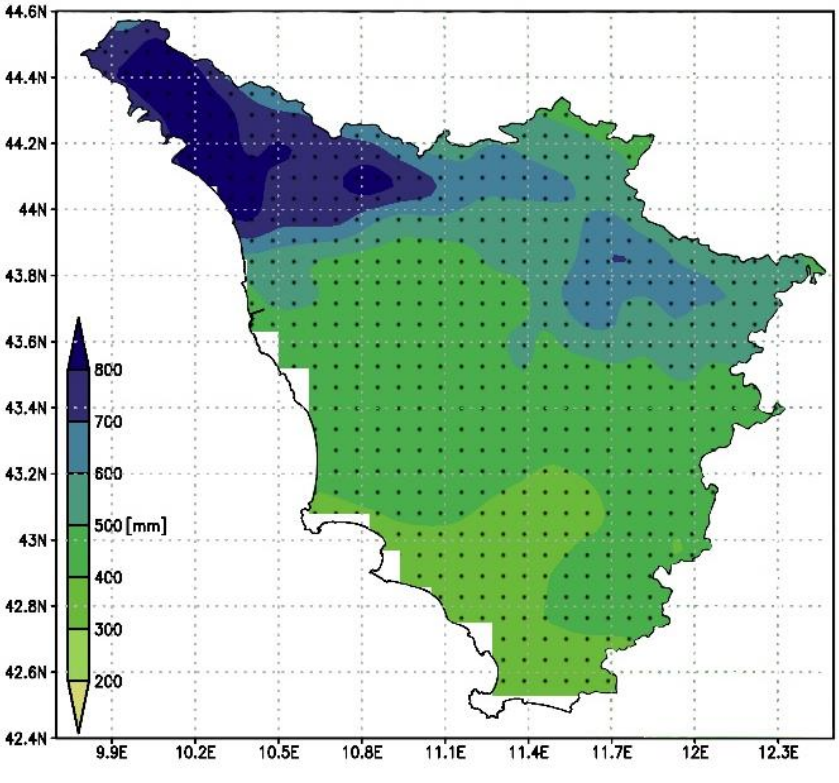
Figure 2. 8 Winkler index (WI) in Tuscany region based on 9 model median ensemble projections, calculated for periods: a) 1990-2019 b) 2031-2060 c) 2070-21002100 under RCP 4.5 scenario. Statistical significance of changes ($p < 0.05$) is presented by dots and calculated by the Student t-test.

2.2.3.2 Precipitation corresponding bioclimatic indices

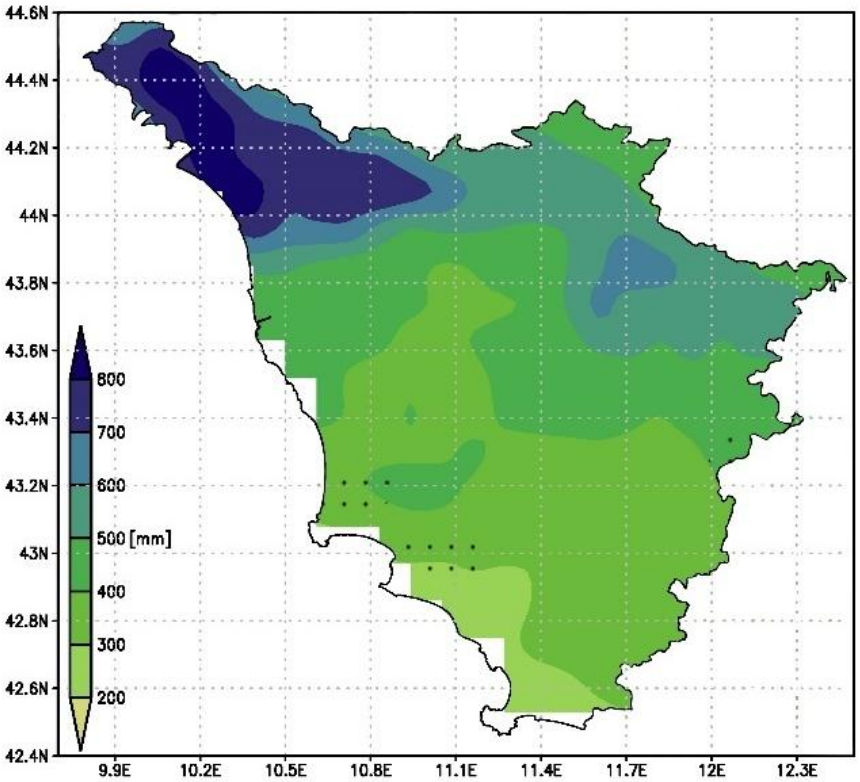
The total precipitation amount during the vegetation period (VEGRR) in Tuscany from 1990 to 2019 ranged mostly from 300 to 400 mm of rainfall. In the period from 2030 – 2061, the precipitation is predicted to increase from 400 to 500 mm in the central part of the region, which has the statistically significant difference ($p<0.05$) in relation to the present period. Afterwards, toward the end of the 21st century (from 2070 to 2100), the precipitation will decrease in most of the DOC parts of Tuscany, without changes that are statistically significant (relative to the present) (**Figure 2.9**). Similar observations were reported in other studies that used CORDEX system under RCP4.5 and RCP8.5 scenarios, stating that total precipitation amount will not bring about statistically significant changes toward the end of 21st century in Europe (Jacob et al., 2014; Teslić et all, 2019).


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
800
700
600
500 [mm]
400
300
200

a)


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
800
700
600
500 [mm]
400
300
200

b)



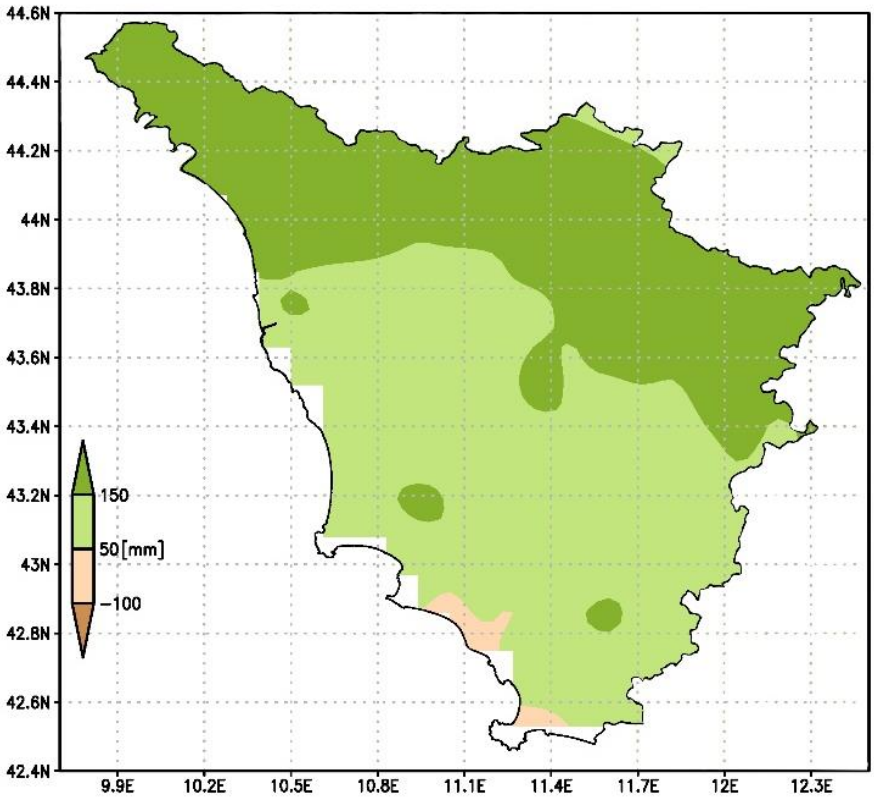
c)

Figure 2. 9 Total precipitation amount during the vegetation period (VEGRR) in Tuscany: a) 1990-2019 b) 2031-2060 c) 2070-2100 calculated under RCP 4.5 scenario. Statistical significance of changes ($p< 0.05$) is presented by dots and calculated by the Student t-test.

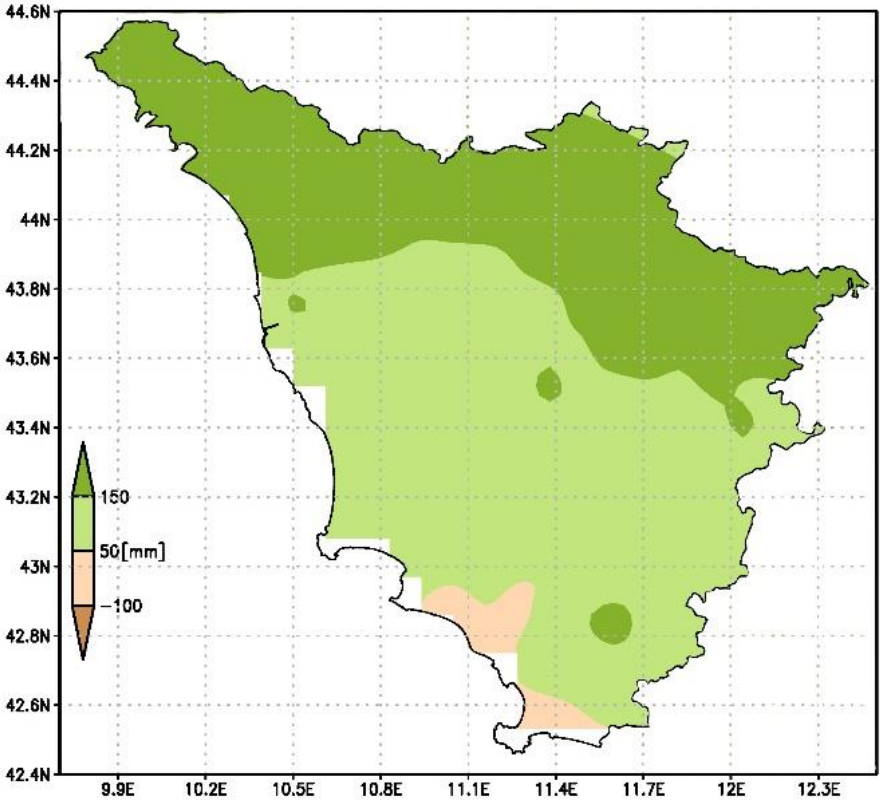
Dryness index (DI) indicates that the northern part of Tuscany will remain "humid" over the 21st century, until 2100, having more than 150 mm of precipitation during the growing season (see the **Table 2.2**). On the other hand, the central part of Tuscany with exception of certain areas will remain "moderately dry" with 50-150 mm of rainfall until the end of 21st century (**Figure 2.10**). The Maremma area, which is placed next to the Tyrrhenian Sea, will transform into "sub-humid "category, having 50-100 mm of precipitation in the studied period from 2071 to 2100, especially in Grosseto district that has an alluvial plain of the Ombrone river.

Interestingly, there are no significant differences in water availability between the present period and the future period under RCP4.5 scenario. In other studies of the dryness index in future conditions, Teslić and collaborators (2019) have found similar water conditions under RCP4.5 scenario in the nearby Emilia Romagna region, thus showing no significant differences in DI when compared to that of the Tuscany region. Fraga and collaborators (2016) reported that the months could be drier during the summer time period causing the loss of the crop yield.

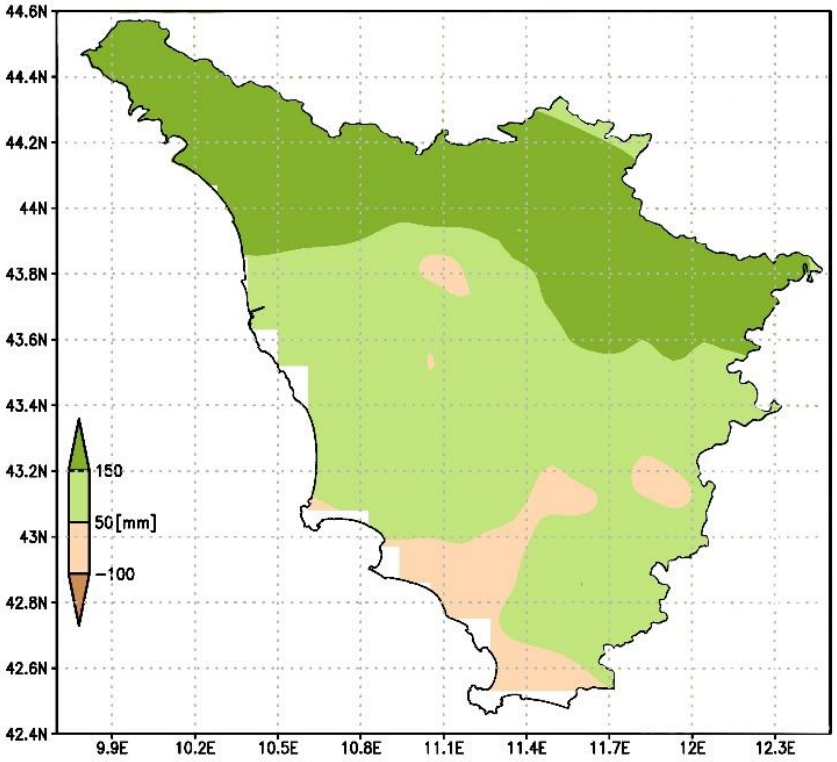
On the other hand, the elevated concentration of the CO_2 may stimulate its absorption, thus reducing the negative impact of the water stress condition and in that way alleviating the crop quantity loss (Salazar-Parra et al., 2012, 2015, Kizildeniz et al., 2015).



a)


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
150
50[mm]
−100

b)


44.6N
44.4N
44.2N
44N
43.8N
43.6N
43.4N
43.2N
43N
42.8N
42.6N
42.4N
9.9E
10.2E
10.5E
10.8E
11.1E
11.4E
11.7E
12E
12.3E
150
50[mm]
−100

c)

Figure 2. 10 Dryness index (DI) in Tuscany based on 9 model median ensemble projections, calculated for periods: a) 1990-2019 b) 2031-2060 c) 2070-2100 under RCP 4.5 scenario. Statistical significance of changes ($p < 0.05$) is presented by dots and calculated by the Student t-test.

2.2.4 Conclusions

The calculation of bioclimatic indices in Tuscany under RCP4.5 scenario showed that the future decades in the 21st century will become warmer, with lower precipitation.

The rise in temperature may be detrimental to the high-quality wine production which was shown by Winkler index. There is high probability that the Tuscany region will be very hot approaching 2100 and the high-quality wine production will be jeopardized only for the table grape production, resin production or low-quality table wine. Sangiovese as the main grape variety may be replaced with other grape varieties that suit the high alcohol wine production.

The water availability will probably decrease which may lead to the yield loss.

We have to take in consideration that the most important DOCG Tuscany's high-quality grape wines Chianti and Chianti classico are traditionally produced out of Sangiovese and their production is regulated by DOCG strict regulations that imply the use of at least 80% of this type of grape. This production is very likely to be endangered by the future upcoming climate changes in Tuscany. That is why it is important to apply the new strategies of adaptation to new climate conditions. Some of the strategies include the translocation of the vineyard areas uphill, while the others imply the substitution of the grape varieties.

2.2.5 References

Abeysinghe, S.K., Greer, D.H. and Rogiers, S.Y. (2019). The effect of light intensity and temperature on berry growth and sugar accumulation in *Vitis vinifera* 'Shiraz' under vineyard conditions. *Vitis*, 58, 7–16.

Alcamo J., Moreno, J.M., Nováky, B., Bindi, M., Corobov, R., Devoy, R.J.N., Giannakopoulos, C., Martin, E., Olesen, J.E. and Shvidenko, A. (2007). Europe, climate change 2007 impacts, adaptation and vulnerability. In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E. (eds) Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. *Cambridge University Press*, Cambridge, UK.

Antcliff, A.J. and Webster, W.J. (1955). Studies on the Sultana vine. I. Fruit bud distribution and budburst with reference to forecasting potential crop. *Australian Journal of Agricultural Research,* 6, 565–588.

Arguez, A., Hurley, S., Inamdar, A., Mahoney, L., Sanchez-Lugo, A. and Yang, L. (2020). Should we expect each year in the next decade (2019-28) to be ranked among the top 10 warmest years globally? *American Meteorology Society*, 655–663.

Arnell, N.W. (1999). Climate change and global water resources. *Global Environmental Change*, 9, 31–49.

Barnett, T., Zwiers, F., Hegerl, G., Allen, M., Crowley, T., Gillett, N., Hasselmann, K., Jones, P., Santer, B., Schnur, R., Stott, P., Taylor, K. and Tett, S. (2005). Detecting and attributing external influences on the climate system: A review of recent advances. *Journal of Climate,*18, 9, 1291–1314.

Bartolini, G., Morabito, M., Crisci, A., Grifoni, D., Torrigiani, T., Petralli, M., Maracchi, G. and Orlandini S. (2008). Recent trends in Tuscany (Italy) summer temperature and indices of extremes. *International Journal of Climatology,* 28,1751–1760.

Berger, A. (1988). Milankovich theory and climate. *Reviews of geophysics*, 26, 4, 624–657.

Buttrose, M.S. and Hale, C.R. (1973). Effect of temperature on development of the grapevine inflorescence after bud burst. *American Journal of Enology and Viticulture*, 24, 14–16.

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

Church, J.A. (2001). Climate change. How fast are sea levels rising? *Science,* 294, 802–803.

Coombe, B. (1987). Influence of temperature on composition and quality of grapes. ISHS *Acta Horticulturae*, 206, 25–35.

Crippen, D.D., Morrison, J. and Janice, C. (1986). The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *American Journal of Enology and Viticulture,* 37, 4, 235–242.

Croll, J. (1889). Discussion on climate and cosmology. *Cambridge University Press*. ISBN10: 1108055303.

Crowley, T.J. (2000). Causes of climate change over the past 1000 years. *Science,* 289, 270–277.

D'Oria, M., Ferraresi, M. and Tanda, M.G. (2017). Historical trends and high-resolution future climate projections in northern Tuscany (Italy). *Journal of Hydrology*, 555, 708–723.

Ebadi, A., Coombe, B.G. and May, P. (1995). Fruit set on small Chardonnay and Shiraz vines grown under varying temperature regimes between budburst and flowering. *Australian Journal of Grape and Wine Research,* 1, 3–10.

Ewart, A., and Kliewer, W.M. (1977). Effects of controlled day and night temperatures and nitrogen on fruit-set, ovule fertility, and fruit composition of several wine grape cultivars. *American Journal of Enology and Viticulture,* 28, 88–95.

Ewing-Mulligan, M., McCarthy, E. (2001). Italian wines for dummies. *Hungry Minds*, ISBN 0-7645-5355-0, 147- 159.

Fraga, H., Atauri, A.R., Malheiro, A.C. and Santos, J.A. (2016). Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. *Global Change Biology*, 22, 3774–3788.

Fraga, H., Malheiro, A.C., Moutinho-Pereira, J. and Santos, J.A. (2013). Future scenarios for viticultural zoning in Europe: ensemble projections and uncertainties. *International Journal of Biometeorology*, 57, 909–925.

Fraga, H., Malheiro, A.C., Moutinho-Pereira, J. and Santos, J.A. (2012). An overview of climate change impacts on European viticulture. *Food and Energy Security*, 1, 94–110.

Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Jones, G.V., Alves, F., Pinto, J.G. and Santos, J.A. (2014). Very high-resolution bioclimatic zoning of Portuguese wine regions: present and future scenarios. *Regional Environmental Change*, 14, 295–306.

Frei, P., Kotlarski, S., Liniger, M.A. and Schär, C. (2018). Future snowfall in the Alps: projections based on the EURO-CORDEX regional climate models. *Cryosphere*, 12, 1–24.

Gladstones, J. (2011). Wine, Terroir and Climate Change. Wakefield Press, Kape Town, Australia.

Greer, D.H., Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Functional Plant Biology*, 37, 206–214.

Hall, A. and Jones, G. V.(2010). Spatial analysis of climate in winegrape-growing regions in Australia. *Australian Journal of Grape and Wine Research*, 16, 389–404.

Holland, T., Smit, B. (2014). Recent climate change in the Prince Edward County winegrowing region, Ontario, Canada: Implications for adaptation in a fledgling wine industry. *Regional Environmental Change*, 14, 1109–1121.

Huglin, P. (1978). Nouveau mode d'évaluation des possibilities héliothermiques d'un milieu viticole. *In: Proceedings of the Symposium International sur l'ecologie de la Vigne. Ministère de l'Agriculture et de l'Industrie Alimentaire,* Contança, 89–98.

Huybrechts, P. and Joughin I. (2005). Ice-sheet and sea-level changes. *Science,* 310, 456–460.

Iglesias, A., Mougou, R., Moneo, M. and Quiroga, S. (2011). Towards adaptation of agriculture to climate change in the Mediterranean. *Regional Environmental Change*, 11, 159–166.

Iland, P., Dry, P., Proffitt, T. and Tyerman, S. (2011). The grapevine from the science to the practice of growing vines for wine. *Patrick Iland Wine Promotions Pty Ltd*, ISBN 13: 9780958160551.

IPCC The Core Writing Team, Pachauri, R.K., Meyer, L. (2014). Climate change 2014. Synthesis report. Contribution of working groups I, II and III to the fifth Assessment Report of the Intergovernmental Panel on Climate Change. *IPCC, Geneva, Switzerland*.

IPCC, (2007). Climate Change 2007. Synthesis Report, Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (Eds.)]. IPCC, Geneva, Switzerland.

Jackson, D.I., Cherry, N.J. (1988). Prediction of a district's grape ripening capacity, using a latitude-temperature index (LTI). *American Journal of Enology and Viticulture*, 1, 19–28.

Jacob, D., Petersen, J., Eggert, B., Alias, A., Christensen, O.B., Bouwer, L.M.,Braun, A., Colette, A., Déqué, M., Georgievski, G., Georgopoulou, E.,Gobiet, A., Menut, L., Nikulin, G., Haensler, A., Hempelmann, N., Jones, C., Keuler, K., Kovats, S., Kröner, N., Kotlarski, S., Kriegsmann, A., Martin, E., van Meijgaard, E., Moseley, C., Pfeifer, S., Preuschmann, S.,Radermacher, C., Radtke, K., Rechid, D., Rounsevell, M., Samuelsson, P., Somot, S., Soussana, J.F., Teichmann, C., Valentini, R., Vautard, R.,Weber, B. and Yiou, P. (2014).EURO-CORDEX: new high-resolution climate change projections for European impact research. *Regional Environmental Change*, 14, 563–578.

Jones, G.V. (2006). Climate and terroir: impacts of climate variability and change on wine. Pp. 1–14, in R. W. Macqueen, L. D. Meinert, eds. Fine Wine and Terroir – The Geoscience Perspective. *Geoscience* Canada, Geological Association of Canada, St. John's, Newfoundland, Canada.

Jones, G.V., White, M.A., Cooper, O.R. and Storchmann, K. (2005). Climate change and global wine quality. *Climate Change,* 73, 319–343.

Kizildeniz, T., Mekni, I., Santesteban, H., Pascual, I., Morales, F. and Irigoyen, J.J. (2015). Effects of climate change including elevated CO_2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. *Agricultural Water Management*, 159, 155–164.

Kliewer, M. and Torres, R. (1972). Effect of controlled day and night temperatures on grape coloration. *American Journal of Enology and Viticulture*, 23, 2, 71–77.

Kliewer, W. M. (1973). Berry composition of *Vitis vinifera* cultivars as influenced by photo- and nycto- temperatures during maturation. *Journal of the American Society for Horticultural Science,* 98, 153–159.

Kliewer, W.M. (1971). Effect of day temperature and light intensity on concentration of malic acid, tartaric acids in *Vitis vinifera* L. grapes. *Journal of the American Society for Horticultural Science,* 96, 372–377.

Kliewer, W.M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of emperor grapes. *American Journal of Enology and Viticulture*, 28, 96–103.

Knežević, Z. (2010). Milutin Milanković and the astronomical theory of climate changes. *Europhysics News*, 41, 3, 17–20.

Kobayashi, A., Yukinaga, H. and Matsunaga, E. (1965). Studies on the thermal conditions of grapes. V. Berry growth, yield and quality of Muscat of Alexandria as affected by night temperature. *Journal of the Japanese Society for Horticultural Science,* 34, 8–13.

Koufos, G.C., Mavromatis, T., Koundouras, S. and Jones, G.V. (2017). Response of viticulture-related climatic indices and zoning to historical and future climate conditions in Greece. *International Journal of Climatology*, 38, 2097–2111.

Kriedeman, P. and Smart, R. (1971). Effects of irradiance, temperature and leaf water potential on photosynthesis of vine leaves. *Photosynthetica*, 5, 6–15.

Lakso, A.N. and Kliewer, W.M. (1978). The influence of temperature on malic acid metabolism in grape berries. II. Temperature responses of net dark CO_2 fixation and malic acid pools. *American Journal of Enology and Viticulture*, 29, 145–149.

Liu, M., Rajagopalan, K., Chung, S.H., Jiang, X., Harrison, J., Nergui, T.,Guenther, A., Miller, C., Reyes, J., Tague, C., Choate, J., Salathé, E.P.,Stöckle, C.O. and Adam, J.C. (2014). What is the importance of climatemodel bias when projecting the impacts of climate change on land surface processes? *Biogeosciences*, 11, 2601–2622.

Loose, S. and Pabst, E. (2019). Prowein business report 2019 "climate change". *Geisenheim University, Institute of Wine and Beverage Business Research*, 1–56.

Luthi, D., le Floch, M., Bereither, B., Blunier, T., Barnola, J.M., Seigenthaler, U., Raynaud, D., Jouzel, J., Fisher, H., Kawamura, K. and Stoker, T.F. (2008). High-resolution carbon dioxide concentration record 650,000-800,000 years before present. *Nature Punblishing Group*, 453, 379–382.

McMichael, A.J., Woodruff, R.E. and Hales, S. (2006). Climate change and human health: Present and future risks. *Lancet*, 367, 859–869.

Mérouge, I., Seguin, G. and Arrouays, D. (1998). Les sols etl'alimentation hydrique de la vigne à Pomerol: état hydrique etcroissance de la vigne en 1995. *Journal International des Sciences de la Vigne et du Vin*, 32, 2, 59–68.

Milanković, M. (1941). Kanon der erdbestrahlung und seine anwendung auf das eiszeitenproblem. *Royal Serbian Academy special publications*, Section of Mathematical and Natural Sciences, 132, v. 33.

Mitrovica, J.X., Tamisiea, M.E., Davis, J.L. and Milne, G.A. (2001). Recent mass balance of polar ice sheets inferred from patterns of global sea-level change. *Nature,* 409, 1026–1029.

Monjo, R., Gaitán, E., Pórtoles, J., Ribalaygua, J. and Torres, L. (2016). Changes in extreme precipitation over Spain using statistical downscaling ofCMIP5 projections. *International Journal Climatology*, 36, 757–769.

Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., Sugaya, S. and Hashizume, K. (2005). Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. *Vitis*, 44, 161–165.

Moriondo,M., Bindi, M., Fagarazzi, C., Ferrise, R. and Trombi, G. (2011). Framework for high-resolution climate change impact assessment on grapevines at a regional scale. *Regional Environmental Change*, 11, 553–567.

Mullins M.G., Bouquet A. and Williams L.E. (1992). Biology of the grapevine. Cambridge University Press, Great Britain. Pages: 239.

NOAA. (2020) http://www.esrl.noaa.gov/gmd/ccgg/trends (online access on 21 November 2020).

OIV. (2019). Statistical report on world vitiviniculture. *International Organisation of Vine and Wine.* Paris, France, 1–23.

Olesen J.E. and Bindi M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy*. 16, 239–262.

Parker, A., Garcia de Cortazar-Atauri, I., van Leeuwen, C. and Chuine, I. (2011). General phenological model to characterise the timing of flowering and véraison of *Vitis vinifera* L. *Australian Journal of Grape and Wine Research*, 17, 2, 206–216.

Parry, M.L., Rosenzweig, C., Iglesias, A., Livermore, M. and Fischer, G. (2004). Effects of climate change on global food production under SRES emissions and socio-economic scenarios. *Global Environmental Change,* 14, 1, 53–67.

Pirie, A.J.C. (1978). Comparison of the climates of selected Australian, French and Californian wine producing areas. *Australian Grape grower and Winemaker*, 172, 74–78.

Rosenzweig, C., Strzepek, K.M., Majorc, D.C., Iglesias, A., Yates, D.N., Alyssa McCluskey, A. and Hillel, D. (2004). Water resources for agriculture in changing climate: international case studies. *Global Environmental Change*, 14, 345–360.

Ruml, M., Vuković, A., Vujadinović, M., Djurdjević, V., Ranković-Vasić, Z., Atanacković, Z., Sivčev, B., Marković, N., Matijašević, S. andPetrović, N. (2012). On the use of regional climate models: implications of climate change for viticulture in Serbia. *Agricultural and Forest Meteorology*, 158–159, 53–62.

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J. and Morales, F. (2012.) Photosynthetic response of Tempranillo grapevine to climate change scenarios. *Annals of Applied Biology*, 161, 277–292.

Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, Á., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L. and Morales, F. (2015). Carbon balance, partitioning and photosynthetic acclimation bin fruit-bearing grapevine (Vitis vinifera L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. *Journal of Plant Physiology*, 174, 97–109.

Santos, J.A., Costa, R. and Fraga, H. (2018). New insights into thermal growing conditions of Portuguese grapevine varieties under changing climates. *Theoretical and Applied Climatology*, 135, 1215–1226.

Santos, J.A., Malheiro, A.C., Pinto, J.G. and Jones, G.V. (2012). Macroclimate and viticultural zoning in Europe: observed trends and atmospheric forcing. *Climate Research*, 51, 89–103.

Santos, J.A., Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Dinis, L-T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., MenzC., Molitor, D., Junk, J., Beyer, M. and Schultz, H.R. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Applied Sciences*, 10, 3092, 1–28.

Schultz, H.R. (2000). Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. *Australian Journal of Grape and Wine Research*, 6, 2–12.

Soares, P.M.M., Lima, D.C.A., Cardoso, R.M., Nascimento, M.L. and Semedo, A. (2017). Western Iberian offshore wind resources: more or less in a global warming climate? *Applied Energy*, 203, 72–90.

Spayd, S., Tarara, J., Mee, D., and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. *American Journal of Enology and Viticulture*, 53, 3, 171–182.

Teslić, N., Vujadinović, M., Ruml, M., Ricci, A., Vuković, A., Parpinello, G.P. and Versari, A. (2019). Future climatic suitability of the Emilia-Romagna (Italy) region for grape production. *Regional Environmental Change*, 19, 599–614.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Marinez Ferreira De Siqueira, Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Townsend Peterson, A., Phillips, O.L. and Williams, S.E. (2004). Extinction risk from climate change. *Nature* 427, 145–148.

Tonietto, J. (1999). Les macroclimatsviticolesmondiaux et l'influence du mésoclimat sur la typicité de la Syrah etdu Muscat de Hambourg dans le sud de la France: méthodologie de caractérisation. Ph.D. Thesis. *Ecole Nationale Supérieure Agronomique*, Montpellier, France, 233p.

Tonietto, J. and Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology,* 124, 81–97.

Van Leeuwen, C. and Darriet, F. (2016). The impact of climate change on viticulture and wine quality. *Journal of Wine Economics*, 11, 1, 150–167.

Vuković, A., Vujadinović, M., Rendulić, S., Djurdjević, V., Ruml, M., Babić, V. and Popović, D. (2018). Global warming impact on climate change in Serbia for the period 1961–2100. *Thermal Science*, 22, 1–14.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, 416, 389–395.

White, M.A., Diffenbaugh, N.S., Jones, G.V., Pal, J.S. and Giorgi, F. (2006). Extreme heat reduces and shifts United States premium wine production in the 21st century. PNAS 103(30):11217–11222

Winkler, A.J. (1962). General Viticulture. *University of California Press, Berkeley*, 633.

Winkler, A.J. and Amerine, M.A. (1944). Composition and quality of musts and wines of California grapes. *Hilgardia*, 15, 6, 493–675.

Winkler, A.J., Cook, J.A., Kliewere, W.M., Lider, L.A. and Cerruti, L. (1974). General Viticulture, 4thEdition. *University of California Press*, Berkely, United States.

Zhu, X., Moriondo, M., van Ierland, E.C., Trombi G. and Bindi M. (2014). A model-based assessment of adaptation options for Chianti wine production in Tuscany (Italy) under climate change. *Regional Environmental Change,* 16, 85–96.

CHAPTER 3

Typicity and quality of Chianti and Chianti Classico wines

3 Typicity of Chianti and Chianti Classico wines

The typicity (or typicality) as a concept was introduced in enology by Sauvageot (1994) and Salette (1997) as a result of general wine characteristics coming from the ground (soil), grape cultivars and winemaking techniques used in production. The typicality should imply the differentiation, the identification and the recognition of the product. A wine typicity that is determined with wine chemical and sensory characteristics should represent an overall image of the terroir and it should provide the originality of the PDO wine production (Letablier and Nicolas, 1994; Salette et al., 1998).

International Organisation of Vine and Wine (OIV) defined the "terroir, as following: "Vitivinicultural "terroir" is a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practises develops, providing distinctive characteristics for the products originating from this area." (OIV, 2010). Eventually, terroir can be described as a multi-parametric assembly of three main bases: the vine plant, the environment including the grape vine (i.e. topography, ground and weather conditions) and social impact (vitiviniculture and social conditions in the region) (Gonzaga et al., 2021).

On the other hand, the Geographical Indication of origin is a notion established on the premise that wine characteristics come from the terroir. Geographical Indication identifies wine with regards to the location of vineyards, guaranteeing the origin of the grapes used for the wine production. In order to label a product with this notion, the producer is obliged to follow a series of regulations that make sure the optimum wine quality is obtained. Geographical classification is used in many countries following the rules in terms of grape variety choice, the minimum amount of alcohol and total acidity in wine, the time and type of wine aging and other winemaking parameters. For instance, the label Protected Designation of Origin (PDO) in France defined as : "Appellation d'Origine Contrôlée" (AOC), or in Italy: Denominazione di Origine Controllata (DOC), may have a product with particular characteristics originating from a geographical environment (République Française, 1993; Markham, 1997). Exceptionally, the wine classification in Bordeaux, which is one of the most eminent wine

regions, started back in 1855 when the categorizing of wine “crus” was established regarding the trading price and its reputation on the market.

3.1 The main appelations of origin in Tuscany: Chianti and Chianti Classico

The first information about wine production in Tuscany dates back to 2000 years ago at Etrurian time. Back to the 13th century, the name “Chianti” was recognised and established the production area, and in 1716 its borders were defined.

Nowadays, Chianti DOCG represents the most extended wine production area in Tuscany and within it there is another small appellation of origin Chianti Classico DOCG that represents a high-quality wine denomination of origin (**Figure 3.1**).

Chianti DOCG and Chianti Classico DOCG regulations are described in detail in their documents called “Disciplinare di produzione”. According to these documents, in order to obtain the name Chianti and Chianti Classico it is necessary to follow the rules regarding the wine denomination, production areas, ampelography and viticulture restrictions, vinification, bottling, aging and labelling.

3.1.1 Chianti DOCG

According to the DOCG regulations written by the Italian Ministry of Agriculture, Food, Forestry and Tourism (2019), Chianti DOCG wine is required to have well defined organoleptic and chemical composition. The sensory characteristics include ruby red color that obtains garnet hues with aging; intensive vinous aromas with scent of violets that become more pronounced during aging. The taste is dry, well balanced, full-bodied, slightly tannic, which evolves to soft velvety mouthfeel. Additionally, the minimum content of chemical compounds that Chianti wine has to possess include total alcohol: 11.50 % v/v (in the case of "reserve" wine the total alcohol content has to be at least 12.0 % v/v), total acidity 4.5 g/L, dry extract 20.0 g/L and dry extract 22.0 g/L.

Entire geographical Chianti area is divided into 7 sub-areas as follows **(Figure 3.1)**:

1) Colli Aretini
2) Colli Fiorentini
3) Colli Senesi
4) Colline Pisane
5) Montalbano
6) Montespertoli
7) Rufina

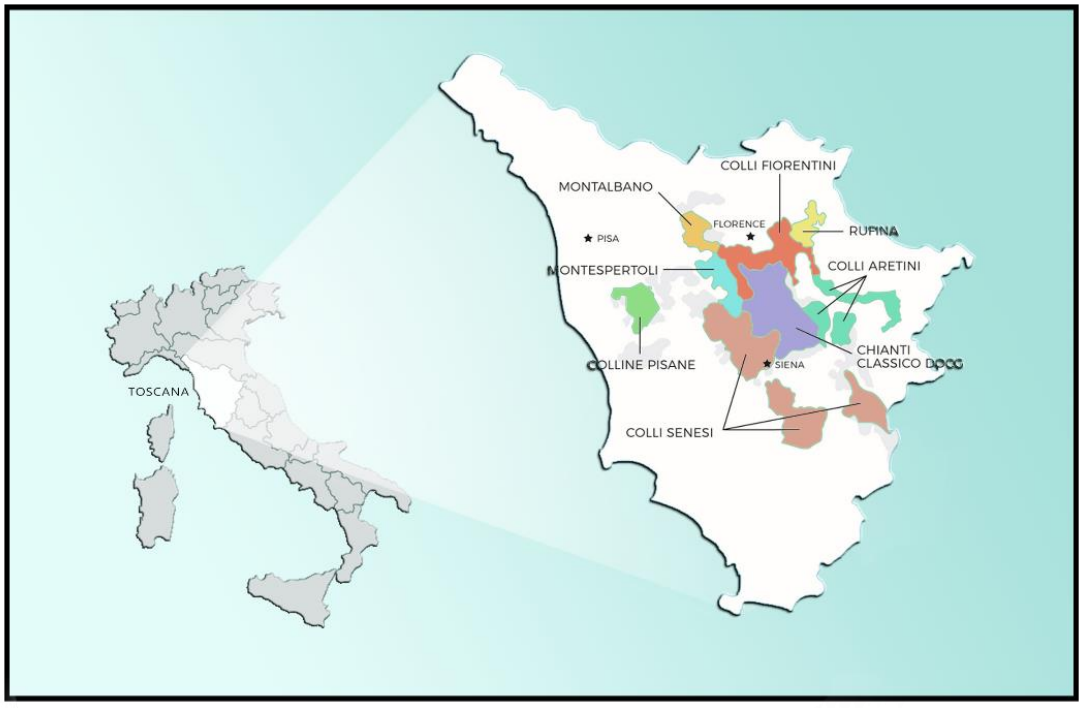


Figure 3. 1 Chianti DOCG sub-areas and Chianti classico DOCG in Tuscany, Italy (modified from w.w.w.vinepair.com).

Each of the sub-areas subjects to slight variation regarding the DOCG regulations of sensory and chemical characteristics and ampelographic specifications.

Chianti Superiore, as the appellation of origin, makes part of the same Chianti DOCG system, having organoleptic attributes corresponding to Chianti DOCG, with variation in minimum level of alcohol 12.0 % v/v.

Ampelographic regulation of the DOCG Chianti wine production contains the grape variety allowed to grow in the well-defined area in the same document "Disciplinare". Thus, the Chianti wines have to be produced from the grapes coming from delimited area of the art. 3 of the same document. The main grape variety for the Chianti DOCG wine production is Sangiovese and its content has to be >70 % of total grapes used in winemaking process. Beside Sangiovese, the other varieties can also be employed in the Chianti wine production, with a specification that they are suitable for grape cultivation in Tuscany region. Furthermore, the white grape cultivars and as well red Cabernet Franc/Cabernet Sauvignon varieties must not exceed the maximum limit of 10 % and 15%, respectively.

The *Consorzio Vino Chianti* (Chianti Wine Consortium) is a sectorial organisation created in 1927 by a group of winegrowers from provinces of the Chianti area: Florence, Siena, Arezzo and Pistoia. Nowadays, the Chianti Wine Consortium gathers about 3,000 producers that produce 800,000 hectolitres of wine with over 15,500 hectares of vineyards. It has a role in protection of producers' interest against wine plagiarism and possible adulteration, in implementation of wine promotion and in providing information for consumers, hence it protects the overall interests related to D.O.C.G. "Chianti" (www.consorziovinochianti.it/en/consorzio).

Chianti wine was recognised as Chianti D.O.C.G. in 1984, because of a dedicated and expertise activity of the winegrowers and wine industry (www.consorziovinochianti.it/en/consorzio).

"Toscana Certificazione Agroalimentare" is an organisation authorised by the relevant Ministry that controls if all the Disciplinare's regulations were respected during the grape and wine production. If so, the produced wine obtains the appellation of origin "Chianti DOCG" that guarantees its quality in terms of chemical and sensory characteristics.

3.1.2 Chianti Classico DOCG

According to "Disciplinare" of Chianti Classico, the production has regulations regarding grape production, vinification and bottle labelling.

As for the grape production regulations, there are vineyard position, maximum yields allowed per hectare and the irrigation rules. All the vineyards that produce Chianti Classico grapes have to be registered on the locations that have hilly positions at an altitude not exceeding 700 meters above the sea level. The pedological characteristics of the terrain consist of the prevalence of arenaceous, calcareous marly substrates, clay schists, sands and pebbles.

The geographical position of the grape production for the Chianti Classico DOCG wines is precisely defined by the Article 3 of Disciplinare. As can be seen in the **Figure 3.2**, the location of the Chianti Classico area is between Florence and Siena provinces in Tuscany and it encompasses the following municipalities: San Casciano in Val di Pesa, Tavernelle Val di Pesa, Greve in Chianti, Barberino val di Pesa, Poggibonsi, Castellina in Chianti, Rada in Chianti, Gaiole in Chianti and Castelnuovo Berardenga.

Figure 3. 2 Chianti Classico DOCG area in Tuscany, Italy and its logo Black rooster (it. Galo Nero) (modified from www.cinellicolombini.it and w.w.w.vinepair.com).

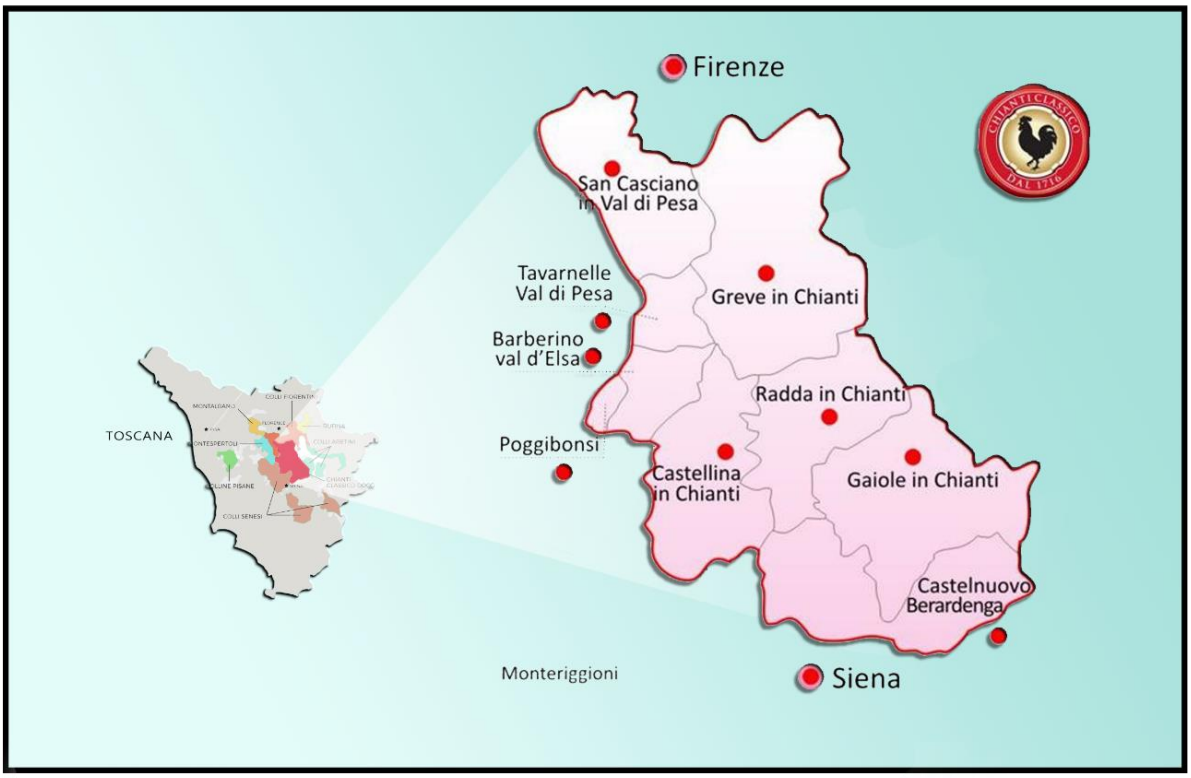


Figure 3. 2 Chianti Classico DOCG area in Tuscany, Italy and its logo Black rooster (it. Galo Nero) (modified from www.cinellicolombini.it and w.w.w.vinepair.com).

Regarding the viticulture regulations, the density of the plant has to be at least 4400 grapevines per ha. The maximum grape production allowed is 7.5 t/ha and the grape load per plant must not exceed 2 kg. The maximum yield of wine in relation to the grapes is 70%. If that is exceeded the wine will lose the right to obtain the designation of origin. Moreover, if the percentage is not respected, a winery will totally lose the right to produce the Chianti Classico wine.

The varietal composition of the wine has to be that of Sangiovese from 80 to 100 %, and the minimum alcohol content in the Chianti Classico wine is 11.5 % v/v.

There are three groups of the Chianti Classico wines according to their aging duration and enological characteristics of wine:

1) Chianti Classico

2) Chianti Classico Riserva

3) Chianti Classico riserva Gran Selezione

According to the wine regulations each of the group has to satisfy the minimum requirements of the chemical compounds like alcohol content (%), total acidity (g/L), sugar free dry extract but also the specific sensory characteristics of wine like color, olfactory attributes and mouthfeel (**Table 3.1**). All the Chianti Classico wines have to age at least three months in bottles before the consumption. Chianti Classico wine can be put on the market in October following the harvest. Chianti Classico Riserva wine has to age 24 months (including 3 months in bottles) before the release on the market and Chianti Classico Gran Selezione 30 months in total.

Table 3. 1 The main sensory and analytical characteristics of Chianti classico wine defined by DOCG regulations.

Wine characteristic	**Chianti Classico**	**Chianti classico Riserva**	**Chianti Classico riserva Gran Selezione**
Color	Medium intense ruby red wine	Intense ruby red, tending to garnet with aging	Intense ruby red, tending to garnet with aging
Olfactory characteristics	Tipical floreal intense aromas	Intense and persistent fruity aromas	Spicy and persistent
Mouthfeel	Dry, fresh, rounded, slightly tannic that refines over time	Dry, well balanced with nice tannins	Dry, persistent, well-balanced
Total alcohol % v/v	12.00	12.50	13.00
Minimum level of total acidity (g/L)	4.5	4.5	4.5
Sugar free dry extract (g/L)	24.0	25.0	26.0
Minimum aging duration	12 months	24 months	30 months

When it comes to the production of the Gran Selezione Chianti Classico wines, the same winery has to conduct all the viticulture and winemaking processes from the grape production until the wine bottling.

The Chianti Classico DOCG wine has its own institution *Consorzio di Tutela* that represents the first Consortium founded in Italy, back in 1924. *Consorzio di Tutela* plays a role in protecting the Chianti Classico brand "Black Rooster" (from plagiarism and unethical competition) and in the implementation of its market promotion. In fact, *Consorzio di Tutela* Chianti Classico ensures the authenticity of the wine by a warranty that the producers fulfilled all the production requirements. Consorzio may also be given the authorisation by the Italian law 2/10/92, no. 164, to perform the activities of vigilance of the production (Malorgio and Grazia, 2007;www.chianticlassico.com/en/consortium).

Valoritalia is an organisation that controls the compliance of the Chianti Classico wines in terms of chemical analysis and sensory characteristics and issues the certificates of the conformity of the product.

3.2 Wine typicity assessment of Chianti and Chianti classico DOCG wines

3.2.1 Introduction

Wine typicity (or typicality) as a notion can identify a specific wine profile and can represent a particular category of wine (Cadot et al., 2010, 2012). Furthermore, wine is typical if some of its attributes that make it unique can be determined since they contribute to a specific wine group, distinctive from the others (Maitre et al., 2010). King and collaborators (2014) suggested that regionality, terroir, or typicality is the distinguishing characteristic of wine coming from the geography, climate and geology, of a specific place. It can present a style specific to an area in a representative wine sample. Regarding the PDO wines, the typicality is integrated within its global quality (Salette, 1997).

According to the World Trade Organization (WTO), the definition of the geographical indication is as follows:"indications which identify a good as originating in the territory of a member country, or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographical origin" (WTO, 2021).

The quality control as a series of legal instructions is one of the indispensable practices developed in many winemaking countries in order to guarantee the safety of excellence of wines that come from historic and specific areas of origin (EC, 2013). Therefore, even in Italy there is a DOP system of wine production, **see** details about Tuscany's regulation of Chianti and Chianti classico wine production in part **3.1.**

Recently, chemometric classification techniques and pattern recognition analysis methods, for wine and other alcoholic beverages, have received great attention as discrimination tools.

A numerous studies have been of use to the wine experts in the assessment of the wine typicality (Ballester et al., 2005; Parr et al., 2010; Perrin and Pages, 2009). In general, wine classification is based on the sensory analysis conducted by trained wine experts, along with the use of instrumental analytical methods. A big amount of analytical data related to wine composition, has been established as a versatile and valuable tool for assessing the wine authenticity, applying some sophisticated statistical techniques, named "chemometrics"(Soto Vázquez et al., 2011; Makris et al., 2006). In this respect, a wine quality assessment may be conducted on the base of the wine flavour and many other wine physicochemical characteristics including total acidity, alcohol content, residual sugars, citric acid, acetic acid, sulphur dioxide, density, pH and sulphates (Sowmya et al., 2019).

Multivariate statistical methods are an effective tool for the study of wine classification, based on large data sets of chemical or sensory information (González and Peña-Méndez, 2000). One of them, the Partial Least square (PLS) analysis, suggests how accurately one variable predicts the variation of another group of data (Noble and Ebeler, 2002; Frank and Kowalski, 1984, Lee and Noble, 2006).

The aim of this study is to build a model of prediction of Chianti and Chianti classico DOCG wine quality based on their chemical composition using the PLS statistical analysis.

3.2.2 Materials and methods

3.2.2.1 Tuscan wine samples

A total of 456 red wines were collected from commercial wine production at Ruffino winery at two winemaking stages: before fining and at bottling over six vintages 2012-2018. The wines were grouped in 6 different DOCG wine denomination categories: Chianti DOCG, Chianti Superiore DOCG, Chianti Riserva DOCG, Chianti Classico DOCG, Chianti Classico Riserva DOCG and Chianti Classico Gran Selezione DOCG (**Table 3.2**) with increasing quality category from 1 (basic) to 6 (top).

Table 3. 2 Wine samples used in this study: 6 quality categories from 2 different wine Tuscany areas: Chianti and Chianti Classico.

Code	Quality	Denomination	"Disciplinare"	Number of samples
CH	1	Chianti DOCG	Chianti	202
CHR	2	Chianti riserva DOCG	Chianti	11
CHS	3	Chianti Superiore DOCG	Chianti	55
CC	4	Chianti Classico DOCG	Chianti Classico	51
CCR	5	Chianti Classico Riserva DOCG	Chianti Classico	98
CCRGS	6	Chianti Classico Riserva Gran Selezione DOCG	Chianti Classico	39

All the wines were produced following Chianti and Chianti classico DOCG regulations **see the section 3.1.1. and 3.1.2**

3.2.2.2 Winemaking practices

The grapes from Chianti and Chianti classico areas were carried to the Ruffino winery, where they were separately vinified in stainless steel tanks. The same oenological protocols were applied as follows: i) grape crushing and destemming; ii)addition of 5 g/hl of potassium metabisulfite and the selected dry yeast (20 g/hl); iii) the average of 17 and 10 days maceration for Chianti classico and Chianti wines respectively at controlled temperature (max 28°C) with punching the cap down six times a day until 3 Babo units; iv) the wines were poured into the stainless steel tanks for the malolactic fermentation at the controlled temperature (22°C) v)

wine ageing according to the DOCG regulations (see the section 3.1.2.).All these operations were repeated every vintage of the trial (2012- 2018).

3.2.2.3 Wine chemical and sensory analysis

All the samples were analysed in two moments during the wine production: before fining and at bottling with FOSS WineScan FT-IR instrument supplied by FOSS (Hilleroad, Denmark) and UV-vis Spectrophotometer Carry 60 produced by Agilent Tecnologies, (CA, USA). FOSS wine analyzer was used to measure wine chemical parameters including total acidity (g/L) and organic acids: tartaric, acetic, malic, lactic, citric, succinic acid expressed in g/L, then alcohol (% vol), anthocyanins (mg/L) and total polyphenols (mg/L), wine density, ashes (g/L), total dry extract (g/L), glycerol (g/L), pH value, potassium (mg/L), sulphates (g/L), enzymatic sugars (g/L) and reducing sugars (g/L). The results provided by FOSS WineScan instrument were relevant and regularly cheked by internal calibration and comparing risults obtained by an external certificated laboratory Isvea in Tuscany.

Agilent Spectrophotometer Carry 60 was used to analyse all the wine samples and to obtain the values of optical density from 200 nm to 800 nm.

All along winemaking process - from the alcoholic fermentation and maceration, through wine fining, aging and bottling - all the wines were tasted by enologists to evaluate their sensory properties. All the enologists have a clear concept of the wine typicality, based on their long working experience in Tuscany. Moreover, all the wines were certificated by appropriate official organisation that issues the Chianti DOCG and Chianti Classico DOCG certificates (see the **section 3.1.1** and **3.1.2**. for more details).

3.2.2.4 Data analysis

RStudio program (version 1.4.1717, 2009-2011, RStudio BPC) and XLSTAT statistical programs were used for the statistical data analysis. Descriptive statistical analysis and principal component analysis (PCA) were performed using RStudio in order to obtain the

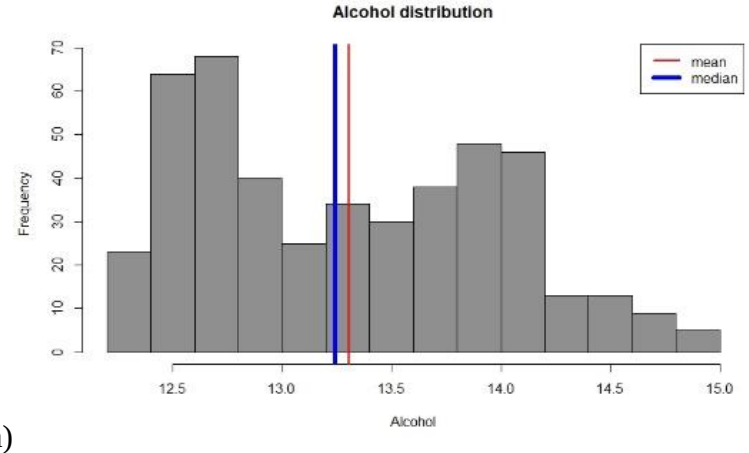
frequency distribution, correlogram, boxplots and PCA graphs of the main chemical parameters related to the wine quality. On the other hand, Partial Least Squares (PLS) analysis were performed by XLSTAT program to find relationship between estimated and observed data of the wine quality.

3.2.3 Results and discussion

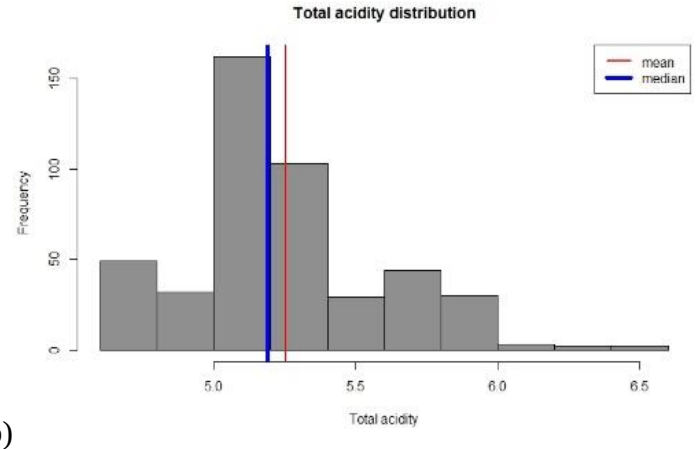
3.2.3.1 Descriptive statistics results of general wine composition

Histograms provide information about samples distribution regarding the main wine chemical compounds such as: alcohol percent, total acidity (g/L), volatile acidity (g/L), tartaric acid (g/L), lactic acid (g/L), pH value, polyphenols (mg/L) and anthocyanins (**Figure 3.3)**. For the other chemical parameters: citric acid (g/L), glycerol (g/L), ash content (g/L), dry extract (g/L), potassium (mg/L) and residual sugar (g/L) see the supplementary data (**Figure S1**). Malic acid was presented only in traces because it was degraded during the malolactic fermentation.

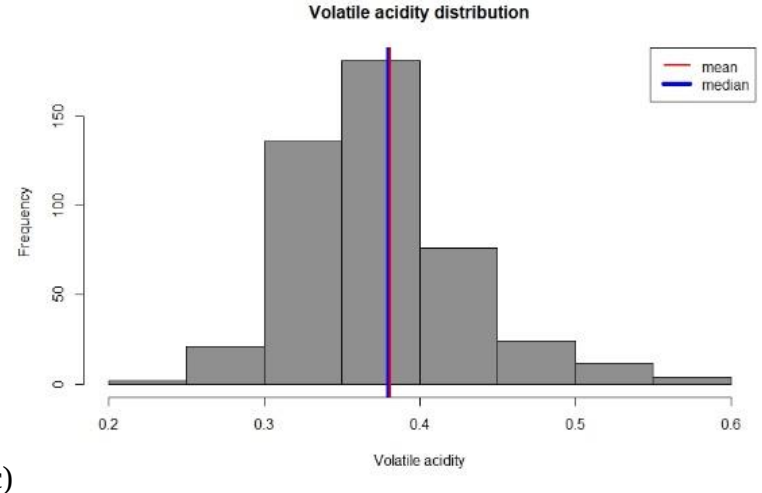
Frequency distribution of each wine chemical property showed the skewness. In particular, the alcohol content, total acidity, volatile acidity, tartaric, lactic, polyphenols and anthocyanins and were skewed to the right with the highest sample frequency at 12.5 % v/v, 5 g /L, 0.4 g/L, 2 g/L, 1.1 g/L, 2750 mg/L and 700 mg/L respectively, while the pH value were skewed to the left with the highest count of samples at 3.5 value.



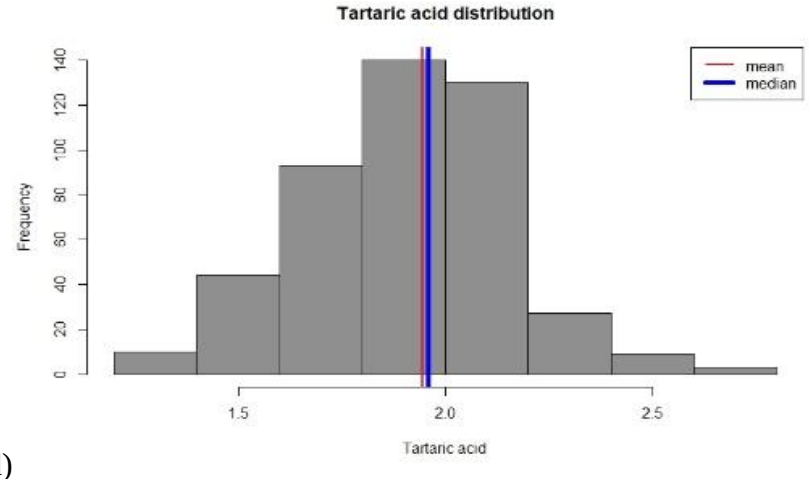
a)


Total acidity distribution
mean
median
Frequency
0
50
100
150
5.0
5.5
6.0
6.5
Total acidity

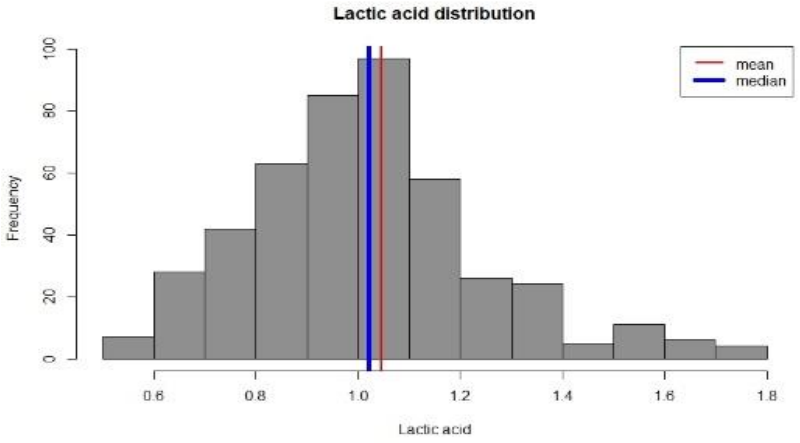
b)


Volatile acidity distribution
mean
median
Frequency
0
50
100
150
0.2
0.3
0.4
0.5
0.6
Volatile acidity

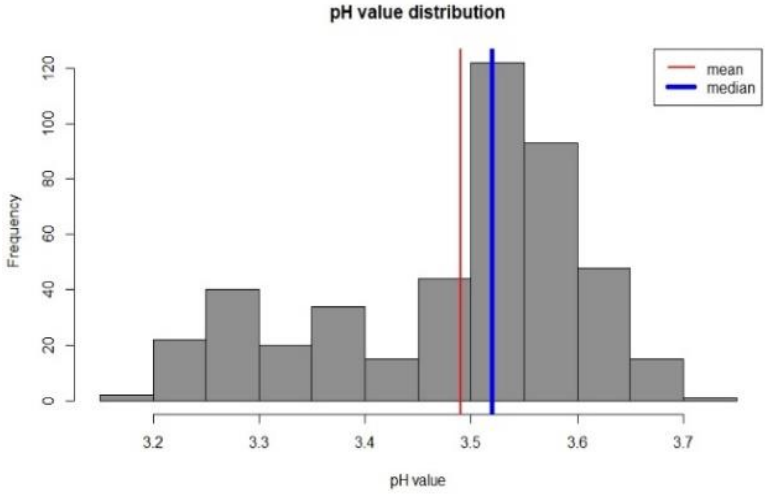
c)


Tartaric acid distribution
mean
median
Frequency
0
20
40
60
80
100
120
140
1.5
2.0
2.5
Tartaric acid

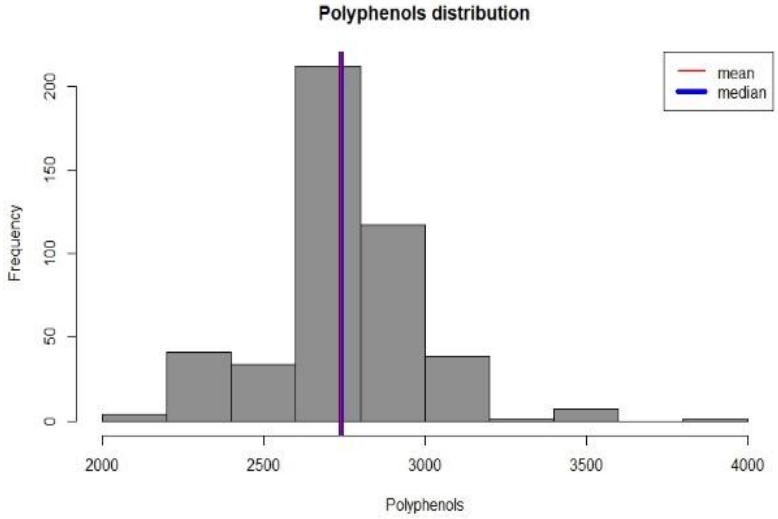
d)


Lactic acid distribution
mean
median
Frequency
0
20
40
60
80
100
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Lactic acid

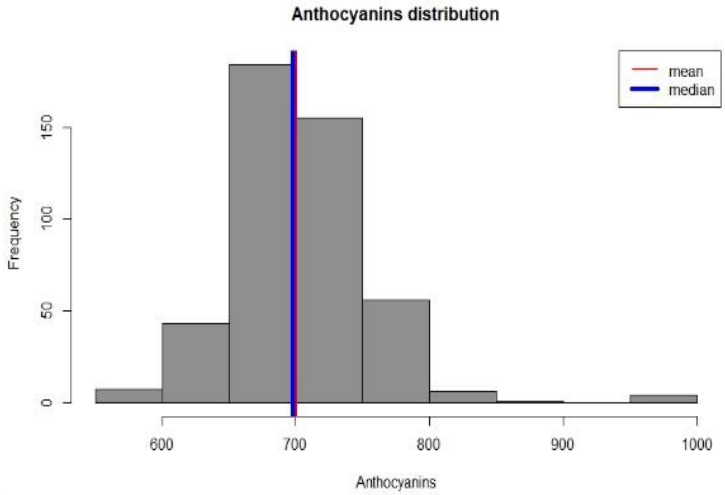
e)


pH value distribution
mean
median
Frequency
0
20
40
60
80
100
120
3.2
3.3
3.4
3.5
3.6
3.7
pH value

f)


Polyphenols distribution
mean
median
Frequency
0
50
100
150
200
2000
2500
3000
3500
4000
Polyphenols

g)



h)

Figure 3. 3 Frequency distribution of the main chemical wine parameters of all wine samples: a) alcohol (% v/v); b) Total acidity (g/L), c) Volatile acidity (g/L), d) tartaric acid (g/L), e) Lactic acid (g/L), f) pH value, g) Polyphenols (mg/L), h) Anthocyanins (mg/L).

A chemical analysis carried out with the FOSS WineScan FT-IR instrument including basic wine compounds: alcohol (%), polyphenols (mg/L), anthocyanins (mg/L), pH value, total acidity (g/L), volatile acidity (g/L), citric acid (g/L), lactic acid (g/L), malic acid (mg/L), tartaric acid (g/L), ashes (mg/L), dry extract (mg/l), glycerine (g/L), potassium (mg/L), sulphates (mg/L), residual sugar (g/L). The basic analysis of wines categorised by wine quality is reported in **Table 3.3**, showing their average values and standard deviation.

As for the alcohol content (%) v/v, its value showed a constant increase from Chianti DOCG to Chianti Classico Riserva Grand Selezione DOCG wines (i.e. from the quality 1 to the quality 6, see the **Table 3.2**), and it ranged from 12.7 to 14.5 % v/v. The polyphenol compounds in wines showed an increase from 2650 mg/L to 2830 mg/L in Chianti DOCG appellation of origin (i.e. from 1 to 3 wine quality). On the other hand, wines from Chianti Classico appellation of origin did not show a significant difference in the polyphenols amount in Chianti classico and Chianti Classico Riserva wines (i.e.4 and 5 wine quality) ranging from 2760 to 2740 mg/L, while the highest quality wine(i.e. 6) had about 3100 mg/L. According to the wine regulation defined by the Chianti and Chianti Classico appellations of origin, no wine sample had the minimum alcohol content below the legal minimum, which is 12, 12.5 and 13% v/v for Chianti, Chianti Classico Riserva and Chianti Classico Riserva Gran Selezione wines respectively (see the section **3.1.1** and **3.1.2.**).These results are in agreement with those

obtained by Rinaldi and collaborators (2020), who reported that the average value of alcohol in Chianti DOCG wine was 12.9 (%), while in Chianti DOCG wine it was 13.6 % v/v for vintages from 2013 to 2015. Furthermore, the total acidity was always above the legal minimum (i.e. 4.5 g/L) and it ranged from 5 to 5.6 g/L (Ministerodellepoliticheagricolealimentariforestali e del turismo, 2020). The average value of pH is quite constant in all wine quality classes, and it is about 3.5.

Table 3. 3 Descriptive statistics for the basic wine chemical analysis determined in Chianti and Chianti classico wines: the mean value and standard deviation categorized by wine quality

Wine quality	1	2	3	4	5	6
N	202	11	55	51	98	39
Alcohol (%)	12.72 ± 0.33	12.91 ± 0.47	13.56 ± 0.46	13.60± 0.32	13.78 ± 0.32	14.51 ± 0.28
Polyphenols (mg/L)	2645 ± 289	2706 ± 397	2827 ± 270	2761 ± 163	2739 ± 117	3092 ± 284
Anthocyanins (mg/L)	667 ± 37	673 ± 23	716 ± 65	703 ± 36	722 ± 32	773 ± 31
pH value	3.51 ± 0.13	3.48 ± 0.12	3.50 ± 0.12	3.43 ± 0.10	3.48 ± .11	3.48 ± .11
Total acidity (g/L)	5.05 ± 0.23	5.16 ± 0.12	5.15 ± 0.11	5.60 ± 0.31	5.44 ± 0.29	5.60 ± 0.33
Volatile acidity (g/L)	0.37 ± 0.04	0.36 ± 0.04	0.33 ± 0.03	0.39 ± 0.05	0.39 ± 0.04	0.49 ± 0.06
Citric acid (g/L)	0.09 ± 0.09	0.10 ± 0.1	0.10 ± 0.09	0.07 ± 0.07	0.07 ± 0.08	0.06 ± 0.08
Lactic acid (g/L)	1.08 ± 0.16	1.04 ± 0.22	0.93 ± 0.22	0.93 ± 0.21	1.06 ± 0.34	1.11 ± 0.30
Malic acid (g/L)	0.01 ± 0.04	0.04 ± 0.07	0.02 ± 0.04	0.05 ± 0.07	0.04 ± 0.05	0.06 ± 0.06
Tartaric acid (g/L)	1.99 ± 0.18	1.87 ± 0.20	1.97 ± 0.19	2.11 ± 0.31	1.83 ± 0.22	1.76 ± 0.34
Ashes (mg/L)	2.62 ± 0.34	2.60 ± 0.27	2.78 ± 0.33	2.58 ± 0.41	2.81 ± 0.27	2.84 ± 0.21
Dry extract (mg/L)	30.19 ± 1.95	29.02 ± 2.07	29.72 ± 2.04	29.63 ± 1.67	29.53 ± 1.83	29.76 ± 2.44
Glicerin (g/L)	7.9 ± 1.3	7.8 ± 1.7	7.9 ± 1.5	8.3 ± 1.5	8.2 ± 1.7	8.7 ± 2.0
Potassium (mg/L)	959 ± 172	926 ± 145	968 ± 176	884 ± 153	939± 160	945 ± 146
Sulfates (mg/L)	0.50 ± 0.18	0.45 ± 0.17	0.54 ± 0.16	0.44 ± 0.14	0.46 ± 0.17	0.54 ± 0.16
Residual sugar (g/L)	3.1 ± 1.3	2.5 ± 1.7	2.2 ± 0.9	1.7 ± 1.1	1.5 ± 0.5	1.2 ± 0.4

3.2.3.2 UV-Vis spectroscopy results

Figure 3.4. shows the average UV-Vis spectrums from replicates of each DOCG type of wine: CH, CHR, CHS, CC, CCR and CCRGS or quality categories 1, 2, 3, 4, 5, and 6 respectively (see Table 3.2 for more details).

As displayed in **Figure 3.4**, the peak values for all wine spectrums are within the range from 350 to 650 nm, in which yellow, red and violet color absorbance can be found. The highest

absorption intensity band is observed in the spectrum of CCRGS wine (brown line) and it was followed by the sample CHS (yellow line). The samples, CHR, CC and CCR showed similar Uv-Vis spectrums, while the sample of Chianti wine (CH) had the lowest absorbance.

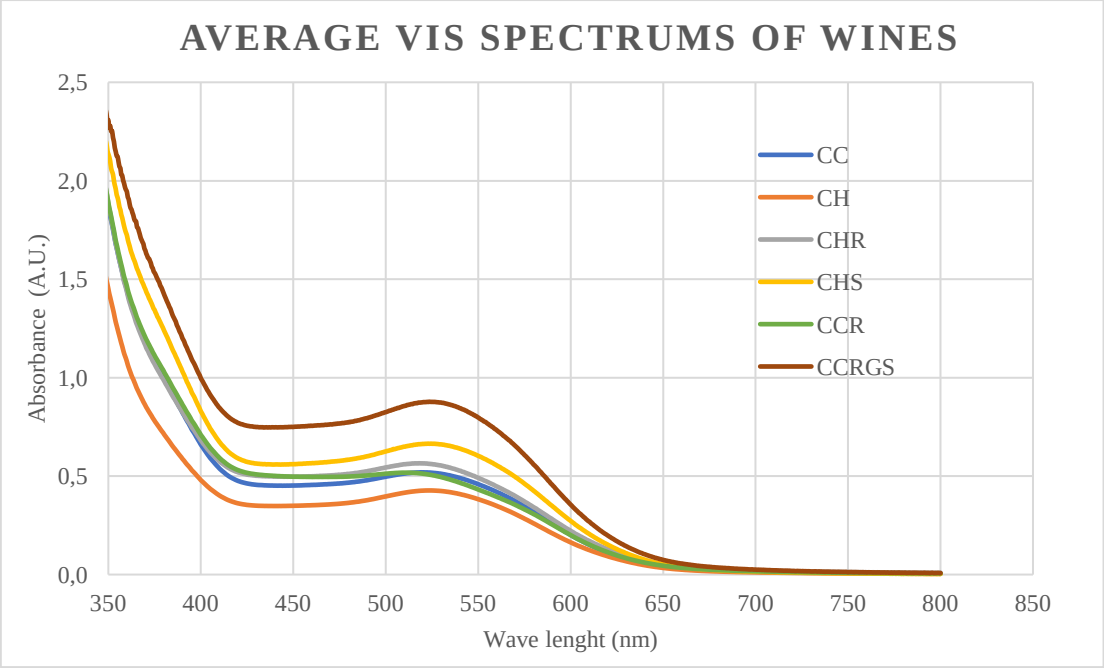


Figure 3. 4 Average VIS spectrum of wines. CH -Chianti, CHS- Chianti Superiore, CHR-Chianti Riserva, CC – Chianti Classico, CCR- Chianti Classico Riserva, CCRGS-Chianti Classico Riserva Gran Selezione.Y axis shows the absorbance values in A.U. and the X axis shows the part of spectrum with wave lengths from 350 to 850 nm.

As proposed by Canuti and collaborators (2020), the differences in absorption in UV-Vis spectrum which indicate the color of the wine, can be explained by the polyphenol content of wine samples. The wine sample CCRGS had 3100 mg/L of total polyphenols, CHS had 2827 mg/l which was followed by CHR, CC and CCR that had 2706, 2761 and 2739 mg/l, respectively. The last sample of CH had the lowest amount of total polyphenols 2645 mg/L (see **Table 3.3** for more details).

3.2.3.3 Classification of wine samples by principal component analysis - Evaluation of the essential chemical quality of Chianti and Chianti Classico DOCG wines

The intrinsic chemical profile describes the common chemical characteristics of all wines, thus representing the suitability and identity of wine properties for all wine samples.

A chemically suitable profile of wines was described by the standard chemical parameters including alcohol content, polyphenols, total acidity, pH, anthocyanins, glicerol, residual sugar, tartaric acid, lactic acid, malic acid, acetic acid, citric acid, succinic acid, dry extract, density, ashes, potassium and sulfates. The statistical analyses of the standard chemical parameters were conducted applying the PCA, and the results were drafted in **Figure 3.5.** and **3.6.**

The correlation circle on axes Dim1 and Dim2 and the projections of the weights of 19 original variables in the factor space is illustrated in **Figure 3.5**.

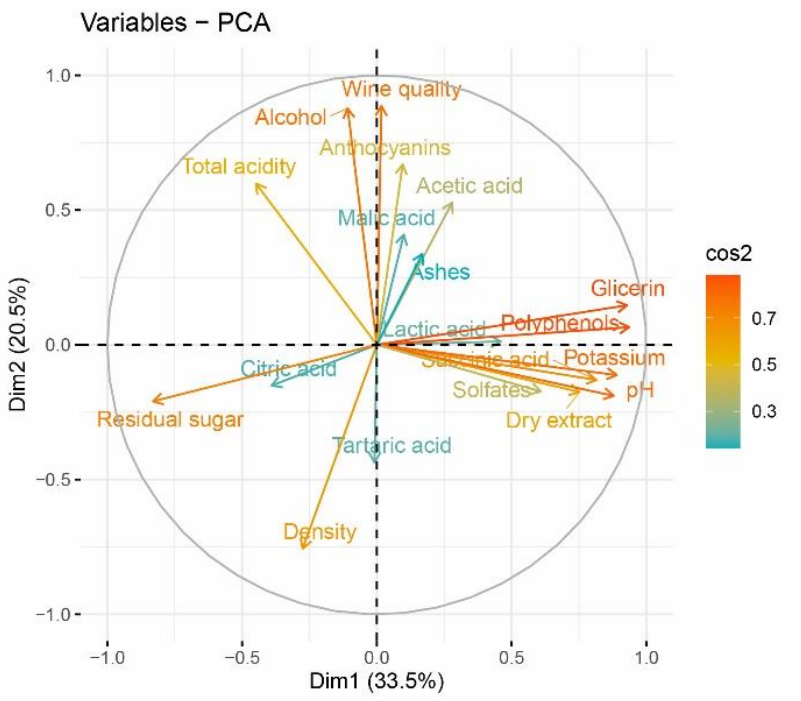


Figure 3. 5 Principal component analysis (PCA) of loadings draft of eligibility profile (for standard chemical parameters) of Chianti and Chianti Classico DOCG wines from Tuscany.

The first two dimensions PC1 (i.e. Dim1) and PC2 (i.e. Dim2) accounted for 54 % of the total variance. The statistical analysis allowed the determination of the squared cosins of variables that were related to both dimensions. As for the first dimension Dim1, the variables that

participated the most are the following: polyphenols (0.936), glycerin (0.928), potassium (0.890), pH value (0.878), residual sugar (0.830), succinic acid (0.814), dry extract (0.752), sulfates (0.608), lactic acid (0.460), total acidity (0.448), and citric acid (0.390). On the other hand, the squared cosins of the second principal component Dim2 are the following: wine quality (see **Table 3.2**) (0.886), alcohol (0.877), anthocyanins (0.669), density (0.756), total acidity (0.598), acetic acid (0.527), tartaric acid (0.436), malic acid (0.408), ashes (0.337). The core plot of the correlation of variables with the principal components is available in the supporting information (see **Figure S2** in the supporting information).

The eigenvalues stand for another parameter that was defined by principal component analysis. It measures the amount of variation held by each principal component. From nineteen principal components obtained, the first five had the eigenvalue higher than 1(6.37, 3.90, 1.94 1.48 and 1.18 respectively) accounting for 78.3 % of the total variance (see **Figure S3** in the supporting information section for more details). The variance percent of principal components is shown in **Figure 3.6**.

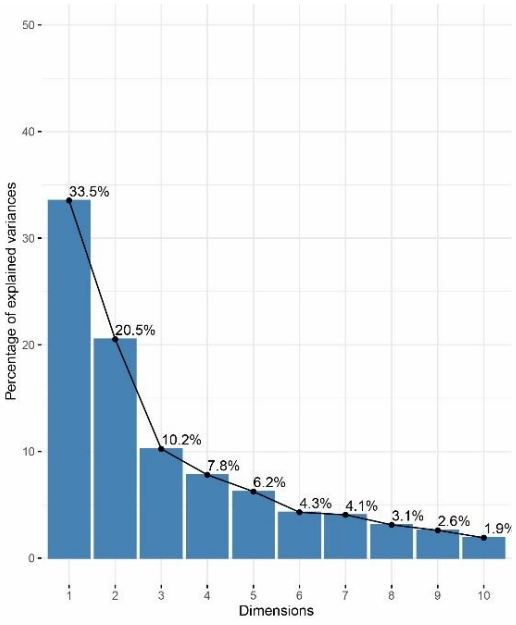


Figure 3. 6 Percentage of variation in principal components of Chianti and Chianti Classico DOCG wines.

The **Figure 3.7** shows the grouping of the wine samples based on two principal components PC1 and PC2. The graph shows the division of samples in two major groups. The first one is

presented by samples 1, 2 and 3 which make part of Chianti appellation of origin, being positioned on the positive part of the first principal component (PC1). The second group is represented by 4, 5, and 6 wine samples which belong to Chianti Classico appellation of origin, and they are placed on the negative part of PC1 scale.

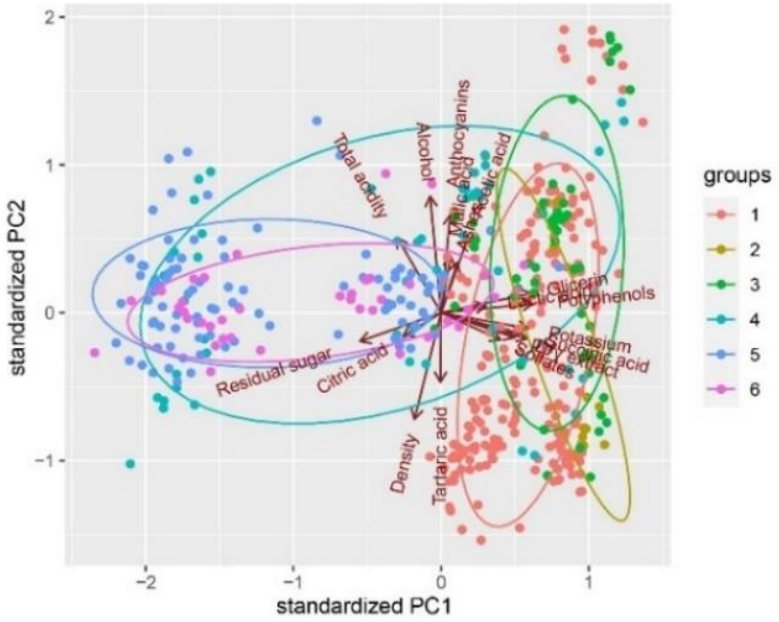


Figure 3. 7 Principal component analysis of Chianti and Chianti Classsico DOCG wines. 1) Chianti, 2) Chianti Riserva, 3) Chianti Superiore, 4) Chianti Classico, 5) Chianti Classico Riserva, 6) Chianti Classico Riserva Gran Selezione.

3.2.3.4 Partial least square analysis (PLS)

Partial least square analysis (PLS) was conducted in order to reveal the link between sensory quality scores and chemical database of Chianti and Chianti Classico wines. The aim of this analysis was to create a model so as to predict the wine quality of new wines.

The prediction PLS regression model was built using the UV-Vis spectrum in the range from 350 to 800 nm and sensory evaluation results (i.e. 6 wine quality categories). Afterwards, results were illustrated in the graph (F**igure 3.8)** where x axis is defined by wine quality scores, and y axis by predicted values. The developed model shows high quality of the predictions. Certain samples are not well predicted, yet the overall correlation is satisfactory:R^2 = 0.805.

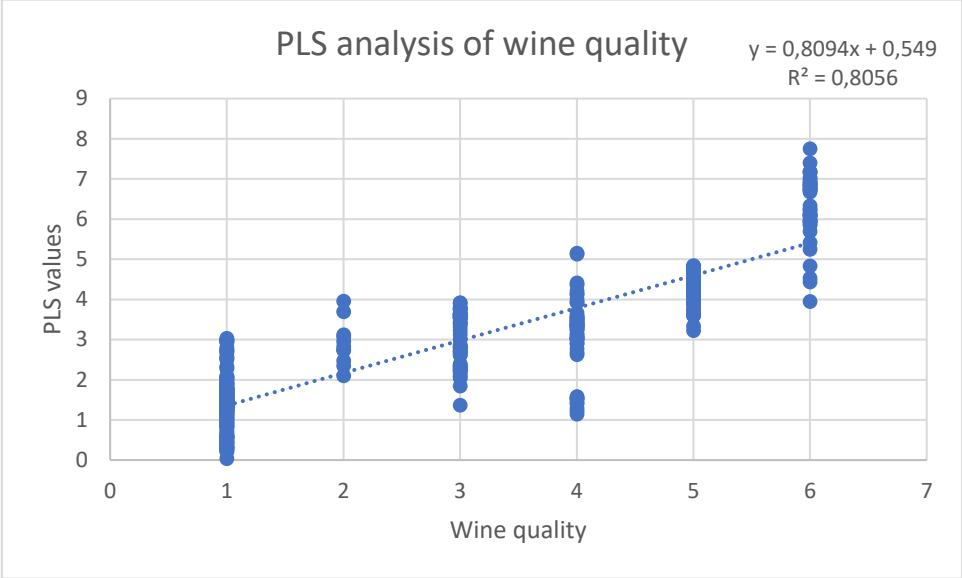


Figure 3. 8 Comparison of predicted wine chemical composition and reference wine quality (PLS).

This finding is in accordance with the study of Canuti and collaborators (2017) that obtained the goodness-of-fit measure (R^2= 0.83) for PLS regression in prediction model of wine typicity based on the chemical analysis and typicity scores of Chianti PDO wine.

The reliability of the model was also examined by RMSE (Root Mean Square Error) for both the training and validation model. The training model contained 356 wine samples and the RMSE value was 0,855. On the other hand, the validation model contained 100 random wine samples (about 28% of total wine samples) and it's RMSE value was 0,745.

The PLS regression model is widely used in prediction of wine quality and typicity. Thus, Frank and Kowalsky (1984) used this statistical model in order to disclose the relation between the sensory (i.e. "subjective") wine characteristics and their chemical compounds (i.e. "objective") in Pinot Noir wine. In a recent study, Canuti et al. (2020) evaluated the typicity of Sangiovese wines coming from two different zones: its country of origin, Italy, and California, using the same statistical analysis.

3.2.4 Conclusion

The specific goal of this study was to examine how physicochemical properties of Chianti and Chianti Classico DOCG wines make a difference in their quality obtained. An essential finding is that wine attributes such as polyphenols, alcohol content, glycerin, potassium, pH value, residual sugar, succinic acid, dry extract, anthocyanins, density and total acidity, had the statistically significant influence on the wines quality and thus their typicity. Multivariate analysis showed a good capability to divide wine samples and to predict the wine quality and typicity of new wine samples based on calculated PLS values.

3.2.5 References

Ballester, J., Dacremont, C., Le Fur, Y., and Etiévant, P. (2005). The role of olfaction in the elaboration and use of the Chardonnay wine concept. *Food Quality and Preference*, 16, 4, 351–359.

Cadot, Y., Caillé, S., Samson, A., Barbeau, G. and Cheynier, V. (2010). Sensory dimension of wine typicality related to a terroir by quantitative descriptive analysis, just about right analysis and typicality assessment. *Analytica Chimica Acta*, 660, 53–62.

Cadot, Y., Caillé, S., Thiollet-Scholtus, M., Samson, A., Barbeau, G. and Cheynier, V. (2012). Characterisation of typicality for wines related to terroir by conceptual and by perceptual representations. An application to red wines from the Loire Valley. *Food Quality and Preference*, 24, 48–58.

Canuti, V., Picchi, M., Zanoni, B., Fia, G. and Bertuccioli, M. (2017). A multivariate methodological approach to relate wine to characteristics of grape composition: the case of typicality. *American Journal of Enology and Viticulture*, 68, 1, 49–59.

EC (2021). European Commission, European Union (EU) Food, Farming, Fisheries, Food safety and quality, Certification, Quality labels, Quality schemes explained (accessed May 30,

2021, In https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/quality-schemes-explained_en).

Frank, I.E. and Kowalski, B.R. (1984). Prediction of wine quality and geographic origin from chemical measurements by partial least-squares regression modelling. *Analitica Chimica Acta*, 162, 241–251.

Gonzaga, L.S., Capone, D.L., Bastian, S.E.P and Jeffery, D.W. (2021). Defining wine typicity: sensory characterisation and consumer perspectives. *Australian Journal of Grape and Wine Research*, 27. 246–256.

González, G., and Peña-Méndez, E.M. (2000). Multivariate data analysis in classification of must and wine from chemical measurements. *European Food Research and Technology*, 212, 100–107.

King, E.S., Stoumen, M., Buscema, F., Hjelmeland, A.K., Ebeler, S.E., Heymann, H. and Boulton, R.B. (2014). Regional sensory and chemical characteristics of Malbec wines from Mendoza and California. *Food Chemistry*, 143, 256–267.

Lee, S.J and Noble, A.C. (2006). Use of partial least squares regression and multidimensional scaling on aroma models of California Chardonnay wines. Am J Enol Vitic 57:363–370.

Letablier, M. T. and Nicolas, F. (1994). Genesis of ‘‘typicality’’. *Sciences Des Aliments*, 14, 5, 541–556.

Maitre, I., Symoneaux, R., Jourjon, F., and Mehinagic, E. (2010). Sensory typicality of wines: How scientists have recently dealt with this subject. *Food Quality and Preference*, 21, 726–731.

Makris, D.P., Kallithraka S. and Mamalos, A. (2006). Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta*, 70, 1143–1152.

Malorgio, G. and Grazia C. (2007). Quantity and quality regulation in the wine sector: the Chianti Classico appellation of origin. *International Journal of Wine Business Research*, 19, 4, 298–310.

Markham, D. (1997). 1855: A History of the Bordeaux Classification; *Wiley: Hoboken, NJ*, USA.

Ministero delle politiche agricole alimentari forestali e del turismo. (2019). Decreto 25 luglio 2019.Modifiche originarie al disciplinare di produzionne della denominazione di origine controllata e garantita dei vini "Chianti". *Gazzeta Ufficiale della Repubblica Italiana,* Serie generale-n.185, 12–33.

Ministero delle politiche agricole alimentari forestali e del turismo. (2020). Disciplinare di produzione della denominazione di origine controllata e garantita dei vini "Chianti Classico". *Gazzeta Ufficiale della Repubblica Italiana,* 1–14.

Noble, A.C. and Ebeler, S.E. (2002). Use of multivariate statistics in understanding wine flavor. *Food Reviews International*, 18, 1–21.

OIV. (2010). Résolution OIV/Viti 333/2010. In AG OIV 25/06/2010 Tbilissi (Georgie).

Parr, W.V., Valentin, D., Green, J. A., and Dacremont, C. (2010). Evaluation of French and New Zealand Sauvignon wines by experienced French wine assessors. *Food Quality and Preference*, 21, 56–64.

Perrin, L. and Pages, J. (2009). A methodology for the analysis of sensory typicality judgements. *Journal of Sensory Studies*, 24, 749–773.

République Française. (1993). Code de la consommation. Article L115-1créé par loi 93-949 1993-07-26 annexe JORF 27 juillet 1993. In Legifrance.gouv.fr. (www.legifrance.gouv.fr).

Rinaldi, A., Moine, A. and Moio, L. (2020). Astringency subqualities and sensory perception of Tuscan Sangiovese wines. *OENO One,* 1, 75–85.

Salette, J. (1997). La typicité: une notion nouvelle au service du produit, de ceux qui l'élaborent et de ceux qui le consomment en l'appréciant. *Revue des Oenologues*, 85, 12–13.

Salette, J., Asselin, C. and Morlat, R. (1998). The relationship between 'terroir' local production and product: An analysis of the 'terroir-vine-wine' system and its analogous application to other products. *Sciences Des Aliments*, 18, 3, 251–265.

Sauvageot, F. (1994). Food-science and the concept of typicality or does researcher in natural-sciences have something to say about food typicality. *Sciences Des Aliments*, 14, 5, 557–571.

SotoVázquez, E., Rìo Segade S., and Cortés Diéguez, S. (2011). Classification of red and white wines by denomination of origin according to phenolic composition and colour characteristics and correlation with standard parameters, *International Journal of Food Science & Technology*, 46, 542–548.

Sowmya, D., Sayyed J., Ganavi, M. and Sankhya N.N. (2019). Analyzing wine types and quality using machine learning techniques. International Journal of Engineering Applied Sciences and Technology, 4, 3, 519–529.

World Trade Organization (2021). Article 22: Protection of geographical indications, Available at: https://www.wto.org/english/docs_e/legal_e/31bis_trips_04b_e.htm. (Accessed May 31, 2021).

www.chianticlassico.com/en/consortium. (online access in December 2020).

www.consorziovinochianti.it/en/consorzio. (online access in December 2020).

CHAPTER 4

Wine quality-oxidation and polyphenols

4 Wine quality-oxidation and polyphenols

4.1 Introduction -wine oxidation and polyphenols

4.1.1 The role of oxygen in winemaking

The role of oxygen in winemaking process is particularly important, which was noticed in the 19th century by a French biologist, microbiologist and chemist Louis Pasteur who declared: "*C'est l'oxygène qui fait le vin"* (translated to Engl.: "It is the oxygen that makes the wine").

The outcome of wine oxidation can be either positive or negative depending on the winemaking process, the oxygen amount added to the wine and the styles of wine. The oxygen management in winemaking is among the most important issues since the wine oxidation can affect the chemical compounds and sensory characteristics of wine. A moderate and controlled oxygen disposal in some winemaking practices, like micro-oxygenation during the aging of wines, may have positive impact enhancing the wine astringency and color stability. On the other hand, a non-controlled oxygenation, either too little or too high, could cause deterioration of wine quality in terms of color stability (i.e. browning), wine structure alteration, the loss of aromas and creation of reductive off-flavors (Waterhouse et al, 2006;Versari et al., 2013;Oliveira et al., 2011;Caillé et al., 2010; Ugliano et al., 2009) The oxidation is generally perceived as a detrimental process in wine production, but it can present a crucial process in creation of a particular wine stiles such as Sherry wine from Spain, Madeira from Portugal, Vinsanto from Italy, and Jura Vin Jaune from France. In these cases, the oxidation is rather desirable, and when controlled it drives specific olfactory and color characteristics of mentioned wines (Atanasova et al., 2002; Waterhouse et al., 2016).

The oxygen uptake decreases during the winemaking process, from the grape harvest until the storage in bottles (**Table 4.1**). At the beginning of production, during the grape harvest and the alcohol fermentation, grapes and wine must get in close contact with the air and the oxygen concentration is measured in mg/L (macro-oxygenation). Afterwards, during the wine aging, the amount of oxygen decreases to µg/L level (micro-oxygenation) and at the end after bottling in ng/L level (Caillé et al., 2010).

Salmon (2006) reported that during the alcoholic fermentation the yeast *Saccharomyces cerevisiae* uses dissolved oxygen from the must to produce lipids (sterol and unsaturated fatty acids – UFA) for the cell walls without negative impact on the wine quality. The yeast needs an oxygen addition of 5-10 mg/L, particularly in the last phase of the cell growth in order to have a regular functionality of their cell metabolisms (Sablayrolles and Barre, 1986). After alcoholic fermentation, from the second month to the third year of the wine aging, the fine lees (i.e. nonviable yeast cells) may have an important role in the protection of wine against oxidation. The lees sterols consume oxygen in the range from 1 to 4 µmole O_2/h x10^{-10} cells, in a decreasing order during the aging (Salmon et al., 2002).

The solubility of oxygen in wine is conditioned by the wine composition including ethanol and soluble solids content. The wine saturation with the air oxygen at the room temperature and atmospheric pressure is about 6 ml/l (i.e. 8.6 mg/L). At 5ºC the solubility of oxygen increases about 10%, conditioning the oenologist to pay attention to the winemaking practices that are carried out at low temperature (Singleton, 1987). In the same work, Singleton estimated the level of oxidation before the wine could show the oxidized characteristics. Thus, the red wine could stand up to 180 mg/L of O_2 during all winemaking steps, but the recommended dosage would be around 80 mg/L (about ten saturations). In a further study Singleton and collaborators (1989) report that the white wines can consume about 10 oxygen saturations before showing the oxidized off-flavours.

Afterwards, numerous researchers have studied the level of oxygen uptake in different winemaking practices and the results are reported in the **Table 4.1**.

Table 4. 1 Oxygen uptake average amount in different winemaking practices.

Winemaking process	**Dissolved oxygen uptake**	**References**
Alcoholic fermentation		
Open pump-over	0,02-0,11 mg/L	Moenne et al., 2014
Closed pump-over	0,6-2,5 mg/L	Moenne et al., 2014
Macro-oxygenetion	5-10 mg/L	Sablayrolles and Barre, 1986

Filtration		
Tangential f.	0,6-2,2 mg/L	Vidal et al., 2001
Diatomeous earth f.	0,1-1,7 mg/L	Vidal et al., 2001
Membrane f.	0,6-2,1 mg/L	Vidal et al., 2003
Plate f.	0,2 mg/L	Vidal et al., 2004
Perpendicular flow polymeric membrane f.	0,1-0,8 mg/L	Valade et al., 2006
Centrifugation		
Centrifugation	up to 8 mg/L	du Toit, 2006
Pumping		
Pumping over	0,1-0,2 mg/L	Vidal et al., 2001
Racking (filling from bottom of the tank)	0,1-0,5 mg/L	Valade et al., 2006
Racking (filling from the top of the tank)	2,1-4,4 mg/L	Valade et al., 2006
Stabilization		
Continious tartaric stabilization	0,1-2,6 mg/L	Valade et al., 2006
Transport		
Transport in full tank	0,4-1,1 mg/L	Valade et al., 2006
Transport in broached tank	1,2 - 6,6 mg/l	Valade et al., 2006
Aging		
Micro-oxygenation	about 5mg/L/month	Caillé et al., 2010
Aging in wood	20-45 mg/L/year	Vivas et al., 2003
Aging in bottles	0,05 - 40 mg/L/year	Karbowiak et al., 2009
Bottling		
Bottling on fixed line	0,2-3,9 mg/L	Vidal et al., 2004
Bottling on moving line	2,0-7,0 mg/L	Valade et al., 2007

The oxidation is a (bio)chemical reaction where electron transfer occurs between reductive and oxidative compounds and oxygen plays a key role. The reactivity of molecular oxygen (O_2) is limited by its diradical nature in the triple ground state and it is not able to directly bound electron pairs coming from wine components at the initial phase of the oxygen consumption. In the presence of transitional metal ions as catalysts, a single electron is added creating a series of intermediates and at the end hydrogen peroxide and water (Waterhouse et al., 2016; Danilewicz, 2003; Miller et al., 1990).

There are two types of oxidations: enzymatic and non-enzymatic. The enzymatic oxidation occurs in the presence of oxidation enzymes (polyphenol oxidase: PPO) that oxidize the wine polyphenols to quinones in the presence of oxygen. As for the latter, in the absence of enzyme,

the transitional metals play the role of catalysts and non-enzymatic oxidation take place (Waterhouse and Laurie, 2006).

One critical step of non-enzymatic wine oxidation is the oxidation of ethanol to acetaldehyde in the presence of hydroxyl radical that has been earlier created in the Fenton reaction (**Figure 4.2**), that stands for the reduction of H_2O_2 to hydroxyl radical with participation of metal iron as catalyst (i.e. ferrous iron (Fe^{2+}) reduces to ferric iron (Fe^{3+})) (Elias and Waterhouse, 2010).

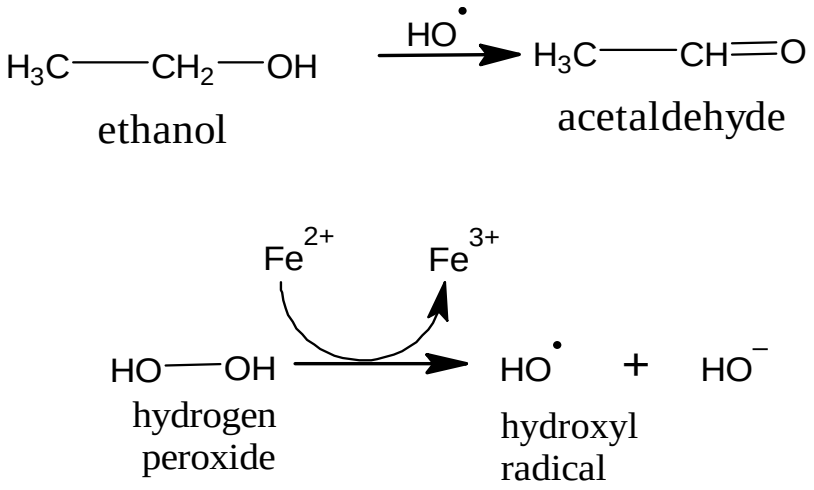


Figure 4. 1 Oxidation reaction of ethanol to acetaldehyde and the Fenton reaction.

The role of iron as a catalyst in the oxidation reaction of organic compounds was discovered by Fenton H.J.H. in the early 1894. He used the $FeSO_4$ salt (Fenton reagent) in a color test to determine the tartaric acid. Later on, Haber Wise proposed the mechanism of oxygen radical formation (**Figure 4.2**), where the first chain reaction is presented by the Fenton reaction (1), the second and third are Haber-Weiss cycle reactions (2 and 3) and the last one is the transformation of the Fe (II) to Fe (III) in the presence of hydroxyl radical (4) (Koppenol, 2001).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + \bullet OH \qquad (1)$$

$$\bullet OH + H_2O_2 \longrightarrow H_2O + \bullet O_2^- + H^+ \qquad (2)$$

$$\bullet O_2^- + H^+ + H_2O_2 \longrightarrow O_2 + \bullet OH + H_2O \quad (3)$$

$$Fe^{2+} + \bullet OH + H^+ \longrightarrow Fe^{3+} + H_2O \quad (4)$$

Figure 4. 2 The mechanism of oxygen radical creation proposed by Haber -Weiss (source: Koppenol, 2001)

4.1.2 O_2 reduction to intermediate reactive oxygen species (ROS)

The molecular oxygen in its triplet state has a low susceptibility to reaction with wine compounds because of its two unpaired electrons in different orbitals. The reactivity of oxygen increases with the loss of unpaired electrons in reaction with iron as catalysts and free radicals coming from wine (Waterhouse and Laurie, 2006).

The reduction chain reactions of molecular oxygen consist of creation of oxygen free radicals i.e. "reactive oxygen species" (ROS) created by stepwise inclusion of single electron presented in **Figure 4.3**.

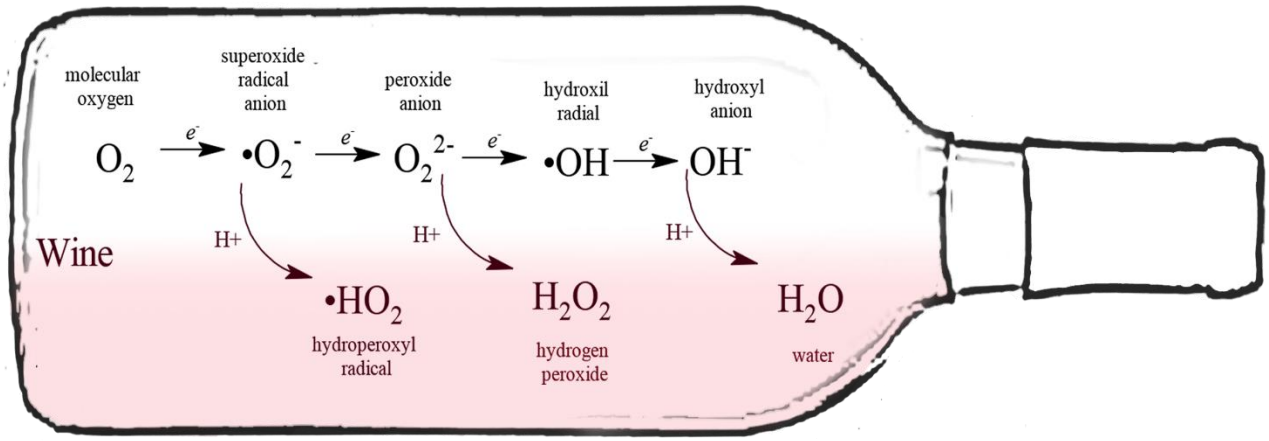


Figure 4. 3 Oxygen reduction to free radicals (Modified from Waterhouse and Laury, 2006)

The cascade of reaction starts when the molecular oxygen in his ground triplet state ($^3O_2$) receives energy from photopigments (i.e. flavins) and passes to its unstable singlet ground state ($^1O_2$). This state of oxygen radical is very unstable with a half-life about 10^{-5} s. Afterwards, the

$^1O_2$ receives one electron which is conducted by means of metal iron generating the superoxide ($•O_2^-$) as a first negatively charged ion with one unpaired electron and half-life time ranged from 10^3 s to 10^4 s. The redox potential of this reaction at pH 7 (E^0) is -0.33V (**Table 4.2**); at the wine pH this ion is presented as hydroperoxide radical (•OOH). The following electron transfer with E^0 1.07 V creates peroxide radical that has all the electrons paired and half-life time about 7s; in the complex wine medium is presented as hydrogen peroxide H_2O_2. The last and most reactive oxidant (l.t. 10^{-9}s) is hydroxyl radical (•OH), formed by reduction of the H_2O_2 in the presence of the ferrous ion salts and this is known as Fenton reaction. The reduction of oxygen concludes with the water creation in the last chain reaction (Boulton, 2003; Danilewicz, 2003; Halliwell and Gutteridge, 1984; Koppenol, 1994; Schwarzwald et al., 1987; Mukhopadhyay Das, 1994).

Table 4. 2 Reduction potentials (V) of reactive oxygen species and metal catalysts at pH 7 (source: Koppenol, 1994, 2010 and Zoecklein et al., 1995).

Inorganic compounds		Organic compounds	
Redox pairs	Eº (V)	Redox pairs	Eº (V)
One *e*- addition reduction potentials			
O2 / •O2-	-0,33		
•HO2 / H2O2	1,07	•ROO / ROOH	1,0
•HO / H2O	2,31	•RO / ROH	1,7
H2O2 / •HO, H2O	0,32	ROOH / •RO, H2O	1,9
Fe3+ / Fe $^{2+}$	0,771		
Cu2+ / Cu+	0,158		
Two *e*- addition reduction potentials			
H2O2 / 2H2O	1,32	ROOH / ROH, H2O	1,8

Inorganic compounds		Organic compounds	
Redox pairs	**E° (V)**	**Redox pairs**	**E° (V)**
One *e*- addition reduction potentials			
O_2 / $\bullet O_2^-$	-0,33		
$\bullet HO_2$ / H_2O_2	1,07	•ROO / ROOH	1,0
•HO / H_2O	2,31	•RO / ROH	1,7
H_2O_2 / •HO, H_2O	0,32	ROOH / •RO, H_2O	1,9
Fe^{3+} / Fe^{2+}	0,771		
Cu^{2+} / Cu^{+}	0,158		
Two *e*- addition reduction potentials			
H_2O_2 / $2H_2O$	1,32	ROOH / ROH, H_2O	1,8

4.1.3 Oxidation substrates of wine

The polyphenols are the main substrates of wine oxidation in the presence of oxygen. They are the first chemical compounds that start-off the chemical reaction of oxidation because they have the lowest redox potential (Oliveira et al., 2011; Waterhouse and Laurie, 2006).

Therefore, we will pay the greatest attention to them in this chapter.

- Wine polyphenol compounds;
- Other chemical compounds coming from wine – ethanol, tartaric acid, glycerol, sugars and organic acids;
- External antioxidants added during wine production - sulphur dioxide, ascorbic acid and glutathione, enological tannins.

4.1.4 Wine polyphenols

Numerous scientists have investigated the polyphenolic compounds because of their high impact on the structure, sensory characteristics and color stability of wine. The major source of polyphenols in wine is grape berry (i.e skin and seed), as well as wood from barrel and oenological tannins added to wine during winemaking processes. The polyphenol is a large group of chemical compounds omnipresent in the plant world, thus beside in grape and wine,

it is possible to find them in coffee, cacao, tea, fruits, and vegetables. As groups of chemical compounds, phenols are molecules that contain at least one aromatic (phenolic or benzoic) ring and one or more hydroxyl groups. Polyphenols are complex groups of polymeric phenol rings (Waterhouse, Sacks & Jaffery, 2016).

The word "tannins" comes from their purpose to tan the animal skin into leather and it does not determinate their chemical structure. Tannins are large groups of polymeric polyphenols with a high molecular weight, with more than 1200 Da divided in two groups: (i) condensed tannins with grape origin and oligomeric and polymeric flavan-3-ol complexes (ii) hydrolysable tannins with oak wood origin as combination of gallic acid (i.e. gallotannins and ellagitannis) (**Figure 4.4**).

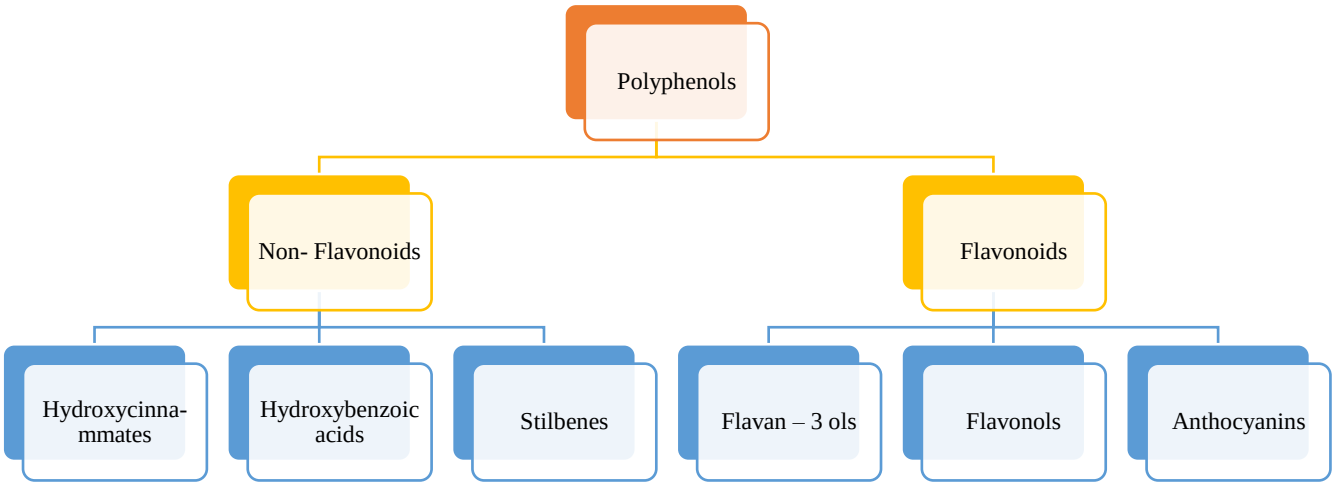


Figure 4. 4 Wine polyphenols classification.

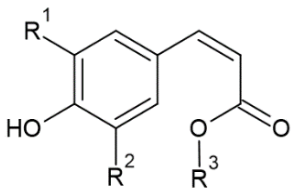
4.1.4.1 Non-flavonoids

4.1.4.1.1 Hydroxycinnamates

Hydroxycinnamates are the phenol compounds found mainly at the beginning of the wine production in the grape must. The main hydroxycinnamic acids are: coumaric, caffeic and ferulic acid, normally presented in their tartaric acid esters: *p*-coutaric acid, caftaric acid and

fertaric acid respectively. They are responsible for the enzymatic oxidation and browning phenomenon.

Table 4. 3. Hydroxycinnamic molecules present in wine.

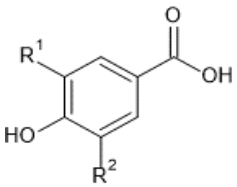
Hydroxycinnamic acids	Compound	R_1	R_2	R_3
	Caffeic acid	OH	H	H
	Caftaric acid	OH	H	tartaric acid
	p-Coumaric acid	H	H	H
	Coutaric acid	H	H	tartaric acid
	Fertaric acid	OCH_3	H	tartaric acid
	Ferulic acid	OCH_3	H	H
	Sinapic acid	OCH_3	OCH_3	H

4.1.4.1.2 Hydroxybenzoic acids

Hydroxybenzoic acids are the minor group of polyphenols created by hydrolysis of wood hydrolysable tannins (i.e. gallic acid). The representative level in young red wines is about 70 mg/L, while in white wines it riches only 10 mg/L.

Hydrolysable tannins: gallotannins and ellagitannins hydrolise to their components gallic and ellagic acid during the wine aging. All hydrolusable tannins including castalagin, vescalagin and roburins accumulate in the range from 2 to 20 mg/L during two or more years in the contact with wood (Garcia et al., 2012).

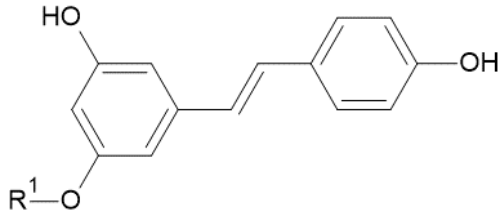
Table 4. 4 Hydroxybenzoic acids present in wine.

Hydroxybenzoic acids	Compound	R_1	R_2
	Gallic acid	OH	OH
	p-Hydroxybenzoic acid	H	H
	Protocatechuic acid	OH	H
	Syringic acid	OCH_3	OCH_3

4.1.4.1.3 Stilbenes

Stilbenes with the most important resveratrol compound created as a plant response to the microbial stress, normally found at the level of 7.2 and 0.5 mg/L in red, rosé, and white wines respectively (Joshi and Devi, 2009; Romero et al., 1996). It seems that resveratrol may have beneficial effects on the human health including antioxidant activity and prevention of some cardiovascular and cancer diseases. As an antioxidant, the resveratrol (**Table 4.5**) protects the macromolecules of human body cells (e.g. DNA, lipids and proteins) against the impact of oxidative reactive species (ROS) that may accumulate in a human body. Afterwards, the Mediterranean diet that includes a moderate red wine consumption is considered for healthy nutrition because of the positive effects of the resveratrol on prevention of some cancer and cardiovascular disease development (Guerrero et al., 2009).

Table 4. 5 Stilbenes present in wine.

Stilbenes	Compound	R1
	Resveratrol	H

4.1.4.2 Flavonoids

Flavonoids are the classes of polyphenols that contain 3 aromatic rings A, B and C, where the central ring C contains oxygen (**Figure 4.5**). The ring A and C are connected along one bond and are linked with the ring B with a single bond. A common feature of all wine and grape polyphenols is that the ring A contains hydroxyl groups attached at the positions 5 and 7, and the most important difference that defines the class of flavonoid compounds is the oxidation

status and the substitution groups of the ring C (Oliveira et al., 2011; Waterhouse et al., 2016). The main classes of flavonoids are flavan-3ols, flavonols and anthocyanins.

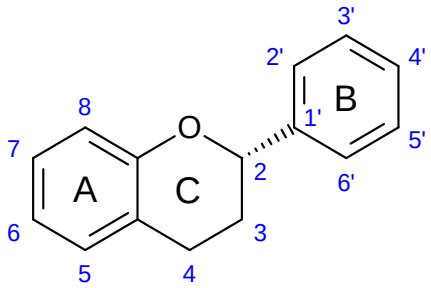


Figure 4. 5 Flavonoid ring structure (Modified from Waterhouse et al., 2016)

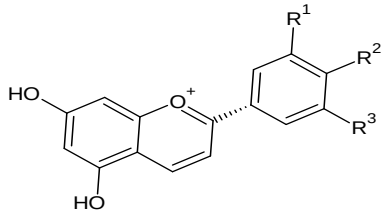
The flavonoids are placed in grape skin and seeds and their extraction in red wine occurs during the maceration period of wine production. They present the most important part of the red wine phenols, while in white wine their content is very low due to the lack of contact between the wine and the grape skin and seed (i.e. the grapes are pressed at the beginning of the vinification) (Waterhouse et al., 2016).

4.1.4.2.1 Flavan–3ols

- Among monomeric catechins (**Table 4.6**), the average amount of catechin and epicatechin (expressed in catechin as the dominant isomer) in Cabernet Sauvignon wine was found in range 37-80 mg/L (Ricardo da Silva et al., 1990). The other flavan-3-ol compounds including gallocatechin, epigallocathechin, and epicatechin gallateare are found in small-scale quantity by HPLC analysis and are useful to monitor the polyphenol extraction in wine maceration (Ritchey and Waterhouse, 1999).

  Saucier and collaborators (1997) investigated the mechanism of flavanol oxidation using monomeric catechin isomer. In that study, a dimer of two catechin groups bounded with the acetaldehyde bridges was discovered.

Table 4. 6 Monomeric flavan-3-ols present in wine.

Monomeric flavan - 3ols	Compound	R1	R2
	Catechin	OH	H
	Epicatechin	H	OH
	Epicatechingallat	H	O-gallic acid

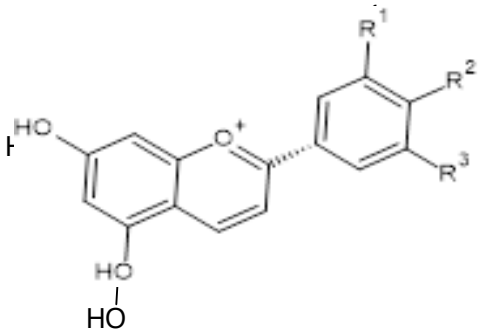
- However, the oligomers of different level of polymerisation and polymers of flavan-3-ols (proanthocyanidins or condensed tannins) account for 1/4 to 1/2 of all wine phenolic compound. The pathway of their polymerisation probably occurs under biochemical condensations of flavan -3-ol units, but this mechanism is still to be disclosed in detail (Dixon et al., 2005; Singleton, 1992).

4.1.4.2.2 Flavonols

Flavonols stand for a non-polymeric group of flavonoids that is created in grape as a response to the light exposure. There are six aglycon molecules in grapes including quercetin, myricetin, laricitrin, kaempferol, isorhamnetin and syringetin. The average amount of the first two compounds found in grapes is about 12 mg/kg, while all the others account for about 1-2 mg/kg. The diversity of the flavonol compounds consists of their bonding with different type of sugars, thus creating a huge family of glycosides, especially 3-O-glucosides and 3-O-glucuronides (Waterhouse et al., 2016; Mattivi et al., 2006). Jeffery et al. (2008) noticed that during the aging of Sangiovese wine, quercetin glucoside is hydrolysed and becomes unstable with a possibility of causing a wine haze and precipitation at the bottom of the wine tanks and bottles. Furthermore, in the recent study Gambuti et al. (2020) suggested that the quercetin concentration higher than 3 mg/L creates the precipitation risk in Sangiovese wine. The Sangiovese wine had the highest concentration of quercetin (from 0.4 to 8.6 mg/L) and quercetin glucoside (from 3.1 to 33.9 mg/L) comparing other 11 monovarietal wines. Anyway, the risk of deposit creation could be significantly decreased (more than 50%) using winemaking

practices like micro-oxygenation and aging in wood. Conversely, the quercetin may interact with the anthocyanin malvidin-3-glucoside in reaction of co-pigmentation enhancing the wine color (Lambert et al., 2011).

Table 4. 7 Flavonols present in wine.

Flavonols	Compound	R1	R2	R3	R4
	Quercetin	OH	OH	H	H
	Quercetin-3-O-galactoside	OH	OH	gal	H
	Quercetin-3-O-glucoside	OH	OH	glc	H
	Quercetin-3-O-glucuronide	OH	OH	glcA	H
	Quercetin-3-O-rhamnoside	OH	OH	rham	H
	Kaempferol	OH	H	H	H
	Kaempferol-3-O-glucoside	OH	H	glc	H
	Isorhamnetin-3-O-glucoside	OH	OCH3	glc	H
	Myrecetin	OH	OH	OH	OH
	Laricitrin	OH	OH	OH	OMe
	Syringetin	OH	OMe	OH	OMe

4.1.4.2.3 Anthocyanins

Anthocyanins are chemical compounds widespread in plants giving a great spectrum of colors depending on the pH value of the environment: form red at pH 1 through violet at pH 7 to blue color in the basic solutions (Fernandes et al., 2017; Wahyuningsh et al., 2016). More specifically, in aqueous solution at pH about 1, the anthocyanins exist in the flavylium cation form that has the red color, whereas at pH 3-4 their color depends on the equilibrium between quinonoidal base (blue-violet color), *cis* and *trans*-chalcone (yellow color) and carbinol pseudobase (non-colored) (**Figure 4.8**) (Pina 1998). Based on this equilibrium, it could be presumed that at the wine acidity level (i.e. pH from 3.2 to 3.9) the anthocyanins color would not be red. Despite that, there are certain mechanisms of color stabilisation where the anthocyanins are involved, including (i) self association where anthocyanin molecules interact between themselves thus increasing the color intensity and the darkness (Gonzalez-Manzano, 2008), (ii) copigmentation that presents the association of wine pigments with non colored

compounds resulting in wine color enhancement, particularly in young wines (Boulton, 2001), (iii) metal ion complexation (Castañeda-Ovando et al., 2009).

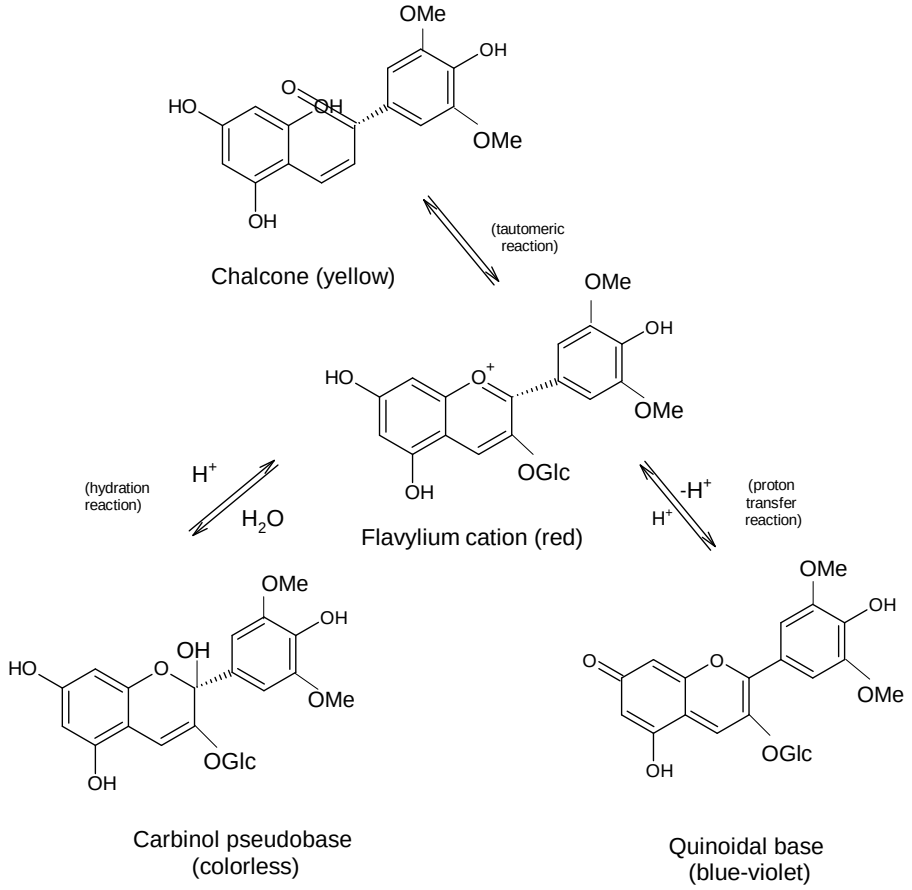
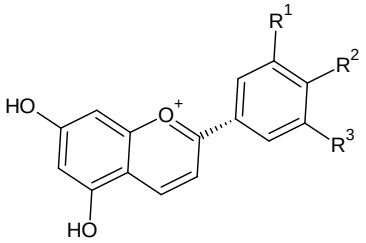


Figure 4. 6 Equilibrium forms of anthocyanins (modified from Waterhouse et al., 2016; Brouillard and Delaporte, 1977)

From the chemical point of view, anthocyanins are glycosylated anthocyanidins (i.e. 3-*O* glucoside of simple flavonoid ring structure), that are the main molecules responsible for the red wine color (Waterhouse, Sacks and Jaffery, 2016). Anthocyanins extraction from the grape skin and their concentration and stability in the grape must are strongly dependent on their concentration in vacuoles and interaction with the cell-wall polysaccharides (Fernandes et al., 2017). The main aglycon compounds found in grapes are: cyanidin, peonidin, delphinidin, petunidin and malvidin that have the variation of the substitutes in the B-ring within the flavonoid ring system (**Table 4.8**) (Waterhouse, Sacks and Jaffery, 2016).

The positively charged C ring of flavylium cation is electrophilic and can bond bisulfilte or water from the wine milieu at C2-C4 position, thus disrupting the double-bond conjugation and losing the red color (Waterhouse, Sacks and Jaffery, 2016).

Table 4. 8 Anthocyanins present in wine.

Anthocyanins	Compound	R1	R2	R3
	Cyanidin	H	OH	OH
	Peonidin	H	OH	OMe
	Delphinidin	OH	OH	OH
	Petunidin	OH	OH	OMe
	Malvidin	OMe	OH	OMe

4.1.5 Other oxidation substrates coming from grape and wine

The other important compounds that are present in extensive concentrations in wine, bearing the wine oxidation, are ethanol, glycerol, polyols, sugars, tartaric acid, malic acid and other organic acids (Danilewicz, 2003; du Toit et al., 2006). These compounds are hydrogen donor species that have an important role in wine aging. After their oxidation they become strong electrophiles and react with wine nucleophiles like thiols and polyphenols (Laurie and Waterhouse, 2006a). All the secondary oxidation products (e.g. aldehydes, ketones etc.) have a role in the further oxidation of the other chemical compounds in wine. More specifically, carbonyl compounds that include acetaldehyde, glyceraldehyde, pyruvic acid, 2-ketoglutaric acid, diacetyl and formaldehyde are instable by-products created during the wine production in microbial fermentation, wine oxidation or in barrel aging (Elias et al., 2008). The range of these compounds found in wine varies from 3 to 494 mg/L of acetaldehyde, 11-460 mg/L of pyruvic acid, and from 0.1 to 7.5 mg/L of diacetyl (Elias et al., 2008).

It is assumed that the **alcohol** oxidation produces acetaldehyde and organic acids that later generate keto acids (Waterhouse and Laurie, 2006). The ethanol oxidises in the presence of hydroxyl radical creating carbon radicals that later in the presence of oxygen will create

hydroxylperoxyl form that will disintegrate in acetaldehyde and hydroperoxyl radical (**Figure 4.7)**.

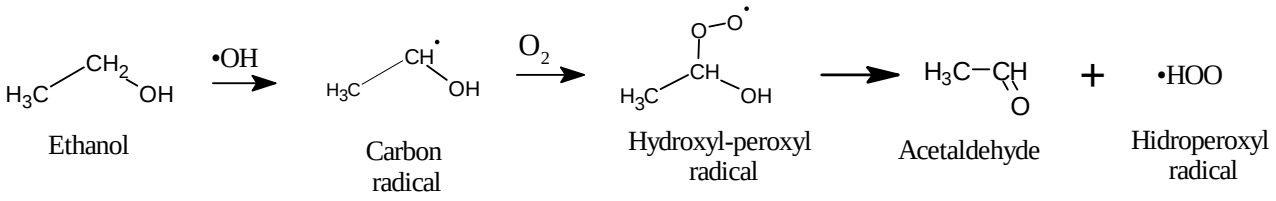


Figure 4. 7 Ethanol oxidation by hydroxyl radical (•OH) to acetaldehyde (modified from Waterhouse and Laurie, 2006).

The acetaldehyde participates in the formation of the ethyl bridges between polyphenol compounds. At the beginning of this process, acetadehyde reacts with flavanols creating a protonated acetaldehyde that is available for further interaction with flavonoids where it creates the benzylic alcohol. Afterwards, the benzylic alcohol in the protonation reaction releases one molecule of water and one intermediate, reactive product – benzylic cation (**Figure 4.8**). Further reactions with other phenolic molecules, result in generating the ethyl linked compounds, which are responsible for the polyphenol polymerisation in wine (Waterhouse and Laurie, 2006).

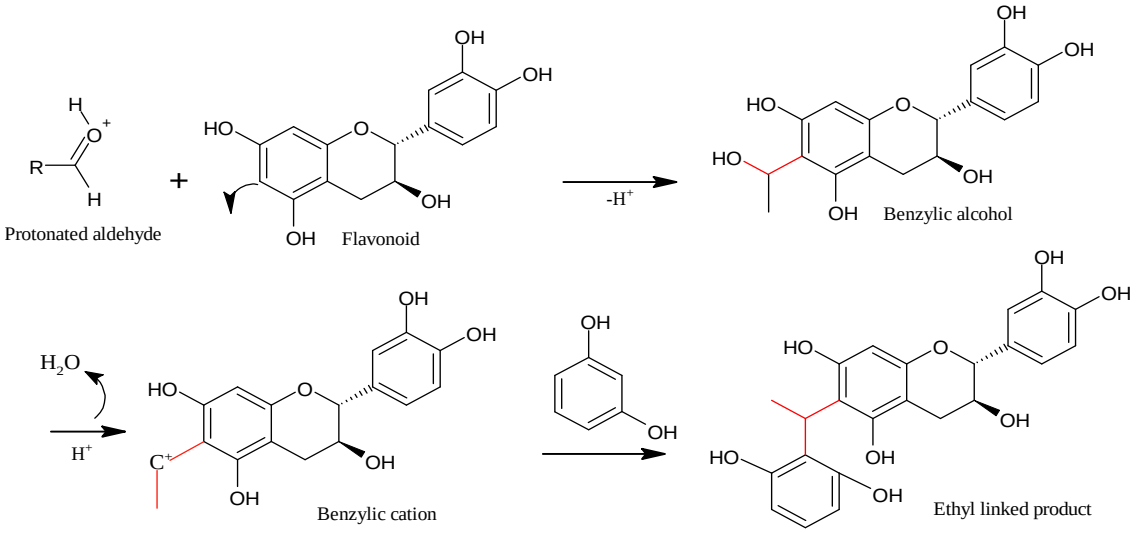


Figure 4. 8 Formation of an ethyl bridge between phenol compounds in wine (Modified from Waterhouse and Laurie, 2006).

The degradation of the tartaric acid due to the oxidation caused by hydroxyl radicals results in creation of a dihydroxyfumaric acid that afterwards links with the molecule of (+)catechin

leading to a xanthylium cation – yellow pigment generation, that changes the wine color (Danilewicz et al., 2003; Clark, 2008). On the other hand, **tartaric** and **malic acids** have an intermediate role in wine oxidation, providing a favourable reduction potential of the Fe(II)/Fe(III) couple and in that way enhance their catalytic activity of the catechin oxidation. Both acids create strong ligands rather with Fe(III), hence the ability of the iron couple to catalyse the wine oxidation changes (Danilewicz, 2014). More specifically, the ferritartrate (Fe^{3+}) ligand (**Figure 4.9)** stimulates the oxidation reactions where the intermediates, dioxytartaric acid and dioxymaleic acid, are created. These compounds can undergo the decarboxylation reactions under specific conditions creating glyoxylic and glycolic aldehydes respectively. The oxidation of malic acid creates pyruvic acid (Fulcrand et al., 1997; Waterhouse and Laurie, 2006).

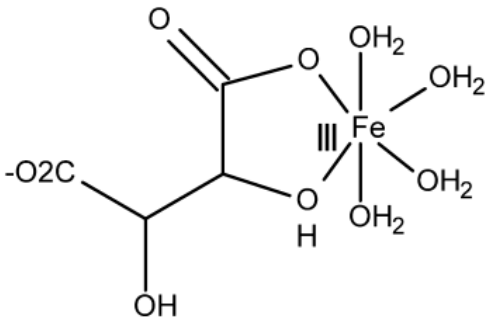


Figure 4. 9 Ferritartrate ligand - monomer (Danilewicz, 2014).

Acetaldehyde, glyoxylic acid and pyruvic acid have an important role in the creation of links between wine flavonoids and thus contribute to the color stabilisation (Fulcrand et al, 1997; Fulcrand et al., 1998; Timberlake and Bridle, 1977).

Glycerol is the other important chemical compound that stands for the oxidation substrate in wine, being present to a certain extent in wine (5-20 g/L). Laurie and Waterhouse (2006a, 2006b) reported that the glycerol oxidation by hydroxyl radical (•OH) creates glyceraldehyde and dixydroxyacetone in the model wine solution that contains hydrogen peroxide and metal iron. Both of yielded molecules are found in aged wines and the wines oxidized by •OH radicals. Later, glyceraldehyde has a role in the creation of condensed polyphenols (i.e. dimers

flavanol-flavanol and anthocyanin- flavanol) between (+)catechin, (-)epicatechin and malvidin -3-glucoside (Laurie and Waterhouse, 2006b).

Sugars would produce appropriate keto and acid functional sugars under oxidative conditions (Waterhouse and Laurie, 2006).

4.1.6 Enological aditives as antioxidans

4.1.6.1 Sulphur dioxide

During the wine production, the potassium metabisulfite is commonly used in the prevention of must and wine oxidation and of the microorganism spoilage. Wines normally contain from 40–200 mg/L of the added SO_2 (Oliveira et al., 2011). At the beginning of the vinification, the sulphur dioxide can inhibit enzymatic browning caused by polyphenol oxidase (PPO) (Embs and Markakis, 1965) and change the quinone product back to phenol molecule, reducing the oxidation level in that manner. It has the same role in a non-enzymatic oxidation process of wine (Waterhouse and Laurie, 2006). Additionally, Danilewicz et al. (2008) reported that the oxidation reaction of 4-metil catechol in the model wine solution that contains sulfur dioxide yields quinone and hydrogen peroxide. The bisulfite ion (HSO_3^-) reacts with both oxidation products: quinone, reducing it back to catechol and sulfonic acid, and hydrogen peroxide, preventing further oxidation of ethanol in Fenton reaction (**Figure 4.10**).

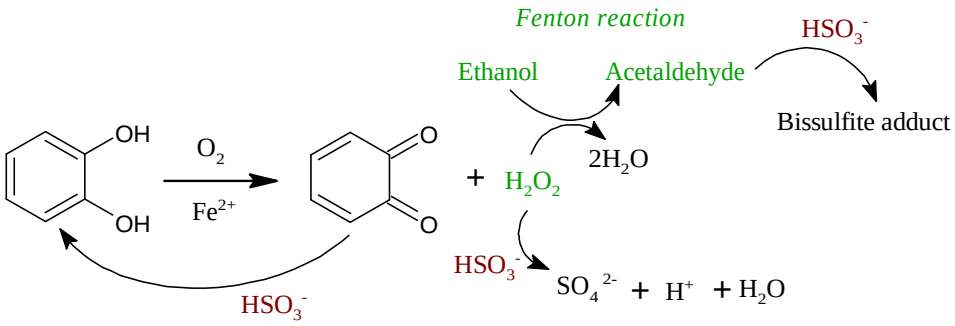


Figure 4. 10 The SO_2 prevention of oxidation: reaction with hydrogen peroxide, acetaldehyde and quinones (Modified from Oliveira et al., 2011).

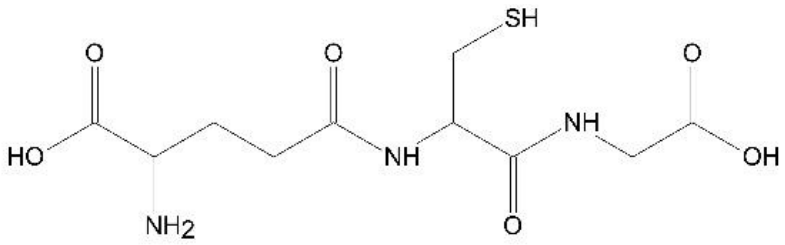
On the other hand, sulfur dioxide reversibly links aldehydes and ketones, which are created as intermediate products of the wine oxidation, thus preventing further oxidation of these wine compounds (Boulton, 2003).

In acidic condition like that of wine, there are two forms of sulfur dioxide: the free SO_2 composed of bisulphite ion HSO_3^- (94–99 % of free SO_2) and molecular SO_2, and total SO_2 that is bound to the unsaturated compounds (Oliverira et al., 2011). In wine like media, the SO_2 is available in its bisulphite specie HSO_3^-.

4.1.6.2 Glutathione

Glutathione (GSH) is a tripeptide molecule which consists of L-glutamate, L-cysteine and glycine. It is a widespread thiol, present in many prokaryotic organisms and mammals. The biochemical role of GSH derives from a free sulfydyil moiety of the cysteine part of molecule which provides redox and nucleophilic attributes (see **Figure 4.11**).

As for the winemaking, the glutathione has recently gained attention as a potential substitutional additive that may reduce the use of the SO_2 since it is organic and non-allergenic antioxidant. In wine must it is used as a protection against undesirable oxidation that can cause the loss of aromas, the creation of oxidative off-flavors and the color changing. The GSH reacts directly with the o-quinones, thus protecting the must against the browning reactions (Singleton, et al., 1985; Du Toit et al., 2006; Ugliano et al., 2011).



a)

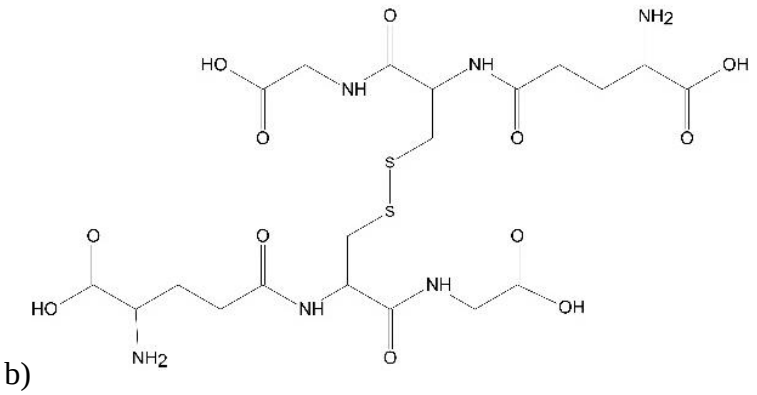
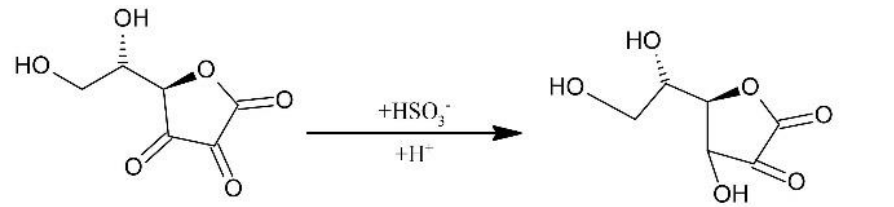


Figure 4.11 Molecule structure of glutathione a) glutathione (GSH), b) glutathione disulfide (GSSH) (Modified from Kritzinger et al., 2013).

4.1.6.3 Ascorbic acid

Ascorbic acid is a chemical compound normally presented in wine which is widely used in winemaking as an antioxidant, especially in white wine production.

It has an ambivalent type of chemical behaviour in wine since it functions both as an antioxidant and a pro-oxidant which is related to the SO_2, oxygen and other chemical compounds present in wine. In the case of low oxygen content and in the presence of SO_2, the ascorbic acid is a highly efficient antioxidant, consuming the oxygen. It protects the wine fruity aromas of white wine, reduces the development of the oxidative off flavors and protect the wine color. On the other hand, its pro-oxidation characteristics may be strengthened when the present SO_2 is nearly depleted. Anyway, there is a wide range of wine components that determine the activity of the ascorbic acid and further studies should be done in order to understand it better (Bradshaw et al., 2011; Barril et al., 2016).



dehydroascorbic acid

monohydroxysulfonate of dehydroascorbic acid

Figure 4.12 Chemical reaction of dehydroascorbic acid with sulfure dioxide (modified from Bradshaw et al., 2011)

4.1.7 Oxidation mechanisms in wine

Based upon the past research, the mechanism of wine oxidation essentially depends on the presence of oxygen and reaction with catalysts, including metals ions of Fe and Cu that start the radical chain reaction at the wine pH. The main reaction pathway is outlined in the **Figure 4.11** (Singleton, 1987; Danilewicz, 2003; Waterhouse and Laurie, 2006).

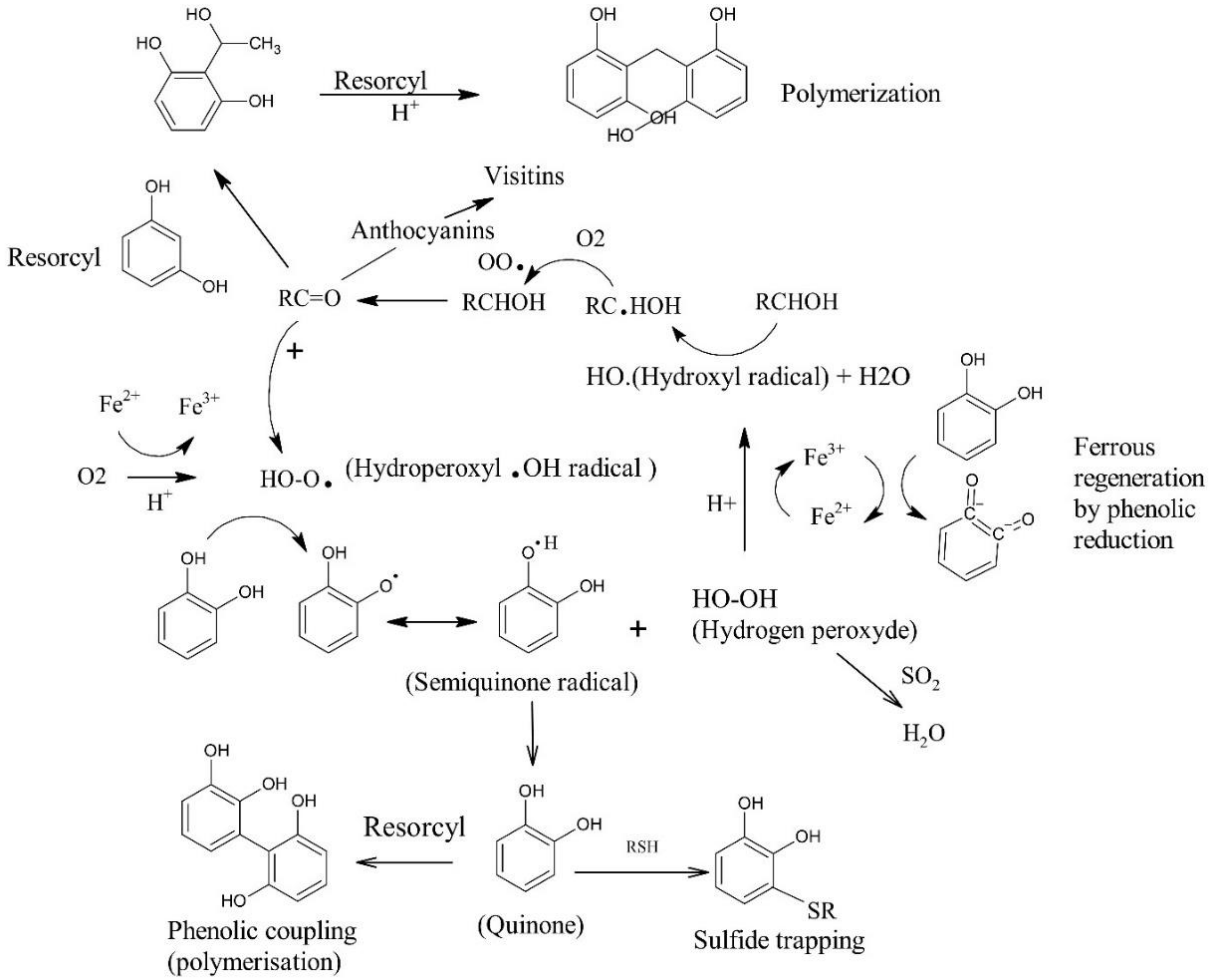


Figure 4. 11 Oxidation mechanism of polyphenols in wine and stepwise hydroxyl radical oxidation proposed by Waterhouse and Laurie (2006).

4.2 References

Atanasova, V., Fulcrand, H., Cheynier, V., and Moutounet M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of winemaking. *Analitica Chimica Acta* 458: 15–27.

Barril, C., Rutledge, D.N., Scollary, G.R. and Clark, A.C. (2016). Ascorbic acid and white wine production: a review of beneficial versus detrimental impacts. *Australian Journal of Grape and Wine Research*, 22,2 169–181.

Boulton, R.B. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*, 67–87.

Boulton, R.B. (2003). A radical view of oxidative reactions in wine. In IX Congreso latinoamericano de viticultura y enología. P. Pszczólkowski (Ed.). *Pontificia Universidad Católica de Chile, Santiago.* pp. 107–123.

Bradshaw, M.P., Barril, C., Clark, A.C., Prenzler, P.D. and Scollary, G.R. (2011). Ascorbic acid: a review of its chemistry and reactivity in relation to a wine environment. *Critical Reviews in Food Science and Nutrition*, 51, 479–498.

Brouillard, R. and Delaporte, B. (1977). Chemistry of anthocyanin pigments. 2. Kinetic and termodinamic study of proton transfer, hydratation and tautomeric reactions of malvidin 3-glucoside. *Journal of American Chemical Society*, 99, 26, 8461–8468.

Caillé, S., Samson, A., Wirth, J., Diéval, J.B., Vidal, S. and Cheynier, V. (2010). Sensory characteristics changes of red Grenache wines submitted to different oxygen exposures pre and post bottling. *Analytica Chimica Acta*, 660, 35–42.

Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, Páez-Hernández, E., Rodríguez, J.A. and Galán-Vidal, C.A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113, 859–871.

Clark, A.C. (2008). The production of yellow pigments from (+)-catechin and dihydroxyfumaric acid in a model wine system. *European Food Research and Technology*, 226, 925–931.

Danilewicz, J.C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture*, 54, 73–85.

Danilewicz, J.C. (2014). Role of tartaric and malic acids in wine oxidation. *Journal of Agricultural and Food Chemistry*, 62, 22, 5149–5155.

Danilewicz, J.C., Seccombe, J.T. and Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen and sulfur dioxide in model wine and wine. *American Journal of Enology and viticulture*, 59, 2, 128–136.

Dixon, R.A., Xie, D.Y., Sharma, S.B. (2005). Proanthocyanidins – a final frontier in flavonoid research? *New Phytologist*, 165, 1, 9–28.

Du Toit, W. J., Marais, J., Pretorius, I. S. and Du Toit, M. (2006). Oxygen in wine: a review. *South African Journal of Enology and Viticulture*, 27, 76–94.

Du Toit, W. J., Marais, J., Pretorius, I. S., and du Toit, M. (2006). Oxygen in must and wine: A review. *South African Journal of Enology & Viticulture*, 27, 1, 76–94.

Elias, R.J. and Waterhouse A.L. (2010). Controlling the Fenton reaction in wine. *Journal of Agricultural and Food Chemistry*, 58, 1699–1707.

Elias, R.J., Laurie, V.F., Ebeler, S.E., Wong, J.W. and Waterhouse A.W. (2008). Analysis of selected carbonyl oxidation products in wine by liquid chromatography with diode array detection. *Analytica Chimica Acta*, 626, 104–110.

Embs, R.J and Markakis, P. (1965). Mechanism of sulfite inhibition of browning caused by polyphenol oxidase. *Journal of Food Science*, 30, 5, 753–758.

Fenton, H.J.H. (1894). LXXIII.-Oxidation of tartaric acid in presence of iron. *Journal of the Chemical Society*, 65, 899–910.

Fenton, H.J.H., and Jackson, H.J. (1899). I.-The oxidation of polyhydric alcohols in presence of iron. *Journal of the Chemical Society,* 75, 1–11.

Fernandes, A., Oliveira, J., Teixeira, N., Mateus, N. and de Freitas, V. (2017). A review of the current knowledge of red wine colour. *Oeno One*, 51, 1, 1–15.

Fernandes, A., Oliveira, J., Teixeira, N., Mateus, N., Freitas, V. (2017). A review of the current knowledge of red wine colour. *Oeno-One*, 51, 1, 1–15.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V. and Moutounet M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry*, 47, 1401–1407.

Fulcrand, H., Cheynier, V., Oszmianski, J. and Moutounet M. (1997). An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde inflavan-3-ol condensation. *Phytochemistry*, 46, 2, 223–227.

Gambuti, A., Picariello, L., Rinaldi, A., Forino, M., Blaiotta, G., Moine, V. and Moio, L. (2020). New insights into the formation of precipitates of quercetin in Sangiovese wines. *Journal of Food Science and Technology*, 57, 2602–2611.

Garcia-Estevez, I., Escribano-Bailon, M.T., Rivas-Gonzalo, J.C., and Alcade-Eon, C. (2012). Validation of a mass spectrometry method to quantify oak ellagitannins in wine samples. *Journal of Agricultural and Food Chemistry*, 60, 6, 1373–1379.

Gonzalez-Manzano S., Santos-Buelga, C., Duenas, M., Rivas-Gonzalo, J.C. and Escribano-Bailon, T. (2008). Colour implications of self-association processes of wine anthocyanins. *European Food and Research Technology, 226*, 483–490.

Guerrero, R.F, García-Parrilla, M.C., Puertasa, B. and Cantos-Villara, E. (2009). Wine, resveratrol and health: A review. *Natural Product Communications*, 4, 5, 635–658.

Halliwell, B., and Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemistry Journal*, 219, 1–14.

Jeffery, D.W., Parker, M., Smith, P.A., (2008). Flavonol composition of Australian red and white wines determined by high-performance liquid chromatography. *Australian Journal of Grape and Wine Research*, 14, 3, 153–161.

Joshi, V.K., and Davi, M.P. (2009). Resveratrol: importance, role, contents in wine and factors influencing its production. *Proceedings of the National Academy of Sciences India Section B-Biological Sciences*, 79, 212–226.

Karbowiak, T., Gougeon, R.D., Alinc J.B., Brachais, L., Debeaufort, F.E., Voilley, A. and Chassagne, D. (2009). Wine oxidation and the role of cork. *Critical Reviews in Food Science and Nutrition*, 50, 1, 20–52.

Koppenol, W.H. (2001). The Haber-Weiss cycle – 70 years later. *Redox Report*, 6, 4, 229-234.

Koppenol, W.H., (1994). Chemistry of iron and copper in radical reactions. C.A. Rice-Evans and R.H. Burdon (Eds.). Free radical damage and its control; Chapter in New comprehensive biochemistry, ISBN: 9780444897169.

Koppenol, W.H., Stanbury, D. and Bounds, P. (2010). Electrode potentials of partially reduced oxygen species, from dioxygen to water. *Free Radical Biology & Medicin*e, 49, 317–322.

Kritzinger, E.C., Bauer, F.F. and du Toit, W.J. (2013). Role of glutathione in winemaking: a review. *Journal of Agricultural and Food Chemistry*, 61, 269–277.

Lambert, S.G., Asenstorfer, R.E., Williamson, N.M., Iland, P.G. and Jones G.P., (2011). Copigmentation between malvidin-3-glucoside and some wine constituents and its importance to colour expression in red wine. *Food Chemistry*, 125, 1, 106–115.

Laurie, V.F. and Waterhouse, A.L. (2006a). Oxidation of glycerol in the presence of hydrogen peroxide and iron in model solutions and wine. Potential effects on wine color. *Journal of Agricultural and Food Chemistry*, 54, 4668–4673.

Laurie, V.F. and Waterhouse, A.L. (2006b). Glyceraldehyde bridging between flavanols and malvidin-3-glucoside in model solutions. *Journal of Agricultural and Food Chemistry*, 54, 9105–9111.

Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M. and Velasco, R. (2006). Metabolite profile of grape: flavonols and anthocyanins. *Journal of Agricultural and food Chemistry*, 54, 20, 7692–7702.

Miller, D.M., Buettner, G.R. and Aust, S.D. (1990). Transition metals as catalysts of "autooxidation" reactions. *Free Radical Biology and Medicine*, 8, 95–108.

Moenne, M.I., Saa. P., Laurie, V.F., Pérez-Correa, J.R. and Agosin, E. (2014). Oxygen incorporation and dissolution during industrial-scale red wine fermentations. *Food Bioprocess Technology*, 7, 2627–2636.

Mukhopadhyay, S.N. and Das, D.K. (1994). Oxygen responses, reactivities, and measurements in biosystems, *CRC Press, Boca Raton, FL*, ISBN 9781003068167. pp. 35-60.

Oliveira, C.M., Ferreira, A.C.S., De Freitas, V., and Silva, A.M.S. (2011). Oxidation mechanisms occurring in wines. *Food Research International*, 44, 5, 1115–1126.

Pina, F., (1998). Thermodynamics and kinetics of flavylium salts malvin revisited. *Journal of the Chemical Society, Faraday Transactions*, 94, 2109–2116.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B., and Lonvaud, A. (2000). Handbook of Enology, Volume 1: The microbiology of winemaking and vinifications. *Chichester: Wiley, England.*

Ricardo da Silva, J.M., Rosec, J.P., Bourzeix, M. and Heredia, N. (1990). Separation and quantitative determination of grape and wine procyanidins by high - performance reversed phase liquid chromatography. *Journal of the Science of Food and Agriculture*, 53, 1, 85–-92.

Ritchey, J.G, and Waterhouse, A.L. (1999). A standard red wine: monomeric phenolic analysis of commercial Cabernet Sauvignon wines. *American Journal of Enology and Viticulture*, 50, 1, 91–100.

Romero-Perez, A.I., Lamuela-Raventos, R.M., Waterhouse, A.L., and de la Torre-Boronat, M.C. (1996). Levels of *cis*- and *trans*-resveratrol and their glucosides in white and rose *Vitis vinifera* wines from Spain. *Journal of Agricultural and Food Chemistry*, 44, 8, 2124–2128.

Sablayrolles, J.M. and Barre, P. (1986). Evaluation des besoins en oxygène de fermentations alcooliques en conditions oenologiques simulées. *Sciences des Aliments*, 6, 373–383.

Salmon, J.M. (2006). Interaction between yeast, oxygen and polyphenols during alcoholic fermentations: Practical implications. Review. *LWT - Food Science and Technology*, 39, 959–965.

Salmon, J.M., Fornairon-Bonnefond, C., Mazauric, J.P. and Moutounet, M. (2002). L'enjeu oenologique de l'élevage sur lie des vins rouges. I- Les lie des levures et la consommation d'oxygène. Connaissances actuelles & Avenir de l'èlevage en barriques (Special issue). *Journal des Sciences et Techniques de la Tonnellerie*, 39–42.

Saucier, C., Little, D. and Glories, Y. (1997). First evidence of acetaldehyde-flavanoll condensation products in red wine. *American Journal of Enology and Viticulture*, 48, 3, 370–373.

Schwarzwald, R., Monkhouse, P., and Wolfrum, J. (1987). Picosecond fluorescence lifetime measurement of the OH radical in an atmospheric pressure flame. *Chemical Physics Letters*, 142, 1, 15–18.

Singleton, V. L., Salgues, J., Zaya, J and Trousdale, E. (1985). Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. *American Journal of Enology and Viticulture*, 36, 50–56.

Singleton, V.L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical im- plications. *American Journal of Enology and Viticulture*, 38, 69–77.

Singleton, V.L. (1992). Tannins and the qualities of wines, in Plant polyphenols (eds Hemingway, R.W. and Laks, P.E.), *Plenum Press*, New York, pp.859–880.

Singleton, V.L., Trousdale, E. and Zaya, J. (1979). Oxidation of wines. I. Young white wines periodically exposed to air. *American Journal of Enology and Viticulture,* 30, 49–54.

Timberlake, C.F. and Bridle, P. (1977). Anthocyanins: colour augmentation with catechin and acetaldehyde. *Journal of the Science of Food and Agriculture*, 28, 539–544.

Ugliano, M., Kwiatkowski, M. J., Vidal, S., Capone, D., Siebert, T., Dieval, J. B., Aagaard, O. and Waters, E. J. (2011). Evolution of 3- mercatohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *Journal of Agricultural and Food Chemistry*, 59, 2564–2572.

Ugliano, M., Kwiatkowski. M., Travis, B., Francis, I.L., Waters, E., Herderich, M. and Pretorius, I. (2009). Post-bottling management of oxygen to reduce off-flavour formation and optimize wine style. *Australian and New Zealand Wine Industry Journal*, 24, 24–28.

Valade, M., Tribaut-Sohier, I., Bunner, D., Laurent, M., Moncomble, D. and Tusseau, D. (2007). Les apports d'oxygène en vinification et leurs impacts sur les vins. Le cas particulier du champagne (2ème partie). ` *Revue Française d'Oenologie,* 222, 17–28.

Valade, M., Tribaut-Sohier, I., Bunner, D., Pierlot, C., Moncomble, D. and Tusseau, D. (2006). Les apports d'oxygène en vinification et leurs impacts sur les vins. Le cas particulier du champagne (1ère partie). *Revue Française d'Oenologie,* 221, 1–8.

Versari, A., Du Toit, W. and Parpinello, G.P. (2013). Oenological tannins: A review. *Australian Journal of Grape and Wine Research,* 19, 1–10.

Vidal, J.C., Boulet, J.C. and Moutounet, M. (2003). Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site, 2ème partie. *Revue Française d'Oenologie,* 201, 32–38.

Vidal, J.C., Boulet, J.C. and Moutounet, M. (2004). Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site, 3ème partie. *Revue Française d'Oenologie,* 205, 25–33.

Vidal, J.C., Dufourcq T., Boulet, J.C. and Moutounet, M. (2001). Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site, 1ere partie. *Revue Française d'Oenologie,* 190, 24–31.

Wahyuningsh, S., Wulandari, L., Wartono, M.W., Munawaroh, H. and Ramelan, A.H. (2016). The effect of pH and color stability of anthocyanin on food colorant. *International Conference on Food Science and Engineering*, 1–9.

Waterhouse A., Sacks G., Jaffery, D. (2016). Understanding Wine Chemistry. ISBN 9781118627808. *Chichester: John Wiley & Sons, Inc.*

Waterhouse, A.L. and Laurie, V.F. (2006). Oxidation of wine phenolics: A critical evaluation and hypotheses. *American Journal of Enology and Viticulture,* 57, 306–313.

Zoecklein, B.W., Fugelsang, K.C., Gumb, B.H. and Nury, F.S. (1995). Wine analysis and production. Chapter 14: oxygen, carbon dioxide and nitrogen. *Springer, Boston, MA.,* Online ISBN: 978-1-4757-6978-4, p. 216–217.

4.3 The oxygen consumption kinetics of commercial oenological tannins in model wine solution and Chianti red wine

Jeremić, J., Vongluangnam, I., Ricci, A., Parpinello, G.P., Versari, A. (2020). The Oxygen Consumption Kinetics of Commercial Oenological Tannins in Model Wine Solution and Chianti Red Wine. *Molecules*, 25 (5)1215.

4.3.1 Introduction

The research work about the oxygen consumption kinetics of commercial oenological tannins in model wine solution and Chianti red wine was published in Molecules scientific journal (Jeremić et al., 2020). Numerous studies have reported that the oxygen plays a very important role in oenology contributing to the wine development in terms of sensory and chemical characteristics (Danilewicz 2003; Caillé et al. 2010; Ferreira et al. 2015). Currently the oxygen management represents a big challenge in winemaking. The moderate and well controlled wine exposure to oxygen like micro-oxygenation (Versari, Du Toit, and Parpinello 2013; Waterhouse and Laurie 2006) seems to enhance the wine quality during the wine aging, while insufficient or very high oxygen exposure can lead to the creation of wine oxidative or reductive off-flavors (Caillé et al. 2010; Carrascón et al. 2018; Ferreira et al. 2015; Oliveira et al. 2011). Nowadays, sulfur dioxide is the main antioxidant used to protect the wine against the detrimental influence of oxygen. However, there is a general concern about the allergic reactions to SO_2 and therefore there is a great interest to find an effective natural antioxidative alternative such as oenological tannins (Li, Guo, and Wang 2008; Ricci et al. 2016). Among tannins, ellagitannin can have beneficial impact on human health because of their antitumor and antiviral activities (Quideau and Feldman 1996). Oenological tannins are authorized in winemaking by the International Organisation de la Vigne et du Vin (OIV 2017) as the clarification coadjutants that prevent the protein instability and iron haze of wine. Nevertheless, oenological tannins have several positive activities, including: (i) antioxidative activity (i.e. wine protection against the chemical oxidation) (Danilewicz, 2007), (ii) antioxidasic activity (i.e. inhibition of laccase activity) (Versari et al. 2013), (iii) improvement of the wine color stability by pigment polymer formation (Canuti et al. 2012; Rinaldi and Moio 2018; Versari et

al. 2013), and copigmentation (Boulton, 2000) (iv) improvement of mouthfeel sensation decreasing the wine astringency and bitterness (Rinaldi and Moio 2018; Versari et al. 2013) However, the misuse of oenological tannins may lead to a negative effect on wine equilibrium (Canuti et al. 2012) emphasizing astringency.

The origin of oenological tannins are different plant sources that contain high level of polyphenolic compounds like grape, quebracho wood, oak, tara, galla chestnut (Ricci et al. 2016; Versari et al. 2013) and myrabolan fruit (Canuti et al. 2012). According to their botanical origin the tannins are divided in two groups: (i) condensed tannins coming from grapes represented by flavan 3-ols oligo- and polymers, and (ii) hydrolysable tannins originating from the oak wood and different plant species that consist of esters of glucose, or other sugars, with gallic acid (gallotannins) and ellagic acid (ellagitannins) (Canuti et al. 2012; Oliveira et al. 2011; Ricci et al. 2016; Versari et al. 2013).

Phenolic compounds of wine are involved in oxidation reaction catalysed by transitional metals such as the iron (in Fenton reaction) and copper (Danilewizc, 2003). The oxygen consumption rate (OCR) of tannins depends on their composition (Ferreira et al. 2015) and on the number of oxygen saturation cycles of wine as well, given that the initial saturations seem to have the fastest oxygen consumption rate compared with those that follow (Carrascón et al. 2018).

The knowledge about the oxygen consumption rate of oenological tannins may have the practical impact in winemaking giving an idea about what type of oenological tannin is to be used in critical moments of winemaking when the oxygen uptake is high. This study aims to provide information about efficiency of condensed and hydrolysable oenological tannins in direct oxygen consumption when added to red wine or to model wine solution.

The optimum level of oxygen exposure during the winemaking and fining processes is difficult to establish because of numerous factors that determinate it, including the duration of oxygen exposure, oxygenation conditions and wine composition. The latest literature considers the method of consecutive cycles of oxygenation-consumption as one of the best techniques to simulate winemaking conditions and forced oxidation levels (Laurie et al., 2014; Petrozziello et al., 2018; Gambuti et al. 2018).

4.3.2 Meterials and methods

Four different oenological tannins were studied including: (i) red grape seed tannin, (ii) skin tannin, (iii) ellagitannins (american oak), (iv) gallotannins (nutgalls). The levels of tannins of each commercial product were given by provider. Each of the selected commercial tannins was added to the model wine solution and the red wine (**Table 4.9**) in order to compare the reactivity of each tannin toward O_2.

Table 4. 9 Composition of samples.

Sample code	Sample composition	Tannin structure
MWs	Model wine solution + seed tannin 1 g/L	Seed tannin: 733 mg TPC/L of which 188 mg tannins/L (as CE)
MWk	Model wine solution + skin tannin 1 g/L	Skin tannin: 856 mg TPC/L of which 172 mg tannins/L (as CE)
MWe	Model wine solution + ellagitannins 1 g/L	Ellagitannin: 478 mg TPC/L of which 53 mg tannins/L (as CE)
MWg	Model wine solution + gallotannins 1 g/L	Gallotannin: 877 mg TPC/L of which 404 mg tannins/L (as CE)
CW	Chianti red wine (control)	total polyphenolic compounds (TPC) 2458 mg/L as catechin equivalent (as CE)
CWs	Chianti red wine + seed tannin 0.1 g/L	Tannin as above
CWk	Chianti red wine + skin tannin 0.1 g/L	Tannin as above
CWe	Chianti red wine + ellagitannins 0.1 g/L	Tannin as above
CWg	Chianti red wine + gallotannins 0.1 g/L	Tannin as above

The model wine solution was prepared with ethanol (12% v/v), tartaric acid (2.5 g/L) purchased from Enartis (Florence, Italy), 5 mg/L of Fe (II) and 0.5 mg/L Cu (II), and addition of 1 g/L of oenological tannin. The pH 3.6 was adjusted with sodium hydroxide (1 M) and hydrochloride (1 M) supplied by Sigma Aldrich Laborchemikalien GmbH (Seelze, Germany). The red wine was provided by Ruffino company, produced in Chianti region in central Tuscany in Italy, with protected Designation of Origin “Chianti”, 2017 vintage with the following chemical

composition: total phenolic compounds 2458 mg/L (expressed as catechin equivalent), pH value 3.55, total sulfur dioxide 107 mg/L, free sulfur dioxide 27 mg/L, iron 3 mg/L and copper 0.1 mg/L.

The oxygen level in samples was measured by oxygen analyzer NomaSense P300 (Nomacorc, Thimister Clermont, Belgium) based on oxy-luminiscence technology. The headspace oxygen (HS) and dissolved oxygen (DO) were revealed by 5 mm diameter of oxo-luminescence dots PSt3 (Nomacorc, Thimister Clermont, Belgium) placed inside of a 0.375 L transparent glass bottles purchased from Zignago Vetro (Portogruaro, Italy). The synthetic cork "Select Green 100" (Nomacorc, Thimister Clermont, Belgium) was used to close the trial bottles because it has a very low oxygen transmission rate (OTR) that ensures an inconsiderable influence of the cork on the head-space oxygen.

4.3.2.1 O_2 saturation method

The O_2 saturation and consumption cycles were repeated up to four times, during the trial time as described in the following. All the samples (model wine and red wine) were saturated by racking at the open air until the dissolved oxygen concentration reached a stable plateau (i.e. saturation), then the samples were poured into a 0.375 L transparent glass bottles that contain oxo-luminescence dots (**Figure 4.11**). The inert gas nitrogen was blown into in the headspace during 1 minute in order to minimize HS oxygen and after the bottles were closed with synthetic cork. The oxygen consumption was monitored over time by its direct measurement on dots until the moment when the DO of the sample dropped to the low stability level. Afterwards, a further O_2 saturations occurred. The second, third and fourth saturation were conducted by inserting a long narrow glass tube into the bottle, blowing the air in order to avoid the loss of the model wine and red wine and to kip the same HS size over trial time.

Figure 4. 11 NomaSense device and tools for the oxygen saturation used in the experiment

4.3.3 Results and discussion

Oxygen consumption monitoring was performed within up to four saturation cycles during nearly 90 days. The measurements were carried out on 36 trials including red wine and model wine solutions. The length of the experiment and amount of tannins added to model wine solution are consistent with the literature (Laguna et al., 2017; Medel-Marabolí et al., 2017; Vazallo-Valleumbrocio et al., 2017).

The oenological tannins exploited in this research are purchased from oenological product market and currently used at the wine industry level. Their composition is consistent with data from literature (Masson et al. 1995; Nonier et al., 2005 and Jordao et al., 2007). Particularly, low molecular weight phenolics (up to dimers) were present in large amounts, which was emphasized by small amounts of tannins compared to total polyphenols, in accordance with the low amount of tannins in the wood. Indeed, Harbertson et al. (2012) reported that gallotannins and quercus hydrolysable tannins have 12% and 27% of tannins, respectively. Furthermore, Vignault et al. (2018) suggested an extended variability in the group of oenological tannins concerning the tannins level that ranges from 16 % to 98.7 % of total product weight.

Taking into consideration that the Chianti red wine already consists of natural polyphenols, the dosage of oenological tannins added to wine samples was low enough to avoid their insolubilization and precipitation that appears to take place at the high dosages (100 ml/L) (Vazallo-Valleumbrocio et al., 2017). On the other hand, it was high enough to cross the sensory threshold as suggested by Glabasnia and Hofmann (2006). As a matter of fact, Rinaldi et al. (2018) reported that the addition of 100 mg/L of external tannin significantly changes the astringency od Sangiovese red wine.

The **Figure 4.12** shows the oxygen consumption over four saturations with ellagitannin added to the Chianti wine; graphs for the other tannins are similar. As can be seen in the **Figure 4.12**, the O_2 consumption of the first saturation was the fastest and afterwards it lasted longer in every following saturation consecutively.

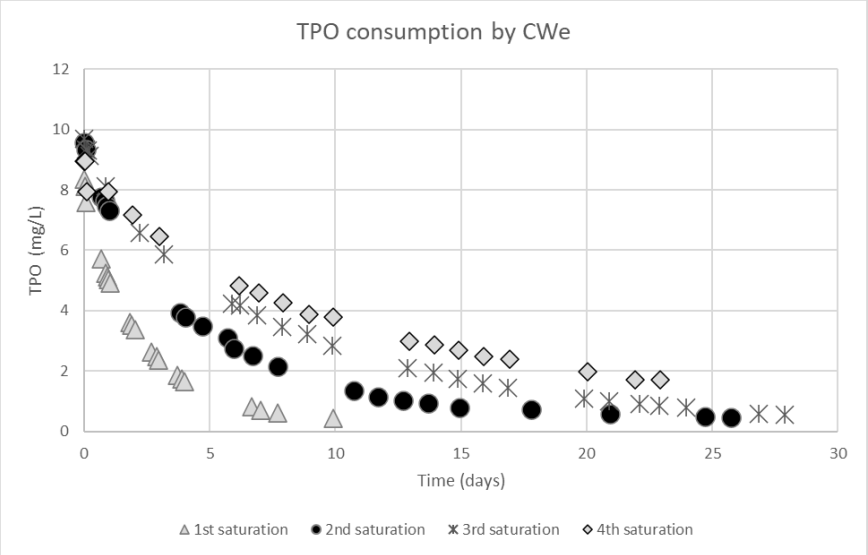


Figure 4. 12 Total packaging oxygen (TPO) consumption by Chianti wine with added ellagitannin over four saturations

Table 4.10 shows the time course of oxygen consumption over three saturation cycles. In particular, the TPO consumption of the first saturation lasted from 7 to 14 days, the second from 16 to 26 days and the third from 27 to 30 days as shown in the samples of Chianti wine (as control) and Chianti wine with added tannins (**Table 4.10)**. As expected, the samples of model wine solution took more time to reach the stable low plateau of O_2, since they have lower polyphenol level.

Table 4. 10 Duration of TPO consumption and stable level of O_2 of all samples.

Experiment	Duration of O_2 consumption (days)			Stable O_2 level (mg/L)		
	1st saturation	2nd saturation	3rd saturation	1st saturation	2nd saturation	3rd saturation
CW	7	16	27	2.31	0.7	0.67
CWs	10	18	27	0.89	0.67	0.58
CWk	10	20	26	1.1	0.16	0.34
Cwe	10	26	28	0.43	0.46	0.56
CWg	14	22	30	1.8	0.45	0.74
MWs	43	29	14	0.87	7.55	7.71
MWk	43	26	14	1.05	6.86	8.81
Mwe	43	29	14	0.33	4.97	6.83
MWg	30	29	14	4.94	4.16	8.27

Figure 4.13 exemplifies the Total Packaging Oxygen (TPO) consumption over oxygenation cycles of all samples, demonstrating the dose response effect of oenological tannins. During the first 30 days of the first oxygenation cycle, the model wine solutions with tannins at 1 g/L entirely consumed the TPO (more than 7 mg/L), excluding gallotanin that consumed only 3.1 mg/L of oxygen. Interestingly, the third saturation of the model wine with gallotannin demonstrated an increase of about 1/5 in the amount of O_2 saturation. This surprising measure might be resulting from the lack of HSO elimination during the third saturation (see Material and Method), nevertheless a peculiar variation in the composition of model wine solution might happen due to evaporation during air blowing according to del Alamo-Sanza et al. (2014).

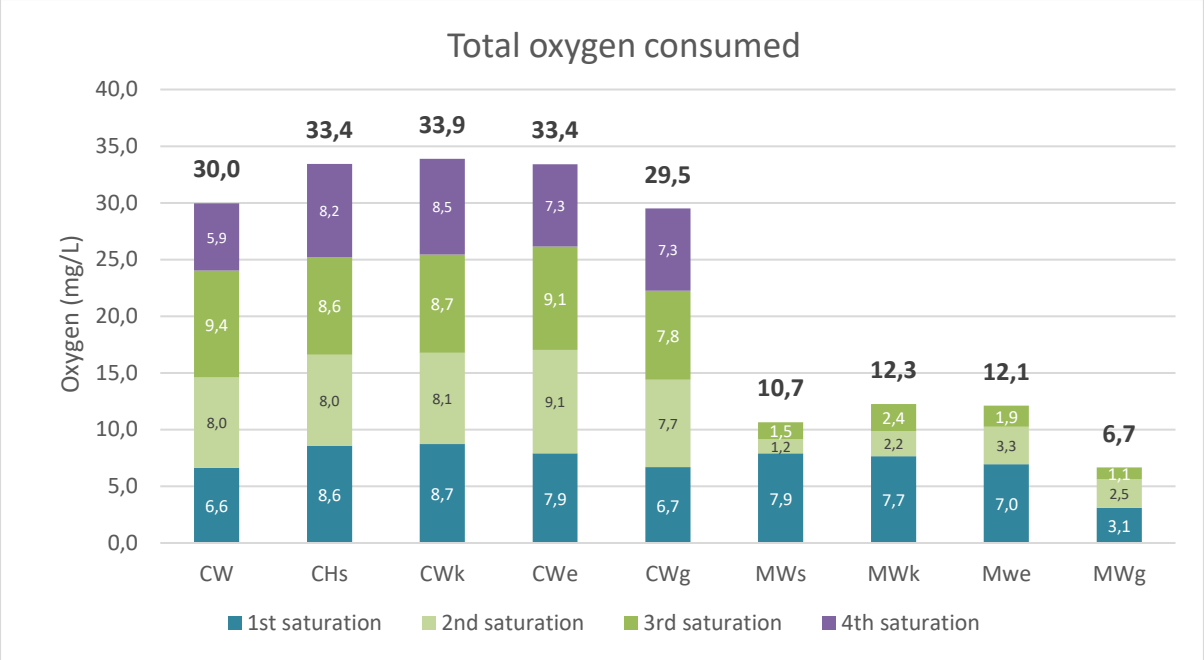
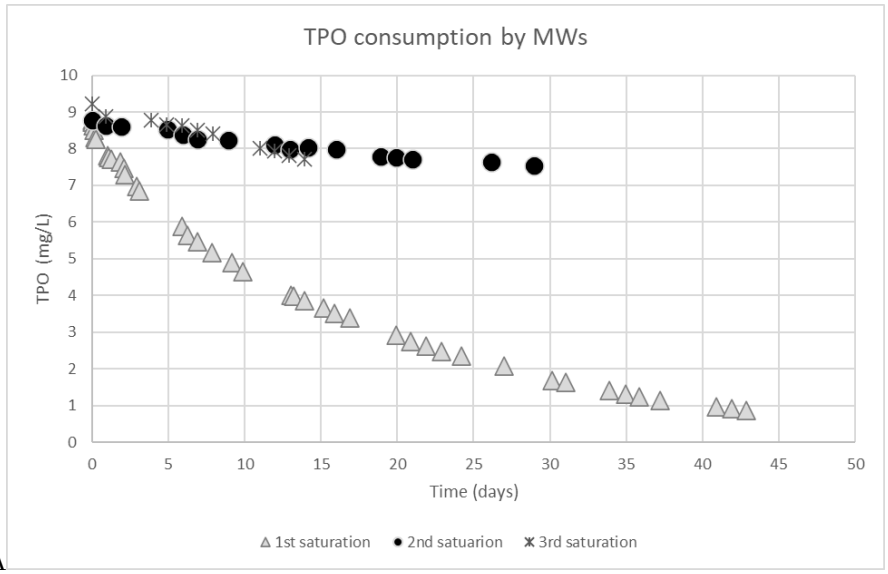


Figure 4. 13 Total oxygen consumption of all samples: CW- Chianti wine-control; CWs- Chianti wine with grape seed tannin; CSk- Chianti wine with grape skin tannin; CWe- Chianti wine with ellagic tannin; CWg -Chianti wine with gallo tannin; MWs- model wine with grape seed tannin; MWk-model wine with grape skin tannin; MWe-model wine with ellagic tannin; MWg -model wine with gallo tannin.

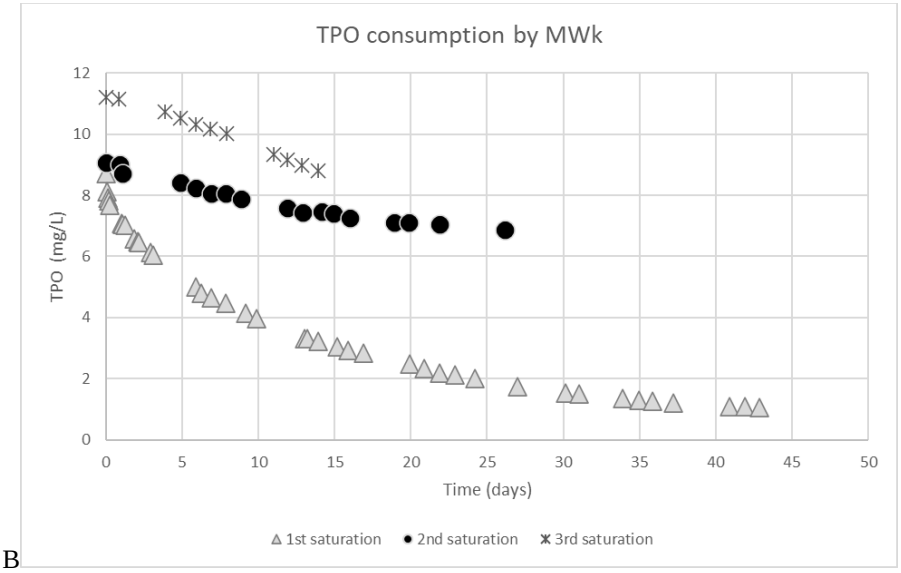
Differently, the studies of the model solutions presented in **Figure 4.14** showed that the O_2 consumption during the first saturation lasted longer than each of the following saturations, hence those trials were monitored for up to 90 days.

In particular, the grape seed tannin and grape skin tannin were able to reach the stable level of about 1 ppm within 43 days, while ellagitannin reaches the same level within 25 days, which means that it combines the oxygen faster than the other tannins. Ellagic tannin reached 0.33 mg/L at 43 days after the first saturation, meaning that it is the fastest tannin and that it has the greatest capacity to combine the oxygen.

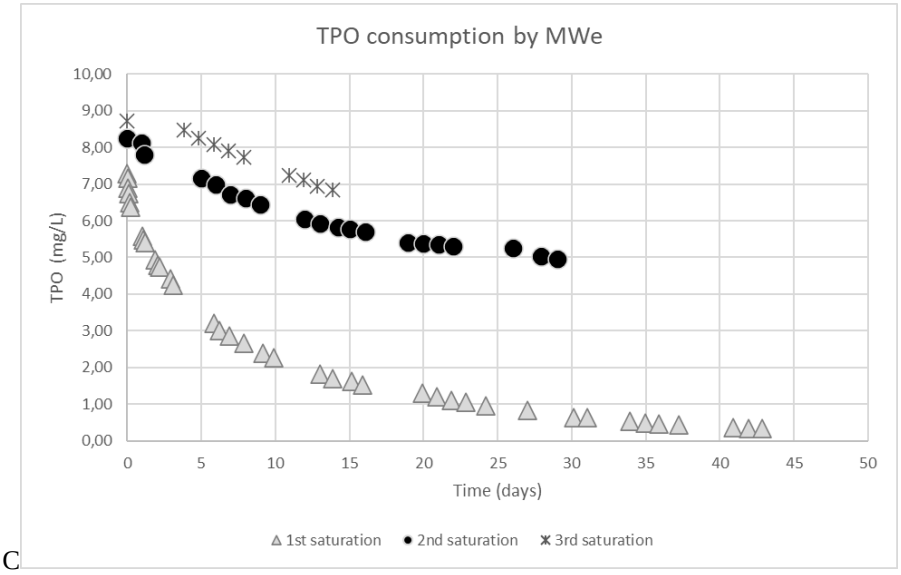
The tannins' efficiency in the model wine solution was corresponding to that in the red wine. Among the commercial tannins, ellagitannin had the fastest TPO consumption and was followed by grape seed tannin, grape skin tannin and gallotannin in decreasing order. This finding is in accordance with the literature, reported by Pasqual et al (2017).


TPO consumption by MWs
TPO (mg/L)
Time (days)
1st saturation
2nd satuarion
3rd saturation

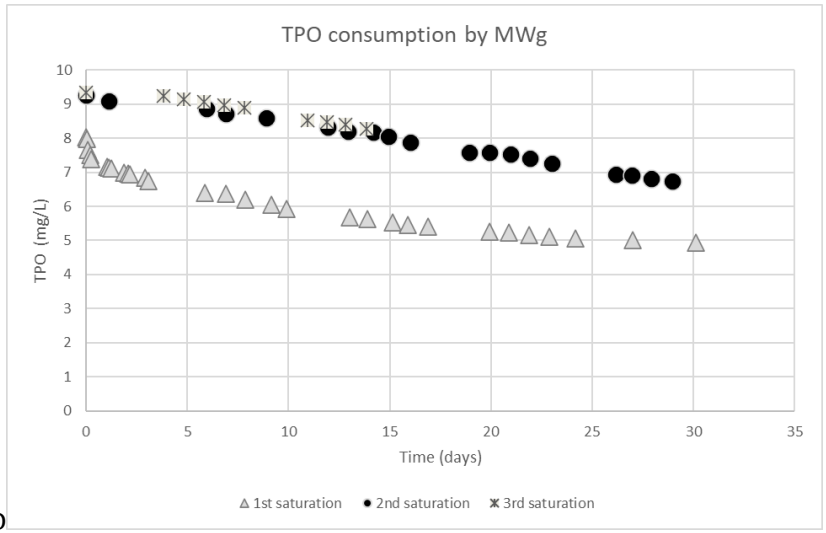
A


TPO consumption by MWk
TPO (mg/L)
Time (days)
1st saturation
2nd saturation
3rd saturation

B


TPO consumption by MWe
TPO (mg/L)
Time (days)
1st saturation
2nd saturation
3rd saturation

C



D

Figure 4. 14 Total packaging oxygen (TPO) consumption by model wine with added tannin over three saturations: A - model wine with grape seed tannin; B - model wine with grape skin tannin; C - model wine with ellagic tannin; D - model wine with gallotannin.

The capacity of tannins to combine the TPO decreases every time when the oxygen is added, as seen in **Figure 4.14**.

After the 3rd saturation cycle, the red wine samples have consumed almost twice as much of the TPO, when compared to the TPO consumed by model wine solutions with grape seed, skin and wood tannins. Thus, a TPO of 20.16 mg/L for red wine and 9.21 mg/L for model wine solution was consumed, while in the case of Chianti wine control (without tannin added) it was 19.76 mg/L. The red wine samples with added tannins showed a decrease in the oxygen consumption rate of each consecutive saturation, meaning that every following saturation took more time to complete the O_2 consumption. Anyway, all the samples of the red wine with added tannins had higher total oxygen consumption, thus corroborating that the oxygen consumption is tannin dependent. It was similar in the model wine samples where the amount of TPO consumed was heterogeneous among the samples, depending on the tannin. The oenological tannins appear to transform in new compounds that have different oxido-reduction characteristics. On the other hand, the red wine samples consumed all the TPO in three consecutive saturations and even in the fourth saturation all the samples with added tannins consumed the entire TPO, except the control sample (**Figure 4.12**). The complete O_2 consumption usually takes long time and for that reason the approach of steady state is applied in experiments (Danilewich and Standing, 2018; Carrascon et al., 2015). As shown in the **Figure 4.14 (D)**, gallo tannin has low capacity to combine the oxygen and during the first

saturation it had quite high steady state (about 5 mg/L) at the end of 30 days of TPO consumption. Every following saturation of the model wine with gallo and other tannins had lower capacity to combine the O_2 and steady states remained above 6 mg/L of Total Packaging Oxygen.

In order to better understand the order of reactions, the kinetics of O_2 consumptions by oenological tannin was modeled based on the TPO measurements at each point of time. All the trials of the kinetic order equation, from zero to third kinetic order, were conducted and graphed in the **Figure 4.15**, upon which the first kinetic order was chosen as the best fit, due to the highest R^2.

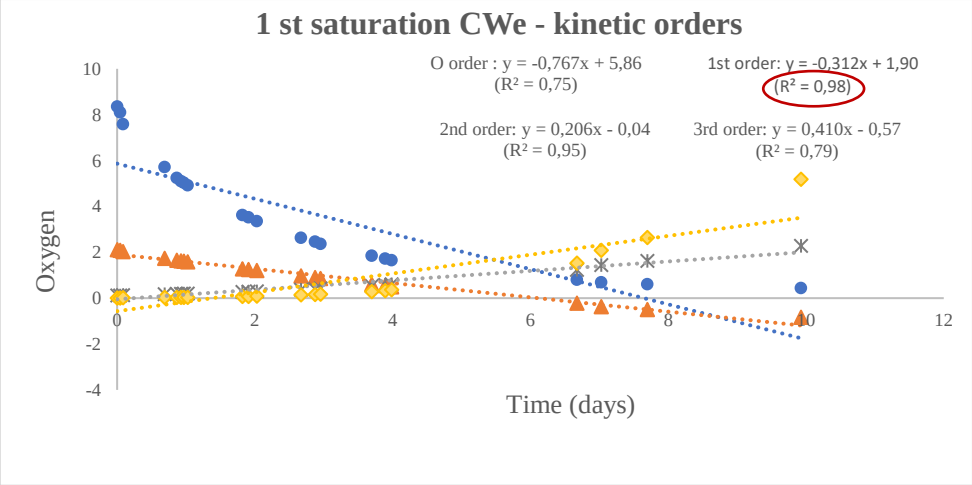


Figure 4. 15 Kinetic orders of oxygen consumption of Chianti wine with ellagitannin: 0 order(●) with equation: $[TPO]_t = -\underline{k}t + [TPO]_0$; 1st kinetic order (▲)with equation $\ln[TPO]_t = -\underline{k}t + \ln[TPO]$; 2nd kinetic order (✖)$1/[TPO]_t = -\underline{k}t + 1/[TPO]_0$;3rd kinetic order (◆)$[1/(TPO)^2]_t = -\underline{k}t + [1/(TPO)^2]_0$

The integral equation for this model was:

$Ln[O_2]_t = -kt + ln[O_2]_0$

where the quotient $[O_2]_0$ was the original concentration of TPO i.e. initial oxygen concentration after each saturation, the quotient $[O_2]_t$ was the concentration of TPO at time t, and *k* was the reaction rate constant that represents the slope of the reaction curve. All the reaction rate constants *k* of model wine samples and Chianti red wine samples of each saturation (total 32 trials) are represented in the **Table 4.11.** Kinetic fitting for linear regression of the two trials

CW and CWg may be improved ($R^2 > 0.9$) by applying the second order reaction: $1/[TPO]_t = -\underline{k}t + 1/[TPO]_0$.

According to Boulton (www.acenologia.com, 2020), the O_2 consumption has the pseudo-first or the first kinetic order when the Fe^{2+} ion reduces rapidly back to Fe^{3+} ion without any change in its concentration due to the consumption. However, Del Alamo-Sanza et al. (2014) reported that OCR could have the second order of kinetic reactions in terms of the type of tannin and the amount of oxygen added to wine. Early research of Lee (2010) showed that the oxidation decomposition of grape anthocyanidins in model wine solution had the second order kinetics of reactions. Indeed, the kinetics of reaction become more complex when hydrolysable tannins, based on pentagalloyglucose and ellagic acid, are oxidised (Bors and Michel, 1999). Specifically, a succeeding study of Poncet-Legrand et al. (2010) showed that both potential dimerization and disproportionation of semiquinones have second order of kinetics. The researchers reported that during the oxidation many oxidative by-products are formed which can later generate new polymerisation reactions. According to this argument, a new kinetics of reaction will probably depend on the ratio between monomers and polymers.

Table 4. 11 Constant rate of oxygen consumption (*k*) and the goodness-of-fit (R^2) for linear regression models of all trials.

Trial	1st saturation		2nd saturation		3rd saturation		4th saturation	
	k	R^2	*k*	R^2	*k*	R^2	*k*	R^2
CW	-0.133	0.77	-0.119	0.92	-0.096	0.96	-0.091	0.99
CWs	-0.242	0.94	-0.148	0.97	-0.103	0.96	-0.078	0.96
CWk	-0.220	0.95	-0.134	0.96	-0.131	0.98	-0.078	0.98
CWe	-0.307	0.98	-0.122	0.94	-0.102	0.99	-0.071	0.99
CWg	-0.105	0.77	-0.133	0.97	-0.081	0.96	-0.087	0.99
MWs	-0.053	0.99	-0.005	0.97	-0.013	0.97	/	/
MWk	-0.049	0.98	-0.011	0.95	-0.020	0.99	/	/
Mwe	-0.071	0.98	-0.017	0.94	-0.020	0.98	/	/
MWg	-0.016	0.93	-0.011	0.99	-0.010	0.96	/	/

As can be seen from the data in **Table 4.11,** the coefficient (*k*) of oxygen consumption was the highest after the first saturation in both red wine samples and model wine solutions, which confirms that the 1st oxidation cycle had the fastest reaction.

The drop in OCR of the red wines in each following oxidation cycle demonstrates the deceleration in redox reactions where polyphenol compounds and metals participate.

In different circumstances, the condensed tannins (i.e. grape seed and skin tannins) in the model wine solutions samples consumed TPO faster after the 3rd saturation compared to the 2nd saturation. In contrast, hydrolysed tannins (i.e. ellagitannins and gallotannins) had a similar OCR after the second and third saturation. According to the literature, this variation is possible probably due to the chemical structure of the condensed tannins (i.e. grape proanthocyanidins) that are likely to generate new intermolecular or intramolecular bonds in oxidative conditions. These new intermediate oxidation products have a lower antioxidant reactivity. Poncet-Legrand (2010) revealed that the condensed tannins at elevated concentration of up to 5 g/L in model wine oxidative conditions create new polymers and higher ratio extension unit/terminal unit. Hence, the condensed tannins during the third saturation are still likely to be active regarding redox reactions.

In the recent study of Lambrusco red wine, Picariello et al. (2018) showed that the samples of red wine with added oenological tannins (i.e. ellagitannins and condensed tannins) produced significantly higher concentration of acetaldehyde, as opposed to the control sample presented by Lambrusco wine without added tannins. After one month, the acetaldehyde was consumed whereas color intensity and pigment polymer concentration increased more in tannin added samples compared with the control. From this perspective, every following saturation cycle after the first one can activate different oxidation pathways of tannins in red wine, thus significantly reducing the reaction sites favourable for the O_2 consumption in each subsequent oxidative cycle.

Both model wine and red wine samples with added enological tannins showed that it is the samples with ellagitannin that had the fastest kinetic reaction of oxygen consumption. The ellagitannin had faster OCR than condensed tannins likely due to a higher number of vicinal

ortho –OH groups which can be oxidized easily (García-Estévez, 2019; Vivas and Glories, 1996).

However, the samples of Chianti wine with added enological tannins showed that the fastest OCR was observed in the sample with ellagitannin after the first saturation, but in every following saturation it had slower OCR than the other tannins. This could probably be due to its combination with nucleophilic wine compounds such as flavanols, ethanol, anthocyanins and thiols consequently decreasing its reactivity (Quideau et al. 2005). On the other hand, ellagitannin in the model wine solution was the fastest, compared with the other tannins after every saturation.

The gallotannin showed the lowest oxygen consumption rate in both the red wine and model wine solution after the first saturation, even though it makes a part of hydrolysable tannins like ellagitannins. Its OCR was even slower than control, which is analogous to the previous report of Pascual et al. (2017). As it can be seen in the **Table 4.11**, the *k* reaction constant rate of gallotannin of the red wine sample was higher after the 2nd and 4th saturation compared to the 1st and 3rd saturations. This is opposed to the other oenological tannins reaction rates that decreased in each wine saturation. These results can be clarified by quinone–phenol dimerization via coupled oxidation, considering that created dimers have lower redox potentials than the original phenols, thus creating two important consequences in wine oxidation patterns: (i) dimers are more reactive to oxygen than the original phenol, (ii) the dimer hydroquinone can reduce the quinone to its original phenol (Boulton et al., 1998; Li et al., 2008). Additionally, Hider et al. (2001) reported that semiquinones, quinones and flavonoids appear to be capable of coordinating metals (Fe, Cu), although normally with low affinity at wine pH. Specifically, at the pH range from 2.0 to 4.5 the green Fe^{2+} semiquinone ligand is dominant, whereas the semiquinone disproportionation to catechol and benzoquinone can be avoided by the coordinated Fe^{2+}.

Nevertheless, having the ability to chelate metals is not equivalent to having a significant antioxidant action. The flavonoid–metal complexing could eliminate the metal from reaction milieu, thus decreasing the catalysts concentration, but could also remain in the reaction media. Anyway, the flavonoid–metal ligand has to be less efficient as an antioxidant compared with the single metal that catalyses the free radical formation (Galleano et al., 2010). Furthermore,

Danilewicz et al. (2019) reported that the Fe(III)/Fe(II) redox couple has the reduction potential about ~350 mV in wine. Thus, the chelates with iron(II) are expected to increase the redox potential of the Fe(III)/Fe(II) couple and in this way to make the oxidation of Fe(II) more thermodynamically unfavorable.

Even though the gallotannin does not react directly with oxygen, it may be a good protector against the enzymatic oxidation, because of its ability to recognize and bind biological macromolecules like proteins (Quideau and Feldman, 1996).

As can be seen from the data in **Table 4.11**, in both the model solution and the red wine, the grape seed tannin increased the oxygen consumption faster than skin tannin, in the first two saturations. Such a kinetic course appears potentially due to the different chemical structures and different mean degrees of polymerization (mDP) of these two condensed tannins. Particularly, the seed tannins are mainly composed of procyanidins with a lower mDP, around 10 units, whereas the skin tannins contain high levels of monomeric flavan-3-ols and oligomers, that have terminal units readily oxidizable. However, skin tannins have a higher mDP, around 30 units, and consist of procyanidins, prodelphinidins and decreasing terminal units, therefore, less oxidizable (Ribereau-Gayon et al., 2006; Sun et al., 1999).

All thing considered, in subsequent oxidation cycles, skin tannin accelerated the oxygen consumption more than seed tannins in both the model wine solution and red wine. This phenomenon occurs probably due to the depolymerization of the long chain skin tannins that released certain units (e.g. epicatechin 3-o-gallate) and increased in catechin as terminal units, hence accelerated the rate of reaction (Carrascón et al., 2018).

4.3.4 Conclusion

The oenological tannins might be an alternative solution to protect wine from oxidation, thus reducing the use of SO_2. As it was shown in this trial, the ellagitannin had the fastest reaction and this tannin may be used in winemaking operations that have high level of O_2 uptake and when it is important to have instant protection against the oxidation. Ellagitannins are very good antioxidants, yet their reactivity could decrease after the first saturation due to combining with some wine components. The skin tannins have more stable reactivity in each saturation

when applied in red wine compared to the other selected tannins. Gallotannins might have the ability to inhibit enzymatic oxidation rather than have a direct reaction with oxygen.

4.3.5 References

Caillé, S., Samson A., A., Wirth, J., Diéval, J.B., Vidal, S., and Cheynier V. (2010). Sensory characteristics changes of red grenache wines submitted to different oxygen exposures pre and post bottling. *Analytica Chimica Acta,* 660 (1–2) 35–42.

Bors, W. and Michel, C. (1999). Antioxidant capacity of flavanols and gallate esters: Pulse radiolysis studies. *Free Radical Biology & Medicine*, 27, 1413–1426.

Boulton, R.B., Singleton, V.L., Bisson, L.F. and Kunkee, R.E. (1998). Principles and Practices of Winemaking. *Bloom, R., Ed.; Aspen Publisher: Maryland, MD, USA*, pp. 413–414.

Canuti, V., Puccioni, S., Giovani, G., Salmi M., Rosi I., and Bertuccioli M. (2012). Effect of oenotannin addition on the composition of sangiovese wines from grapes with different characteristics. *American Journal of Enology and Viticulture,* 63 (2) 220–231.

Carrascón, V., Fernandez-Zurbano, F., Bueno, M., Ferreira, V. (2015). Oxygen consumption by red wines. PartII: Differential effects on color and chemical composition caused by oxygen taken in different sulfurdioxide-related oxidation contexts. *Journal of Agricultural and Food Chemistry*, 63, 10938–10947.

Carrascón, V., Vallverdú-Queralt, A., Meudec E., Sommerer N., Fernandez-Zurbano P., and Ferreira, V. (2018). The kinetics of oxygen and SO_2 consumption by red wines. What do they tell about oxidation mechanisms and about changes in wine composition? *Food Chemistry,* 241, 206–214.

Danilewicz, John. (2003). Review of reaction mechanisms of oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper. *American Journal of Enology and Viticulture,* 542, 73–85.

Danilewicz, J. C. (2007). Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *American Journal of Enology and Viticulture*, 58, 53–60.

Danilewicz, J.C. and Standing, M.J. (2018). Reaction mechanisms of oxygen and sulfite in red wine. *American Journal of Enology and Viticulture*, 69, 189–195.

Danilewicz, J.C., Tunbridge, P. and Kilmartin, P.A. (2019). Wine reduction potentials: Are these measured values really reduction potentials? *Journal of Agricultural and Food Chemistry*, 67, 4145–4153.

del Alamo-Sanza, M., Pando, V. and Nevares, I. (2014). Investigation and correction of the interference of ethanol, sugar and phenols on dissolved oxygen measurement in wine. *Analytica Chimica Acta*, 809, 162–173.

Ferreira, V., Carrascon V., Bueno, M., Ugliano, M., and Fernandez-Zurbano, P. (2015). Oxygen consumption by red wines. Part I: consumption rates, relationship with chemical composition, and role of SO_2. *Journal of Agricultural and Food Chemistry*, 63 (51) 10928–10937.

Galleano, M., Verstraeten, S.V., Oteiza, P.I. and Fraga, C.G. (2010). Antioxidant actions of flavonoids: Thermodynamic and kinetic analysis. *Archives of Biochemistry and Biophysics*, 501, 23–30.

Glabasnia, A. and Hofmann, T. (2006). Sensory-directed identification of taste-active ellagitannins in American (Quercus alba L.) and European oak wood (Quercus robur L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *Journal of Agricultural and Food Chemistry*, 54, 3380–3390.

Gambuti, A., Picariello, L., Rinaldi, A. and Moio, L. (2018). Evolution of Sangiovese wines with varied tannin and anthocyanin ratios during oxidative aging. *Frontiers in Chemistry*, 6, 1–11.

García-Estévez, I., Escribano-Bailón, M.T. and Alcalde-Eon, C. (2019). Effect of the presence of different oak ellagitanninsin their own disappearance under oxidative or inert atmosphere. *Food Chemistry*, 286, 43–50.

Harbertson, J.F., Parpinello, G.P., Heymann, H., Downey, M.O. (2012). Impact of exogenous tannin additions on wine chemistry and wine sensory character. *Food Chemistry, 131*, 999–1008.

Hider, R.C., Liu, Z.D. and Hhodr, H.H. (2001). Metal chelation of polyphenols. *Methods in Enzymology*, 335, 190–203.

Jordao, A.M., Ricardo-da-Silva, J.M. and Laureano, O. (2007). Ellagitannins from Portuguese oak wood (Quercus pyrenaica Willd.) used in cooperage: Influence of geographical origin, coarseness of the grain and toasting level. *Holzforschung*, *61*, 155–160.

Laguna, L., Sarkar, A., Bryant, M.G., Beadling, A.R., Bartoloméa, B. and Moreno-Arribas, M.V. (2017). Exploring mouthfeel in model wines: Sensory-to-instrumental approaches. *Food Research International*, *102*, 478–486.

Laurie, V.F., Salazar, S., Campos, I., Cáceres-Mella, A., Peña-Neira, Á. (2014). Periodic aeration of red wine compared to microoxygenation at production scale. *American Journal of Enology and Viticulture, 65*, 254–260.

Lee, J. (2010). Degradation kinetics of grape skin and seed proanthocyanidins in a model wine system. *Food Chemistry*, 123, 51–56.

Li, H., Guo, A., and Hua Wang, H. (2008). Mechanisms of oxidative browning of wine. *Food Chemistry*, 108 (1) 1–13.

Masson, G., Moutounet, M. and Puech, J.L. (1995). Ellagitannin content of oak wood as a function of species and of sampling position in the tree. *American Journal of Enology and Viticulture*, *46*, 262–268.

Medel-Marabolí, M., Romero, J.L., Obreque-Slier, E., Contreras, A., Peña-Neira, A. (2017). Effect of a commercial tannin on the sensorial temporality of astringency. *Food Research International*, *102*, 341–347.

Nonier, M.F., Vivas, N., De Gaulejac, N.V., Absalon, C., Vitry, C., Fouquet, E. (2005). Global fractionation of oak heartwood extractable polymers (lignins, polysaccharides and ellagitannins) by selective precipitations. *Journal of the Science of Food and Agriculture, 85*, 343–353.

Oliveira, C.M., Silva Ferreira A.C., De Freitas V., and Silva, A.M.S. (2011). Oxidation mechanisms occurring in wines. *Food Research International,* 44 (5) 1115–1126.

Quideau, S., Michael, J., Lefeuvre, D., Montaudon, D., Saucier, C., Glories, Y., Pardon, P., and Pourquier, P. (2005). The chemistry of wine polyphenolic c-glycosidic ellagitannins targeting human topoisomerase II. *Chemistry - A European Journal*, 11 (22) 6503–13.

Pascual, O., Vignault, A., Gombau, J., Navarro, M., Gómez-Alonso, S., García-Romero, E., Canals, M.J., Hermosín-Gutíerrez, I., Teissedre, P.-L., and Zamora, F. (2017). Oxygen consumption rates by different oenological tannins in a model wine solution. *Food Chemistry*, 234, 26–32.

Petrozziello, M., Torchio, F., Piano, F., Giacosa, S., Ugliano, M., Bosso, A. and Rolle, L. (2018). Impact of increasing levels of oxygen consumption on the evolution of color, phenolic and volatile compounds of Nebbiolo wines. *Frontiers of Chemistry, 6*, 1–15.

Picariello, L., Gambuti, A., Petracca, F., Rinaldi, A. and Moio, L. (2018). Enological tannins affect acetaldehyde evolution, colour stability and tannin reactivity during forced oxidation of red wine. *International Journal of Food Science & Technology,* 53, 228–236.

Poncet-Legrand, C., Cabane, B., Bautista-Ortín, A.B., Carrillo, S., Fulcrand, H., Pérez, J. and Vernhet, A. (2010). Tannin oxidation: Intra- versus intermolecular reactions. *Biomacromolecules*, 11, 2376–2386.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2006). Phenolic compounds. In handbook of oenology vol. 2—The chemistry of wine stabilization and treatments, 2nd ed. *John Wiley & Sons: Chichester, UK*, 141–203.

Ricci, A., Kenneth J. O., Parpinello, G.P., Mattioli A.U., Teslić, N., Kilmartin, P.A., and Versari, A. (2016). Antioxidant activity of commercial food grade tannins exemplified in a wine model. *Food Additives and Contaminants* - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 33 (12) 1761–74.

Rinaldi, A. and Moio, L. (2018). Effect of enological tannin addition on astringency subqualities and phenolic content of red wines. *Journal of Sensory Studies*, 33 (3) 1–11.

Sun, B.S., Pinto, T., Leandro, M.C., Ricardo-da-Silva, J.M. and Spranger, M.I. (1999). Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine. *American Journal of Enology and Viticulture*, 50, 179–184.

Vazallo-Valleumbrocio, G., Medel-Marabolí, M., Peña-Neira, A., López-Solís, R. and Obreque-Slier, E. (2017). Commercial enological tannins: Characterization and their relative impact on the phenolic and sensory composition of Carménère wine during bottle aging. *LWT Food Science and Technology*, *83*, 172–183.

Versari, A., du Toit, W., and Parpinello, G.P. (2013). Oenological tannins: A review. *Australian Journal of Grape and Wine Research,* 19 (1) 1–10.

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

Vivas, N. and Glories, Y., (1996). Modelisation et etimation du bilan des apports d'oxyène au cours de l'élevage des vins rouge. I – les apports technologiques et lies au mode d'élevage. *Le Progrès Agricole et Viticole*, 113, 222–227.

Waterhouse, A.L. and Laurie, V.F. (2006). Oxidation of wine phenolics: a critical evaluation and hypotheses. *American Journal of Enology and Viticulture*, 57 (3) 306–313.

Www.acenologia.com. Available online: http://www.acenologia.com / enfoques / roger _ boulton _enf0612_eng.htm (accessed on 20 November 2020).

4.4 The kinetics of oxygen consumption of six Sangiovese red wines from Tuscany

4.4.1 **Introduction**

Sangiovese (*Vitis vinifera* L., ssp. sativa) is the most widespread grape cultivar in Italy, produced on about 54.000 ha (Vouillamoz et al., 2007). In Tuscany, its cultivation dates from The Roman time and the name "Sangiovese" derives from Latin "Sanguis Jovis", meaning the "blood of Jupiter" (Robinson et al., 2012). According to the recent research, the parent varieties of this grape cultivar originate from both Tuscany (the central part of Italy) and Calabria (The south of Italy). Vouillamoz et al., (2007) reported in their study of Sangiovese grape variety origin, based on the DNA profiling using microsatellite markers, that Sangiovese is an offspring from an ancient Tuscany variety "Ciliegiolo" and "Calabrese di Monenuovo" variety that almost certainly comes from Calabria. Later research of Bergamini et al. (2013) using the grape cultivars from south Italy suggested that the variety "Negrodolce" might be one of the parents of Sangiovese beside the well known Ciliegiolo, thus confirming the southern part of origin of Sangiovese. Sangiovese is cultivated on an area of about 38.000 ha in Tuscany (i.e. 64 % of total Tuscany grape production), where near 30.000 ha are used for the DOC/DOCG wine production and 8000 ha for the IGT production (www.inumeridelvino.it).

Sangiovese has always been among the varieties that are suitable for the high-quality wine production, possessing a great number of clones: 130 in 2020. Having a long history in wine production it became a symbol of foundation of appellation of origins that are internationally recognized as DOC (Denominazione di origine Controllata) and DOCG (Denominazione di Origine Controllata e Garantita). Beside Tuscany, where is the main grape variety cultivated, it is produced even in other countries including Argentina, California (USA), France and other countries (available online: Ministero delle Politiche Agricole, Alimentari e Forestali). Considering as the most important grape variety in Italy, it is of great interest to study the enological potential of Sangiovese.

The oxygen has a crucial role in wine aging and wine evolution leading the enologists to pay a great attention to its management during all winemaking processes. It was noticed by a French chemist and microbiologist Louis Pasteur a long time ago in 1864, who said: "*Wine is made*

and matures essentially transforming itself from a young wine to an older wine almost exclusively by the influence of air" (Valade et al., 2006). The contact of oxygen with wine can have both positive and negative outcomes depending on its amount added to wine and as well on winemaking step, i.e. timing. Thus, well-controlled and balanced exposure to the oxygen specially during the winemaking practices like micro-oxygenation and wine aging in barrels can improve the wine quality. The most important wine parameters include the color stability, the improvement of wine mouthfeel by decreasing astringency and bitterness involving the reactions of tannins, the melioration of olfactory characteristics by the loss of reductive and vegetative aromas (Atanasova et al., 2002; Cano- López et al. 2008; Cejudo Bastante et al., 2011; Gambuti et al. 2012, 2017). On the contrary, an excessive exposure to oxygen may cause detrimental effect on the wine quality regarding increase of wine color instability, the appearance of the oxidation off-flavours and the creation of microorganism's spoilage (du Toit et al., 2006; Waterhouse and Laurie, 2006).

The modelling of wine oxidation reactions, especially for the main chemical compounds, becomes a crucial study in winemaking, allowing a prediction of wine evolution in the presence of oxygen. Understanding of oxygen consumption by wine provides a preliminary assessment of its capability to sustain certain amount of oxygen without significant change of quality. The main chemical compounds reported to have a good correlation with the oxygen consumption include wine polyphenolic compounds, specifically the tannin and anthocyanin, then the catalytic metals like iron and copper, pH value and sulphur dioxide (Carrascon et al., 2017; Ferreira et al., 2015; Koppenol, 1994; Vivas and Glories, 1996).

Wine color is one of the most important wine quality parameters that supply information about wine style, eventual faults like oxidation, evolution during aging and general perception by wine consumers (Peynaud, 1987).

CIELab is a system of color measurement based on the spectrophotometric measurement of spectrum from 380 to 780 nm. It can be applied for color determination of all visible with human eye and it is widespread used in different types of industry that have to determine and define a specific color of their product (e.g. car industry, editorial activity, photography etc.). It is developed by "Commission Internationale de L'Eclairage" (CIE) that in 1986 developed a new CIELab system of three-dimensional color space CIE XYZ consisted of tristimulus

values that are non-linearly transformed to coordinates *L**, *a**, *b**, *C** and *h* (hue angle). Each color is defined by these coordinates (CIE, 1986). The vertical axis *L** represents the measurement of lightness going from completely opaque (0) to completely white (100). In horizontal plane the coordinate *a** defines a redness as positive values or greenness as negative value, while *b** coordinate defines yellowness as positive or blueness as negative **(Figure 4.11).**

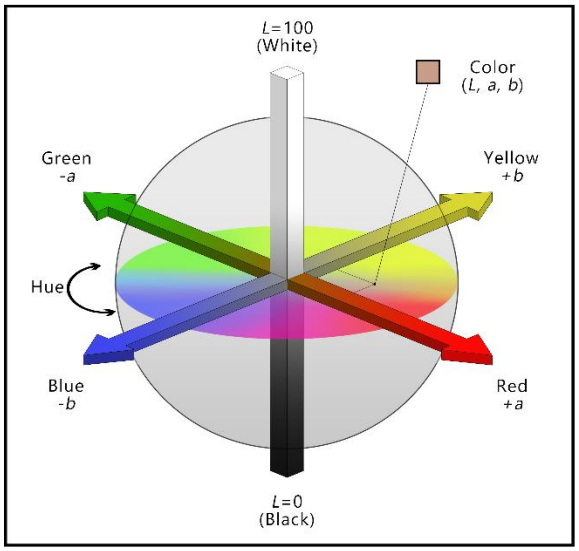


Figure 4. 16 CIELab color space coordinates: *a** (from -*a** green, to +a*red), *b** (from -*b** blue, to +*b** yellow), *L**lightness (from *L*=0 black, to *L*=100 white), hue-angle.

it is suitable for the wine pigment analysis A precise description of color can be conducted by CIELab color coordinates

The aim of this study is to develop a model of Sangiovese oxidation kinetics for improving the comprehension of its behaviour under controlled oxidation condition and its ability to resist to detrimental effects of excessive oxidation. The main attention was dedicated to the kinetics of oxidation reactions that elucidate the time needed by the wine to consume oxygen. The ongoing of reactions were monitored by selected parameters, including wine color, sulphur dioxide total polyphenols content and tannins.

4.4.2 Materials and methods

4.4.2.1 Wine samples

Six red wines vintage 2016 from different locations in Tuscany (**Figure 4.17**.) are used in this experiment (code A-F).

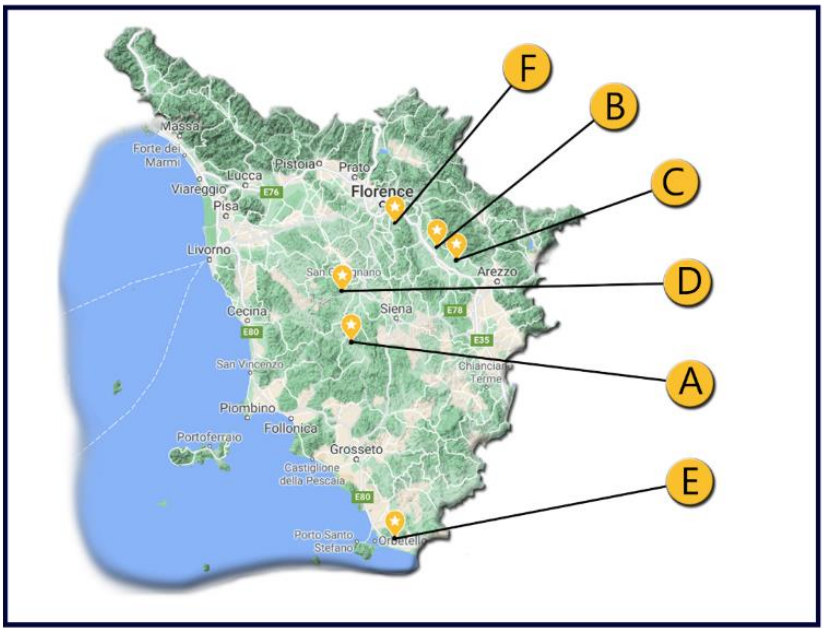


Figure 4. 17 Map of Tuscany with six location of grape origin (A-F).

All wines are vinificated with Sangiovese grape variety only (i.e. monovarietal) using a commercial winemaking standard protocol from Ruffino winery (Monteriggioni, Italy). The grape processing initiated with destemming and crashing using destemmer Delta E/F produced by Bucher Vaslin, France. Six stainless steel tanks of 120 hl were used for the separated fermentation where the grape must was inoculated with *Saccharomyces cerevisiae* yeast (20 g/hL) from commercial use at the moment of tank filing. Afterwards, the fermentation was conducted at the automatic controlled temperature, macro-oxygenation and pumping over conditions using Parsec software SAEN 5000. The alcoholic fermentation with contemporaneous maceration was performed at the temperature from 26 °C to 28 °C, using the macro-oxygenation in the first phase of alcoholic fermentation adding 1 mg O_2/L/day. The

pumping-over was conducted twice a day all long the maceration time. At the end, the tanks were drained, and only the free run wine was used for the trials. The malolactic fermentation started at the controlled temperature, set at 20 °C after inoculation with *Oenococcus oeni* bacterias from the commercial use. After racking, the wine is aged in the stainless-steel tanks (100 hl) for 6 months and after that prepared for bottling with syntetic corc Nature 100 made by Nomacorc. by adding of metabisulfite of potassium to reach the level of 50 mg free SO_2/L.

4.4.2.2 Chemical analysis of wine

The chemical analysis of ethanol, total acidity, volatile acidity, titratable acidity as tartaric acid, dry extract, pH, glycerine and residual sugar were performed using FTIR & Uv-vis spectrophotometer - multiparametric analyser Bacchus (Steroglass, Perugia, Italy). Metals are analysed by ICP-MS 7700 Series (Agilent Technologies, Japan). The total metal content in wine was analysed including Al, Ag, Ar, Ba, Cd, Ca, Co, Fe, Li, Mg, Mn, Ni, Pb, K, Cu, Ru, Na, Sr, Ti, V and Zi chemical elements. The analysis of wine color was performed by Agilent UV-vis spectrophotometer Carry 60 (Agilent Tecnologies, CA, USA) measuring the optical density at 200 nm to 800 nm wavelengths. The samples were not filtered previously. The wine color intensity (CI) was calculate as following: Abs 420 nm + Abs 520 nm + Abs 620 nm, while the color hue (H) was defined as Abs 420 nm/Abs 520 nm. The CIE Lab color parameters: *L**, *a**, *b**, *C* and *h* were obtained using the Software "Cary WinUV Color Application" version 5.0.0.1000 (Agilent Technologies) implementing CIE 1964 standard observer of 10 degrees visual field and the CIE standard illuminant D 65 which is considered to be standard of daylight. The color differences (ΔE) were calculated performing the following equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Free and total SO_2 were analysed by Titrex ACT 2 instrument (Steroglass, Perugia, Italy) using direct titration with iodine. The color analysis (trials of accelerated oxidation) were conducted in Termostatic chamber Climatic Hood 810 (Enrico Bruno S.r.l., TO, Italy).

4.4.2.3 The oxygen consumption measurements

The oxygen content in wine was analysed with the oxygen analyzer NomaSense P300 (Nomacorc, Thimister Clermont, Belgium) based on oxy-luminiscence technology. The transparent glass bottles of 0.375L (Zignago Vetro, Portogruaro, Italy) supplied by glued 5 mm diameter oxo-luminescence dots PSt3 (Nomacorc, Thimister Clermont, Belgium). The oxygen sensors PSt3 were positioned and fixed by glue in the inner part of bottle, particularly in the medium level of bottle and in the neck of bottle for the measurement of dissolved oxygen (DO) and head-space oxygen (HS) respectively. For the measurement of dissolved oxygen of the first saturation was used dipping probe (Nomacorc, Thimister Clermont, Belgium) made of a polymer optical fiber cable and oxygen sensitive coat located at one end. The trial bottles were closed by synthetic cork "Select Green 100" (Nomacorc, Thimister Clermont, Belgium) that provides low oxygen transmission rate (OTR) of O_2 of about 0.4 mg/L after 3 months.

4.4.2.4 Oxygen saturation trial

All the samples were saturated with air up to five consecutive cycles (min. 3 cycles). The first saturation was performed by racking of the wine from one recipient to another until the oxygen level measured by dipping probe reached the stable maximum level. Afterwards, the wines were poured in the 0.375L bottle with oxygen sensor, the head-space of each bottle was inertized by blowing of nitrogen for 60 sec to decrease the level of HS oxygen and finally closed by synthetic cork. Conversely, the following saturations were carried out by blowing of the air through a narrow glass pipette immersed in the wine reaching the bottom of the bottle to ensure a homogeneous distribution of oxygen in wine (**Figure 4.18**). Indeed, the different methods of oxygen saturation, i.e. racking and pipetting, was adopted to improve the accuracy if measurement (as the level of the wine would decrease during the racking affecting the measurement of the head-space O_2). The decrease of oxygen level in samples i.e. oxygen consumption was followed measuring by Nomasense P300 instrument. The end of the oxygen consumption cycle was considered as the moment when the DO attained the low stable level (plateau), the bottles were opened, and all the procedure of oxidation was done over again, as previously described in part **4.3**.

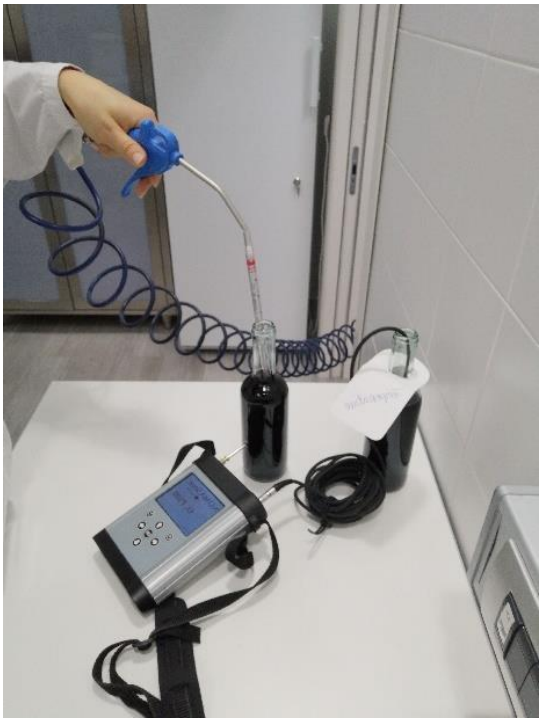


Figure 4. 18 Tools used for the wine saturation (pipette and the air piston), sampling bottle dotted with the oxygen sensors PSt3, Nomasense P300 instrument

4.4.3 Results and discussion

4.4.3.1 Oxygen consumption rate

Wines were poured in individual bottles with sensors and their oxygen consumption was monitored over three to five saturation cycles, during total duration of trial up to 261 days. All the samples are divided in two groups according to their number of saturations: (i) the first group was saturated three times only (samples A, F and E), whereas (ii) the second group (samples B, C and D) had 5 saturations during the same period of experiment due to high O_2 consumption rate.

The samples with slow oxygen consumption rate and 3 saturations **(Fig. 4.19, plot I)** showed a rapid 1st saturation followed by 3rd and 2nd saturation, respectfully. The O_2 consumption time course of samples E and F was similar (data not shown). **Figure 4.19**, **plot II**, shows the time course of oxygen consumption with five saturations in which the 5th saturation appeared the fastest and the 1st saturation the slowest. The samples B and C followed the same model of oxygen consumption (data not shown).

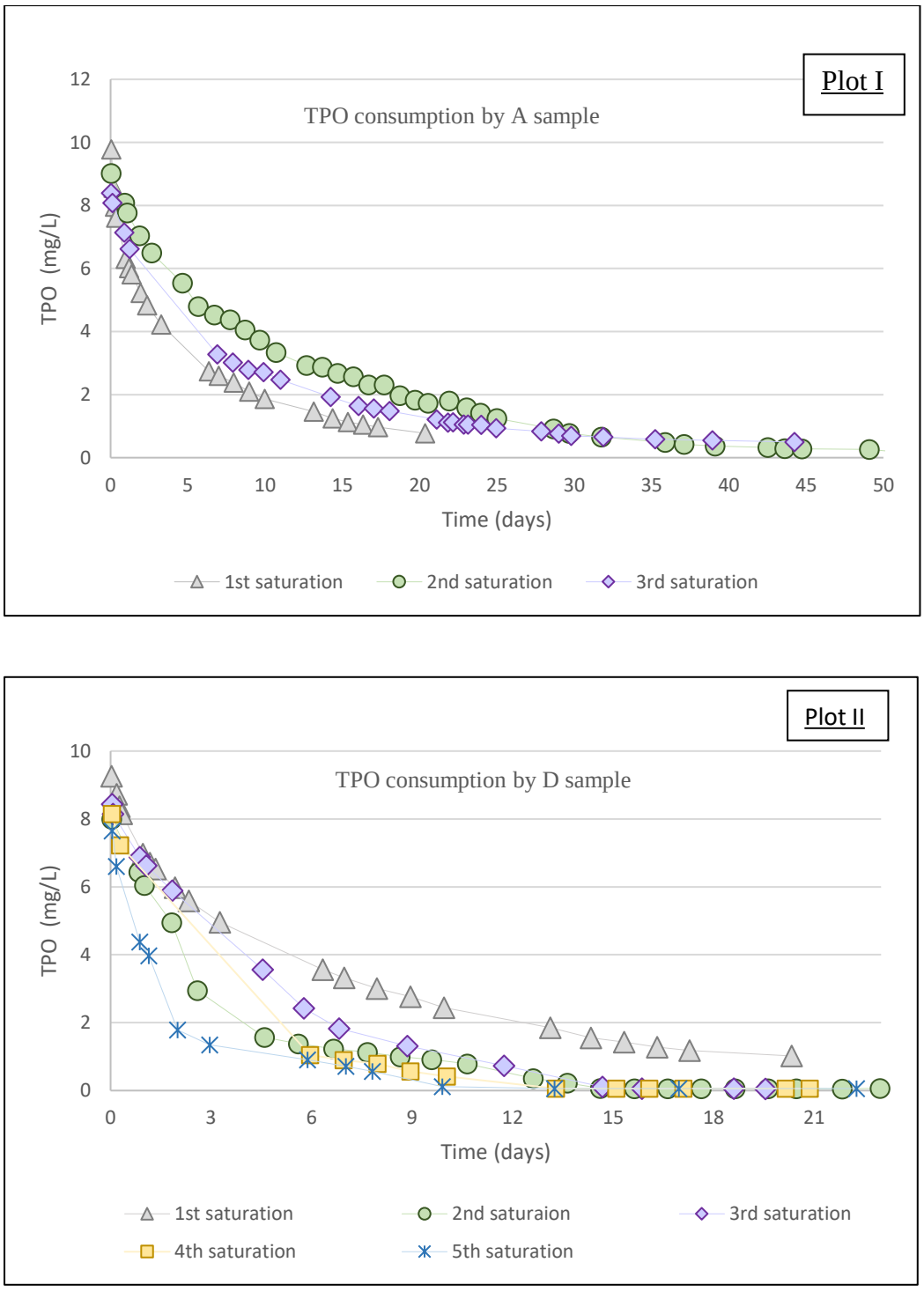


Figure 4. 19 Total oxygen consumption. Plot I: consumption of TPO versus time over three saturations of sample A (wine F and E showed a similar trend); Plot II: consumption of TPO versus time over 5 saturations of sample D (wines B and C showed a similar trend).

In previous work Jeremić et al. (2020) showed the oxygen consumption kinetics of the commercial tannins added to red wine Chianti and model wine solution. All tannins including

seed tannin, grape skin tannin, gallotannin and ellagitannin under condition of model wine solution had the fastest oxygen consumption during the first saturation. Therefore, in the samples A, E and F, the oxygen consumption could derive probably from these types of compounds, especially from grape skin and seed tannin, since the wine has not been aged in oak.

On the contrary, the samples B, C and D showed a different pathway of oxygen consumption in consecutive saturations: the first saturation is the slowest and the last one is the fastest. The hypothesis of initial lack of intermediate reactive products (i.e radicals) if formulated. Particularly, the O_2 molecule is not available to react with wine organic compounds at as soon as added to wine. It is rather necessary to receive one electron from iron in catalysing reaction, changing its ground state to triplet that is more reactive and ready to enter in chain reaction of creating oxygen reactive species albe to oxidise several wine nucleophilic compounds including polyphenols, ethanol, thiols and other (Waterhouse et al., 2016; Danilewicz, 2003; Miller et al 1990).

Table 4.12 shows details of the amount of consumed oxygen by wines in terms of dissolved oxygen, head space oxygen, total packaging oxygen and duration of the saturation cycles. Thus, dissolved and head space oxygen consumed by wines ranged from 6 to 7 mg/L, and 1 to 3 mg/L in each saturation cycle, respectively. The duration of the first oxidation cycle was 20 days and all the following cycles had different length, depending on the speed of O_2 consumption. The overall O_2 consumption was obtained by addition of TPO of all saturation cycles of each sample. As expected, the biggest difference of overall O_2 consumption is found between samples saturated three times (from 23 mg/L to 26.5 mg/L) and samples saturated five times that consumed from 38 to 43 mg/L of O_2.

Table 4. 12 The oxygen consumption of head space (HS), dissolved oxygen (DO), total packaging oxygen (TPO) over five saturation of samples A, B, C, D, E and F.

		A	B	C	D	E	F
1st saturation	D.O.	7.115	7.04	7.67	7	6.52	6.97
	H.S.	1.89	1.8	1.37	1.25	0.99	1.82
	T.P.O.	**9.01**	**8.84**	**9.04**	**8.24**	**7.51**	**8.8**
	Days	20	20	20	20	21	21
2nd saturation	D.O.	6.65	6.38	6.1	6.46	6.41	6.52
	H.S.	2.23	3.02	2.62	1.48	1.66	2.68
	T.P.O.	**8.89**	**9.4**	**8.71**	**7.94**	**8.05**	**9.27**
	Days	65	14	9.5	29	68	68
3rd saturation	D.O.	6.39	6.3	6.16	6.48	6.58	6.4
	H.S.	1.49	2.47	0.803	1.9	0.67	1.99
	T.P.O.	**7.89**	**8.77**	**6.97**	**8.39**	**7.25**	**8.39**
	Days	44	19	20	20	43	43
4th saturation	D.O.	/	6.27	6.06	6.34	/	/
	H.S.	/	2.39	1.29	1.74	/	/
	T.P.O.	/	**8.66**	**7.35**	**8.09**	/	/
	Days	/	22	21	21	/	/
5th saturation	D.O.	/	6.09	6.09	6.06	/	/
	H.S.	/	1.72	0.71	1.5	/	/
	T.P.O.	/	**7.82**	**6.82**	**7.59**	/	/
	Days	/	22	22	22	/	/
Total TPO consumed:		**25.78**	**43.49**	**38.88**	**40.25**	**22.81**	**26.46**

To better examine how the rate of TPO consumption changes in wines, the kinetic of reactions of oxygen consumptions were modelled using linear regression. The equations $y = kx + b$, contain the rate constant k that presents the slope of the trend line of the graph. The determination coefficient (R^2) is a goodness-of-fit measure for linear regression models where number 1 represents to best fit, and 0 no fit.

The criterium how to choose the right kinetic order of TPO consumption was decided by creating regression plots using the time (expressed in days) for the x axis and TPO, Ln TPO,

1/TPO and $1/(TPO)^2$ as the y axis for zero, first, second and third kinetic order, respectively and their equations are presented below [1-4]. In these equations, TPO_0 presents the initial concentration, while TPO_t is the concentration of TPO at time t.

Kinetic order equations:

Zero order: $[TPO]_t = -\underline{k}t + [TPO]_0$ [1]

First order: $\ln[TPO]_t = -\underline{k}t + \ln[TPO]_0$ [2]

Second order: $1/[TPO]_t = -\underline{k}t + 1/[TPO]_0$ [3]

Third order: $[1/(TPO)^2]_t = -\underline{k}t + [1/(TPO)^2]_0$ [4]

Figure 4.20 shows the plot with all types of kinetic orders for the sample D at the first saturation cycle (the other saturations of sample D are presented in supporting information in **Figure S4).** The plots of every saturation cycles for each sample were performed in the same way and the data of the best fitting first order reactions are summarized in **Table 4.13**. The best fit as R^2 was showed by the first order of kinetic reaction, which agrees with literature (Ferreira et al., 2015 and Jeremić et al. 2020). Nevertheless, other authors proposed that the oxygen consumption rate is affected by the oxygen and tannins concentration following the second order of kinetic reaction (Pascual et al., 2017). As it can be seen in the **Figure 4.20**, the first order of the kinetic equation has the best fit R^2=0.99. It is similar in all the other simples (data not shown).

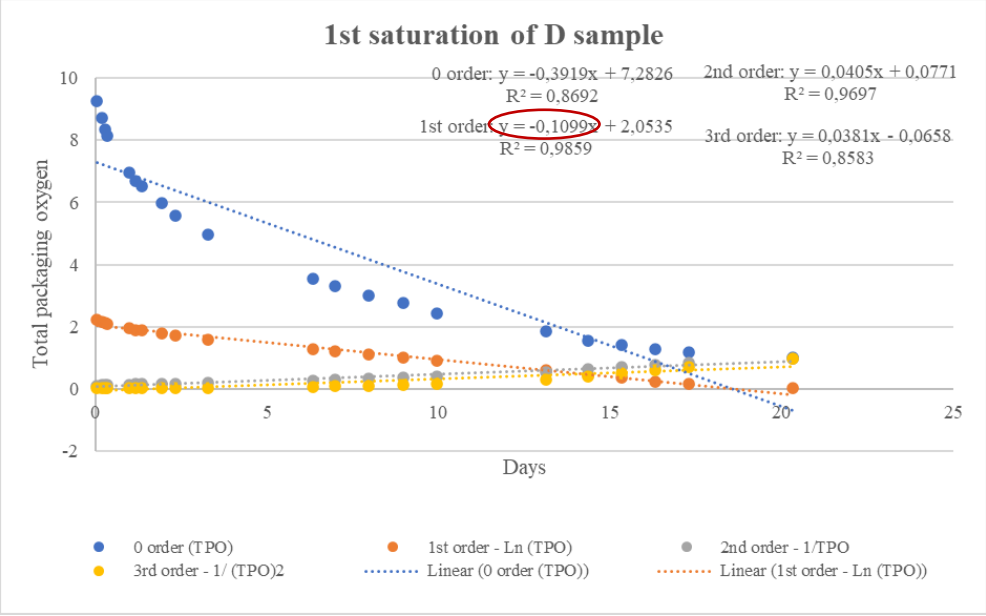


Figure 4. 20 Linear regression plot of zero, first, second and third kinetic order of the first saturation cycle for the wine sample D.

As showed by **Table 4. 13,** the constant rate *k* of the OCR of the first saturation was the highest in the first group of samples (A, E and F), validating that the oxygen consumption rate of the first oxidation cycle was the fastest. Afterwards, the kinetic of reaction decreased in 3rd and 2nd saturation. In this case, the oxidation mechanism probably started earlier in wine that probably had many nucleophiles as oxidative reactants active already (Boulton et al. 1996).

Table 4. 13 A summary of kinetic equations for the first order TPO consumption over five saturations

Trial	1st saturation	2nd saturation	3rd saturation	4th saturation	5th saturation
A	y = **-0.120x** + 1.962 (R² = 0.97)	y = **-0.067x** + 1.958 (R² = 0.98)	y = **-0.068x** + 1.749 (R² = 0.94)		
B	y = **-0.134x** + 1.887 (R² = 0.92)	y = **-0.333x** + 2.276 (R² = 0.86)	y = **-0.291x** + 2.256 (R² = 0.97)	y = **-0.254x** + 1.791 (R² = 0.95)	y = **-0.281x** + 1.492 (R² = 0.88)
C	y = **-0.124x** + 2.008 (R² = 0.97)	y = **-0.446x** + 1.976 (R² = 0.87)	y = **-0.305x** + 1.427 (R² = 0.92)	y = **-0.298x** + 1.695 (R² = 0.92)	y = **-0.259x** + 0.783 (R² = 0.80)
D	y = **-0.110x** + 2.053 (R² = 0.99)	y = **-0.205x** + 1.582 (R² = 0.89)	y = **-0.278x** + 2.314 (R² = 0.97)	y = **-0.261x** + 1.732 (R² = 0.92)	y = **-0.244x** + 1.392 (R² = 0.88)
E	y = **-0.093x** + 1.957 (R² = 0.98)	y = **-0.058x** + 1.999 (R² = 0.99)	y = **-0.088x** + 1.964 (R² = 0.92)		
F	y = **-0.110x** + 1.858 (R² = 0.93)	y = **-0.066x** + 1.817 (R² = 0.97)	y = **-0.096x** + 1.786 (R² = 0.96)		

Oppositely, the second group of samples (B, C and D) were characterised by low *k* values, therefore confirming that the first oxidation cycle was the slowest, whereas the other saturation cycles were fast. The oxidation where the first saturation was the slowest, may follow the oxidation pattern as described above.

To better understand the oxygen consumption rate (mg O_2/L/day), the calculation was performed as follows: the oxygen concentration at time t $[O_2]_t$, was subtracted from the initial

dissolved oxygen $[O_2]_0$ - $[O_2]_t$ and then divided with number of days for that period of time. As it can be seen in the **Figure 4.21,** the OCR in the initial phase was higher than the rest of the time of O_2 consumption and it lasts first four days. In the first day, the OCR was from 3,8 to 2.6 mg/L/day. During the mid-phase, the OCR was 0.9 – 0.64 mg/L/day, and at the end in prefinal phase 0.5 – 0.35 mg/L/day. The findings are consistent with previous work reported by Ferreira et al. 2015.

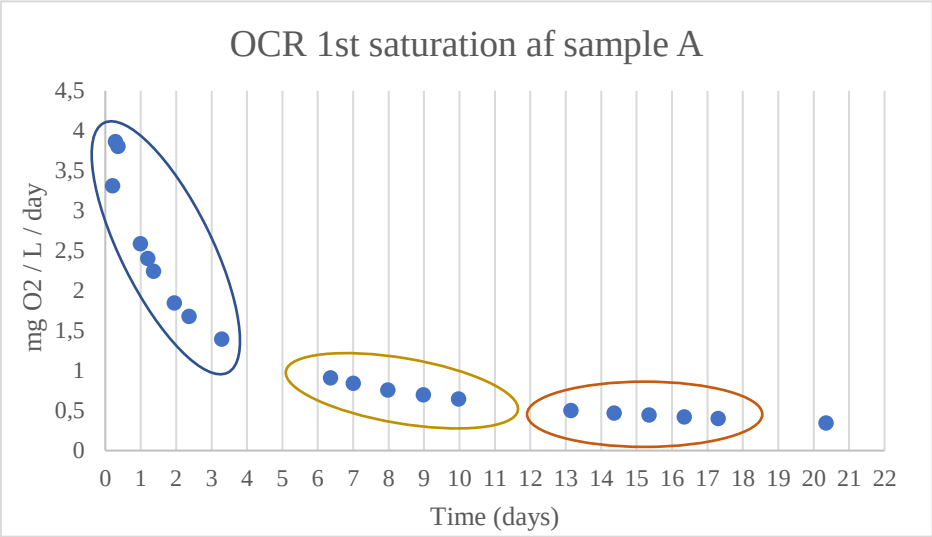


Figure 4. 21 Oxygen consumption rates (OCR) in initial time (blue ellipse), the average OCR (brown ellipse) and prefinal phase (red ellipse).

Furthermore, the head-space oxygen showed slow tendency of consumption in respect to dissolved oxygen and its concentration was always greater than the O_2 dissolved in wine (**Figure 4.22)**. The slopes *k* values of the first order of kinetic reaction of dissolved oxygen and head space consumption of the sample B are respectively: -0.306, -0.066 (1 st saturation), -0.393, -0.310 (2^{nd} sat.), -0.351, -0.250 (3^{rd} sat.), -0.256, -0.236 (4^{th} sat.), -0.273, -0.274 (5^{th} sat.). As expected, the *k* value was always high in DO, thus wines consume DO faster than HSO due to the tendency of head-space oxygen to transfer toward DO establishing an equilibrium of the partial pressure of these two phases through the gas-liquid surface, as proposed by Vidal and Moutounet (2006). Therefore, the HSO can be considered as a resource of additional oxygen that decreases continuously depending on the rate of DO reactions with the wine compounds.

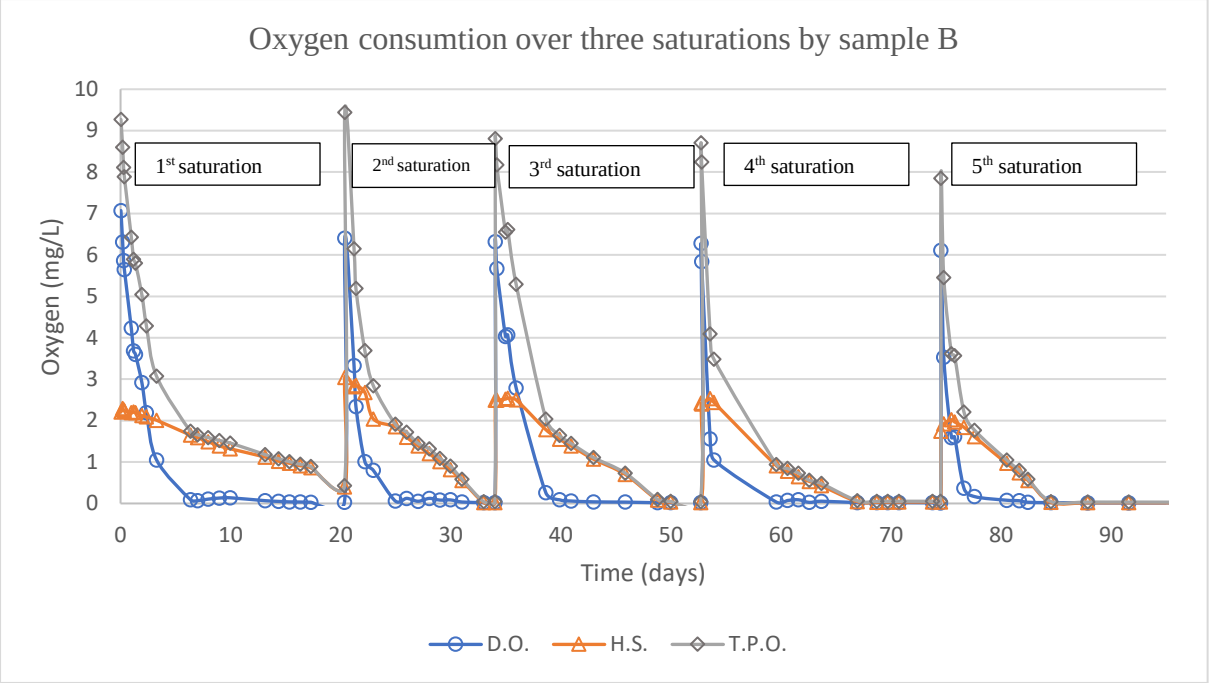


Figure 4. 22 The drop of dissolved oxygen (DO), head-space oxygen (HSO) and total packaging oxygen (TPO) over five saturations of the sample B.

4.4.3.2 Chemical analysis

The base chemical compounds in wine were analysed before and after trial by FT IR spectrophotometer Bacchus and Titrex ACT 2 instruments and results are reported in the **Table 4.14.** The alcohol level remained unchanged after wine oxidation. The total acidity decreased only in sample B, while the volatile acidity decreased in all samples after the oxidation trial. The level of the tartaric acid increased, and consequently the pH value decreased in all wine samples. Furthermore, the dry extract content decreased in all wine samples. As expected, both free and total sulphur dioxide diminished in all wine samples. The residual sugar raised in the samples A, B and F, while in samples C, D and E declined after the oxidation.

Table 4. 14 The main chemical compounds in wine: alcohol, total acidity, volatile acidity, tartaric acid, dry extract, glycerine, pH value, free SO_2, total SO_2 and residual sugar.

Sample	Vintage	Alcohol (% vol)	Total acidity (g/L)	Volatile acidity as acetic a. (g/L)	Tartaric acid (g/L)	Dry extract (g/L)	Glycerine (g/L)	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Residual sugars (glu+fru) (g/L)
A	2016	=13.2	=5.54	↓0.26	↑2.13	↓28.0	=7.1	↓3.45	↓9	↓44	↑0.4
B	2016	=12.5	↓5.48	↓0.26	↑2.19	↓27.1	↓7.3	↓3.45	↓9	↓44	↑0.9
C	2016	=11.9	=5.57	↓0.12	↑2.49	↓26.8	↓7.3	↓3.41	↓11	↓46	↓0.9
D	2016	=13.5	=5.05	↓0.35	↑1.34	↓28.3	=7.9	↓3.40	↓9	↓46	↓1.0
E	2016	=15.0	↓4.98	=0.21	↑1.59	↓28.4	↓9.6	↓3.38	↓9	↓43	↓2.6
F	2016	=13.0	=5.05	↓0.23	↑1.81	↓32.2	=9.5	↓3.36	↓11	↓49	↑1.0

4.4.3.3 Sulphur dioxide consumption

It is well known that sulphur dioxide as the main antioxidant in winemaking protect the wine against the detrimental influence of oxidation that causes the loss of wine aromas, appearing of oxidation off-flavours, color instability and browning (Blouin, 2017).

The amounts of both free and total SO_2 are presented in the **Table 4.15**. The values of total SO_2 and free SO_2 in six wines ranged from 43 - 49 mg/L, and from 9 - 11 mg/L respectively. The drop of free sulphur dioxide was from 1 to 6 mg/L, while total SO_2 decreased ca. 10-21 mg/L.

Table 4. 15 Free and total sulphur dioxide consumptions during oxidation trials

Samples	Initial free SO_2 (mg/L)	Free SO2 after oxidation (mg/L)	Δ Free SO_2 (mg/L)	Initial total SO_2 (mg/L)	Total SO_2 after oxidation (mg/L	Δ total SO_2 (mg/L)	Total O_2 uptake (mg/L)	Time (days)
A	9	8	1	44	32	12	26	129
B	9	5	4	44	34	10	43	97
C	11	5	6	46	25	21	39	93
D	9	5	4	46	29	17	40	112
E	9	5	4	43	25	18	23	132
F	11	6	5	49	32	17	26	132

The SO_2 consumption is important for understanding of various mechanisms of oxidation. The sulphur dioxide does react directly with oxygen as previously supposed (Ribereau-Gayon, 2000). The mechanism of wine oxidation starts with the activation of oxygen catalyzed by an Fe(II)–tartrate complex which generates Fe^{3+} and H_2O_2; afterwards the Fe^{3+} reacts with *o*-diphenol producing quinones. The SO_2 in his active form HSO_3^- bounds H_2O_2 and in this way protects ethanol and other the wine chemical compounds that could enter in Fenton reaction and additionally bounds other quinone producing sulfonate stable adducts or reduce them to initial catechol (**Figure 4.23**). However, when the free SO_2 is lower than 16 mg/L, the Fenton reaction becomes the major mechanism of oxidation. Furthermore, the consumption of the first half of O_2 amount in red air- saturated wine lasts about 40 h, which is significantly faster than the oxidation reaction where the SO_2 takes part (Danilewicz, 2011; 2013; 2016a; Danilewicz and Standing, 2018; Elias and Waterhouse, 2010).

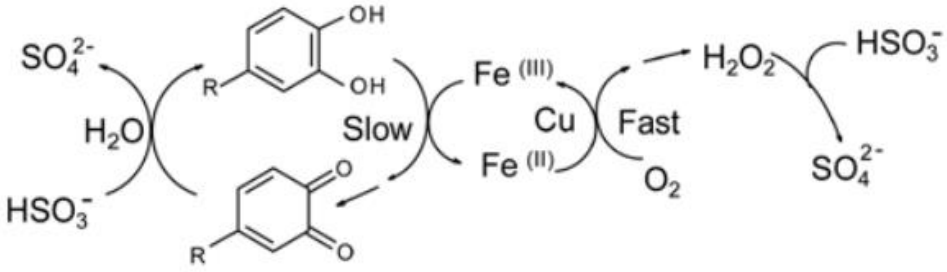


Figure 4. 23 The role of SO_2, iron and copper in polyphenol oxidation mechanism proposed by Danilewicz (2018).

The balance between the initial TSO_2 and its final amount at the end of all saturation cycles did not show any meaningful relationship, as proposed by Ferreira et al. (2015) already.

According to Danilewich and Standing (2018), the main loss of total sulphur dioxide is due to its reaction with hydrogen peroxide and quinones coming from the wine oxidation. Anyway, other electrophiles resulting from non-oxidative reactions, like tryptophan derivates and flavanol carbocation released by acid-catalysed cleavage of tannin interflavanic linkage, may

also consume sulphur dioxide (Arapitsas et al., 2018; Ma et al., 2018). Noteworthy, proanthocyanidin depolymerization might results in carbocations formation partially converted into red cyanidins (with a specific absorption at 550 nm) if the medium is sufficiently conducive to oxidation (Ribéreau Gayon and Stonestreet, 1966).

4.4.3.4 Chromatic analysis

Figure 4.25 shows the spectra of red wine before and after the accelerated aging test, respectively: 0.529 – 0.707 AU (sample A), 0.545 – 0.640 AU (B), 0.520 – 0.636 AU (C), 0.489 – 0.638 AU (D), 0.514 – 0.639 AU (E), and 0.670 – 0.836 AU (F). As expected the AU@420 nm always increased due to wine browning caused by the oxidation of polyphenols, as suggested reported by Sigleton and Kramling (1976). The samples that consumed low amount of total oxygen (A, E and F) showed the highest level of the absorbance at 420 nm demonstrating that those samples were more susceptible to oxidation comparing to the others.

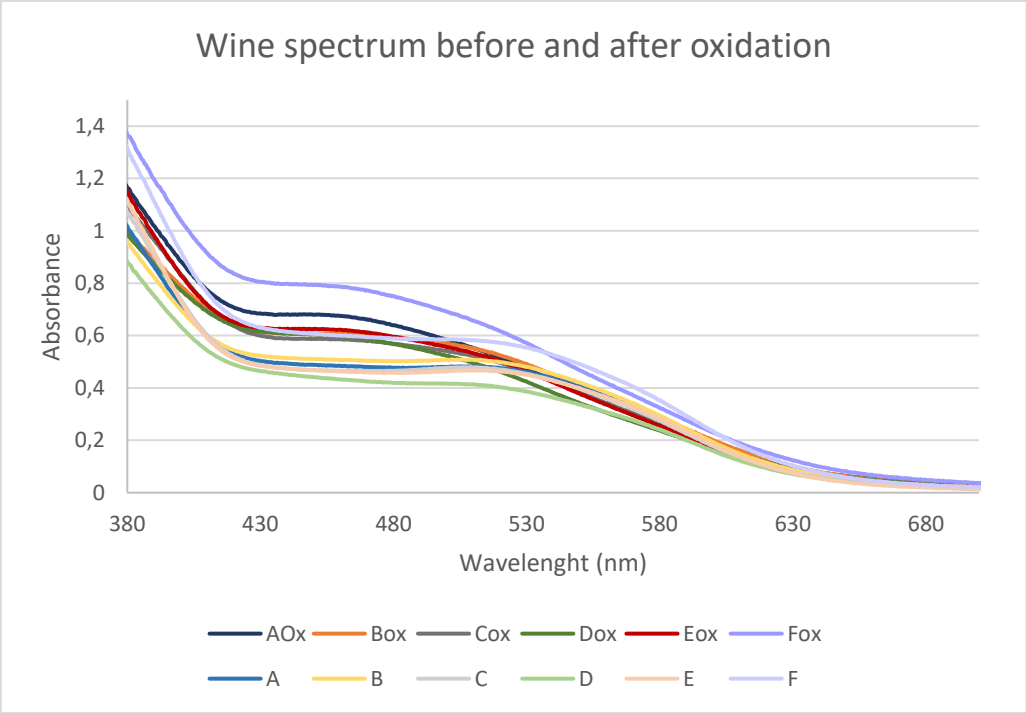


Figure 4. 24 Vis spectra at 380-680 nm for six red wines before oxidation (A, B, C, D, E and F) and after accelerated oxidation trial (Aox, Box, Cox, Dox, Eox, Fox).

Existing literature supports our assumption that the wine browning pigments come from non-enzymatic oxidation of polyphenols i.e. flavanols that occurs in different pathways. In the first one the condensed tannins undergo the C-C bonds creation and breaking in acidic conditions,

and in the second one is presented by autoxidation of flavanols (Cheynier et al. 1989; Haslam, 1980; Simpson, 1982). In another hand, during the enzymatic oxidation of catechin, the intermediate colorless compounds are formed, that in further oxidation can give the brown pigments (Guyot et al., 1995). Furthermore, Oszmianski, Cheynier and Moutounet (1996) in their study of the (+) catechin oxidation in the model wine solution that contains 0-20 mg/L of iron ferrous studied the mechanism of oxidation and their degradation products that can be revealed on the different λ of the UV-vis spectra. Thus, yellow pigments released during the enzymatic oxidation or autoxidation in the absence of iron have the maximum absorption at 385 – 415 nm, and those created in metal catalysed autoxidation have λ_{max} at 440-460 nm. Particularly, the sample F before and after oxidation at our study showed the highest absorbance at 385-415 nm, probably due to the described mechanism of enzymatic oxidation that normally occurs at the initial phase of the wine production.

The differences of wine color parameters were calculated by simply subtraction of values after oxidation from values before oxidation. The color intensity (CI) and color hue (H) increased in all wines (the numbers are negative because the values before oxidation were lower than the values after oxidation), thus probably due to the increasing of the yellow color (*b**) (**Table 4.16**). The red wine color measured as *a** parameter decreased in almost all samples suggesting in this way the loss of red pigments, probably due to their engagement in formation of other compounds with different absorption property. Furthermore, the parameter *b** showed a significant increase probably due to the formation of new yellow pigments during the oxidation of polyphenols like flavanols (Pérez-Magariño et al., 2004).

The samples with 5 saturations (B, C and D) showed decrease *L**-value, meaning that became dark. Alcalde-Eon et al. (2014) observed a decreasing lightness (*L**) when oenological tannin was added to wine. Indeed, a continuous reduction in wine pigments during the wine aging increases the lightness of wine (Heras-Roger et al., 2014; Rivas et al. 2006).

Table 4. 16 Wine color parameters and their differences before and after oxidation: Δ CI $_{(beforeox\text{-}afterox)}$ – color intensity, Δ H $_{(beforeox\text{-}afterox)}$ - color hue, $\Delta L^*_{(beforeox\text{-}afterox)}$ -lightness, $\Delta a^*_{(beforeox\text{-}afterox)}$ -red color, $\Delta b^*_{(beforeox\text{-}afterox)}$ - yellow color, $\Delta E^*_{(beforeox\text{-}afterox)}$ -color difference

Wine	ΔCI	ΔH	Δa*	ΔL*	Δb*	ΔE*
A	-1,2	-0,3	2,1	0,0	-16,4	16,5
B	-0,7	-0,1	1,2	0,3	-8,3	8,4
C	-0,8	-0,2	1,5	0,2	-10,4	10,5
D	-1,1	-0,2	-0,1	1,0	-13,4	13,5
E	-1,0	-0,2	1,2	-0,2	-14,3	14,4
F	-1,1	-0,2	1,9	0,0	-14,7	14,8

The color difference between the samples before and after all oxidation cycles was calculated using the equation of ΔE as described above. The scale of color difference visibility was described by Mokrzycki and Tatol (2012) as follows (i) 0-1: the difference is not noticeable, (ii) 1-2: the difference can be perceived exclusively by an experienced observer, (iii) 2-3.5: the difference is noticeable even for non-experienced observer, (iv) 3.5-5: the difference is very clear for everyone, (v) 5-6: gives the impression that these are two different colors. All the samples have remarkably high ΔE value, meaning that the color difference was very visible.

The effect of oxidation on the wine color was further evaluated by a correlation analysis in R studio program as presented in the **Figure 4.25**.

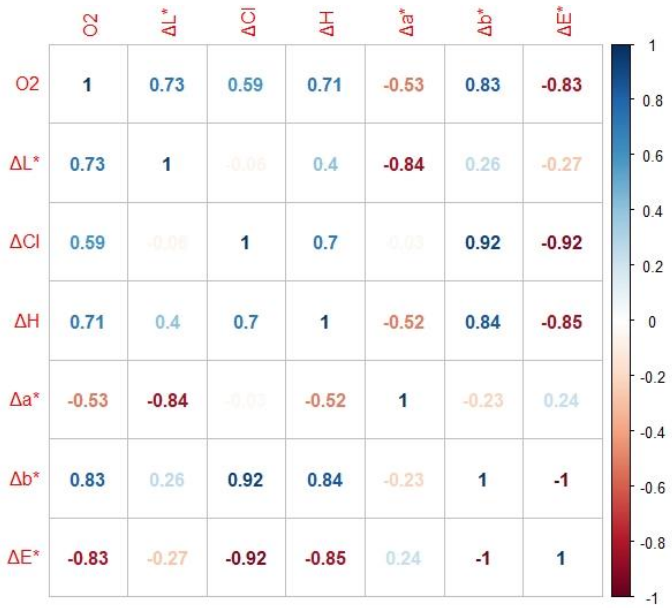


Figure 4. 25 Correlogram of influence of oxidation on wine color: Correlations between total oxygen consumption and wine color parameters CIE Lab, CI and H before and after oxidation

The strongest positive correlation of 0.83 was found between total O_2 uptake and $\Delta\ b^*_{(beforeox-afterox)}$, meaning that the more oxygen is consumed, the great amount of yellow and orange pigment is created **(Figure 4.25)**. Conversely, the negative correlation between the oxidation and total color changing $\Delta E^*_{(beforeox-afterox)}$ showed that samples with high O_2 consumption changed little their color during the oxidation. This remark suggest that the samples B, C were more resistant to oxidation impact. The high correlation between ΔE and Δb* (R^2=1) enforce the great contribution of the yellow pigments on the wine color.

4.4.3.5 Metal analysis

The total metal analysis did not reveal any significant correlation between the total consumed O_2 and the metal amount analysed before and after wine oxidation. The results are synthetised in the *correlogram* created in R studio program (available in supporting information as **Figure S5).** In our results only the total metal content was analysed, without metal ions, specifically Fe^{2+}, Fe^{3+}, Cu^{2+}, Cu^{3+}, Mn^{2+} and Mn^{3+} that are involved in oxidation mechanisms (Danilewicz, 2013; 2016 b). The non-enzymatic oxidation of polyphenols that cause the oxidative browning of wines is catalysed by metals iron and copper (Li et al., 2008). Particularly, the oxidation of catechol to semiquinones and quinones is facilitated by Fe (II) and Fe (III) cations as catalysts, where molecular oxygen is reduced to hydroperoxide radical (•OOH) and H_2O_2 (Danilewicz, 2003). Hawkins and Davies (1997) suggested that copper can create the hydroxyl radical (•OH) and several other radicals reacting with hydrogen peroxide. In another hand, the copper enhance the Fe catalysing capacity of wine oxidation (Danilewicz, 2016b). Manganese has also been studied by Danilewicz (2016b) in the model wine solution where Fe and Cu are present in high concentrations. It resulted that Mn^{3+} can easily oxidise the tartaric acid, while Mn^{2+} speeds up the oxidation of Fe^{2+} cation. In another hand, manganese is a strong catalyst of sulfite autoxidation as well.

4.4.4 Conclusion

All things considered, it can be concluded that Sangiovese wine samples coming from different areas of Tuscany had a distinct capacity of oxygen consumption. The efficiency of the SO_2 is

not high as it was expected to be and there are other antioxidants present in wine. The analysis of metals did not show any important correlation between their content in wine and the total amount of consumed oxygen. On the other hand, the CIE Lab analysis of Sangiovese color showed a strong correlation between the consumed oxygen level in wine and yellow color formation, while the level of the red color decreased with the wine oxidation.

4.4.5 References

Alcalde-Eon, C., García-Estevez, I., Puente, V., Rivas-Gonzalo, J.C. and Escribano-Bailoń M.T. (2014). Color stabilization of red wines. A chemical and colloidal approach. *Journal of Agricultural and Food Chemistry*, 6984 – 6994.

Arapitsas, P., Guella, G. and Mattivi, F. (2018). The impact of SO2 on wine flavanols and indoles in relation to wine style and age. *Scientific Reports*, 8, 858. 1–13.

Atanasova, V., Fulcrand, H., Cheynier, V. and Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta,* 458, 15–27.

Bergamini, C., Caputo, A.R., Gasparro, M., Perniola, R., Cardone, M.F. and Antonacci, D. (2013). Evidences for an alternative genealogy of 'Sangiovese'. *Molecular Biotechnology*, 53, 278–288.

Blouin, J. (2017). La SO_2 in enologia. Proprietà e limiti. Effetti tecnologici. Utilizzo pratico. Soluzioni alternative. *Eno-one*. EAN: 9788888792132.

Boulton, R.B., Singleton, V.L., Bisson, L.F. and Kunkee, R.E. (1996). Principles and Practices of Winemaking. Davis, CA: *Springer; Science and Business Media.* ISBN: 978-1-4419-5190-8.

C.I.E. (1986). Colorimetrie, 2nd ed. Publication C.I.E. No. 15,2. *Central Bureau of the Commission Internationale de L'Eclairage.* Viena, Austria.

Cano-López, M., Pardo-Mínguez, F., Schmauch, G., Saucier, C., Teissedre, P. L., López-Roca, J. E., et al. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *Journal of Agricultural and Food Chemistry*, 56, 5932–5941.

Carrascon, V., Fernandez-Zurbano, P., Bueno, M. and Ferreira, V. (2015). Oxygen consumption by red wines. part II: differential effects on color and chemical composition caused by oxygen taken in different sulfur dioxide-related oxidation contexts. *Journal of Agricultural and Food Chemistry*, 63, 10938–10947.

Casey, J. (2002). A commentary on the AWRI closure report. *Aust NZ Grapegrow Winemak*, 457, 65–69.

Cejudo-Bastante, M. J., Pérez-Coello, M. S. and Hermosín-Gutiérrez, I. (2011). Effect of wine micro-oxygenation treatment and storage period on colour- related phenolics, volatile composition and sensory characteristics. *LWT-Journal of Food Science and Technology*, 44, 866–874.

Cheynier, V., Rigaud, J., Souquet, J.-M., Barillère, J.-M., Moutounet, M. (1989). Effect of pomace contact and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. *American Journal of Enology and Viticulture,* 40. 1, 36–42.

Cilliers, J. J. L., Singleton, V. L. (1989). Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. *Journal of Agricultural and Food Chemistry*, 37, 890–896.

Coelho, J.M., Howe, P.A., and Sacks, G.L. (2015). A headspace gas detection tube method to measure SO_2 in wine without disrupting SO_2 equilibria. *American Journal of Enology and Viticulture*, 66, 257–265.

Danilewicz, J.C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: central role of iron and copper. *American Journal of Enology and Viticulture*, 54, 2, 73–85.

Danilewicz, J.C. (2011). Mechanism of autoxidation of polyphenols and participation of sulfite in wine: Key role of iron. *American Journal of Enology and Viticulture,* 62, 319–328.

Danilewicz, J.C. (2012). Review of oxidative processes in wine and value of reduction potentials in enology. *American Journal of Enology and Viticulture,* 63,1–10.

Danilewicz, J.C. (2013). Reactions involving iron in mediating catechol oxidation in model wine. *American Journal of Enology and Viticulture,* 64, 316–324.

Danilewicz, J.C. (2016 b). Chemistry of manganese and interaction with iron and copper in wine. *American Journal of Enology and Viticulture,* 67, 4, 377–384.

Danilewicz, J.C. and Standing, M.J. (2018). Reaction Mechanisms of Oxygen and Sulfite in Red Wine. *American Journal of Enology and Viticulture,* 6, 3, 189–195.

Danilewicz, J.C., Seccombe, J.T. and Whelan, J. (2008). Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *American Journal of Enology and Viticulture,* 59, 128–136.

Danilewicz, JC. (2016 a). Reaction of oxygen and sulfite in wine. *American Journal of Enology and Viticulture,* 67, 13–17.

du Toit, W. J., Marais, J., Pretorius, I. S. and Du Toit, M. (2006). Oxygen in must and wine: a review. *South African Journal of Enology and Viticulture,* 27, 76–94.

Elias, R.J. and Waterhouse, A.L. (2010). Controlling the Fenton reaction in wine. *Journal of Agricultural and Food Chemistry*, 58, 1699–1707.

Ferreira, V., Carrascon, V., Bueno, M., Ugliano, M. and Fernandez-Zurbano P. (2015). Oxygen consumption by red wines. Part I: consumption rates, relationship with chemical composition, and role of SO_2. *Journal of Agricultural and Food Chemistry*, 63, 51, 10928–37.

Gambuti, A., Picariello, L., Rinaldi A. and Moio, L. (2018). Evolution of Sangiovese wines with varied tannin and anthocyanin ratios during oxidative aging. *Frontiers in Chemistry*, 1-11.

Gambuti, A., Rinaldi, A., Ugliano, M. and Moio, L. (2012). Evolution of phenolic compounds and astringency during aging of red wine: effect of oxygen exposure before and after bottling. *Journal of Agricultural and Food Chemistry,* 61, 1618–1627.

Gambuti, A., Siani, T., Picariello, L., Rinaldi, A., Lisanti, M. T., Ugliano, M., et al. (2017). Oxygen exposure of tannins-rich red wines during bottle aging. influence on phenolics and color, astringency markers and sensory attributes. *European Food Research and Technology*, 243, 669–680.

Guyot, S., Chewier V., Souquet, J.M. and Moutounet, M. (1995). Influence of pH on the enzymatic oxidation of (+)-catechin in model systems. *Journal of Agricultural and Food Chemistry,* 43, 2458–2462.

Haslam, E. (1980). In vino veritas: oligomeric procyanidins and the ageing of red wine. *Phytochemistry*, 19, 2577–2582.

Hawkins, C.L. and Davies, M.J. (1997). Oxidative damage to collagen and related substrates by metal ion/hydrogen peroxide systems: Random attack or site-specific damage? *Biochimica et Biophysica Acta*, 1360, 1, 84–96.

Heras-Roger, J., Pomposo-Medina, M., Díaz-Romero, C. and Darias-Martín, J. (2014). Copigmentation, colour and antioxidant activity of single-cultivar red wines. *European Food Research and Technology*, 239, 13–19.

Iland, P. (2004). Chemical analysis of grapes and wine: techniques and concepts. *Patrick Iland Wine Promotions Pty Ltd*, Campbelltown, SA, Australia, ISBN: 0958160511 9780958160513.

Jeremić, J., Vongluangnam, I., Ricci, A., Parpinello, G.P. and Versari, A. (2020). The oxygen consumption kinetics of commercial oenological tannins in model wine solution and chianti red wine. *Molecules*, 25, 5, 1215, 1–13.

Koppenol, W.H., (1994). Chemistry of iron and copper in radical reactions. Chapter in new comprehensive biochemistry. *Free Radical Damage and its Control,* 3–24.

Li H., Guo, A. and Wang, H. (2008). Mechanisms of oxidative browning of wine. Review. *Food Chemistry,* 108, 1–13.

Ma, L., Watrelot, A.A., Addison, B. and Waterhouse, A.L. (2018). Condensed tannin reacts with SO_2 during wine aging, yielding flavan-3-olsulfonates. *Journal of Agricultural and Food Chemistry,* 66, 9259-9268.

Miller, D., M., Buettner, G. R. and Aust, S. D. (1990). Transition metals as catalysts of "autooxidation" reactions. *Free Radical Biology & Medicine,* 8, 95-108.

Mokrzycki, W. and Tatol, M. (2012). Color difference delta E - A survey. *Machine Grape Vision*, 20, 383–411.

Oszmianski, J., Cheynier, V. and Moutounet M. (1996). Iron-catalyzed oxidation of (+)-catechin in model systems. *Journal of Agricultural and Food Chemistry,* 44, 1712−1715.

Pascual, O., Vignault, A., Gombau, J., Navarro, M., Gómez-Alonso, S., García-Romero, E., Canals, M. J., Hermosín-Gutíerrez I., Teissedre, P-L. and Zamora, F. (2017). Oxygen consumption rates by different oenological tannins in a model wine solution. *Food Chemistry*, 234, 26–32.

Pérez-Magariño, S. and González-San José, M.L. (2004). Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *Journal of Agricultural and Food Chemistry*, 52, 1181–1189.

Peynaud, E. (1987). El gusto del vino. *Ed: Mundi-Prensa. Madrid, Spain.*

Registro Nazionale delle Varietà di Vite, Ministero delle Politiche Agricole, Alimentari e Forestali. Available online: http://catalogoviti.politicheagricole.it/catalogo.php (accessed on 20 November 2020).

Ribéreau Gayon, P. and Stonestreet, E. (1966). Le dosage des tannins du vin rouge et la détermination de leur structure. *Chimica Analitica,* 48, 188–196.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000). Handbook of enology. Vol 2. The chemistry of wine stabilization and treatments. *Wiley & Sons, New York,* ISBN-13: 978-0471973638.

Rivas, E.G.P., Alcalde-Eon, C., Santos-Buelga, C., Rivas-Gonzalo, J.C. and Escribano-Bailón, M.T. (2006). Behaviour and characterisation of the colour during red wine making and maturation. *Analytica Chimica Acta,* 563, 215–222.

Sacks, G.L., Howe, P. A., Standing, M. and Danilewicz J.C. (2020). Free, bound, and total sulfur dioxide (SO2) during oxidation of wines *American Journal of Enology and Viticulture,* 71, 4, 266–277.

Simpson, R. F. (1982). Factors affecting oxidative browning of white wine. *Vitis*, 21, 233–239.

Sinleton, V.L. and Kramling T.E. (1976). Browning of white wines and an accelerated test for browning capacity. *American Journal of Enology and Viticulture*, 27, 4, 157–160.

Vidal, J.C. and Moutounet, M. (2006). Monitoring of oxygen in the gas and liquid phases of bottles of wine at bottling and during storage. *Journal International des Sciences de la Vigne et du Vin, Publications Internationales*, Bordeaux, France, 40, 1, 35–45.

Vivas, N. and Glories, Y. (1996). Role of oak wood ellagitannins in the oxidation process of red wines during aging. *American Journal of Enology and Viticulture,* 47, 103–107.

Vouillamoz, J.O.S., Monaco, A., Costantini, L., Stefanini, M., Scienza, A. and Grando, M.S. (2007). The parentage of ‘Sangiovese’, the most important Italian wine grape. *Vitis*, 46, 19–22.

Waterhouse, A. L. and Laurie, V. F. (2006). Oxidation of wine phenolics: a critical evaluation and hypotheses. *American Journal of Enology and Viticulture*, 57, 306–313.

CHAPTER 5

Voltammetry

5 Voltammetry

Jeremić, J., Ricci, A., Tacconi, G., Lagarde-Pascal, C., Parpinello, G.P. and Versari, A. (2020). Monitoring oxidative status in winemaking by untargeted linear sweep voltammetry. *Foods*, 9, 728, 1–10.

5.1 Monitoring of oxidative status in winemaking by untargeted sweep voltammetry

5.1.1 Introduction

The most important part of vinification, the alcoholic fermentation, is the moment where many chemical compounds get transformed thus creating a wine structure and organoleptic characteristics of wine. In this perspective, there is demand for an instrument that performs fast analysis of fermentation allowing an effective monitoring of the process thus obtaining the best results from every grape variety enhancing the wine quality and preventing the wine faults (Killeen et al. 2018; Shrake et al., 2014). It is common knowledge that the oxygen can have both positive and negative influence on wine chemical structure, aromas and mouthfeel, according to its use in terms of moment and dosage of addition along all winemaking processes from fermentation to bottling (Castellari et al., 2004; Day et al. 2015). Specifically, the oxidation is principal problem in enology since it can cause the wine color instability creating a browning effect, the appearance of wine bitterness, the loss of wine aromatic compounds and flovor (Boulton et al., 1998).

Sulphur dioxide is the main antioxidant used in winemaking to prevent the wine from oxidation, nonetheless a recent solicitude regarding wine allergens conducted scientific studies toward substitute winemaking protocols implementing new natural antioxidants like oenological tannins (Giacosa et al., 2019; Santos et al., 2012; Stockley, 2005; Versari et al., 2013). Over the past ten years, the grape and wine polyphenols acquired considerable attention from researchers and oenologists as result of their encouraging antioxidant activity herein described as any substance that postpones, prevents or eliminates oxidative harm to a target

molecule (Halliwell and Gutteridge, 1998; Sacchi et al., 2005; Smith et al., 2015). The antioxidant performance of these compounds depends strongly on morphological properties of each molecule and it is fundamentally associated to the presence of hydroxyl function(s) in the aromatic fraction. The examples of these compounds are tannins, phenolic acids, flavan-3-ols, flavonols, stilbenes and others (Haslam, 1988; Rice-Evans et al., 1996; Singleton and Esau, 1969).

From the analytical standpoint, the strong correlation exists between total phenolic compounds and antioxidant capacity experimented as ABTS, DPPH and FRAP (Paixão et al., 2007). Difficulty of monitoring of each winemaking process in order to predict the oxidative response of wine still remains, notwithstanding numerous recent progress in wine oxidation studies. Hence, a quotidian winemaking presents a challenge due to the uncertainty of the terminal wine quality (Danilewicz, 2012; Waterhouse and Laurie, 2006). From this perspective, it is necessary to create a rapid and trustworthy method to examine the oxidation throughout whole vinification. The selection of method may have an important influence on obtained results because of the difference in chemical structure of each antioxidant and sample tasted. There are several common spectrophotometric methods for antioxidant testing in use (e.g. ABTS, FOX, FRAP, DPPH, TEAC, TRAP, etc.) (Craft et al., 2012; Frankel and Meyer, 2000). Anyway, there is a need to create a rapid antioxidant test for grape and wine polyphenols, kipping in mind their distinct chemistry. To rich this goal, electrochemical methods supply as well a great capability for the exploration of antioxidants, evaluation of antioxidant capacity and measurement of electrochemical indexes employing several types of electrodes like glassy carbon and modified gold electrodes (Sochor et al., 2013).

Kilmatrin and collaborators successfully introduced cyclic voltammetry to examine antioxidant qualities of wine antioxidants like polyphenols, ascorbic acid, glutathione, sulphur dioxide (Kilmatrin et al., 2001; Ricci et al. 2019). Furthermore, linear sweep voltammetry (LSV) – an electrochemical method was used by Medvidović et al. in their study of the mechanism of the electrochemical oxidation of rutin. The method is based on measurement of a cell current as a function of time and as a function of the potential between the indicator and reference electrodes. Concurrently, the studies of the sulphites in fruit juice and wine were performed. (Romão et al., 2012; Scampicchio et al., 2008). Albeit it presents a great prospective in the science, voltammetric technique has a significant disadvantage due to the electrode fouling by

wine phenolics that is timewasting due to the necessity to clean it manually which can be bypassed by using of new generation of disposable single-use electrodes. Recent studies have demonstrated that voltammetry with single-use electrodes can be effectively used to determine oxidative mechanisms of white wines (Gonzalez, Vidal and Ugliano, 2018; Ugliano, 2016).

The purpose of this study is to implement for the first time in industry the monitoring of antioxidant models of red, rosé and white wine throughout alcohol fermentation and maceration only for the red wines, by using linear sweep voltammetry associated with a new disposable single-use electrode, designed for polyphenol testing.

5.1.2 Materials and methods

Experimantal Design. Experiments were developed to monitor the influence of selected winemaking steps (maceration, alcoholic fermentation and fining) on the chemical composition of red, rosé and white wines regarding collective phenolic compounds tested by linear sweep voltammetry, associated with a disposable single-use electrode. A total of 116 measurement were done, particularly 45 were during red wine maceration and the remaining in white and rosé fining.

Samples. The wines and musts (vintage 2019) used in this research were acquired at industrial scale from different grape cultivars comprising Sangiovese, Pinot gris, Chardonnay and Vermentino. Standard winemaking procedure was applied for all grape immediate processing at the winery Ruffino (Monteriggioni and Castellina in Chianti, Italy). The samples were analysed on-site during the trials using the portable electrochemical device as described in the following sections.

Winemaking practices. The on-site monitoring of standard winemaking practices in progress at Ruffino wineries included grape destemming and crushing (5 trials), fining (7 trials), maceration during red wine fermentation at 27 ± 2 ◦C (5 trials), white and rosé wine alcoholic fermentation at 17 ± 2 ◦C (20 trials) and red wine draining and pressing (10 trials). Individual trials were monitored several times for a total of 116 samples. The selection of both grape

cultivars and processing were determined by the actual needs at the winery since the experimental trials were conducted at production level, i.e., in a real winemaking company.

In Ruffino winery, a classification of Chianti Classico in A and B quality groups starts in vineyards that give different grape quality depending on the type of soil, solar exposition and grapevine plant vigour. Some parameters of grape maturation are monitored in vineyard including plant vigour, grape sugar content and phenolic maturity to create vineyard maps with different zones of grape quality. These maps are used for precise viticulture grape harvest conducted by New Holland harvester machine that automatically separates the grape quality A and B. Thus, the quality A comes from defined areas where the grape maturity reaches the optimum level regarding the sugar accumulation and polyphenol maturation, so this type of wine is suitable for production of Chianti Classcico Gran Riserva wines. On the other hand, the B quality grape comes from Chianti Classico vineyards that fulfil all the DOCG Chianti Classico regulation requirements.

The classification of Chianti Classico wines into A and B qualities comes from vineyards where the selection of the grape quality is conducted on the base of grape harvest parameters like: plant vigour, chemical composition of the grapes and sensory characteristics. These parameters are analysed and followed by agronomist and enologists before the harvest in order to define the right level of grape maturation that will ensure the production of required wine quality (for more details, see the **Chapter 1** – Chianti and Chianti Classico Appellations of Origin).

Later, the maceration of the separated red grapes is conducted in the cellar until the moment when the enologists notice through wine tasting that there is no further changing in the polyphenol extraction. Once the alcoholic fermentation and maceration are finished, the winemakers verify again the wine quality by wine tasting, chemical analysis and this trial the sample A will be dedicated to Chianti Classico Gran Selezione production, while B wine will make part of Chianti Classico basic wine.

Voltammetry. Electrochemical measurements were carried out on-site using a portable device (**Figure 5.1**) according to the method proposed by Ugliano (2016). Concisely, a commercial Nomasense Polyscan P200 electrochemical analyzer (Vinventions, Schio, Italy) equipped with a disposable, miniaturized screen-printed triple-sensor—i.e., a three-electrode system

including a single strip rectangular working electrode of 3.3 mm^2, counter and reference electrodes—was employed.

Figure 5. 1 NomaSense Polyscan P200, electrochemical analyser of polyphenols (Source: www.winequalitysolutions.com).

Under ambient conditions, one drop of a sample (ca. 50 µL) was poured on the sensor and the linear sweep voltammogram for every single analysis recording signal in the range 0–1200 mV at a scan rate of 100 mV/s. A new sensor was used each time for measurements that were carried out without dilution of the sample. All potentials are reported against the Ag/AgCl reference electrode. Apart from the LSV signal, the Polyscan is self-calibrated to provide four unitless compositional indexes including:

1) EasyOx: easily oxidized compounds like anthocyanins, that are quickly involved in the oxidation reactions;
2) PhenOx: group of all oxidizable compounds correlated with the index of Folin–Ciocalteu;
3) IPT: Total Polyphenol Index commonly used by winemaker and
4) TAN/ACN ratio: support for the oenologists to add the right amount of O_2 to wine during wine production relying on the tannin/anthocyanin ratio.

The results of the Polyscan P200 calibration are available online (Ferias, 2018).

Data Analysis. One hundred sixteen samples were included in data matrix (red, rosé and white wines), the entire electrochemical signal up to 1200 mV with a scan rate every 10 mV for each

sample (i.e., 120 data points) and 4 compositional indexes (EasyOx, PhenOx, IPT and TAN/ACN ratio). The program for the data analysed were XLSTAT v. 2018.3 (Addinsoft, Paris, France) and The Unscrambler X v. 10.3 (Camo ASA, Oslo, Norway), for the plots creation to visualize the effects of selected winemaking practices on the wine composition. Design of the difference between the classes of data was performed by linear discriminant analysis (commonly used when groups are known a priori) in order to evaluate the suitability of a classification of white wine during alcoholic fermentation, and to reveal the most important electrochemical signal performing a stepwise selection of variables as described by Babin et al. (2011).

5.1.3 **Results and Discussion**

5.1.3.1 Linear Sweep Voltammetry

In this trial, the maceration of Sangiovese red musts of Chianti Classico A and B quality were monitored up to 21 days and 8 day respectively, from grape destemming and crushing until wine draining (see Materials and Methods).

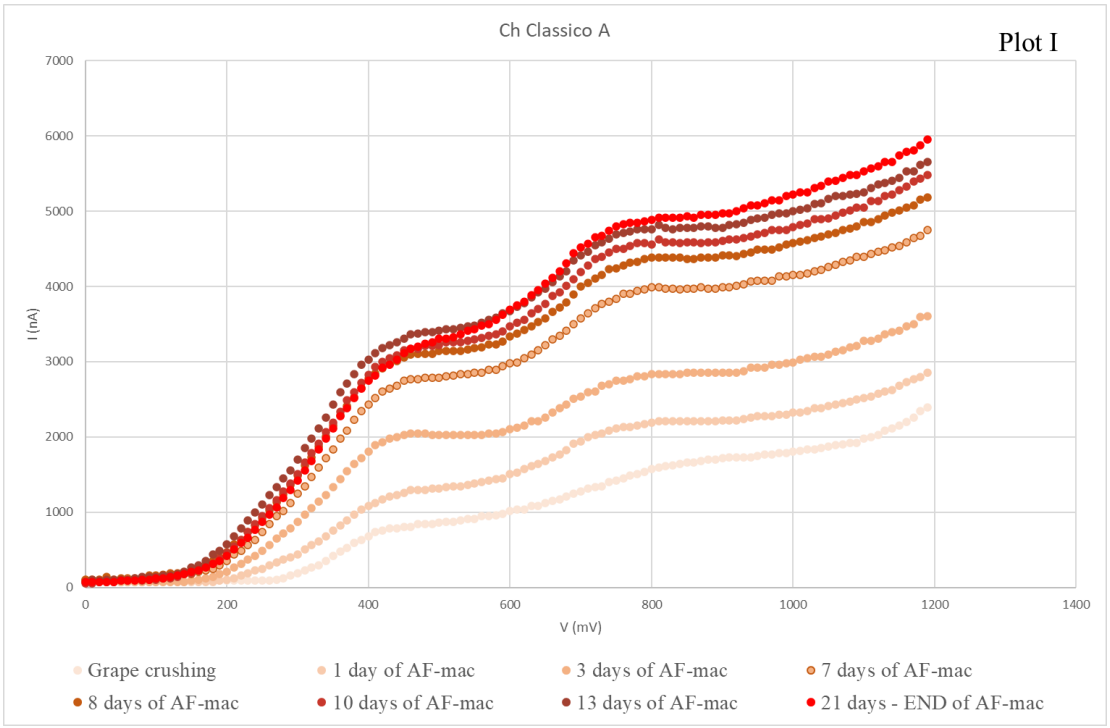
Figure 5.2 (Plot I and Plot II) represents the linear sweep voltammetry associating the electrode potential (E/mV) and the resulting current (I/nA) of the samples Chanti Classico A and B. The progressive oxidation of wines created a signal in the range from 0 to 1200 mV where the high potential values were used for oxidizing of entire antioxidant composition of Sangiovese wines. The voltammograms revealed a peculiar pattern with peaks at about 440 mV and 780 mV, which is consistent with work of Kilmartin and collaborators (2001), where suggested that the most easily oxidizable wine compounds including catechol and gallate-polyphenolic have oxidative peaks with the current recorded at the same mV values. Current extents had different potentials in wine samples depending on variation in total phenolic compounds concentrations, including anthocyanins (Aguirre et al., 2010; Janeiro and Oliveira Brett, 2007).

Furthermore, in the trial of the Chianti Classico A wine (Plot I), the signals at 13 and 21 days showed an intersection at about 600 mV, where the signal of later measurement (day 21) had lower values until 600 mV and afterwards it increased to its maximum value at 1200 mV. It should be emphasized that simple phenolic compounds are oxidized at low values, while the other more complex polyphenols that contain more difficulty oxidizable groups create peaks at higher potential values.

During maceration, the LSV signals showed an initial rapid rise (up to day 7) followed by a slowdown, thus approaching a plateau at day 21 (Figure 5.2, Plot I) in the case of Chianti Classico A sample. The observed trend is consistent with the timeline of phenolic extraction from red grapes, where "free" compounds from the grape surface leach almost instantaneously to the grape must and subsequently compounds from the berry interior dissolves into the must (Zanoni et al., 2010). Likewise, extraction of anthocyanins from the solid berry parts increases at the beginning of the maceration and reaches a peak and afterwards, a following drop takes place due to degradation of anthocyanins and their condensation with tannins (Andrich et al., 2005).

On the other hand, the trial of Chianti Classico B wine (Plot II) had the intersection at day 7 and 8 at about 450 mV wherein the signal of the last day of maceration (day 8) had a higher value until 450 mV and later the signal decreased, and it was lower than the signal of the previous day 7.

This decreasing of the signal after 7^{th} day was probably due to the less stable anthocyanins mentioned above (Andrich et al., 2005). The Chianti Classico B sample is used for the "Chianti Classico" quality production that is characterised by lighter red color and by less complex wine structure in respect to Chianti Classico Gran Selezione wine (see the section **3.1.1** and **3.1.2** - Chianti Classico DOCG regulations).



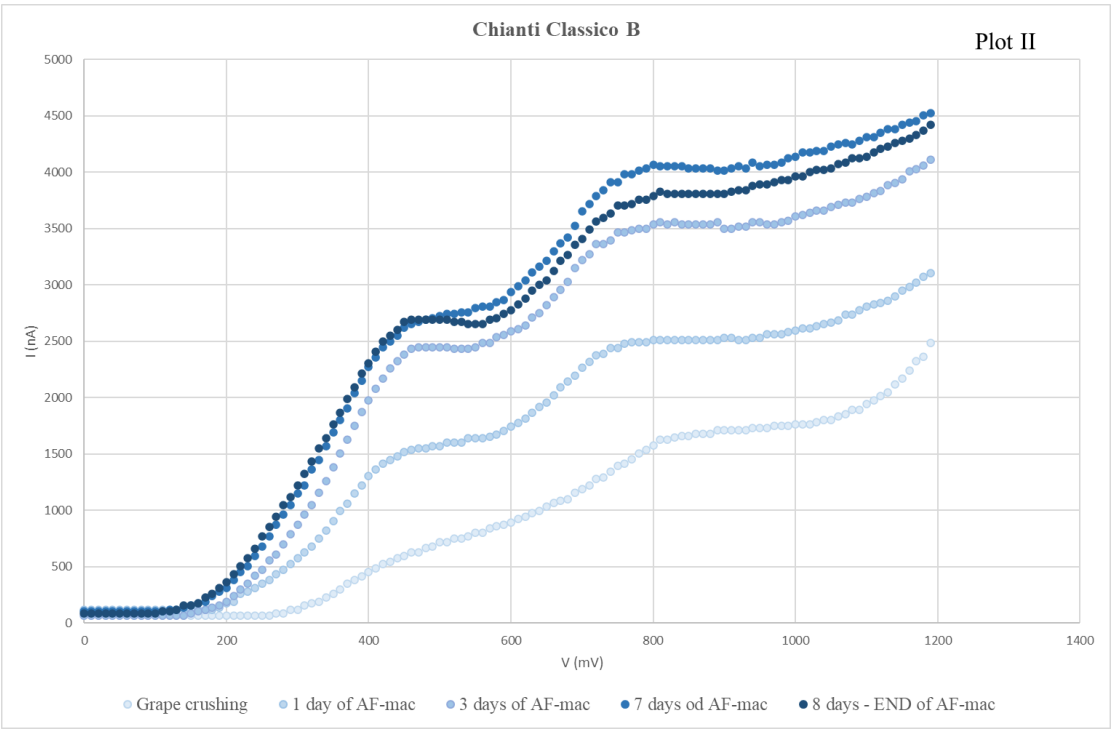


Figure 5. 2 Spectrum of linear sweep voltammetry measured during maceration of Sangiovese wine: Plot I- Chianti Classico A; Plot II – Chianti Classico B.

Nonetheless, the voltammograms of red wines show the first voltammetric anodic peak at approximately 440 mV related to the oxidation of flavan-3-ols, oligomeric and polymeric

tannins which represents the oxidation of catechol group on the B-ring, while the second peak at approximately 800-900 mV represents the oxidation of the resorcinol group on the A-ring. Furthermore, the peak at~ 680 mV is principally related to anthocyanins (Makhotkina and Kilmartin, 2010; Kilmartin et al, 2001).

High correlation (R^2 = 0.72) was found between the entire scanned region (0-1200 mV) that is related to total phenolic compounds (PhenOx) and the region related to low potentials (from 200 to 600 mV) that corresponds to more easily oxidizable compounds (easyOx) such as catechins and caffeic, caftaric and gallic acids. Easy oxidizable compounds represent approximately 30 % of the total polyphenol compounds.

5.1.3.2 Maceration Trials

The change of selected phenolic indexes was evaluated on Sangiovese trials during the maceration of solid parts of berry into juice during fermentation (**Figure 5.3**), which is the most important process for creation of red wine style related to suitable extraction of polyphenol compounds. Four electrochemical indexes that are related to phenolic composition of the wine including EasyOx, PhenOx IPT and TAN/ACN were followed up to 29 days. During this time, Sangiovese demonstrated a particular extraction pattern where the amount of polyphenols increased from the initial phase of maceration until the moment when it reached a maximum plateau (it had exponential increasing of the values) until the final level.

Unsurprisingly, the average percent of easily oxidable polyphenols (EasyOx) presented as a part of the total polyphenolic compounds (PhenOx) was about 30 %, while the ratio TAN/ACN was around 6, which is common value for Sangiovese grape variety (**Fig.5.3**). These latter results can be explained by the presence of an equilibrium between the anthocyanins content of the grape and the wine (i.e. their adsorption – desorption). Once, when the equilibrium has been established, no further anthocyanins can be extracted from grape skin into the wine (Boulton, 2001).

Furthermore, these parameters decrease as a result of oxidation, modification in their structure, precipitation and adsorption in yeast cell walls. Tannin extraction is a similar process that has different extraction kinetics between skin and seed tannins. The skin tannins are promptly solubilized together with anthocyanin compounds, while the seed tannins have a slow

extraction rate that speed up with the alcohol formation, since the alcohol helps dissolution of the seed cuticle (Boulton et al., 1998).

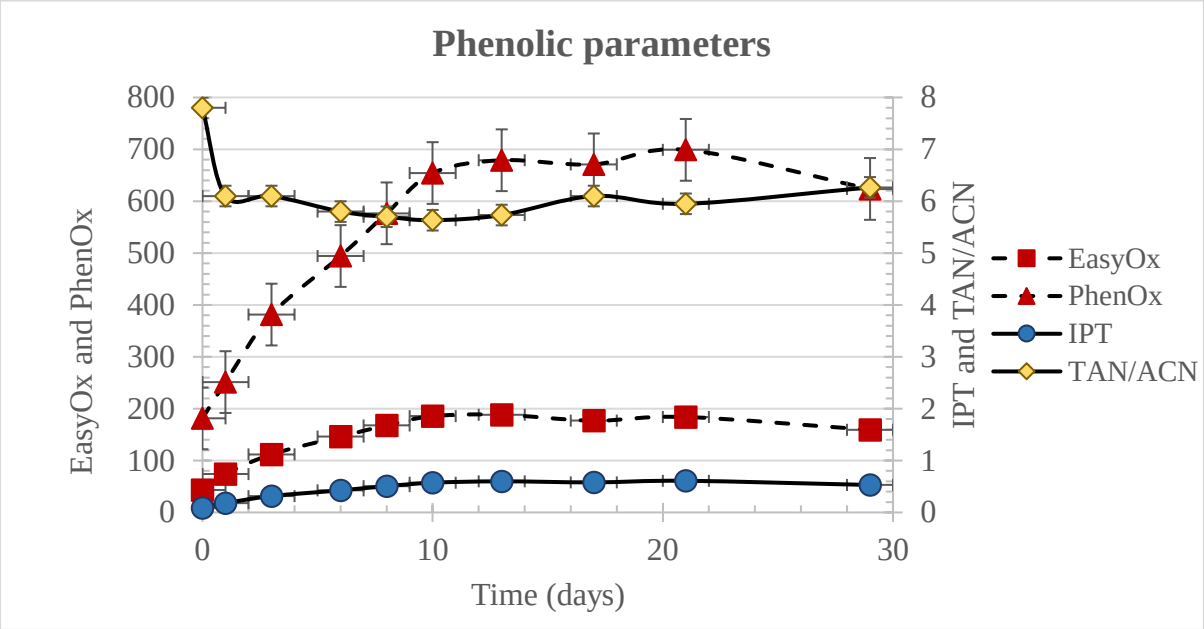


Figure 5. 3 Phenolic extraction of Sangiovese wine over 29 days during fermentative maceration.

For what concern the length of the maceration of Chianti Classico A and Chianti Classico B wine samples, it is worth mentioning that the B quality wine samples remained in the contact with the grape skin for 8 days, while the A quality samples lasted for 21 days (**Figure 5.4**). At the moment when the winemakers decided to drain the Chianti Classico B wine, the wine sensory characteristics started to be more astringent and harsher in the mouthfeel. The Phenox value started to decrease after the 7th day of maceration from 580 to 552, which corresponds to the increasing of the negative organoleptic characteristics. On the other hand, the EasyOx values reached the stable level after the 7th day of maceration of the Chianti Classico B wine and it had the value about 167.

On the contrary, during the maceration of the Chianti Classico A wine sample, it is possible to notice a constant rising of the PhenOx values until the end of the maceration, up to value 775. The must presented very positive organoleptic characteristics during all the process of maceration increasing the complexity of the mouthfeel and having smooth tannins. The end of the maceration presented the moment when the wine remained the same regarding its total organoleptic complexity two days in a row.

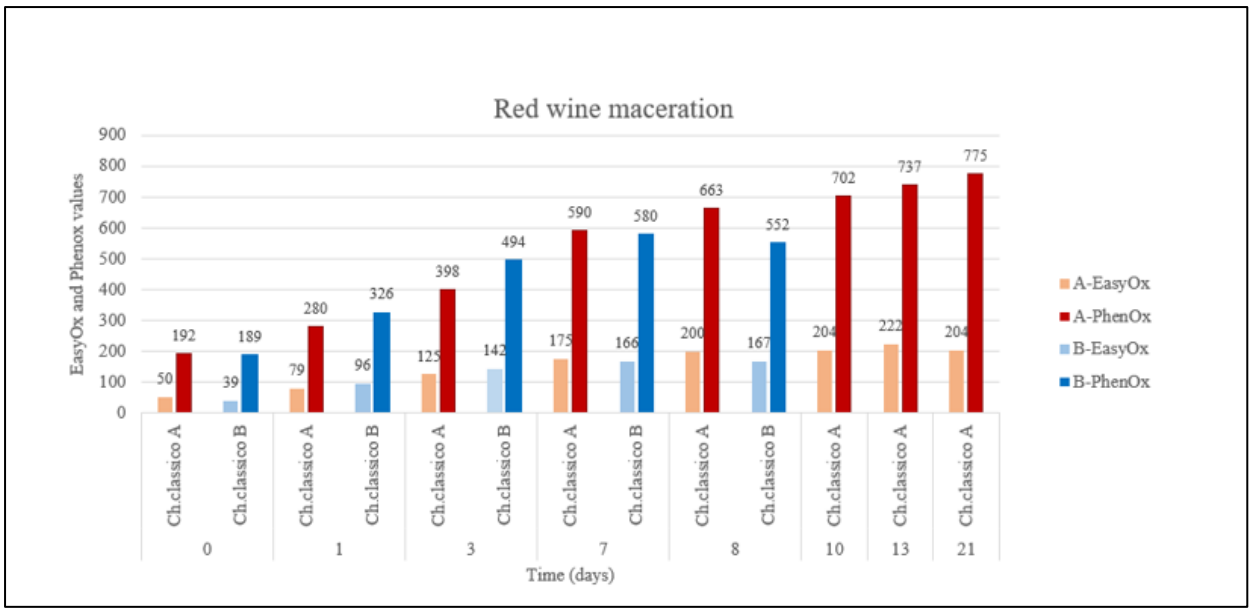


Figure 5. 4 Easy oxidizable phenols (EasyOx) and Total polyphenolic compounds (PhenOx) of Chianti Classico A and Chianti Classico B samples.

5.1.3.3 Alcoholic Fermentation Trials

The voltammetric technique was tested during the white wine alcoholic fermentation of 3 white wines including Pinot gris (14 samples), Chardonnay (10 samples) and Vermentino (4 samples). Discriminant analysis was used on electrochemical signals in order to create groups of the wine samples and afterwards to better understand eventual relations between the variables. For the purpose to reduce the background noise, the range of electrochemical signal was selected from 300 to 1200 mV, and discriminant analysis used on that range successfully grouped all 28 white wine samples according to their cultivar (**Figure 5.4**). Selection of variables was done using stepwise procedure, where the first 10 signals (at 300, 370, 670, 680, 770, 780, 940, 970, 1000 and 1190 mV) presented a good enough result with only one Chardonnay sample misclassified. Remarkably, most of the selected signals were associated to a high oxidation voltage presented by chemical compounds that are more difficult to oxidize, which appears to be the main driver for creation of specific qualitative pattern between grape varieties. The presented method seems to be appropriate to follow the effect of wine oxidation also. In fact, the applying of voltametric method of analysis as a successful tool for monitoring oxidation of white wines was previously proposed using cyclic voltammetry (200 – 1200 mV

at a scan rate of 100 mV/s) in association with supervised multivariate analysis (Martins et al., 2008).

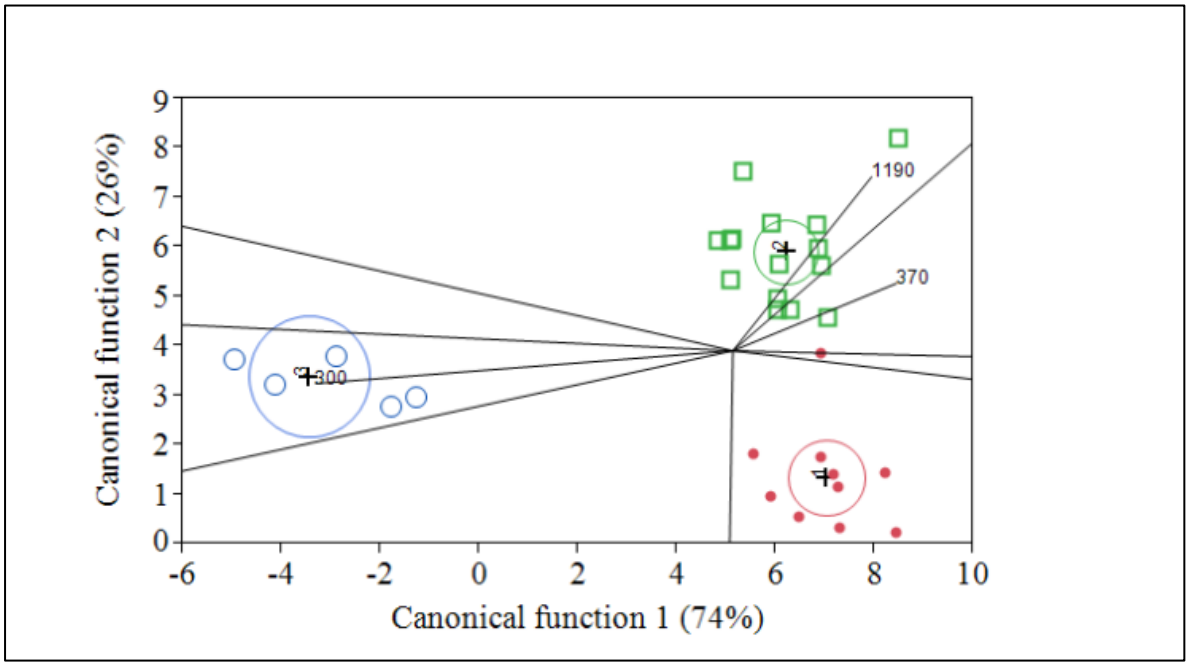


Figure 5. 4 Linear discriminant analysis of white grape varieties; 1) Chardonnay (red), 2) Pinot gris (green), and 3) Vermentino (blue) during alcoholic fermentation (adapted from Jeremić et al., 2020).

However, as it was predicted, the content of polyphenols analysed in white wines during the fermentation (**Figure 5.5**) was lower than those found in Sangiovese red wines (**Figure 5.3**) having an average ratio PenOx/EasyOx of about 4.7, 5.0, 3.6 for Chardonnay, Pinot gris and Vermentino, respectively. There results encourage further association with literature trying to better understand the astringent characteristics of wine. In fact, the skin contact with juice increase the amount of polyphenolic compounds in wine, and the contact time will define the wine composition during the prefermentative maceration (Ough, 1969), as well as temperature (Hernanz et al., 2007). More specifically, Giordano and collaborators (2012) in a comparative study on the vinification of Vermentino grapes reported that the total polyphenolic compounds were: 82, 112, 141, 162 and 522 mg/L for hyperoxygenation, control, reductive conditions, prefermentative cryomaceration (at 5 ºC during 24h) and extending maceration, respectively. On the other hand, a similar study on Chardonnay white wine was conducted by Naviglio and collaborators (2018) confirming that below 6 ºC the increase in phenolics in wine is negligible and its concentration was 440 mg/L.

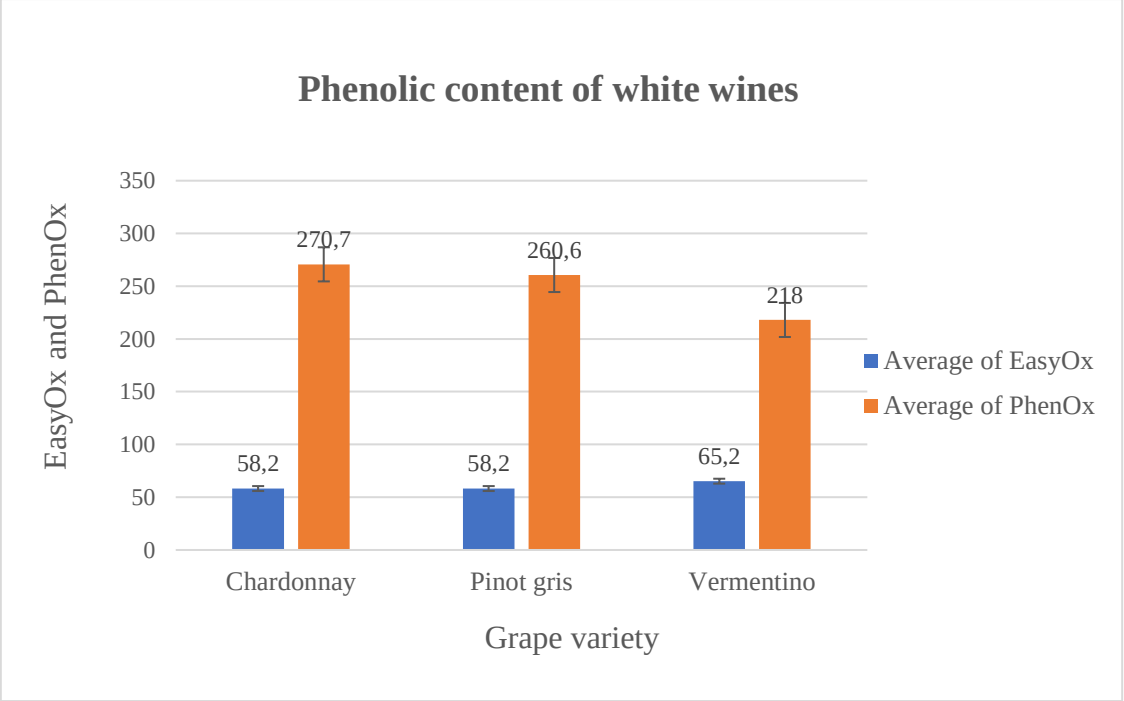


Figure 5. 5 EasyOx and PhenOx (with error bars) of white wine grape varieties: Chardonnay, Pinot gris and Vermentino during alcoholic fermentation at 17 ºC.

5.1.3.4 Fining Trials

Pinot gris must were clarified during the early phase of fermentation with activated carbon from charcoal, rather than later in wine (i.e. after fermentation) in order to eliminate oxidizable phenol compounds before any influence on color is caused, including pinking and to obtain the stability of the rosé wine color.

It is well known that the total phenolic content is positively correlated (up to R^2= 0.94) with antioxidant activity of wine (Frankel et al., 1995; Paixão et al., 2007). There is a positive correlation (up to R^2 = 0.99) between total polyphenol compounds measured by spectrophotometer and voltametric instrument (Ricci et al., 2019; Kilmartin et al., 2002), including Polyscan device where used (R^2 = 0.91) (Ugliano et al., 2016). More specifically, Ugliano and collaborators (2014) reported that the sum of each oxidation current per potential increasing is defined as "antioxidant power" of the wine or grape must, which is highly correlated (R^2 = 0.98) with 420 nm AU values.

Figure 5.6 shows that the total oxidizable polyphenols (PhenOx) in wine decreased with use of activated carbon up to 40 g/hL, while the influence on the EasyOx appeared to be insignificant. Singleton and Draper (1962) reported that commercial activated carbons from charcoal vary in level of uptake of anthocyanins from red grapes and excess tannins as well. These differences in polyphenolic compounds elimination are related to grape cultivar and method of preparation of activated carbon (Corcho-Corral et al., 2005).

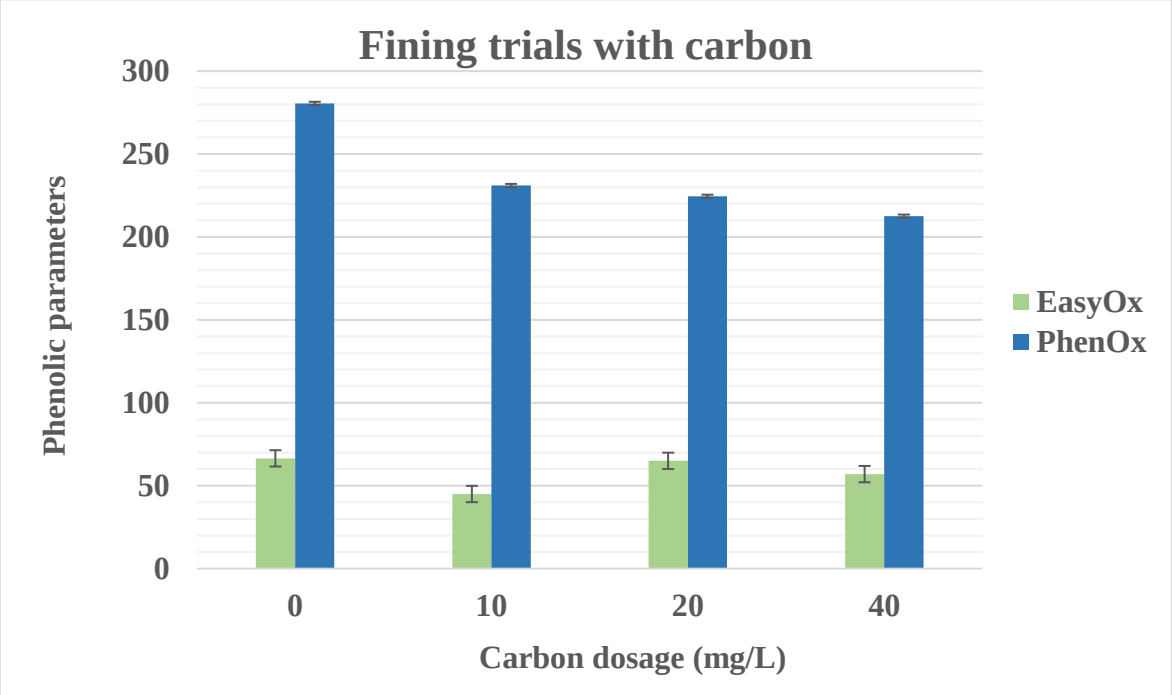


Figure 5. 6 Active carbon effect on the Pinot gris must composition during finig.

As can be seen from data in **Figure 5.6**, the negligible effect on EasyOx index that includes anthocyanins as well is probably associates with very low amount of these pigments in Pinot gris samples, making it difficult to absorb them with carbon for obtaining a considerable removal. It is well known that Pinot gris may develop pinking phenomenon that is the creation of salmon-red blush color in oxidative conditions, that may appear in white wines made from white grape varieties. The threshold of the anthocyanin level for the pink color appearance is 0,3 mg/L (Andrea-Silva et al., 2014).

5.1.4 **Conclusions**

It is very important to use suitable devices to monitor vinification processes in the cellar to guarantee the success of high-quality wine production. In this view an easy-to-use and prompt method based on disposable screen-printed voltametric sensors was used for *in situ* measurements of the polyphenols content of white and red wines during initial phase of the winemaking process in the cellar. The collection of the compositional data of wine is an useful support for enhancement of the wine quality, specially for skin maceration and must fining processes, giving to enologists an input to take prompt corrective measures.

The grape musts having low EasyOx and/or PhenOx values would most likely benefit from pressing under intert conditions and would probably need a mild fining treatment. On the other hand, the grape musts with higher values of EasyOx and PhenOx would probably need a strong fining treatment and possibly a hyperoxigenation or microxygenation of wines following a selected strategy. Moreover, monitoring of the phenolic extraction during the maceration of red wine aids to establish the duration of the process, as well as extent of saignée of rosé wines and racking of red wines. In summary, monitoring grape phenolic content, vinification samples and finished wines would permit for creation of quality control graphs for improving complete product quality management.

5.1.5 References

Aguirre, M.J., Chen, Y.Y., Isaacs, M., Matsuhiro, B., Mendoza, L. and Torres, S. (2010). Electrochemical behaviour and antioxidant capacity of anthocyanins from Chilean red wine, grape and raspberry. *Food Chemistry*, 121, 44–48.

Andrea-Silva, J., Cosme, F., Ribeiro, L.F., Moreira, A.S.P., Malheiro, A.C., Coimbra, M.A., Rosário, M., Domingues, M. and Nunes, F.M. (2014). Origin of the pinking phenomenon of white wines. *Journal of Agricultural and Food Chemistry*, 62, 5651–5659.

Andrich, G., Zinnai, A., Venturi, F. and Fiorentini, R. (2005). A tentative mathematical model to describe the evolution of phenolic compounds during the maceration of Sangiovese and Merlot grapes. *Italian Journal of Food Science*, 17, 45–58.

Babin, B.G.; Watson, M.P. (2011). *Discriminant Analysis: An Overview*; Lovric, M., Ed.; International Encyclopedia of Statistical Science, *Springer: Berlin/Heidelberg, Germany*.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, 52, 67–87.

Boulton, R.B., Singleton, V.L., Bisson, L.F., Kunkee, R.E. (1998). Principles and Practices of Winemaking. *Bloom, R., Ed., Aspen Publisher*: Frederick, MD, USA.

Corcho-Corral, B., Olivares-Marín, M., Valdes-Sánchez, E., Fernández-González, C., Macías-García, A. and Gómez-Serrano, V. (2005). Development of activated carbon using vine shoots (*Vitis vinifera*) and its use for wine treatment. *Journal of Agricultural and Food Chemistry*, 53, 644–650.

Craft, B.D., Kerrihard, A.L., Amarowicz, R. and Pegg, R.B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety*, 11, 148–173.

Danilewicz, J.D. (2012). Review of oxidative processes in wine and value of reduction potentials in enology. *American Journal of Enology and Viticulture*, 63, 1–10.

Day, M.P., Schmidt, S.A., Smith, P.A. and Wilkes, E.N. (2015). Use and impact of oxygen during winemaking. *Australian Journal of Grape and Wine Research*, 21, 693–704.

Frankel, E.N. and Meyer, A.S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, 80, 1925–1941.

Frankel, E.N., Waterhouse, A.L. and Teissedre, P.L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of. Agricultural and Food Chemistry*, 43, 890–894.

Frías, D. Seguimiento de la maceración de uvas tintas mediante polyscan P200. 2018. Available online: https://www.vinventions.com/assets/4d4a0795-b6b2-49dd-9d05-12d7a6eb2eb8/4-danielfrias-presentacion-polyscan-vinventions.pdf (accessed on 20 November 2020).

Giacosa, S., Río Segade, S., Cagnasso, E., Caudana, A., Rolle, L., and Gerbi, V. (2019). Chapter 21—SO_2 in Wines: Rational use and possible alternatives. In Red Wine Technology. Morata, A., Ed., *Elsevier: Amsterdam, The Netherlands*, 309–321.

Giordano, M., Caudana, M., Hock, M., Zeppa, G., Rolle, L. and Gerbi, V. (2012). First experiences of winemaking for the increasing of the varietal character of Vermentino wines. *Rivista di Viticoltura e di Enologia*, 2, 17–28.

Gonzalez, A.; Vidal, S.; Ugliano, M. (2018). Untargeted voltammetric approaches for characterization of oxidation patterns in white wines. *Food Chemistry, 269*, 1–8.

Halliwell, B., Gutteridge, J.M.C. (1998). Free Radicals in Biology and Medicine. *Oxford University Press: Oxford, UK*.

Haslam, E. (1988). Practical Polyphenolics. In from structure to molecular recognition and physiological action. *Cambridge University Press: New York, NY, USA*.

Hernanz, D., Recamales, Á.S., González-Miret, M.L., Gómez-Míguez, M.J., Vicario, I.M. and Heredia, F.J. (2007). Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. *Journal Food Engineering*, 80, 327–335.

Janeiro, P. and Oliveira Brett, A.M. (2007). Redox behaviour of anthocyanins present in *Vitis vinifera* L. *Electroanalysis*, 19, 1779–1786.

Killeen, D.J.; Boulton, R.; Knoesen, A. (2018). Advanced monitoring and control of redox potential in wine fermentation. *American Jouranl of Enology and Viticulture, 69*, 394–399.

Kilmartin, P.A., Zou, H. and Waterhouse, A.L. (2001). A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *Journal of. Agriculture and Food Chemistry*, 49, 1957–1965.

Kilmartin, P.A., Zou, H. and Waterhouse, A.L. (2001). A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *Journal of Agricultural and Food Chemistry*, 49, 1957–1965.

Kilmartin, P.A., Zou, H., Waterhouse, H.L. (2002). Correlation of wine phenolic composition versus cyclic voltammetry response. *American Journal of Enology and Viticulture,* 53, 294–302.

Makhotkina, O. and Kilmartin, P.A. (2010). The use of cyclic voltammetry for wine analysis: Determination of polyphenols and free sulfur dioxide. *Analytica Chimica Acta*, 668, 155–165.

Martins, R.C., Oliveira, R., Bento, F., Geraldo, D., Lopes, V.V., Guedes de Pinho, P., Oliveira, C.M. and Silva Ferreira, A.C. (2008). Oxidation management of white wines using cyclic voltammetry and multivariate process monitoring. *Journal of Agricultural and Food Chemistry*, 56, 12092–12098.

Medvidović -Kosanović, M., Šeruga, M., Jakobek, L., Novak, I. (2010). Electrochemical and antioxidant properties of Rutin. *Collection of Czechoslovak Chemical Communications*, *75*, 547–561.

Naviglio, D., Formato, A., Scaglione, G., Montesano, D., Pellegrino, A., Villecco, F. and Gallo, M. (2018). Study of the grape cryo-maceration process at different temperatures. *Foods*, 7, 107, 1–13.

Ough, C.S. (1969). Substances extracted during skin contact with white musts. I. General wine composition and quality changes with contact time. *American Journal of Enology and Viticulture*, 20, 93–100.

Paixão, N., Perestrelo, R., Marques, J.C., Cãmara, J.S. (2007). Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. *Food Chemistry*, 105, 204–214.

Ricci, A., Parpinello, G.P., Teslić, N., Kilmartin, P.A. and Versari, A. (2019). Suitability of the cyclic voltammetry measurements and DPPH• spectrophotometric assay to determine the antioxidant capacity of food-grade oenological tannins. *Molecules,* 24, 2925, 1–12.

Ricci, A., Teslic, A., Petropolus, V.-I., Parpinello, G.P. and Versari, A. (2019). Fast analysis of total polyphenol content and antioxidant activity in wines and oenological tannins using a flow injection system with tandem diode array and electrochemical detections. *Food Analytical Methods*, 12, 347–354.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1996). Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, 933–956.

Roginsky, V., de Beer, D., Harbertson, J.F., Kilmartin, P.A., Barsukova, T. and Adams, D.O. (2006). The antioxidant activity of Californian red wines does not correlate with wine age. *Jouranl of the Science of Food and Agriculture*, 86, 834–840.

Romão Sartori, E. and Fatibello-Filho, O. (2012). Simultaneous voltametric determination of ascorbic acid and sulfite in beverages employing a glassy carbon electrode modified with carbon nanotubes within a Poly (Allylamine Hydrochloride) film. *Electroanalysis, 24*, 627–634.

Sacchi, K.L., Bisson, L.F. and Adams, D.O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Jouranl of Enology and Viticulture*, *56*, 197–206.

Santos, M.C., Nunes, C., Saraiva, J.A., Coimbra, M.A. (2012). Chemical and physical methodologies for thereplacement/reduction of sulfur dioxide use during winemaking: Review of their potentialities and limitations. *European Food Research and Technology*, 234, 1–12.

Scampicchio, M., Lawrence, N.S., Arecchi, A. and Mannino, S. (2008). Determination of sulfite in wine by linear sweep voltammetry. *Electroanalysis*, *20*, 444–447.

Shrake, N.L., Amirtharajah, R., Brenneman, C., Boulton, R., Knoesen, R. **(2014)**. A. In-line measurement of color and total phenolics during red wine fermentations using a Light-emitting eiode sensor. *American Jouranl of Enology and Viticulture, 65*, 463–470.

Singleton, V.L. and Draper, D.E. (1962). Adsorbents and wines. I. Selection of activated charcoals for treatment of wine. *American Journal of Enology and Viticulture*, 13, 114–125.

Singleton, V.L., Esau, P. (1969). Phenolic substances in grapes and wine, and their significance. In Advance in Food Research. *Academic Press: New York, NY, USA*.

Smith, P.A., McRae, J.M. and Bindon, K.A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Australian Journal of Grape and Wine Research*, 21, 601–614.

Sochor, J., Dobes, J., Krystofova, O., Ruttkay-Nedecky, B., Babula, P., Pohanka, M., Jurikova, T., Zitka, O., Adam, V., Klejdus, B. and Kizek R. (2013). Electrochemistry as a tool for studying antioxidant properties. *International Journal of Electrochemical Science*, 8, 8464–8489.

Stockley, C.S. (2005). Wine and health: Sulfur dioxide and the wine consumer. *The Australian & New Zealand. Grapegrower and Winemaker, 501*, 73–76.

Ugliano, M. (2016). Rapid fingerprinting of white wine oxidizable fraction and classification of white wines using disposable screen-printed sensors and derivative voltammetry. *Food Chemisty*, 212, 837–843.

Ugliano, M., Dieval, J.-B., Vidal, S. and Tacchini, P. (2014). Electroanalytical method for predicting the oxidability of a wine or a grape must. Patent US 2014/0141120 A1, 22 May 2014.

Ugliano, M., Wirth, J., Begrand, S., Dieval, J.-B. and Vidal, S. (2016). A novel voltammetric approach for rapid analysis of white grapes polyphenols and monitoring of pre-fermentative operations. *The Wine & Viticulture Journal,* 31, 28–31.

Versari, A., du Toit, W., and Parpinello, G.P. (2013). Oenological tannins: A review. *Australian Journal of Grape and Wine Research, 19*, 1–10.

Waterhouse, A.L. and Laurie, V.F. (2006). Oxidation of wine phenolics: A critical evaluation and hypotheses. *American Jouranl of Enology and Viticulture*, 57, 306–313.

Zanoni, B., Siliani, S., Canuti, V, Rosi, I. and Bertuccioli, M. (2010). A kinetic study on extraction and transformation phenomena of phenolic compounds during red wine fermentation. *International Journal of Food Science & Technology*, 45, 2080–2088.

CHAPTER 6

Cellar trials

6 Cellar trials

6.1 Nitrogen sparging trial to remove CO_2 in excess from Chianti 2018 red wine

6.1.1 Introduction

The study aims to remove the CO_2 in excess found in young red wine DOCG Chianti for improving its sensory characteristics. The problem arises from short period of storage from vintage to bottling (min. 6 months) of the wine, with limited number of racking, therefore some high values of CO_2 may occur – usually around 1 g/L (**Figure 6.1**) – which was found to modify negatively the sensory characteristic of the wine.

To solve the problem the approach includes the sparging of wine with inert gas (N_2), during which process the wine is sampled with time, i.e. at different level of CO_2, and evaluated by physico-chemical and sensory analysis to ascertain the optimal CO_2 content that should be present in the wine and the corresponding process conditions.

As can be seen in the **Figure 6.1,** the amount of dissolved carbon dioxide increased significantly, specially in the past 5 years.

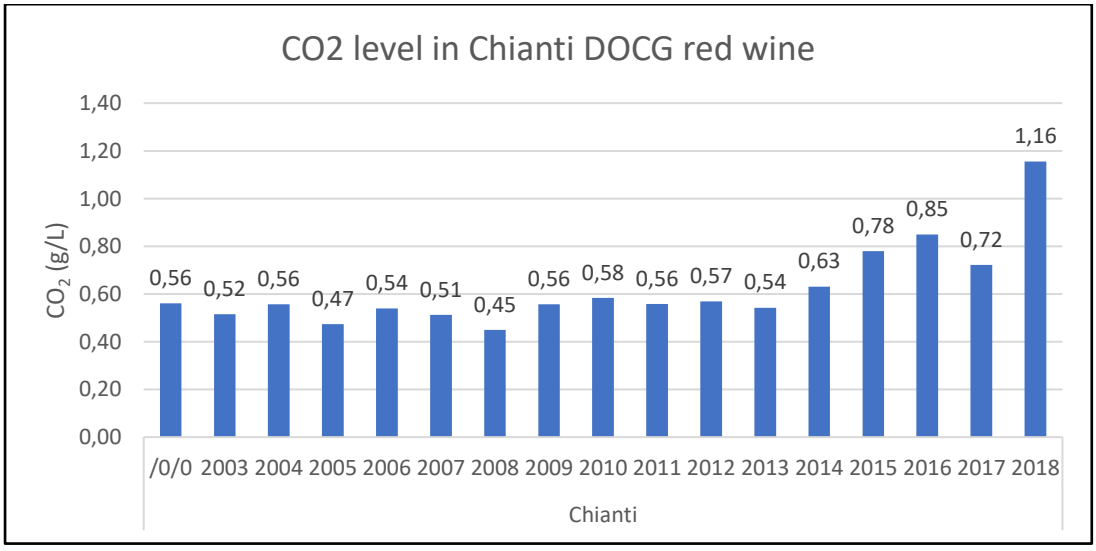


Figure 6. 1 CO_2 content in Chianti red wines over 17 years.

Some issues are necessary to clarify to set-up the sparging process:

- When sparge (the best moment during the winemaking: wine clarification, filtration, bottling preparation etc.);
- How to sparge? (the optimal nitrogen/wine flow rate (v/v));
- How long treat the wine? (time course of CO_2 removal);
- The optimal level of residual CO_2 in wine? (Influence on the sensory characteristics of wine);

Two preliminary trials were carried out to understand the influence of different conditions on results and their efficiency.

1st trial:

6.1.2 Materials and methods

The Chianti red wine 2018 vintage stored in rectangular concrete tank of 684 hL was sparged with nitrogen during the pump over process soon before the bottling (i.e. during the addition of the oenological products before bottling).

Carbon dioxide and dissolved oxygen were measured directly from the tanks with the instrument CboxQC At-line produced by Anton Paar in Graz, Austria. For the measurements in the laboratory, it is used the same instrument connected it with SFD Filling Device, a pressure chamber made by Anton Paar.

6.1.2.1 Gas parameters:

According to the literature, to remove the CO_2 from wine, the ratio gas/wine (v/v) should be from 0.1 to 0.8 v/v with a porosity of the gas diffuser less than 0.03 mm to ensure an ideal gas bubble size to efficiently remove CO_2 from wine (Dharmadhikari, 2019)

In this first trial, the used sparger had a porosity of 0.004 mm and the nitrogen flow rate was set up at 5400 L/h N_2 by using the gas reducer and the flow meter (**Figure 6.2**).

The wine flow-rate was set at 90 hL/h (i.e. 150 L/min) in order to obtain the gas/wine ratio of 0.6 (v/v).

The line of pumping over was made of the first 4 m long cellar hose (60 mm diameter) connected to the lower tank valve, where the wine comes from and to the pump on another side. At the exit of the pump Francesca produced in Faenza in Italy the gas sparger (diffuser) was connected at the beginning of the 25 m long wine hose (60 mm diameter), whose another side was immerged into the wine on the top of the tank (**Figure 6.3**).


A


B

Figure 6. 2 A - Pump with sparger; B- Flow meter

All the process was conducted during the wine preparation for bottling, when the oenological products for the tartaric stabilization are added to wine.

During the sparging the wine was sampled at 3 points: 1) On the higher tank valve (B), where the instrument Anton Paar was attached for online measurements of CO_2 and O_2 level; 2) Tank tap (C), 3) On the wine surface, on the top of the tank (D), where the wine mixed with the nitrogen enters the tank (**Figure 6.3**).

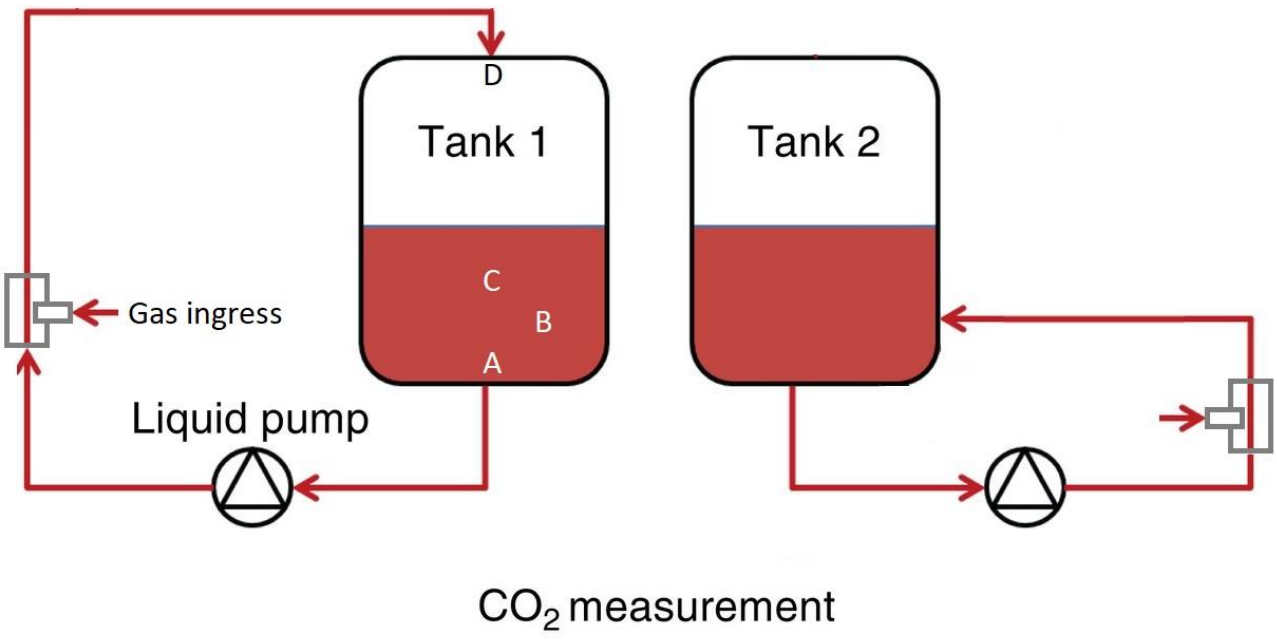


Figure 6. 3 Points of the wine sampling. Legend: (A) Low tank valve; (B) High tank valve; (C) Wine sample tap; (D) Lid on the top of the tank. (*)

6.1.3 Results

Before sparging, the initial CO_2 value was 0.90 g/L for the wine sampled on the top of the tank (**Figure 6.3-D**), and 1.04 g/L measured online (**Figure 6.3-B**) (**Table 6.1**). The difference is probably due to different sampling procedure and method of analysis.

Table 6. 1 The CO_2 and O_2 content change during the sparging trial.

Time (min)	Time of sparging (min)	CO_2 sampled from B (mg/L)	O_2 sampled from B (mg/L)	CO_2 sampled from D (mg/L)	Comments
10:04		1.05	0.176	0.93	
10:07		1.05	0.103		
10:17		1.05	0.091		
10:28	00:00				Start of N_2 sparging
10:42	00:14	1.05	0.075		
10:54	00:26	1.05	0.066		
11:23	00:55	1.04	0.108		
11:37	01:09	1.04	0.162		
11:44	01:16	1.03	0.181	0.82	A.P. rinsed with 4 L of wine
11:54	01:26	0.88	0.205		

11:56	01:28	0.88	0.207		
12:09	01:41	0.88	0.112		
13:11	02:43	0.85	0.229		
13:13	02:45	0.84	0.246		
13:26	02:58	0.83	0.228		A.P. rinsed with 4 L of wine
13:33	03:05	0.73	0.187	0.72	Oenological products addition
14:00	03:32			0.66	
14:30	04:02			0.60	
14:45	04:17				End of sparging (although the pumping over continues until 17:00)
17:00	06:32			0.56	End of the pumping over

The time course of CO_2 removal showed an initial lag-phase during which the CO_2 content remained almost stable (lasted 1:16 hrs and corresponding to approx ¼ of the tank volume pumped over) (see **Fig. 6.4**). Then the CO_2 content started to drop for reaching the targeted value of 0.6 g/L in 4h and 15 minutes (255 min). At this time (i.e. 255 min) about 382.5 hL of wine was pumped over and 22950 L of nitrogen was used, which correspond to a gas/wine ratio of 0.6 (v/v) (22950/38250=0.6)

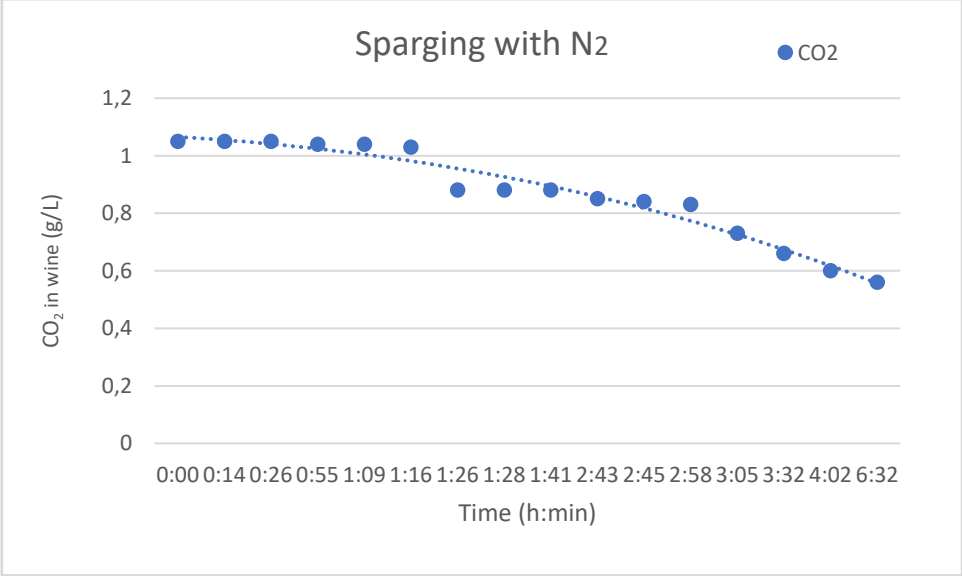


Figure 6. 4 Time course of CO_2 decrease in wine during sparging with N_2 at the flow rate 90 L/min.

II trial

The aim of the second trial was to repeat the sparging process and in this way to obtain further information for different tank volumes.

6.1.4 Materials and methods

In the second trial the volume of the tank was 215 hL and the pumping over was set from-down-to-down (**Fig. 6.3:** tank 2), whereas all the other parameters remained the same as in the previous trial. In the trial II a hose 4 m long connected the exit of the pump with the nearly bottom of the tank (**Fig. 6.5**), whereas in trail I the hose was 25 m long, since it was placed on the top of the tank (and 4 m was not enough to reach the point D, see the **Fig. 6.3**).

Two replicates of wine samples were taken at different level of the CO_2 for tasting, directly from the tank during the sparging trial in real time: at 0,96 g/L, 0,80 g/L, 0,68 g/L and 0,56 g/L. Three experienced winemakers from Ruffino winery tasted all the samples in order to reveal the best level of the CO_2 in Chianti DOCG wine, which provides the soft mouthfeel. The wine was sampled from the tank tap (C) every 15 minutes during the sparging trial and the CO_2 content was measured with the Anton Paar instrument in the laboratory (in the chamber).

Figure 6. 5 Pumping over line of the second trial

6.1.5 Results

The lag-phase last 15 minutes, then the CO_2 content dropped to targeted level (0.6 mg/L) in approximately 80 minutes only (**Table 6.2**). It is necessary to pump over 56 % of wine volume during the sparging using the gas and wine flow parameters described in the section 6.1.2.1. to obtain the result of 0.6 g/L of CO_2.

Table 6. 2 The CO_2 content in wine during the sparging trial.

Time of trial II (min)	CO_2 sampled from C (mg/L)	Comments
0	0.96	Sparging start
15	0.96	
30	0.80	
45	0.77	
60	0.68	
75	0.62	
90	0.56	The end of the sparging

CO_2 gas is liberating from wine slowly from the beginning of production (i.e. after the alcoholic fermentation) when it reaches the highest level until the bottling (data not shown). From the practical point of view of winemaking in the cellar, the best moment to do the sparging with the nitrogen is the wine preparation for the bottling, because in that moment the CO_2 level will be the lowest comparing the all the other winemaking practeces, thus the pump over will be the shortest and the use of nitrogen lowest.

6.1.5.1 Sensory characteristics of wine:

The control wine has more reductive aromas and it is less fruity comparing the treated wine. For what concern the mouthfeel, the control wine is more astringent and it has more expressed acidity, while the treated wine was described by winemakers as more soft, sweet and round and less astringent and acid. Following parameters (scaled from 0 to 9) are proposed to be included in the future wine tastings:

1) Mouthfeel: Acidity, astringency, sweetness, roundness, wine body, bitterness, alcohol perception
2) Olfactory characteristics: fruity, floral, spiciness, minerality, balsamic, aroma intensity, herbaceous, reductive, ethereal.

On the base of these characteristics, it is possible to analyse the wine aroma compounds that would give the precise idea of the sparging impact on wine sensory characteristics.

A summary of the trial parameters of both trials including the wine volume, the gas flow rate, the sparging time, the initial and the final level of the carbon dioxide and the gas/wine ratio are presented in the **Table 6.3**.

Table 6. 3 Trial parameters.

Tank n.	Tank volume (hL)	Red wine	Gas flow rate (N_2)	Trial time	Initial CO_2 (mg/L)	CO_2 (mg/L) at the end of trial	Gas/wine ratio (v/v)
471	648	Chianti 2018	90 l/min	T1: 4:15	1.05	0.56	0.6
417	215	Chianti 2018	90 l/min	T2: 1:15	0.94	0.57	0.6

6.1.6 Conclusion

The initial questions were answered during the cellar trial:

- When sparge: the best moment during the winemaking suitable for the sparging with nitrogen is bottling preparation;
- The optimal nitrogen/wine flow rate is to be discovered yet in further researches. The trials in this research were done using only one N_2/wine flow rate of 0.6 (v/v));
- The time course of CO_2 removal is related to the volume of the wine which has to be sparged with the gas. Its calculation is possible to make taking in consideration a time that is necessary to pump over 50% of wine volume, if the nitrogen flow rate is set at 90 L/min.
- The optimal level of residual CO_2 in wine that does not have a negative impact on the sensory characteristics of wine is 0.6 g/L.

6.1.7 References

Dharmadhikari M, (2019). Use of inert gases. Retrieved from https://www.extension.iastate.edu/wine/use-inert-gases

Chapter 7

General conclusion

7 General conclusion

During the PhD program a significant amount of the wine quality data and climate change information was collected and analyzed with the aim to assess of the enological potential of Sangiovese grape variety. It allowed for a valorization of Chianti and Chianti Classico red wine from Tuscany. In this regard, the two main initial research questions were answered. The first one dealth with understand the impact of climate changes at a regional level on the grape growing and winemaking practices with the aim of improving the wine quality in the future, with respect to the traditional regional winemaking regulations. The understanding of the future grape growing and winemaking conditions allows the producers to better organize their activity and to maintain and improve wine quality under the traditional winemaking regulations. The objective of the second research question was to establish the quality control procedures and analytical protocols to improve and assure the composition of wine from grape growing, through winemaking and aging processes.

As for the climate change, the main study was conducted in collaboration with the University of Belgrade (Serbia) developing the predictions of the future climate conditions in Tuscany, from the present until 2100. a result, the study showed that the Tuscany region will be warmer with lower precipitation amount in the 21st century, under 4.5 RCP scenario. Winkler index showed the increase in temperature which may be detrimental to the high-quality wine production. There is high probability that the Tuscany region will be very hot toward 2100 and the high-quality wine production will be threatened. Hence, the chances are that Tuscany will be suitable only for the table grape production, resin production or low-quality table wine. The water availability will probably decrease which may lead to the yield loss. The high-quality wine production is very likely to be endangered by the future upcoming climate changes in Tuscany. That is why it is important to apply the new strategies of adaptation to new climate conditions. Some of the strategies include the translocation of the vineyard area uphills, while the others suggest the substitution of the main grape variety with other grape varieties suitable for the high alcohol wine production. As for the future research, developing the prediction of the future climate conditions under 6.0 and 8.5 RCP scenarios .

When it comes to wine quality, the wine descriptive analysis of FT-IR and UV-vis Spectrometry properties of the past 8 vintages of Chianti and Chianti Classico DOCG allowed for an examination of their physicochemical properties and hence the differences in their quality. A fundamental finding was that wine properties like polyphenols, alcohol content, glycerin, potassium, pH value, residual sugar, succinic acid, dry extract, anthocyanins, density, and total acidity, had the statistically significant influence on the wine quality and its their typicity. The PLS multivariate statistical analysis demonstrated a good ability to separate wine samples and to predict the wine quality and typicity of new wine samples.

In the second study related to wine quality, the oxygen consumption kinetics of commercial oenological tannins in model wine solution and Chianti red wine were examined. Based on the research, the oenological tannins might become a substitute for SO_2 in wine protection from oxidation. As it was reported in this trial, the ellagitannin had the fastest reaction with the oxygen and it can be used as an efficient protector against oxidation. Ellagitannins are good antioxidants, even though their reactivity could diminish after the first saturation. The skin tannins have a stable reactivity in each saturation. Gallotannins might have the ability to inhibit enzymatic oxidation rather than having a direct reaction with oxygen.

In the third study of wine quality, the kinetics of oxygen consumption of six Sangiovese red wines from Tuscany were examined. Sangiovese wine samples coming from different areas of Tuscany had a different capacity of oxygen consumption. The analysis of metals did not show any important correlation between their content in wine and the total amount of consumed oxygen. Furthermore, the CIE Lab analysis of Sangiovese color showed a strong correlation between the consumed oxygen level in wine and yellow color formation, while the level of the red color decreased with the wine oxidation.

In the last study related to wine quality, the sweep voltammetry method was examined as a tool for monitoring the oxidative status in winemaking. The collection of the compositional data of wine provided a significant support for the enhancement of wine quality, especially for skin maceration and must fining processes, providing the enologists with a base to take immediate corrective measures. Monitoring the phenolic extraction during the maceration of red wine helps defining the duration of the process, as well as the extent of saignée in rosé wines and racking of red wines. In brief, monitoring the grape phenolic content, vinification samples and

finished wines would allow for development of quality control charts for enhancing the entire product quality management.

At the end, the cellar trials allowed the company to improve the wine sensory characteristics by eliminating the excessive CO_2 from Chianti DOCG wine.

8 Abstract

This PhD program was developed in collaboration between the Ruffino winery and the University of Bologna and the University of Belgrade. All the work is divided in three sections as follows:

The first part is related to the climate change in Tuscany. The ongoing climate change may affect the agriculture to a large extent since all the crop production is influenced by the weather conditions. Thus, even viticulture and wine production may subject to certain changes. The new meteorological conditions may have a strong impact to the DOP wine production that follows their appropriate production regulations. This is the reason why it is important to better understand the climate conditions of the last century and to estimate the future climate conditions in order to be able to adapt the wine production to the newly formed environment, keeping the same wine DOP style and quality characteristics. In this work, the climate conditions in Tuscany where assessed from 1990 until future 2100 year.

When it comes to the second part, the typicity and quality of the Chianti and the Chianti Classico appellation of origin were studied. These appellations are the main DOP wines from Tuscany, the production of which was initiated back in the 18th century. Nowadays, their style and the typicity are well known to the consumers worldwide. Therefore, it is particularly important to better understand their enological potential in order to be able to keep their style in the future. The main grape variety used for these wines is Sangiovese which is employed in at least 75% of the total production of the mentioned wines and it was studied by a multiparametric approach in this PhD research.

As for the third part, the wine oxidation, as one of the most important chemical reactions that influences the wine quality and aging, was studied. In the beginning of the wine production, during the alcoholic fermentation, the red wine composition is shaped during the maceration of the grape juice on the grape skin. In this moment it is important to monitor the polyphenol extraction that determinates compounds for the wine quality. Polyphenols are one of the main substrates of the wine oxidation and their content was assessed using the linear sweep voltammetry in the initial part of vinification.

In addition, the response of the Sangiovese red wines and model wines, with added oenological tannins to the oxygen saturation, was studied. These studies are important as they give us an image of the response to the oxidation process and how it influences the wine quality and the wine evolution.

Figure 7. 1 Drought and wine – climate change (source: www.winemag.com20200203wine-climate-change).

9 Abstract (Italian version)

Questo programma PhD è stato sviluppato in collaborazione con l'azienza Ruffino, l'Università di Bologna e l'Università di Belgrado. Tutto il lavoro è stato diviso in tre sezioni come segue:

La prima parte riguarda il cambiamento climatico in Toscana. Il cambiamento climatico in corso può avere conseguenze in larga scala sull'agricoltura perché tutta la produzione agricola è influenzata dalle condizioni meteorologiche. Pertanto, anche la viticoltura e la produzione di vino potranno subire dei cambiamenti. Le nuove condizioni meteorologiche potrebbero avere un forte impatto sulla produzione di vini DOP, la quale segue le sue proprie regole di produzione. Questa è la ragione per cui è importante comprendere meglio le condizioni climatiche dell'ultimo secolo e fare una valutazione delle condizioni climatiche future per poter adattare la produzione di vino all'ambiente di nuova formazione pur mantenendo le stesse caratteristiche stilistiche e la stessa qualità del vino DOP. Nel presente lavoro, le condizioni climatiche in Toscana sono state valutate a partire dal 1990 fino al futuro 2100.

Per quanto riguarda la seconda parte, sono state studiate la tipicità e la qualità del Chianti e della denominazione di origine Chianti Classico. Queste denominazioni sono le principali DOP vinicole in Toscana, la produzione delle quali è iniziata nel XVIII secolo. Oggigiorno, il loro stile e la loro tipicità sono ben note ai consumatori di tutto il mondo. Pertanto, è particolarmente importante capire bene il loro potenziale enologico per poter mantenere il loro stile anche in futuro. La principale varietà di uva usata per questi vini è il Sangiovese che è impiegata per almeno il 75% della produzione totale dei summenzionati vini e che è stata studiata con un approccio multiparametrico in questa tesi di dottorato.

Infine, la terza parte riguarda lo studio dell'ossidazione del vino, la quale è una delle più importanti reazioni chimiche che influenzano la qualità e l'invecchiamento del vino. All'inizio della produzione, durante la fermentazione alcolica, la composizione del vino rosso si plasma durante la macerazione del mosto d'uva sulla buccia della stessa. In questo momento è importante monitorare l'estrazione dei polifenoli la quale determina i composti per la qualità del vino. I polifenoli sono uno dei più importanti sostrati dell'ossidazione del vino e il loro

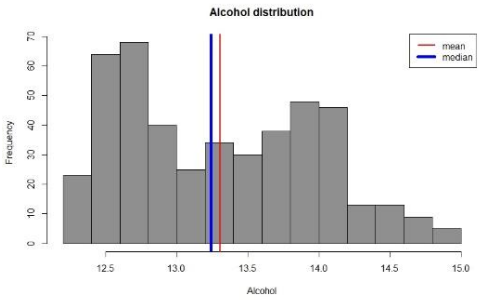
contenuto è stato valutato usando la voltammetria a scansione lineare nella parte iniziale della vinificazione.

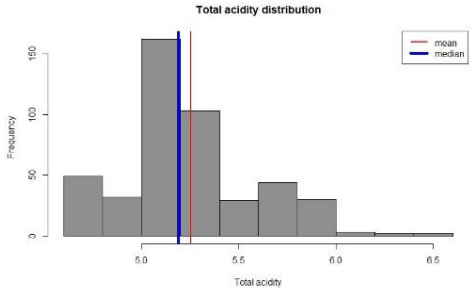
In aggiunta, è stata studiata la risposta dei vini rossi Sangiovese e delle soluzioni simil-vino, con l'aggiunta di tannini enologici alla saturazione dell'ossigeno. Questi studi sono importanti perché ci danno un'immagine della risposta al processo di ossidazione e di come ciò influenzi la qualità e l'evoluzione del vino.

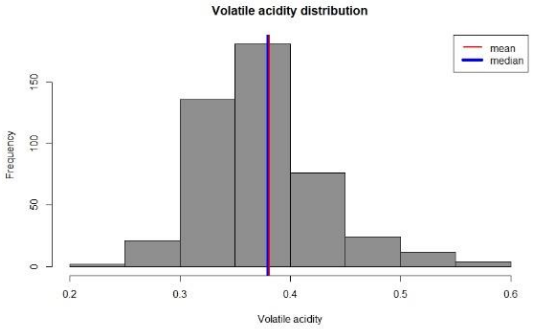
10 Appendix

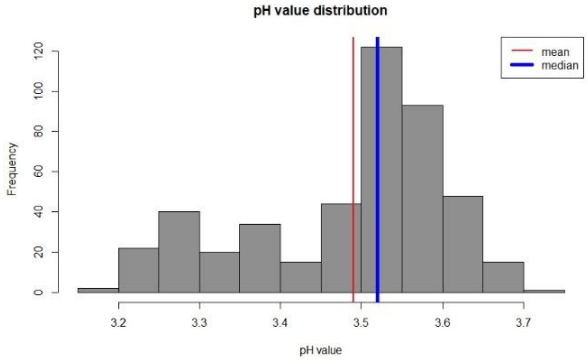
SUPPORTING INFORMATION

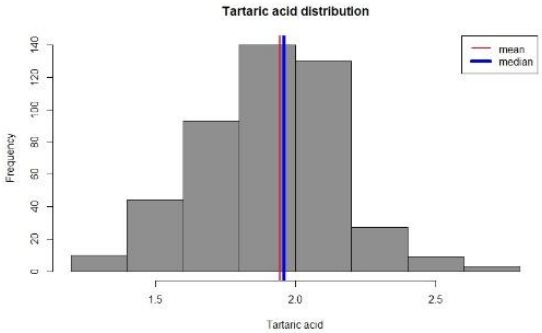
Chapter 3

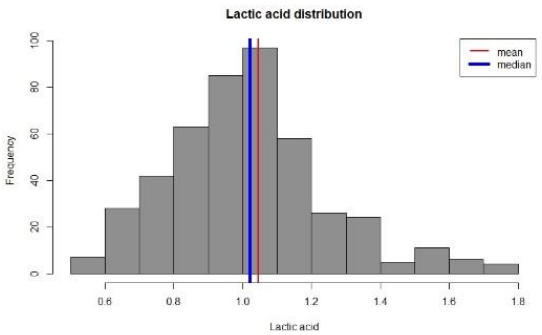


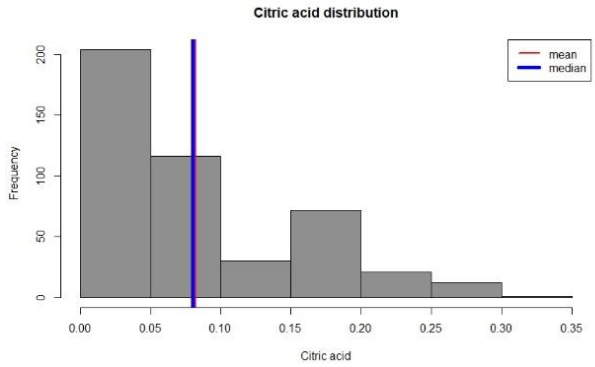


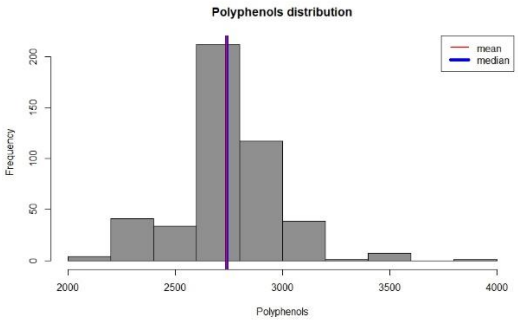


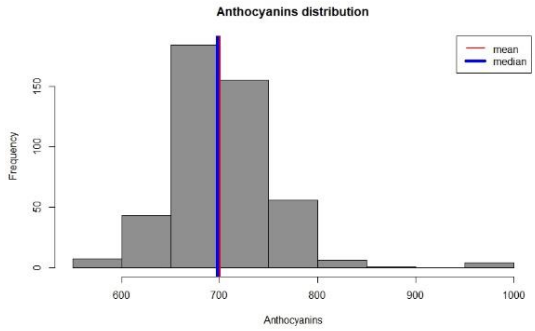


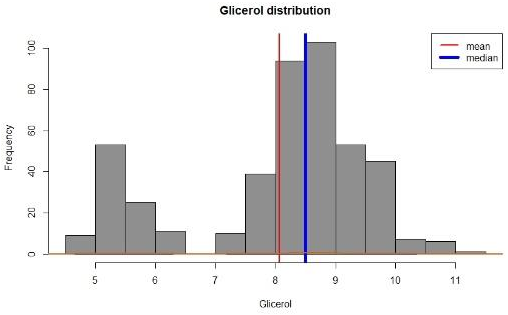


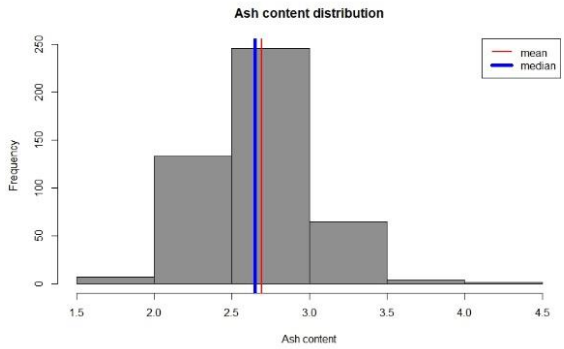


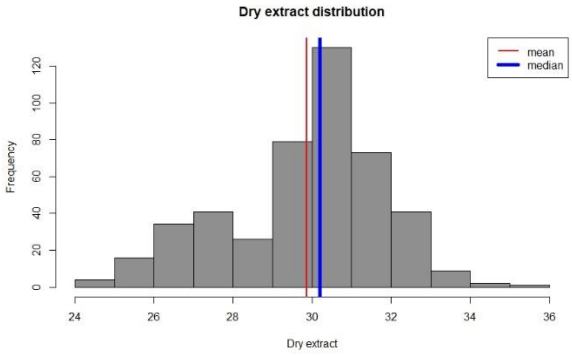

Citric acid distribution
mean
median
Frequency
Citric acid

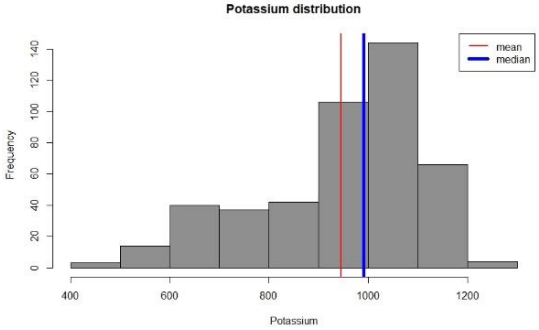

Polyphenols distribution
mean
median
Frequency
Polyphenols


Anthocyanins distribution
mean
median
Frequency
Anthocyanins


Glicerol distribution
mean
median
Frequency
Glicerol


Ash content distribution
mean
median
Frequency
Ash content


Dry extract distribution
mean
median
Frequency
Dry extract


Potassium distribution
mean
median
Frequency
Potassium

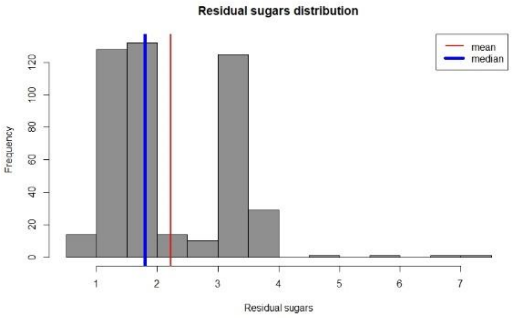

Residual sugars distribution
mean
median
Frequency
Residual sugars

Figure S1. Distribution of wine samples by the main chemical properties

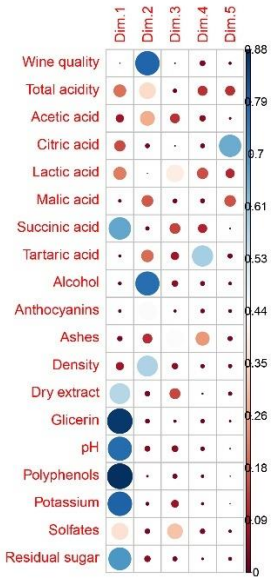


Figure S2. Corrplot of the variables that determine the principal components of Chianti and Chianti Classico wines.

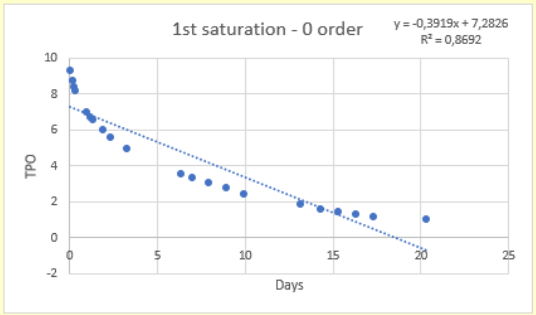
```
> get_eigenvalue(res.pca)
       eigenvalue variance.percent cumulative.variance.percent
Dim.1  6.37140943       33.5337338                    33.53373
Dim.2  3.90074488       20.5302362                    54.06397
Dim.3  1.94507014       10.2372113                    64.30118
Dim.4  1.48336405        7.8071792                    72.10836
Dim.5  1.18584319        6.2412799                    78.34964
Dim.6  0.81666882        4.2982570                    82.64790
Dim.7  0.77145138        4.0602704                    86.70817
Dim.8  0.59231834        3.1174649                    89.82563
Dim.9  0.49545689        2.6076678                    92.43330
Dim.10 0.36274291        1.9091732                    94.34247
Dim.11 0.28098364        1.4788612                    95.82134
Dim.12 0.18487831        0.9730437                    96.79438
Dim.13 0.14906156        0.7845345                    97.57891
Dim.14 0.12322775        0.6485671                    98.22748
Dim.15 0.10273485        0.5407098                    98.76819
Dim.16 0.09737406        0.5124951                    99.28069
Dim.17 0.06863359        0.3612294                    99.64191
Dim.18 0.04445120        0.2339537                    99.87587
Dim.19 0.02358500        0.1241316                   100.00000
```

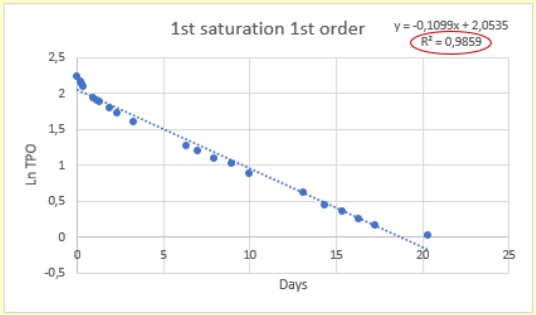
Figure S3. Eigenvalues of PCA of Chianti and Chianti Classico wines.

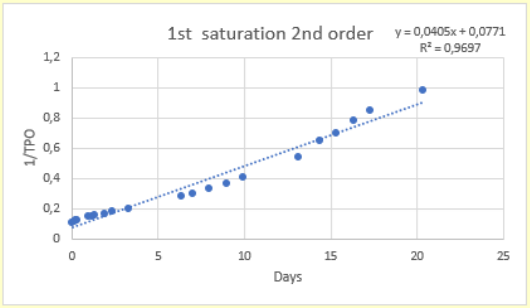
Chapter: 4

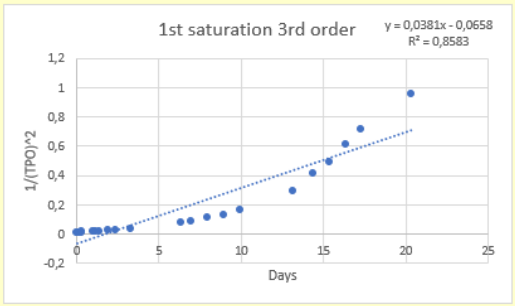
I

1st saturation kinetic order graphs



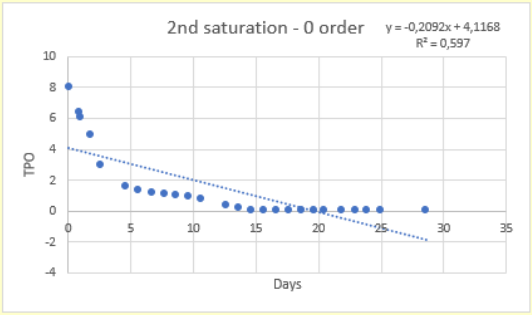


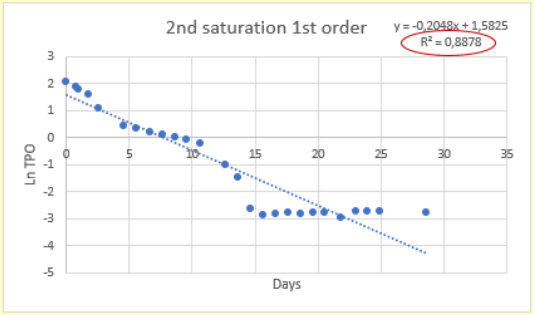


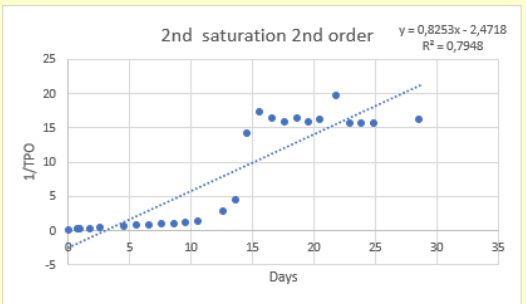


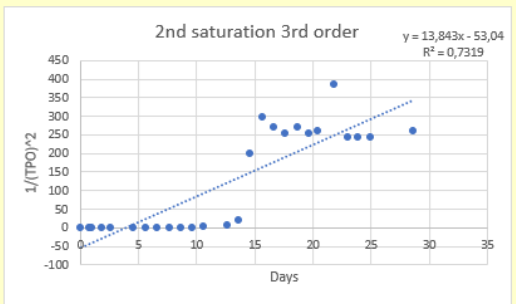
II

2nd saturation kinetic order graphs



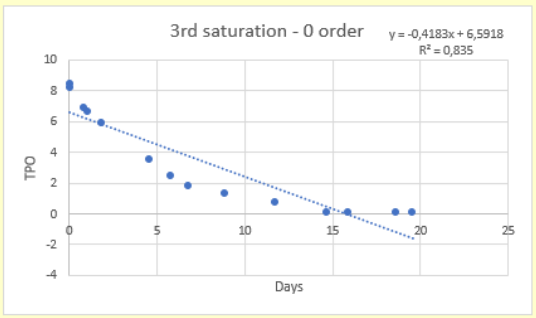


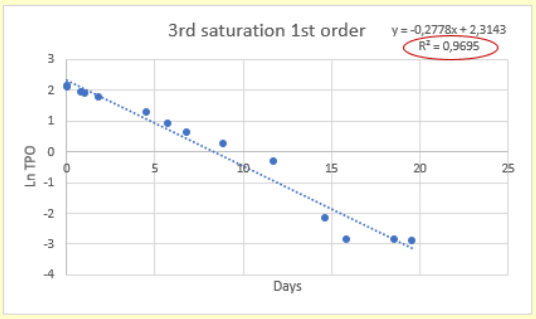


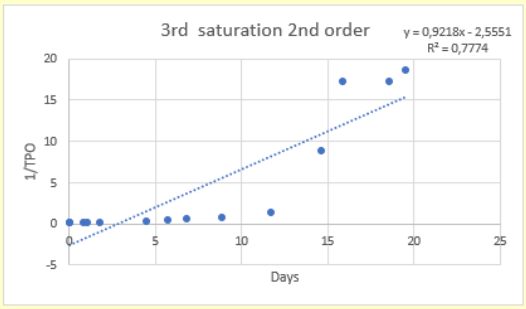


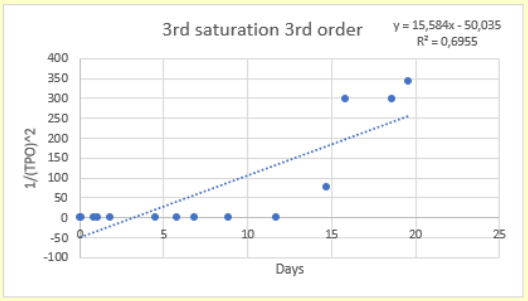
III

3rd saturation kinetic order graphs



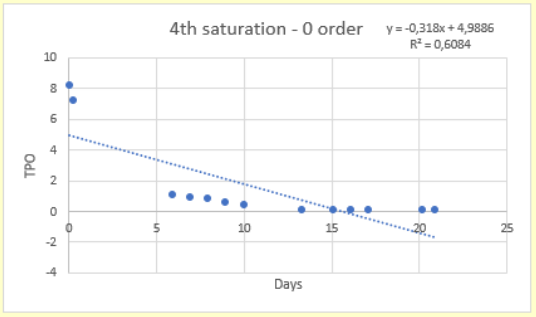


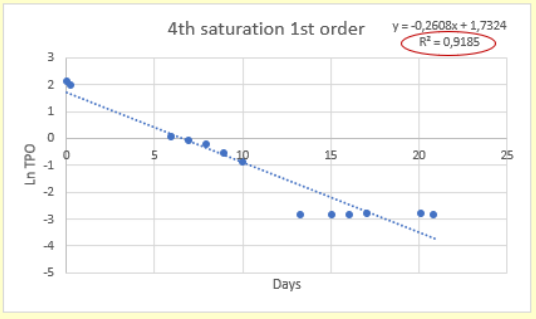


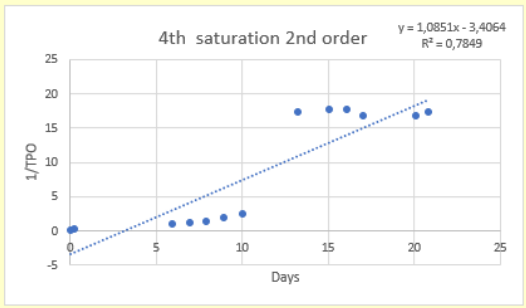


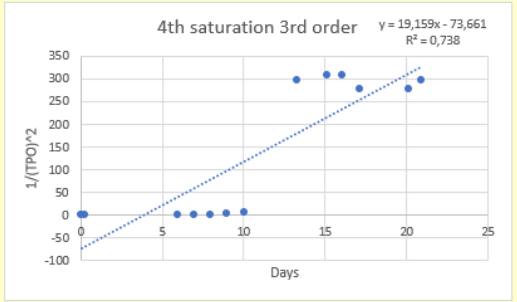
IV

4th saturation kinetic order graphs









V

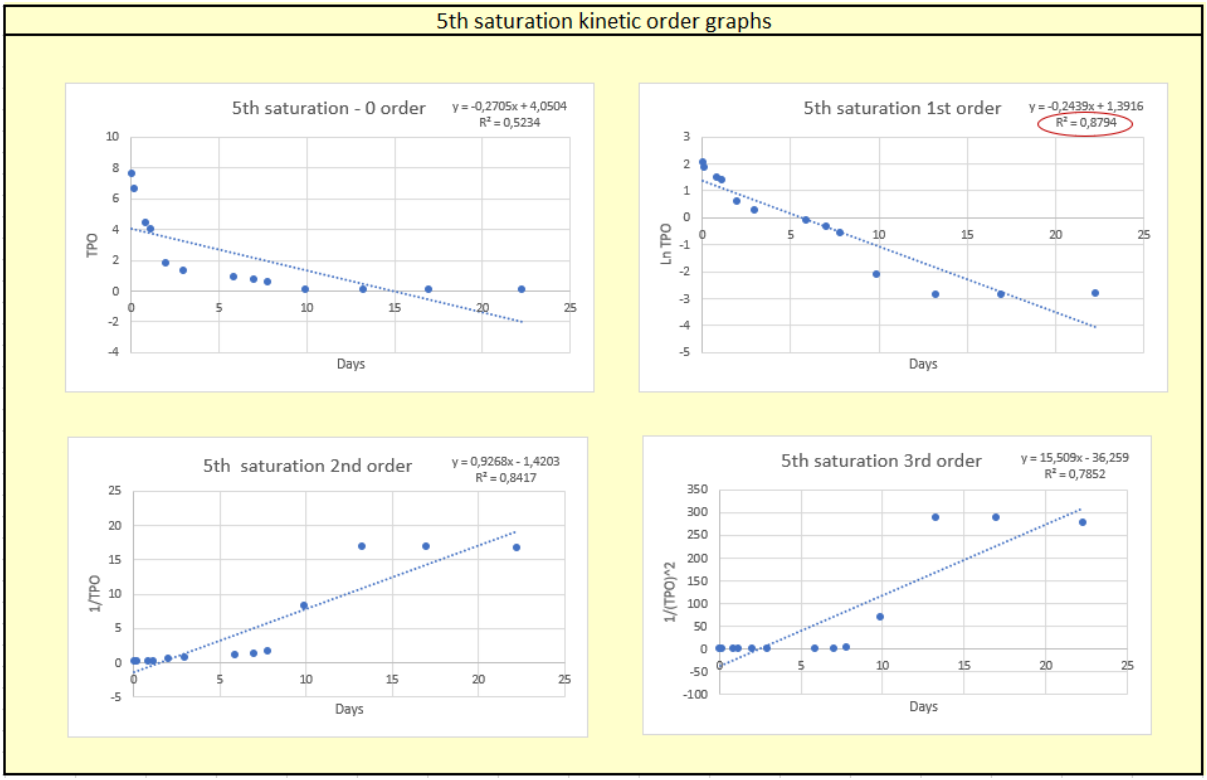


Figure S4. Linear regression plots of zero, first, second and third order of kinetic reaction of the sample D. I kinetic orders of the first saturation; II kinetic orders of the second saturation; III kinetic orders of the third saturation; IV kinetic orders of the fourth saturation; V kinetic orders of the fifth saturation

Chapter : 4

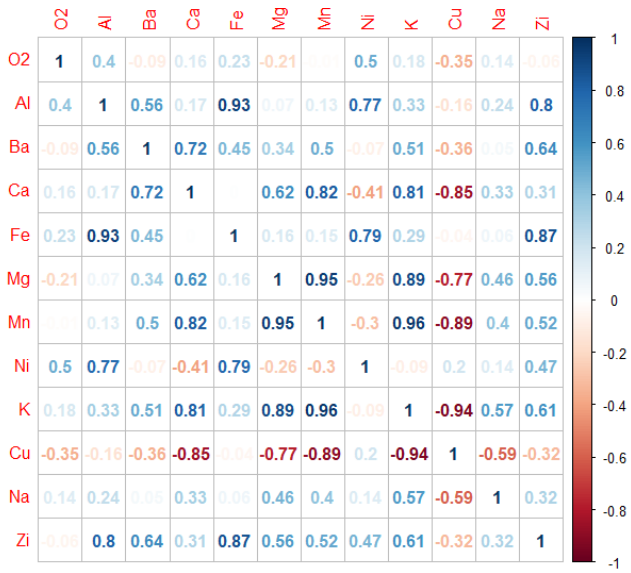


Figure S5. Corrplot of oxygen consumption and metals analysed by ICP-MS.