



**ORIGINAL RESEARCH ARTICLE**

# UV-C-induced changes in a white wine: Evaluating the protective power of hydrolysable tannins and SO<sub>2</sub>

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## ABSTRACT

UV-C treatment is a non-thermal technology that could be used for the microbiological stabilisation of must and wine. However, light - in this case UV-C light - has an impact on wine. UV-C is a high-energy light source and can promote photo-induced oxidation reactions. The formation of reactive oxygen species during the UV-C treatment of wine can be prevented via supplementation with antioxidants. These have the ability to neutralise reactive oxygen species or directly interact with the products of photo-induced oxidation reactions. This study investigates the impact of different UV-C doses on the chemical and sensory characteristics of Chardonnay wine and examines the protective effect of two antioxidants: sulphur dioxide (SO<sub>2</sub>) and hydrolysable tannins. A sensory evaluation revealed that UV-C doses higher than 1 kJ/l increased the *colour intensity* and the *oxidised* and *burnt* odour attributes of the wine. The *peach* odour attribute decreased with UV-C treatment. On a chemical level, increasing UV-C doses promoted the formation of compounds such as acetaldehyde and 2-aminoacetophenone (2-AAP), while causing the degradation of C13-norisoprenoids, higher alcohols, monoterpenes and esters. Contrary to expectations, SO<sub>2</sub> did not act as an antioxidant when wine was treated with UV-C. It intensified the *burnt* odour attribute and did not mitigate the intensity of the *oxidised* odour attribute, which can be explained by the formation of acetaldehyde. However, hydrolysable tannins acted as antioxidants when combined with UV-C treatment, effectively mitigating the formation of 2-AAP and acetaldehyde, while preventing the wine from becoming *oxidised*.

**KEYWORDS:** UV-C, white wine, 2-aminoacetophenone, SO<sub>2</sub>, hydrolysable tannins, tryptophan, acetaldehyde

## INTRODUCTION

Both traditional and modern vinification methods exist for protecting wine from harmful organisms. One of the methods, which is discussed for grape must and wine microbiological stability, involves the use of UV-C. UV-C technology has generally been described as an environmentally friendly, easy to install, safe and highly scalable method for food preservation (Atılgan *et al.*, 2021). It is also compatible with other technologies in food processing. Scientific research has been carried out in various areas, such as water treatment (Bukhari *et al.*, 1999), food and beverage treatment, as well as wine treatment (Diesler *et al.*, 2019; Falguera *et al.*, 2011; Fredericks *et al.*, 2011; Ünlütürk *et al.*, 2008). UV-C treatment is based on the absorption of UV-C light by the DNA of microorganisms in the range of 250 to 260 nm. UV-C alters the DNA of microorganisms: it cross-links two adjacent thymine bases, which leads to the formation of thymine dimers; this, in turn, disrupts the replication process of the microorganisms. In addition, UV-C can lead to the formation of cyclobutane-pyrimidine-dimers, and as a consequence, to cell death. (Rastogi *et al.*, 2010; Ravanat *et al.*, 2001). The UV-C treatment of fluid products, such as fruit juice (Food and Drug Administration, 2012), apple juice, cider (Health Canada, 2004) and milk (EU Regulation 2017/2470, 2017) has been approved by the FDA, Health Canada and the European Union.

The first law of photochemistry states that photochemical reactions occur when chemical substances absorb light. Sunlight can promote a number of radicals and initiate photochemical reactions in wine; for instance, the photo-oxidation of phenols and anthocyanins, followed by the polymerisation of the oxidised products, which in turn leads to non-enzymatic browning (Li *et al.*, 2008). Several studies examining the effect of UV-C light on different foods, such as apple juice and grape juice, have found that UV-C treatment can lead to significant changes in colour. Falguera *et al.* (2011) and Falguera *et al.* (2012) have reported the photodegradation of pigments in apple and grape juice under UV light and associated colour brightening. Other studies have reported browning in apple and grape juice due to the photo-oxidation of phenolic compounds following UV-C light exposure (Müller *et al.*, 2014; Pala and Toklucu, 2012). Golombek (2019) detected yellow xanthylum cation pigments in model wine after UV-C treatment. In addition, Dias *et al.* (2013) and Maury *et al.* (2010) have reported the development of xanthylum cation pigments in white wine under light exposure. Xanthylum cation pigments occur in the presence of catechin or epicatechin through the degradation of tartaric acid under oxidative conditions. Their formation is promoted by light in the presence of iron (Clark and Scollary, 2003; Clark *et al.*, 2011). Depending on the composition of the wine, UV-C light will either brighten or intensify the wine colour.

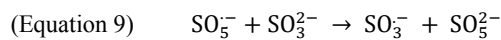
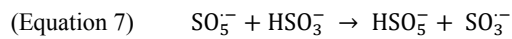
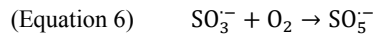
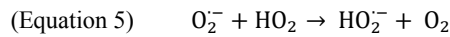
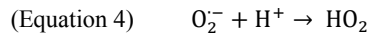
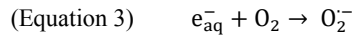
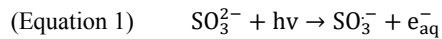
Light can also cause the formation of off-flavours, such as 2-aminoacetophenone (2-AAP), methional and dimethyl disulfide, through riboflavin-sensitized photo-

oxidation (Fracassetti *et al.*, 2019; Golombek *et al.*, 2021; Horlacher and Schwack, 2014; Maujean and Seguin, 1983) and the photo-Fenton reaction (Grant-Preece *et al.*, 2017). The latter can result in the production of acetaldehyde, which is a compound responsible for the oxidation off-flavour in wine and can result in a higher SO<sub>2</sub> demand (Waterhouse *et al.*, 2016). Furthermore, it has been reported that light can degrade aliphatic esters or alcohols in wine, which contribute to wine typicity (D'Auria *et al.*, 2009; Kim *et al.*, 2021), via riboflavin-sensitized degradation (Cellamare *et al.*, 2009), and that it can also degrade monoterpenes and C13-norisoprenoids (Carlin *et al.*, 2022; Golombek *et al.*, 2021).

UV-C is shortwave and high-energy light that can initiate various photochemical reactions, ranging from isomerisation to photo-induced oxidation. Golombek *et al.* (2021) have reported higher concentrations of 2-AAP in white wine produced from must treated with an overdose of UV-C light. Riboflavin, a yellow, fluorescing compound that acts as a photosensitizer, plays an important role in many light-induced reactions. Most riboflavin in wine results from alcoholic fermentation due to the metabolic activity of *Saccharomyces cerevisiae* (Fracassetti *et al.*, 2021a). The average concentration of riboflavin in wine is 150 µg/l (Mattivi *et al.*, 2000). Several reviews have been published on the riboflavin-sensitized degradation mechanisms of riboflavin: the excited singlet and triplet states of riboflavin are involved in the reaction, and a distinction is made between type I and type II pathways (Choe *et al.*, 2005).

Bradshaw *et al.* (2011) and Fracassetti *et al.* (2021b) have found that the effects of light on wine can be mitigated by a variety of antioxidants, such as glutathione, ascorbic acid, tannins and SO<sub>2</sub>, which serve the purpose of scavenging free radicals and hydrogen peroxide to prevent photochemical oxidation reactions. The most widely used antioxidant in the wine industry is SO<sub>2</sub>. As well as scavenging hydrogen peroxide, it reacts with molecules such as acetaldehyde or methional (Danilewicz, 2016; Fracassetti *et al.*, 2019). However, SO<sub>3</sub><sup>2-</sup> - a dissociated form of SO<sub>2</sub> in wine - absorbs UV-C light at 254 nm with a molar absorption coefficient of 25.5/M/cm at pH 2.5 and is transformed into hydrated electrons (*e*<sub>aq</sub><sup>-</sup>) and sulfite radicals (SO<sub>3</sub><sup>-</sup>) (Equation 1 and Equation 2), which cause oxidation-reduction coupling processes to occur (Cao *et al.*, 2021, Vellanki and Batchelor, 2013). As a strong reducing agent with a redox potential of 2.9 V (Buxton *et al.*, 1988), *e*<sub>aq</sub><sup>-</sup> is highly reactive towards unsaturated bonds, as well as O<sub>2</sub> (Buxton *et al.*, 1988; Cao *et al.*, 2021). This can lead to the formation of further radicals like the superoxide (O<sub>2</sub><sup>-</sup>) and the hydroperoxyl radical (HO<sub>2</sub><sup>-</sup>) (Equations 3, 4 and 5). SO<sub>3</sub><sup>-</sup> is an oxidant that can promote a range of reactions, leading to, for example, the formation of the peroxymonosulfate radical (SO<sub>5</sub><sup>-</sup>) and the sulfate radical (SO<sub>4</sub><sup>-</sup>) (Equations 6, 7, 8 and 9) (Cao *et al.*, 2021). SO<sub>4</sub><sup>-</sup> has a high redox potential between 2.5 V and 3.1 V (Neta *et al.*, 1988), and is a strong oxidising radical that can oxidise a high variety of organic compounds (Botlaguduru *et al.*, 2015; Cao *et al.*, 2021).

In water treatment, this process is called Advanced Reduction Process (ARP).



Other antioxidants recommended by the International Organization of Vine and Wine (OIV) for stabilising wine are oenological tannins (Resolution OIV-OENO 624-2022). A study by Fracassetti *et al.* (2021b) has demonstrated the efficacy of using hydrolysable tannins, a specific type of oenological tannin, for preventing the light-struck taste in wine from occurring. Tannins have a high antioxidant potential due to their ability to donate hydrogen atoms or electrons, as well as their ability to chelate metal ions (Magalhães *et al.*, 2014; Vignault *et al.*, 2018). As an important constituent of hydrolysable tannins, gallic acid is a radical scavenger and can react with a wide variety of ROS, such as singlet oxygen, hydroxyl radicals and sulphate radicals in different model solutions (Aruoma *et al.*, 1993; Marino *et al.*, 2014). The mechanisms associated with the antioxidant activity of tannins are based on several processes, including hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SETPT), sequential proton loss electron transfer (SPLET), transition metals chelation (TMC) and radical adduct formation (RAF) mechanisms (Molski, 2023).

In this study, the impact of increasing UV-C doses on chemical and sensory changes in white wine was investigated, focusing on sensory attributes, identified by a trained panel, as well as the formation and degradation of 2-AAP, tryptophan, acetaldehyde, aliphatic esters, higher alcohols, C13-norisporenoids and terpenes. The selected UV-C doses were chosen according to Hirt *et al.* (2022), with 1.0 kJ/l as the microbial relevant UV-C dose for white wine and 2.0 and 3.0 kJ/l as UV-C overdose treatments, in order to determine the relationship between the UV-C dose and the induced photo-chemical changes. The effectiveness of using the antioxidants  $\text{SO}_2$  and hydrolysable tannins for preventing the adverse effects of increasing UV-C doses was investigated. The hypothetical mitigating effect of these antioxidants was analysed, assessed and discussed in order to determine the role of these antioxidants in the reaction mechanisms forming and degrading odour-active substances in wine.

## MATERIALS AND METHODS

### 1. Grape must and winemaking

*Vitis vinifera L. cv.* Chardonnay grapes were harvested from the experimental vineyards of the Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz (Neustadt an der Weinstraße, Germany) during the 2020 vintage. After destemming, the grapes were crushed and transferred to a 1000l stainless-steel fermenter. The must was inoculated with *Saccharomyces cerevisiae* (Lalvin CY 3079 YSEO, Lallemand, Montreal, Canada) and fermented at 18 °C until < 1 g/l of residual sugar was reached. After alcoholic fermentation, the wine was inoculated with *Oenococcus oeni* (Lalvin VP41, Lallemand, Montreal, Canada). After malolactic fermentation, the wine was stabilised with 20 mg/l free  $\text{SO}_2$  (Bisulfite 15, Laffort, Bordeaux, France). The wine was stored in the dark at 18 °C for 2 months before the start of the experiment. The wine had an absorption of 8.7 AU at 254 nm. For purpose of the experiments, the wine was separated into three batches and supplemented with antioxidants as follows: control (20 mg/l free  $\text{SO}_2$ , 0 g/hl hydrolysable tannin),  $\text{SO}_2$  (55 mg/l  $\text{SO}_2$ , 0 g/hl hydrolysable tannin) and hydrolysable tannins (20 mg/l  $\text{SO}_2$ , 20 g/hl hydrolysable tannins). The chosen hydrolysable tannins belong to the class of gallotannins and they are used to prevent light struck according to the manufacturer (EnartisTan Blanc, Enartis, Trecate, Italy).

### 2. UV-C treatment of wine

A series of five connected annular thin-film reactors developed by the Max RubnerInstitut (Karlsruhe, Germany), each equipped with a 20W low-pressure mercury lamp and fluid-guiding elements, was used for the UV-C experiments (Hirt *et al.*, 2022). The reactors were equipped and connected to each other with Tygon® standard thermoplastic soft polyvinyl chloride (PVC) tubes (wall thickness 2.5 mm, Heidolph, Schwabach, Germany). A peristaltic pump (Pumpdrive 5206, Heidolph, Schwabach, Germany) with a flow rate of 100 l/h was used to treat the batches. The volume of every batch was 3 l. The batches were exposed to three different UV-C doses: 1.0 kJ/l, 2.0 kJ/l and 3.0 kJ/l, as well as 0.0 kJ/l, with the lamps turned off. The duration times of the treatment were 1.5 min to achieve a UV-C dose of 1.0 kJ/l, 3.4 min to achieve a UV-C dose of 2.0 kJ/l and 5.3 min to achieve a UV-C dose of 3.0 kJ/l. Fast processing lasting a few minutes and the additional argon sparging of pipes and reception containers prevented oxygen from entering the system (the dissolved oxygen was always between 1.7 and 1.9 mg/l). Oxygen concentration was determined using oxygensensitive spots (PSt3; PreSens Precision Sensing GmbH, Regensburg, Germany). A Julabo F30VC/3 cooling system (Julabo Labortechnik, Seelbach, Germany) was included to reduce the effects of the heat from the UV-C lamps. The maximum temperature fluctuation during the experiments was  $\pm 1.0$  °C. The UV-C doses were controlled by chemical actinometry using an iodide/iodate solution according to the method of Rahn (1997). All UV-C treatments were conducted in triplicate. After the UV-C

experiment, 0.75-l Alsace-style wine bottles were filled with all treatments and sealed with screw caps. Chemical and sensory evaluations took place one week after bottling.

### 3. Chemical analysis

Alcohol, pH and residual sugar were measured using Fourier-transform infrared spectroscopy (FT-IR) (WineScan™ FT120 Basic, FOSS GmbH, Hamburg, Germany). Calibration was performed using the method provided by the manufacturer. Total phenols were determined using the Folin-Ciocalteu assay, which was carried out as described by Singleton and Rossi (1965) in Method OIV-MA-AS-210: R2009. The antioxidant capacity of the wines was determined as described by van den Berg *et al.* (2000) in Trolox equivalents (Trolox equivalent antioxidant capacity; TEAC). Acetaldehyde was quantified using a commercially available enzymatic assay (Thermo Fischer Scientific, Waltham, USA). The Folin-Ciocalteu assay, the antioxidant capacity measurement and the enzymatic determination of acetaldehyde were carried out using an automated Konelab 20i (Thermo Fisher Scientific, Waltham, USA). Free SO<sub>2</sub> was determined by the Ripper method (Ripper, 1892) using Titrator T50 (MettlerToledo, Columbus, USA). Tryptophan was quantified by high-performance liquid chromatography (HPLC) according to the method described by Golombek *et al.* (2021).

The HPLC was composed of four main modules: autosampler (AS4150, Jasco, Gross-Umstadt, Germany), pump (PU-4180, Jasco, Gross-Umstadt, Germany), column oven (CO-4060, Jasco, Gross-Umstadt, Germany) and fluorescence detection (FP-4025, Jasco, Gross-Umstadt, Germany). The aroma compounds were quantified by headspace solid-phase microextraction with gas chromatography coupled with quadrupole mass spectrometry (HS-SPME-GC-qMS) based on the published method by Golombek *et al.* (2021). The HS-SPME-GC-qMS was composed of three main modules: an autosampler (Combi PAL, CTC Analytics, Zwingen, Switzerland), gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, Waltham, USA), and a quadrupole mass spectrometer (Trace DSQ, Thermo Fisher Scientific, Waltham, USA). The quantitative determination of 2-AAP was carried out following the published method by Schmarr *et al.* (2016), using a headspace solid-phase microextraction gas chromatography coupled with a triple quadrupole mass spectrometer (HS-SPME-GC-MS/MS). The HS-SPME-GC-MS/MS was composed of three main modules: autosampler (TriPlus RSH, Thermo Fisher Scientific, Waltham, USA), gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, Waltham, USA), mass spectrometer (MS TSQ Quantum XLS Ultra, Thermo Fisher Scientific, Waltham, USA). All analytical measurements were carried out in duplicate.

**Table 1.** Reference standards for aroma and taste attributes.

Attribute	Reference standards
Aromas	
citrus	1 ml lemon juice, 2 ml grapefruit juice (Granini)
pear	30 ml pear juice (Alnatura)
peach	5 ml peach juice (Granini), a quarter of medium fresh peach
pineapple	2 pineapple-flavoured gummy bears (Tropifrutti, Haribo), 2 ml tinned pineapple juice (Del Monte)
nutty	4 medium-sized mortars walnuts, 7 medium-sized mortars hazelnut
honey	1 g fruit-blossom honey (Langnese), 0.5 ml vanilla syrup (Monin)
acacia blossom	20 µl 2aminoacetophenone (0.2 ml/10 ml)
oxidised	14 ml Sherry (Emilio Lustau San Emilio PX Pedro Ximenez), 1 ml acetaldehyde
burnt	60 µl benzyl mercaptan, one burnt rubber ring (Vivess)
Taste	
sweet	600 mg fructose
sour	200 mg tartaric acid
mouthfeel	verbal description: soft mouthfeel: harmonious, balanced; hard mouthfeel: inharmonious, early dominance of bitterness, acidity and/or rough, unripe, hard tannins
astringency	80 mg tannic acid
bitter	80 mg caffeine
body	verbal description: thin body: watery; dense body: creamy, full

All compounds were added to 100 mL base wine (2020 Riesling, Dienstleistungszentrum Ländlicher Raum).

## 4. Sensory evaluation

The sensory evaluation comprised a descriptive analysis (DA) as described by Lawless and Heymann (2010). It was carried out by an expert sensory panel of 16 panellists (nine females and seven males) with an age range of 24 to 64 years old. Before the training and evaluation sessions, the three experimental replicates of each batch were pre-evaluated via triangle testing to determine if there were any sensory differences among the experimental replicates. The triangle tests were carried out on each experimental batch separately as described by ISO 4120:2021. The statistical evaluation revealed that none of the nine batches showed significant differences in the experimental replicates. The panellists attended three training sessions prior to the evaluation sessions. During the initial training session, the participants performed a check-all-that-apply (CATA) test with pooled replicates of all nine batches. Nine aroma attributes were identified based on the CATA results (Table 1), which were discussed by the panellists and used later for the DA. In the second training session, the participants were trained to recognise the nine aroma attributes. The third training session focused on the intensity evaluation of the unstructured line scales of the nine aroma attributes presented in different dilutions with the base wine. In addition to the nine aroma attributes, the participants were trained to recognise and evaluate the intensity of four taste attributes (sweet, sour, bitter and astringency) in different concentrations, as well as to evaluate the colour. A list of all the attributes, including the reference standards recipes, is provided in Table 1.

The sensory evaluation, which included two sensory repetitions, was carried out using a fully randomised design in three sessions held on different days. Each session comprised six wine samples presented in random order to each judge. The wine samples and the nine aroma reference standards were presented in clear DIN 10960 wine glasses (Schott Zwiesel, Germany), each covered with a plastic lid. The volume of each wine sample was 30 ml. The wine temperature during the wine tasting was 16 °C. All samples were labelled with a three-digit code. During the training and evaluation sessions, the taste and aroma attributes were rated based on unstructured line scales (valued from 0.0 to 10.0) anchored with the terms “not perceptible” (0.0) and “very intense” (10.0), except for the attributes *colour intensity*, *mouthfeel* and *body*. The attributes were evaluated based on the following ranges: “light yellow” (0.0) to “golden” (10.0) for *colour intensity*, “soft” (0.0) to “hard” (10.0) for *mouthfeel*, and “thin” (0.0) to “dense” (10.0) for *body*. The reference standards were prepared in neutral white base wine (2020 Riesling, Dienstleistungszentrum Ländlicher Raum). The training and formal evaluation were conducted in an air-conditioned sensory laboratory equipped with individual white booths and maintained at a temperature of 18 °C. Data acquisition and evaluation were performed using FIZZ (Version 2.51 c 02, Biosystemes, Couternon, France).

## 5. Data analysis

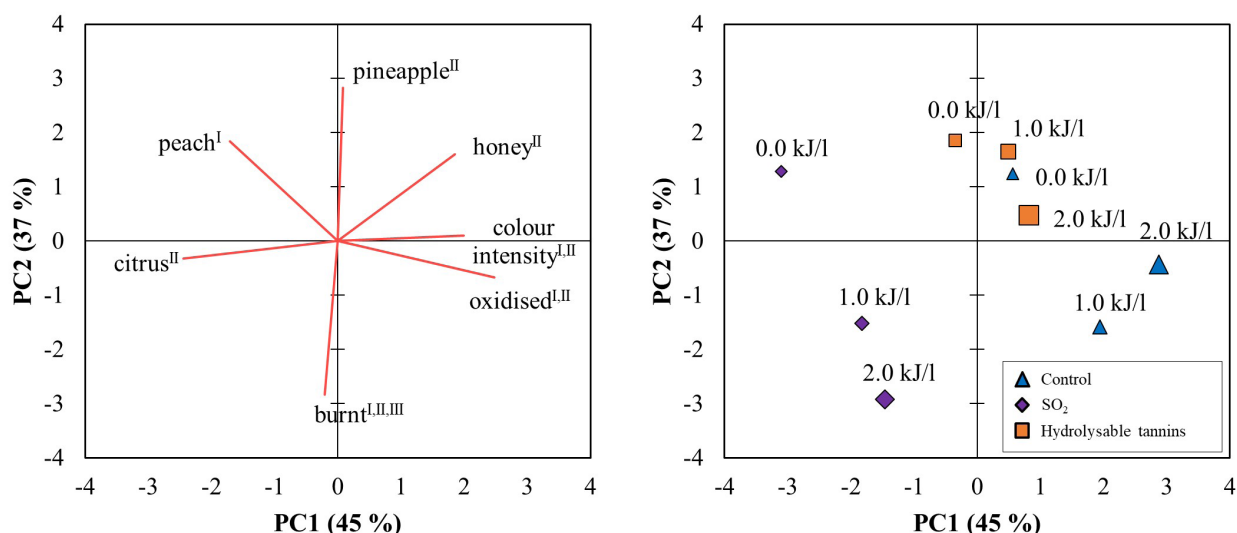
The statistical analysis was performed using XLSTAT (Version 2021.2.2.1147 (32-bit), Addinsoft SARL, Paris, France). The normality of discrete data was tested using the Shapiro-Wilk method ( $p \leq 0.05$ ). The triangle test data were examined for significant changes across replicates using the ClopperPearson method and the Thurstonian model ( $p \leq 0.05$ ). Chemical and DA data were analysed for significant differences between treatments using analysis of variance (ANOVA,  $p \leq 0.05$ ). For DA data, a three-way mixed model ANOVA was applied: panellists were treated as a random effect, while replication and wine were treated as fixed effects. The UV-C effect and the effect of antioxidant supplementation were considered separately, and their interaction was computed using two-way ANOVA. Fisher’s least significant difference (LSD) post-hoc test ( $p \leq 0.05$ ) was used for all data. Principal component analysis (PCA) was performed on the sensory data using Pearson correlation (n-1).

# RESULTS AND DISCUSSION

## 1. Sensory analysis results

UV-C light was applied in increasing doses to Chardonnay wine to which different concentrations of SO<sub>2</sub> and hydrolysable tannins had been added. The sensory attributes (loadings) that were significantly influenced by UV-C treatment and/or supplementation with antioxidants to the Chardonnay wine (scores) are shown in the PCA (Figure 1). PC1 and PC2 explain 82 % of the total variance. An increasing UV-C dose caused a decrease in the *peach* odour attribute, an increase in *oxidised* and *burnt* odour attributes, and a change in *colour intensity* to golden yellow. The formation of xanthylum cation pigments (Maury *et al.*, 2010; Golombek, 2019) and/or the formation of riboflavin-degradation products (Dias *et al.*, 2013) during the UV-C treatment might explain the colour intensification. The observed changes in wine odour due to the UV-C treatment are in agreement with earlier research by Golombek *et al.* (2021), who showed that an overdose of UV-C caused *oxidative* and *burnt* odour in Riesling wine after grape must treatment. It is thought that a number of photo-induced reactions are initiated by UV-C treatment, forming reactive oxygen species (ROS). Such ROS-forming reactions may eventually result in the formation of new aroma active compounds, such as methional 2-AAP, and acetaldehyde, from different precursors, such as methionine, tryptophan and ethanol (Fracassetti *et al.*, 2019; Golombek *et al.*, 2021; Kim *et al.*, 2021; Maujean and Seguin, 1983).

The supplementation with antioxidants caused changes in the wine irrespective of UV-C treatment (Figure 1). An increase in SO<sub>2</sub> concentration caused an increase in the *citrus* and *burnt* attributes and a decrease in the *colour intensity*, *oxidised* and *honey* attributes. SO<sub>2</sub> is known to have an antioxidant effect on wine; it reacts with aldehydes and gives the wine more freshness (Waterhouse *et al.*, 2016). Supplementation with hydrolysable tannins caused an increase in the *pineapple* attribute. It is likely that hydrolysable tannins



**FIGURE 1.** Principal component analysis of descriptive sensory data of Chardonnay wine treated with different UV-C doses and supplemented with antioxidants: control (20 mg/l free SO<sub>2</sub>, 0 g/hl hydrolysable tannins), SO<sub>2</sub> (55 mg/l SO<sub>2</sub>, 0 g/hl hydrolysable tannins), hydrolysable tannins (20 mg/l SO<sub>2</sub>, 20 g/hl hydrolysable tannins). The data were provided by a trained sensory panel (n=16×2) and processed by a two-way ANOVA to obtain significantly changed attributes. I = significantly different attributes due to UV-C treatment, II = significantly different attributes due to antioxidant supplementation, and III = significantly different attributes due to UV-C treatment\*antioxidant supplementation interaction.

act as nucleophilic substances that react with electrophilic substances in wine (such as β-damascenone in the present study, which decreased with hydrolysable tannin addition (Figure 4)), thus affecting the wine matrix and altering the perception of wine aroma.

The data in Table 2 give a detailed assessment of UV-C-induced changes to the *colour intensity*, *peach*, *oxidised* and *burnt* attributes in Chardonnay wine with or without supplemented antioxidants. Regarding the *colour intensity* and *peach* attributes, the interaction of the factors UV-C treatment\*antioxidant supplementation resulted in *p*-values of 0.956 and 0.925 respectively. A higher SO<sub>2</sub> content in the wine and the addition of hydrolysable tannins to the wine neither enhanced nor mitigated the UV-C effect in relation to the control wine. As regards the *oxidised* odour attribute, the interaction of the factors UV-C treatment\*antioxidant supplementation resulted in a *p*-value of 0.411. A higher SO<sub>2</sub> content in the wine decreased the perception of the *oxidised* odour attribute in the untreated wine; however, the UV-C effect on this odour attribute remained similar to the control wine. The UV-C effect on the wine with a higher SO<sub>2</sub> content was not found to be mitigated. In contrast, the addition of hydrolysable tannins did mitigate the formation of the *oxidised* odour attribute with UV-C treatment. A very low *p*-value of 0.001 was obtained for the interaction of the factors UV-C treatment\*antioxidant supplementation, affecting the *burnt* odour attribute. A higher SO<sub>2</sub> content in the wine enhanced the formation of the *burnt* odour attribute with UV-C treatment, while the addition of hydrolysable tannins mitigated it.

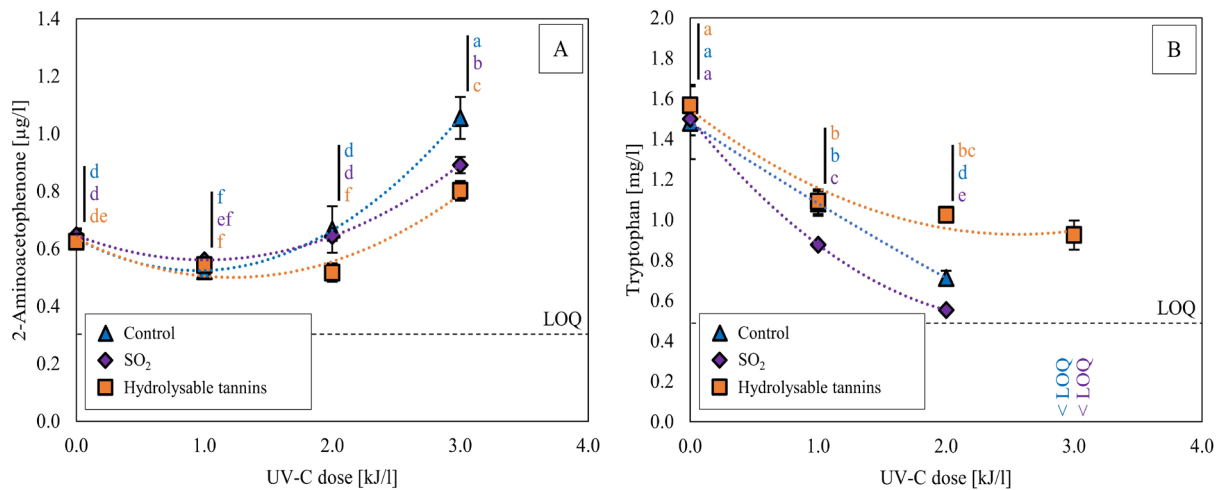
A possible explanation for the adverse effect of SO<sub>2</sub> during the UV-C treatment is the ability of SO<sub>2</sub> to absorb UV-C light at 254 nm and produce a series of SO<sub>3</sub><sup>2-</sup> reactive oxidative species (Cao *et al.*, 2021). These findings suggest that UV-C causes SO<sub>2</sub> to lose its ability to act as an antioxidant. These results are in accordance with those of Fracassetti *et al.* (2020), who found SO<sub>2</sub> to have a pro-oxidative effect on methionine degradation during light exposure. UV-C might also be responsible for SO<sub>2</sub> causing the formation of substances associated with the *burnt* aroma attribute. Possible odour-active substances that can be formed in the process are thiophenols (Tomasino *et al.*, 2023). The mitigation of the UV-C effect in wine was achieved by the addition of hydrolysable tannins. Hydrolysable tannins may have prevented the increase in the *oxidised* odour attribute after UV-C treatment through proton transfer and the neutralisation of ROS.

## 2. 2-Aminoacetophenone formation and tryptophan degradation

Golombek *et al.* (2021) reported that Riesling produced from must treated with an overdose of UV-C light (21 kJ/l) contained more 2-AAP than control wine. On the basis of those results, the sample in the present study was analysed for the concentration of 2-AAP at different UV-C doses (Figure 2). *Acacia blossom* is related to the odour-active substance 2-AAP, which results from the degradation of indol-3-acetic acid, a process commonly referred to as “atypical aging” (Hoenicke *et al.*, 2002). Aside from this pathway, 2-AAP can also be formed through photo-induced oxidation of tryptophan (Horlacher and Schwack, 2014). A significant increase in 2-AAP was observed at a UV-C dose of 3.0 kJ/l

**TABLE 2.** Scores for the colour intensity, peach, oxidised, and burnt sensory attributes in Chardonnay wine treated with different UV-C doses and supplemented with antioxidants: control (20 mg/l free SO<sub>2</sub>, 0 g/hl hydrolysable tannins), SO<sub>2</sub> (55 mg/l SO<sub>2</sub>, 0 g/hl hydrolysable tannins), hydrolysable tannins (20 mg/l SO<sub>2</sub>, 20 g/hl hydrolysable tannins). The data were provided by a trained sensory panel (n=16×2) and processed by two-way ANOVA to obtain significantly changed attributes. Different letters show significant differences for one attribute due to UV-C treatment and/or antioxidant supplementation, according to Fisher's test with p ≤ 0.05.

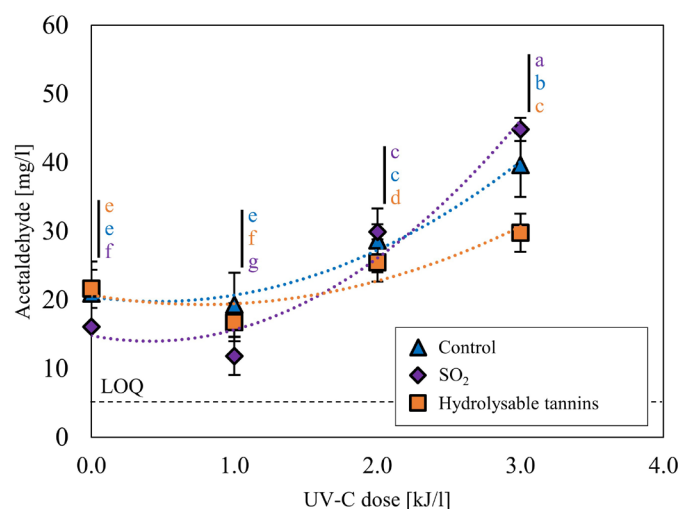
Antioxidant supplementation	UV-C dose [kJ/l]	Colour intensity [0-10]	Peach [0-10]	Oxidised [0-10]	Burnt [0-10]
Control	0.0	3.4 <sup>bc</sup>	3.0 <sup>abc</sup>	4.4 <sup>bc</sup>	1.9 <sup>d</sup>
	1.0	4.2 <sup>ab</sup>	2.4 <sup>bc</sup>	4.9 <sup>ab</sup>	4.0 <sup>ab</sup>
	2.0	4.5 <sup>a</sup>	2.0 <sup>c</sup>	6.0 <sup>a</sup>	2.5 <sup>cd</sup>
SO <sub>2</sub>	0.0	3.0 <sup>c</sup>	3.3 <sup>ab</sup>	2.3 <sup>d</sup>	1.9 <sup>d</sup>
	1.0	3.3 <sup>bc</sup>	2.8 <sup>abc</sup>	3.6 <sup>c</sup>	3.6 <sup>bc</sup>
	2.0	3.8 <sup>abc</sup>	2.8 <sup>abc</sup>	4.0 <sup>bc</sup>	4.9 <sup>a</sup>
Hydrolysable tannins	0.0	4.0 <sup>abc</sup>	3.5 <sup>a</sup>	4.1 <sup>bc</sup>	2.0 <sup>d</sup>
	1.0	4.5 <sup>a</sup>	3.4 <sup>ab</sup>	3.8 <sup>bc</sup>	2.2 <sup>d</sup>
	2.0	4.7 <sup>a</sup>	2.7 <sup>abc</sup>	4.5 <sup>bc</sup>	2.2 <sup>d</sup>
p-value UV-C treatment		0.017	0.048	0.003	0.001
p-value antioxidant supplementation		0.003	0.067	0.001	0.001
p-value UV-C treatment*antioxidant supplementation		0.956	0.925	0.411	0.001



**FIGURE 2.** Effects of UV-C treatment on 2-aminoacetophenone (A) and tryptophan (B) concentrations in Chardonnay wine in its original form (control), with increased SO<sub>2</sub> content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C treatment according to Fisher's test with p ≤ 0.05.

only (Figure 2A). The sensory analysis did not show any significant differences in the *acacia blossom* odour attributes, because during the sensory evaluation the panellists only evaluated wines that had been treated with UV-C at doses of 1.0 kJ/l and 2.0 kJ/l. The presence of hydrolysable tannins in the wine, as well as high SO<sub>2</sub> content, mitigated the formation of 2-AAP during the UV-C treatment (Figure 2A).

This is in agreement with the findings of Fracassetti *et al.* (2021a) and Fracassetti *et al.* (2019): these studies showed that hydrolysable tannins mitigated the formation of methional during the exposure of white wine and model wine to visible light. The formation of methional and 2-AAP in wine can be induced by light. In both cases, riboflavin and oxygen play an important role. Furthermore, a study by



**FIGURE 3.** Effect of UV-C treatment on the concentration of acetaldehyde in Chardonnay wine in its original form (control), with increased SO<sub>2</sub> content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C treatment according to Fisher's test with  $p \leq 0.05$ .

Remucal and McNeill (2011) has shown that methionine, tryptophan, tyrosine and histidine degrade in water through the same riboflavin-mediated photodegradation pathway. The latter results, along with those of the present study and those of Horlacher and Schwack (2014), Fracassetti *et al.* (2019) and Maujean and Seguin (1983), allow us to assume that the formation of both methional and 2-AAP follows the same reaction pathway via riboflavin-sensitised photo-oxidation.

With a low dose of 1.0 kJ/l UV-C the concentration of 2-AAP was found to decrease, indicating that 2-AAP was degraded as a result of the UV-induced Mannich reaction (Shi *et al.*, 2021). The Mannich reaction involves a primary or secondary amine, an enolisable carbonyl compound (donor) and a non-enolisable carbonyl compound (acceptor) in a polar protic solvent (Carey and Sunberg, 2007). In this case, the amino group of 2-AAP may have acted as a primary amine. The Mannich reaction leads to the formation of  $\beta$ -amino carbonyl compounds - the so-called Mannich bases. With an increasing UV-C dose and an increasing concentration of radicals, it is likely that the formation rate of 2-AAP outpaces the Mannich reaction and causes an accumulation of 2-AAP. Further studies are necessary to prove the hypotheses that 2-AAP can be degraded as a result of the Mannich reaction.

A decrease in tryptophan concentration was observed with increasing UV-C dose (Figure 2B). As expected, the hydrolysable tannins protected tryptophan from degradation due to their ability to provide protons formation (Magalhães *et al.*, 2014) when being oxidised, which would explain the mitigation of 2-AAP formation in wine. Interestingly, a faster degradation of tryptophan was observed with higher SO<sub>2</sub> concentrations, despite 2-AAP formation being diminished. It is possible that radicals produced by SO<sub>2</sub> promote the degradation of tryptophan and produce other tryptophan degradation products, such as kynurenic acid,

which rapidly forms dimers under the influence of UV light (Bellmaine *et al.*, 2020).

### 3. Acetaldehyde formation

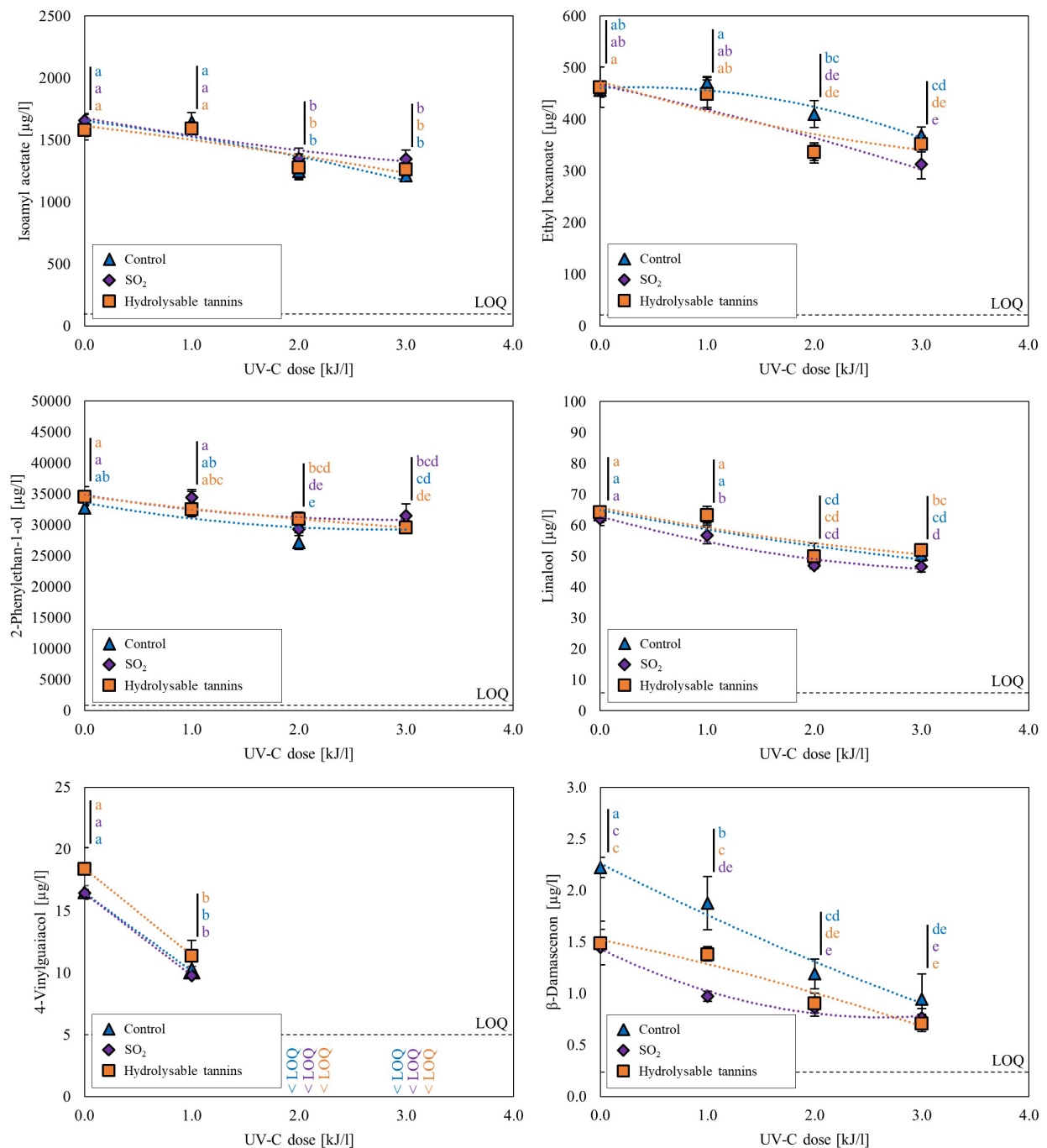
Acetaldehyde can contribute to the *oxidised* odour attribute in wine. To investigate the effect of UV-C treatment in combination with the protective power of hydrolysable tannins and SO<sub>2</sub>, the concentration of acetaldehyde was measured (Figure 3). A UV-C treatment of 2.0 kJ/l and higher caused an increase in the acetaldehyde concentration in the wine, likely due to the oxidation of ethanol to acetaldehyde via the photo-Fenton reaction (Grant-Preece *et al.*, 2017). At a dose of 3.0 kJ/l, the effects of hydrolysable tannins and SO<sub>2</sub> on acetaldehyde formation became significant, while hydrolysable tannins mitigated acetaldehyde formation and increased SO<sub>2</sub> accelerated it. These results are in agreement with the findings from the sensory analysis (Figure 1, Table 2). The increase in acetaldehyde at high SO<sub>2</sub> levels can be explained by sulphur-containing radicals formed by UV-C light (Equation 1- Equation 5), which promote the formation of  $HO_2^{\cdot-}$  and  $O_2^{\cdot-}$  and subsequently that of acetaldehyde. The mitigation of acetaldehyde formation by hydrolysable tannins can be explained by their radical-scavenging properties. The lower initial concentration of acetaldehyde in the wine with increased SO<sub>2</sub> before UV-C treatment is due to the ability of SO<sub>2</sub> to bind acetaldehyde (Waterhouse *et al.*, 2016).

With a low dose of 1.0 kJ/l UV-C, acetaldehyde concentrations were found to decrease. As has been suggested for 2-AAP (Figure 2A), it is likely that the degradation of acetaldehyde occurred as a result of the Mannich reaction.

### 4. Changes in volatile compounds

An increase in UV-C dose resulted in decreasing concentrations of all the classes of investigated volatile compounds, namely





**FIGURE 4.** Effect of UV-C treatment on the concentration of volatile compounds in Chardonnay wine in its original form (control), with increased SO<sub>2</sub> content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C treatment according to Fisher's test with  $p \leq 0.05$ .

C13-norisoprenoids, monoterpenes, higher alcohols and esters (Figure 3). Carlin *et al.* (2022), Cellamare *et al.* (2009) and D'Auria *et al.* (2009) found that visible light can induce the photodegradation of higher alcohols and esters in wine. Cellamare *et al.* (2009) demonstrated that aliphatic esters are degraded by riboflavin-induced photodegradation in an aqueous ethanol solution during UV exposure. It is assumed that a similar effect occurred here in the Chardonnay wine

during UV-C treatment. A study by Carlin *et al.* (2022) also demonstrated that light exposure led to a significant decrease in beta-damascenone, linalool and 4-vinylguaiacol in Chardonnay wines stored in flint bottles and coloured bottles under typical supermarket shelf conditions. Additionally, Golombek *et al.* (2021) demonstrated that UV-C light can induce the photodegradation of beta-damascenone (C13-norisoprenoids) and linalool (monoterpenes) via riboflavin in model wine.

**TABLE 3.** Basic chemical parameters of UVC treated Chardonnay wine in its original form (control), with increased SO<sub>2</sub> content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UVC treatment according to Fisher's test with  $p \leq 0.05$ .

PART 1/2												
UVC dose [kJ/l]	Alcohol [% vol.]				pH				Residual sugar [g/l]			
	Control	SO <sub>2</sub>	Hydrol. tannins	Control	Control	Hydrol. tannins	Control	Control	SO <sub>2</sub>	Hydrol. tannins	Control	Hydrol. tannins
0.0	12.3 ± 0.1 a	12.3 ± 0.2 a	12.2 ± 0.2 a	3.58 ± 0.02 a	5.0 ± 0.1 a	3.60 ± 0.02 a	1.0 ± 0.2 a	1.0 ± 0.2 a	0.8 ± 0.5 a	0.7 ± 0.3 a		
1.0	12.2 ± 0.3 a	12.0 ± 0.3 a	12.3 ± 0.1 a	3.59 ± 0.02 a	5.0 ± 0.1 a	3.60 ± 0.03 a	1.0 ± 0.4 a	1.0 ± 0.4 a	1.0 ± 0.3 a	0.7 ± 0.5 a		
2.0	12.3 ± 0.1 a	12.2 ± 0.1 a	12.3 ± 0.1 a	3.59 ± 0.01 a	5.0 ± 0.1 a	3.58 ± 0.01 a	0.9 ± 0.2 a	0.9 ± 0.2 a	0.8 ± 0.2 a	0.9 ± 0.4 a		
3.0	12.1 ± 0.2 a	12.1 ± 0.2 a	12.3 ± 0.1 a	3.60 ± 0.03 a	4.9 ± 0.1 a	3.59 ± 0.02 a	0.9 ± 0.5 a	0.9 ± 0.5 a	0.8 ± 0.2 a	0.7 ± 0.2 a		
p-value	0.639	0.526	0.843	0.812	0.513	0.697	0.977	0.977	0.864	0.901		

PART 2/2														
UVC dose [kJ/l]	Free SO <sub>2</sub> [mg/l]				Antioxidant capacity [mmol Trolox eq./l]				Gallic acid [mg/l]				Total phenols [mg/l GAE]	
	Control	SO <sub>2</sub>	Hydrol. tannins	Control	Control	SO <sub>2</sub>	Hydrol. tannins	Control	Control	SO <sub>2</sub>	Hydrol. tannins	Control	SO <sub>2</sub>	Hydrol. tannins
0.0	19.7 ± 0.9 a	55.4 ± 0.4 a	19.8 ± 0.7 a	5.0 ± 0.1 a	5.3 ± 0.2 a	10.8 ± 0.3 a	1.7 ± 0.2	2.1 ± 0.3	76.9 ± 0.5 a	235 ± 2 a	268 ± 6 a	329 ± 1 a		
1.0	13.8 ± 1.2 b	48.0 ± 1.1 b	18.4 ± 1.0 a	5.0 ± 0.1 a	5.1 ± 0.1 a	10.3 ± 0.3 b	< LOQ	< LOQ	74.7 ± 1.2 a	229 ± 1 b	245 ± 1 b	329 ± 3 a		
2.0	10.9 ± 0.7 c	44.5 ± 0.2 c	15.4 ± 0.5 b	5.0 ± 0.1 a	5.2 ± 0.1 a	9.9 ± 0.2 b	< LOQ	< LOQ	64.6 ± 0.4 b	231 ± 1 b	245 ± 1 b	323 ± 1 b		
3.0	8.3 ± 0.3 d	36.8 ± 1.0 d	14.6 ± 0.8 b	4.9 ± 0.1 a	5.1 ± 0.1 a	9.6 ± 0.3 b	< LOQ	< LOQ	60.1 ± 0.7 c	227 ± 1 b	240 ± 3 c	318 ± 2 c		
p-value	0.0001	0.0001	0.0001	0.513	0.206	0.015	-	-	0.001	0.002	0.001	0.033		

The addition of antioxidants did not prevent a UV-C-induced loss of volatile compounds. The addition of hydrolysable tannins was expected to reduce the riboflavin-sensitised photo-oxidation type I mechanism, in which hydrogen can be abstracted from hydrolysable tannins and not from volatile compounds. However, the data suggest that the degradation of volatile substances occurred via riboflavin-sensitised photo-oxidation type II mechanism with molecular oxygen (Choe *et al.*, 2005). The addition of SO<sub>2</sub> and hydrolysable tannins decreased the concentration of β-damascenone regardless of the UV-C treatment; this may be due to reactions between β-damascenone and nucleophilic wine components (Daniel *et al.*, 2004).

## 5. Basic wine parameters

No significant changes in alcohol, pH and residual sugar were observed with UV-C treatment (Table 3). Furthermore, antioxidant supplementation did not influence these parameters. Free SO<sub>2</sub>, gallic acid, total phenols and antioxidant capacity decreased with UV-C treatment. Free SO<sub>2</sub> decreased with UV-C treatment in the wines with high SO<sub>2</sub> content (-18.6 mg/l at 3.0 kJ/l UV-C dose), followed by the control wines (-11.4 mg/l at 3.0 kJ/l UV-C dose), then the wines with hydrolysable tannins (-4.9 mg/l at 3.0 kJ/l UV-C dose), indicating that SO<sub>2</sub> is light sensitive. The SO<sub>2</sub> decrease suggests that radical formation and radicals can promote riboflavin-sensitised photo-oxidation (Fracassetti *et al.*, 2020). It is also possible that the radicals react with nucleophilic compounds in wine, and that hydrolysable tannins decelerate SO<sub>2</sub> loss, meaning that they may have antioxidant properties. Hydrolysable tannins are known to be capable of scavenging radicals produced during light exposure (Vignault *et al.*, 2018).

The antioxidant capacity of the wine doubled after supplementation with hydrolysable tannins (from 5 to over 10 mmol Trolox eq./l). Gallic acid and total phenols increased after supplementation with hydrolysable tannins. The increase in total phenols caused by the addition of SO<sub>2</sub> is regarded as a false-positive overestimation of total phenols, as described by Abramovič *et al.* (2015). UV-C treatment with increasing doses did not cause any decrease in the antioxidant capacity of the wine without hydrolysable tannins. Meanwhile, it caused minor decreases in concentration of gallic acid and total phenols in the wine without hydrolysable tannins, but major decreases in the wine with hydrolysable tannins. These observations are in agreement with the findings of Pala and Toklucu (2012), who investigated the effect of UV-C on white and red grape juice and did not detect any changes in antioxidant capacity and total phenols. The antioxidant capacity of wine with hydrolysable tannins decreased significantly during UV-C treatment, which is related to the properties of hydrolysable tannins: they efficiently react with radicals in wine - in this case light-induced radicals - and the products may have different antioxidant capacity (Arts *et al.*, 2004; Magalhães *et al.*, 2014). The UV-C induced decrease in antioxidant capacity suggests that hydrolysable tannins prevent light struck, which is in agreement with the findings of Fracassetti *et al.* (2021a).

## CONCLUSION

Due to its low antioxidant capacity, white wine exhibits sensitivity to UV-C. Doses higher than 1.0 kJ/l induce changes in the sensory and chemical properties of wine. Here, overdose UV-C treatment caused a decrease in the *peach* odour attribute, and an increase in *oxidised* and *burnt* aroma as well as in *colour intensity*. The supplementation of the wine with SO<sub>2</sub> did not mitigate the UV-C effects. Instead, sulphite seems to have absorbed the UV-C light and produced a series of sulphur-containing radicals that are thought to be responsible for the *burnt* aroma. The supplementation of the wine with hydrolysable tannins mitigated the sensory impact of UV-C.

The UV-C treatment led to an increase in the concentration of 2-AAP and acetaldehyde. The supplementation with SO<sub>2</sub> mitigated the formation of 2-AAP but accelerated the degradation of tryptophan, suggesting that SO<sub>2</sub> has a major impact on reaction pathways leading to unknown reaction products. In contrast to 2-AAP, acetaldehyde formation was accelerated by SO<sub>2</sub> supplementation, thus indicating that it promoted the photo-Fenton reaction. Overall, in combination with UV-C, SO<sub>2</sub> loses its antioxidant properties and becomes a supplement with pro-oxidative characteristics. On the other hand, hydrolysable tannins mitigated both the formation of 2-AAP and the degradation of tryptophan, indicating that hydrolysable tannins act as powerful proton donors when being oxidised. Furthermore, hydrolysable tannins demonstrated their antioxidant properties by mitigation the UV-C induced formation of acetaldehyde.

Increasing UV-C doses caused the degradation of C13-norisoprenoids, monoterpenes, higher alcohols and esters. Supplementation with SO<sub>2</sub> and hydrolysable tannins did not mitigate their degradation. This suggests that the degradation of volatile compounds follows the riboflavin-sensitised photo-oxidation type II reaction pathway.

Further research is needed to investigate the protective power of other antioxidants, such as ascorbic acid, glutathione, resveratrol and condensed tannins, in the context of the UV-C treatment of wine. Further studies are necessary to determine which substances formed during UV-C treatment are responsible for the *burnt* odour of wine. It is necessary to identify the unknown degradation products of tryptophan and to verify the reaction pathway of riboflavin-sensitised photo-oxidation of volatile compounds.

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