UNIVERSITY OF NAPLES FEDERICO II

DEPARTMENT OF AGRICULTURAL SCIENCE

Ph.D. in Agricultural and Food Science

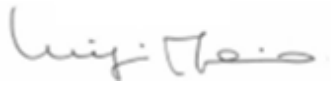
XXIX Cycle (2014-2017)

Oxygen contribution to phenolic evolution during aging of red wines

Ph.D. Luigi Picariello

Tutor

Prof. Luigi Moio



Co-tutor

Prof.ssa Angelita Gambuti

Coordinator

Prof. GuidoD'Urso

“*C’est l’oxygene qui fait le vin*”

Louis Pasteur

Introduction

The effects of oxidation in foods and wines are usually considered detrimental and they include degradation of vitamins or lipids, loss of nutritional value, development of off-flavors, and browning (Bradshaw & Prenzler 2001). The oxidation can be distinguished in enzymatic and chemical depending on the involvement or less of specific oxidases. In the case of wine enzymatic oxidation almost entirely occurs in grape musts while the chemical oxidation is dominant during wine aging. In grape must, the enzymatic browning is correlated with the content of hydroxycinnamates such as caffeoyltartaric acid (caftaric acid) and para-coumaroyltartaric acid (coutaric acid) (Cheynier, et al. 1988) and it is due to the action of polyphenoloxidases tyrosinase and laccase (Li, Guo & Wang, 2008).The enzymatic browning is largely limited by a proper use of SO_2 because it inhibits tyrosinase and, in less measure, laccase activities. Less manageable and, if excessive, dangerous for the wine quality and for its longevity, is instead chemical oxidation.

The chemical oxidation is often associated with a sensory degradation consisting in: the production of new pigments as well as the xanthylium cation pigments (Es-Safi et al., 2000, Clark & Scollary, 2002; Labrouche et al., 2005, Clark et al., 2007) and, the formation of off-odour such as aldehydes or sotolon (Ugliano, 2013). Wines with a peculiar aroma profile obtained by aging under oxidative conditions, for example Madeiras, Cherry and Jerez, are an exception. On the other hand, many reactions that favor wine stability, soften tannin harshness and improve aroma, could not take place without moderate contact with oxygen (Castellari et al., 1998; Atanasova et al., 2002, Gambuti et al., 2012; Ugliano, 2013). Although it is now clear that a moderate oxygen supply is a fundamental step to improve wine quality and its shelf life, it remains difficult to effectively assess oxygen demand of each wine and how to avoid excessive oxidation. This is due to the fact that, though important researches on wine oxidation chemistry and in the role of antioxidants has been achieved in recent years

(Danilewicz, 2003; Laurie & Waterhouse 2006; Nikolantonaki & Waterhouse 2012), the mechanistic chemistry of the action of each wine components is not entirely outlined.

Another important aspect is the use of antioxidants to prevent excessive wine oxidation. Sulfur dioxide is widely used in winemaking to inhibit oxidation. However, SO_2 is toxic (Yang & Purchase 1985) to some groups of people and may cause allergic reactions. As a consequence numerous efforts have been made to limit the use of SO_2 (Peng, et al., 1998, Roussis, et al., 2007 Guerrero, & Cantos-Villar, 2015). In the past, ascorbic acid has been considered as an alternative antioxidant (Zoecklein et al., 1995; Rankine, 2002) but, issues regarding its subsequent pro-oxidant activity, strongly limit its applicability (Peng, et al., 1998). An alternative option for the prevention of the oxidation during wine production and aging, is furnished by glutathione which is efficient in protecting white wines against browning and loss of flavor (Singleton et al., 1985; Kritzinger et al., 2012 and Roussis et al., 2007; Cheynier & Van Hulst, 1988). Although several researchers have showed that sulfur dioxide, glutathione and ascorbic acid are all excellent reducing agents that are capable of interacting with oxidation products, the influence of these compounds, on the outcome of winemaking techniques involving a moderate uptake of oxygen such as micro-oxygenation and nano-oxygenation, still require more study. It is also important to underline that the needing of antioxidant protection of a wine is strictly linked to its phenolic composition, being differences between white and red wine longevity, the most notable example. The most proper way to preserve quality of a wine is therefore to start considering its phenolic composition and the possible effect that, first the moderate oxygen uptake and, then the antioxidants addition, can determine on its quality and longevity. The mains endogenous antioxidants in wine are in fact the phenolics compounds. These compounds, particularly anthocyanins, flavanols and condensed tannins, have also a fundamental role in wine sensory quality (Mazza et al., 1999; Waterhouse, 2002). Anthocyanins being mainly responsible for red wine color, flavanol and condensed tannins for bitterness and astringency. All these

classes undergo numerous chemical reactions, more or less triggered by oxygen during red wine aging, that determine the formation of new colored and uncolored polymers (Hayasaka, & Kennedy, 2003) and changes of the bitterness and astringency characteristics of wine (Monagas, et al., 2005).

Numerous are therefore the endogenous (e.g. anthocyanins, proanthocyanidins and pH,) and exogenous (e.g. sulfur dioxide, hydrolysable tannins and glutathione) factors that are worth investigation for the important implication for red wine quality and longevity. For all these reasons the first part of this study has been aimed to furnish a contribution to better understanding red wine oxidation, by evaluating the role of main wine phenolics and exogenous antioxidants on the evolution of sensory active phenolics during the oxidation of red wines. In this part of the PhD thesis three experiments evaluating the role of anthocyanins, condensed and hydrolysable tannins, glutathione and sulfur dioxide on the outcome of wine oxidation have been performed. The second part has been focused on industrial experiments in which practices that allow measured amounts of oxygen supply to wine during aging, the micro-oxygenation MOx (oxygen supply into the range 1-20 mg/L/month) and the nano-oxygenation NOx (oxygen supply into the range 1-5 mg/L/year), were evaluated. Positive effects of MOx can be obtained when the treatment is applied correctly but, when the oxygen dosage is too high, the appearance of oxidative off-flavour is very detrimental. The presence of exogenous antioxidants as well as the relationships with expected shelf-life of red wines are critical points that have been investigated in the second part of this PhD thesis. In a second experiment, controlled exposure of wine to small quantities of oxygen during bottle aging (1–5 mg/L/year O2), has been evaluated. Although the use of closures with specific oxygen permeability is an important opportunity for winemakers, data in the literature are still related to few red grape varieties and not very rich in tannins. Because Ultra-Premium wines are usually rich in polyphenols (Ritchey & Waterhouse 1999; Fanzone et al., 2012) the

investigation on the effect of NOx on the evolution of phenolic composition and sensory profile of tannins-rich red wines has been proposed.

1. BACKGROUND

1.1 Reactions of oxygen in wine

Phenolic compounds are primary substrates for wine oxidation. Starting from their oxidation a cascade of chemical transformations occurred and significant changes of sensory characteristics of wine were determined. The first mechanistic pathway of the chemical reactions involved in wine oxidation was defined by Vernon Singleton in 1974 who stated that reaction of oxygen was with phenolic substrates, and that the initial phenolic oxidation products lead to the subsequent reactions. Although last decades have seen a growing interest of scientific community in mechanisms involved in wine oxidation, the management of these transformations is still a critical point to the production of wine.

Under the chemical point of view the oxidation is a gain of oxygen or a loss of electrons, whereas reduction is a loss of oxygen or a gain in electrons. Molecular oxygen (O_2) does not directly react with most wine components (triplet state); it needs to be activated to the singlet state to become reactive and then be reduced to water (Ribéreau-Gayon et al., 2000; Danilewicz, 2003). In wines, this process requires reducing species with a cathecol-like structure (o-diphenol groups) and the presence of transition metal ions, particularly Fe(II). The oxidized polyphenol (quinone) is generated, and oxygen is reduced to hydrogen peroxide. Quinones and hydrogen peroxide are main player of further processes of oxidation. Quinones are electrophiles (Singleton, 1987) and react directly with nucleophilic compounds present in wine, like thiols (Singleton, 1985; Lavigne et al., 2007), bisulfite and flavan-3-ols by a Michael addition reaction. Hydrogen peroxide is instead quenched by sulfur dioxide, if present. When sulfur dioxide is consumed the hydrogen peroxide reacts with Fe^{2+} via the

Fenton reaction to produce highly reactive hydroxyl radicals, which, in turn, oxidize additional wine constituents, including the most abundant, the ethanol to produce acetaldehyde (Wildenradt & Singleton, 1974). Aldehydes then react with bisulfite, phenolics, and other nucleophilic species (Peterson & Waterhouse, 2016). When all nucleophiles are consumed aldehydes and by-products accumulate in wine contributing to oxidation spoilage (Waterhouse et al., 2016).

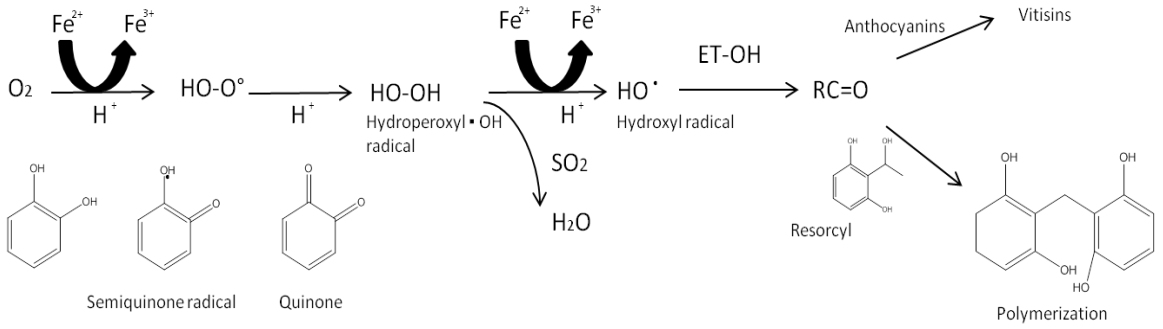


Fig.1. Wine phenolic oxidation pathway and subsequent hydroxyl radical oxidation.

In further paragraphs the key compounds involved in first steps of wine oxidation cascade, molecular oxygen, hydrogen donor molecules, transition metal catalysts and quinones will be described more in detail.

1.2. Oxygen

During wine production and aging wine comes into contact with air determining O_2 dissolving in the medium. The solubility of oxygen in must and wine is influenced mainly by temperature and, in less measure, by the solid particulate and ethanol content (Singleton,

1987). At room temperature and atmospheric pressure the oxygen solubility in air-saturated wine is 6.0 mL/L (8.6 mg/L) (Singleton 1987). As an example du Toit et al., (2006) reported that between 5 and 35 °C the amount of O_2 necessary to saturate wine drops from 10.5 mg/L to 5.6 mg/L. Each phase of wine production determine a dissolution of O_2. More in detail: during the crushing and pressing of fresh grapes, must is almost saturated with O_2 (Schneider, 1998); fermentation determines production of CO_2 which probably remove O_2 outside the tank (Boulton et al., 1999); winery operations determine different supplies of O_2: pumping wine from tank to tank resulted in an oxygen addition from 0.1 mg/L up to 6 mg/L (du Toit et al., 2006); racking resulted in 3-5 mg/L; centrifugation added from 1.0 mg/L. up to 8 mg/L (du Toit, 2006); protected pump-overs added 2.2 mg/L while 7.4 mg/L for those with deliberate aerative splashing were observed; filtration determine an addition of 4-7 mg/L. Aging containers, stainless steel tank, wood barrel and bottle, strongly affect the evolution of wine due to O_2 transferred to wine x year. In each kind of tank oxygen is distributed in layers, reaching levels close to saturation at the interface with the tank headspace and down to almost zero oxygen below the first 10 to 20 cm of wine volume (Moutounet & Mazauric 2001). During barrel aging the amount of oxygen added to wine varies in a wide range (from 8 to 45 mg/L/year), depending on the humidity of the wood and the thickness and the grain of the staves. The lower the humidity, the tighter the grain and the thinner the staves, the higher the O_2 permeating into the wine (Vivas et al., 2003; Del Alamo-Sanza et al, 2017). Micro-oxygenation, a practice that simulate barrel oxygen transfer delivering oxygen in the form of bubbles through a porous diffuser, allowed a controlled amount of O_2 to be added. Once a wine is bottled wine is exposed to O_2 coming from the bottling procedures used (dissolved and headspace O_2), and the ingress through the closure (ranging from 0.3 to 4.8 mg/L/year) (Oliveira et al., 2013). As expected, for each phase of winemaking and aging, the level of oxygen reached quickly decreased after the oxygenation ceased.

1.3. Activation of Oxygen in wine

1.3.1. Transition metals catalysts

As previous stated oxygen cannot directly react with wine compounds but needs to be activated. At wine pH 3.5 the oxidation of catechol to quinone, along with that of reduction of oxygen to H_2O_2 cannot occur in thermodynamics ($\Delta E_{3.5}$ = - 0.01 V) (Main, 1992; Li et al., 2008) but transition metals, particularly iron and copper, can increase the reaction rate, and initiate the reactions (Danilewicz, 2003). Iron and copper, the wine metal ions involved in oxidative spoilage, not only activate molecular oxygen but also catalyze the degradation of hydrogen peroxide to the hydroxyl radical in the Fenton reaction. These transition metal ions are present in wine due to the soil where vines were grown and the release from equipment used during winemaking.

As recently reported by Waterhouse et al. (2016) at the beginning of past century the importance of iron (Fe) and copper (Cu) as catalysts of wine oxidation was already presumed by Ribéreau-Gayon (1931) who observed that the removal of Fe and Cu with potassium ferrocyanide stopped the oxidation. At that times the contamination of wines by transition metals was high and most wines resulted dramatically oxidized. Nowadays, thanks to the modern vineyards equipment and practices and the growing use of stainless steel tanks in winery, wines contain lower average iron levels below 5 mg/L and of copper below 0.3 mg/L (Li et al., 2005). These low amounts are however enough to catalyze the oxidation processes. It is now clear that the process depends also on metal speciation. The reduced species dominate likely due to the acidic, reducing environment of wine and the presence of phenolics (Elias & Waterhouse, 2010). Cu and, in less extend Mn, accelerate Fe(II) oxidation and determine an higher oxidation of wine (Peynaud 1984; Cacho et al., 1995; Danilewicz, 2016).

1.3.2. Wine phenolics as hydrogen donor molecules

Phenolic compounds are primary substrates for oxidation. Among the wide group of wine phenolics, the compounds with catechol functionality containing a 1,2,3-trihydroxyl group (pyrogallol), a 1,2- dihydroxy aromatic ring (catechol) are easily oxidized to yield the resulting phenoxyl semiquinone radical that can be stabilized by a second oxygen atom to give the quinone. Reversibly, the quinones can be reduced again to the hydroquinones. These compounds are involved in main steps of oxidation cascade: at the beginning to start oxidation process and later, to react with ROS such as the hydroperoxyl radical produced by Fenton reaction. Not all wine phenolics contain characteristics catechol functionality, for example the main anthocyanin present in red wine, malvidin-3-glucoside, is not readily oxidized while oligomeric and polymeric phenolics (procyanidins and condensed tannins) that present a catechol group in the B-ring can both trap radicals and chelate metals acting also as antioxidant (Lotito et al. 2000).

1.3.3 Quinone reactions

Quinones are key reactive chemical intermediates formed in abundance during the oxidation of wine. As previously stated these compounds can be reduced back to hydroquinones but they can also strongly react with nucleophilic compounds. All these reactions are of fundamental importance for wine aging determining important consequences for wine longevity and aging. Wine nucleophiles like bisulfite, nucleophilic thiols, amino acids and flavan-3-ol nuclephiles give condensation reactions with them determining several effects on wine flavor as the loss of varietal aromas (e.g. Sauvignon blanc volatile thiols), the disappearance of undesirable reductive aromas due to some thiols, the formation of oxidative off-flavors originated from the oxidative deamination process (Strecker degradation) of

amino acids (Bittner, 2006; Ugliano, 2013; Waterhouse & Nikolantonaki, 2015) and the formation of brown pigments (Cheynier et al., 1989). The antioxidants sulfur dioxide and ascorbic acid also played an important role in reducing quinones back (Cheynier et al., 1995). Another antioxidant, the glutathione has been investigate for its nucleophilic reaction with quinones. As glutathione is capable of performing nucleophilic reactions with quinone it can protect important aroma compounds, such as volatile thiols, competing with them for electrophilic quinones. At the same manner it can limit the formation of off- flavor aldehydes from Strecker degradation (Nikolantonaki & Waterhouse, 2012).

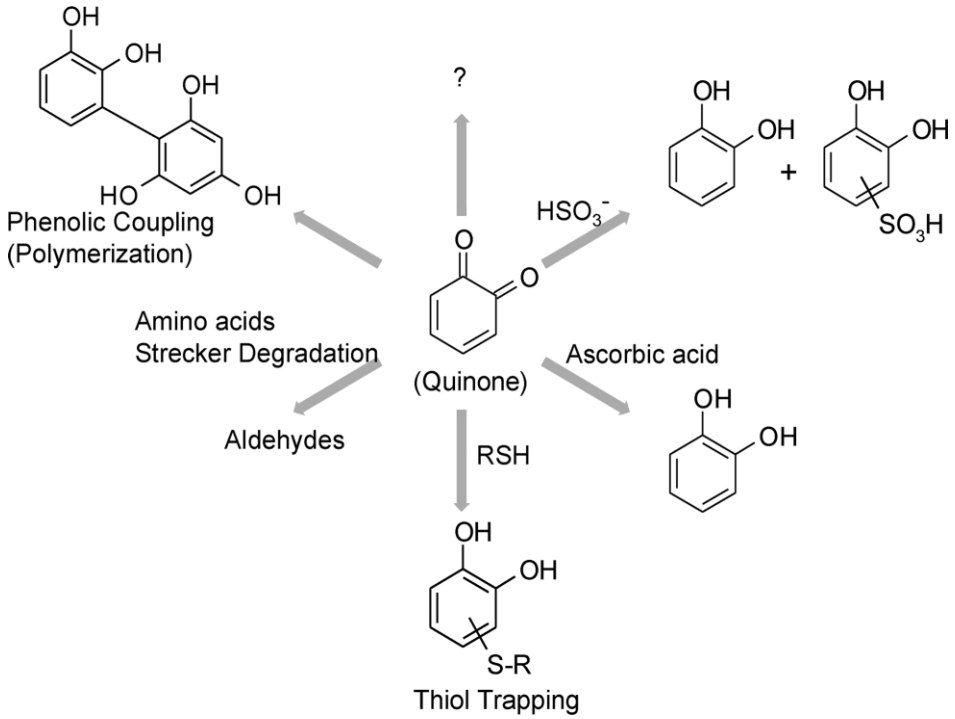


Fig.2. Structural Hypothesis of Reaction Products between quinone and wine relevant nucleophiles

1.4 Main exogenous antioxidants

1.4.1Sulfur dioxide

Sulfur dioxide (SO_2) is the most widespread used chemical preservative due to its antimicrobial and antioxidant properties. It is a gas very soluble in H_2O and in wine it exists as several species in wine:

- SO_2(aq) also called molecular SO_2 in equilibrium with SO_2(g)
- Free bisulfite ion ($HSO^{3}-$) which prevents wine oxidation
- Bound bisulfite: they are covalent adducts deriving from the reaction of bisulfite ion with aldehydes and other electrophilic wine components; Weakly bound adducts can contribute to free SO_2 pool following loss of free bisulfite.
- Sulfite ion ($SO_3{}^{2}-$) wich represents <1% of SO_2 species at wine pH.

Under the analytical point of it is possible to distinguish the free SO_2= molecular SO_2 + free bisulfite ion HSO_3- and the total SO_2 as the sum of all species present in wine.

Unfortunately, the addition of SO_2 to wines raises health-related objections due to serious allergic reactions incurred by sulfite-sensitive individuals (Yang and Purchase, 1985; Warner et al., 2000; Zhou et al., 2004), and concerns over sulfites have resulted in regulatory restrictions set by World Health Organization (WHO) and International Organization of Vine and Wine (O.I.V.). The US FDA estimates that as many as 1% of the general population show an increased degree of sensitivity to sulfites (Papazian, 1996). Starting from 2004 wines must prominently display on the bottle the presence of total sulfites in excess of 10 mg/l (European Union Regulation 1991/2004). Furthermore excessive quantities of SO_2 can give the wine unpleasing aroma or may favor the wine to turn cloudy during its keeping (Ribereau-Gayon et

al., 2006; Li et al., 2005). For all these reasons the market of wines with low SO2 levels have been increased in last decades (Azabagaoglu, et al., 2007).

The knowledge of specific activity of each species in wine is an important step to better manage SO_2 addition preventing wine spoilage without alter wine healthiness and sensory quality. It has been reported that molecular SO_2 is the most active against wine microbial spoilage (Boulton et al., 1996) because it restrict growth of a wide range of microorganisms, including yeasts and bacteria (Beech & Thomas, 1985). The antiseptic action is due to several effects such as the reduction of co-factors and vitamins (NAD+, FAD, thiamin) (Waterhouse et al., 2016), the reduction of disulfide bridges in proteins (Waterhouse et al., 2016), and the reaction with nucleic acids (Fugelsang, & Edwards, 2007). The level of SO_2 as molecular form of SO_2 depends on wine pH, ethanol content and temperature. A pH decrease or an ethanol content or temperature increase causes an increase in molecular SO_2. Concerning the reaction of SO_2 with oxygen, the direct interaction is very unlikely to occur because it should involve a metal-catalyzed radical chain reaction in which bisulfite is oxidized to the sulfite radical ($SO_3^{\bullet -}$). Because of the radical scavenging activity of polyphenols present in wine, this direct interaction of oxygen and bisulfite is quite impossible (Danilewicz, 2007). The main specie involved in SO_2 antioxidant action is instead the free bisulfite ion which reacts with both key compounds involved in oxidation cascade: quinones and hydrogen peroxide. More in detail free bisulfite reacts with quinones giving nucleophilic addition and reduction to regenerate hydroquinones (Danilewicz, 2007); the nuclophilic displacement of HSO_3^- by H_2O_2 with consequent production of H_2O and sulfuric acid inhibits, instead, the Fenton reaction (McArdle & Hoffmann, 1983).

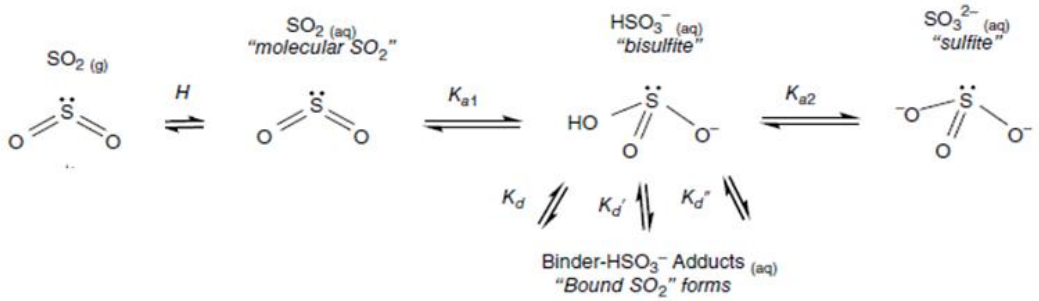


Fig.3. Equilibria of sulfur dioxide species.

Total SO_2 concentration is limited by current regulation but part of them is not really "active" in wine. This is because carbonyl and keto compounds present in wine form covalent adducts (sulfonates) with $HSO3^-$ ion. Bound forms of SO_2 show decreased antiseptic activity compared to free SO_2 while has no antioxidant effect (Boulton, 1996). SO_2 binders can be classified as "weak" or "strong" based on the dissociation constant, acetaldehyde being the strongest, (Kd = 2.06 × 10−6 at pH 3.5)(Burroughs at al., 1973).

1.4.2 Ascorbic Acid

Due to healthy and sensory criticisms about the presence of sulfur dioxide in wine other antioxidants has been proposed. Among them one of most used especially for white wine production, is ascorbic acid. This antioxidant is also present in grapes at concentrations ranging between 5 and 150 mg/kg of fruit (Zoecklein et al., 1999). The addition of 250 mg/l of ascorbic acid is allowed (OIV – Code International des Pratiques Œnologiques, 2016). While no replacement for the anti-microbial role of sulfur dioxide was observed, ascorbic acid and its optical isomer, erythorbic acid, could replace sulfur dioxide as anti-oxidant in wine defence against oxidation.

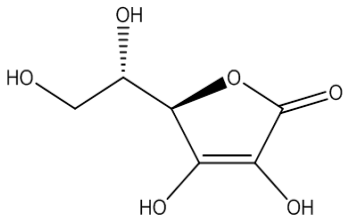


Fig.4. Ascorbic acid.

The efficacy of ascorbic acid as an antioxidant in winemaking is mainly due to its capacity to scavenge molecular oxygen in the presence of iron as a catalyst (Danilewicz, 2003; Kilmartin, 2010). In addition, in presence of oxidized phenolics, ascorbic acid is thought to be able to recycle o-quinones back to o-dihydroxyphenols (Isaacs & Van Eldik, 1997; Nikolantonaki & Waterhouse 2012). The reaction between ascorbic acid and oxygen results in hydrogen peroxide and dehydroascorbic acid. The former can become really dangerous for wine oxidation if is not anymore quenched by sulfur dioxide. This is why ascorbic acid can only briefly delayed oxidative spoilage, as long as a wine contains sufficient free SO_2 to consume any H_2O_2 formed (Flanzy 1959; Fessler 1961). Otherwise hydrogen peroxide is produced and a dramatic pro-oxidative effect can be observed (Ough 1992; Peng & Sefton, 1998).

1.4.3 Glutathione

Glutathione GSH, is a tripeptide comprised of three amino acids (cysteine, glutamic acid, and glycine) that is the most abundant non-protein thiol compound widely present in living organisms, from prokaryotes to eukaryotes (Anderson, 1998). As it exists in the reduced GSH and oxidized form as glutathione disulfide (GSSG) it acts as an antioxidant, a free radical scavenger and a detoxifying agent in living cells.

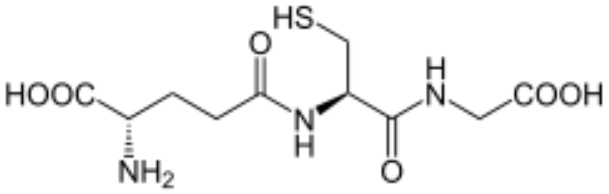


Fig.5. Glutathione

In grape wine the glutathione content varies from 17 to 114 mg/kg in grapes and from 14 to 102 mg/L in musts (Cheynier et al., 1989). Fracassetti et al. 2011 showed that concentration of GSH in grape juice ranged from 1.1 to 42.3 mg/ L, which correlates well with values previous reported (Maggu et al., 2007; Janes et al., 2010). In wine, the highest GSH concentration detected was 27.4 mg/L;during wine ageing, GSH concentrations are known to decrease, leading to lower concentrations of this compound in older wines (Lavigne et al., 2007).

GSH strongly influence wine oxidation pattern because of thiolic group can regenerate quinones to the native phenolics (Singleton, et al., 1985). The first practical evidences of its important contribution to wine stability is linked to its ability to limit must browning due to polyphenoloxidase activity (Cheynier, et al., 1989; Dubourdieu & Lavigne-Cruege 2003). During grape crushing polyphenoloxidase catalyze the oxidation of hydroxycinnamates to the corresponding o-quinones. GSH, with its thiolic group serving as an electron-rich nucleophilic center, gives nucleophilic reactions with quinones (Salgues, M. et al., 1986), The product, is a thioether, the 2-Sglutathionyl caftaric acid, called also grape reaction product (GRP) taht is not a substrate for further oxidation. As GSH gives nucleophilic reaction with quinones, an important role in chemical oxidation is also expected (Nikolantonaki & Waterhouse 2012). It prevents the loss of varietal wine flavor during the aging of wines such as the Sauvignon blanc (Ugliano, et al., 2011) and of terpene alcohols such as linalool in Muscat wines (Papadopoulou & Roussis, 2001) and Debina white wines (Papadopoulou & Roussis, 2008). Also a synergic action between glutathione and SO_2 in prevent the oxidative evolution of phenolic and volatile compounds during micro-oxygenation of red wine (Gambuti et al., 2015) and the aging of sparkling wines (Webber et al., 2017) has been observed. In contrast, a more recent study showed that the oxidation of glutathione leaded to the formation of its disulfide (Arapiptas et al., 2016) which in turn reacted with SO_3H^- to provide S-sulfonated glutathione ($GSSO_3H$) and then, decreasing the available $HSO3^-$.

However molar ratio GSH/SO2 usually occurring in real wines are much lower that those studied by Arapiptas et al., (2016) suggesting the existence of other competitive reactions. Due to all these positive effects, the addition of GSH in must (maximum up to 20 mg/L) has been recently allowed by the *Organization Internationale de la Vigne et du Vin* – OIV (OIV – Code International des Pratiques Œnologiques, 2016).

1.5 Sensory active Phenolics

Phenolic compounds play a major role in wine quality because they contribute to sensory characteristics of wines, in particular color, astringency bitterness and to wine aging behavior. The higher the content of phenolic compounds in a wine (usually ranges between 2000 to 6000 mg/L), the higher the capacity of wine to tolerate oxygen over time. They are synthesized in grape tissues and transferred to must and wine during winemaking. Wine phenolics are grouped into two categories, the flavonoids (C6 – C3 – C6) and non-flavonoids (C6 – C1 the *p*-hydroxybenzoic group, C6 – C3 the cinnamic group and C6 – C2 – C6 stilbenes).

Under the enological and sensory point of view the most important groups belong to the flavonoids category and are: the anthocyanins which are responsible for red wine color and, the flavan-3-ols and condensed tannins which are responsible for bitterness and astringency of wines. Flavonoids accumulate in grape as a defense tool against stress because they are potent antioxidants because they scavenge reactive oxygen species (Pannala et al., 2001). Structural characteristics, as the o-dihydroxy structure in the B ring and the 2,3 double bond in conjugation with the 4–oxo function in the C ring, are essential for effective free radical scavenging activity (Rice-Evans, et al., 1996; Lien, et al., 1999).

1.5.1 Anthocyanins

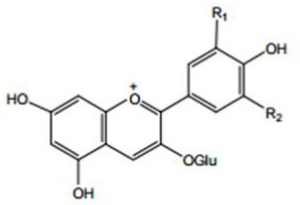
Anthocyanins are water soluble flavonoid pigments that confer color to red grapes skins. Only the "teinturier" grapes have anthocyanins also in the pulp (Cabezas, et al., 2003; Santiago, et al., 2008). Grape anthocyanins have at least a carbohydrate esterified at the 3 position. The aglycone is called anthocyanidin. Color of red wine come from the anthocyanins or their further derivatives that are extracted or formed during the vinification process. Anthocyanins have a positive charge on the molecule and, the fully conjugated 10 electron A–C ring π - system enables it to absorb light and thus have color. If the conjugation is disrupted, the color is lost.

The typical concentrations of free anthocyanins in full-bodied young red wines are around 500 mg/L, but can in some cases be higher than 2,000 mg/L (Burns, et al., 2002; Budić-Leto, et al., 2009). In wines made from V. vinifera grapes, the main native anthocyanins are the 3-O-monoglucosides of the six anthocyanidins, pelargonidin, cyanidin, delphinidin peonidin, petunidin and malvidin (Mazza, et al., 1995; Jackson, 2008; Castillo-Muñoz, et al., 2009), with the latter usually the most abundant.

The six anthocyanins can be also found in grapes also acylated with acetic acid, coumaric acid, caffeic acid (Mattivi, et al., 2006) and, rarely with lactic acid and ferulaic acid (Alcalde-Eon, et al., 2006). The acylation has been showed to increase their stability. Some V. vinifera red varieties, such as 'Pinot noir' does not contain acylated anthocyanins and related wines suffer an easier loss of color over time (Boss, et al., 1996; Mazza, et al., 1999).

The concentration of monomeric native anthocyanins in red wines declines constantly owing to the numerous reactions in which they are involved. Most of these reactions determine the formation of new more stable pigments assuring a long enduring color to red wine. Essentially native anthocyanins undergo three main processes: a small fraction disappears by degradation, oxidation and precipitation (Waterhouse et al., 2016), others undergo reactions of

self-association and copigmentation which determine an short-term enhancement of their chromatic properties, and another part is involved in the formation of more stable low molecular weight new pigments (e.g. pyranoanthocyanins) and polymeric anthocyanins with flavan-3-ols and proanthocyanidins (Brouillard, et al., 2003; Wrolstad, et al., 2005; Jackson, 2008).



Anthocyanidin	R_1	R_2
Malvidin	OCH_3	OCH_3
Cyanidin	H	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Delphinidin	OH	OH

Fig.6. Main Native anthocyanins of red grape.

More in detail, the phenomenon of copigmentation causes hyperchromic and bathochromic shifts of the absorption spectrum of anthocyanins with an enhancement of the color in young red wines ranging from 30% to 50% (Boulton, 2001). It is due to molecular associations between pigments and other organic molecules present in the solution that can or less be covalently linked to the pigment (Boulton, 2001). Molecules involved in copigmentation are called cofactors and include a wide group of compounds, such as phenolic acids, and anthocyanins themselves (self-association) (Rustioni, et al., 2012). New low molecular weight pigments high stable over time and to sulfite bleaching, derive from cycloaddition processes between anthocyanins and some compounds containing a polarizable double bond such as acetaldehyde (Benabdeljalil, et al., 2000), pyruvic acid (Fulcrand, et al., 1998)and vinyl phenol (Fulcrand, et al., 1996) and/or from reactions between anthocyanins and flavanols mediated by acetaldehyde (Doco, et al., 1996; Timberlake, & Bridle, 1976; Fulcrand et al., 1996). Polymeric pigments are formed via direct reaction between and anthocyanins (A) and

flavan-3-ols or condensed proanthocyanidins (T) leading to products, denoted A–T and T–A, according to the position of the anthocyanin moiety (Salas et al., 2004).

1.5.2 Flavanols and condensed tannins

Flavanols are a wide group of compounds belonging to flavonoid class that exist in grape and wine as monomers oligomers and polymers. The polymeric structures are also called condensed tannins, leucoanthocyanidins or proanthocyanidins. Condensed tannins origin from condensation of monomeric units. Flavan-3-ol monomers found in grapes and wine are (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate and (-) epigallocatechin. The first three units are present in both skins and seeds, (-)-epigallocatechin is exclusively present in skins. These monomeric unites are also referred as "catechins". The levels of total monomeric flavan-3-ols in red wine are in the range of 40–120 mg/L with the majority usually being (+)-catechin and (-)-epicatechin. Condensed tannins derive from condensation of monomeric flavan-3-ols. They are polymers showing a very wide range in molecular weight and monomeric composition. Polymers with up to 80 units have been reported in grape skin tannins (Souquet et al., 1996) while lower polymerization degree have been reported fin grape seeds (Foo & Porter, 1981 and Prieur et al., 1994). Concentrations of condensed tannins in wines can reach 1.5 g/L (Waterhouse et al., 2016). In agreement with Singleton (1992) significant portion, 25–50%, of phenolic compounds in a typical red wine exist as oligomers and polymers (condensed tannins) of flavan-3-ols.

These compounds are responsible for bitterness and astringency of wine; these sensory properties change with structure of flavanol (Vidal et al., 2004): increasing the polymerization degree the bitterness decrease and the astringency increase (Robichaud & Noble 1990; Noble, 1994). However, due to difficulties in purifying and determining the chemical structure of the whole pool of condensed tannins, the relationship between astringency and structure of

condensed tannins is still nor well known. Some authors reported that astringency of proanthocyanidins increase with chain length, up to the decamer level, and to decrease beyond this value, because the polymers become insoluble (Lea, 1990). Other authors instead reported that higher molecular weight proanthocyanidins (>20 flavanol units) were soluble in wine (Sarni-Manchado, et al., 1999; Maury, et al., 2001). Usually, during aging of wine in contact with little amount of oxygen as during wood and bottle aging, a loss of astringency occur. This is ascribed to polymerization of proanthocyanidins (Mouls & Fulcrand 2012) or to the formation of higher molecular weight proanthocyanidin-anthocyanin adducts. The two common oxidation reactions are oxidative flavanol polymerization and aldehyehyde-mediated polycondensations of anthocyanins and flavanols. (Salas, et al., 2005). However also a decrease in tannins molecular mass with aging and oxygen (McRae, et al., 2015) as has been also reported for red wines.

1.6 Techniques of management of oxygen

1.6.1 Micro-oxygenation

The positive effects observed during maturation of wine in barrels such as color stabilization, disappearance of reduction taste and reduction of vegetal characteristics, have been ascribed to oxygen passing through the wood and being dissolved in wine. Microoxygenation (MOx) is an alternative method for accelerating the maturation process of wine providing the same improvements in wine quality, but spending less time and a lower cost of barrel aging (Carlton, et al., 2007). This technique, developed in France by Patrick Ducournau at the beginning of the 1990s (Roig & Yerle, 2003), is essentially a technology aimed to supply oxygen in wine-making simulating barrel aging (Parish, et al., 2001; Cano-Lopez, ,et al., 2007).

MOx allows the introduction of controlled, small and continuous amounts (few millilitres of gas per litre of wine per month) of oxygen into wine via a porous diffuser (Parish, 2001; Moutounet, et al., 2001). (Perez-Magarino, et al., 2007). Oxygen bubbles velocity and size are critical for the optimum application of MOX: it is necessary to prevent loss of oxygen if bubbles reach the top of the tank too fast; it has been estimate the maximum bubble rise velocity for oxygen in wine around 0.28 m s−1. Bubbles and tank sizes are important to be sure that oxygen is well diffused in the wine. Dykes and Kilmartin, 2007 estimated bubble diameter during micro-oxygenation of wine has been determined to be 310 μm and 668 μm at the bubble release point for diffusers with pore sizes of 1μm and 10 μm respectively at an oxygen dose rate of 25 mg L−1 month−1. The rising path and oscillatory behavior of the bubble is determined by the size of the bubbles at their point of detachment from the diffuser used for injecting gas into the liquid phase (Martìn et al., 2007). It is nowadays well known that oxygen can affect the quality of wine, either positively or negatively (Vilela-Moura, et al., 2010) and it can be highly beneficial only when well controlled (Flecknoe-Brown, 2006). Oxidative spoilage, loss of color, higher astringency and adverse microbial activity are the results of excessive addition of oxygen to wine (Parish, et al., 2000). Parameters as temperature, dissolved oxygen rate (Nevares & Del Alamo, 2008), free sulfur dioxide level should be monitored during the process to avoid excessive oxygen supply.

The optimum temperature range for MOx process is between 14 and 17 °C. Higher temperature can result in poor solubility of oxygen; decreases in temperature can cause accumulation of oxygen in the headspace of the tank (Paul, 2002; Du Toit, et al., 2006; Gomez-Plaza, et al., 2011). About the oxygenation dosage it is important that the rate and amount of oxygen supplied to the wine has to be equal to or lower than the rate at which it is consumed by the wine. This avoid oxygen accumulation and excessive oxidation of wine (Ortega-Heras, et al., 2008; Blauw, 2009). The optimal oxygenation dosage for each wine depend on intrinsic ability of the wine to consume this oxygen due to its phenolic and volatile

composition as well as to the level of free sulfur dioxide present (McCord, 2003). As sulfur dioxide quenches hydrogen peroxide produced during first steps of oxidation cascade is of fundamental importance to monitor the level of this antioxidant during the treatment (Gambuti et al., 2015).

1.6.1.1 Effects of Micro-oxygenation

Micro oxygenation favors the formation of new polymeric pigments, thus MOX determines wine color stabilization (Castellari, et al., 2000; Perez-Magarino, et al., 2007; Lessica, & Kosmerl, 2009; Gonzalez-del Pozo, et al., 2010). Numeros studies confirmed that micro oxygenated wines show a higher color intensity than untreated wines (Atanasova, et al.2002; Du Toit, et al., 2006 ; Lopez, et al., 2007; Cejudo-Bastante, et., al., 2011.). In some cases no effect on color intensity has been observed (Bautista-Ortin, et al., 2005; Versari, et al., 2013) indicating that the effect is strongly linked to initial phenolic composition of wine. Some of most important reactions occurring during MOx are them resulting in the formation of ethyl-linked compounds and pyranoanthocyanins (Lopez, et al., 2006). The rate of these reactions is pH-dependent (Schmidtke, et al., 2011); The lower the pH is, the higher the formation of B-type vitisins and polymeric pigments (Kontoudakis, et al, .2011). Microoxygenation stimulates the polymerization of proanthocyanidins, thus an increase of the mean degree of polymerization of wine has been observed (Del-Carmen Llaudy, et al., 2006; Lopez, et al., 2008), likely resulting in a decrease of wine astringency (Atanasova, et al., 2002; Arfelli, et al., 2011; Gambuti et al., 2012; Parpinello, et al., 2012; Cejudo-Bastante, et al., 2012).

Concerning olafctory characteristics of wines, MOx wines showed an increase in the intensity of plum/currant, spicy (Cejudo-Bastante, et al., 2011), toasting, spices and coffee attributes (Del-Carmen Llaudy, et al., 2006) and the appearance of tobacco and nutty notes, which were absent in the non-treated wines (Cejudo-Bastante, et al., 2011). MOX results also in the

oxidation of some thiols, resulting in less vegetal character (Vidal & Aagaard, 2008) decreasing the unwanted sulphur compounds, methanethiol and ethanethiol to levels below their odour thresholds McCord, (2003). MOx is a powerful tool to mature wines decreasing costs of production, and this is the reason why it is widely used nowadays in wineries all over the world. However the initial composition of wine, the correct management of all factors involved as well as the end-use and the wine shelf-life have to be carefully considered.

1.6.2 Nano-oxygenation

From winery to the consumer all wines underwent a bottle aging and, for many premium wines, this step can be long years. During bottle aging oxygen cames in contact with wine. It derives from the bottling process or enters the package during storage. The amount of oxygen dissolved in wine and originated from the air which came in contact with wine during bottling operations is called dissolved oxygen (DO) (Kielhöfer & Würdig 1962, Perscheid & Zürn 1978, Kettern 1985, Schneider 2005). During aging, the ingress of air through the closure is responsible for additional oxygen supply (Perscheid & Zürn 1978, Schneider 2005, Müller-Späth 1977, Skouroumounis et al., 2005, Lopes et al., 2006). Every closure allowed a specific ingress of oxygen identified as closure oxygen transmission rate (OTR) value. During the last decade the importance of closure oxygen transmission rate (OTR) has been largely evaluated by scientific community (Ugliano, et al., 2013). As during bottle aging nano amount of oxygen are transferred to wine, the selection and use of closures with specific oxygen barrier properties has been called nano-oxygenation. Nowadays the range of closures currently available covers a rage of approximately 0.5 to 5 mg O_2 per bottle per year (Ugliano et al., 2013). Therefore the total oxygen uptake of a wine after bottling is the sum of the dissolved (DO) and headspace (HS) oxygen at bottling, the oxygen that is contained in the closure itself, and the amount of oxygen that diffuses into the bottle through the closure (OTR).

Several studies showed that wines bottled with closures with different OTR possess different chemical and sensory characteristics (Skouroumounis et al., 2005; Lopes et al. 2006, Godden et al., 2002, Hart & Kleinig 2005, Lopes et al., 2009, O'Brien et al., 2009). First studies demonstrated that a white wine sealed with screwcap retained higher sulfur dioxide (SO_2) compared to the same wine sealed under synthetic closures, natural corks, and technical corks (Godden et al. 2002). Successively other authors observed that closures with higher OTR could be used to prevent formation of reductive off-flavors such as hydrogen sulfide (H_2S) (Ugliano., et al. 2011) or decrease the astringency in wine (Gambuti et al., 2013). In contrast a lower OTR could help to limit the loss of fruitiness attributes due to oxidation (Lopes et al., 2009, O'Brien et al., 2009). Although this technique is very important for winemaker and more studies have been performed, the relationship between original phenolic composition of wines and the impact of closure OTR is still not elucidated. The study of the impact of wine matrix on the evolution during nano-oxygenation of wines could help to better manage the aging behavior of bottled wines.

2. AIMS

Oxygen influences phenolic composition and determines changes in sensory characteristics linked to phenolics, such as colour and astringency, all of which determine wine quality. It is known that the presence of a proper ratio between native anthocyanins and tannins could affect the reactions of pigment stabilization and changes in this ratio could be important for ~~a~~ proper wine aging (Singleton & Trousdale, 1992; Francia-Aricha et al., 1997); Morel-Salmi, et al., 2006). This is why the addition of enological tannins is an accepted enological practice (OIV: ENO 5/2008), aimed at stabilizing wine color and improving wine structure (Obreque-Slíer et al., 2009). Although several studies evaluating the effect of adding enological tannins to red wine have been reported (Bautista-Ortin, et al., 2005; Harbertson, et al., 2012; Versari,

et al., 2013), never has a study been undertaken to understand and evaluate the impact of different anthocyanin tannin ratios during oxidation. For this reason in the first part of this thesis a mixture of oligomeric tannins (OT) was added to a wine rich in anthocyanins so as to have wine with three different anthocyanin tannin ratios. Samples were then treated with hydrogen peroxide to trigger the Fenton reaction; color, phenolic compounds and acetaldehyde were evaluated. In a second experiment, with the aim to evaluate the influence of three different commercial tannins (condensed, gallo- and ellagitannins) on acetaldehyde production and color and phenolic compounds evolution during oxidation of red wine. A third experiment has been performed considering the action of antioxidants. Oxidation is a long-standing problem in wine industry and sulfur dioxide SO_2 is the generally used chemical to control it. SO_2 acts as antioxidant in three ways, scavenging hydrogen peroxide, reacting with ortho-quinones acting as sacrificial nucleophiles and binding carbonyl compounds produced by Fenton reaction (Adachi et al., 1979; Danilewicz & Wallbridge, 2010). However, concerns over its ability to induce severe allergic reactions have created a great need for its reduction or replacement. In last decade, although it is not yet an authorized wine additive, the tripeptide glutathione (GSH) has been proposed in winemaking as alternative antioxidant to decrease the use of SO_2 (Kritzinger, Bauer and Du Toit, 2012). In this part of thesis, to better understand if GSH can be proposed as an alternative to SO_2 to scavenge hydrogen peroxide, inhibit Fenton reaction and prevent grape native anthocyanins loss during wine aging, the oxidative degradation of malvidin 3-monoglucoside was studied in model solutions and in red wines treated with hydrogen peroxide and added with increasing concentration of sulfur dioxide and glutathione. Moreover, because pH is one of main wine feature affecting wine oxidation owing to its role on the formation of new stable polymeric pigments from native anthocyanins (Pechamat, et al., 2014; Kountoudakis et al., 2011) and on SO_2 speciation in hydroalcoholic solution, the experiments were carried out at two typical wine pH: 3.2 and 3.8.

The last experiment conducted in this thesis was carried out using three monovarietal wines and applying a controlled nano-oxygenation. Nowadays preference towards wines from native grapes increases with respect to wines obtained from widely spread international cultivars (Chrysochou, et al., 2012). In this scenario, certain red wines from Southern Italy, namely Aglianico, Casavecchia and Pallagrello are gaining international recognition. Preliminary data indicate that these wines are very rich in phenolic compounds, in particular Aglianico is characterized by the high content of astringent tannins (Rinaldi, et al., 2014) while Casavecchia and Pallagrello resulted also rich in anthocyanins (Masi, et al., 2001). It appears therefore of great interest to investigate the potential of NOx as a tool to modulate composition of these wines and the related sensory attributes. In particular, their peculiar phenolic composition allows comparing different wine phenolic models, with the aim of obtaining additional insights in the contribution of oxygen exposure to wine development. In this last part of thesis, the effect of post-bottling oxygen exposure on wines made from Aglianico, Pallagrello and Casavecchia grapes was investigated during bottle aging. Wines were aged with closures at increasing oxygen ingress rate (OTR). Two levels of oxygen ingress were considered for each wine (4.5 and 3.2 mg/L/year) and a further closure having lower oxygen ingress (1.6 mg O_2/L/year) was used for Aglianico. Wines were analysed after 0, 7 and 15 months of aging. Chromatic characteristics, anthocyanins and low and high molecular weight tannins and pigments were analyzed in order to assess the role of NOx on the evolution of wine phenolics and related chromatic characteristics during aging. Reactivity of wine tannins towards proteins and sensory olfactive descriptive analysis were performed in order to evaluate the slight sensory differences due to closures and aging time.

3. MATERIALS AND METHODS

3.1 Spectrophotometric analyses

Colorant intensity, Abs 420 nm, Abs 520 nm, Abs 620 nm and hue were evaluated according to (Glories, 1984). Total anthocyanins and vanillin reactive flavans were determined by a spectrophotometric method described by Di Stefano & Guidoni (1989). Briefly: two microfuge tubes were used, and a first 1.5-mL microfuge tube was made up by dispensing 125 µL of diluted wine (diluted 1 to 10 with pure methanol) and then adding 750 µL of a solution of vanillin (4 % in methanol). After 5 min, the tube was placed in cold water (4 °C) and 375 µL of concentrated hydrochloric acid was added. After a 15 min incubation of the mixture at room temperature (20 °C), the absorbance was determined at 500 nm. For a second tube, the procedure was the same apart the fact that 750 µL of pure methanol was used instead of the solution of vanillin. The 500 nm absorbance of this tube is considered the blank. Concentrations were calculated as (+)-catechin (mg/L) by means of a calibration curve. For total tannins analysis, the Ribereau-Gayon P., & Stonestreet E. (1966) method was used. This assay is based on the conversion of proanthocyanidins into colored anthocyanidins by oxidative cleavage under hot and acidic conditions (100 °C for 30 min in a mixture of chlorhydric acid 50 %, n-butanol and ferric sulfate $Fe_2(SO_4)$. After the reaction, the absorbance was measured at 550 nm. A sample prepared with all reagents but not heated and just stored at room temperature and in the darkness was used as a control. Small polymeric pigments (SPP) and large polymeric pigments (LPP) were determined by Harbertson–Adams assay (HAa) (Harbertson et al., 2003). In this assay, by combining protein precipitation by using bovine serum albumin BSA (SIGMA, Life Science Saint Louis MO, USA) and bisulfite bleaching, two kinds of polymeric pigments in wines were determined: large polymeric pigments (LPP) that precipitate with protein, small polymeric pigments (SPP) that do not

precipitate and , pH changes allowed the evaluation of total anthocyanins. A Shimadzu UV-1800 (Kyoto, Japan) UV spectrophotometer was used; 10-mm plastic cuvettes were used. All analyses were carried out in duplicate.

3.2 High-performance liquid chromatography determination of acetaldehyde

Acetaldehyde was determined using the method of Han, et al., (2015). Briefly, wine sample aliquots (100 mL) were dispensed to a vial, followed by addition of 20 mL of freshly prepared 1120 mg/L SO2 solution. Next, 20 mL of 25% sulfuric acid (Carlo Erba reagent 96%) was added, which was followed by 140 mL of 2 g/L 2,4-dinitrophenylhydrazine reagent (Aldrich chemistry). After mixing, the solution was allowed to react for 15 min at 65 C and then promptly cooled to room temperature. Analysis of carbonyl hydrazones was conducted by HPLC (HPLC Shimadzu LC10 ADVP apparatus (Shimadzu Italy, Milan), consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 50 mL loop. A Waters Spherisorb column (250 4.6 mm, 4 mm particles diameter) was used for separation. The chromatographic conditions were: sample injection volume, 50 mL; flow rate, 0.75 mL/min; column temperature, 35 C; mobile phase solvents, (A) 0.5% formic acid (Sigma Aldrich 95%) in water milli-Q (Sigma Aldrich) and (B) acetonitrile (Sigma Aldrich 99,9%); gradient elution protocol, 35% B to 60% B (t ¼ 8 min), 60% B to 90% B (t ¼ 13 min), 90% B to 95% B (t ¼ 15 min, 2-min hold), 95% B to 35% B (t ¼ 17 min, 4-min hold), total run time, 21 min. Eluted peaks were compared with derivatized acetaldehyde standard. All analyses, were conducted through two experimental replicas and two analytical replicas.

3.3 High-performance liquid chromatography analyses of anthocyanins

The separation of anthocyanins was carried out according to the OIV Compendium of International Methods of Analysis of Wine and Musts (2007). Analyses were performed in a HPLC Shimadzu LC10 ADVP apparatus (Shimadzu Italy, Milan), consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 50 mL loop. AWaters Spherisorb column (250 4.6 mm, 4 mm particles diameter) with pre-column was used. Fifty mL of wine or calibration standards were injected onto the column. Detection was performed by monitoring the absorbance signals at 518 nm. All the samples were filtered through 0.45 mm, Durapore membrane filters (Millipore - Ireland) into glass vials and immediately injected into the HPLC system. The HPLC solvents were: solvent A: water milli-Q (Sigma Aldrich)/formic acid (Sigma Aldrich 95%)/acetonitrile (Sigma Aldrich 99,9%) (87:10:3) V/V; solvent B: water/formic acid/acetonitrile (40:10:50) V/V. The gradient used was: zero-time conditions 94% A and 6% B, after 15 min the pumps were adjusted to 70% A and 30% B, at 30 min to 50% A and 50% B, at 35 min to 40% A and 60% B, at 41 min, end of analysis, to 94% A and 6% B. After 10-min equilibrium period the next sample was injected. The flow rate was 0.80 mL/min. For calibration the external standard method was used: the calibration curve was plotted for malvidin-3-monoglucoside (Extrasynthese, Lyon, France) on the basis of peak area and the concentration was expressed as mg/L of malvidin-3-monoglucoside. All analyses, were conducted through two experimental replicas and two analytical replicas.

3.4 Saliva precipitation index (SPI) determination

The SPI was determined by the densitometric analysis of selected bands in the SDS–PAGE pattern of saliva before and after the interaction with wine (Rinaldi, et al. 2012). It represents an estimation of the percentage of proteins that have been precipitated by tannins. Human saliva used for binding reactions was obtained by mixing resting saliva samples from different individuals. Saliva was collected from six non-smoking volunteers (three males and three females) by expectorating saliva into a preweighted ice-cooled tube for 5 min. The resulting mix was centrifuged at 10,00 g for 10 min at 4 °C to remove any insoluble material and salivary proteins in the supernatant were used for the analyses. Interaction mixtures (75 µL final volume) contained 50 µL of saliva and 25 µL of wine previously filtered at 0.45 µm (Millipore; Rome, Italy) and diluted with synthetic wine (ethanol 12 %, tartaric acid 4 g/L, pH 3.2 with NaOH 1 N). Binding reactions were performed in Eppendorf tubes kept at 37 °C for 5 min. Mixtures were then centrifuged at 4 °C for 10 min at 10,000 g. Electrophoresis was performed on the resulting saliva supernatant. The SDS–PAGE electrophoresis of saliva was performed on a Bio-Rad Protean II xi Cell electrophoresis apparatus (Bio-Rad, Milano, Italy) using a PowerPac 1000 Bio-Rad power supply set at 150 and 180 V for the stacking and the resolving gel, respectively. After mixing with an equal volume of 2 × electrophoresis sample buffer (0.125 M Tris–HCl, 4 % SDS; 20 % v/v glycerol, 0.2 M DTT, 0.02 % bromophenol blue, pH 6.80) and heated at 95 °C for 4 min, saliva samples were analyzed by SDS–PAGE using 30 % acrylamide/bisacrylamide (37.5:1) solution. Resolving gels were 14 % acrylamide, while stacking gels were 5 % acrylamide. Gels were fixed with a mixture of ethanol, acetic acid and deionized water (40:10:50) for 1 h. After washing in water for 5 min, gels were stained with Coomassie Brilliant Blue R250 staining solution (Bio-Rad, Milano, Italy). Destaining was performed by incubation in the Coomassie Blue R250 destain solution (Bio-Rad, Milano, Italy). Densitometric tracing of gels was performed with a Bio-Rad GS800 densitometer, and electrophoretic data were analyzed by Quantity One analysis software,

version 4.5 (BioRad). The SPI values were expressed as g/L of gallic acid equivalent (GAE). The calibration curve was obtained plotting the percentage of selected bands reduction as a function of increasing condensed tannins concentrations (Biotan, Bordeaux, France) concentrations (g/L) The equation of the calibration curve is y = 19.026x + 15.858, where x represents the condensed tannins concentration and y the SPI value. The coefficient of determination for the SPI was R^2 = 0.9907. As total phenolics of condensed tannins solutions were quantified by the Folin–Ciocalteu method, the correlation between SPI and the total phenolics concentration (y = 1.0899x – 0.2128; R^2 = 0.9728) permitted to express the reactivity of salivary proteins toward tannins in g/L of gallic acid equivalent (GAE). All the data are expressed as mean ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

3.5 Sensory analysis

The odor profiles of the experimental wines (AGL, CAS, PALL) were obtained after 15 months from bottling. The panel was composed of eight judges (four males and four females, 22–45 years of age), recruited from the staff and the students of the Department of Food Science of the University of Naples Federico II. All judges were specifically trained in performing olfactory profiling of red wine and were selected on the basis of their sensory abilities. The performance of the sensory panel was evaluated by principal component analysis which was conducted for all assessors on the mean judge score for each odor attribute (King. et al. 2001), The analyses were conducted in individual sensory booths. The samples (30 mL) were presented at room temperature (20 °C) in black tulip-shaped glasses, covered with glass Petri dishes and coded with random three-digit codes. Two replicates for each wine and each closure were analyzed. In each session, the order of presentation of the samples was randomized among the judges in order to minimize any possible order and carryover effects. The evaluated odor attributes were determined by consensus after the panel had evaluated the

experimental wine samples and had discussed to reduce the number of descriptors. The intensity of the descriptors was rated using a 9-point scale (0 = not detected, 1 = weak, 2 = medium, 3 = strong, 4 = very strong), half values being allowed. The order of presentation of the descriptors on the sheets was randomized among panelists, and, for the same panelist, it was different in the two replicate sessions. This was done to balance the fact that the last descriptors could be less accurately evaluated, due to fatigue.

3.6 Experimental

3.6.1 *First study:* Effect of tannin addition on the evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine.

The experiment was performed on a red wine produced in South Italy with thermovinification technique, which allowed a wine with a high concentration of native anthocyanins to be obtained. The base parameters were: alcohol 13.59 ± 0.3 %V/V, titratable acidity 5.28 ±0.2 g/L of tartaric acid, pH 3.55 ± 0.04, volatile acidity 0.50 ± 0.06 g/Lof acetic acid, the sum of native anthocyanins were 3.43 ± 0.1 g/L (at least 5 times higher than normal concentration of native anthocyanins in a red wine) and BSA reactive tannins were 683.2 ± 18.8 mg/L. Base parameters were determined according to the OIV compendium of international methods of wine and must analysis (2007). The samples were treated to change the anthocyanin tannin ratios. Oligomeric Tannins O.T. (Tannin VR grape Biotan, Laffort Oenologie, France) were added to red wine so as to have three wines with three different anthocyanin/tannin ratios: W (anthocyanin/tannin ratio 1:0.5) WT (anthocyanin/tannin ratio 1:1) WTT (anthocyanin/tannin ratio 1:3). The concentration of O.T. has been reported by (Fontoin, Saucier, Teissedre, & Glories, 2008). Wines were prepared by adding different concentration of tannin in 5 liter flask previously saturated with nitrogen. Three hours late hydrogen peroxide 3% V/V was added to wines to eliminate the present total sulfur dioxide and to add an extra amount of 74 mg/l of oxygen equivalent. This quantity was chosen to simulate two year of aging in barrel

(Nevares & Del Alamo-Sanza, 2015) or wine micro oxygenation (Tao, Dykes, & Kilmartin, 2007). After the addition, samples were stored at the temperature of 18° C and monitored for thirty days. Two replicates of the experiment were performed.

3.6.2 *Second study*: Effect of different enological tannins on color stability and tannins evolution during forced oxidation of red wine.

The experiment was performed on a red wine produced in Southern Italy with the thermovinification technique, which allowed to obtained a wine with a high concentration of native anthocyanins. Three tannin treatments using three different commercial tannin products were carried out. T1, T2 and T3 were added to red wine so as to have the three correspondent experimental wines (T1, T2 and T3). Wines were prepared adding different tannins (2 g/L) to 1 L flask previously saturated with nitrogen. After 1 hour wines were added with hydrogen peroxide 1% $_{V}$/V (1 mL/L) with an amount of 21 mg/L of oxygen equivalent. Three replicates of the experiment were performed. Treatments were compared to the control (C) added only with hydrogen peroxide. Two replicates of the experiment were performed each time.

3.6.3 *Tirth study*: Oxidative degradation of malvidin 3-monoglucoside in model solution and real wine.

Oxidation reactions were performed in 10 mL reagent bottles. The bottles were purged with nitrogen and placed in darkness at 20 °C. In the first experiment the effect of antioxidants and pH was evaluated in model solutions (Fig. 7). All solutions contained malvidin 3-monoglucoside 100 mg/L (203 mM), ethanol (12% v/V) and tartaric acid (q. s.). The oxidation Ox was performed adding hydrogen peroxide at a concentration of 39.2 mg/L of O_2 eq (1.225 mM) to trigger Fenton reaction (Elias and Waterhouse, 2010). Because this reaction involves hydrogen peroxide and metal ions at concentration (< 0.2 μM) much lower than expected in all commercial water supplies (Clark et al., 2007) the occurrence of Fenton

reaction in our experimental conditions is guaranteed. Six oxidized (Ox) samples were obtained: Ox, adding only hydrogen peroxide; Ox+SO_2 low, adding hydrogen peroxide and 37.4 mg/L of SO_2 (0.584 mM); Ox+SO_2 high, adding hydrogen peroxide and 202 mg/L of SO_2 (3.16 mM); Ox+GSH, adding hydrogen peroxide and 30 mg/L of glutathione (0.098 mM); Ox+SO_2 low+GSH, adding hydrogen peroxide, 37.4 mg/L of SO_2 (0.584 mM) and 30

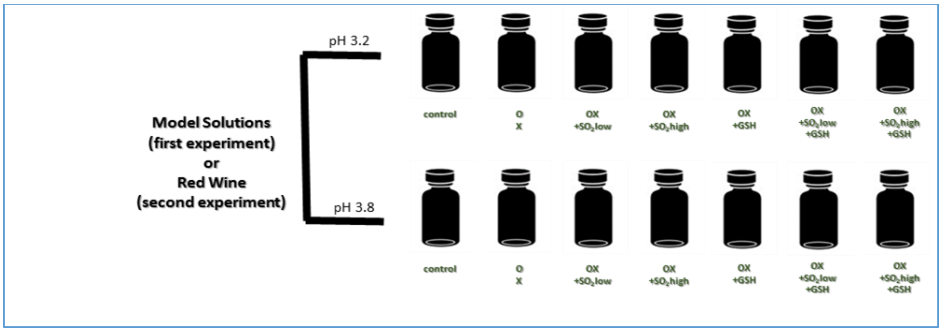


Fig. 7. Experimental plan.

mg/L of glutathione (0.098 mM); Ox+SO_2high+GSH, adding hydrogen peroxide, 202 mg/L of SO_2 (3.16 mM) and glutathione (0.098 mM). Samples were prepared at two pH: 3.2 and 3.8 and monitored after 0, 16 and 72 hours of incubation at 20 °C. Dilution coefficient were considered to compare treated sample with control one. In the second experiment the same treatments were performed on a red wine produced in South Italy (*Vitis vinifera* L. Casavecchia). The pH of wine was adjusted to pH 3.2 by adding tartaric acid. All samples (model solutions and wines) were prepared in duplicate.

3.6.4 *Fourth study*: Oxygen exposure of tannins-rich red wines during bottle aging. Influence on phenolics and color, astringency markers and sensory attributes.

All wines were produced during 2012 vintage. Monovarietal wines were produced with Aglianico (AGL), Casavecchia (CAS) and Pallagrello (PALL) grapes produced in Campania region (Italy) with a standard industrial process. Before bottling, according with standard

winery procedure, the content of SO_2 was adjusted to have a molecular SO_2 of 1.8 mg/L to avoid wine microbial spoilage during bottle aging. The base parameters (mean ± standard deviation) of wines at bottling are reported in Table 1.

Tab. 1. The base parameters (mean ± standard deviation) of wines at bottling .

	ethanol content (%v/V)	pH	residual sugars (g/L)	free SO_2 (mg/L)	total SO_2 (mg/L)	Total anthocyanins A (mg/L)	Total tannins (T) (mg/L)	T/A
AGL	12.48±0.07	3.49±0.02	2.95±0.05	28.8±0.0	108.8±0.6	198.89±4.28	3813±61	19
CAS	13.30±0.08	3.71±0.03	1.86±0.09	60.8±0.6	121.6±0.6	674.83±3.37	4025±35	6
PALL	13.40±0.10	3.62±0.06	2.19±0.06	48±0.6	109.0±0.3	591.82±4.50	4187±18	7

All the data are expressed as means ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

Two levels of NOx were applied to each wine: 3.2 and 4.5 mg O_2/L/year, by means of Nomacorc Select 300 and Select 700 closures respectively (Nomacorc SA, Thimister Clermont, Belgium). Because AGL wines contained less anthocyanins (Tab. 1) and they are strongly influenced by closure OTR (Gambuti, et al., 2013) a closure having lower oxygen ingress (1.6 mg O_2/L/year) was also tested (Nomacorc Select 100). Total package oxygen (TPO) is the sum of dissolved and headspace oxygen after bottling. TPO at bottling was measured by means of oxo-luminenscence, using a Nomasense oxygen analyzer (Nomacorc SA, Thimister Clermont, Belgium). Measurements were taken approximately 30 min after bottling and data are given in Table 8. Wines were analyzed after 0, 7 and 15 months of aging in bottle. At each sampling time two bottles for each treatment were analyzed.

3.7 Statistical analysis

Data were compared using Fisher's least significant differences (LSDs) procedure. When the variances were not homogeneous, data were analyzed using Kruskall–Wallis test. When

results of the “Kruskal–Wallis” test were significant ($P < 0.05$), the significance of between-group differences was determined by the “Bonferroni–Dunn” test (5 % significance level). For sensory analysis of Casavecchia and Pallagrello, quantitative data relative to odor profiles were analyzed by Wilcoxon signed-rank test (differences of $p < 0.05$ were considered significant). For sensory analysis of Aglianico, Kruskal–Wallis test (differences of $p < 0.05$ were considered significant) and Dunn test (post hoc test) were used. Multifactorial ANOVA with third-order interactions was used to evaluate the relationships among factors. Differences of $p < 0.05$ were considered significant. These analyses were performed using XLSTAT (Addinsoft, XLSTAT Version 2013.6.04).

The statistical analyses were performed using XLSTAT (Addinsoft). All data are means of four values (2 experimental replicates X 2 analytical replicates).

4. RESULTS AND DISCUSSION

Part 1 New insights into wine oxidation

4.1 Effect of tannins addition on the evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine:

A comparison among the absorbances of wines during oxidation treatment is shown in (Fig.8). The C.I. increased for all samples after the addition of hydrogen peroxide. For sample W, C.I. reached a maximum after 7 days of oxidation while for sample WT and WTT the level of absorbance increased throughout the experiment. The increase of C.I. during oxidation is consistent with the findings of Atanasova et al., (2002), showing that micro-oxygenated wines have a higher C.I. than untreated wines. Samples with added OT (WT-WTT), showed an increase in absorbance at 420 nm consistent with the observations of other authors on the effect of tannins on yellow tint (Bautista-Ortin et al., 2005). Just after the addition of oxidant the values of abs at 420 nm increased for WT and WTT; for sample W the abs at 420 nm increased only after the 21th day. The increase in abs at 420 nm due to the formation of yellow pigments is consistent with the trend reported by Somers, (1971) during wine maturation. Significant differences were detected for abs at 520 nm: it increased throughout the experiment for samples WT and WTT while sample W tended to quickly increase during the first 7 days and then stabilized. Interestingly, by increasing tannin concentration, the C.I. and the absorbance at 520 nm increase: which is consistent with findings reporting that oligomeric proanthocyanins are the principal cofactors in young red wines favoring copigmentation (Boulton, 2001).

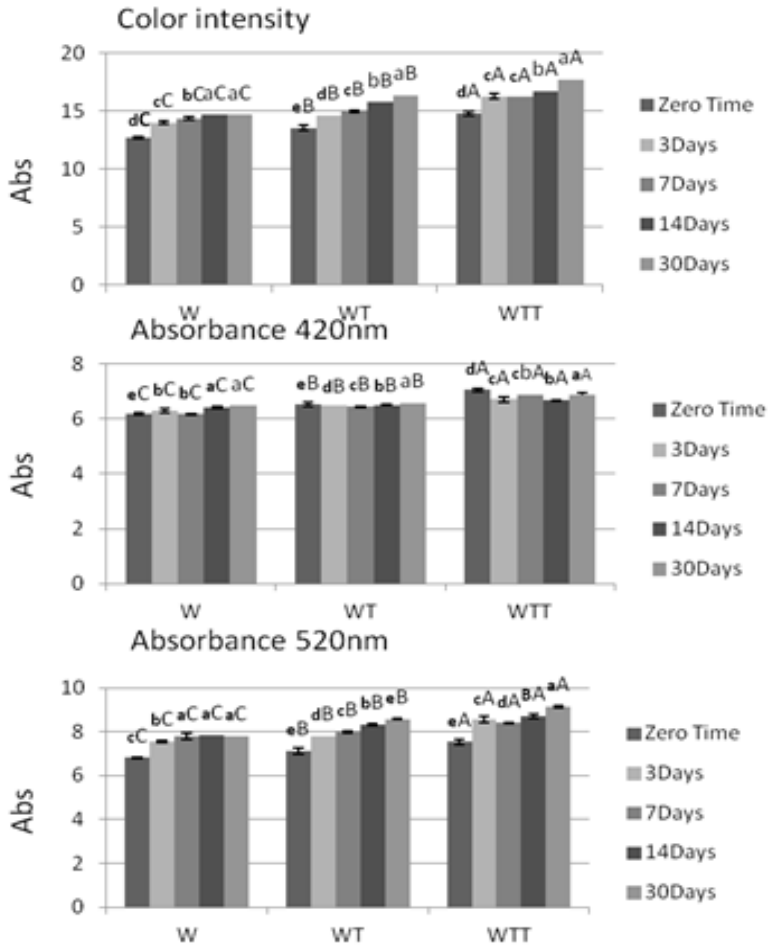


Fig. 8. Evolution of color intensity and absorbance 420 at and 520nm in the three samples (W, WT, WTT) during oxidation. Wines differed for anthocyanins/tannins ratio: W (ratio anthocyanins/tannins 1: 0.5) WT (ratio anthocyanins/tannins 1: 1) WTT (ratio anthocyanins/tannins 1: 3).Wine type (a, b, c, d, e) and oxidation time (A, B, C) sharing the same letters are not significantly different ($P < 0.05$).

The involvement of native anthocyanins in copigmentation reactions is consistent with the decrease of native anthocyanins in Fig. 9, and with similar effects observed in red wines during micro oxygenation (López, et al., 2006; Gambuti, et al., 2015). Simultaneously, an increase in the amount of polymeric pigments (LPP + SPP (large and small polymeric pigments)) determined by Adams method was observed in the three samples. Polymeric pigments tended to increase during the entirely period of the experiment with a growth WTT > WT> W. Concerning the chemical nature of these pigments, Versari et al., (2008) observed that the sum of SPP and LPP estimated by the Adams assay showed a good correlation (R^2= 0.9511) with total polymeric pigments evaluated by HPLC. Moreover Peng et al., (2001),

showed that a large proportion of these molecules were oligomers (5 units and higher). Our results confirm that with moderate oxygen exposure, these polymeric pigments becomes of paramount importance for wine color (Boulton, 2001; González-del Pozo, et al., 2010; Gambuti, et al., 2012).

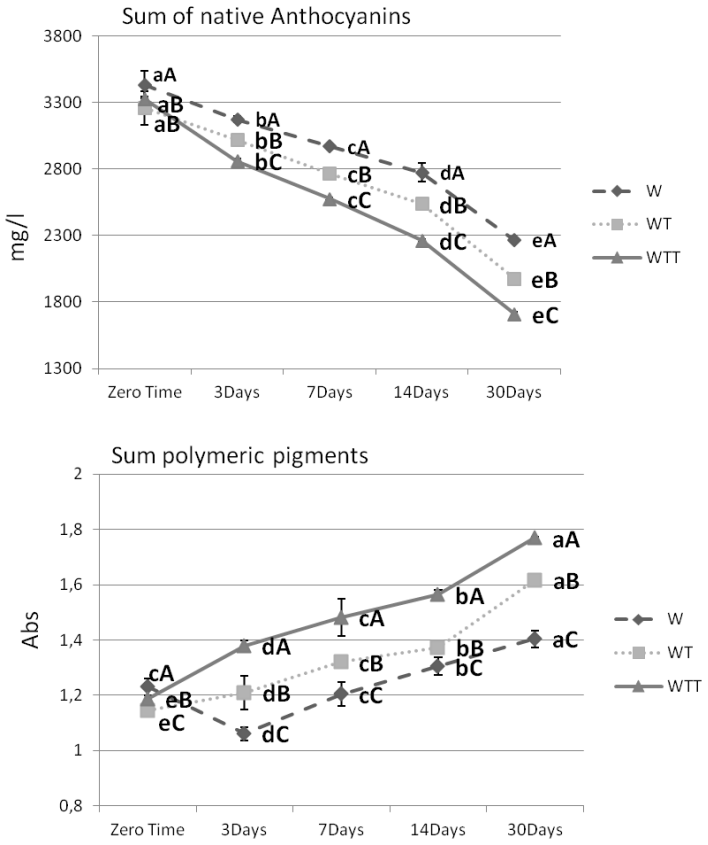


Fig. 9 Evolution of anthocyanins and total sum of the polymers (SPP+LPP) in the three samples (W, WT, WTT) during oxidation. Data on SPP+LPP are related to samples diluted 1:2. Wines differed for anthocyanins/tannins ratio: W (ratio anthocyanins/tannins 1: 0.5) WT (ratio anthocyanins/tannins 1: 1) WTT (ratio anthocyanins/tannins 1: 3).Wine type (a, b, c, d, e) and oxidation time (A, B, C) sharing the same letters are not significantly different ($P < 0.05$).

As expected, the three different samples exhibited different tannin concentrations at zero time (Fig.10). Over time all samples showed a significant increase in the concentration of BSA reactive tannins. This surprising result can be explained taking into account that Harbertson et al., (2014) showed that BSA reactive tannins do not involve all the tannins present in wine but only high molecular weight tannins capable of precipitating with BSA. Therefore the increase

of this value during the experiment could be due to an increase in the polymerization of tannin polymer making them precipitable by BSA and then quantifiable by this assay.

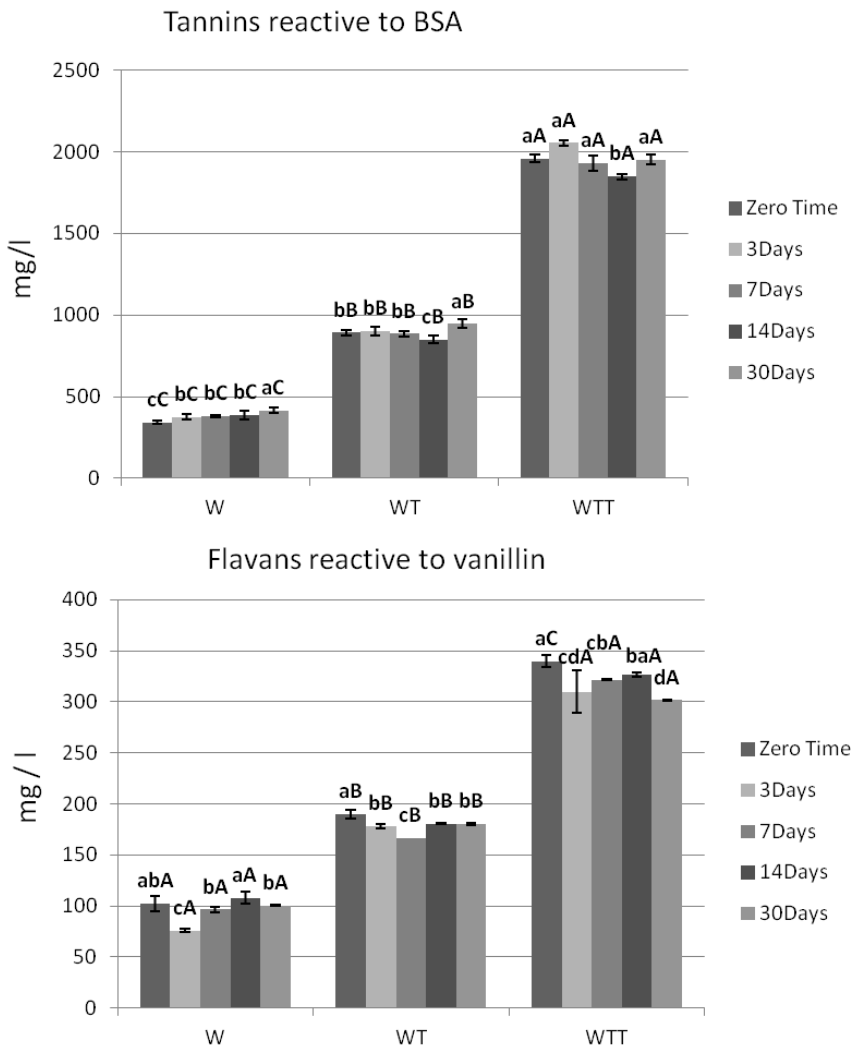


Fig. 10. Evolution of tannins reactive to BSA (data are related to samples diluted 1:2), and flavans reactive to vanillin (data are related to samples diluted 1:10), in the three samples (W, WT, WTT) during oxidation. Wines differed for anthocyanins/tannins ratio: W (ratio anthocyanins/tannins 1: 0.5) WT (ratio anthocyanins/tannins 1: 1) WTT (ratio anthocyanins/tannins 1: 3).Wine type (a, b, c, d, e) and oxidation time (A, B, C) sharing the same letters are not significantly different ($P < 0.05$).

The vanillin reactive flavans showed a significant decrease for the three samples (Fig.10). The vanillin assay uses aldehyde vanillin as a reactant which reacts with the same positions on the flavanol A-ring to produce a colored product (Hagerman et al., 1998). The decrease of VRF values with oxidation in experimental samples is consistent with the minor presence of

nucleophile sites on the flavanol molecules due to the significant effect of polymerization. The measurement of VRF may be therefore considered an indirect, inverse measure of the oxidative polymerization of flavanols and the VRF assay could be used to evaluate tannin oxidation (Gambuti et al., 2012). The trend detected for W, WT and WTT wines during oxidation confirms this hypothesis.

A key parameter for monitoring wine aging is acetaldehyde because it is expected to increase in response to continuous oxygen exposure due to non-enzymatic oxidation of ethanol (Wildenradt & Singleton, 1974); excessive levels of acetaldehyde negatively affected wine sensory quality (Carlton et al., 2007). The drastic formation of acetaldehyde after the addition of hydrogen peroxide (Fig.11) is consistent with the well-known Fenton reaction stated by Casellato, Tamburini, Vigato, Vidali, & Fenton (1984) and with the quick oxidation of ethanol.

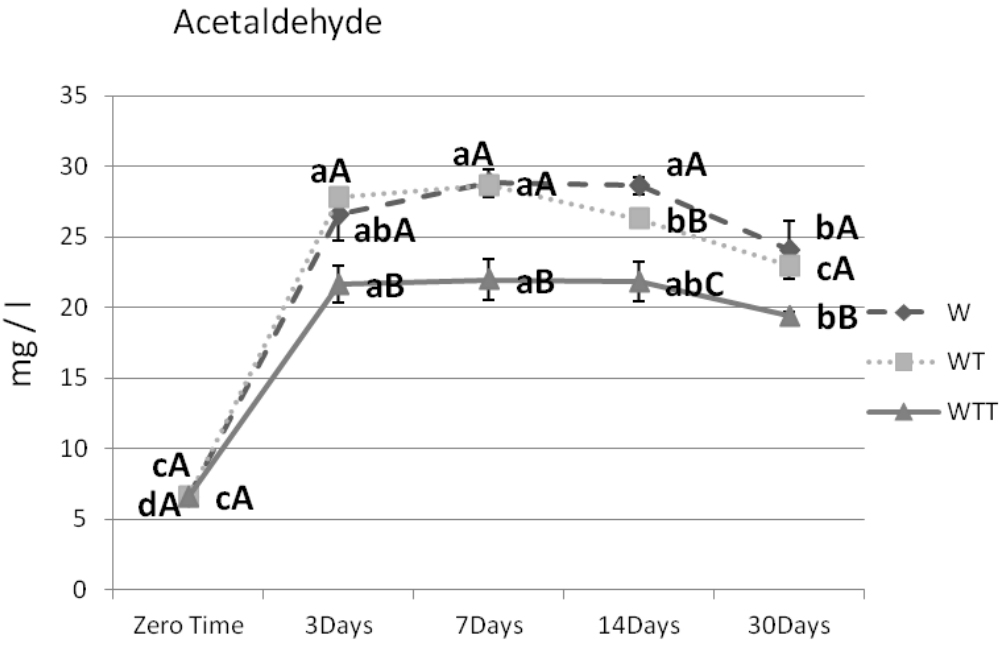


Fig. 11. Evolution of acetaldehyde in the three samples (W, WT, WTT) during oxidation. Wines differed for anthocyanins/tannins ratio: W (ratio anthocyanins/tannins 1:0.5) WT (ratio anthocyanins/tannins 1:1) WTT (ratio anthocyanins/tannins 1: 3).Wine type (a, b, c, d, e) and oxidation time (A, B, C) sharing the same letters are not significantly different ($P < 0.05$).

The evolution of this molecule during the experiment in the three samples is slightly different. Sample W showed an increase until the seventh day of contact with hydrogen peroxide and a decrease after fourteen days. The WT and WTT samples showed the same trend as W until the fourteenth day. However, a lower production of acetaldehyde was observed for WTT and the decrease of acetaldehyde during the last phases of oxidation was significant only for W and WT. The lower formation of acetaldehyde in WTT could be due to a lower yield of reaction between ethanol and high reactive radicals produced by Fenton reaction. This is likely due to the fact that these reactions are unspecific and OT could compete with ethanol for radicals decreasing the formation of acetaldehyde (Laurie & Waterhouse, 2006). The consumption of acetaldehyde observed in the last days of the experiment for W and WT can be due to: 1) direct reactions of acetaldehyde with malvidin-3-glucoside to produce vitisin- B (Bakker & Timberlake, 1997), 2) the flavanol association cross-linked by glyoxylic acids (Es-Safi, et al., 2000), and 3) the formation of ethyl-bridged anthocyanins and flavonols (Atanasova et al., 2002). Evidence of ethyl-linked anthocyanins-flavanols was first reported by (Timberlake & Bridle, 1976). Successively, acetaldehyde-flavanol condensation products were observed in model solution and in red wine (Fulcrand, et al., 1996; Saucier, et al., 1997; Salas, et al., 2004). Other important reactions for wine quality could determine a decrease in acetaldehyde content, such as the reaction between acetaldehyde and nucleophilic wine alcohols to give a class of compounds called acetals that are less volatile and reactive then acetaldehyde (da Silva Ferreira, et al., 2002; Peterson, et al., 2015). In particular, glycerol is one of the most abundant chemical compounds in wine and heterocyclic acetals deriving from the reaction between acetaldehyde and glycerol can accumulate over time. These compounds are responsible for oxidation and development of off flavors of wine (Schneider, et al., 1998). A greater and faster production of cyclo addition products and ethyl bridged polymers could determine a more elevated consumption of acetaldehyde in WTT than in the other samples.

4.2. Effect of tannins addition on the evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine.

Color intensity C.I. and hue are important indicators of color quality of red wines. After the addition of this strong oxidant, the C.I. starts to increase for all samples (Table 2). At the same time, slight significant changes of hue were detected. For the C sample, unspiked with enological tannins, a decrease of C.I. and 520nm abs during last phases of oxidation was observed. In contrast, C.I. and 520 nm abs of T3 wines increased until the 14th days of oxidation and then stabilized. For T1 and T2 samples a slight increase has been detected.

Tab. 2. Absorbance at 420, 520, 620 nm, in the four samples (T1, T2, T3 and C) after the oxidation.

Zero Time	T1	T2	T3	C
Absorbance 420nm	10,01±0,02 cC	10,52±0,12 dB	11,44±0,06 dA	11,66±0,18 aA
Absorbance 520nm	14,07±0,01 aC	13,94±0,09 bC	14,63±0,07 cB	15,68±0,15 aA
Absorbance 620nm	2,78±0,02 dD	2,94±0,02 cC	3,40±0,02 cB	3,69±0,12 bA
Color Intensity	26,85±0,03 bD	27,4±0,18 dC	29,46±0,12 cB	31,2±0,44 aA
Hue	0,71±0,00 dD	0,75±0,00 dB	0,78±0,00 dA	0,74±0,00 bcC
3days				
Absorbance 420nm	10,36±0,13 bC	11,17±0,06 bcB	11,69±0,15 cA	11,56±0,04 aA
Absorbance 520nm	13,98±0,1 abD	14,17±0,03 abC	14,44±0,12 dB	14,97±0,08 bA
Absorbance 620nm	2,8±0,05 dD	3,02±0,03 cC	3,27±0,09 dB	3,3±0,47 bcA
Color Intensity	27,14±0,28 bD	28,39±0,1 bcC	29,31±0,31 cB	30,06±0,11 bcA
Hue	0,74±0,00 cC	0,79±0,00 bB	0,81±0,01 bA	0,7±0,00 aD
7days				
Absorbance 420nm	10,29±0,01 bC	11,03±0,05 cB	11,36±0,12 dA	11,26±0,29 bAB
Absorbance 520nm	13,98±0,02 aC	14,19±0,09 abB	14,2±0,05 eB	14,83±0,11 bA
Absorbance 620nm	2,83±0,01 cD	3,01±0,03 cC	3,13±0,05 eB	3,43±0,06 cA
Color Intensity	27,09±0,03 bC	28,23±0,18 cB	28,69±0,22 dB	29,51±0,47 cA
Hue	0,74±0,00 cD	0,78±0,00 cB	0,8±0,01 cA	0,76±0,01 bC
14days				
Absorbance 420nm	10,33±0,09 bC	11,33±0,19 bB	13,53±0,01 aA	11,63±0,23 aB
Absorbance 520nm	13,73±0,15 cD	14,24±0,15 aC	15,91±0,08 aA	14,88±0,27 bB
Absorbance 620nm	2,88±0,01 bD	3,25±0,09 bC	4,66±0 aA	3,86±0,07 aB
Color Intensity	26,94±0,25 bD	28,82±0,43 abC	34,09±0,08 aA	30,36±0,56 abB
Hue	0,75±0,00 bD	0,8±0,01 bB	0,85±0,00 aA	0,78±0,00 aC
30days				
Absorbance 420nm	10,81±0,14 aC	11,80±0,19 aB	13,18±0,04 bA	10,33±0,23 cD
Absorbance 520nm	13,79±0,10 bcC	14,22±0,12 aB	14,45±0,01 bA	13,99±0,14 cC
Absorbance 620nm	2,98±0,00 aC	3,39±0,09 aB	4,34±0,00 bA	2,91±0,14 dC
Color Intensity	27,58±0,22 aC	29,40±0,39 aB	32,97±0,05 bA	27,22±0,050 dC
Hue	0,78±0,01 aC	0,83±0,01 aB	0,85±0,00 aA	0,74±0,01 cD

Different letters indicate statistical differences ($p<0.05$). Wine type (a, b, c, d) and oxidation time (A, B, C, D) sharing the same letters are not significantly different.

As previously observed by other authors (Cejudo-et al., 2011; Gambuti et al., 2016), the addition of oxygen can determine different results on 520nm absorbance and C.I., being the effect mainly related to wine initial composition and richness in phenolic compounds. Optical densities at 620 nm, indicating the blue colour of wine (Glories, 1984), were higher in oxygenated wines (Tab. 2), probably due to the formation of co-pigmented complexes and to the polymerization phenomena involving the formation of ethyl bridges, which generated red-violet compounds (Oliveira et al., 2010).

For all samples a decrease of native anthocyanins through all the experiment was observed (Fig. 12), being this trend less pronounced for C and T1. This behavior is in agreement with the trend observed by numerous authors during micro-oxygenation in red wines (Atanasova, et al., 2002; Cano-López, et al., 2006; Gambuti, et al., 2015).

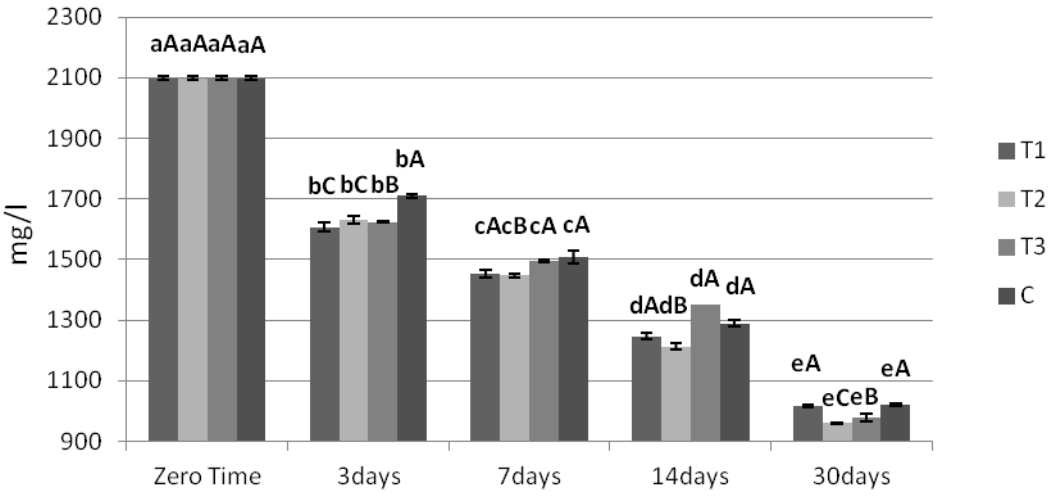


Fig. 12. Evolution of anthocyanins in the experimental samples during the oxidation. Different letters indicate statistical differences ($p<0.05$). Wine type (a, b, c, d) and oxidation time (A, B, C, D) sharing the same letters are not significantly different.

It is known that, as a red wine ages, the proportion of monomeric anthocyanins quickly decreases as the colored polymeric anthocyanins are formed. As a consequence the anthocyanins equilibrium is affected (Kunsági-Máté, et al., 2011). The monomeric anthocyanins in young red wines diminish for their involvement in: i) direct reaction between free anthocyanins and oxidation by-products, such as acetaldehyde and glyoxylic acid (González-Neves, et al., 2007; Puértolas, et al., 2011); ii) reactions mediated by ethanal with condensed tannins (Es-Safi, et al., 1999; Atanasova, et al., 2002; Es-Safi, et al, 2002) and with ellagitannins (Chassaing et al., 2010; García-Estevez et al., 2013).

It is likely that the four samples showed different evolution of the polymeric pigments (LPP + SPP = large + small polymeric pigments). Concerning the chemical nature of these pigments, Versari, et al., (2008) have observed that the sum of SPP and LPP estimated by the Adams assay showed a good correlation (R2= 0.9511) with total polymeric pigments evaluated by HPLC, having a molecular weight m/z <1400 (Peng et al., 2001). Results obtained in this experiment confirm that the presence of enological tannins can determine color stabilization (Versari, et al., 2013).

Fig. 13 shows the evolution of tannins. At zero time a higher content of tannins was observed in all samples spiked with commercial tannins. At molecular level the mechanisms involved in the binding of tannins of different origin with proteins are similar.

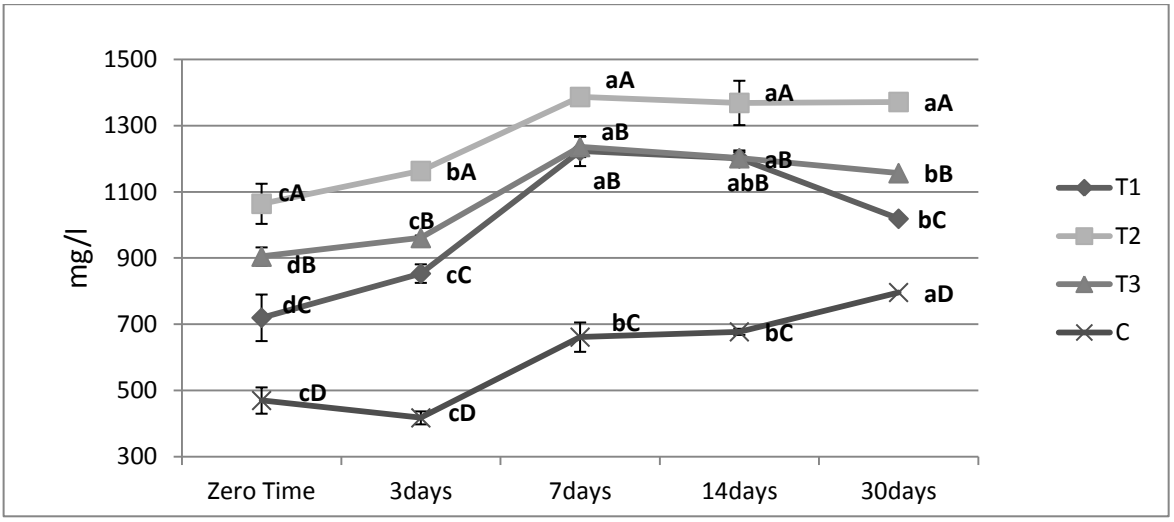


Fig. 13 Evolution of tannins in the four samples (T1, T2, T3 and C) during the oxidation. Different letters indicate statistical differences ($p<0.05$). Wine type (a, b, c, d) and oxidation time (A, B, C, D) sharing the same letters are not significantly different.

During the oxidation, the increase of the level of tannins in C and T2 is probably due to a polymerization of tannin structures (Fulcrand, et al., 1997; Saucier, et al., 1997; Es-Safi, et al., 1999). In T1 and T3 wines a decrease of tannins after 15 days of oxidation has been observed. This is due to the fact that, after the addition of a strong oxidant, the acetaldehyde increases drastically consistent with the occurring of Fenton reaction and with the quick oxidation of ethanol (Waterhouse, & Laurie, 2006; Gambuti, et al., 2015). It is expected that enological tannins determine an increase in the production of acetaldehyde. The reasons can be different: a higher production could be due to the sinergic action of H_2O_2 and traces of metal catalysts present as contaminants of enological tannins preparations; in addition, the presence of more O-diphenols could dramatically increase the production of radicals that could oxidize ethanol (Danilewicz, 2003 and Waterhouse, & Laurie, 2006). The free acetaldehyde should then decreases consistent with the involvement in numerous reactions owing to the great reactivity of this electrophile compound (Peterson, & Waterhouse, 2016).

4.3. Oxidative degradation of malvidin 3-monoglucoside in model solution and real wine.

4.3.1 Model solution experiment

In the first part of this study a model solution containing malvidin 3-monoglucoside was analyzed by detection at 520 nm; as expected by the chemistry of anthocyanins in aqueous solution (Tab. 3), a lower content of malvidin 3-monoglucoside was observed at higher pH. A degradation of malvidin 3-monoglucoside after the addition of hydrogen peroxide occurred and it is slightly enhanced increasing pH. Zhang, et al., (2000) found, in agreement with our results, a minor loss of litchi anthocyanins by H_2O_2 at lower pH. The direct chemical degradation of malvidin 3-monoglucoside is not the only reaction trigged by hydrogen peroxide. The oxidation of other compounds present in solution, ethanol and tartaric acid, by Fenton chemistry (Elias & Waterhouse, 2010).

Tab.3. Effect of SO_2 and GSH on the degradation of malvidin 3-monoglucoside (detected by analysis of the Abs at 520 nm) by hydrogen peroxide in model solution at pH 3.2 and pH 3.8.

	0h	16h	72h
pH3.2	Abs 520 nm	Abs 520 nm	Abs 520 nm
Control	1,12 ± 0,02 Aa	1,02 ± 0,01 Ab	0,98 ± 0,00 Ac
OX	1,12 ± 0,00 Aa	0,68 ± 0,03 Cb	0,26 ± 0,00 Cc
OX+SO2	1,12 ± 0,01 Aa	0,88 ± 0,02 Bb	0,35 ± 0,02 Bc
OX+GSH	1,12 ± 0,01 Aa	0,59 ± 0,01 Db	0,24 ± 0,01 Dc
OX+MIX	1,12 ± 0,00 Aa	0,82 ± 0,05 Bb	0,37 ± 0,01 Bc
	0h	16h	72h
pH3.8	Abs 520 nm	Abs 520 nm	Abs 520 nm
Control	0,66 ± 0,01 Aa	0,64 ± 0,02 Aa	0,62 ± 0,00 Ab
OX	0,66 ± 0,01 Aa	0,28 ± 0,02 Cb	0,27 ± 0,06 Cb
OX+SO2	0,66 ± 0,00 Aa	0,58 ± 0,01 Bb	0,42 ± 0,00 Bc
OX+GSH	0,66 ± 0,00 Aa	0,28 ± 0,01 Cb	0,27 ± 0,01 Cb
OX+MIX	0,66 ± 0,01 Aa	0,59 ± 0,01 Bb	0,40 ± 0,00 Bc

Different letters indicate statistical differences ($p<0.05$). Wine type (A, B, C, D) and oxidation time (a, b, c, d) sharing the same letters are not significantly different.

At pH 3.2 an antioxidant effect of SO_2 is detected regardless the concentration used. At higher pH an anti-Fenton activity was detected only at higher SO_2 concentration (Tab 3). At pH 3.8 low concentration of SO_2 determined a loss of malvidin 3-monoglucoside. It is possible that in these conditions SO_2 did not protect the ethanol but promoted its oxidation, which is in agreement with the fact that a substantial proportion of SO_2 is oxidized to produce highly oxidizing SO radicals such as the peroxomonosulfate radical (Danilewicz, 2007). To understand if GSH may fulfil the antioxidant roles of SO_2 in wine conditions, such as to bind aldehyde compounds and to scavenge hydrogen peroxide, GSH and a combination of both antioxidant (SO_2 and GSH) were used. An increase in the degradation of malvidin 3-monoglucoside was detected when GSH was used alone and in combination with low concentration of SO_2 at both pH. The scavenging effect of SO_2 was instead dominant when its concentration was high and, in this case, no significant acitivity of GSH was detected. These results seems in disagreement with previous results obtained during micro-oxygenation of wine (Gambuti, et al., 2015) where a little protective effect of GSH against anthocyanins degradation was detected. The reason of the different behavior can be found in the fact that in the present experiment only the anti-Fenton activity of GSH was determined while in the previous study the oxidation was obtained adding directly oxygen (in a micro-oxygenation experiment). The small loss of malvidin 3-monoglucoside may be due to the formation, in a strong oxidant medium, of oxidized glutathione GSSG that may acts as oxidant even if this activity has been reported only in living systems and in presence of enzymes (Ceballos-Picot et al., 1996). It is also possible that GSH forms chemical adducts as that observed by Sonni, Moore, Clark, Chinnici, Riponi & Scollary (2011) between GSH and catechin. However no new compounds were detected at 280 nm in the chromatographic condition we used to

analyze anthocyanins. Further investigation can help to elucidate the reason of the disappear of malvidin 3-monoglucoside in presence of GSH at lower pH.

4.3.2 Red wine experiment

In contrast with model solution, in wine lower the pH slightly higher and faster pigment oxidation (Tab 4). This is in agreement with previous results obtained on wine (Pechamat et al., 2014) and it is probably due to the occurrence of reactions involving malvidin 3-monoglucoside, the glyoxylic acid produced by tartaric acid oxidation and, other flavanols present in wine and not in model solution (Es Safi et al., 2003).

Tab.4. Effect of SO_2 and GSH on the degradation of Total anthocyanins (detected by HPLC analysis) by hydrogen peroxide in red wine at pH 3.2 and pH 3.8.

	0h	16h	72h
pH3.2	Total anthocyanins	Total anthocyanins	Total anthocyanins
Control	997,1 ± 14,3 Aa	1000,5 ± 22,2 Aa	977,0 ± 7,8 Aa
OX	997,1 ± 14,3 Aa	537,4 ± 7,8 Cb	487,0 ± 8,6 Cc
OX+SO2 low	997,1 ± 14,3 Aa	660,5 ± 0,8 Bb	613,2 ± 13,6 Bc
OX+SO2 high	997,1 ± 14,3 Aa	1009,0 ± 21,0 Aa	1012,6 ± 6,4 Aa
OX+GSH	997,1 ± 14,3 Aa	546,0 ± 11,0 Cb	459,7 ± 55,9 Ca
OX+SO2low+GSH	997,1 ± 14,3 Aa	662,5 ± 12,6 Ba	635,8 ± 33,1 Ba
OX+SO2high+GSH	997,1 ± 14,3 Aa	979,4 ± 24,0 Aa	943,9 ± 1,4 Aa
	0h	16h	72h
pH3.8	Total anthocyanins	Total anthocyanins	Total anthocyanins
Control	962,4 ± 37,5 Aa	967,7 ± 28,6 Aa	940,7 ± 0,5 Ab
OX	962,4 ± 37,5 Aa	596,5 ± 14,9 Bb	533,5 ± 6,5 Ec
OX+SO2 low	962,4 ± 37,5 Aa	685,1 ± 14,2 Bb	630,1 ± 2,6 Dc
OX+SO2 high	962,4 ± 37,5 Aa	992,1 ± 7,1 Aa	975,8 ± 6,7 Aa
OX+GSH	962,4 ± 37,5 Aa	551,6 ± 10,4 Bb	500,6 ± 12,7Cc
OX+SO2low+GSH	962,4 ± 37,5 Aa	616,9 ± 5,5 Bb	670,2 ± 5,6 Ca
OX+SO2high+GSH	962,4 ± 37,5 Aa	968,2 ± 16,0 Aa	932,2 ± 14,6 Bb

Different letters indicate statistical differences ($p<0.05$). Wine type (A, B, C, D) and oxidation time (a, b, c, d) sharing the same letters are not significantly different.

As expected a positive action of SO_2 against malvidin 3-monoglucoside degradation at both pH was detected and it resulted correlated with concentration and higher with respect to model solution. In contrast with data on model solution no oxidant activity and no significant effect of pH was detected in wine. Departure from model solution in the activity of SO_2 in

real wine has been also recently observed by Danilewicz (2015). These results are not surprising and indicate that in real wine ethanol, malvidin 3-monoglucoside and tartaric acid are not the only target of radicals produced by Fenton but other compounds such as other phenolics, malic acid, glyceraldehyde and volatile compounds compete with them (Elias & Waterhouse, 2010). In contrast with model solution never an oxidant activity of SO_2 was detected. About the GSH, as observed in model solution, a slight oxidative action was observed when it was used alone or in combination with high concentration of SO_2 to contrast hydrogen peroxide action. Thus, also in real wine, GSH is not capable to inhibit Fenton reaction and/or to bind efficiently carbonyls as SO_2 while it seems that, in presence of hydrogen peroxide it can instead contribute to oxidation and/or reacts with flavonoids. A significant effect of GSH was observed and it resulted in a slight loss of anthocyanin in presence of hydrogen peroxide.

Part 2 Post bottling management of oxygen

4.4. Oxygen exposure of tannins-rich red wines during bottle aging. Influence on phenolics and color, astringency markers and sensory attributes

Results obtained in first experiments of PhD thesis showed that wine phenolic composition and its antioxidant protection by SO_2 are of fundamental importance to regulate all reactions of stabilization and to contrast the action of oxygen during wine aging. The use of closures allowing a controlled exposure to oxygen of wine can be therefore a useful practice to manage wine evolution and shelf-life. Table 5 shows the results of SO_2 analyses of wines exposed to increasing amounts of oxygen after 15 months of bottle aging. As expected, both free SO_2 and

total SO_2 decreased during NOx (Kwiatkowski M.J., et al 2007; Ugliano, et al., 2012) mainly due to the reaction of wine with oxygen. The theoretical values of consumed SO_2 can be evaluated considering a simplified model of wine oxidation in which for each mole of oxygen, in presence of metals, one mole of phenolic compound is oxidized to the corresponding quinone and one mole of hydrogen peroxide is produced (Elias & Waterhouse 2010). SO_2 reacts with both products of this reaction because it reduces the quinone back to the catechol and scavenges hydrogen peroxide (Danilewicz, et al., 2008). Therefore a O_2:SO_2 molar reaction ratio of 1:2, corresponding to a concentration ratio of 1mg/L:4mg/L is usually considered for the estimation of SO_2 consumption during wine oxidation (Han, et al., 2015). In our experiment total oxygen exposure after 15 months of bottle aging was maximum 6.6 mg/L for AGL, 10.7 mg/L for CAS and 7.6 mg/L for PALL (Table 5) corresponding to a theoretical SO_2 consumption of almost 28, 44 and 24 mg/L of SO_2

Tab. 5. SO_2 Values and loss of $SO_2$of the wines after15 months of aging in bottle closed with synthetic closures at different oxygen ingress.

	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Free SO_2 loss (mg/L)	Tot SO_2 loss (mg/L)
AGL100	15 ± 0a	67 ± 1a	14	42
AGL300	12 ± 0b	66 ± 3a	17	43
AGL700	12 ± 1b	67 ± 0a	17	42
CAS300	34 ± 1a	52 ± 5a	27	70
CAS700	32 ± 0a	57 ± 3a	29	65
PALL300	28 ± 1a	60 ± 1a	20	49
PALL700	31 ± 3a	61 ± 1a	17	48

Different letters indicate statistical differences ($p<0.05$). Latin small letters (a, b, c) are used to compare wines aged with closures at different oxygen ingress. All the data are expressed as means ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

Assuming that all oxygen was consumed, which is reasonable considering that wines still contained relatively large amounts of oxidizable substrates such as SO_2 and phenolics (Ugliano, 2013; Ferreira, et al., 2015) it is evident that for these wines more than 4 mg/L of total SO_2 were consumed for each mg/L of oxygen (Table 6).

Tab.6 Wine codes, total package oxygen (TPO) at bottling, closure contribution and total oxygen exposure (TOE) for the three wines and the closures used.

	TPO at bottling (mg/L)[a]	Closure contribution (mg/L)[b]	TOE (mg/L)
AGL100	1.0	2.0	3.0
AGL300	1.0	4.0	5.0
AGL700	1.0	5.6	6.6
CAS300	5.0	4.0	9.0
CAS700	5.1	5.6	10.7
PALL300	1.1	4.0	5.1
PALL700	2.0	5.6	7.6

This result is in agreement with results of a previous study on red wines aged for 1 year under similar controlled oxygen exposure (Ugliano, et al., 2012). Previous data on SO_2 consumption and related oxygen exposure of white wines also deviated from the ratio 1 to 4 mg/L (Barril, et al., 2012; Barril, et al., 2016) indicating that this model is not always sufficient to explain the effective consumption of SO_2 because does not allow for numerous other reactions that affect SO_2 fate in wine. Several oxygen-independent mechanisms may determine an higher loss of molecular SO_2. They include the irreversible reactions of SO_2 with the middle ring of flavan-3-ols (Arapitsas, et al., 2014) and α, β-unsaturated aldehydes (Daniel, et al., 2004) and, the loss of SO_2 as vapor through the closure when bottled wines are stored long time at high temperature (Boulton, et al., 1999). No significant differences were observed in total SO_2 decrease of wines with closure oxygen ingress. This result is in agreement with previous results on three Shiraz wines analyzed after 12 months of aging with similar closures (Ugliano, et al., 2012) and with Cabernet Sauvignon wines monitored along 24 months of bottle aging with synthetic and natural closures (Kwiatkowski, et al., 2007). Free SO_2 values within AGL wines were significantly different when a closure having a lower oxygen ingress (AGL100) was used, confirming that, depending on wine initial composition, the postbottling oxygen exposure can alter the overall oxidative state of the wine and the consumption of SO_2 (Ugliano, et al., 2012; Kwiatkowski, et al., 2007).

For all wines, changes of color intensity and hue were observed during aging (Tab. 7). The most relevant change was observed for the color intensity of Aglianico that slightly increased between 7 and 15 months of aging due to the increase of 420 nm, 520 and 620 nm abs and it is related to a general increase of absorbance values typical of aged wine (Boulton 2001). It is due to the involvement of native anthocyanins in reactions giving pigments with different molar absorptivity values (Gómez-Míguez, et al., 2006) as confirmed by the correspondent decrease of monomeric native anthocyanins (Tab. 8). In contrast the variations detected over time for color intensity of CAS and, in slight measure of PALL, were negligible (an increase of color intensity of 1% in the case of CAS while for AGL was of 9%).

Oxygen ingress through closure showed generally a minor influence on color intensity, and only in few cases (AGL after 7 months of aging and PALL after 15 months of aging). In a previous study a positive correlation between color intensity of red Grenache wine and oxygen ingress through closure was detected (Wirth, et al., 2012). The fact that no all wines showed the same behavior is due to differences in phenolic composition as well as in other wine parameters affecting oxidation and copigmentation reactions such as the iron content, the pH and the tartaric and malic acids (King, et al., 2001; Danilewicz, 2014).

Tab. 8. Chromatic characteristics and total anthocyanins of wines aged in bottle for 15 months

		Ads 420 nm				Ads 520 nm				Ads 620 nm				Color Intensity				Hue			
AGLIANICO	bottling	3.11	±	0.05	BBB	3.97	±	0.03	BBB	0.93	±	0.02	BBB	8.01	±	0.07	BBB	0.78	±	0.01	AAA
AGL100	7*	3.00	±	0.03	B	3.86	±	0.03	Bb	0.92	±	0.01	Bb	7.78	±	0.07	Bb	0.78	±	0.00	Aa
AGL300	7*	3.02	±	0.06	B	3.91	±	0.04	Bab	0.93	±	0.02	Bab	7.86	±	0.12	Bab	0.75	±	0.01	Bb
AGL700	7*	3.06	±	0.01	B	3.92	±	0.02	Ba	0.96	±	0.02	Ba	7.93	±	0.03	Ba	0.78	±	0.00	Aa
AGL100	15*	3.27	±	0.13	A	4.25	±	0.15	A	1.19	±	0.09	A	8.71	±	0.37	A	0.77	±	0.01	Ab
AGL300	15*	3.25	±	0.07	A	4.23	±	0.07	A	1.21	±	0.10	A	8.68	±	0.24	A	0.77	±	0.01	Ab
AGL700	15*	3.28	±	0.07	A	4.19	±	0.08	A	1.17	±	0.04	A	8.64	±	0.19	A	0.78	±	0.00	Aa
CASAVECCHIA	bottling	3.86	±	0.05	BB	5.55	±	0.05	AA	1.45	±	0.02	B	10.86	±	0.11	BA	0.70	±	0.00	AB
CAS300	7*	3.77	±	0.06	C	5.29	±	0.05	B	1.49	±	0.02	A	10.55	±	0.07	C	0.71	±	0.02	B
CAS700	7*	3.83	±	0.01	C	5.34	±	0.01	B	1.50	±	0.01	A	10.68	±	0.01	B	0.72	±	0.00	A
CAS300	15*	3.98	±	0.02	A	5.57	±	0.02	A	1.46	±	0.01	B	11.00	±	0.04	A	0.71	±	0.00	A
CAS700	15*	3.95	±	0.02	A	5.61	±	0.12	A	1.44	±	0.01	B	11.00	±	0.14	A	0.70	±	0.01	AB
PALLAGRELLO	bottling	3.83	±	0.03	BA	5.57	±	0.02	AA	1.39	±	0.02	BB	10.78	±	0.07	ABA	0.69	±	0.00	CC
PALL300	7*	3.95	±	0.07	A	5.43	±	0.08	B	1.59	±	0.04	Aa	10.97	±	0.19	A	0.73	±	0.01	Aa
PALL700	7*	3.86	±	0.04	A	5.39	±	0.06	B	1.47	±	0.03	Ab	10.72	±	0.12	A	0.72	±	0.01	Ab
PALL300	15*	3.87	±	0.02	B	5.48	±	0.03	Bb	1.35	±	0.01	Bb	10.71	±	0.05	Bb	0.71	±	0.00	B
PALL700	15*	3.88	±	0.03	A	5.54	±	0.02	Aa	1.38	±	0.01	Ba	10.79	±	0.01	Aa	0.70	±	0.01	B

.*months of bottle aging. AGL100, AGL 300 and AGL 700: Aglianico wines bottled with Nomacorc Select 100, Select 300 and Select 700 closures respectively. CAS300 and CAS700: Casavecchia wines bottled with Nomacorc Select 300 and Select 700 closures respectively. PALL300 and PALL700: Pallagrello wines bottled with Nomacorc Select 300 and Select 700 closures respectively. Different letters indicate statistical differences ($p<0.05$). Latin small letters (a, b, c) are used to compare wines aged with closures at different oxygen ingress. Capital letter (A, B, C) are used to compare wines aged with the same closure over time; the second capital letter near the sample at bottling indicate the statistical differences among AGL300, CAS700 and P700 over time; the third capital letter near AGL sample at bottling indicate the statistical difference among AGL 700 over time. All the data are expressed as means ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

For all samples, wine aging resulted in a significant decrease of monomeric anthocyanins (Tab. 8). The loss of anthocyanins occurs during aging due to autoxidation (Francis & Markakis 1989) and/or to the formation of other more complex pigments and higher in molecular weight (Boulton 2001). The relevant loss of native monomeric anthocyanins was expected given the high sensitivity to oxygen and the greater involvement in copigmentation reactions of native pigments. Two different trends can be observed: the loss of total monomeric anthocyanins of CAS and PALL is higher and almost constant over the 15 months of aging, in the case of AGL the major loss was observed in the first 7 months. The percentages of loss detected for CAS and PALL (ranging between 53 and 47%) are in agreement with a previous study carried out on a Cabernet Franc, Merlot and Blaufrankisch

wine blend, where a decrease comprised between 38 and 44 % of total monomeric anthocyanins was observed after one year of aging in bottle closed with closures at the same OTR (Gambuti A., et al 2013). It is possible that AGL in the first 7 months of aging is

Tab. 8. Monomeric anthocyanins of red wines closed with synthetic closures at different oxygen ingress.

		Dp3glc	Pt3glc	Pn3glc	Mv3glc	Mv3acglc	Mv3cmglc	tot. mon. anth.	% decrease tot. mon. anth.
AGL	0*.	29.5 ± 0.48 AAA	33.89 ± 5.54 AAA	14.71 ± 3.2 AAA	238.36 ± 5.02 AAA	31.53 ± 0.67 AAA	14.89 ± 0.89 AAA	362.88 ± 6.08 AAA	
AGL100	7*	13.04 ± 1.27 Ba	14.27 ± 0.52 Ba	4.25 ± 0.62 B	99.22 ± 1.27 Ba	12.8 ± 1.27 Ba	4.35 ± 0.55 Ba	147.93 ± 2.35 Ba	59
AGL300	7*	11.07 ± 0.22 Bb	13.05 ± 1.2 Ba	4.05 ± 0.87 B	86.85 ± 1.53 Bb	10.06 ± 0.91 Bb	5.02 ± 0.19 Ba	130.10 ± 2.00 Bb	64
AGL700	7*	10.66 ± 0.85 Bb	11.64 ± 0.37 Bb	4.23 ± 0.66 B	81.77 ± 1.17 Bc	9.94 ± 0.56 Bb	2.54 ± 0.41 Bb	120.78 ± 1.73 Bc	67
AGL100	15*	7.71 ± 0.47 C	7.75 ± 0.29 Ca	2.51 ± 0.11 Ca	58.9 ± 1.69 Ca	8.17 ± 0.18 C	nd	85.04 ± 1.77 Ca	77
AGL300	15*	7.06 ± 0.85 C	7.63 ± 0.3 Ca	2.52 ± 0.16 Ca	60.44 ± 2.06 Ca	8.03 ± 0.76 C	nd	85.68 ± 2.36 Ca	76
AGL700	15*	6.72 ± 0.99 C	6.32 ± 0.35 Cb	1.52 ± 0.9 Cb	53.2 ± 4.3 Cb	7.53 ± 0.87 C	nd	75.29 ± 4.59 Cb	79
CAS	0*	75.81 ± 0.5 AAA	125.08 ± 2.81 AAA	79.55 ± 0.86 AAA	1164.4 ± 5.78 AAA	239.06 ± 30.12 AAA	135.69 ± 0.03 AAA	1819.6 ± 30.69 AAA	
CAS300	7*	64.99 ± 3.57 B	98.22 ± 1.16 B	47.2 ± 2.04 B	895.95 ± 11.93 B	188.86 ± 2.27 B	10.86 ± 0.69 B	1306.1 ± 12.84 B	28
CAS700	7*	63.67 ± 2.64 B	97.11 ± 1.68 B	45.97 ± 0.64 B	888.47 ± 8.75 B	189.3 ± 2.15 B	12.04 ± 1.33 B	1296.6 ± 9.50 B	29
CAS300	15*	42.87 ± 0.35 C	74.47 ± 1.24 C	32.71 ± 0.45 C	588.62 ± 6.31 Cb	105.82 ± 4.75 C	nd	844.49 ± 7.92 C	54
CAS700	15*	43.45 ± 0.65 C	75.85 ± 0.83 C	30.63 ± 5 C	597.8 ± 6.8 Ca	111.47 ± 5.83 C	nd	859.2 ± 10.28 C	53
PALL	0*	97.26 ± 2.11 AAA	141.52 ± 3.09 AAA	50.2 ± 2.06 AAA	1082.5 ± 20.78 AAA	255.53 ± 6.94 AAA	115.85 ± 3.39 AAA	1742.9 ± 22.36 AAA	
PALL300	7*	74.81 ± 1.53 Bb	107.44 ± 1.9 B	33.24 ± 0.71 B	806.3 ± 6.94 Bb	175.19 ± 2.57 B	11.49 ± 0.84 B	1208.5 ± 7.64 B	31
PALL700	7*	78.05 ± 1.38 Ba	110.27 ± 1.96 B	34.38 ± 0.94 B	825.21 ± 12.27 Ba	180.4 ± 3.41 B	13.28 ± 1.11 B	1241.6 ± 12.89 B	29
PALL300	15*	53.53 ± 0.53 C	76.52 ± 7.18 C	23.24 ± 0.51 C	546.46 ± 9.28 C	97.8 ± 0.54 C	nd	797.55 ± 9.32 C	54
PALL700	15*	54.34 ± 3.47 C	81.69 ± 6.01 C	23.75 ± 2.03 C	559.73 ± 43.2 C	95.8 ± 1.79 C	nd	815.31 ± 43.42 C	53

*months of bottle aging. Different letters indicate statistical differences ($p<0.05$). AGL100, AGL 300 and AGL 700: Aglianico wines bottled with Nomacorc Select 100, Select 300 and Select 700 closures respectively. CAS300 and CAS700: Casavecchia wines bottled with Nomacorc Select 300 and Select 700 closures respectively. PALL300 and PALL700: Pallagrello wines bottled with Nomacorc Select 300 and Select 700 closures respectively. Latin small letters (a, b, c) are used to compare wines aged with closures at different oxygen ingress. Capital letter (A, B, C) are used to compare wines aged with the same closure over time; the second capital letter near the sample at bottling indicate the statistical differences among AGL300, CAS700 and P700 over time; the third capital letter near AGL sample at bottling indicate the statistical difference among AGL 700 over time. All the data are expressed as means ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

more sensitive to oxygen owing to the lower protection of SO_2. Concerning the kind of monomeric anthocyanins, as previously reported by other authors (Francis & Markakis 1989), the acylated monoglucoside disappears faster than the other monoglucosides owing to a greater reactivity and/or to the hydrolytic degradation of the acylated anthocyanins to

anthocyanidin-3-glucoside (Tab. 8). In the case of AGL wines the loss of acylated anthocyanins resulted also affected by the OTR of closures. These data not only confirm that anthocyanins are among targets of oxidation (Vivas & Glories 1993) but also that the effect of NOx on anthocyanins degradation strongly depends on a wide range of parameters such as: the anthocyanins concentration, nature (acylated or not acylated) and the presence of copigmentation cofactors such as phenolic acids, flavaonols and flavanols and medium characteristics (pH, content of iron, iron speciation) (Boulton, 2001).

Table 9 shows the effect of bottle aging on polymeric SO_2-resistant pigments and phenolics reactive towards vanillin. The levels of large polymeric pigments LPP and small polymeric pigments SPP found in Aglianico, Casavecchia and Pallagrello wines were in agreement with the values reported on local Italian wines (Boselli, et al., 2004) and on Pinot Noir, Cabernet Sauvignon and Syrah (Harbertson J.F., et al 2003). Consistent with previous studies (De Beer, et al., 2004; Versari, et al., 2007), during bottle aging an increase of polymeric pigments (SPP for PALL and CAS and LPP for AGL) occurred (Tab. 9). As well known the formation of SO_2-resistant pigments may balance the loss of native anthocyanins contributing to the stability of color intensity of wines (Boulton 2001). Because of during winemaking and aging a greater formation of LPP compared to SPP is usually observed (Harbertson., et al., 2003; De Beer, et al., 2004; Versari, et al., 2007), it is likely that during red wine aging the formation of LPP succeeds that of SPP. This can indicate that AGL wine is in an advanced oxidative state than PALL and CAS.

Tab. 9. Evolution of short SPP and large LPP polymeric pigments and vanilline reactive flavans VRF during nano-oxygenation

		SPP				LPP				VRF (mg/L)			
AGLIANICO	bottling	0.692	±	0.004		0.267	±	0.032	C	1704	±	5	AAA
AGL100	7*	0.689	±	0.003		0.351	±	0.025	B	1580	±	19	B
AGL300	7*	0.695	±	0.009		0.359	±	0.027	B	1611	±	32	B
AGL700	7*	0.687	±	0.009		0.362	±	0.030	B	1568	±	33	B
AGL100	15*	0.676	±	0.008	Bb	0.446	±	0.006	A	1464	±	34	Ca
AGL300	15*	0.701	±	0.014	a	0.438	±	0.012	A	1403	±	30	Cab
AGL700	15*	0.699	±	0.019	a	0.463	±	0.014	A	1385	±	65	Cb
CASAVECCHIA	bottling	0.579	±	0.022	C	0.307	±	0.012		1433	±	13	BB
CAS300	7*	0.682	±	0.039	B	0.316	±	0.035		1684	±	3	A
CAS700	7*	0.711	±	0.057	B	0.315	±	0.055		1606	±	39	A
CAS300	15*	0.835	±	0.014	Ab	0.293	±	0.003	a	1321	±	47	C
CAS700	15*	0.904	±	0.017	Aa	0.232	±	0.016	b	1323	±	29	C
PALLAGRELLO	bottling	0.593	±	0.026	C	0.297	±	0.026		1504	±	10	BA
PALL300	7*	0.687	±	0.019	B	0.275	±	0.017		1639	±	92	A
PALL700	7*	0.666	±	0.008	B	0.305	±	0.034		1504	±	40	A
PALL300	15*	0.756	±	0.014	A	0.306	±	0.003		1371	±	33	C
PALL700	15*	0.742	±	0.011	A	0.313	±	0.016		1377	±	45	B

*months of bottle aging. AGL100, AGL 300 and AGL 700: Aglianico wines bottled with Nomacorc Select 100, Select 300 and Select 700 closures respectively. CAS300 and CAS700: Casavecchia wines bottled with Nomacorc Select 300 and Select 700 closures respectively. PALL300 and PALL700: Pallagrello wines bottled with Nomacorc Select 300 and Select 700 closures respectively. Different letters indicate statistical differences ($p<0.05$). Latin small letters (a, b, c) are used to compare wines aged with closures at different oxygen ingress. Latin capital letter (A, B, C) are used to compare wines aged with the same closure over time; the second capital letter near the sample at bottling indicate the statistical differences among AGL300, CAS700 and P700 over time; the third capital letter near AGL sample at bottling indicate the statistical difference among AGL 700 over time. All the data are expressed as means ± standard deviation of four replicates (two experimental replicate x two analytical replicate).

The occurrence of polymerization reactions involving monomeric and condensed flavanols can be indirectly evaluated determining vanillin reactive flavans VRF values over time. During aging a decrease of VRF was observed for all wines (Tab 9). Because the essential structural requirements for reaction of the flavonoids with the vanillin reagent are a single bond at the 2, 3 position and free meta-oriented hydroxy groups on the B ring, this assay can be considered a measurement of flavan-3,4-diol, flavan-3-ol, and condensed tannins. Vanillin

reacts with the A ring of flavanols at one of positions 6 or 8 but, also the oxidation by-product acetaldehyde reacts with the same positions on A ring of flavanols . Therefore a decrease of VRF may be considered an indirect measure of the oxidative polymerization of flavanols linked to reactions trigged by acetaldehyde and involving tannins and anthocyanins (Fulcrand, et al., 2004). Also in this case different behaviors were observed: a constant loss was detected for AGL indicating a continuous involvement of flavanols and small tannins in condensation reactions while no definite trend over time was detected for CAS and PALL. About AGL it is possible that condensation reactions are favored by and by an higher presence of carbonyls originated by oxidation reactions due to the lower SO_2 protection. The higher sensitivity to oxygen of AGL can account also for the significant differences among samples with different oxygen ingress. No significant changes with oxygen ingress were instead observed for PALL and CAS. In previous studies both, a decrease in tannins molecular mass with aging and oxygen (McRae, et al.,.2013; McRae, et al., 2015) as well as an increase has been reported for red wines (Vernhet, et al., 2011; Mouls & Fulcrand 2012). Therefore, apart differences due to analytical methods used to determine the molecular mass of tannins, it is still not clear how and to which extent aging and oxygen affect the balance between de-polymerization and condensation reactions of tannins. The percentage of decrease of VRF detected after 15 months of aging for the three wines analyzed followed the trend: AGL (17%)>>PALL (8%) > CAS (7%). It is interesting to observe that the tannins (T) to anthocyanins (A) ratio T/A of the three red wines follow the same trend AGL (19)>> PALL (7) > CAS(6). Because this ratio has been proposed as a key parameter for wine pigment evolution (Mayén, et al., 1995) it is possible that lower amounts of anthocyanins with respect to tannins favor the formation of large polymeric structures. However previous studies carried out on Monastrell wine (Bautista-Ortín, et al., 2007) indicated that not only the T/A ratio but also the specific nature of phenolic compounds alters the trend in forming polymeric pigment. Future studies could

help to understand how the kinetic of the production of highly reactive oxidation products and the formation of polymeric phenolics (pigments and not) are influenced by this ratio.

Apart from color stabilization and formation of polymeric pigments, understanding whether variations in polyphenolic composition trigged by bottle aging at different NOx levels can help to diminish tannins astringency is important. In recent years, more information is available about the changes in chemical structure of tannins with oxidation (Mouls & Fulcrand 2012); however, these data do not provide information on modifications in their sensory characteristics, in particular about their astringency. Changes in astringent characteristics of tannin molecules can be instead easily investigated evaluating their reactivity toward salivary proteins determining the saliva precipitation index SPI (Gambuti, et al., 2013). Data on SPI (Fig. 14) showed that, in agreement with (Muccillo, et al., 2014), Aglianico wine was characterized by the higher content of tannins reactive toward proteins. For all wines, the SPI diminished over time and the higher decrease after 15 months of aging were detected for AGL, followed by CAS and PALL.

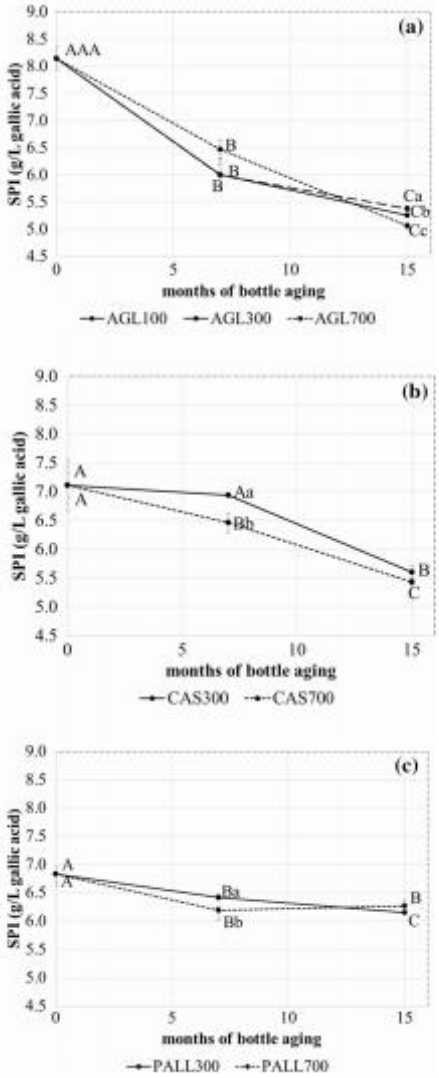


Fig. 14. Evolution of saliva precipitation index SPI of (a) Aglianico, (b) Casavecchia and (c) Pallagrello wines during bottle aging with different oxygen exposure. AGL100, AGL300 and AGL700: Aglianico wines bottled with Nomacorc Select 100, Select 300 and Select 700 closures, respectively. CAS300 and CAS700: Casavecchia wines bottled with Nomacorc Select 300 and Select 700 closures respectively. PALL300 and PALL700: Pallagrello wines bottled with Nomacorc Select 300 and Select 700 closures respectively. Different letters indicate statistical differences ($p < 0.05$). Latin small letters (a, b, c) are used to compare wines aged with closures at different oxygen ingress. Capital letters (A, B, C) are used to compare wines aged with the same closure over time. All the data are expressed as mean ± standard deviation of four replicates (two experimental replicate × two analytical replicate)

This confirms that during aging tannin macromolecules are involved in numerous reactions (due to wine pH, matrix components and oxygen intake) such as (1) the acetaldehyde-mediated polymerization of flavanols, anthocyanidins and proanthocyanidins, (2) the

aldehydes and glyoxylic acid-mediated polymerization of flavanols (Fulcrand, et al., 2004; He, et al., 2008) and (3) the formation of high polymerized and branched tannins by intra- and intermolecular reactions of native proanthocyanidins (Vernhet, et al., 2011; Mouls, & Fulcrand 2012) that determine a change in their capability to form bonds with salivary proteins and precipitate them. Due to the high number of possible tannin structures present in a wine and the great number of reactions that they underwent during aging and oxidation, the reasons of differences can arise from: (a) differences in tannin characteristics, concentrations and extractability from different grapes, (b) different winemaking practices applied and (c) different levels of oxygen exposure during fermentation and post-fermentative practices and aging. The recent finding that about 30 oxidation markers were identified after oxidation of flavanols monomer, dimers and apple and cranberry proanthocyanidins (McRae, et al., 2015) gives an idea of how complex is the pool of reactions triggered by oxygen. In real wine in fact, besides the wide range of structures deriving from the oxidation of monomers, oligomers and more complex tannin molecules, also molecules yielded by anthocyanins–tannins oxidation should be considered. As observed for red pigments, the impact of oxygen exposure on SPI evolution was larger for AGL than for CAS and PALL. AGL showed lower SO_2 protection at bottling and, as in a recent study it has been showed that this antioxidant limits tannin polymerization during micro-oxygenation (Gambuti, et al., 2015), this could justify the rapid decrease detected during bottle aging of AGL. Differences into the chemical nature of tannins, their reactivity toward salivary proteins and in anthocyanins/tannins ratio of wine can also contribute (Versari, et al., 2013). A smaller but significant decrease in SPI over time was detected for CAS and PALL. It is interesting to observe that for these wines reactions involving saliva reactive tannins, as well as pigments, occurred although CAS and PALL were bottled under an high SO_2 protection confirming recent studies showing the inability of SO_2 to completely protect polyphenols during oxidation (Danilewicz, 2016). After 7 months of bottle aging for CAS and PALL and, in higher measure, 15 months of aging for AGL, significant

lower values ($p < 0.005$) of SPI were detected in wines bottled with closures at higher OTR confirming previous findings on the role of NOx on the decrease in tannins reactivity and wine astringency (Gambuti A., et al. 2013).

Beside chromatic characteristics and astringency, NOx is able to influence the olfactory profile of wine (Ugliano, 2013). In order to evaluate in which way the oxygen ingress of closures could have affected the odour profile of the experimental wines, sensory analysis was performed 15 months after bottling. For all the experimental wines, the bottle ageing with different oxygen ingress determined significant differences in their odour profiles (Figure 15).

In agreement with previous studies (Caillé, et al., 2010; Wirth, et al., 2012) higher oxygen exposure determined a significantly higher intensity of fruity odours ("red fruits" for CAS and PALL; "red fruits" and strawberry jam" for AGL). The fact that in our case this behavior was observed for all wines and that other authors (Caillé, et al., 2010; Wirth, et al., 2012) have observed similar outcomes suggest that increases in red wine fruity attributes could be a common response to NOx. The increase of black and red fruit aromas associated with higher oxygen exposure could be linked to formation of aroma compounds such as furaneol, previously associated with red wine fruity aromas and forming under oxidative conditions (Balboa-Lagunero, et al., 2011). It is also possible that, under conditions of increased oxygen exposure, certain aroma compounds potentially binding to SO_2 are in part released due increased SO_2 consumption, as it could be the case for β-damascenone (Roullier-Gall, et al., 2015).

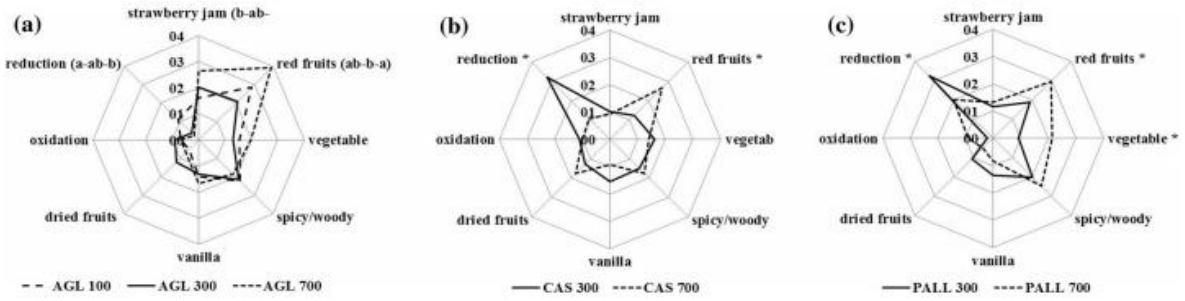


Fig. 15. Odor profiles of (a) Aglianico, b Casavecchia and c Pallagrello wines after 15 months of aging. AGL100, AGL300 and AGL700: Aglianico wines bottled with Nomacorc Select 100, Select 300 and Select 700 closures, respectively. CAS300 and CAS700: Casavecchia wines bottled with Nomacorc Select 300 and Select 700 closures, respectively. PALL300 and PALL700: Pallagrello wines bottled with Nomacorc Select 300 and Select 700 closures, respectively. For Casavecchia (CAS) and Pallagrello (PALL), asterisk (*) near a descriptor indicates a statistically significant difference ($p < 0.05$) between the two wines. For Aglianico (AGL), letters in brackets near a descriptor indicate a statistically significant difference ($p < 0.05$) and refer to AGL100–AGL300–AGL700, in this order. All the data are expressed as mean ± standard deviation of four replicates (two experimental replicate × two analytical replicate)

Aglianico wine aged with the lower oxygen ingress closure (AGL100) showed a higher intensity of reduction attribute (Fig. 15). Volatile sulfur compounds are responsible for the perceived 'reductive' off-aromas and the appearance of these odors during bottle aging, particularly with low levels of oxygen, has been documented (Ugliano, et al., 2012). Their accumulation has been associated to "de novo" formation of sulfur volatiles from several different precursors through specific chemical routes (Ugliano, 2013). Olfactory profiles reveal that the closures with different oxygen ingress determined a different evolution of this odour. In particular, CAS wine showed the most dramatic difference between the two closures, as the CAS300 wine (closed with the closure giving lower oxygen ingress) showed a rating of 3.2 for reduction odour, (referring to the measure scale 1 to 5 points), while in the CAS700 wine, the reduction odour was perceived as "weak" with a rating of 1. A similar result, but with a smaller difference, was found for Pallagrello wine. These data, indicating a significant positive role of oxygen exposure on the decrease of sulfur off-odor, are in agreement with data detected by Ferreira et al., (2014) on 16 Spanish red wines during bottle

aging. The evidence that higher intensity of reduction off-odor are related to wines richer in proanthocyanidins (CAS and PALL) is also in agreement with the previous study (Ferreira, et al., 2014) showing a that increments of a strong sulfur off-flavour, the methanethiol, correlate to proanthocyanidins levels.

Apart from chemical reactions triggered by oxygen and determining a contemporary decrease of sulfur off-odor and an increase of fruity notes, it is also possible that the loss of the masking effect of the reduction odour could have contributed to determine the higher intensity of red fruit odour in PALL700 and CAS700.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

During wine-making and aging, the phenolic composition gradually changes, mainly owing to oxidation reactions which may result in a decrease in astringency as well as in color stabilization. Anthocyanins and tannins are the main compounds involved in these changes while the acetaldehyde, thanks to its electrophilic properties, is one of the key compounds that can track the limit between a moderate positive oxidation and the appearance of oxidative off-flavour. The study of literature evidenced that, although in last decades important new insights have been done in this field (Wildenradt, & Singleton, 1974; Danilewicz, 2003; Laurie, & Waterhouse, 2006; Gambuti, et al., 2015), two main issues remain to be investigate in more detail. First the role of initial wine composition in terms of phenolic composition, pH and antioxidants used in winemaking. In addition, although nowadays the techniques allowing an exposure of wine to controlled amount of oxygen have been proposed and widely evaluated by scientific community, the relationship between the initial wine composition (phenolic compounds and preservatives eventually presents) and the wine shelf-life needs still more investigation.

In the first experiment made in this PhD work, with the aim to investigate the role of wine initial phenolic composition on the evolution of wine during the oxidation, the effect of the anthocyanins/tannins ratio on the production of acetaldehyde and on the evolution of chromatic characteristics and native phenolic compounds has been evaluated. Oligomeric tannins were added to a red wine rich in anthocyanins so as to have wines with three different anthocyanin A/tannin T ratios W (ratio 1A: 0.5T) WT (ratio 1A: 1T) WTT (ratio 1A: 3T). Samples were then treated with hydrogen peroxide to trigger the Fenton reaction. The samples treated with a higher concentration of tannins showed a clear improvement of color intensity, with oxidation mainly due to an increase in polymeric pigments. The higher the content of tannins, the lower the production of acetaldehyde. These are the first data showing the effect

of different A/T ratios on the production of acetaldehyde during an oxidation process. May be that, as wine was very rich in anthocyanins, the addition of tannins is well tolerate and increases the consumption of acetaldehyde in reactions stabilizing color. In this study high concentration of anthocyanins were considered, further studies should consider wines with lower concentration of anthocyanins to have a wider picture of the effect of this ratio on wine evolution.

Results obtained in the first study suggested that the addition of tannins to red wine is a concrete possibility to improve the wine aging behavior. To stabilize wine color and determine improvements in mouthfeel properties and longevity the addition of enological tannins preparations obtained from grapes (condensed tannins) and wood (gallotannins and ellagitannins) are allowed in wine industry. However, no study has considered the effect of these tannins on the outcome of red wine oxidation. Therefor in this study enological tannins were added to a red wine so as to have wines with three different phenolic composition. Samples, as for the first study, were then treated with hydrogen peroxide to trigger the Fenton reaction. All tannins added have determined a greater production of acetaldehyde that was consumed in 30 days. In addition an increase of the reactivity of wine tannins towards proteins during first phases of oxidation was observed for all wines.

To explore other possibilities to manage wine oxidation, the use of exogenous antioxidants has been evaluated in the third experiment. As pH is a fundamental parameter for all reactions involved in wine evolution, the experiment was performed at two pH covering the range usually found in wines. The oxidative degradation by hydrogen peroxide of native grape anthocyanin was studied in model solutions and in red wines added with increasing concentration of sulfur dioxide and glutathione GSH. The presence of hydrogen peroxide and metal ions in traces has allowed to investigate the possibility to use GSH to prevent the Fenton reaction in wine conditions. Two wines pH were considered: 3.2 and 3.8. The protective effect of sulfur dioxide on malvidin 3-monoglucoside degradation was higher at

lower pH in model solution. No effect of pH on sulfur dioxide action towards the native anthocyanin in real wine was detected. Surprisingly GSH has determined an increase in the degradation of malvidin 3-monoglucoside regardless of pH. A recent study (Arapitsas et al., 2016) showed that, in the presence of oxygen, GSH gives glutathione disulfide (GSSG), and GSSG reacts with SO_3H^- to provide S-sulfonated glutathione (GSSO3H); as a result, in these conditions GSH can decrease the protective action of sulfur dioxide. Therefore GSH is not effective in prevent native anthocyanins loss due to the Fenton reaction during red wine aging. It is likely that, during wine oxidation, its action is only limited to its ability to react with quinones. Results obtained in the fourth experiment carried out in this PhD thesis support this hypothesis. These results confirm that a proper use of sulfur dioxide, taking into account the pH of treated wines, remains the best possibility to manage wine oxidation.

In the second part of PhD thesis experiments, a technique allowing a controlled management of oxygen exposure in wine industries, the NOx, has been considered. In an industrial trial performed on three Southern Italy red wines Aglianico (AGL), Casavecchia (CAS) and Pallagrello (PALL) with high content of total tannins but different initial anthocyanins composition. These wines were aged in bottle for 15 months with closures allowing different degrees of wine oxygen exposure. In all wines oxygen exposure resulted in a progressive decrease of monomeric anthocyanins, vanillin reactive flavans and content of tannins reactive towards salivary proteins. In contrast no significant decrease in color intensity was detected due to the formation of polymeric pigments. For all wines, the closure with the highest oxygen ingress determined a higher intensity of red fruit notes.

6. REFERENCES

- Adachi, T., Nonogi, H., Fuke, T., Ikuzawa, M., Fujita, K., Izumi, T., ... & Iwaida, M. (1979). On the combination of sulphite with food ingredients (aldehydes, ketones and sugars). II. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *168*(3), 200-205.
- Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing: a comprehensive study. *Analytica Chimica Acta*, *563*(1), 238-254.
- Anderson, M. E. (1998). Glutathione: an overview of biosynthesis and modulation. *Chemico-biological interactions*, *111*, 1-14.
- Arapitsas, P., Speri, G., Angeli, A., Perenzoni, D., & Mattivi, F. (2014). The influence of storage on the "chemical age" of red wines. *Metabolomics*, *10*(5), 0.
- Arapitsas, P., Ugliano, M., Perenzoni, D., Angeli, A., Pangrazzi, P., & Mattivi, F. (2016). Wine metabolomics reveals new sulfonated products in bottled white wines, promoted by small amounts of oxygen. *Journal of Chromatography A*, *1429*, 155-165.
- Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta*, *458*(1), 15-27.
- Bakker, J., & Timberlake, C. F. (1997). Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *Journal of Agricultural and Food Chemistry*, *45*(1), 35-43.
- Balboa-Lagunero, T., Arroyo, T., Cabellos, J. M., & Aznar, M. (2011). Sensory and olfactometric profiles of red wines after natural and forced oxidation processes. *American journal of enology and viticulture*, *62*(4), 527-535.
- Barril, C., Clark, A. C., & Scollary, G. R. (2012). Chemistry of ascorbic acid and sulfur dioxide as an antioxidant system relevant to white wine. *Analytica Chimica Acta*, *732*, 186-193.
- Barril, C., Rutledge, D. N., Scollary, G. R., & Clark, A. C. (2016). Ascorbic acid and white wine production: a review of beneficial versus detrimental impacts. *Australian Journal of Grape and Wine Research*.
- Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., & Gómez-Plaza, E. (2007). The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *Journal of Food Composition and Analysis*, *20*(7), 546-552.
- Bautista-Ortin, A. B., Martinez-Cutillas, A., Ros-Garcia, J. M., Lopez-Roca, J. M., & Gomez-Plaza, E. (2005). Improving colour extraction and stability in red wines: the use of maceration enzymes and enological tannins. *International Journal of Food Science and Technology*, *40*(8), 867–878.
- Benabdeljalil, C., Cheynier, V., Fulcrand, H., Hakiki, A., Mosaddak, M., & Moutounet, M. (2000). Evidence of new pigments resulting from reaction between anthocyanins and yeast metabolites [hemisynthesis, fermentation products]. *Sciences des Aliments (France)*.
- Bittner, S. (2006). When quinones meet amino acids: chemical, physical and biological consequences. *Amino acids*, *30*(3), 205-224.
- Blaauw, D. A. (2009). *Micro-oxygenation in contemporary winemaking* (Doctoral dissertation, Thesis, Cape Wine Academy, Stellenbosch, South Africa).
- Boselli, E., Boulton, R. B., Thorngate, J. H., & Frega, N. G. (2004). Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. *Journal of agricultural and food chemistry*, *52*(12), 3843-3854.
- Boss, P. K., Davies, C., & Robinson, S. P. (1996). Anthocyanin composition and anthocyanin pathway gene expression in grapevine sports differing in berry skin colour. *Australian Journal of Grape and Wine Research*, *2*(3), 163-170.
- Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (2013). *Principles and practices of winemaking*. Springer Science & Business Media.
- Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (1999). Preparation of musts and juice. In *Principles and practices of winemaking* (pp. 65-101). Springer US.
- Bradshaw, M. P., Prenzler, P. D., & Scollary, G. R. (2001). Ascorbic acid-induced browning of (+)-catechin in a model wine system. *Journal of agricultural and food chemistry*, *49*(2), 934-939.
- Budić-Leto, I., Vrhovšek, U., Gajdoš Kljusurić, J., & Lovrić, T. (2009). Anthocyanin pattern of skin extracts from the babić and plavac mali grapes and anthocyanin pattern of the produced wine. *Acta alimentaria*, *38*(1), 67-75.
- Burns, J., Mullen, W., Landrault, N., Teissedre, P. L., Lean, M. E., & Crozier, A. (2002). Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes. *Journal of agricultural and food chemistry*, *50*(14), 4096-4102.

- Burroughs, L. F., & Sparks, A. H. (1973). Sulphite-binding power of wines and ciders. I. Equilibrium constants for the dissociation of carbonyl bisulphite compounds. *Journal of the Science of Food and Agriculture*, *24*(2), 187-198.
- Cabezas, J. A., Cervera, M. T., Arroyo-García, R., Ibáñez, J., Rodríguez-Torres, I., Borrego, J., ... & Martínez-Zapater, J. M. (2003). Garnacha and Garnacha Tintorera: genetic relationships and the origin of teinturier varieties cultivated in Spain. *American Journal of Enology and Viticulture*, *54*(4), 237-245.
- Cacho, J., Castells, J. E., Esteban, A., Laguna, B., & Sagristá, N. (1995). Iron, copper, and manganese influence on wine oxidation. *American Journal of Enology and Viticulture*, *46*(3), 380-384.
- Caillé, S., Samson, A., Wirth, J., Diéval, J. B., Vidal, S., & Cheynier, V. (2010). Sensory characteristics changes of red Grenache wines submitted to different oxygen exposures pre and post bottling. *Analytica Chimica Acta*, *660*(1), 35-42.
- Cano-López, M., Pardo-Minguez, F., López-Roca, J. M., & Gómez-Plaza, E. (2007). Chromatic characteristics and anthocyanin profile of a micro-oxygenated red wine after oak or bottle maturation. *European Food Research and Technology*, *225*(1), 127-132..
- Cano-López, M., Pardo-Mínguez, F., Schmauch, G., Saucier, C., Teissedre, P. L., López-Roca, J. M., & Gómez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *Journal of agricultural and food chemistry*, *56*(14), 5932-5941.
- Carlton, W. K., Gump, B., Fugelsang, K., & Hasson, A. S. (2007). Monitoring acetaldehyde concentrations during micro-oxygenation of red wine by headspace solid-phase microextraction with on-fiber derivatization. *Journal of agricultural and food chemistry*, *55*(14), 5620-5625.
- Casellato, U., Tamburini, S., Vigato, P. A., Vidali, M., & Fenton, D. E. (1984). Binuclear oxovanadium(IV) complexes as catalyst for the oxygenation of the catechols. *Inorganica Chimica Acta*, *84*(1), 101–104.
- Castellari, M., Arfelli, G., Riponi, C., & Amati, A. (1998). Evolution of phenolic compounds in red winemaking as affected by must oxygenation. *American Journal of Enology and Viticulture*, *49*(1), 91-94.
- Castellari, M., Matricardi, L., Arfelli, G., Galassi, S., & Amati, A. (2000). Level of single bioactive phenolics in red wine as a function of the oxygen supplied during storage. *Food Chemistry*, *69*(1), 61-67.
- Castillo-Muñoz, N., Fernández-González, M., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2009). Red-color related phenolic composition of Garnacha Tintorera (Vitis vinifera L.) grapes and red wines. *Journal of agricultural and food chemistry*, *57*(17), 7883-7891.
- Ceballos-Picot, I., Witko-Sarsat, V., Merad-Boudia, M., Nguyen, A. T., Thévenin, M., Jaudon, M. C., ... & Descamps-Latscha, B. (1996). Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radical Biology and Medicine*, *21*(6), 845-853.
- Cejudo-Bastante, M. J., Pérez-Coello, M. S., & Hermosín-Gutiérrez, I. (2011). Effect of wine micro-oxygenation treatment and storage period on colour-related phenolics, volatile composition and sensory characteristics. *LWT-Food Science and Technology*, *44*(4), 866-874.
- Chassaing, S., Lefeuvre, D., Jacquet, R., Jourdes, M., Ducasse, L., Galland, S., ... & Quideau, S. (2010). Physicochemical Studies of New Anthocyano-Ellagitannin Hybrid Pigments: About the Origin of the Influence of Oak C-Glycosidic Ellagitannins on Wine Color. *European Journal of Organic Chemistry*, *2010*(1), 55-63.
- Cheynier, V. F., & Van Hulst, M. W. (1988). Oxidation of trans-caftaric acid and 2-S-glutathionylcaftaric acid in model solutions. *Journal of Agricultural and Food Chemistry*, *36*(1), 10-15.
- Cheynier, V., Basire, N., & Rigaud, J. (1989). Mechanism of trans-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *Journal of Agricultural and Food Chemistry*, *37*(4), 1069-1071.
- Cheynier, V., Fulcrand, H., Guyot, S., Oszmianski, J., & Moutounet, M. (1995). Reactions of enzymically generated quinones in relation to browning in grape musts and wines..
- Cheynier, V., Souquet, J. M., & Moutounet, M. (1989). Glutathione content and glutathione to hydroxycinnamic acid ratio in Vitis vinifera grapes and musts. *American Journal of Enology and Viticulture*, *40*(4), 320-324.
- Chrysochou, P., Krystallis, A., Mocanu, A., & Leigh Lewis, R. (2012). Generation Y preferences for wine: an exploratory study of the US market applying the best-worst scaling. *British Food Journal*, *114*(4), 516-528.
- Clark, A. C., Prenzler, P. D., & Scollary, G. R. (2007). Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid. *Food chemistry*, *102*(3), 905-916.
- da Silva Ferreira, A. C., Barbe, J.-C., & Bertrand, A. (2002). Heterocyclic Acetals from Glycerol and Acetaldehyde in Port Wines: Evolution with Aging. *Journal of Agricultural and Food Chemistry*, *50*(9), 2560–2564.

- Daniel, M. A., Elsey, G. M., Capone, D. L., Perkins, M. V., & Sefton, M. A. (2004). Fate of damascenone in wine: the role of SO2. *Journal of agricultural and food chemistry*, *52*(26), 8127-8131.
- Danilewicz JC (2014) Role of tartaric and malic acids in wine oxidation.*Journal of agricultural and food chemistry* 62(22):5149-5155
- Danilewicz JC, Seccombe JT, 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
- 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. (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*(1), 53-60.
- 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*(3), 319–328.
- Danilewicz, J. C. (2016). "Reaction of Oxygen and Sulfite in Wine." *American Journal of Enology and Viticulture* 67 (1): 13–17.
- Danilewicz, J. C., & Wallbridge, P. J. (2010). Further studies on the mechanism of interaction of polyphenols, oxygen, and sulfite in wine. *American Journal Of Enology And Viticulture*, *61*(2), 166-175.
- De Beer D, Harbertson JF, Kilmartin PA, Roginsky V, Barsukova T, Adams DO, Waterhouse AL (2004) Phenolics: A comparison of diverse analytical methods. *American Journal of Enology and Viticulture* 55(4):389-400
- Del Alamo-Sanza, M del, and LM Cárcel.(2017). "Characterization of Oxygen Transmission Rate of Oak Wood Species Used in Cooperage." *Journal of Agricultural.*
- del Carmen Llaudy, M., Canals, R., González-Manzano, S., Canals, J. M., Santos-Buelga, C., & Zamora, F. (2006). Influence of micro-oxygenation treatment before oak aging on phenolic compounds composition, astringency, and color of red wine. *Journal of agricultural and food chemistry*, *54*(12), 4246-4252.
- Di Stefano, R.; Guidoni, S. (1989). La determinazione dei polifenoli totali nei mosti e nei vini. *Vignevini*, *16*(12), 47–52.
- Doco, T., Es-Safi, N. E., Cheynier, V., & Moutounet, M. (1996). Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *Journal of Chromatography A*, *752*(1-2), 85-91.
- Du Toit, W. J., Lisjak, K., Marais, J., & du Toit, M. (2006). The effect of micro-oxygenation on the phenolic composition, quality and microbial composition of South African red wines. *South African Journal of Enology and Viticulture*, *27*, 57-67.
- Dubourdieu, D., Moine-Ledoux, V., Lavigne-Cruege, V., & Tominaga, T. (2003). Recent advances in white wine aging: the key role of the lees. *Progrès Agricole et Viticole (France)*.
- Elias, R. J. & Waterhouse, A. L. (2010). Controlling the Fenton Reaction in Wine. *Journal of Agricultural and Food Chemistry*, *58*, 1699-1707.
- Es-Safi, N. E., Cheynier, V., & Moutounet, M. (2003). Implication of phenolic reactions in food organoleptic properties. *Journal of Food Composition and Analysis*, *16*(5), 535-553.
- Es-Safi, N. E., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-O-glucoside in a model solution system. *Journal of agricultural and food chemistry*, *47*(5), 2096-2102.
- Es-Safi, N. E., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-O-glucoside in a model solution system. *Journal of agricultural and food chemistry*, *47*(5), 2096-2102.
- Es-Safi, N. E., Le Guernevé, C., Cheynier, V., & Moutounet, M. (2000). New phenolic compounds formed by evolution of (+)-catechin and glyoxylic acid in hydroalcoholic solution and their implication in color changes of grape-derived foods. *Journal of agricultural and food chemistry*, *48*(9), 4233-4240..
- Fanzone, M., Peña-Neira, A., Gil, M., Jofré, V., Assof, M., & Zamora, F. (2012). Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Research International*, *45*(1), 402-414.
- Ferreira V, Bueno M, Franco-Luesma E, Culleré L, Fernández-Zurbano P. (2014). Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *Journal of agricultural and food chemistry* 62(41):10015-10027
- Ferreira V, Carrascón V, Bueno M, Ugliano M, Fernandez-Zurbano P.(2015). Oxygen consumption by red wines. Part I: consumption rates, relationship with chemical composition and role of SO2. *Journal of agricultural and food chemistry* 63 (51):10928–10937
- Fessler, J.H. (1961). "Erythorbic Acid and Ascorbic Acid as Antioxidants in Bottled Wines." *American Journal of Enology and Viticulture* 12: 20–24.

- Flanzy, M. (1959). Recherches sur la vinification des vins doux naturels. In *Annales de Technologie Agricole* (Vol. 8, pp. 285-320).
- Flecknoe-Brown, A. (2006). " Diffusive" methods of oxygenating wine: sismpler, better, lower-cost barrel replacement. *Australian and New Zealand grapegrower and winemaker*, (511), 64-69.
- Fontoin, H., Saucier, C., Teissedre, P. L., & Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference*, *19*(3), 286–291.
- Foo, L. Y., & Porter, L. J. (1981). The structure of tannins of some edible fruits. *Journal of the Science of Food and Agriculture*, *32*(7), 711-716.
- Francia-Aricha, E. M., Guerra, M. T., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (1997). New Anthocyanin Pigments Formed after Condensation with Flavanols. *Journal of Agricultural and Food Chemistry*, *45*(6), 2262–2266.
- Francis, F. J., & Markakis, P. C. (1989). Food colorants: anthocyanins. *Critical Reviews in Food Science & Nutrition*, *28*(4), 273-314.
- Fulcrand H, Atanasova V, Salas E, Cheynier V .(2004) .The fate of anthocyanins in wine: Are there determining factors? In ACS symposium series (Vol. 886, 68-88) Oxford University Press
- Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry*, *47*(7), 1401-1407.
- Fulcrand, H., Cheynier, V., Oszmianski, J., & Moutounet, M. (1997). An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry*, *46*(2), 223-227.
- Fulcrand, H., dos Santos, P. J. C., Sarni-Manchado, P., Cheynier, V., & Favre-Bonvin, J. (1996). Structure of new anthocyanin-derived wine pigments. *Journal of the Chemical Society, Perkin Transactions 1*, (7), 735-739.
- Gambuti, A., Han, G., Peterson, A. L., & Waterhouse, A. L. (2015). Sulfur dioxide and glutathione alter the outcome of microoxygenation. *American Journal of Enology and Viticulture*, ajev-2015.
- Gambuti, A., Rinaldi, A., Ugliano, M., & 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*(8), 1618–1627.
- Gambuti, A., Siani, T., Picariello, L., Rinaldi, A., Lisanti, M. T., Ugliano, M., ... & Moio, L. (2016). 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*, 1-12.
- García-Estévez, Ignacio, Rémi Jacquet, Cristina Alcalde-Eon, Julián C. Rivas-Gonzalo, M. Teresa Escribano-Bailón, and Stéphane Quideau. 2013. "Hemisynthesis and Structural and Chromatic Characterization of Delphinidin 3- O -Glucoside–Vescalagin Hybrid Pigments." Journal of Agricultural and Food Chemistry 61 (47). American Chemical Society: 11560–68. doi:10.1021/jf4033188.
- Glories, Y. (1984). La couleur des vins rouges. 1° e 2° partie. *Connaiss. Vigne Vin*, *18*, 253–271.
- Godden, P., Francis, L., Field, J., Gishen, M., Coulter, A., Valente, P., ... & Robinson, E. (2002, September). Closures–Results from the AWRI three years post bottling. In *Romeo Bragato Conference* (pp. 12-14).
- Gómez-Míguez, M., González-Manzano, S., Escribano-Bailón, M. T., Heredia, F. J., & Santos-Buelga, C. (2006). Influence of different phenolic copigments on the color of malvidin 3-glucoside. *Journal of Agricultural and Food Chemistry*, *54*(15), 5422-5429.
- Gómez-Plaza, E., & Cano-López, M. (2011). A review on micro-oxygenation of red wines: Claims, benefits and the underlying chemistry. *Food Chemistry*, *125*(4), 1131-1140.
- González-del Pozo, A., Arozarena, Í., Noriega, M.-J., Navarro, M., & Casp, A. (2010). Short- and long-term effects of micro-oxygenation treatments on the colour and phenolic composition of a Cabernet Sauvignon wine aged in barrels and/or bottles. *European Food Research and Technology*, *231*(4), 589–601.
- González-Neves, G., Franco, J., Barreiro, L., Gil, G., Moutounet, M., & Carbonneau, A. (2007). Varietal differentiation of Tannat, Cabernet-Sauvignon and Merlot grapes and wines according to their anthocyanic composition. *European Food Research and Technology*, *225*(1), 111-117.
- González-Sanjosé, M. L., Ortega-Heras, M., & Pérez-Magariño, S. (2008). Microoxygenation treatment and sensory properties of young red wines. *Food Science and Technology International*, *14*(5), 123.
- Guerrero, R. F., & Cantos-Villar, E. (2015). Demonstrating the efficiency of sulphur dioxide replacements in wine: a parameter review. *Trends in Food Science & Technology*, *42*(1), 27-43.
- Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., & Riechel, T. L. (1998). High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. *Journal of Agricultural and Food Chemistry*, *46*(5), 1887–1892.

- Han, G., Ugliano, M., Currie, B., Vidal, S., Diéval, J. B., & Waterhouse, A. L. (2015). Influence of closure, phenolic levels and microoxygenation on Cabernet Sauvignon wine composition after 5 years' bottle storage. *Journal of the Science of Food and Agriculture*, *95*(1), 36-43.
- Han, G., Wang, H., Webb, M. R., & Waterhouse, A. L. (2015). A rapid, one step preparation for measuring selected free plus SO2-bound wine carbonyls by HPLC-DAD/MS. *Talanta*, *134*, 596–602.
- Harbertson, J. F., Kilmister, R. L., Kelm, M. A., & Downey, M. O. (2014). Impact of condensed tannin size as individual and mixed polymers on bovine serum albumin precipitation. *Food Chemistry*, *160*, 16–21.
- 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*(3), 999-1008.
- Harbertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture*, *54*(4), 301-306
- Hart, A., & Kleinig, A. (2005). The role of oxygen in the aging of bottled wine. *The Australian and New Zealand Wine Industry Journal*, *20*(2), 46-50.
- Hayasaka, Y., & Kennedy, J. A. (2003). Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Australian Journal of Grape and Wine Research*, *9*(3), 210-220.
- He, F., Pan, Q. H., Shi, Y., & Duan, C. Q. (2008). Chemical synthesis of proanthocyanidins in vitro and their reactions in aging wines. *Molecules*, *13*(12), 3007-3032.
- Isaacs, N. S., & van Eldik, R. (1997). A mechanistic study of the reduction of quinones by ascorbic acid. *Journal of the Chemical Society, Perkin Transactions 2*, (8), 1465-1468.
- Jackson, R. S. (2008). *Wine science: principles and applications*. Academic press.
- Janeš, L., Lisjak, K., & Vanzo, A. (2010). Determination of glutathione content in grape juice and wine by high-performance liquid chromatography with fluorescence detection. *Analytica chimica acta*, *674*(2), 239-242.
- Kettern, W. (1985). Bedeutung von SO2, CO2 und Sauerstoff bei der Abfüllung. *Weinwirtschaft*, *5*, 142-148.
- Kielhöfer, E., & Würdig, G. (1962). Die Oxidationsvorgänge im Wein. *Weinwissenschaft*, *9*, 217-232.
- Kilmartin, P. A. (2010). Understanding and controlling non-enzymatic wine oxidation. In *Managing wine quality* (Vol. 2, pp. 432-458). Woodhead Publishing Limited Cambridge, UK.
- King, G., & Zeng, L. (2001). Logistic regression in rare events data. *Political analysis*, 137-163.
- Kontoudakis, N., González, E., Gil, M., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Influence of wine pH on changes in color and polyphenol composition induced by micro-oxygenation. *Journal of Agricultural and Food Chemistry*, *59*(5), 1974-1984.
- Kritzinger, E. C., Bauer, F. F., & Du Toit, W. J. (2012). Role of glutathione in winemaking: a review. *Journal of Agricultural and Food Chemistry*, *61*(2), 269-277.
- Kunsági-Máté, S., May, B., Tschiersch, C., Fetzer, D., Horváth, I., Kollár, L., & Nikfardjam, M. P. (2011). Transformation of stacked π–π-stabilized malvidin-3-O-glucoside—Catechin complexes towards polymeric structures followed by anisotropy decay study. *Food Research International*, *44*(1), 23-27.
- Kwiatkowski, M. J., Skouroumounis, G. K., Lattey, K. A., & Waters, E. J. (2007). The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a Cabernet Sauvignon wine during two years' storage. *Australian Journal of Grape and Wine Research*, *13*(2), 81-94.
- Labrouche, F., Clark, A. C., Prenzler, P. D., & Scollary, G. R. (2005). Isomeric influence on the oxidative coloration of phenolic compounds in a model white wine: Comparison of (+)-catechin and (−)-epicatechin. *Journal of agricultural and food chemistry*, *53*(26), 9993-9998.
- Laurie, V. F., & Waterhouse, A. L. (2006). 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*(13), 4668–4673.
- Lavigne, V., Pons, A., & Dubourdieu, D. (2007). Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection: changes in concentration in dry white wines during alcoholic fermentation and aging. *Journal of Chromatography A*, *1139*(1), 130-135.
- Lea, A. G. H. (1990). Bitterness and astringency: the procyanidins of fermented apple ciders. *Developments in food science*.
- Lessica, M., & Kosmerl, T. (2009). Micro-oxygenation of red wines. *Acta Agriculturae Slovenica*, *93*(3), 327-336.
- Li, H., Guo, A., & Wang, H. (2008). Mechanisms of oxidative browning of wine. *Food chemistry*, *108*(1), 1-13.
- Li, H., Wang, H., Yuan, C., & Wang, S. (2005). *Wine chemistry*. Beijing: Scientific Publishing Company.

- Lien, E. J., Ren, S., Bui, H. H., & Wang, R. (1999). Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Biology and Medicine*, *26*(3), 285-294.
- Lopes, P., Saucier, C., Teissedre, P. L., & Glories, Y. (2006). Impact of storage position on oxygen ingress through different closures into wine bottles. *Journal of agricultural and food chemistry*, *54*(18), 6741-6746.
- Lotito, S. B., Actis-Goretta, L., Renart, M. L., Caligiuri, M., Rein, D., Schmitz, H. H., ... & Fraga, C. G. (2000). Influence of oligomer chain length on the antioxidant activity of procyanidins. *Biochemical and Biophysical Research Communications*, *276*(3), 945-951.
- Maggu, M., Winz, R., Kilmartin, P. A., Trought, M. C., & Nicolau, L. (2007). Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *Journal of Agricultural and Food Chemistry*, *55*(25), 10281-10288.
- Main, G. L. (1992). *Juice fining treatments to reduce browning and use of sulfur dioxide in white wine production* (Doctoral dissertation, University of Arkansas, Fayetteville).
- Martín, M., Montes, F. J., & Galán, M. A. (2007). Oxygen transfer from growing bubbles: Effect of the physical properties of the liquid. *Chemical Engineering Journal*, *128*(1), 21-32.
- Masi, E., Vignani, R., Di Giovannantonio, A., Mancuso, S., & Boselli, M. (2001). Ampelographic and cultural characterisation of the Casavecchia variety. *Advances in horticultural science*, 47-55.
- Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., & Velasco, R. (2006). Metabolite profiling of grape: flavonols and anthocyanins. *Journal of agricultural and food chemistry*, *54*(20), 7692-7702.
- Maury, C., Sarni-Manchado, P., Lefebvre, S., Cheynier, V., & Moutounet, M. (2001). Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. *American journal of enology and viticulture*, *52*(2), 140-145.
- Mayén, M., Mérida, J., & Medina, M. (1995). Flavonoid and non-flavonoid compounds during fermentation and post-fermentation standing of musts from Cabernet Sauvignon and Tempranillo grapes. *American Journal of Enology and Viticulture*, *46*(2), 255-261.
- Mazza, G., & Francis, F. J. (1995). Anthocyanins in grapes and grape products. *Critical Reviews in Food Science & Nutrition*, *35*(4), 341-371.
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., & Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *Journal of agricultural and food chemistry*, *47*(10), 4009-4017.
- McArdle, J. V., & Hoffmann, M. R. (1983). Kinetics and mechanism of the oxidation of aquated sulfur dioxide by hydrogen peroxide at low pH. *The Journal of Physical Chemistry*, *87*(26), 5425-5429.
- McCord, J. (2003). Application of toasted oak and micro-oxygenation to ageing of Cabernet Sauvignon wines. *Aust. NZ Grapegrower Winemaker*, *474*, 43-53.
- McRae, J. M., Day, M. P., Bindon, K. A., Kassara, S., Schmidt, S. A., Schulkin, A., ... & Smith, P. A. (2015). Effect of early oxygen exposure on red wine colour and tannins. *Tetrahedron*, *71*(20), 3131-3137.
- McRae, J. M., Kassara, S., Kennedy, J. A., Waters, E. J., & Smith, P. A. (2013). Effect of wine pH and bottle closure on tannins. *Journal of Agricultural and Food chemistry*, *61*(47), 11618-11627.
- Monagas, M., Bartolome, B., & Gomez-Cordoves, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Critical reviews in food science and nutrition*, *45*(2), 85-118.
- Mouls, L., & Fulcrand, H. (2012). UPLC-ESI-MS study of the oxidation markers released from tannin depolymerization: Toward a better characterization of the tannin evolution over food and beverage processing. *Journal of Mass Spectrometry*, *47*(11), 1450–1457.
- Moutounet, M., & Mazauric, J. P. (2001). L'oxygène dissous dans les vins: Les gaz en oenologie. *Revue française d'oenologie*, (186), 12-15.
- Muccillo, L., Gambuti, A., Frusciante, L., Iorizzo, M., Moio, L., Raieta, K., ... & Aversano, R. (2014). Biochemical features of native red wines and genetic diversity of the corresponding grape varieties from Campania region. *Food chemistry*, *143*, 506-513.
- Müller-Späth, H. (1977). Neueste Erkenntnisse über den Sauerstoffeinfluß bei der Weinbereitung aus der Sicht der Praxis. *Weinwirtschaft*, *113*(6), 144-157.
- Nevares, I., & Del Alamo-Sanza, M. (2015). Oak stave oxygen permeation: A new tool to make barrels with different wine oxygenation potentials. *Journal of Agricultural and Food Chemistry*, *63*(4), 1268–1275.
- Nikolantonaki, M., & Waterhouse, A. L. (2012). A method to quantify quinone reaction rates with wine relevant nucleophiles: a key to the understanding of oxidative loss of varietal thiols. *Journal of Agricultural and Food Chemistry*, *60*(34), 8484-8491.
- Noble, A. C. (1994). Bitterness in wine. *Physiology & behavior*, *56*(6), 1251-1255.
- O'Brien, V., Colby, C., & Nygaard, M. (2009). Managing oxygen ingress at bottling. *Wine Industry Journal*, *24*(1), 24-29.

- Obreque-Slíer, E., Peña-Neira, A., López-Solís, R., Ramírez-Escudero, C., & Zamora-Marín, F. (2009). Phenolic characterization of commercial enological tannins. *European Food Research and Technology*, *229*(6), 859–866.
- OIV, 2007. Compendium of International Methods of Wine and Must Analysis. Of- fice International de la Vigne et du Vin, Paris.
- OIV. 2016. "International Code Oenological Practices.International Organization of Vine and Wine."
- Oliveira, C. M., Ferreira, A. C. S., De Freitas, V., & Silva, A. M. S. (2011). Oxidation mechanisms occurring in wines. *Food Research International*.
- Oliveira, J., Azevedo, J., Silva, A. M., Teixeira, N., Cruz, L., Mateus, N., & de Freitas, V. (2010). Pyranoanthocyanin dimers: A new family of turquoise blue anthocyanin-derived pigments found in port wine. *Journal of agricultural and food chemistry*, *58*(8), 5154-5159.
- Oliveira, V., Lopes, P., Cabral, M., & Pereira, H. (2013). Kinetics of oxygen ingress into wine bottles closed with natural cork stoppers of different qualities. *American Journal of Enology and Viticulture*, ajev-2013.
- Pannala, A. S., Chan, T. S., O'Brien, P. J., & Rice-Evans, C. A. (2001). Flavonoid B-ring chemistry and antioxidant activity: fast reaction kinetics. *Biochemical and Biophysical Research Communications*, *282*(5), 1161-1168.
- Papadopoulou, D., & Roussis, I. G. (2001). Inhibition of the decline of linalool and α-terpineol in muscat wines by glutathione and n-acetyl-cysteine. *Italian journal of food science*, *13*(4), 413-419.
- Papadopoulou, D., & Roussis, I. G. (2008). Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by glutathione and N-acetylcysteine. *International journal of food science & technology*, *43*(6), 1053-1057.
- Papazian, L., Bregeon, F., Thirion, X., Gregoire, R., Saux, P., Denis, J. P., ... & Gouin, F. (1996). Effect of ventilator-associated pneumonia on mortality and morbidity. *American journal of respiratory and critical care medicine*, *154*(1), 91-97.
- Parish, Matthew, David Wollan, and Robert Paul. 2001. "Micro-Oxygenation: A Review."
- Paul, R. (2002). Micro-oxygenation–Where now. In *Australian Society of Viticulture and Oenology, Uses of Gases in Winemaking Seminar Proceedings* (pp. 18-30).
- Pechamat, L., Zeng, L., Jourdes, M., Ghidossi, R., & Teissedre, P. L. (2014). Occurrence and formation kinetics of pyranomalvidin-procyanidin dimer pigment in merlot red wine: Impact of acidity and oxygen concentrations. *Journal of Agricultural and Food Chemistry*, *62*(7), 1701-1705.
- Peng, Z., Duncan, B., Pocock, K. F., & Sefton, M. A. (1998). The effect of ascorbic acid on oxidative browning of white wines and model wines. *Australian Journal of Grape and Wine Research*, *4*(3), 127-135.
- Peng, Z., Hayasaka, Y., Iland, P. G., Sefton, M.A., P., & Waters, E. J. (2001). Quantitative analysis of polymeric procyanidins (tannins) from grape (Vitis vinifera) seeds by reverse phase high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *49*(1), 26–31.
- Pérez-Magariño, S., Sánchez-Iglesias, M., Ortega-Heras, M., González-Huerta, C., & González-Sanjosé, M. L. (2007). Colour stabilization of red wines by microoxygenation treatment before malolactic fermentation. *Food Chemistry*, *101*(3), 881-893.
- Perscheid, M., & Zürn, F. (1978). Bedeutung der Sauerstoffaufnahme bei der Weinbereitung. *Deutsche Weinbau*, *25*, 1109-1116.
- Peterson, A. L., & Waterhouse, A. L. (2016). 1H NMR: A Novel Approach To Determining the Thermodynamic Properties of Acetaldehyde Condensation Reactions with Glycerol,(+)-Catechin, and Glutathione in Model Wine. *Journal of Agricultural and Food Chemistry*, *64*(36), 6869-6878.
- Peterson, A. L., Gambuti, A., & Waterhouse, A. L. (2015). Rapid analysis of heterocyclic acetals in wine by stable isotope dilution gas chromatography–mass spectrometry. *Tetrahedron*, *71*(20), 3032–3038.
- Peynaud, E. 1984. Knowing and Making Wine. Edited by John Wiley&amp. NewYork.
- Prieur, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry*, *36*(3), 781-784.
- Puértolas, E., Álvarez, I., & Raso, J. (2011). Changes in phenolic compounds of Aragón red wines during alcoholic fermentation. *Food Science and Technology International*, *17*(2), 77-86.
- Rankine, B. 2002. Making GoodWine. Pan Macmillan Australia, Sydney. Sydney.
- Ribérean-Gayon, P., Maujean, A., Dubourdieu, D., & Glories, Y. (2000). Handbook of Enology (Vol. 2): The Chemistry of Wine Stabilization and treatment. *Chichester: John Wiley & Sons. McCarter, JD and Withers, SG (1994). Mechanisms of enzymatic glycoside hydrolysis. Current Opinion in Structural Biology*, *4*, 885-892.
- Ribéreau-Gayon, J. 1931. "Thèse Doctorat Ès Sciences Physiques."
- RibéreauGayon, P., & Stonestreet, E. (1966). Dosage des tanins du vin rouge et détermination de leur structure. *Chimie analytique*, *48*(4), 188-+.

- Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Handbook of enology—the chemistry of wine, stabilization and treatments 2006. *Varietal aroma*, 205-230.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.
- Rinaldi, A., Gambuti, A., & Moio, L. (2012). Application of the SPI (Saliva Precipitation Index) to the evaluation of red wine astringency. *Food chemistry*, *135*(4), 2498-2504.
- Rinaldi, A., Jourdes, M., Teissedre, P. L., & Moio, L. (2014). A preliminary characterization of Aglianico (Vitis vinifera L. cv.) grape proanthocyanidins and evaluation of their reactivity towards salivary proteins. *Food chemistry*, *164*, 142-149.
- Ritchey, J. G., & 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.
- Robichaud, J. L., & Noble, A. C. (1990). Astringency and bitterness of selected phenolics in wine. *Journal of the Science of Food and Agriculture*, *53*(3), 343–353.
- Roig, G., & Yêrle, S. (2003). Balance y perspectivas de 10 años de microoxigenación. *ACE: Revista de enología*, (30), 2.
- Roullier-Gall C, Witting M, Tziotis D, Ruf A, Lucio M, Schmitt-Kopplin P, Gougeon RD (2015) Advances in Wine Research. SE Ebeler, G Sacks, S Vidal, P Winterhalter (Eds.) American Chemical Society.
- Roussis, I. G., Lambropoulos, I., & Tzimas, P. (2007). Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *American Journal of Enology and Viticulture*, *58*, 274−278.
- Rustioni, L., Bedgood, D. R., Failla, O., Prenzler, P. D., & Robards, K. (2012). Copigmentation and anti-copigmentation in grape extracts studied by spectrophotometry and post-column-reaction HPLC. *Food chemistry*, *132*(4), 2194-2201.
- Sáenz-Navajas, M. P., Avizcuri, J. M., Ferreira, V., & Fernández-Zurbano, P. (2014). Sensory changes during bottle storage of Spanish red wines under different initial oxygen doses. *Food research international*, *66*, 235-246.
- Salas, E., Fulcrand, H., Meudec, E., & Cheynier, V. (2003). Reactions of anthocyanins and tannins in model solutions. *Journal of Agricultural and Food Chemistry*, *51*(27), 7951-7961.
- Salas, E., Fulcrand, H., Poncet-LeGrand, C., Meudec, E., Köhler, N., Winterhalter, P., & Cheynier, V. (2005). Isolation of flavanol-anthocyanin adducts by countercurrent chromatography. *Journal of chromatographic science*, *43*(9), 488-493.
- Salas, E., Guernevé, C. Le, Fulcrand, H., Poncet-Legrand, C., & Cheynier, V. (2004). *Structure determination and colour properties of a new directly linked flavanol–anthocyanin dimer*. *Tetrahedron Letters* (Vol. 45).
- Salgues, M., Cheynier, V., Gunata, Z., & Wylde, R. (1986). Oxidation of Grape Juice 2-S-Glutathionyl Caffeoyl Tartaric Acid by Botrytis cinerea Laccase and Characterization of a New Substance: 2, 5-di-S-Glutathionyl Caffeoyl Tartaric Acid. *Journal of Food Science*, *51*(5), 1191-1194.
- Santiago, J. L., González, I., Gago, P., ALONSO-VILLAVERDE, V., Boso, S., & Martínez, M. C. (2008). Identification of and relationships among a number of teinturier grapevines that expanded across Europe in the early 20th century. *Australian journal of grape and wine research*, *14*(3), 223-229.
- Sarni-Manchado, P., Deleris, A., Avallone, S., Cheynier, V., & Moutounet, M. (1999). Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agent in enology. *American Journal of Enology and Viticulture*, *50*(1), 81-86.
- Saucier, C., Little, D., & Glories, Y. (1997). Research note first evidence of acetaldehyde-flavanol condensation products in red wine. *American Journal of Enology and Viticulture*, *48*(3), 370–373.
- Schmidtke, L. M., Clark, A. C., & Scollary, G. R. (2011). Micro-oxygenation of red wine: techniques, applications, and outcomes. *Critical reviews in food science and nutrition*, *51*(2), 115-131.
- Schneider, R., Baumes, R., Bayonove, C., & Razungles, A. (1998). Volatile Compounds Involved in the Aroma of Sweet Fortified Wines (Vins Doux Naturels) from Grenache Noir. *Journal of Agricultural and Food Chemistry*, *46*(8), 3230–3237.
- Schneider, V. (1998). Must hyperoxidation: a review. *American Journal of Enology and Viticulture*, *49*(1), 65-73.
- Schneider, V. (2005). Aufnahme von Sauerstoff in Keller und Flasche. *Winzer*, *12*, 6-9.
- Singleton, V. L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *American Journal of Enology and Viticulture*, *38*(1), 69-77.
- Singleton, V. L., Salgues, M., Zaya, J., & Trousdale, E. (1985). Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *American Journal of Enology and Viticulture*, *36*(1), 50-56.
- Skouroumounis, G. K., Kwiatkowski, M. J., Francis, I. L., Oakey, H., Capone, D. L., Duncan, B., ... & Waters, E. J. (2005). The impact of closure type and storage conditions on the composition, colour and

flavour properties of a Riesling and a wooded Chardonnay wine during five years' storage. *Australian Journal of Grape and Wine Research*, *11*(3), 369-377.

- Sonni, F., Moore, E. G., Clark, A. C., Chinnici, F., Riponi, C., & Scollary, G. R. (2011). Impact of glutathione on the formation of methylmethine-and carboxymethine-bridged (+)-catechin dimers in a model wine system. *Journal of Agricultural and Food Chemistry*, *59*(13), 7410-7418.
- Souquet, J. M., Cheynier, V., Brossaud, F., & Moutounet, M. (1996). Polymeric proanthocyanidins from grape skins. *Phytochemistry*, *43*(2), 509-512.
- Tao, J., Dykes, S. I., & Kilmartin, P. A. (2007). Effect of SO2 concentration on polyphenol development during red wine micro-oxygenation. *Journal of Agricultural and Food Chemistry*, *55*(15), 6104–6109.
- Timberlake, C. F., & Bridle, P. (1976). Interactions Between Anthocyanins, Phenolic Compounds, and Acetaldehyde and Their Significance in Red Wines. *American Journal of Enology and Viticulture* , *27* (3), 97–105. Retrieved from
- Ugliano, M. (2013). Oxygen contribution to wine aroma evolution during bottle aging. *Journal of agricultural and food chemistry*, *61*(26), 6125-6136.
- Ugliano, M., Dieval, J. B., Dimkou, E., Wirth, J., Cheynier, V., Jung, R., & Vidal, S. (2013). Controlling oxygen at bottling to optimize post-bottling development of wine. *Pract. Winery Vineyard*, *34*, 44-50.
- Ugliano, M., Dieval, J. B., Siebert, T. E., Kwiatkowski, M., Aagaard, O., Vidal, S., & Waters, E. J. (2012). Oxygen consumption and development of volatile sulfur compounds during bottle aging of two Shiraz wines. Influence of pre-and postbottling controlled oxygen exposure. *Journal of agricultural and food chemistry*, *60*(35), 8561-8570.
- Ugliano, M., Kwiatkowski, M., Vidal, S., Capone, D., Siebert, T., Dieval, J. B., ... & Waters, E. J. (2011). Evolution of 3-mercaptohexanol, 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*(6), 2564-2572.
- Vernhet, A., Dubascoux, S., Cabane, B., Fulcrand, H., Dubreucq, E., & Poncet-Legrand, C. (2011). Characterization of oxidized tannins: comparison of depolymerization methods, asymmetric flow field-flow fractionation and small-angle X-ray scattering. *Analytical and bioanalytical chemistry*, *401*(5), 1559-1569.
- Versari, A., Boulton, R. B., & Parpinello, G. P. (2007). Analysis of SO2-resistant polymeric pigments in red wines by High-Performance Liquid Chromatography. *American journal of enology and viticulture*, *58*(4), 523-525.
- Versari, A., Boulton, R. B., & Parpinello, G. P. (2008). A comparison of analytical methods for measuring the color components of red wines. *Food Chemistry*, *106*(1), 397–402.
- Versari, A., du Toit, W., & Parpinello, G. P. (2013). Oenological tannins: a review. *Australian Journal of Grape and Wine Research*, *19*(1), 1–10.
- Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta*, *513*(1), 57-65.
- Vilela-Moura, A., Schuller, D., Falco, V., Mendes-Faia, A., & Côrte-Real, M. (2010). Effect of refermentation conditions and micro-oxygenation on the reduction of volatile acidity by commercial S. cerevisiae strains and their impact on the aromatic profile of wines. *International journal of food microbiology*, *141*(3), 165-172.
- Vivas, N., & Glories, Y. (1993). Les phénomènes d'oxydoréduction liés à l'élevage en barrique des vins rouges: aspects technologiques. *Revue française d'oenologie*, *33*(142), 33-38.
- Vivas, N., Debeda, H., Menil, F., Vivas de Gaulejac, N., & Nonier, M. F. (2003). Demonstration of oxygen transfer through the staves of the barrels by using an original system to measure wood porosity. First results. *Sciences des Aliments (France)*.
- Warner, C. R., Diachenko, G. W., & Bailey, C. J. (2000). Sulfites: An Important Food Safety Issue. An Update on Regulatory Status and Methodologies. *Food Safety Magazine*.
- Waterhouse, A. L. (2002). Wine Phenolics. *Annals of the New York Academy of Sciences*, *957*(1), 21–36.
- Waterhouse, A. L., & Nikolantonaki, M. (2015). Quinone reactions in wine oxidation. In *Advances in Wine Research* (pp. 291-301). American Chemical Society.
- Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding Wine Chemistry*. John Wiley & Sons.
- Webber, V., Dutra, S. V., Spinelli, F. R., Carnieli, G. J., Cardozo, A., & Vanderlinde, R. (2017). Effect of glutathione during bottle storage of sparkling wine. *Food Chemistry*, *216*, 254-259.
- Winterbourn, C. C., & Metodiewa, D. (1999). Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide: Free Radical Biology and Medicine, *27*, 322-328.

- Wirth, J., Morel-Salmi, C., Souquet, J. M., Dieval, J. B., Aagaard, O., Vidal, S., ... & Cheynier, V. (2010). The impact of oxygen exposure before and after bottling on the polyphenolic composition of red wines. *Food Chemistry*, *123*(1), 107-116.
- Wrolstad, R. E., Durst, R. W., & Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science & Technology*, *16*(9), 423-428.
- Yang, W. H., & Purchase, E. C. (1985). Adverse reactions to sulfites. *Canadian Medical Association Journal*, *133*(9), 865.
- Zhang, Z. Q., Duan, X. W., Ji, Z. L., & Pang, X. Q. (2000). Influence of pH and active oxygen on the stability of anthocyanins from litchi pericarp. In *I International Symposium on Litchi and Longan 558* (pp. 339-342).
- Zhou, S., Koh, H. L., Gao, Y., Gong, Z. Y., & Lee, E. J. D. (2004). Herbal bioactivation: the good, the bad and the ugly. *Life sciences*, *74*(8), 935-968.
- Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (1995). Sulfur dioxide and ascorbic acid. In *Wine analysis and production* (pp. 178-191). Springer US.

8. PUBLICATIONS

- Gambuti, A., Siani, T., Picariello, L., Rinaldi, A., Lisanti, M. T., Ugliano, M., ... & Moio, L. (2016). 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*, 1-12.

- Picariello, L., Gambuti, A., Picariello, B., & Moio, L. (2017). Evolution of pigments, tannins and acetaldehyde during forced oxidation of red wine: Effect of tannins addition. *LWT-Food Science and Technology*, *77*, 370-375.

- Picariello L., Francesca P., Alessandra R., Moio L., Gambuti A. (submitted 2017) Enological tannins affect acetaldehyde evolution, color stability and tannins reactivity during forced oxidation of red wine. *LWT-Food Science and Technology.*

- Gambuti Angelita, Picariello Luigi, Rolle Luca, Moio Luigi (submitted 2017) Evaluation of the use of sulfur dioxide and glutathione to prevent oxidative degradation of malvidin3-monoglucoside by hydrogen peroxide in the model solution and real wine. *Food Research International*.