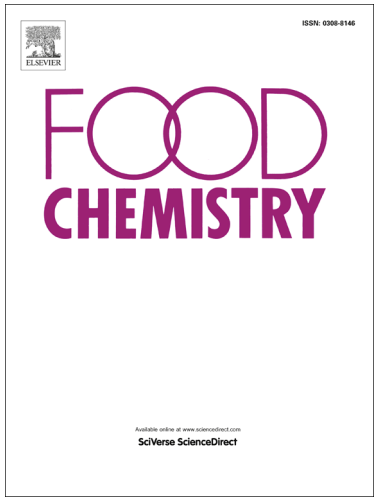

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Daniela Fracassetti, Sara Limbo, Luisa Pellegrino, Antonio Tirelli

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Light-induced reactions of methionine and riboflavin in model wine: effects of hydrolysable tannins and sulfur dioxide

Daniela FRACASSETTI*, Sara LIMBO, Luisa PELLEGRINO and Antonio TIRELLI

Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Milan, Italy

Corresponding author at Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy. E-mail address: daniela.fracassetti@unimi.it.

Abstract

The riboflavin-mediated photo-degradation of methionine in white wine has been related to onset of undesired light-struck taste. This research investigated the effects of different concentrations of riboflavin and methionine, hydrolysable tannins from various sources (nut galls, chestnut and oak woods) and sulfur dioxide on methionine degradation in a model wine exposed to light. Increased methionine concentration resulted in its increased degradation with the consequent formation of volatile sulfur compounds, namely methanethiol, dimethyl disulphide and dimethyl trisulphide. Tannins, especially nut gall tannin, were effective in limiting both methionine degradation and the production of volatile sulfur compounds. Sulfur dioxide enhanced the methionine degradation although the light-struck taste was not perceived when sulfur dioxide concentration was higher than 50 mg/L. In conclusion, the use of hydrolysable tannins can represent a promising tool for protecting white wine against the light-struck taste also limiting the use of sulfur dioxide.

Keywords: photo-degradation; light-struck taste; methanethiol; dimethyl disulphide; light exposure.

1. Introduction

Riboflavin is a vitamin behaving as a photosensitizer in many food and beverages. The riboflavin level in grape is usually lower than few tens of micrograms per litre of grape juice (Riberau-Gayon, Glories, Maujean, & Dubourdieu, 2006), but it can increase during the winemaking mainly due to the metabolic activity of *Saccharomyces cerevisiae* (Santos, García-Ramírez, & Revuelta, 1995). Values approaching 150 µg/L (Mattivi, Monetti, Vrhovšek, Tonon, & Andrés-Lacueva, 2000) or even higher can eventually occur in wine depending on the yeast strain (Fracassetti, Gabrielli, Encinas, Manara, Pellegrino, & Tirelli, 2017).

In bottled wines, riboflavin is involved in light-induced reactions affecting changes of volatile compounds, colour and flavour (Benitez, Castro, Natera, & García Barroso, 2006; Dias, Smith, Ghiggino, & Scollary, 2012; Grant Preece, Barril, Schmidtke, Scollary, & Clark, 2017). A further photochemical reaction occurring in white wine is the photo-degradation of tartaric acid, in presence of iron, to glyoxylic acid and the following browning due to the formation of xanthylium cation pigments (Grant-Preece, Barril, Schmidtke, & Clark, 2018). Wavelengths in the range 370-450 nm can excite riboflavin to the short-lived singlet state and then to the triplet state which is then involved in photo-oxidation reactions through diverse mechanisms (Grant Preece et al., 2017). Triplet riboflavin is a strong oxidant and reacts with electron-donor compounds, such as methionine. The methionine concentration in wine is 3 mg/L on average (Amerine & Ough, 1980; Riberau-Gayon et al., 2006; Grant-Preece et al., 2017), i.e. a molar concentration around 40 times higher than that of riboflavin, and its oxidation leads to the formation of methional. The latter is unstable, photosensitive and readily decomposes to methanethiol (MeSH) and acrolein *via* a retro Michael reaction. Two molecules of MeSH can yield dimethyl disulphide (DMDS) (Maujean & Seguin, 1983a). This mechanism has been investigated in white wines being responsible of an off-aroma, usually named sunlight

flavour or light-struck taste (LST) (Maujean, Haye, & Feuillat, 1978; Maujean & Seguin, 1983b; Andrés-Lacueva, Mattivi, & Tonon, 1998), due to MeSH which has a low olfactory perception threshold (0.3 µg/L in model wine; 2-10 µg/L in wine) and a rotten egg- or cabbage-like odour (Pripis-Nicolau, de Revel, Bertrand, & Lanvaud-Funel, 2004; Solomon, Geue, Osidacz, & Siebert, 2010). DMDS has a higher perception threshold (20-45 µg/L) and it gives cooked-cabbage or onion-like notes (Mestres, Busto, & Guash, 2000). These negative olfactory notes may develop in white wine bottled in clear-glass bottles along with higher color intensity and browning thus causing relevant economical losses and waste of product (Cáceres-Mella, Flores-Valdivia, Laurie, López-Solís, & Peña-Neira, 2014). While the risk of this fault was proved to decrease when riboflavin concentration in white wine was lower than 80-100 µg/L (Pichler, 1996; Mattivi et al., 2000), little is known about the impact of methionine concentration in LST appearance in spite both MeSH and DMDS arisen from the oxidation of this amino acid.

A few technological approaches have been proposed to prevent LST in wine. Among these, the use of low riboflavin -producing *Saccharomyces* strains for the alcoholic fermentation may prevent high amount of riboflavin in wine (Santos et al., 1995; Fracassetti et al., 2017). An effective removal of riboflavin was achieved treating wine with insoluble adsorbing materials, such as charcoal or bentonite, but their use should be limited as also desired flavouring compounds are removed, thus detrimentally affecting the wine flavour (Pichler, 1996; Fracassetti et al., 2017). Some compounds can hamper the oxidizing behaviour of riboflavin. Oxygen itself (1-9 mg/L in bottled wine; Ugliano et al., 2013) can either oxidize the reduced riboflavin to its ground state with the production of the hydroperoxyl radical (Grant-Preece at al., 2017) or act as quencher of triplet riboflavin, as proved by Goldsmith, Rogers, Cabral, Ghiggino, and Roddick (2005) in beer. The effect of sulfur dioxide (SO_2) in the LST development is still unknown though it might either deplete molecular oxygen or react with singlet oxygen (Grant-Preece et al., 2017). Some phenols

proved to be effective quenchers of singlet oxygen (Briviba, Devasagayam, Sies, & Steenken, 1993; Lagunes, Vázquez-Ortega, & Trigos, 2017). In particular, flavan-3-ols in white wine are able to hamper the rise of LST (Maujean & Seguin, 1983b). However, these phenols can bring bitter taste and/or woody texture to white wine, especially when galloylated forms are involved (Robichaud & Noble, 1990). Maujean and Seguin (1983b) also suggested that the condensed tannins (proanthocyanidins) might prevent the LST, due to their ability in shading the riboflavin by acting as shield. To the best of our knowledge, the efficacy of wood-derived hydrolysable tannins, namely gallotannin and ellagitannin, in preventing the LST has not been investigated, so far.

This research aimed to understand the effect of different concentrations of riboflavin and methionine on the photo-degradation mechanisms in model wine. The addition of hydrolysable tannins from chestnut, oak woods, and nut galls, were investigated in order to clarify the influence of these antioxidants on the photo-degradative mechanisms. Moreover, the effect of SO_2 was also evaluated. The appearance of LST was considered in terms of formation of MeSH and DMDS, and sensory evaluation. Based on the current knowledge of the photo-degradative mechanisms in oenological conditions, we can hypothesize that i) methionine content have a major role in the rise of LSF and ii) hydrolysable tannins effectively prevent the methionine degradation and, as a consequence, limit the appearance of this fault.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, ethanol, acetonitrile, riboflavin, citric acid, tartaric acid, tetrahydrofuran (THF), boric acid, mercaptoethanol, *o*-phtaldehyde (OPA), L-methionine, d_6-dimethyl sulphide (d_6-DMS), isopropyl disulphide, dimethyl disulphide (DMDS), dimethyl trisulphide (DMTS), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrochloric

acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals were of analytical grade, at least. HPLC grade water was obtained by a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA).

Commercial hydrolysable tannins from nut galls, chestnut and oak woods intended for oenological use were provided by Dal Cin (Concorezzo, Italy).

The model wine contained 5.0 g/L tartaric acid and 12% ethanol (v/v), adjusted to pH 3.2 with sodium hydroxide (Merck, Darmstadt, Germany).

2.2. Photo-degradation kinetic of riboflavin

The light treatment was carried out using a laboratory-made apparatus consisting of three fluorescence light bulbs placed 40 cm from each other. Three 100 mL bottles containing the test sample (triplicate trials) were each positioned between two light bulbs, i.e. at 20-cm distance (Supplementary S1). The compact fluorescent light bulbs (Philips) emitted cold light (6500 K) with a luminous flux of 3172 Lumen with high emission in the absorption wavelengths of riboflavin (370 and 440 nm). The apparatus was kept in a dark room with air conditioning set at 22 °C. The temperature was monitored in the proximity by a thermometer dipped in water in a 100 mL bottle placed in the centre of the apparatus.

The degradation kinetic of riboflavin was evaluated in both model wine and white wine. The white wine was a sulfur-free Chardonnay wine (vintage 2015), collected at a private winery, containing riboflavin 175 µg/L and total SO_2 lower than 10 mg/L determined by direct titration with iodine in accordance to the method OIV-MA-AS323-04B (2010). Both model wine and white wine were spiked with riboflavin up to 400 µg/L. The samples were continuously illuminated and aliquots were collected at 30, 60, 120, 240 min; control aliquots were stored in the dark. The degradation kinetics were assessed as triplicate trials.

2.3. Experimental plan

Aliquots of model wine were prepared by adding: i) 3 mg/L methionine and 0, 50, 75, 100, 200, 300 µg/L riboflavin; ii) 200 µg/L riboflavin and 0, 1.4, 3.5, 7.0, 13.0 mg/L methionine; iii) 3 mg/L methionine, 200 µg/L riboflavin without or with 40 mg/L of each of the hydrolysable tannins (tannins from nut galls, chestnut and oak); iv) 3 mg/L methionine, 200 µg/L riboflavin, 50 mg/L SO_2 without or with 40 mg/L of the hydrolysable tannins listed at the previous point; v) 3 mg/L methionine, 200 µg/L riboflavin, 40 mg/L nut gall tannin and 10, 25, 50, 75, 100 mg/L SO_2. All the solutions were contained in 100 mL clear glass bottles tightly closed without head space and exposed for 120 min to the fluorescent light. A set of bottles was also filled with the solutions iii) and nitrogen was sparged for 180 min to attain anoxic conditions (dissolved oxygen < 0.2 mg/L determined with OxySenseTM 101 (DecisionLink, Inc., Las Vegas, NV)). The model wine solutions were maintained at 20±2°C and protected from light before and after the controlled light exposure by covering the bottles with an aluminium foil. Control samples for each trial were stored at 20±2°C in the dark for 120 min in order to determine whether the degradation of methionine and riboflavin was light-dependant. In any case, neither methionine nor riboflavin changed under storage in the dark. Triplicate determinations were carried out for each experiment. For each condition tested, the molar ratio between degraded methionine and degraded riboflavin was calculated.

2.4. Determination of riboflavin

The method reported by Fracassetti et al. (2018) was applied for assessing the riboflavin content with some modifications. Briefly, sample solutions were passed through a 0.22-µm PVDF filter (Millipore, Billerica, MA, USA) and 50 µL aliquot was injected

without further treatment. An Acquity HClass UPLC (Waters, Milford, MA, USA) system equipped with a photo diode array detector 2996 (Waters) was used. The detection wavelength was 440 nm. The separation was carried out with (A) 90% 50 mmol citrate buffer atpH2.5 and 10%methanol (v/v) and (B) 10% 50 mmol citrate buffer at pH 2.5 and 90% methanol (v/v) in gradient mode (70% B in 8 min) at a flow rate of 0.6 mL/min. Calibration curves were obtained for riboflavin concentrations spanning from 20 µg/L to 500 µg/L. Data acquisition and processing were performed by Empower 2 software (Waters).

2.5. Determination of methionine

Methionine concentration was assessed by HPLC as *o*-phthalaldehyde (OPA) derivative under the conditions described by Fracassetti et al (2017) with some modifications. The derivatization solution was prepared in a 10 mL volumetric flask by dissolving 250 mg of OPA in 1.5 mL of ethanol, adding 200 µL of 2-mercaptoethanol, and making up to the volume with borate buffer 0.4 M at pH 10.5. The pre-column derivatization procedure was performed automatically by the autosampler. The chromatographic separation of OPA-methionine derivative was carried out using a Waters Alliance 2695 chromatographer (Milford, MA, USA) equipped with a VWR Hitachi L2480 fluorescence detector (Milan, Italy). The column was a Nova-Pak C18 (150 mm x 3.9 mm column, 4 µm particle size stationary phase) (Waters). Eluting solvents were A) 1% of tetrahydrofuran, 8% methanol and 91% citrate buffer (10 mM, pH 7.3); B) 80% methanol and 20% citrate buffer (10 mM, pH 7.3). The separation run was carried out in 17 min, including 9 min column rinsing and conditioning. The elution gradient was 0.5 min, 60% A; 0.5-8 min, 20% A. The flow rate was set to 1 mL/min and the column temperature was 35°C. The OPA-methionine derivative was detected by monitoring fluorescence at 335 nm and 440 nm as excitation and emission wavelengths, respectively. Methionine

quantification was carried out by the external standard method (0.1-15 mg/L). Data acquisition and processing were performed by Empower 2 software (Waters).

2.6. Analysis of volatile sulfur compounds

The analysis of volatile sulfur compounds (VSCs) was assessed by Solid Phase Micro Extraction (SPME)-GC/MS as reported by Nguyen, Nicolau, and Kilmartin (2009) with some modifications. Ten millilitres of sample were added with 2.5 g of magnesium sulphate heptahydrate and placed in a glass-vial that was immediately hermetically closed. The fibre was a carboxen-polydimethylsiloxane-divinylbenzene (CAR-PDMS-DVB; 50/30 µm x 1 cm) (Supelco, Bellefonte, PA, USA). The SPME was carried out with an autosampler (HTA autosampler, Brescia, Italy) set at the following conditions: incubation for 5 min at 40°C; agitation 10-s on and 3-s off; extraction for 30 min; desorption for 20 min. The GC/MS equipment was a Perkin Elmer Autosystem XL Gas Chromatograph coupled with a Turbomass Mass Spectrometer (Perkin Elmer, Italy). The separation was achieved by a Stabilwax-MS column (30 m x 0.250 mm x 0.25 µm) (Restek, Bellefonte, PA, USA) and using helium as carrier gas at 1 mL/min flow rate. The oven temperature was initially set at 40°C and held for 5 min, ramped at 1.5°C/min up to 60 °C, ramped at 4°C/min up to 150°C and held for 5 min, finally ramped at 40°C/min up to 230°C and held for 10 min. The transfer line temperature was set at 230°C and the source temperature at 250°C. The MS detector registered the *m/z* in the range from 33 up to 350 Da. The ions used for identification of target molecules were chosen according to the NIST library and Nguyen et al. (2009). Duplicate injections were carried out for each sample. Moreover, the relative standard deviations (RDS%) were evaluated by triplicate injections in three different days (n=9). RDS% were 9.1%, 8.1% and 11.2% for MeSH, DMDS and dimethyl trisulfide (DMTS), respectively. Results are expressed as relative concentration (µg/L) for MeSH referred to d_6-DMS; DMDS and DMTS amounts were determined by the external

standard method (0.5-100 µg/L). The ratio between the moles of sulfur atom formed obtained by summing MeSH, DMDS and DMTS concentrations, and the moles of sulfur lost as degraded methionine was calculated.

2.7. Sensory evaluation

A panel constituted by five expert judges (3 males, 2 females, aged 25-40) carried out the olfactory scoring for the “cooked cabbage” descriptor. The score ranged from 1 (not perceived) to 5 (extremely perceived). The model wine spiked with methionine (3 mg/L) and two different levels of riboflavin (200 µg/L or 400 µg/L) and exposed to light for four hours was used to train the panellists. The judges were calibrated by sniffing model wine solutions spiked with methionine (3 mg/L) and riboflavin (200 µg/L) exposed to light for increasing time up to two hours. Each sample was evaluated just after the bottle opening.

2.8. Characterization of commercial hydrolysable tannins

The total phenols index (TPI) of the commercial hydrolysable tannins (from nut gall, chestnut and oak woods) was assessed colorimetrically by the Folin-Ciocalteu method (Scalbert, Monties, & Janin, 1989). A tannin solution (1 g/L) in methanol/water 50/50 (v/v) was serially diluted in the same solvent. The Folin-Ciocalteu reagent was diluted 10 times with water (v/v) and 2.5 mL was added to 0.5 mL of diluted tannin solution together with 2 mL of 75 g/L sodium carbonate solution. The reaction mixtures were kept 1 h at room temperature in the dark prior the absorbance measurement at 765 nm. Gallic acid solutions in the range 5-100 mg/L were used for calibration and the results were expressed as g gallic acid/g powder. Triplicate determinations were carried out.

The antioxidant capacity of the hydrolysable tannins was assessed according to the DPPH assay, following the method of Brand-Williams, Cuvelier, and Berset (1995) with

some modifications. The DPPH solution was diluted with methanol to obtain 1.00±0.03 absorbance units at 515 nm. The hydrolysable tannins were dissolved (20 g/L) in 70% methanol (v/v) and serially diluted. An aliquot (2.94 mL) of the DPPH solution was placed in a cuvette, then 60 µL of sample were added and carefully mixed. The absorbance values were measured after incubation for 50 min at 20±2°C. A calibration curve of Trolox at concentrations from 50 to 1000 mM was prepared. Results were expressed as mmol Trolox/g powder. Triplicate determinations were carried out.

2.9. Statistical analysis

The one-way ANOVA was carried out by means of SPSS Win 12.0 program (SPSS Inc., Chicago, IL). The equations of the calibration curves were calculated by the linear regression analysis. Differences related to the methionine degraded and the formation of VSCs were evaluated by the T-test ($p < 0.05$).

3. Results and discussion

3.1. Photo-degradation kinetic of riboflavin

The laboratory-made illuminating apparatus allowed the photodegradation of riboflavin to occur in a reasonably short time and under standardized and controlled conditions. Temperature fluctuations were lower than 2°C throughout the light treatments and thus considered negligible.

The riboflavin degradation kinetics in model wine and white wine were compared (Figure 1). In both cases, degradation followed a first order kinetic and riboflavin was completely degraded after two hours of exposure to the light, in spite of the different composition of the two tested matrices. However, the degradation rate was lower in white wine ($k=0.021$ min^{-1}) than in model wine ($k=0.039$ min^{-1}), likely due to the presence of

quenching compounds, including flavan-3-ols (Maujean & Seguin 1983b) in the latter. To avoid such interferences and accurately follow the light-induced reactions of riboflavin and methionine, the model wine was adopted in this study. Based on these data, the subsequent treatments were carried out by illuminating the samples for two hours.

3.2. Influence of concentrations of methionine and riboflavin on their photo-degradation

The degradation rate of methionine in presence of riboflavin as photo-sensitizer is not known, to the best of our knowledge. Methionine proved to be stable in riboflavin-free hydro-alcoholic acidic solution, as no degradation occurred in the model wine spiked with methionine (3 mg/L) and exposed to light for two hours. On the contrary, the presence of riboflavin led to methionine degradation up to 27%, depending on the initial concentrations of the two compounds (Table 1). When the concentration of methionine was kept constant and that of riboflavin was progressively increased from 0 to 300 µg/L, the relative amount of degraded methionine increased in parallel, with a negligible level (1.2%) observed when riboflavin was 50 µg/L. In a second experiment, riboflavin concentration was kept at 200 µg/L whereas methionine concentration was increased from 0 to 13.2 mg/L (0-90 µM). Under these conditions, the relative amounts of degraded methionine were much higher (Table 1). In particular, for comparable levels of riboflavin, an increase of methionine of about 0.5 mg/L increased degradation of this amino acid, further suggesting the strong influence of methionine concentration on its own degradation (Table 1). The molar ratio degraded methionine/degraded riboflavin was calculated for all the tested conditions and the values roughly ranged between 2 and 35. Our data strongly disagree with the reaction scheme proposed by Maujean and Seguin (1983a) where a 1:1 ratio between riboflavin and methionine was expected (Supplementary S2). These authors adopted a model wine comparable to ours (6 g/L tartaric acid and sodium tartrate, and 11% (v/v) ethanol,

adjusted to pH 3.15) but unfortunately did not give detail concerning the adopted experimental conditions.

Our results confirm that riboflavin has a key role in inducing the methionine degradation. Moreover, chemical pathways to explain such a behaviour have not been extensively studied in wine. When exposed to light at wavelengths spanning from 370 nm to 450 nm, riboflavin excites from its ground state (S_o) to the more reactive singlet state (S_1) which may reach the triplet state (T_1) through an intersystem crossing (Figure 2). In this step, known as photo-oxidation Type II, the singlet oxygen is generated, an electrophile able to react with alkenes, amines, sulfides (Foote, 1976; DeRosa & Crutchely, 2002). Methionine was listed among the amino acids primarily reacting with this oxygen species (Min & Boff, 2002). In the second step, called photo-oxidation Type I, riboflavin goes from the high-energy state T_1 to a lower energy state through the formation of reduced riboflavin. In this step, methionine acts as electron donor, being oxidized to methional. Besides methional, other compounds can originate from oxidative pathways involving methionine (Barata-Vallejo, Ferreri, Postigo, & Chatgilialoglu, 2010) that could explain the molar ratio degraded methionine/degraded riboflavin higher than the expected 1:1. Moreover, the degradation of methionine could also be enlightened by the cyclic mechanism of reduced riboflavin /ground state riboflavin (Figure 2). However, no residual riboflavin was detected in our light-treated samples.

Based on the above considerations, the formation of volatile sulfur compounds (VSCs) deriving from methionine degradation was evaluated in model wine spiked with either methionine or riboflavin before light exposure. Additionally, the sensory analysis was carried out by a panel test to evaluate the olfactory perception of cooked cabbage as indicator of LST. The formation of MeSH was rather low, whereas DMDS and DMTS were the prevailing compounds and, as expected, they reached levels up to10 times higher in the samples where methionine concentration was increased compared to samples where

riboflavin concentration was increased (Table 1). These behaviours agree with those observed for methionine degradation. Higher sulfur conversion yields were found for increasing levels of methionine; their values, in the range 2.9-13.4%, indicated that not only methionine acted as electron-donor to reduce riboflavin but was likely involved in other chemical pathways, i.e. reacting with singlet oxygen (Figure 2). The "cooked-cabbage" perception increased as the concentrations of riboflavin increased (Table 1), but to a lower extent in comparison to the samples where methionine was increasingly added (Table 1). Even though riboflavin concentrations lower than 100 µg/L are reported to decrease the risk of triggering the LST in wine (Pichler 1996; Mattivi et al. 2000), the minimum levels of riboflavin and methionine needed to give rise to the LST fault are still unclear. The LST perception increased linearly as the methionine concentration increased (Table 1). DMTS can arise from both methional and methanethiol oxidation (Gijs, Perpète, Timmermans, & Collin, 2000) and confers cabbage, onions, cooked vegetable-like odours, as well as potato and earthy-like notes (Mestres et al., 2000; Gijs, Chevance, Jerkovic, & Collin, 2002). As already mentioned, a few milligrams per litre of methionine is usually found in wine deriving from yeast cells lysis (Riberau-Gayon et al., 2006, Fracassetti et al., 2017). It is noteworthy that low-riboflavin producer yeasts can be also low-methionine producers (Fracassetti et al., 2017). These findings showed that, besides riboflavin, methionine plays a strong role in the formation of LST. Moreover, LST in white wine is avoided when concentrations below 50 µg/L and 1.5 mg/L are present for riboflavin and methionine, respectively.

3.3. Effect of hydrolysable tannins on photo-degradation

Prevention of LST is essential in preserving the sensory properties of white wine. Maujean and Seguin (1983b) suggested flavan-3-ols to be effective at this regard likely because of their shading properties. Other phenols, belonging to the group of hydrolysable

tannins (gallotannin and ellagitannin), have been previously used in winemaking due to their protective effect on wine stability and sensory character (Obreque-Slíer, Peña-Neira, López-Solís, Ramírez-Escudero, & Zamora-Marín, 2009). In order to assess their effectiveness in preventing the light-induced degradation of riboflavin and methionine, different wood hydrolysable tannins were tested in model wine in order to understand their possible protective effect against LST. Both anoxic and oxic conditions were considered. Tannin at 40 mg/L were used in order to avoid the perception of astringency and bitterness (Robichaud & Noble, 1990). Only nut gall tannin effectively decreased methionine degradation under oxic conditions (Table 2). Such a behaviour can be ascribed to the two-fold higher total phenol index (TPI) compared to the other tannins tested, although the specific antioxidant abilities (AC/TPI) were comparable to each other (Supplementary S4). Similarly, Vignault and co-authors (2018) found two-folds higher TPI, expressed as gallic acid, in nut gall tannins in comparison to chestnut and oak tannins. In particular, among phenolics, gallic acid showed the ability of quenching the singlet oxygen (Lagunes & Trigos, 2015; Lagunes et al., 2017) and, as a consequence, higher amounts of gallic acid could limit the methionine degradation (Figure 2). Consistently, the concentration of gallic acid equivalents we found in nut gall tannin was more than twice (6.01 mg/g) in comparison to the two ellagitannins (2.84 mg/g and 2.26 mg/g for chestnut and oak tannins, respectively).

When oxygen-free conditions were applied, the methionine degradation increased whatever the hydrolysable tannin source but nut gall tannin showed a protective effect on methionine even under this condition. When no oxygen is present, only the Type I mechanism can take place (Figure 2). The nut gall tannin could compete with methionine in accepting the electrons from T_1 state riboflavin, which turns into reduced riboflavin. The capability of hydrolysable tannin, in particular nut galls, to quench the T_1 state riboflavin

could be also assumed (Vaish & Tollin, 1970). Further investigations are necessary to confirm these hypotheses in real wine.

The levels of VSCs found following to the light exposure under oxic conditions readily increased when anoxic conditions were applied (Table 2). Under anoxic condition, the concentrations of MeSH and DMTS were up to six-folds higher compared to the oxic condition, and even eighty-four folds higher for DMDS. This is because oxygen can both quench excited riboflavin (Grant-Preece et al., 2017) and produce stable methionine sulfoxide and other methionine-oxidative compounds (Barata-Vallejo et al., 2010). No DMTS was detected when hydrolysable tannins were added, indicating their protective effect against the oxidation of methional and MeSH to DMTS (Gijs et al., 2000). The nut gall tannin was also effective in avoiding the formation of DMDS under oxic condition (Table 2). The protective effect of the hydrolysable tannins resulted more evident under anoxic condition: lower concentrations were found for MeSH, DMDS and DMTS in comparison to the model wine with no addition (Table 2). This was confirmed by the sensory analysis: the perception of the “cooked cabbage” descriptor was lower under oxic condition as well as where the hydrolysable tannins were added (Table 2). The effectiveness of the three tannins was nut gall tannin > oak tannin ≥ chestnut tannin. Absorbance in the range 370-450 nm of samples added with tannins was measured before and after the light treatment and negligible differences (< 0.02 AU) were found. Consequently, the possible light shielding effect of tannins suggested by Maujean and Seguin (1983b) could be excluded. The reaction between quinones, deriving from the reaction between singlet oxygen and tannins (Briviba et al., 1993), and the compounds containing a free thiol group is well known (Cilliers & Singleton, 1990). Quinones can readily react with the MeSH formed from methionine and thus lead to lower amount of DMDS (Figure 2).

3.4. Effect of sulfur dioxide on photo-degradation

The role of SO_2 in photo-degradation was investigated as this compound is the most common antioxidant used in winemaking. Methionine degradation was greatly enhanced in the model wine in presence of SO_2 (Table 2), maybe due to the increased oxidizing power of oxygen when SO_2 is present without phenols (Danilewicz and Wallbridge, 2010). In this condition, about 10% of riboflavin remained after the light exposure (Table 3). A quenching effect of singlet state riboflavin allowing spin conversion back to the ground state might explain this finding. However, degradation of methionine was higher in the presence than in the absence of SO_2 (Table 2), even in presence of hydrolysable tannins (Table 3). The ability of SO_2 to indirectly consuming oxygen is well known (Riberau-Gayon et al., 2006). The consequent lower amount of dissolved oxygen favours the Type I mechanism, thus increasing methionine degradation. This was more evident when also hydrolysable tannins were added, since their ability to quench singlet oxygen could further promote the Type I mechanism involving methionine (Figure 2). The effect of increasing concentrations of SO_2 in presence of nut gall tannin is not easy to explain. In these conditions, the degradation of methionine was highest (53%) when the SO_2 concentration was below 10 mg/L (Table 3). In spite of these results, the olfactory perception of LST decreased in presence of SO_2 concentration higher than 50 mg/L (score 1 out of 5), likely due to the formation of either sulfonated methional or sulfonated MeSH (Figure 2). Further investigation is needed to clarify the effect of SO_2 against the LST in white wine. The transition metals (iron and copper) can oxidize phenolics generating hydrogen peroxide which is removed by SO_2 and, at the same time, the radicals from SO_2 are removed by phenols (Danilewicz and Wallbridge, 2010). A possible limited effectiveness of SO_2 against the appearance of LST in white wine cannot be excluded.

Conclusions

Our findings highlighted that the degradation of methionine induced by photo-degradation of riboflavin does not occur when the concentration of this last is below 50 μg/L. When riboflavin concentration was increased, higher levels of VSCs formed and, consequently, LST perception increased. Methionine concentration itself was also detrimental, even more than riboflavin, as an increase of methionine level caused a strong increase of both VSCs' amount and ratio degraded methionine/degraded riboflavin. Besides riboflavin, the level of methionine in white wine needs to be considered for preventing the LST. Nut gall tannin can effectively hamper the methionine degradation and VSCs formation under oxic conditions. In fact, anoxic conditions (oxygen lower than 0.2 mg/L) can enhance methionine degradation and the addition of nut gall tannin might not prevent the LST. Sulfur dioxide enhanced methionine degradation, even at low concentration, but its addition did not lead to the sensory perception of LST. The positive influence of hydrolysable tannins on photo-degradative mechanisms was shown as well as their role in preventing the LST in model wine. The latter proved to be an effective medium for specifically investigating the light-induced degradation of riboflavin since many compounds dissolved in wine can interfere in the related chemical pathways. However, further investigation will evaluate the effectiveness of other gallic acid-rich tannins in preventing the LST in bottled white wine.

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Figures' captions

Figure 1: Degradation kinetics of 400 µg/L riboflavin in model wine (red triangle) and white wine (blue circle) exposed to a fluorescence light emitting 3172 Lumen at 6500 °K. Solid lines show the first order kinetic (ln $[RF_t]/[RF_0]$); dotted lines show the riboflavin decay ($[RF_t]/[RF_0]$).

Figure 2. Reaction scheme of methionine and riboflavin photo-degradation (Maujean & Seguin, 1983a; Grant-Preece et al, 2017). The outcomes deriving from the present study and supported by previous literature are indicated through dotted lines and underlined text. [a]: Barata-Vallejo et al., 2010. [b]: Briviba et al., 1993; Lagunes & Trigos, 2015; Lagunes et al., 2017. [c]: Gijs et al., 2000.

Supplementary S1. Scheme of the illumination apparatus developed and used in this study.

Supplemetary S2. Reaction scheme of methional formation due to light exposure reported by Maujean and Seguin (1983a).

Table 1: Decrease of methionine (Met; mg/L) and riboflavin (RF; µg/L), formation of methanethiol (MeSH), dimethyl disulphide (DMDS) and dimethyl trisulfide (DMTS), and sensory scores (1: not perceived; 5: extremely perceived). for increasing concentrations of RF (0-300 µg/L) and Met (0-13.0 mg/L) under oxic condition. Results are expressed as average ± standard deviation. The relative decrease (%) is reported in bracket. Different letters mean significant difference ($p < 0.05$). Sulfur conversion yield was calculated as ratio between the moles of sulfur atom formed obtained by summing MeSH, DMDS and DMTS concentrations, and the moles of sulfur lost as degraded methionine. Legend: n.a.: not added; n.d.: not detected.

RF (µg/L), Met constant (3.02±0.14 mg/L)	**Photo-degradation**		**Molar ratio degraded Met/ degraded RF**	**Volatile sulfur compounds (µg/L)**			**Sulfur conversion yield (%)**	**Sensory score**
	Met degraded mg/L (%)	*RF degraded µg/L (%)*		*MeSH*	*DMDS*	*DMTS*		
0	0	n.a.	--	--	--	--	--	1.0±0.0[a]
47.2±2.6	0.04±0.00[a] (1.2)	47.2±2.6 (100)	1.8	0.41±0.04[a]	n.d.	n.d.	3.2	1.1±0.0[a]
74.8±6.6	0.26±0.00[b] (8.7)	74.8±6.6 (100)	8.9	0.56±0.05[b]	n.d.	n.d.	0.7	1.2±0.1[b]
104.8±5.7	0.31±0.00[c] (10.3)	104.8±5.7 (100)	7.1	1.94±0.18[c]	n.d.	n.d.	0.8	1.5±0.2[b]
207.5±11.2	0.32±0.00[c] (10.6)	207.5±11.2 (100)	3.4	1.30±0.12[d]	1.87±0.15[a]	4.09±0.46[a]	7.6	2.3±0.3[c]
325.7±19.6	0.68±0.01[d] (22.5)	325.7±19.6 (100)	4.2	1.82±0.17[e]	3.03±0.25[b]	7.38±0.83[b]	6.1	3.0±0.2[d]
Met (mg/L), RF constant (194.7±5.5 µg/L)	**Photo-degradation**		**Molar ratio degraded Met/ degraded RF**	**Volatile sulfur compounds (µg/L)**			**Sulfur conversion yield (%)**	**Sensory score**
	Met degraded mg/L (%)	*RF degraded µg/L (%)*		*MeSH*	*DMDS*	*DMTS*		
0	n.a.	194.7±5.5 (100)	--	--	--	--	--	1.0±0.0[a]
1.40±0.07	0.17±0.00[a] (11.6)	194.7±5.5 (100)	2.1	0.41±0.04[a]	0.73±0.06[a]	1.25±0.14[a]	4.7	1.1±0.0[a]
3.50±0.18	0.67±0.00[b] (18.2)	194.7±5.5 (100)	8.5	0.96±0.09[b]	1.57±0.13[b]	3.23±0.36[b]	2.9	2.2±0.2[b]
6.91±0.36	1.40±0.00[c] (18.6)	194.7±5.5 (100)	16.7	1.94±0.18[c]	10.36±0.84[c]	41.99±4.70[c]	13.4	3.2±0.3[c]
13.19±0.70	3.52±0.02[d] (26.7)	194.7±5.5 (100)	35.5	2.37±0.22[d]	21.63±1.75[d]	59.30±6.64[d]	8.1	4.1±0.3[d]

Table 2. Decrease of methionine (Met; mg/L) and riboflavin (RF; µg/L), and formation of methanethiol (MeSH), dimethyl disulphide (DMDS) and dimethyl trisulfide (DMTS), and sensory scores (1: not perceived; 5: extremely perceived) in model wine containing 40 mg/L hydrolysable tannins from different wood sources and under different oxygen conditions. Results are expressed as average ± standard deviation. The relative decrease (%) is reported in bracket. Different letters mean significant difference ($p < 0.05$). Sulfur conversion yield was calculated as ratio between the moles of sulfur atom formed obtained by summing MeSH, DMDS and DMTS concentrations, and the moles of sulfur lost as degraded methionine. The average concentrations of methionine and riboflavin at dark were, 2.98±0.08 mg/L and 200.8±12.8 µg/L respectively. Legend: n.d.: not detected.

Oxic condition								
	Photo-degradation		**Molar ratio**	**Volatile sulfur compounds (µg/L)**			**Sulfur**	
Hydrolysable tannin	*Met degraded mg/L (%)*	*RF degraded µg/L (%)*	**degraded Met/ degraded RF**	*MeSH*	*DMDS*	*DMTS*	**conversion yield (%)**	**Sensory scores**
None	0.53±0.03^{ae} (18.0)	200.8±12.8 (100)	6.8	0.84±0.08^{a}	0.85±0.07^{a}	4.04±0.45^{a}	3.4	2.3±0.3^{a}
Chestnut	0.55±0.02^{ae} (18.1)	200.8±12.8 (100)	6.8	0.71±0.06^{a}	0.31±0.03^{b}	n.d.	0.6	1.8±0.1^{b}
Nut galls	0.33±0.02^{b} (11.0)	200.8±12.8 (100)	4.2	0.57±0.05^{b}	n.d.	n.d.	0.5	1.3±0.2^{c}
Oak	0.65±0.05^{ac} (21.5)	200.8±12.8 (100)	8.2	0.74±0.07^{a}	0.39±0.03^{b}	n.d.	0.5	1.9±0.2^{b}
Anoxic condition								
	Photo-degradation		**Molar ratio**	**Volatile sulfur compounds (µg/L)**			**Sulfur**	
Hydrolysable tannin	*Met degraded mg/L (%)*	*RF degraded µg/L (%)*	**degraded Met/ degraded RF**	*MeSH*	*DMDS*	*DMTS*	**conversion yield (%)**	**Sensory scores**
None	0.85±0.01^{d} (28.5)	200.8±12.8 (100)	9.2	5.30±0.48^{c}	70.95±5.75^{c}	25.26±2.83^{b}	37.0	5.0±0.3^{a}
Chestnut	0.90±0.08^{d} (30.0)	200.8±12.8 (100)	9.8	2.91±0.26^{d}	9.59±0.78^{d}	9.41±1.05^{c}	7.5	3.2±0.2^{b}
Nut galls	0.61±0.11^{e} (20.2)	200.8±12.8 (100)	8.2	2.18±0.20^{e}	2.18±0.18^{e}	1.69±0.19^{d}	3.1	2.0±0.3^{c}
Oak	0.72±0.09^{cd} (24.1)	200.8±12.8 (100)	9.6	2.75±0.25^{d}	8.06±0.65^{f}	4.15±0.46^{e}	6.4	3.0±0.3^{b}

Table 3: Decrease of methionine (Met; mg/L) and riboflavin (RF; µg/L) in model wine containing sulfur dioxide (SO_2, 50 mg/L) and hydrolysable tannins (40 mg/L), and nut gall tannin (40 mg/L) and increasing concentration of SO_2. Results are expressed as average ± standard deviation. The relative decrease (%) is reported in bracket. Different letters mean significant difference ($p < 0.05$). The average concentrations of methionine and riboflavin at dark were, 3.18±0.12 mg/L 206.6±6.8 µg/L respectively.

Hydrolysable tannin (40 mg/L), SO_2 (50 mg/L)	**Photo-degradation**		**Molar ratio**
	Met degraded mg/L (%)	*RF degraded µg/L (%)*	**degraded Met/degraded RF**
None	0.86±0.10^{a} (29.6)	185.2±1.1^{a} (90)	11.9
Chestnut	1.66±0.03^{b} (55.2)	206.6±6.8^{b} (100)	21.1
Nut galls	1.41±0.16^{c} (44.7)	206.6±6.8^{b} (100)	17.2
Oak	1.77±0.01^{d} (59.0)	206.6±6.8^{b} (100)	22.3
SO_2 (mg/L), nut gall tannin (40 mg/L)	**Photo-degradation**		**Molar ratio**
	Met	*RF*	**degraded Met/degraded RF**
10	1.58±0.15^{a} (52.9)	206.6±6.8 (100)	20
25	1.01±0.39^{b} (33.8)	206.6±6.8 (100)	13
50	1.24±0.16^{c} (41.4)	206.6±6.8 (100)	16
75	1.07±0.07^{bc} (35.7)	206.6±6.8 (100)	13
100	1.08±0.05^{bc} (35.9)	206.6±6.8 (100)	14

Figure 1

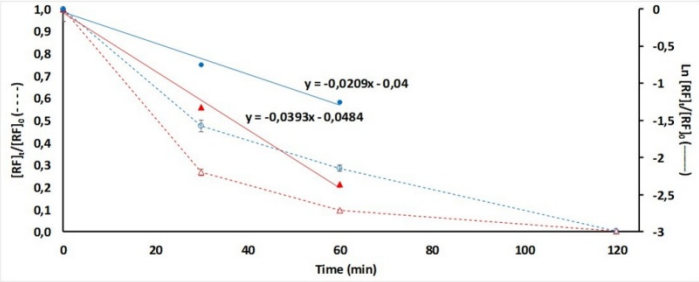
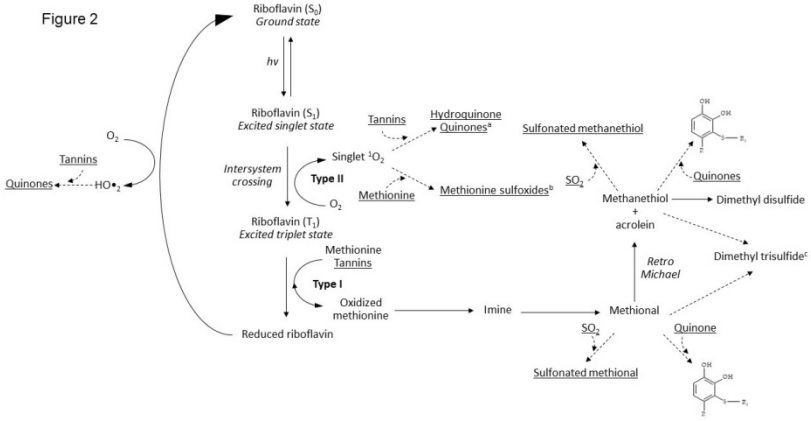


Figure 2



Highlights

- Photo-oxidation of riboflavin and methionine was investigated in model wine
- Hydrolysable tannins and sulfur dioxide were studied to limit the photo-oxidation
- Methionine degradation increased as its concentration increased
- Higher volatile sulfur compounds were found in anoxic condition
- Hydrolysable tannins limited methionine degradation and light struck-taste