- 1 Light-struck taste in white wine: reaction mechanisms, preventive strategies and
- 2 future perspectives to preserve wine quality
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Abstract

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- 12 Background
- The light-dependent reactions involving riboflavin (RF) and methionine (Met) as substrates
- are responsible for the light-struck taste (LST). This fault is associated to cabbage-like odors
- due to the formation of methanethiol and dimethyl disulfide impacting negatively on the
- sensory properties of white wine. The reaction can occur for a relatively short period of white
- wine bottled in clear glass under both natural and artificial lights.
- 18 Scope and Approach
- This review aimed to point out the aspects related to the mechanisms of light-dependent
- reactions and the oenological strategies applicable to counteract the appearance of this
- 21 detrimental fault.
- 22 Key Findings and Conclusions
- LST can be prevented through the proper choice of the fermenting yeast, the addition of
- certain adjuvants being able to remove RF, and additives with a protective effect, such as
- 25 hydrolysable tannins. As the use of these oenological tools plays an important role in limiting
- the detrimental change, they represent the strategies applicable in productive approaches.
- In this context, the more recent findings are summarized also to update the knowledge about
- the complex reaction mechanisms allowing to overcome the formation of this fault and
- 29 supporting the wine industry.

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Keywords: light exposure; riboflavin; methionine; sulfur compounds; off-flavors; storage.

1. Introduction

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The quality maintenance of wine during its storage is a fundamental aspect for both wine producers and consumers. This purpose can be achieved controlling certain environmental factors, such as light exposure and temperature, which increase can be responsible for faster oxidative reactions and quicker aroma decay. Nowadays, more attention has been given to the light exposure that can cause detrimental changes in wine where the lightinduced off-flavours are associated to the light-struck taste (LST) or "Goût de Lumière" (Dozon & Noble, 1989). This fault is mainly due to photochemical oxidation processes in which riboflavin (RF), a highly photosensitive vitamin, plays an important role. The photosensitisation process of RF proceeds with photo-oxidative degradation of methionine (Met) generating methanethiol (MeSH) and dimethyl disulphide (DMDS) (Maujean & Seguin, 1983a). Both volatile sulfur compounds (VOCs) give unpleasant cabbage and onion-like odours that make wine undrinkable. The prevention of LST appearance is crucial for the winemakers as the wine quality needs to be ensured and maintained. In order to understand the impact of LST, a national survey was carried out (https://www.survio.com/survey/d/K6Y3T0W6C5S5Z1G2J) and 231 responses from Italian wineries were collected. The results showed that LST occurred 1011 times which makes 4.4 incidents per winery, and the wine was recalled from the market in 15 wineries (6.5%), with consequent loss of income. Many producers utilized flint bottles for their white and rosé wines (even 80% of the total production). Moreover, we asked the technological procedures and treatments the winemakers usually apply to prevent LST. Most of the winemakers (50%) use bentonite as a prevention tool; other treatments include the addition of polyvinylpolypyrrolidone (PVPP) which is ineffective in RF removal as a recent research showed (Fracassetti et al., 2017).

Beside white wine, the light exposure also affects the sensory characteristics of other foods, such as milk (Patton, 1954) and beer. Particularly in the latter, the formation of 3-methyl-2-butene-1-thiol induces a unique "skunky" odour and taste (Cardoso et al., 2012). Riboflavin is the photosensitizer that causes the photolysis of iso- α -acids leading to the beer spoilage in the presence of sulfur-containing amino acids (Gunst & Verzele, 1978; Cardoso et al., 2012). In order to clarify and point out LST-related aspects, this review focuses on (i) the reaction mechanisms responsible for the defect formation, (ii) the light-induced faults in wine and (iii) the other light-dependant effects. The (iv) oenological strategies are also examined

2. Reaction mechanisms involved in light-dependent changes

considering both microbiological and technological approaches.

LST is a wine fault recognizable as a distinctive, unpleasant sulfide-like aroma resembling onion and cooked cabbage; it appears after light exposure with emission spectra within UV-Vis, particularly between 370 and 450 nm. The photodegradative reactions may occur within minutes or days. This spoilage concerns white wines, both still and sparkling, usually bottled in flint glass which is ineffective in shielding the harmful wavelengths. Since 1970, the new marketing approaches have led to an increased use of clear bottles to show the wine colour to the customers, because it is considered as an important feature of wine quality (i.e., its oxidative status). Moreover, wine has begun to be increasingly available at the large-scale retail trade or grocery stores where the protection against the light exposure could hardly be ensured (Dozon & Noble, 1989).

RF, or vitamin B₂, is characterized by a bright, yellow colour and it is sensitive to UV-Vis light radiation. Chemically it is composed of tricyclic heterocycle isoalloxazine, involved in redox reactions, and ribitol, an alcohol responsible for protein binding. RF is a constituent of

prosthetic groups, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which play essential roles in oxidoreductase enzymes like dehydrogenases, oxidases, or electron transferase (Choe et al., 2005). They take part in redox reactions due to their ability to transfer single electrons and hydrogen atoms. RF shows the highest light absorption at 225, 275, 370 and 450 nm at pH 7 (Drössler et al., 2003; Abbas & Sibirny, 2011). Once RF is exposed to blue or UV-A light, it acts as a photosensitizer. A photosensitizer is a species that reaches a high energy state when exposed to light at specific wavelengths inducing chemical reactions or physical changes in a target substrate. Specifically, when RF is exposed to light, it reaches the excited triplet state, a bi-radical with powerful oxidant effect (E⁰ ~ +1,7 V) inducing the direct oxidation of many biomolecules (Cardoso et al., 2012). Photo-oxidation can occur in two possible pathways. In Type I mechanism, the triplet sensitizer reacts directly with compounds able to donate electrons, such as phenols and amino acids (Figure 1). In this pathway, RF acts as electron or hydrogen acceptor, leading to produce either free radicals or free radical ions. The reaction between triplet RF and a substrate can occur through three different mechanisms: electron transfer (ET), hydrogen atom transfer (HAT) and proton-coupled electron transfer (PCET) (Cardoso et al., 2012). The proceeding of reaction depends on the thermodynamic properties of both excited triplet RF and substrates. For ET, one-electron oxidation potential below 1.8 V for the reducing substrate is the limiting thermodynamic property, whereas for HAT mechanism, bond dissociation energy is the limiting factor, which should be of less than 300 kJ. PCET is an intermediate mechanism depending on both electron oxidation potential and dissociation energy. The formation of free radicals initiates free-radical chain reactions by attracting hydrogen or electrons from other compounds. As a consequence, oxygen may be reduced to peroxyl radical which generates hydrogen peroxide and therefore hydroxyl radical (Min & Boff, 2002; Cardoso et al., 2012) triggering the possible further oxidation processes in wine. In direct reaction between excited triplet

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RF and various substrates, reduced RF radical or anionic RF radical are formed (Figure 1). These RF radicals can be oxidized by oxygen, regenerating ground state RF, and forming superoxide anion radical. The latter may then form hydrogen peroxide, a strong oxidising species, by dismutation that causes depletion of antioxidants present in wine, including sulfur dioxide and polyphenols. Furthermore, the reaction between hydrogen peroxide and superoxide anion (Haber-Weiss reaction) may occur, leading to hydroxyl radical formation (Choe et al., 2005). Moreover, hydrogen peroxide may undergo Fenton reaction catalysed by iron (II) ions, leading to hydroxyl radical formation as well. Hydroxyl radical with a reduction potential of + 2.3 V is one of the strongest oxidizing species, that may induce the oxidation of wine and the appearance of sensory fault. It has been demonstrated that in Type I pathway the excited triplet RF may also react directly with triplet oxygen, transferring an electron to form superoxide anion. However, only less than 1% of the reaction of triplet sensitizer and triplet oxygen produces superoxide anion (Kepka & Grosswiener, 1972). Such reaction causes the conversion of triplet oxygen to singlet oxygen (¹O₂) and ground state RF (Type II pathway) (Figure 1). Singlet oxygen directly and rapidly gives rise to nonradical reactions with electron rich compounds (e.g., with double bonds) that result oxidised (Min & Boff, 2002). Singlet oxygen can find different substrates in wine, such as amino acids or phenolic compounds (Thomas & Foote, 1978; DeRosa & Crutchley, 2002). Regarding amino acids, singlet oxygen primarily reacts with tryptophan, histidine, tyrosine, Met and cysteine at significant rates, forming peroxides (Min & Boff, 2002; Huvaere & Skibsted, 2015). Tryptophan, histidine and tyrosine contain double bonds in their structure, which make them promptly attacked by singlet oxygen. Methionine and cysteine contain a sulfur atom with four nonbonding electrons which are rapidly attracted by electrophilic singlet oxygen. Remucal and McNeill (2011) demonstrated that in aqueous solution at pH 7.4 tyrosine, tryptophan, Met and histidine react with singlet oxygen at similar rates, but the amount of

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amino acid degradation which can be attributed to single oxygen reaction varies from 10% for tyrosine and tryptophan up to 100% for histidine. In the presence of RF, singlet oxygen is responsible for about 33% of Met degradation (Remucal & McNeill, 2011). One of known compounds arising from the reaction between Met and singlet oxygen is methionine sulfoxide (Fracassetti et al., 2020), a stable compound which does not undergo further oxidation or degradation processes (Barata-Vallejo et al., 2010). Singlet oxygen can react with certain di-phenols (e.g., cathecol) firstly generating hydroquinones as well as benzoquinones as secondary oxidation product (Briviba et al., 1993). Flavonoids present in wine can interact with singlet oxygen through both chemical and physical processes. The latter pathway prevails with increasing abundance of phenolic functions as well as, with the presence of catechol and pyrogallol rings (Huvaere & Skibsted, 2015). Chemical quenching leads to the transfer of energy from a molecule in high-energy state to another molecule, the latter can be oxidized or its spin is modified. When chemical quenching occurs, the generated quinones can trigger oxidation chain reactions. Consequently, quinones can bind to other phenols producing brown dimers or polymers which may precipitate when high molecular weight is reached (Ribéreau-Gayon et al., 2006). Furthermore, xanthylium derivatives can be produced thus inducing the browning of white wine (Li et al., 2008). The occurrence of the two photo-oxidative mechanisms depends on the concentration of oxygen in the reaction environment. In anoxic conditions only Type I occurs, whereas in the presence of oxygen, both mechanisms may take place to a different extent (Min & Boff, 2002; Grant-Preece et al., 2017b). Compounds that are easily oxidised, like certain phenolics or amino acids, promote Type I pathway, while species not prone to oxidising like alkenes, favour Type II. In any case, the factor mainly influencing the reaction mechanism is the presence or absence of oxygen, as some compounds degraded via Type I at low

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oxygen concentrations may be degraded via Type II when the oxygen concentration increases (Min & Boff, 2002).

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3. The light-induced faults in wine

RF and Met are the two factors mainly affecting LST formation. RF concentration in grape and must seldom exceeds a few tens micrograms per liter (Riberau-Gayon et al., 2006), but its content can rise to 110-250 µg/L during alcoholic fermentation (Mattivi et al., 2000; Fracassetti et al., 2017), due to the activity of Saccharomyces cerevisiae. RF amount can be further increased to 160-318 µg/L when wine remains in contact with yeast lees once the alcoholic fermentation is completed (Andrés-Lacueva et al., 1998). A concentration of RF lower than 50-80 µg/L greatly decreases the risk of developing the LST (Pichler, 1996; Mattivi et al., 2000; Fracassetti et al., 2019a). Therefore, the measurement of RF concentration in finished wine may be a suitable chemical marker for the susceptibility of wine to develop LST (Andrés-Lacueva et al., 1998; Mattivi et al., 2000). In the study carried out by Mattivi et al. (2000), sensory evaluation of 85 white wines from Italy, Slovenia and Spain was performed. Each wine was prepared in double, with and without the addition of RF (1 mg/L), then both samples were exposed to light for 48 h. After that, trained panelists rated the intensity of the off-flavour aroma. Results showed that the intensity of the defect was significantly higher in the samples with the RF added, confirming that RF is a crucial factor for the appearance of this off-flavour. RF concentration in wine and sensory values of the LST were positively correlated, but the low correlation coefficient (r = 0.3544) indicated that RF concentration did not allow to predict the intensity of the off-flavour formed. Noteworthy, there were also some samples with a high concentration of RF which did not develop the off-flavour, as well as some wines that did get spoiled despite their low concentration of RF. These results evidenced that there are some other wine features that

can affect the stability to light and that the occurrence of LST is due to complex mechanisms that cannot be fully explained only considering the presence of RF. In fact, other factors can affect the light stability of wine including the transition metals, as they are involved in redox reactions, polyphenols, precursors of the off-flavour, sulfur-containing amino acids, sulfur dioxide (Mattivi et al., 2000). The latter plays an important role on the rate of light-induced radical reaction chains (Fracassetti et al., 2020). LST in wine was first described by Maujean et al. (1978), who focused their study on the effect of light on Champagne. They found that the molecules responsible for the fault formation were sulfur-containing amino acids, as Met and cysteine, and RF as a photosensitizer. According to these authors, the addition of RF, Met and cysteine to Champagne, increased the off-flavour formation after exposure to light in anoxic conditions. The proposed mechanism of the light-dependent reaction concerns the formation of triplet state RF (Maujean & Seguin, 1983a). This species is characterized by having high energy, unstable electronic distribution, weaker bonds and high capacity to accept electrons making triplet state RF more reactive compared to its equivalent ground state (Wardle, 2009). Met is among the wine components able to donate electrons. Met is firstly decarboxylated and it loses a hydrogen atom generating an imine. In this way, RF is fully reduced by acquiring two electron-equivalents and two hydrogen atoms (Figure 2). The imine is not stable and easily hydrolyses to methional. The latter is also chemically unstable, photosensitive and, through a retro Michael reaction, decomposes to MeSH and acrolein. Two molecules of MeSH can yield dimethyl disulfide (DMDS) (Maujean & Seguin, 1983a). Both these sulfur compounds are highly volatile and have a low perception threshold, as 0,3 µg/L in model solution and 2-10 µg/L in wine for MeSH and 20-45 µg/L in wine for DMDS (Mestres et al., 2000; Pripis-Nicolau et al., 2004; Fracassetti & Vigentini, 2018). MeSH is characterized by rotten egg or cabbage-like olfactory notes, whereas DMDS gives cooked cabbage and onion-like odour. These two molecules are responsible for the LST.

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Met concentration also affects LST formation. Fracassetti et al. (2019a) found that in model wine solution with a constant concentration of Met (3 mg/L) and increasing concentration of RF (0-300 µg/L), the amount of degraded Met rose in parallel after light exposure. On the other hand, when RF concentration was constant (200 µg/L) and Met concentration increased (0–13 mg/L), Met degraded to a much greater extent. The average content of Met in wine is about 3-5 mg/L (Amerine & Ough, 1980; Riberau-Gayon et al., 2006; Grant-Preece et al., 2017; Sartor et al., 2021), nonetheless it can vary depending on different factors such as grape cultivar, vineyard treatments, winemaking conditions, yeast performing the alcoholic fermentation and its autolysis, and it can reach about 15 mg/L (Soufleros et al., 2003; Fiechter & Mayer, 2011). The molar ratio of degraded Met/degraded RF ranged between 2 and 35, which disagrees with the 1:1 ratio previously thought to occur between these two substrates (Maujean & Seguin, 1983b). This could be explained by the formation of other products originating from Met in photo-degradative reactions (Barata-Vallejo et al., 2010). Additionally, the concentrations of DMDS and dimethyl trisulfide (DMTS), product of the reaction between MeSH and DMDS, were about ten times higher in the samples where Met concentration increased, compared to samples where RF concentration increased. These results are in agreement with the sensory evaluation performed by the expert panelists who perceived cooked cabbage odour with higher intensity in the set of samples with increasing Met concentration. This study clearly shows that LST formation in terms of concentration of VOCs and perception of this defect, linearly increased as Met concentration grew indicating that this amino acid can be more detrimental than RF. Therefore, besides RF, the level of Met in white wine needs to be considered for preventing LST. The mechanism of LST formation is very complex and certain aspects of the reactions occurring in wine matrix need further clarification. Recently, a new possible pathway of MeSH formation was described by using the proton-transfer-reaction mass spectrometry (PTR-MS) technique (Asaduzzaman et al., 2020). In the experimental conditions adopted

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(Met 75 mg/L, RF 1.7 mg/L pH 8.0, light exposure at wavelengths 405-640 nm with maximum at 430, 550 and 640 nm for six hours), MeSH was the first compound appearing just after only 2.3 min under light, ant it rapidly increased. DMDS was found, after 3.5 min of light exposure. These results confirm that two molecules of MeSH yield to DMDS. The third detected compound was methional (at 5.30 min) that is surprising and disagrees to the light-dependent mechanism previously proposed by Maujean and Seguin (1983a). Other coproducts of MeSH formation via methional, such as formic acid and 2-propenal, occurred only after 29 and 22.5 min of light exposure, respectively. A further proof that MeSH can be directly formed from Met under light exposure conditions was obtained when an aldehyde group blocker (sodium cyanoborohydride) was added prior to light exposure. In this case MeSH increased rapidly, similarly to the previous conditions without aldehyde blocker (Asaduzzaman et al., 2020). According to Asaduzzaman et al. (2020), during the early steps of photo-oxidation, MeSH can be generated by an alternative and fast pathway that does not include methional and involves a direct cleavage of Met side chain. However, no data related the occurrence of sulfur compounds in model wine or wine was reported which acidic pH can influence RF behaviour under light (Sheraz et al., 2014). Besides Met, Maujean and Seguin (1983a) investigated also another sulfur amino acid, cysteine, which may also take part in the off-flavour development. Hydrogen sulfide may also have a role in unpleasant odour perception (Haye et al., 1977; Maujean et al., 1978; Maujean & Seguin 1983a). As previously stated, the photodegradation reactions of RF can occur in two possible pathways depending mainly on the oxygen concentration. In bottled wine, the concentration of oxygen ranges between 1 mg/L and 9 mg/L (Ugliano et al., 2013). The oxygen-free environment is rapidly obtained in bottled wines especially if the bottling is carried out using inert gases and the wine is added with SO₂. That is why in wine Type I mechanism is favoured, where excited triplet RF reacts with electron rich compounds like Met, leading to unpleasant sulfur compounds formation. Besides Met, RF can also react with other electron-

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rich amino acids like tyrosine, tryptophan, histidine or cysteine (Choe et al., 2005; Huvaere & Skibsted, 2015). RF quenching leads to the formation of substrate radicals, which fate in wine is not fully understood yet (Cardoso et al., 2012).

Among wine components phenolic compounds are also efficient quenchers of triplet RF (Huvaere & Skibsted, 2015). The reaction between phenols and flavins is a chemical type of quenching, resulting in oxidative degradation of phenols (Cardoso et al., 2012). In particular, flavonoids with a catechol-like B ring have high quenching rates, probably due to the stabilisation of o-hydroxy phenoxyl radical anion. Deactivation of excited triplet RF by flavonoids results in their consumption with generation of dimeric compounds and other oxidised species. These may affect the colour and antioxidant capacity of wines.

3.1 Other consequences of light exposure of wine

After light exposure modifications of wines composition other than the sulfur compounds-related off-flavours can occur. Dozon and Noble (1989) conducted a sensory analysis of still and sparkling white wines, exposed to 40-watt fluorescent light bulbs with spectrum similar to that of sunlight. The results of descriptive analysis of wines kept under light for 24 and 72 hours showed that after longer light exposure, not only the perception of cooked cabbage and wet dog odours increased, but also citrus and honey aroma perception was significantly lower. This could be due to either the masking effect of LST appearance or the chemical changes in the composition of other aroma, such as esters or terpenes, undergoing to degradation or hydrolysis (Benítez et al., 2003; D'Auria et al., 2009). Indeed, the exposure to UV light of Champagne for 24 hours completely changed the esters profile. The content of certain esters decreased, like ethyl acetate, ethyl butanoate, 3-methyl-1-butanol acetate, ethyl hexanoate, or disappeared like ethyl decanoate. In order to clarify the possible influence of RF on depletion of these molecules, ethyl hexanoate (14 mg) was irradiated in

the presence of RF (2 mg) for one hour. At the end of illumination, the concentration of the ester was measured, and it showed a decrease up to 9%. This observation proved that the photo-oxidation of RF can induce changes in concentrations of other aromas, including esters (D'Auria et al., 2003). Since the sensory analysis was not carried out, we cannot state if such a difference had a relevant impact on the overall flavour perception. Light exposure can also induce the formation of other undesirable aroma compounds such as acetaldehyde, which is considered one of the main markers of oxidative reactions (Danilewicz, 2003; Han et al., 2019). Dias and co-authors (2013) found that the dominating descriptor identified in Chardonnay wine stored under light for 18 days was acetaldehyde which increase was significant after 10 days of exposure. This compound was also present in model wine samples left outdoor during Australian summer. On the contrary, acetaldehyde was not detected in the samples kept in the dark neither at room temperature nor at 45°C (Clark et al., 2007). The light exposure was crucial for the formation of acetaldehyde and it had a greater affect than temperature. Similarly, in different Spanish white wines saturated with oxygen and left one week in the dark, there was not a significant change in acetaldehyde concentration (Escudero et al., 2002). These results highlight the light exposure is required for the formation of acetaldehyde at least in terms of triggering the oxidation processes in short periods of time. As the light exposure promotes also the formation of radicals, other wine compounds, e.g. tartaric acid, amino acids other than Met, phenolics, can be also oxidized. Tartaric acid (TA) is among the most abundant compounds of wine (Clark et al., 2007; Riberau-Gayon et al., 2006). Its oxidation leads first to the formation of dihydroxyfumaric acid. By its further oxidation and decarboxylation, glyoxal and glyoxylic acid (GA) are formed (Clark, 2008). Both these molecules can bind two flavanol units such as (+)-catechin or (-)epicatechin, forming a dimer with the units bound through a carboxymethine bridge. Afterwards, the dimer undergoes a dehydration and an oxidation leading to a formation of

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yellow pigments, known as xanthylium ions (Li et al., 2008). These compounds contribute to the oxidative browning spoilage of wine. The oxidative degradation of TA is favoured in the presence of iron ions. Iron (III) forms complexes with α -hydroxy carboxylic acids, such as TA, which are relatively stable when stored in darkness, but are photodegraded once exposed to light (Grant-Preece et al., 2017a; Grant-Preece et al., 2017b). The mechanism of photochemical degradation of α -hydroxy acids includes their oxidative decarboxylation in the presence of iron (III) acting as catalyzer of oxidation (Balzani & Carassiti, 1970). According to this pathway, TA degrades to 2-hydroxy-3-oxo-propanoic acid and its possible tautomers, 2,3-dihydroxypropenoic acid and hydroxypyruvic acid. These compounds can react with hydrogen peroxide present in the solution due to Fenton reaction, leading to GA formation among other degradation compounds (Figure 3). Moreover, in the irradiated TA solution, the dissolved oxygen consumption accelerates (Grant-Preece et al., 2017a). This is because transition metal ions in their reduced forms can reduce oxygen to radical species. The superoxide radical anion in acidic condition forms hydroperoxyl radical, which in turn yields hydrogen peroxide. The latter can be further reduced by iron (II) generating hydroxyl radical that is a very powerful oxidant and even at low concentrations can readily oxidise TA. In this pathway, known as Fenton reaction, TA forms a radical which is then oxidised by molecular oxygen or iron (III) to dihydroxymaleic acid which is in equilibrium with its tautomers, hydroxyoxaloacetic acid and dihydroxyfumaric acid (Clark & Scollary, 2003). These compounds can be further oxidised and decarboxylated to glyoxylic and other acids. The light exposure may increase the browning potential of white wines. The oxidation of TA probably proceeds through both photodegradation of iron (III) tartrate and Fenton reaction. GA formation requires the light exposure as well as the presence of oxygen and iron which plays a major role as initiator of both photodegradation and Fenton reaction (Clark et al., 2007). As for LST, the protection of wine against the light can prevent the oxidation of TA and the consequent browning of wine. Nonetheless, a lower content of iron, being the

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catalyser of Fenton reaction, can also limit the TA-related oxidative pathways. Iron can derive from grape and it can be introduced in wine through the adsorption from winemaking equipment as well as the treatment with fining agents, such as bentonite (Hopfer et al., 2015; Bekker et al., 2019). It is among the metal ions that may change during winemaking as a consequence of processes (e.g., filtering, pH adjustments, yeast hull addition and bentonite fining) (Castineira Gomez et al., 2004; Nicolini et al., 2004). Some operations in winemaking process including the treatment with polyvinylpyrrolidone or chelating resin (Loubser & Sanderson, 1985), ion exchange technique (Benítez et al., 2002) as well as the use of bentonite, the proper filtration system and the yeast lees (Bekker et al., 2019) can allow to keep lower the concentration of iron and slow down the oxidative rate of Fenton reaction.

4. Oenological strategies preventing the light-struck taste

4.1 Microbiological approaches: lowering riboflavin and methionine levels

Microbiological strategies suitable for limiting the concentration of compounds involved in LTS defect are not available, yet. Fracassetti and collaborators (2017) observed that some commercial strains of *S. cerevisiae* released different amount of RF in wine, revealing that is probably a strain-dependent character. This evidence paves the way for the development of new approaches that exploit the natural biodiversity of the strains in terms of RF and Met production. To this purpose, a better understanding of RF and Met pathways under oenological conditions is advised. This section illustrates the metabolism of LTS-associated molecules in *S. cerevisiae* with particular attention to the involved genes and enzymatic reactions. Besides *S. cerevisiae*, non-*Saccharomyces* yeasts are gaining interest due to their positive impact on aromatic profiles of certain wines. For this reason, sequential inoculum or co-inoculum schemes can be carried out. A focus on non-*Saccharomyces*

yeasts is presented as well as the possible role of lactic acid bacteria on RF and Met production and release.

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4.1.1 Riboflavin

RF is one of the essential vitamins for the growth of animals, being involved as a coenzyme in a great variety of metabolic reactions. In particular, FAD and FMN, the two RF active forms, act as cofactors for oxidoreductases and as prosthetic groups for enzymes in the βoxidation pathway (Massey, 2000). Since mammals have lost the ability to produce RF, it assumed a great commercial value; thus, lower organisms such as yeasts have been exploited in the industrial processes to produce this vitamin (Garcia-Ramírez et al., 1995). Some microorganisms and all animal cells are capable of uptake riboflavin, while riboflavin overproducers have distinct systems for riboflavin excretion (Abbas et al., 2011). Partly for this reason, yeast metabolism determines a significant contribution to RF concentration in wine and to study if the RF production is dependent on the used yeast strain become relevant in the LST occurrence (Fracassetti et al., 2017). RF biosynthetic pathway has been widely studied in S. cerevisiae both for academic and biotechnological interests. RF precursors are GTP and ribulose 5-phosphate and in the first step of the pathway, GTP is converted by GTP cyclohydrolase II, (Rib1) to 2,5-diamino-6-(ribosylamino)-4-(3H)pyrimidinone 5'-phosphate (DRAP); then, it is reduced by Rib7p to 2,5-diamino-6-(ribitylamino)-4-(3H)-pyrimidinone 5'-phosphate (Gudipati et al. 2014). Afterwards, a deamination occurs to obtain the 5-amino-6-ribitylamino-2,4-(1H,3H)-pyrimidinedione-5'phosphate catalysed by Rib2p (Urban et al., 2003). The two following steps consist of a dephosphorylation through an unidentified phosphatase and of a condensation with ribulose 5-phosphate, obtaining the 3,4-dihydroxy-2-butanone-4-phosphate (DHAB) by the DHBP synthase (RIB3). The latter step is catalysed by lumazine synthase (RIB4) and provides the

formation of the 6,7-dimethyl-8-(1-D-ribityl) lumazine (Jin et al., 2003; García-Ramírez et al., 1995). Finally, the last reaction uses two molecules of 6,7-dimethyl-8-(1-D-ribityl)-lumazine and the riboflavin synthase (RIB5) catalyses the generation of one molecule of riboflavin (Santos et al. 1995) (Figure 4). Hence, starting from RF, FMN and FAD are synthesized by riboflavin kinase (Fmn1p) and FAD synthetase (Fad1p), respectively (Santos et al. 2000; Wu et al. 1995). The characterization of the *S. cerevisiae* biosynthetic pathway was carried out studying the deletion mutants for all the interesting genes: RIB1, RIB2 and RIB7 (Oltmanns et al., 1972), RIB3 (Bacher et al., 1977), RIB4 (Garcia-Ramirez et al., 1995) and RIB5 (Santos et al., 1995). All the obtained mutants revealed an auxotrophic behaviour for RF, suggesting that S. cerevisiae strains could be genetically modified to get strains not RF producers. Since it is not currently acceptable to propose genetically modified organisms (GMOs) in oenology, these mutants could be used to obtain yeast extracts suitable as nutrients during the winemaking process or as an additive to prevent the anti-fermentative activity of medium-chain fatty acids, as described in Ribéreau-Gayon et al. (2006). In 2017, Fracassetti and collaborators evaluated the impact of several conventional oenological nutrients derived from yeast, usually containing vitamins, on RF concentration during vinification. Results revealed a higher RF level in wine samples added with the nutrients (76 and 72 µg/L, for yeast extract and yeast lysate, respectively) in comparison to the unfortified ones (55 µg/L); in particular, RF increase of 21 µg/L detected in the presence of yeast extract can be ascribed to the RF found in the tested additive (70 µg/g) (Fracassetti et al., 2017). Spontaneous fermentations are characterized by a succession of several non-Saccharomyces yeasts resulting from their natural presence on grapes as well as promoted by oenological practices, technology and cellar hygiene (Jolly et al., 2014). Despite these findings, non-Saccharomyces yeasts usually disappear during the early stages of fermentation (Fleet et al., 1984; Henick-Kling et al., 1998) because of their slow growth and inhibition due to SO₂, high ethanol and low oxygen concentrations (Jolly et al., 2014).

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Though no evidence is reported in literature, it may be hypothesized that the lysis of non-Saccharomyces cells can contribute to the release of RF, even during the early stage of the fermentation process. Concerning the wine-related lactic acid bacteria (LAB), the genera Oenococcus and Lactobacillus were compared by Terrade and co-authors (2009) to determine their vitamins requirements for growth conditions. The authors discovered that the tested Lactobacillus strains were auxotroph for RF as opposed to the O. oeni strains, revealing the ability of the latter species to synthesize vitamin B₂.

4.1.2 Methionine

In grape and must 28-39% of the total nitrogen is represented by free amino acids (Rapp & Versini, 1991). Nitrogen content increases during grape maturation and at grapevine harvest it corresponds to 70% of the organic nitrogen. Amino acids are the main part of nitrogen source for yeasts during the alcoholic fermentation and for lactic bacteria in malolactic fermentation. Moreover, they can be a source of undesirable compounds in wines, such as ethyl carbamate, biogenic amines, and ß-carbolines (Herraiz & Ough, 1993). In addition, free amino acids are a source of assimilable sulfur, essential for yeast growth and relevant in winemaking, since sulfur metabolism can generate unpleasant compounds, such as hydrogen sulphide and mercaptans (Rauhut, 1993).

For all these reasons the free amino acids concentration must be taken into account, especially at the end of the alcoholic fermentation since the autolysis of the yeast cells leads to their discharge (Moreno-Arribas et al., 2009).

Particularly, Met, a sulfur-containing amino acid, plays several roles in yeast metabolism (Henschke & Jiranek, 1991) and in wine off-flavour (Fracassetti et al., 2019). Its catabolism

tetrahydropteroyltriglutamate-homocysteine S-methyltransferase (Met6) catalyses the transformation of a molecule of 5-methyltetrahydropteroyltri-L-glutamate and one of Lhomocysteine to Met and tetrahydropteroyltri-L-glutamate (Ugliano & Henschke, 2009). Met catabolism proceeds by the conversion of methionine to a-keto-g-(methylthio)butyrate (KMBA). KMBA is then decarboxylated to methional, that is subsequently reduced to methionol (Perpète et al., 2006). Moreover, Met can be transformed to methanethiol by a demethiolase and then esterified to methanethioacetate (Ugliano & Henschke, 2009). Usually, around 3-4 mg/L of methionine is found in wine and it derives from yeast cells lysis (Riberau-Gayon et al., 2006, Fracassetti et al., 2017). Met is stable in RF-free conditions, while RF presence induces Met degradation to form MeSH and DMDS (Maujean et al., 1978; Maujean and Seguin, 1983b; Andrés-Lacueva et al., 1998). Also non-Saccharomyces yeasts could contribute to increase Met concentration in wine during the winemaking process with similar release mechanisms, even though this aspect was not previously described for non-Saccharomyces yeasts. Specific attention has to be given to O. oeni, because this species contributes to wine sensorial properties producing VOCs deriving from Met degradation in significant quantities, such as methanethiol and dimethyl disulfide (Pripis-Nicolau et al., 2004; Vallet et al., 2007). It is probable that also cell lysis of LAB could discharge significant amount of Met and its

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4.2 Technological approaches

derivatives at the end of the malolactic fermentation.

The prevention of LST can be achieved by applying some oenological procedures in winery once the alcoholic fermentation is completed. Several approaches have been investigated with the aim either to reduce the RF concentration, or to add compounds that could act as a triplet RF quencher and/or as a Met competitor for the reaction with the excited triplet RF,

thus reducing the formation of Met-derived spoilage molecules. In any case, the technologies applied in wine production should not take part in the formation of LST and, in general, in any wine fault. In this context, attention is required in case of using UV-C light. UV radiation is widely used in food processing due to its antimicrobial effect (Bintsis et al., 2000). Even if the treatment is not approved by the International Organization of Vine and Wine (OIV), it was successfully applied for decreasing the microbial population in must (Fredericks et al., 2011; Falguera et al., 2013; Fracassetti et al., 2019) and wine (Matias et al., 2016; Mijowska et al., 2017). The UV radiation of wine containing RF can cause its photo-degradation and the formation of VOCs leading to the detrimental change of the sensory property.

4.2.1 Riboflavin removal

Because RF is one of triggering factor of LST formation, its depletion should reduce wine defect. A level of RF lower than 80-100 μ g/L decrease the risk of LST appearance (Pichler, 1996; Mattivi et al., 2000). RF removal from wine can be attained by the use of fining agents, such as bentonite. Bentonite is a montmorillonite clay that assumes negative charge at wine pH and is commonly used in winemaking process as a clarifying agent. Its main task in winemaking is the adsorption of wine proteins that are positively charged, in order to avoid the formation of protein-related haziness. Its usual dosage ranges between 2 g/L and 8 g/L that should be specifically calculated for each wine (Riberau-Gayon et al., 2006). Bentonite shows the capacity to adsorb RF, but its action is not specific, thus the amount of clay necessary for an adequate RF depletion is high (Pichler, 1996). In the study of Pichler (1996), 2 g/L of bentonite were needed for the removal of 95 μ g/L of RF. However, such bentonite dosage can lead to a significant depletion of wine aromas, thus the reduction of its overall quality (Riberau-Gayon et al., 2006).

More recently Fracassetti et al. (2017) investigated the effectiveness of different fining agents (bentonite, zeolite, polyvinylpolypyrrolidone [PVPP], kaolin, colloidal suspension of pure silica, egg albumin charcoal) in terms of RF removal from model wine solution and white wine. In a model wine solution RF was added up to 350 µg/L. The results pointed out that the RF depletion was proportional to the increased concentrations of bentonite added and at the highest concentration of bentonite (1 g/L), 35% of RF was removed. Another effective adjuvant was zeolite even if its use is not approved by OIV. An addition of 1 g/L zeolite decreased the RF concentration by 40%; this adjuvant was tested because it represents a possible replacement of bentonite to improve protein and tartrate stability (Mercurio et al., 2010). Large pore-sized charcoal was able to adsorb 70% and 94% of RF at the concentration of 5 mg/L and 10 mg/L, respectively. Doses higher than 10 mg/L completely removed RF in model solution. The small pore-sized charcoal was less active, it removed 50% of RF. PVPP, silica, kaolin and egg white were not effective in RF depletion. Bentonite, charcoal and zeolite were then evaluated in Chardonnay wine containing 350 μg/L of RF. Their capacity to remove RF resulted lower compared to that in a model wine solution, with RF removed corresponding to 10% for zeolite, 25% for bentonite and 70% for charcoal. This could be due to the role played by other wine components such as proteins, phenols, lipids, polysaccharides that can be adsorbed by the clarifying agents which active sites are less available to adsorb RF (Ribéreau-Gayon et al., 2006). The adsorption mechanism is not RF-specific, that is why the compounds present at higher concentrations in wine compete with RF for the active sites of charcoal, preventing the vitamin from being adsorbed. Moreover, both charcoal and high doses of bentonite, can adsorb flavouring compounds, thus making the wine poorer in terms of aroma complexity and sensory characteristics. With the usually applied doses the amount of RF removed is not high enough to ensure the total prevention of LST.

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4.2.2 The use of antioxidants in LST prevention

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Some antioxidants are present in wine, those originated from yeast metabolism, such as glutathione (GSH), or added during the winemaking procedures, such as sulfur dioxide (SO₂), and phenolics. Among the latter, flavan-3-ols showed the ability to inhibit the lightinduced fault. Specifically, the addition of (+)-catechin and (-)-epicatechin monomers and dimers slowed down the formation of LST (Maujean & Seguin, 1983b). These authors suggested that flavan-3-ols and maybe the condensed tannins, which building blocks are represented by flavan-3-ols, can shield RF from light. Additionally, phenolic compounds demonstrate the capacity to quench triplet RF in model beer solutions (Grant-Preece at al., 2017). The use of phenols, both condensed and hydrolysable tannins, is a promising way to limit the appearance of LST thanks to their antioxidant properties as well as to their ability in quenching the singlet oxygen (Briviba et al., 1993; DeRosa & Crutchley, 2002). Nevertheless, the phenolics need to be adequately chosen because of their bitter taste and astringency, and also woody texture in case of hydrolysable tannins (Vivas et al., 2003). Therefore, their addition to white wine should be thoroughly evaluated in order not to alter the sensory properties of wine. The hydrolysable tannins are commonly used in winemaking in order to improve wine stability and sensory characteristics (Vivas et al., 2003; Pascual et al., 2017; Vignault et al., 2018). Hydrolysable tannins have also been assessed as a potential effective measure to prevent LST, as proved by Fracassetti et al. (2019a) who investigated the influence of the addition of chestnut, oak and nut gall tannins in model wine solution. Their capacity to prevent LST was investigated at the concentration of 40 mg/L, since this amount of tannins added to wine does not influence the astringency or bitterness perception (Robichaud & Noble, 1990). The experiments were carried out both in oxic and anoxic conditions. In the presence of oxygen, nut gall tannin was able to decrease Met degradation (11% of degraded

Met), compared to either the tannin-free conditions (18% of degraded Met) or samples added with chestnut or oak tannins (18.1 and 21.5%, respectively). The major Met protection with nut gall tannin compared to other tannins, could be due to its two-fold higher total phenol index (TPI) and high concentration of gallic acid. This phenolic acid is a singlet oxygen quencher (Lagunes & Trigos, 2015), therefore, it can reduce singlet oxygen thus preventing Met degradation. In anoxic conditions, Met degradation increased in all samples, but once again nut gall tannin showed a major protective effect (20.2% of degraded Met in solution with nut gall tannin, compared to 28.5% degraded in control sample). In air-free conditions, Type I mechanism occurs, where triplet excited RF reacts directly with Met. The authors suggested that nut gall tannin can compete with Met for the reaction with RF, thus limiting its degradation. In terms of VSCs formation, both MeSH and DMTS concentrations were six-folds higher in anoxic conditions compared to oxic ones. DMDS concentration was even eighty-four folds higher. These results are in agreement with the theoretical LST mechanism: in the presence of oxygen, both Type I and II mechanisms can take place (Min & Boff, 2002; Grant-Preece et al., 2017). Oxygen can quench triplet excited RF and can also react with Met giving its stable oxidation products, including methionine sulfoxide (Fracassetti et al., 2020). As a consequence, less VSC are formed in oxic conditions. In the presence of oxygen, the addition of hydrolysable tannins hampered the DMTS formation, as this compound was not found in the solution. DMDS production was significantly lower when chestnut and oak tannins were added, while with nut gull tannin it was not detected at all. The most significant differences in VSC formation were even more evident in anoxic conditions, where lower concentrations of MeSH, DMDS and DMTS were found in the presence of tannins in comparison to control sample. These differences were also sensorially perceived and significantly lower scores were found in the presence of tannins, accordingly to VSC content.

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The possible mechanisms of LST prevention performed by tannins can be due to their ability in RF quenching, thus protect Met from degradation, or they can react with singlet oxygen forming quinones (DeRosa & Crutchley, 2002), electrophile molecules able to react with amine group of Met or with free thiol group of MeSH protecting from DMDS and DMTS formation. As regard to the addition of tannins in white wine, the effectiveness of chestnut tannin was investigated (Fracassetti et al., 2019b). A RF-enriched wine was exposed to light in presence of chestnut tannin. After two hours of illumination and consequent twenty-four months of storage in the dark the analysis showed that MeSH and DMDS were not detected or found in concentrations lower than a perception threshold as the sensory analysis did not reveal an olfactory defect. Hydrolysable tannins showed to successfully limit both Met degradation and VSC formation. They may not totally prevent LST, but their addition prior to bottling can limit the risk of the formation of light-dependent spoilage. An important aspect to take into consideration when applying this preventive method is the dosage of tannins in order not to affect the taste of wine. It is noteworthy to mention that tannins at concentrations studied (50 mg/L) did not lead to any colour alterations after light exposure (Fracassetti et al., 2019b; Fracassetti et al., 2019c). Other antioxidants, including GSH and SO₂, were tested individually or in combinations, together with chestnut tannin. In model wine, the most effective condition preventing the appearance of LST was the addition of all three antioxidants together, as only negligible amount of MeSH and DMTS were detected, while no DMDS was detected. On the other hand, the results obtained in white wine slightly differed from the model solution. In the presence of chestnut tannin and GSH alone or in combination with SO₂, MeSH and DMDS were undetected or detected at concentrations below their perception threshold. MeSH was present in concentrations over the perception threshold in samples of white wine without any added antioxidant but with SO₂. These results suggest that SO₂ could not be

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able to protect from LST during wine storage, contrarily to what was observed in model wine solution. The formation of sulfonate compounds was showed in white wine where SO₂ was added and in an environment where oxygen is present (Arapitsas et al., 2016). The concentration of these compounds increased for higher amounts of oxygen dissolved on bottling. We can assume SO₂ favoured the formation of VOCs in the experimental conditions adopted even because a medium richer in radicals could be generated as a consequence of the photo-degradation of RF. In fact, SO₂ showed to favour the oxidative pathways when an acidic solution containing RF and Met is exposed to light (Figure 5). The photo-oxidation, monitored by NMR, led to strong and fast increase of Met sulfoxide which formation was limited when gallic acid was present instead of SO₂ (Fracassetti et al., 2020). This suggests the ability of phenolics in the overall prevention of the light-dependent oxidations which have a detrimental impact on desired sensory characteristics of wine. To the best of our knowledge no data have been reported on RF and Met decay with regards to the wood ageing and batonnage. Even if these winemaking practices are less common for white wine than for red wine, the ageing in oak containers is becoming a common practice in some winegrowing regions (González-Centeno et al., 2020) increasing and improving the availability of white wines aged in wood in the market, adapting to the demands of the international trade (Alañón et al., 2018). During the wood ageing, amino acids can undergo through oxidative deamination generating higher alcohols (Câmara et al., 2006; Carpena et al., 2020). However, no specific result was reported on Met and its susceptibility to oxidative

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4.3 Packaging and storage approaches

deamination in such winemaking condition.

Glass bottle is a physical barrier shielding wine from light. Its choice is of crucial importance when it comes to limiting the risk of LST. Consumers often prefer flint glass bottles because

the colour of wine, a marker of its oxidative status, is visible. Moreover, the production and recycling costs are lower for clear bottles and they have a lower carbon footprint (Hartley, 2008). In survey carried out among 231 Italian wineries (https://www.survio.com/survey/d/K6Y3T0W6C5S5Z1G2J), up to 80% of total white wine production per winery is bottled in clear glass. The protection from UV/Vis light offered by clear glass is significantly lower compared to other commonly used bottle colours, such as amber or green. Flint glass allows to pass about 90% of the harmful light (under 500 nm), whereas green and amber bottles allow to pass 50% and 10% of light, respectively (Clark et al., 2011). The importance of wine bottle colour in LST prevention is a well-known aspect needing a further consideration. Dozon and Noble (1989) carried out a sensory study to evaluate the effect of light on white wines, both sparkling and still, which were bottled in flint and green bottles, and then exposed to light. Panel of trained judges determined the minimum time of exposure necessary to produce a difference in aroma compared to a control kept in the dark. The results showed that for samples in flint bottles, the time required to develop an off-odour was significantly shorter than for samples kept in green bottles. For flint bottles it was 3.4 and 3.3 hours for still and sparkling wine, respectively. In green bottles, an off-odour appeared after 18 and 31.1 hours for still and sparkling wine, respectively. Green bottles do not provide a total protection against LST, but they definitely increase the time necessary for the fault perception, which resulted six- and ten-fold longer for still and sparkling wines, respectively. It is noteworthy to mention that in this study wine samples were kept 35 cm from the light source, while in the commercial retail conditions, wines usually are placed farther from the light source. In real life conditions, time of light exposure necessary to produce the olfactory defect, probably would have to be longer. Differences between flint and green bottles in preventing LST were also investigated by Arapitsas et al. (2020). In their study, wines were kept in a specially arranged room

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simulating supermarket conditions, with windows with curtains, shelves, air conditioning and lamps kept on 12 hours/day. Sensory analysis managed by an expert panel, who evaluated wines within fifty days of light exposure, showed that most of flint bottles developed LST after four weeks. The more intense perception of the defect was observed for longer time of light exposure, proving this factor to be mainly causative to LST occurrence. Moreover, all the wines bottled in flint glass developed LST, whereas the wines bottled in green glass never exceeded four out of 10 point-scale in sensory analysis, meaning that the spoilage was not clearly developed. These results confirm the previous ones, regarding the capacity of green bottles to protect wines from LST better than flint ones (Dozon & Noble,1989). Moreover, wines in green bottles were resistant to LST development up to 50 days of light exposure which confirms that in retailing conditions, due to further wine positioning from the light source, LST needs more time to develop (Arapitsas et al., 2020). Another interesting outcome from this work is that LST is irreversible; the sensory evaluation conducted after one year of wine storage in the dark showed the same results as the analysis performed immediately after the light exposure (Arapitsas et al., 2020). The light can be both absorbed by the glass and partially reflected. As a consequence, the shape of the bottle and the direction of the light can also affect the occurrence of LST in wine. It has been proved that long neck bottles with a shallow angle on the bottle shoulder such as Rhenish bottles, show a better protection from light compared to short neck or sharp shoulder angle bottles (Hartley, 2008). Nonetheless, even if dark bottles can shield the light and prevent the appearance of LST, the light exposure of white wine bottled in dark glasses (Antique Green and French Green) can have an impact on its characteristics. In this condition, highest concentration of xanthylium ions causing a major browning was found indicating a more evidenced oxidation. This occurred as a consequence of the increase of temperature due to the dark bottle (Maury et al., 2010). For this reason, taking into account the overall quality of white wine, oenological

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approaches built up by means of technology, chemistry and microbiology are crucial to counteract the detrimental light-dependent faults.

Nowadays, in order to protect the wine bottles in flint bottles, plastic or paper films are used to cover the bottles. These devices are able to absorb the wavelengths that cause LST, but they increase the cost of wine production in terms of necessary material and labouring hours, since each bottle has to be wrapped individually. Moreover, the bottle wine wrapping is associated with the waste disposal problems and the increase of plastic material production.

5. Conclusions

Several factors are involved into the light-dependent spoilage of wine. LST depends mainly on the concentration of RF and Met, but also to the chemical composition of wine (i.e. iron, copper, phenolic compounds, antioxidants added). The type of light source in terms of wavelengths emitted by the lamps, the duration of the light exposure during wine storage, the distance of the lamp as well as the bottle shape and color need to be taken into account. As many aspects can affect the occurrence of wine faults, it is clear that the entire winemaking process, from the must production and alcoholic fermentation up to the storage and selling conditions, are important aspects that have to be considered in order to maintain and ensure the quality of the wine till the bottle opening.

In an oenological perspective, technological aspects including the use of specific adjuvants having the ability of bind sulfur-containing off-flavors and quench excited RF or oxidized Met as well as removing RF represent a challenge to counteract this wine fault. As the same time, the use of yeast able to release low amounts of RF and Met can carry out a protective effect. In this scenario, investigation on oenological tannins of different origin will support the wine industry with affective tool against the detrimental effect of LST. Contemporarily.

the selection of low RF and Met producing yeast strains can also open up new applications to positively tackle this problem of current oenology and a further improvement of precision oenology.

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701 Figures' Captions

- Figure 1: Riboflavin photosensitization by Type I or Type II mechanisms (Cardoso et al.,
- 703 2012).
- Figure 2: Reaction scheme of methional formation due to light exposure (Maujean & Seguin,
- 705 1983).

- Figure 3: Scheme of the proposed tartaric acid degradation through the Fenton reaction
- and under light exposure (Clark et al., 2007).
- Figure 4: Riboflavin pathway in Saccharomyces cerevisiae (Gudipati et al. 2014).
- 709 Figure 5: Photo-catalytic cycle for the riboflavin-mediated oxidation of methionine in the
- 710 presence of gallic acid and sulfur dioxide through Type I and Type II mechanisms
- 711 (Fracassetti et al., 2020).

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