Influence of UV treatment to inactivate microorganisms on the molecular-chemical and sensory properties of wine

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Publications

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Cvetkova, S., Hirt, B., Stahl, M., Scharfenberger-Schmeer, M., & Durner, D. (2023). UV-C treatment: A non-thermal inactivation method for microbiological stabilisation of must and wine. *BIO Web of Conferences*, *56*, 02035. https://doi.org/10.1051/bioconf/20235602035

Oral Presentations

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Abbreviations used

Abbreviations	Description
2-AAP	2-aminoacetophenone
ANOVA	Analysis of variance
ARP	Advanced reduction process
ATA	Atypical aging note
BER	Base excision repair
CPD	Cyclobutane-pyrimidine dimers
DNA	Deoxyribonucleic acid
E_e	Electron energy level
E_v	Vibrational energy level
E_{photon}	Photon energy level
EFSA	European Food Safety Authority
FDA	U.S. Food and Drug Administration
GC	Gas chromatography
GMP	Good Manufacturing Practice
h	Planck constant
HACCP	Hazard analysis and critical control points
$HO^{\cdot}$	Hydroxyl radical
$HO_2^{\cdot}$	Hydroperoxyl radical
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography
HS	Headspace
HSO_3^-	Hydrogen sulfite
IC	Internal conversion
ISC	Intersysteme crossing
λ	Wavelength
LED	Light-emitting diode
LMBestrV	German food irradiation regulation
LOD	Limit of detection
LOQ	Limit of quantification
M	Multiplicity value
m_s	Spin quantum number
MS	Mass spectrometry
ν	Frequenz
N_A	Avogadro constant
N_0	Initial cell counts
N	Cell counts according to the specified irradiation time
NER	Nucleotide excision repair
$O_2^{\cdot -}$	Superoxide radical anion
$^1O_2^*$	Excited singlet state of oxygen
OIV	International Organization of Vine and Wine
PPs	Pyrimidine pyrimidone photoproducts

Abbreviations	Description
R^2	Coefficient of determination
RF	Riboflavin
$^3RF^*$	Excited triplet state of riboflavin
RMSE	Root mean squared error
RNA	Ribonucleic acid
S	Total spin quantum number
S_0	Singlet ground state
S_n	Excited singlet state
S_1	First excited singlet state
S_2	Second excited singlet state
SO_2	Sulfur dioxide
SO_3^{2-}	Sulfite
SPME	Solid phase microextraction
t	Time
T_n	Excited triplet state
T_1	First excited triplet state
TRP	Tryptophan
TrinkwV	German potable water regulation
UV	Ultraviolet radiation
UV-A	Ultraviolet radiation in the wavelength range between 400 – 315 nm
UV-B	Ultraviolet radiation in the wavelength range between 315 – 280 nm
UV-C	Ultraviolet radiation in the wavelength range between 280 – 200 nm
YPD	Yeast extract peptone dextrose
ΔE_{00}	Color difference

Zusammenfassung

Die UV-C-Technologie ist ein chemikalienfreies, nicht-thermisches Verfahren zur mikrobiellen Stabilisierung von Lebensmitteln und Getränken. Es wird als mögliche Alternative zu weinüblichen Stabilisierungsmethoden auf Basis von Zusatzstoffen diskutiert. Ziel dieser Arbeit war es, die UV-C-Technologie zur mikrobiellen Stabilisierung von Wein zu untersuchen, wobei der Schwerpunkt auf der Untersuchung des Einflusses der UV-C-Behandlung auf die chemischen und sensorischen Eigenschaften von Wein lag. Es könnte gezeigt werden, dass die UV-C-Behandlung die typischen Schadorganismen im Wein effektiv inaktivieren kann. Die Weibull-Funktion wurde als das geeignete Modell zur Beschreibung der Inaktivierungskinetik von Schadorganismen im Wein identifiziert. Daher sollte diese Funktion verwendet werden, um die erforderliche mikrobielle UV-C Dosis zu bestimmen. Faktoren wie die Absorption des Weins, die Zellzahl und die Art der Schadorganismen wurden dabei als signifikante Parameter bestimmt. Steigende UV-C Dosen verursachten mehrere photochemische Reaktionen im Wein, die schließlich zur Bildung von Fehlaromen wie 2-Aminoacetophenon (2-AAP) und Acetaldehyd als Produkte der lichtinduzierten Reaktionen geführt haben. Darüber hinaus führte die Erhöhung der UV-C Dosis zum Abbau von unterschiedlichen aromaaktiven Substanzklassen. Auch phenolische Verbindungen wurden durch die steigende UV-C-Behandlung beeinflusst. Hydrolysierbare Tannine und Schwefeldioxid (SO_2) wurden als mögliche Antioxidantien untersucht, um die photoinduzierten oxidativen Reaktionen zu reduzieren. Entgegen der Erwartung förderte die Zugabe von SO_2 den oxidativen Prozess und verursachte einen Anstieg der 2-AAP und Acetaldehyd Konzentration im Wein. Die Zugabe von hydrolysierbaren Tanninen zeigte eine starke antioxidative Wirkung. Darauf aufbauend sollte eine hohe Konzentration von SO_2 im Wein vor der UV-C-Behandlung vermieden werden. Alternativ können hydrolysierbare Tannine verwendet werden. Weiterhin wurde eine detaillierte Untersuchung des photoinduzierten Reaktionswegs der Bildung von 2-AAP in Kombination mit Sauerstoff und Übergangsmetallen im Modelwein durchgeführt. Die Experimente haben gezeigt, dass eine Erhöhung der Sauerstoffkonzentration die Bildung von 2-AAP fördert und den Abbau des Photosensibilisators Riboflavin (RF) verringert. Zum ersten Mal wurde ein Zusammenhang zwischen der photoinduzierten Bildung von 2-AAP und der Photo-Fenton-Reaktion gefunden. Diese Reaktion steht in direkter Konkurrenz zur 2-AAP-Bildung. Um den langfristigen mikrobiellen Schutz von UV-C zu gewährleisten, wurde die mikrobielle Stabilität von UV-C behandeltem Wein über einen Zeitraum von 12 Wochen untersucht. Eine erneute Zunahme von *Brettanomyces bruxellensis* nach UV-C-Behandlung konnte während der Lagerzeit im Wein nicht festgestellt werden. Die Anwendung der alternativen Wellenlänge von 280 nm anstatt 254 nm wurde untersucht. Die Studie hat eine höhere Inaktivierungseffizienz bei 280 nm gezeigt. Chemische und sensorische Analysen zeigten, dass aromaaktive Verbindungen bei beiden Wellenlängen in gleicher Weise beeinflusst wurden. Allerdings ist die Absorption von Phenolen bei 280 nm stärker ausgeprägt und daher wurden sie bei 280 nm stärker beeinflusst als bei 254 nm. Das hat zu einem höheren Einfluss auf die sensorischen Eigenschaften des Weins bei 280 nm geführt. Dementsprechend sollte die Inaktivierung des Schadorganismen im Wein nicht mit einer UV-Behandlung bei 280 nm erfolgen. Da die Anwendung der UV-C-Technologie bei 254 nm als mögliche alternative Methode erwiesen wurde, sollte in darauf aufbauenden zukünftigen Studien die Technologie im Großmaßstab untersucht werden.

Abstract

UV-C technology is a chemical-free, non-thermal method for microbial stabilization of food and beverages. It is discussed as a possible alternative for wine preservation methods based on additives. The objective of this work was to examine the UV-C technology for microbial stabilization of wine with a focus on investigating the influence of UV-C treatment on the chemical and sensory characteristics of wine. It was evident that UV-C treatment can effectively inactivate the typical harmful microorganisms in wine, such as *Brettanomyces bruxellensis* or *Acetobacter aceti.* The Weibull function was identified as the appropriate model to describe the inactivation kinetics of microorganisms in wine. Therefore, it should be used to determine the microbial required UV-C dose. Factors like wine absorption, cell count, and type of microorganisms could be determined as parameters influencing the effectiveness of UV-C treatment. Increasing UV-C doses caused several photochemical reactions in wine, which eventually could result in the formation of off-flavors such as 2-aminoacetophenone (2-AAP) and acetaldehyde as products of light-induced reactions. Besides that, increasing UV-C doses caused the degradation of aroma-active substance classes such as C13-norisoprenoide, monoterpene, and esters. Phenolic compounds were also affected by UV-C, as shown by decreasing concentrations of hydroxycinnamic acids and flavanoids. Hydrolyzable tannins and sulfur dioxide (SO_2) were examined as possible antioxidants to mitigate the photo-induced oxidative reactions. Counterintuitively, the addition of SO_2 promoted oxidative processes, and caused an increase of 2-AAP and acetaldehyde levels, while hydrolyzable tannins showed strong antioxidative effects, reducing oxidative reactions. To ensure the best quality of wine, high concentrations of SO_2 should be avoided before UV-C treatment. Vice versa, hydrolyzable tannins are preferable to be added before UV-C application. In detail, the photo-induced reaction pathway of the formation of 2-AAP was investigated in combination with oxygen and transition metals. Research has shown that increasing oxygen concentrations promotes the formation of 2-AAP and decreases the degradation of photosensitizer riboflavin (RF). For the first time, a link between the photo-induced formation of 2-AAP and the photo-Fenton reaction was found: the photo-Fenton reaction, which is catalyzed by transition metals, stands in direct competition with 2-AAP formation. To ensure the long-term microbial protection of UV-C, the microbial stability of UV-C treated wine was investigated over a 12-week storage. A re-increase of *Brettanomyces bruxellensis* cells after UV-C treatment could not be observed in wine during the storage period. UV-C application at 280 nm instead of 254 nm was exanimated as an alternative wavelength. The wavelength of 280 nm enables the use of LEDs instead of ecologically harmful mercury-vapor lamps. The research has shown higher inactivation efficiency at 280 nm. Chemical and sensory analysis revealed that aroma-active compounds were similarly affected at both wavelengths. However, the absorbance of phenolics is more pronounced at 280 nm, and therefore, they were more strongly affected at 280 nm than at 254 nm, which in turn led to a higher impact on the sensory properties of the wine at 280 nm. Accordingly, the inactivation of harmful microorganisms in wine should not be conducted with UV treatment at 280 nm. Since the application of UV-C technology at 254 nm can be safely used for wine preservation, the next step of future studies should investigate the technology on an industrial application scale.

1. Introduction

UV-C technology is a promising alternative method for food preservation (Singh et al., 2020). In prior studies, UV-C light in the wavelength range of 200 to 280 nm has been found to have a germicidal effect against harmful microorganisms (fungi, bacteria, and viruses) (Demirci et al., 2020). It is a long-established method successfully applied to ensure microbial stability in various fields, such as water treatment or air and surface disinfection, for example, in operating rooms (Hadžikadić & Avdaković, 2018). Numerous scientific studies have demonstrated the application of UV-C technology using the 254 nm wavelength for microbial stabilization in food and beverage production without significant changes in the authenticity characteristics of the product under optimal conditions (Keyser et al., 2007; Guerrero-Beltran et al., 2008; Tran & Farid, 2004; Caminiti et al., 2010; Ünlütürk et al., 2008; Delorme et al., 2020). In 2000, this method was classified as a safe method for food processing by the U.S. Food and Drug Administration (FDA) and is currently approved by the FDA (21 CFR 179.39), Health Canada (Health Canada, 2004), and the European Union (EU Regulation 2017/2470, 2017) for the preservation of beverages such as fruit juice, cider, and milk. In Germany, the UV-C treatment of potable water (TrinkwV, 2001, § 11) and fruit and vegetable surfaces are permitted (LMBestrV, 2022, § 1).

Despite the natural acids and alcohols that give it a certain natural protective effect, wine is naturally a perishable product. For example, harmful microorganisms such as *Brettanomyces bruxellensis* or *Acetobacter aceti* are often responsible for the spoilage of the wine during vinification and make it undrinkable (Bartowsky, 2009; Loureiro, 2003). There are several methods for microbial stabilization of wine, each of which has advantages and disadvantages. SO_2 is a widely used additive in winemaking based on antimicrobial and antioxidative properties (Lisanti et al., 2019). However, climate change is causing wines to become low in acidity due to rising the average temperatures during grape growth (Van Genuchten, 2023). This reduces the antimicrobial effect of SO_2 and favors the growth conditions of harmful microorganisms in wine, so the amount of SO_2 used must be significantly increased (Waterhouse et al., 2016). In 2022 the European Food Safety Authority (EFSA), based on the evaluation of a large number of studies, concluded that dietary intake of sulfites can have health consequences for consumers, especially if they consume large quantities of foods with this additive (Younes et al., 2022). This led to an increased public critique, especially of the increasing SO_2 use in wine. Some other chemical-free alternative microbial stabilization methods of wine exist on the market, such as layer filtration or flash pasteurization (Lisanti et al., 2019). However, these methods also have disadvantages and, therefore, cannot always be used since some types of vinification lead to undesirable changes in the authenticity characteristics of the wine (Rosária et al., 2022; Waterhouse et al., 2016). For example, in some cases, flash pasteurization can impair the nutritional and sensory quality of foods, such as changing the aroma and taste profile and/or reducing the content of certain nutrients in wine (Rahman, 2020).

An alternative method could be UV-C technology. In winemaking, there was only a limited number of publications investigating the use of UV-C technology for the microbial stability of wine. Fredericks et al. (2011), Diesler et al. (2019), and Junqua et al. (2020) showed the first promising results related to the use of UV-C technology at the wavelength of 254 nm for the

stabilization of must and wine. Nevertheless, these studies primarily focused on the fundamental consideration of the possible uses of the method.

Some studies suggested that the effectiveness of UV-C technology in a liquid medium depends on a variety of divergent factors, such as the optical or physiochemical properties of the treated product (Atilgan et al., 2020). In addition, UV-C light is a high-energy radiation that can promote a variety of reactions through interactions with the substances in the must (Golombek et al., 2020). Furthermore, recent studies showed a potential approach of the wavelength 280 nm in combination with LED as a light source for microbial stabilization (Popovic et al., 2023). This approach can offer a great advantage in terms of effectiveness and environmental friendliness compared to the commercially used wavelength of 254 nm with the mercury-vapor lamp as the light source.

This dissertation is dedicated to a comprehensive investigation of the effect of increasing UV-C radiation on the microbial, chemical, and sensory properties of wine. Its goal is to provide a justified overview of the possibility of applying UV-C technology for microbial stabilization of wine, based on research, to determine possible limitations, influencing factors, and potential enhancements.

2. Literature overview

2.1 UV-C technology

2.1.1 Fundamental principle of the germicidal effect

According to different wavelengths and energy, electromagnetic radiation is divided into several spectra: radio wave, microwave, infrared, visible, ultraviolet, X-ray, and gamma radiation. Ultraviolet radiation is further subdivided into four ranges: UV-A (400 - 315 nm), UV-B (315 - 280 nm), UV-C (280 - 200 nm), and Vacuum UV (200 – 100 nm) (Kowalski, 2010). The wavelength ranges of UV-C and UV-B are also known as the germicidal ranges of inactivating bacteria and viruses. In particular, the wavelength at 254 nm is nowadays preferred for UV-C technology, as this wavelength is close to the absorption maximum at 260 nm of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and is technically efficient at the same time (Koutchma et al., 2009).

Absorption, reflection, diffraction, refraction, and scattering are ways how electromagnetic radiation can interact with materials. UV irradiation has a germicidal impact based on the absorption of UV light by DNA and RNA of microorganisms. They include nucleobases, which can absorb UV radiation from 200 to 310 nm with a maximum molar absorption coefficient between 7500 and 13000 $M^{-1}cm^{-1}$. Figure 1 shows the UV absorption spectra of adenine, cytosine, guanine, and thymine in water. The absorption of UV treatment led to a series of photochemical reactions that induce the damage of nucleic acids, which in turn inhibits the reproduction processes of microorganisms (Koutchma et al., 2009; Kowalski, 2010). The nucleic acids are crucial for the storage and transferability of genetic information in living organisms.

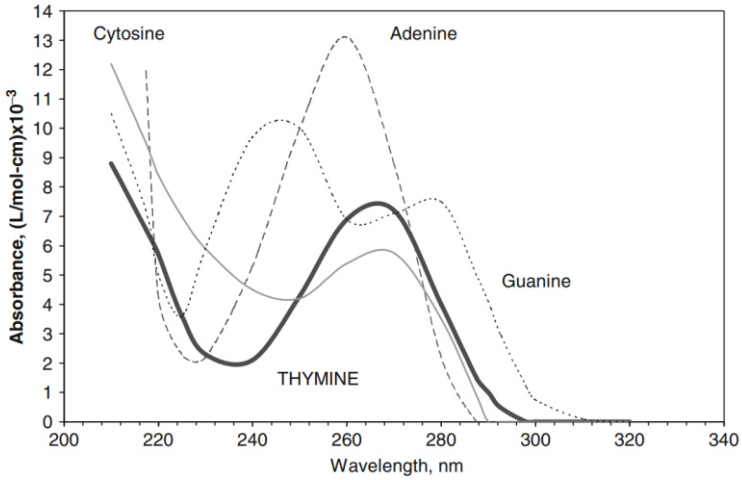


***Figure 1**: UV absorbance spectra of adenine, cytosine, guanine, and thymine in water (Kowalski, 2010).*

The main reactions, which are caused by the absorption of UV-C irradiation, are the formation of cyclobutane-pyrimidine dimers (CPD) by 2+2 cycloaddition between C5-C6 double bonds of pyrimidine and pyrimidine pyrimidone (6-4) photoproducts (6-4PPs) via the Paternó-Büchi reaction. The C5-C6 of one pyrimidine base is linked through two covalent bonds with C4 carbonyl groups of the adjacent second pyrimidine base (Figure 2) (Harm, 1980; Rastogi et al., 2010). The UV-C radiation at 254 nm has an irradiant energy of 112.8 kcal/Einstein (Koutchma et al., 2009). Consequently, the irradiation has enough energy to split the O-H, C-C, C-H, C-N, and S-S bonds. Dimers formed after UV irradiation disrupt DNA replication, preventing cell reproduction (Koutchma et al., 2009*;* Kowalski, 2010). The 6-4PPs can still react to Dewar isomers via photoisomerization upon interaction with light at wavelengths above 290 nm (Figure 2) (Taylor & Cohrs, 1987; Douki & Sage, 2015). Furthermore, UV-B treatment promotes dimerization reactions between two adenine bases or the formation of adenine-thymine adducts (Kumar et al., 1987). CPD and 6-4PPs represent the majority of the products formed after UV treatment (Kowalski, 2010).

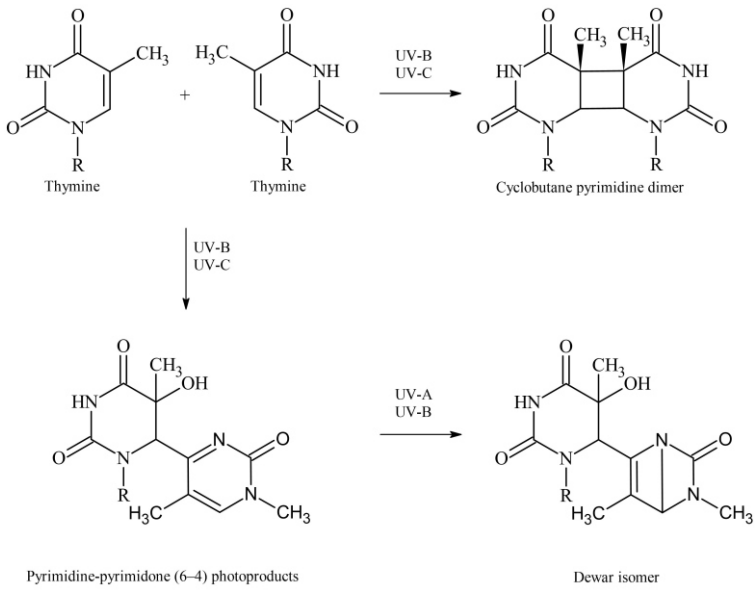


***Figure 2**: Schematic representation of cyclobutane pyrimidine dimer, pyrimidine-pyrimidone photoproducts, and dewar isomer formation after DNA exposure to UV light (Kowalski, 2010).*

Proteins and enzymes of cells can also absorb UV irradiation. This can lead to the formation of covalent bonds between DNA and proteins which can contribute to the inactivation of harmful microorganisms (Akgün & Ünlütürk, 2017; Pattison et al., 2011). Furthermore, the indirect, germicidal properties of UV radiation are related to membrane damage, growth retardation, and DNA damage through the formation of reactive oxygen species (ROS). For example such as superoxide radical anions ($O_2^{\cdot -}$). They are a product of photoinduced reactions that can lead to the formation of 8-hydroxy-2'-deoxyguanosine in DNA (Cadet & Wagner, 2013; Schenk et al., 2010). The combination of different damages suggests that more than the wavelength at 254 nm is suitable for germicidal effectiveness and microbial inactivation.

The study by Bowker et al. (2010) indicates that emitted UV light at the 280 nm range can also lead to higher microbial inactivation. Furthermore, the study by Akgün and Ünlütürk (2017) shows that the synergistic effect of the 280 nm and 365 nm wavelengths has a higher inactivation efficiency than 254 nm. Thus, current research is concerned not only with the wavelength of 254 nm but with various other wavelengths, such as 280 nm.

Some microorganisms have developed mechanisms to reduce UV damage and protect genetic information. Repair mechanisms such as photoreactivation and dark repair play an important role (Sanz et al., 2007). Several types of repair mechanisms are distinguished in dark repair, including nucleotide excision repair (NER) and base excision repair (BER). During photoreactivation, the enzyme photolyase binds specifically to CPDs and, under the influence of light in the range of 310 - 480 nm, splits the thymine dimers into monomers so that they become capable of complementary base pairing again (Harm 1980; Sinha & Häder, 2002). In contrast to photoreactivation, dark repair does not involve direct DNA repair but a replacement of damaged DNA with new, undamaged DNA (Sinha & Häder, 2002; Wood, 1996; Patrick et al., 1964). This process does not require any further light irradiation as with photoreactivation. Some studies have shown that UV-C damaged microorganisms use these mechanisms to repair the damage caused by UV-C and can grow again under certain conditions (Chan & Killick, 1995; Lindenauer & Darby, 1994; Jungfer et al., 2006; Sanz et al., 2007).

2.1.2 UV-C applications and their influencing factors

Food spoilage is most often caused by yeasts, bacteria, or molds. Such harmful microorganisms as *Kluyveromyces, Galactomyces, Hyphopichia Brettanomyces, Brochothrix thermosphacta, Carnobacterium spp., Lactobacillus spp., Candida spp., Rhodotorula spp.,* and *Pseudomonas spp.* are the most common cause of food decay (Lorenzo et al., 2017; Koutchma et al., 2009). UV-C technology has proven to be an effective method for microbial stabilization. Applications for UV-C technology include areas such as healthcare, water treatment, air purification, agriculture and the food industry (Koutchma et al., 2009). UV-C technology in food industry is used for different purposes, such as meat and vegetable processing (surface disinfection of fruits, vegetables, and meat) and beverage treatment (juices, cider, and milk) (Tchonkouang et al., 2023). This technology is described as an environmentally friendly, chemical-free, easy-to-install, safe, and scalable method for microbial stabilization of food (Atilgan et al., 2020). The historical use of UV-C technology for microbial stabilization began in 1910 when UV radiation was applied for water disinfection (Henry et al., 1910). Nowadays, UV-C technology is a widely used method in potable water treatment, often in combination

with in situ-generated ROS, for example, by adding hydrogen peroxide or sulfites to simultaneously disinfect and remove organic pollutants from the water. This process is known as an advanced reduction process (ARP) for the degradation of pollutants in water. The process is based on the absorption of light, for example, SO_2 at 254 nm (molar absorption coefficient: 25.5/M/cm at pH 2.5). That process causes the formation of sulfite radicals and various other ROS (Equation 1-9) (Cao et al., 2020; Vellanki & Batchelor, 2013).

$$SO_3^{2-} + hv \rightarrow SO_3^{\cdot-} + e_{aq}^- \quad \textit{Equation 1}$$

$$HSO_3^- + hv \rightarrow SO_3^{\cdot-} + H^\cdot \quad \textit{Equation 2}$$

$$e_{aq}^- + O_2 \rightarrow O_2^{\cdot-} \quad \textit{Equation 3}$$

$$O_2^{\cdot-} + H^+ \rightarrow HO_2 \quad \textit{Equation 4}$$

$$O_2^{\cdot-} + HO_2 \rightarrow HO_2^- + O_2 \quad \textit{Equation 5}$$

$$SO_3^{\cdot-} + O_2 \rightarrow SO_5^{\cdot-} \quad \textit{Equation 6}$$

$$SO_5^{\cdot-} + HSO_3^- \rightarrow HSO_5^- + SO_3^{\cdot-} \quad \textit{Equation 7}$$

$$SO_5^{\cdot-} + HSO_3^- \rightarrow H^+ + SO_4^{\cdot-} + SO_4^{2-} \quad \textit{Equation 8}$$

$$SO_5^{\cdot-} + SO_3^{2-} \rightarrow SO_3^{\cdot-} + SO_5^{2-} \quad \textit{Equation 9}$$

UV-C technology is permitted in the USA, Canada, the European Union, and South Africa. For example, the U.S. Food and Drug Administration (FDA) has approved this method for inactivating microorganisms in water, juices, and surfaces that come into contact with food, using low-pressure mercury-vapor lamps that emit 90% of their emission at a wavelength of 253.7 nm (FDA 2000, 21 CFR § 179.39). Commercially, a mercury-vapor lamp with a wavelength of 254 nm is used for microbial stabilization due to its easier technical implementation (Koutchma et al., 2009). However, such lamps have some disadvantages, including high energy consumption, limited lifespan, high operating temperatures, and mercury content (Vilhunen et al., 2009). Due to regulation (EU) 2017/852, based on the Minamata Convention to reduce anthropogenic emissions of mercury, there are significant efforts to reduce mercury consumption. Current research approaches are focused on replacing mercury lamps with light-emitting diodes (LEDs) (Beck et al., 2016). The advantages of LEDs include lower energy costs, direct application possibilities, and mercury-free operation.

The FDA does not specify minimum or maximum values for the UV dose. The dose should be determined on a case-by-case basis, taking into account Good Manufacturing Practice (GMP) and individual circumstances (FDA 2000, 21 CFR § 179.39). A large number of studies demonstrate broader applications of UV-C technology for microbial stabilization in the food sector. Among others, this technology has been used for the microbial stabilization of foods such as coconut water (Donsingha & Assatarakul, 2018), liquid egg whites (Ünlütürk et al., 2010), grape juice (Pala & Toklucu, 2012), orange juice (Feliciano et al., 2018), and apple juice (Islam et al., 2016). The use of UV-C technology has a great potential as an alternative method with a wide range of applications in the food industry.

In viticulture and enology, the application of UV-C technology was investigated in different steps of winemaking. It was successfully applied for suppressing powdery mildew infections in vineyards (McDaniel et al., 2024), for enhancing anthocyanin concentration and sensory properties of wine by treating grape postharvest (Gindri et al., 2022), as well as for microbial stabilization of must and wine (Fredericks et al., 2010; Diesler et al., 2019; Junqua et al., 2020). The studies on the microbial stability of must and wine have shown the effectiveness of the method against harmful microorganisms such as *Metschnikowia pulcherrima, Acetobacter aceti,* and *Pediococcus acidilactici*. No mutagenic effect of different *Salmonella typhimurium* strains could be detected in the UV-C treated must (Diesler et al., 2019). Furthermore, the studies showed no significant influence on the sensory properties of must and wine. Nevertheless, Diesler et al. (2019) reported that increasing UV-C doses can lead to the degradation of compounds such as linalool, ß-damascenone, and caftaric acid in must. This indicates oxidative processes must be performed during UV-C treatment.

Various factors can influence the performance of UV-C inactivation. These factors can be divided into three groups: process parameters, food parameters, and type of harmful microorganisms. The flow ratio of liquid food during UV-C treatment plays a critical role (Atilgan et al., 2020; Tchonkouang et al., 2023). Food parameters such as physicochemical (e.g., viscosity, density, solubility) and optical (e.g. turbidity, absorption coefficients of food ingredients) characteristics can influence the effectiveness of UV-C inactivation. Harmful microorganisms also show different sensitivity to UV-C treatment. Factors such as cell wall structure, composition, and cell wall thickness determine the sensitivity of the harmful microorganism to UV-C treatment. For example, yeasts show increased resistance to UV-C treatment due to their lower number of pyrimidine bases and differently structured cell membranes (Tran & Farid, 2004). The harmful microorganisms can be classified into the following categories with increasing resistance to UV-C treatment: Gram negative < Gram positive < yeasts < bacterial spores < molds < viruses (Guerrero-Beltrán & Barbosa-Cánovas, 2004). Other factors, such as cell count and growth stage of the microorganisms, can also influence the effectiveness of UV-C technology (Atilgan et al., 2020; Guerrero-Beltrán & Barbosa-Cánovas, 2004).

Several models were examined to predict microbial inactivation by considering multiple influencing factors, describing UV-C inactivation kinetics. The models offer the possibility to determine several influencing factors and to compare them with each other. There are several different modeling approaches used to describe the inactivation process, such as Log-Linear, Weibull, Log Logistic, and Gompertz models (Atilgan et al., 2020). The Weibull model (Equation 10) describes the course of UV-C inactivation while tailing or shoulder effects can occur simultaneously (Atilgan et al., 2020). The p - parameter of the Weibull model describes the concavity (downwards ($p > 1$) and upwards ($p < 1$) of the curve; the δ - parameter describes the first decimal reduction time of the surviving cells; N_0 - the initial cell counts, N - the cell counts according to the specified irradiation time, and t - the irradiation duration time (Atilgan et al., 2020).

$$\frac{N}{N_0} = 10^{-\left(\frac{t}{\delta}\right)^p} \qquad \textit{Equation 10}$$

Nevertheless, UV-C technology has some limitations. For example, high turbidity or a high concentration of absorbing molecules in food can lead to a low penetration depth of UV-C treatment, and the inhomogeneity of the product can also cause inconsistent irradiation of the product (Guerrero-Beltrán & Barbosa-Cánovas, 2004). The method is challenging to standardize and must be investigated for each product before serial processing. Since UV-C is a high-energy radiation source, it can have an impact on the food ingredients (Bhat, 2016; De Souza et al., 2012; Golombek et al., 2020).

2.2 Wine

According to EU legislation (Reg. (EU) 1308/2013), wine is defined as "a product obtained exclusively by the complete or partial alcoholic fermentation of fresh grapes, whether or not mashed, or from grape must". The wine consists of 86% water, followed by ethanol with 12%. The remaining 2% is made up of a large number of chemical compounds and elements whose composition can vary due to a variety of factors such as grape variety, terroir, weather conditions, and production process. The most important groups of substances of this 2% are organic acids, polysaccharides, minerals, nitrogen compounds, antioxidants, phenolics, and volatile compounds, the part naturally present, part of which is added during the vinification process (Waterhouse et al., 2016).

2.2.1 Harmful microorganisms in wine

Microorganisms are usually associated with the fermentation process of alcoholic beverages. For the vinification process, usually, the vintners use *Saccharomyces cerevisiae.* Depending on the style of wine, malolactic fermentation is sometimes carried out as a second fermentation after alcoholic fermentation. Lactic acid bacteria such as *Oenococcus oeni* or *Lactobacillus* are used to convert the sharp malic acid into softer lactic acid (Jackson, 2008). Nevertheless, this process is not always desirable. On the contrary, the uncontrolled growth of some microorganisms can have negative effects on the sensory properties of the wine, such as color, aroma, taste, and mouthfeel. In wine, the foremost common harmful microorganisms are yeast *Brettanomyces, Hansenula, Hanseniaspora/Kloeckera, Pichia,* and *Zygosaccharomyces;* lactic acid bacteria *Lactobacillus, Leuconostoc,* and *Pediococcus;* and the acetic acid bacterial genera *Acetobacter* and *Gluconobacter* (Du Toit & Pretorius, 2019). There are three stages in vinification where harmful microorganisms may diminish the quality of wine. The first stage is grapes. They can be infected with yeast, molds, lactic acid and acetic acid bacteria. The second stage is during the alcoholic fermentation. Yeasts are naturally present in the grape or winery environment and can contribute to the fermentation process. The third stage is the period of storage. After alcoholic fermentation, the wine has a low resistance to harmful microorganisms (Fleet, 1993). The undesirable microbial metabolism can cause the formation of off-flavors such as vinegar, horsy, mousy, geranium notes, and buttery, as well as some harmful microorganisms that cause visual effects such as film formation, viscosity, or formation of sediments (Cosme et al., 2018). Figure 3 shows the main harmful microorganisms in wine leading to the spoilage aroma of wine and their secondary metabolism. Sources of harmful

microorganisms could be grapes, vineyards, and poor cleaning and sanitation methods in wineries.

Brettanomyces bruxellensis is considered to be a major harmful microorganism of wine spoilage (Boulton et al., 1996; Loureiro, 2003). This yeast has shown a negative and positive contribution to the aroma profile of wine, beer or tea Kombucha (Schifferdecker et al., 2014). The *Brettanomyces* genus was first isolated by Hjelte Claussen in 1904 from beer, and the name is derived from 'British brewing fungus' (Claussen, 1904). The flavor resulting from the yeast metabolism lends the beer characteristic fruity, pineapple aromas and floral, apricot, and tropical fruit aromas. The *Brettanomyces* genus in the wine was first determined by Barret et al. (1950). *Brettanomyces bruxellensis* belongs to the grape microflora (Guerzoni & Marchetti, 1987). Specifically, the wines are particularly susceptible to *Brettanomyces bruxellensis* growth during maturation in oak barrels. During metabolism, *Brettanomyces bruxellensis* catalyzes the formation of volatile phenols such as 4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol through the enzymatic conversion of p-coumaric acid, fertaric acid, and caftaric acid (Milheiro et al., 2017). In low concentrations, the presence of these compounds can contribute to the aroma complexity of wine. At high concentrations of compounds, wine develops a distinctive, unpleasant aroma associated with attributes such as phenolic, leather, and horse sweat. The *Brettanomyces* genus can adapt to harsh environmental conditions such as high ethanol concentrations, low pH values, and low nitrogen sources (Rozpedowska et al., 2011). It is estimated that the cell counts of 10^5 cell mL^{-1} trigger the off-flavor character of wine (Fugelsang & Zoecklein, 2003).

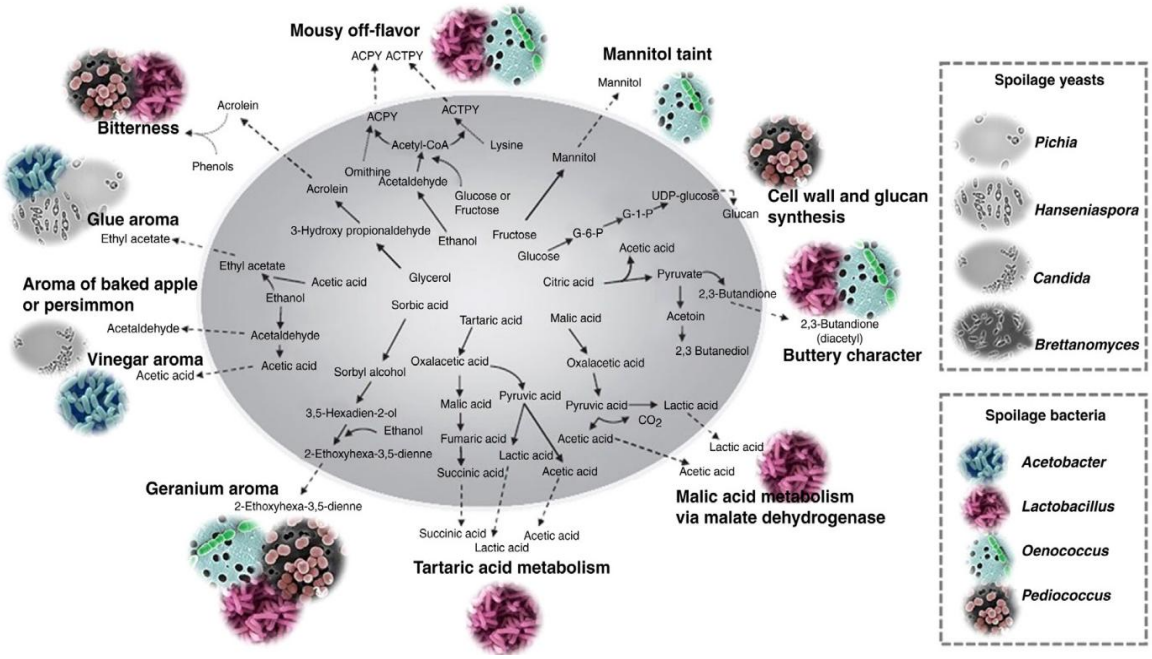


Figure 3*: Main harmful microorganisms in wine leading to spoilage aroma of wine and their secondary metabolism (Cosme et al., 2018).*

2.2.2 Non-volatile wine compounds

Organic acids are the most important (mainly) non-volatile components of wine, primarily responsible for the composition, microbial and physicochemical stability, and organoleptic quality of wine. The concentration of titratable acids in wine varies between 4 and 9 g/L (Kliewer et al., 1967). The three main acids, which make up about two-thirds of the amount of all acids in wine, are tartaric acid, malic acid, and citric acid, which are extracted from the grape. During alcoholic fermentation and malolactic fermentation, additional acids are formed: pyruvic acid, lactic acid, succinic acid, acetic acid, oxalacetic acid, and fumaric acid (Ribéreau-Gayon et al., 2006). Carbohydrates such as glucose and fructose occur in small quantities in fully fermented wine. During alcoholic fermentation, greater amounts of glucose and fructose are broken down into alcohol and several by-products, among other organic acids. The average ratio of glucose to fructose in residual sugar is 0.58 (Belitz et al., 2001). In addition to hexoses, the following pentoses (0.3 - 2.0 g/L) are always present in wine, such as arabinose, rhamnose, and xylose. The pentoses are not fermentable by yeasts and are more abundant in red wines (Moreno-Arribas & Polo, 2008). The list of minerals contained in wine includes chlorides (1 g/L), phosphates (70 - 500 mg/L), potassium (0.5 - 2.0 g/L), calcium (80 - 40 mg/L), sodium (10 - 40 mg/L), magnesium (60 - 150 mg/L), manganese (1 - 3 mg/L), as well as iron (1 - 3 mg/L) and copper (0.3 - 0.4 mg/L) (Belitz et al., 2001). The typical nitrogen compounds in wine are oligopeptides, amino acids, biogenic amines and proteins.

2.2.2.1 Amino acids

Amino acids play a significant role in the taste and sensory properties of wines by influencing the aroma and mouthfeel and contributing to the acidic-alkaline buffering capacity of the wine. Their interaction of amino acids with other compounds can improve the quality of different types of wines, including sparkling and red wines (Nandorfy et al., 2022). The total amount of free amino acids is between 1 and 4 g/L (Lehtonen, 1996). The typical representatives of the amino acids in wine are alanine, arginine, cysteine, histidine, leucine, lysine, methionine, proline, and tryptophan (TRP) (Figure 4). Most of the amino acids in grape and wine are primary α-amino acids and are proteinogenic (Waterhouse et al., 2016). The concentration of amino acids is influenced by several factors such as grape variety, maturity, vintage, temperature and alcoholic content (Gutiérrez-Escobar et al., 2023; Lorenzo et al., 2017). Amino acids are one of the most essential grape juice and wine nutrients. During alcoholic fermentation, yeast consumes the amino acids and transforms them into volatile compounds. Studies have shown that nitrogen composition, including the amino acid composition, can biochemically affect the volatile composition and concentration in wine (Hernández-Orte et al., 2002; Gutiérrez-Gamboa et al., 2019). A positive correlation between most amino acids and the volatile compound ethyl octanoate in sparkling wine was observed by Sun et al. (2023). The study has also shown that an increased concentration of proline has promoted the stronger expression of attributes such as sweetness, viscosity, and red fruits in wine (Nandorfy et al., 2022). Furthermore, amino acids such as phenylalanine and methionine can react with quinones to form Strecker aldehydes contributing to wine oxidation (Oliveira et al., 2017). Under light influence, methionine and TRP can promote the formation of methional and 2-AAP (Fracassetti et al., 2019; Horlacher & Schwack, 2014).

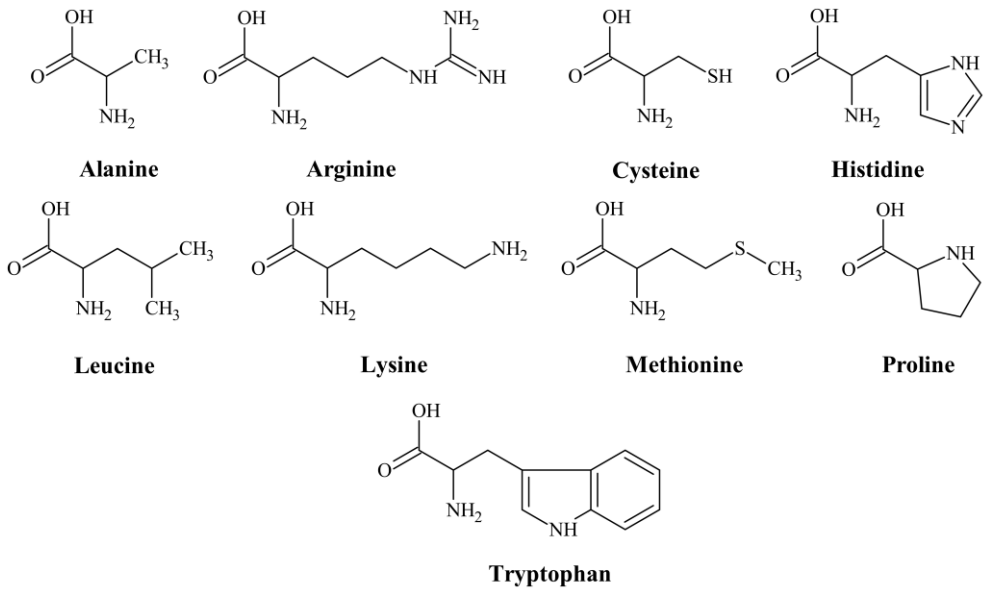


Figure 4*: Structure of alanine, arginine, cysteine, histidine, leucine, lysine, methionine, proline, and tryptophan (Berg et al., 2017).*

2.2.2.2 Polyphenols

Polyphenols belong to the secondary plant substances and contain at least one hydroxyl group (Stagos, 2021). These groups of substances largely determine the sensory properties and color of wine, and have antibacterial as well as antioxidant properties (Weber et al., 2012). The polyphenol content is influenced by the grape varieties used, geographical location, technological processes, the type of yeasts used during alcoholic fermentation and the maceration period (Ribéreau-Gayon et al., 2006). The typical polyphenol concentration in white wine is between 0.2 - 0.5 g/L, and in red wine, between 1 – 5 g/L (Gutiérrez-Escobar et al., 2021). Based on their carbon structure, the polyphenols are divided into two groups: flavonoids with a C6-C3-C6 backbone and non-flavonoids, which usually have a C6-C1 or a C6-C3 basic body. The fruit pulp contains mainly polyphenols of the non-flavonoid class, while the peel, seeds, and stems contain mainly polyphenols of the class flavonoids. The non-flavonoids are further divided into three groups: hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes (Durner, 2011).

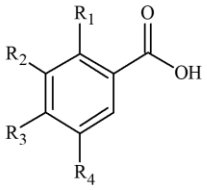
Hydroxybenzoic acids are extracted into the wine mainly by breaking grape skins and seeds during fermentation (Figure 5). They contribute, along with other phenols, to wine color, astringency, and bitterness. Gallic acid is the most important in this group, with a concentration between 10 mg/L in white and 70 mg/L in red wine (Waterhouse, 2002). Gallic acid effectively scavenges various reactive species of nitrogen, oxygen, and sulfur and chelates metal ions. Computational studies have shown that the antioxidant activity of gallic acid is primarily based on proton loss electron transfer mechanisms in aqueous solutions (Molski, 2023).

Hydroxycinnamic acids belong to the third most common group of polyphenols in grapes and are mainly found in bound form in the grapes, mainly as tartaric acid esters (Ong & Nagel, 1978). During winemaking and wine aging, these esters can be split and released as free acids. Hydroxycinnamic acids can be easily oxidized and are associated with the wine's tanning process. To this substance group belong compounds such as caffeic acid, ferulic acid, and

coumaric acid (Figure 5). The average amount of hydroxycinnamic acids in white and red wines is about 30 and 100 mg/L, respectively (Visioli et al., 2020).

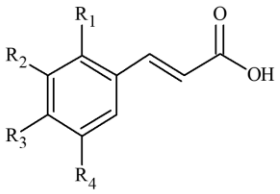
Stilbenes are bioactive compounds consisting of two aromatic rings connected by a methylene group, including trans-piceid and trans-resveratrol (Figure 5). They occur naturally in wine but in low concentrations (0 - 5 mg/L). However, when grapes are subjected to biotic or abiotic stress, the levels of resveratrol, its glycoside called piceid, and its dimeric and trimeric forms (e.g., pallidol, viniferine) can increase from negligible to more than 100 mg/L (Visioli et al., 2020; Gutiérrez-Escobar et al., 2021).

Hydroxybenzoic acids



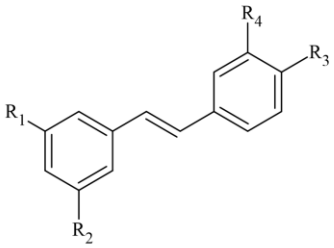
Compounds	R_1	R_2	R_3	R_4
Gallic acid	H	OH	OH	OH
Gentisic acid	OH	H	H	OH
Syringic acid	H	OCH_3	OH	OCH_3
Vanillic acid	H	OCH_3	OH	H

Hydroxycinnamic acids



Compounds	R_1	R_2	R_3	R_4
Caffeic acid	H	OH	OH	H
Ferulic acid	H	OCH_3	OH	H
p-Coumaric acid	H	H	OH	H
Sinapinsäure	H	OCH_3	OH	OCH_3

Stilbenes



Compounds	R_1	R_2	R_3	R_4
trans-Resveratrol	OH	OH	OH	H
trans-Piceid	OGlc	OH	OH	H
Piceatannol	OH	OH	OH	OH

Figure 5*: Structure of principal hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes in wine (Gutiérrez-Escobar et al., 2021).*

Flavonoids are more or less intense yellow pigments, which are made up of a benzopyran, a phenyl ring, and a phenyl residue. The flavonoid group will be divided into further subgroups: anthocyanidins, flavanols (flavan-3-ols), flavonols, and tannins (Gutiérrez-Escobar et al., 2021).

Anthocyanins are water-soluble phenolic pigments that form the basis of the color of red wine and are present in wine as glycosides (Waterhouse et al., 2016). The five most common anthocyanidins formed in grapes are delphinidin, cyanidin, petunidin, peonidin, and malvidin (Figure 6) (Gutiérrez-Escobar et al., 2021). The color of anthocyanins changes depending on the wine's pH, sulfur dioxide concentration, temperature, and co-pigmentation. At a pH below 3, all anthocyanidins are present in the flavan (red) cation form (Ribéreau-Gayon et al., 2006).

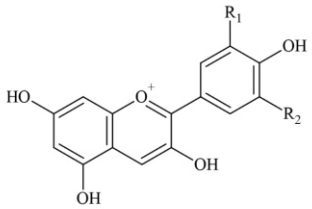
Through reactions with other phenols, co-pigmentation occurs, which increases color intensity (hypochromic effect), shifts the absorption maximum to a higher wavelength (bathochromic effect), and stabilizes the molecule (Belitz et al., 2001; Baranac et al., 1996).

Flavanols or flavan-3-ols are found in the skin and seeds of grapes in monomeric, oligomeric, or polymeric forms; the latter two forms are also known as proanthocyanidins or condensed tannins (Waterhouse et al., 2016). Flavanols stabilize wine's color and sensory properties (especially astringency and bitterness) (Noble, 1994). The concentration of flavanols in white wine is significantly lower than in red wine. According to Goldberg et al. (1999) research, white wine's flavanol content ranges from 5% to 25% of red wine. The main compounds in the flavanol class are catechin, epicatechin, epigallocatechin, epigallocatechin-3-gallate and epigallocatechin-3-gallate (Figure 6). During wine storage, flavan-3-ols can react with SO_2 to form sulfonate-modified flavanols or react with acetaldehyde to ethylidene-bridged flavan-3-ols. These reactions can reduce the astringency of the wine and stabilize its color (Ma et al., 2018).

Flavonols are yellow pigments located in grape skins. They are usually in glycosidic form, bound to a sugar (Flamini et al., 2013). The main flavonols found in grapes and wine are myricetin, quercetin, laricitrin, kaempferol, isorhamnetin, and syringetin (Figure 5) (Mattivi et al., 2006). The typical concentration of flavonols in red wine is 100 mg/L (Durner, 2011).

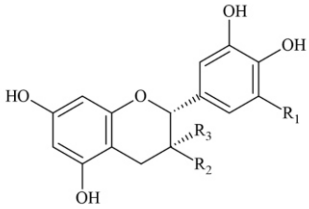
Tannins are complex phenolic compounds formed by polymerizing elementary molecules with phenolic functions. The biosynthetic pathway of all tannins is based on Calvin cycle products that provide precursors for tannin synthesis via the shikimate pathway or acetate/malonate pathway (Molino et al., 2023). The molecular weight of tannins ranges between 600 and 3500 Da (Ribéreau-Gayon et al., 2006). Tannins influence wines structure, taste, and aging potential and can precipitate proteins. Furthermore, tannins have high antioxidant potential due to their ability to release hydrogen atoms or electrons, as well as their capacity to chelate metal ions (Keulder, 2006). They are categorized into condensed and hydrolyzable tannins (Figure 7). Condensed tannins are formed by the condensation reaction of flavanols (flavan-3-ols) and can be present in wine as dimers, trimers and oligomers. During wine storage, condensed tannins tend to undergo further polymerization reactions, leading to a reduction in wine astringency (Waterhouse et al., 2016). The typical concentration of tannins is 100 mg/L in white wine and 1 to 4 g/L in red wine (Monagas et al., 2003). Hydrolyzable tannins are mainly found in wine stored in oak barrels. After six months of barrel aging, hydrolyzable tannin content in white wine reaches 100 mg/L, while in red wine aged for two or more years, it reaches 250 mg/L (Waterhouse, 2002). Hydrolyzable tannins are divided into two subclasses. Gallotannins consist of sugar molecules, usually glucose, esterified with one or more gallic acid units. Ellagitannins consist of glucose units esterified with one or more ellagic acid units (Resolution OIV-OENO 624-2022). Through hydrolysis, these tannins can be degraded into their primary structures.

Anthocyanidins



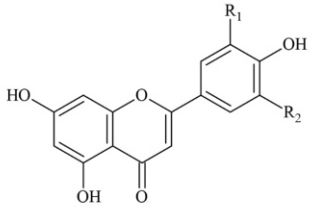
Compounds	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pentunidin	OH	OCH_3
Peonidin	OCH_3	OCH_3

Flavanols



Compounds	R_1	R_2	R_3
(+)-Catechin	H	OH	H
(-)-Epicatechin	H	H	OH
(-)-Epigallocatechin	OH	H	OH
(-)-Epicatechin-3-gallate	H	H	Gallic acid
(-)-Epigallocatechin-3-gallate	OH	H	Gallic acid

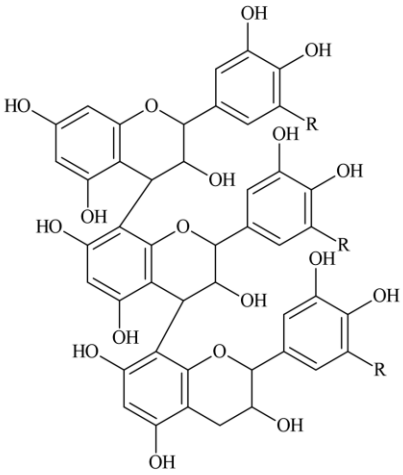
Flavonols



Compounds	R_1	R_2
Myricetin	OH	OH
Quercetin	OH	H
Laricitrin	OCH_3	OH
Kaempferol	H	H
Syringetin	OCH_3	OCH_3
Isorhamnetin	OCH_3	H

***Figure 6**: Structure of principal athocyanins, flavanols, and flavonols in wine (Gutiérrez-Escobar et al., 2021).*

Condensed tannins



Hydrolyzable tannins

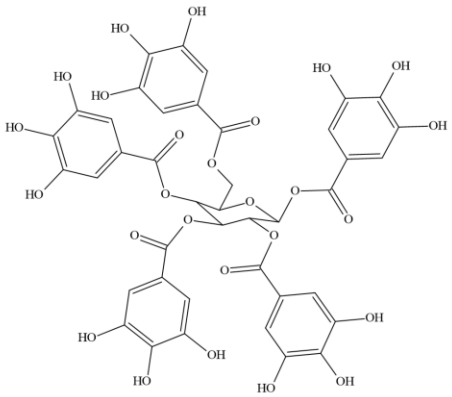


***Figure 7**: Structure of condensed and hydrolyzable tannins (Versari et al., 2012).*

2.2.3 Volatile wine compounds

Many volatile compounds are present in wine, with a total concentration of 0.8 to 1.2 g/L (Belitz et al., 2001). A distinction is made between primary aromas, which are extracted directly from the grape; secondary aromas, which are formed during fermentation and the first stages of aging; and tertiary aromas, which are formed during storage and aging. The aroma profile of the wine is influenced by a wide variety of parameters, including the grape variety, the terroir, climatic conditions, the production process, and the storage conditions (Van Leeuwen et al., 2020; Styger et al., 2011). Based on their structure, volatile compounds are divided into different organic substance classes (Ribéreau-Gayon et al., 2006; Waterhouse et al., 2016). Classes of compounds relevant for this work are monoterpenes, C13-norisoprenoids, esters, and higher alcohols. Monoterpenes are a subgroup of the substance class isoprenoids, including diterpenes, semiterpenes, and sesquiterpenes, and play an important role in the aroma profile of wine (Ashour et al., 2010). Monoterpenes are mainly formed in the grape during ripening and fermentation through the metabolic process and are the precursor compounds of mevalonate, a metabolite derived from acetyl-CoA (Styger et al., 2011). In the grapes, monoterpenes are present in free and glycosidically bound forms. The monoterpene's glycosidically bound forms are odorless and released during alcoholic fermentation by enzymatic activity (Wilson et al., 1986). The most important monoterpenes in wine are geraniol (sensory threshold 32 µg/L), linalool (sensory threshold 25 g/L), nerol (sensory threshold 300 µg/L), and α-terpineol (sensory threshold 250 µg/L) (He et al., 2023). Monoterpenes significantly contribute to the characterization of fruity and floral aromas in wines, especially Muscat and Gewurztraminer (Mateo & Jiménez, 2000; Waterhouse et al., 2016).

C13-norisoprenoids are formed by carotenoid degradation by enzymatic or chemical cleavage. Carotenoids are yellowish pigments that act as antioxidants against photooxidative damage in the facades due to their highly conjugated double formation (Styger et al., 2011). In the beginning of winemaking, the C13-norisoprenoids are in free form or as glycoconjugates. The glycoconjugates will then be released into their volatile aglycon during fermentation through enzymatic and acid hydrolysis processes (Mendes-Pinto, 2009). The most important C13-norisoprenoids in wine are β-damascenone (sensory threshold 0.05 µg/L), β-ionon (sensory threshold 0.09 µg/L) and 1,1,6-trimethyl-1,2-dihydronaphthalene (sensory threshold 2 ng/L) (Waterhouse et al., 2016). The conditions such as pH, temperature and oxygen content during the fermentation and maturation process of the wine can greatly influence the concentration of C13-norisoprenoids in the wine. C13-norisoprenoids contribute to floral, fruity, and ripe notes in wine and are particularly relevant in Riesling and Chardonnay grape varieties (Mendes-Pinto, 2009).

Esters are considered one of the most important classes of substances that contribute significantly to the aroma profile of wine, especially in young wines. This class of substances is mainly formed during alcoholic fermentation - by the condensation of an organic acid and an alcohol catalyzed by acetyl-CoA and esterase (Styger et al., 2011). Furthermore, bound esters are present as glycosides in the grapes, which are released by enzymatic hydrolysis during alcoholic fermentation (Saerens et al., 2009). The most common esters produced during alcoholic fermentation are ethyl acetate (odor threshold 12 mg/L), ethyl butanoate (odor threshold 20 µg/L), ethyl hexanoate (odor threshold 14 µg/L), and ethyl octanoate (odor threshold 5 µg/L) (Waterhouse et al., 2016). Esters can be degraded in wine over time by

hydrolysis. Esters are chemically unstable in an aqueous solution, and their degradation can be promoted by a low pH, temperature, and oxygen increase (Bundgaard et al., 1988; Waterhouse et al., 2016; Ramey & Ough, 1980). During hydrolysis, esters break down into their initial acids and the corresponding alcohol. This process plays a significant role in developing the aroma profile of the wine during maturation (Ramey & Ough, 1980).

Higher alcohols, known as fusel alcohols, are produced during alcoholic fermentation as a by-product of the catabolism of amino acids (Moreno-Arribas & Polo, 2008). This process is called the Ehrlich pathway. During the process, pyruvate is formed, which is then converted to higher alcohols via decarboxylation and reduction. Factors such as higher temperature, higher concentrations of suspended solids, and low concentrations of nitrogen assimilable by yeast lead to increased formation of higher alcohols in wine (Bell & Henschke, 2005). The main higher alcohols in wine are 3-methyl-1-butanol (sensory threshold 1.2 mg/L), 2-methyl-1-propanol (sensory threshold 40 mg/L), and 2-phenylethanol (sensory threshold 14 mg/L) (Ferreira et al., 2000). In medium-high concentrations, higher alcohols contribute to a complex, fruity, and floral aromatic profile of the wine. In high concentrations, higher alcohols lead to a pungent and fuzzy taste (De-La-Fuente-Blanco et al., 2016).

2.2.4 Formation of acetaldehyde in wine

Acetaldehyde is one of the most important aroma-active substances of the volatile acid class. It is formed during alcoholic fermentation, bacterial activity, and oxidative conditions. The typical concentration of acetaldehyde in wine is around 30 mg/L (red wine) and 80 mg/L (white wine) (McCloskey & Mahaney, 1981). The sensory threshold for acetaldehyde in wine is between 100 and 125 mg/L (Berg et al., 1955). At high concentrations, acetaldehyde is associated with odor descriptions such as grassy, nutty or ripe apple. During alcoholic fermentation, acetaldehyde is formed by the decarboxylation of pyruvate (Romano et al., 2019). The oxidative formation of acetaldehyde in wine is catalyzed by iron (Figure 8), known as the Fenton oxidation reaction (Elias & Waterhouse, 2010). The reaction could be caused by other metals such as copper, which is known under the name Fenton-like reaction.

In the first step, oxidation of oxygen to a superoxide radical anion ($O_2^{\cdot-}$) occurs through the transition to Fe^{3+}. In an acidic medium, the superoxide reacts with phenols to form a hydroperoxyl radical ($HO_2^{\cdot}$), which then forms hydrogen peroxide (H_2O_2) and, via further interaction with Fe^{2+}, forms the hydroxyl radical ($HO^{\cdot}$). The part with redox reaction between Fe^{2+} and H_2O_2 is known under the name - Fenton reaction, which Hanry John Hostman Fenton found in 1894 (Fenton, 1894). $HO^{\cdot}$ is a highly reactive oxidant that can react with most organic molecules (Danilewicz, 2003). Based on the high ethanol concentration in wine, $HO^{\cdot}$ primarily reacts with ethanol to form acetaldehyde. Acetaldehyde can react in wine with phenols such as anthocyanins and flavanols to form methylmethine-bridged compounds and ethyl-linked oligomers (Timberlake & Bridle, 1976; Es-Safi et al., 1999). These reactions products can affect the wine's color, flavor, and astringency (Han et al., 2019). The Fenton reaction can be induced through light exposure. This variant of the Fenton reaction is known under the reaction name photo-Fenton reaction (Grant-Preece et al., 2015). For wastewater treatment, the photo-Fenton and photo-Fenton-like reactions have shown promise for reducing toxic compounds in combination with UV-C light at 254 nm. Light improves the reaction reactivity through acceleration of the reduction of Fe^{3+} to Fe^{2+} (Shah et al., 2022).

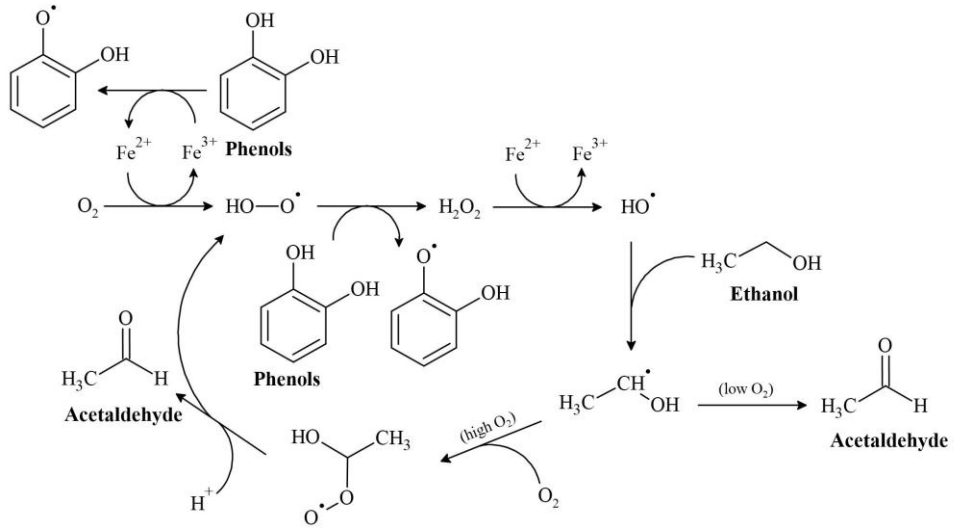


***Figure 8**: Metal-catalyzed nonenzymatic formation of acetaldehyde under high and low dissolved oxygen concentrations in wine (Elias & Waterhouse, 2010).*

2.2.5 Formation of 2-aminoacetophenone in wine

In addition to aroma substances that contribute to a typical aroma profile in wine, atypical aromas also occur, referred to as off-flavors. Relevant examples of this atypical aroma include 2-aminoacetophenone (2-AAP), which is considered the critical component for the "atypical aging note" (ATA) in wine (Rapp et al., 1993). 2-AAP is associated with odor attributes such as wet wool, naphthalene, and acacia flower, with an odor threshold between 0.5 and 1.5 µg/L in wine (Christoph et al., 1995). 2-AAP, as an off-flavor, occurs not only in wine but also in other foods such as cheese, beer, condensed milk, and powdered milk (Hoenicke et al., 2002). Water deficit, insufficient nitrogen during viticulture, and the influence of light can promote the formation of 2-AAP. It can be produced by the oxidative degradation of indole-3-acetic acid for example, by radical co-oxidation of sulfites through the cleavage of the pyrrole ring via intermediates such as scatole, 3-(2-formylaminophenyl)-3-oxopropionic acid and n-formyl-2-aminoacetophenone (Hoenicke et al., 2002; Dollmann et al., 2015) (Figure 9A). The study of Huang et al. (2014) showed that 2-AAP can react further under special conditions via copper-catalyzed reaction to indoline-2,3-diones. Indole-3-acetic acid is one of the natural growth regulators of plants and can be enzymatically formed from TRP in the plant via indole-3-pyruvate and indole-3-acetaldehyde (Zhao, 2011). The typical concentration of indole-3-acetic acid in wine is between 3 and 90 µg/L (Hoenicke et al., 2002). To reduce the formation of 2-AAP in wine, preventive steps such as crop and leaf thinning as well as lowering fermentation temperature during alcoholic fermentation are recommended (Schwab et al., 1996).

Another mechanism for the formation of 2-AAP is the light-induced degradation of TRP via N-formylkynurenine and kynurenine as intermediates (Figure 9B). Horlacher and Schwack (2014) have shown that the presence of RF significantly influences the formation of 2-AAP. RF (vitamin B_2) is known as a photosensitizer and, in combination with light, can cause the off-flavor "light-struck taste" in wine (Fracassetti et al., 2021).

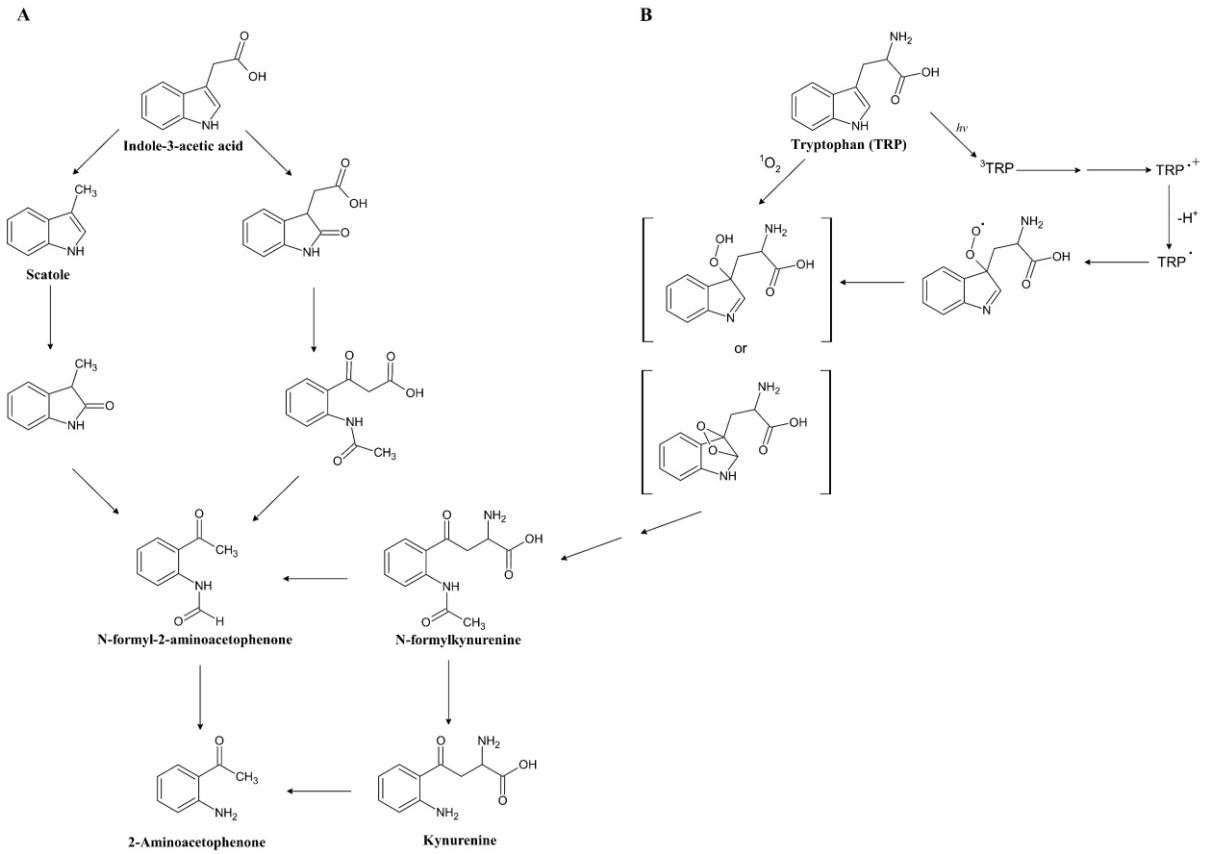


***Figure 9**: Formation of 2-aminoacetophenone from indole-3-acetic acid (A) and tryptophan (B) (Horlacher & Schwack, 2014).*

2.2.6 Wine antioxidants: SO_2 and enological tannins

Oxidation is one of the main phenomena affecting wine quality and is a limiting factor in wine storage. Oxidation can occur at different stages of winemaking and storage due to enzymes, oxygen, temperature fluctuations, and sunlight exposure (Waterhouse et al., 2016). There are several antioxidants that can be added to wine to reduce oxidative processes (Oliveira et al., 2011). The antioxidants relevant to this work are SO_2 and tannins. SO_2 is a widely used product in vinification due to its antimicrobial and antioxidant properties (Boulton et al., 1996). SO_2 acts as a weak acid in aqueous environments and forms conjugate bases (Figure 10). Thus, SO_2 occurs in wine in three chemical forms: molecular SO_2, hydrogen sulfite (HSO_3^-), and sulfite (SO_3^{2-}) (Coelho et al., 2015).

The equilibrium between the different chemical forms of SO_2 in wine depends on several parameters such as pH, alcohol content, and storage temperature of the wine. The typical pH of wine is between 3 - 4. At this pH, the majority amount of SO_2 exists in HSO_3^- form. Molecular SO_2 has an antimicrobial effect and inhibits the growth of harmful organisms in wine by inhibiting metabolic processes, causing DNA damage, or reducing the cell membrane potential (Waterhouse et al., 2016). HSO_3^- is a soft nucleophile, which reacts directly with ROS such as hydrogen peroxide, via nucleophilic addition with acetaldehyde, anthocyanins, and quinones or via Michael addition with unsaturated ketones to form sulfonates (Beech et al., 1979; Burroughs & Sparks, 1973; Elias & Waterhouse, 2010; Danilewicz et al., 2008). The addition of SO_2 reduces the oxidation of phenols in wine, which leads to color stability and contributes to the

preservation of fresh and fruity aromas (Oliveira et al., 2011). On the other hand, the excessive amount of SO_2 in wine can cause negative effects on the organoleptic characteristics of wine.

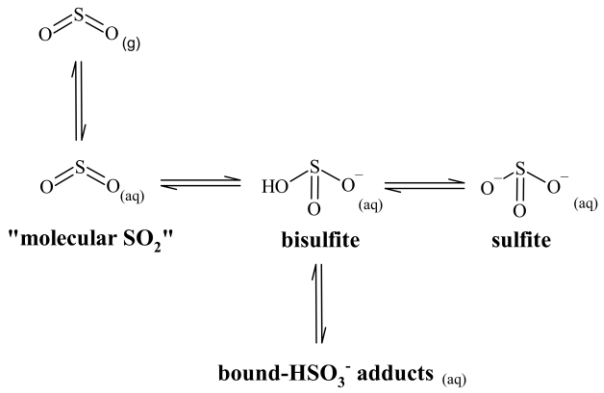


***Figure 10**: Equilibria of sulfur dioxide species at 20 °C in water (Coelho et al., 2015).*

Enological tannins are other antioxidants approved by the International Organization of Vine and Wine (OIV) to stabilize wine (Resolution OIV-OENO 624-2022). Tannins, based on their chemical structure, are strong antioxidants. They are powerful nucleophiles due to the benzene ring skeleton and phenolic hydroxyl groups. The structure of tannins allows for the formation of stable phenolic radicals that effectively break oxidative chain reactions (Cadenas & Packer, 2001). Furthermore, tannins tend to form chelation complexes with transition metals such as iron and copper, which, in turn, leads to a reduction in the oxidation reactions promoted by transition metals, such as the Fenton and Haber-Weiss reactions (Vignault et al., 2018; Cadenas & Packer, 2001; Lopes et al., 1999). Furthermore, the study by Fracassetti et al. (2021) has shown that the addition of hydrolyzable tannins, in particular gallotannins, is suitable as a prevention against the formation of the light-struck taste in wine.

2.3 Photochemistry

2.3.1 Fundamentals of photochemistry

Photochemistry is a part of chemistry that deals with the chemical reactions that occur through the interaction of light with molecules. Light is electromagnetic radiation that consists of discrete light quanta (also called photons) and is carried by an electromagnetic field (Atkins et al., 2022). A photon is an elementary quantum with neither a rest mass nor a charge. It moves through space at the speed of light and has wave and particle properties. The energy of a photon (E_{photon}) is directly proportional to its frequency (υ) via Planck's constant (h) and inversely proportional to is wavelength (λ) (Equation 11) (Latscha et al., 2008).

$$E_{photon} = h \cdot \upsilon = \frac{h \cdot c}{\lambda} \qquad \textit{Equation 11}$$

The photons can interact with matter in different ways. The photon can be absorbed during the interaction, raising the atom or molecule from the ground state to an excited state (Wöhrle et al., 2012). Alternatively, the photon can be reflected, scattered, or passed through without

interaction. That depends on the properties of the material/molecule and the wavelength of the photon. The absorption process takes place in the form of an electronic transition between quantized energy levels of the electrons (Turro et al., 2009). Generally, between the electronic singlet ground state (S_0) in an excited electronic state. This is either an excited singlet state (S_n) or an excited triplet state (T_n). The S_0 of a molecule is defined as the state in which the system has the lowest possible energy in the electron configuration in the corresponding molecular orbital. Thus, the system has the lowest total energy; the electrons are arranged in the electron configuration with the lowest potential energy (Diem, 2021; Persico & Granucci, 2018). The electrons are assigned to the orbitals, considering the Pauli exclusion principle, Hund's rule, and the Aufbau principle (Atkins et al., 2022; Balzani et al., 2024).

The Pauli exclusion principle states that the two electrons within an atom must not have the same quantum numbers (principal quantum number, minor quantum number, magnetic quantum number of angular momentum, and spin quantum number (m_s)) within an atom. A maximum of two electrons can occupy each orbital, and the orbitals are occupied according to their energetic potential. If they are filled by electrons with opposite spin state, resulting in the total spin quantum number of electrons (S) (Equation 12) out of 0. The spin is an intrinsic form of angular momentum, which is defined by the m_s and can take the value of $+ 1/2$ or $- 1/2$ (Atkins et al., 2022).

$$S=\sum_{s} m_s \qquad \textit{Equation 12}$$

During the transition from S_0 to S_n, an electron excitation to the higher energy level occurs when the raised electron changes the paired spin orientation of S_0 (Figure 11). In this state, S corresponds to the value of 0 with a multiplicity value (M) of 1. The multiplicity value (Equation 13) describes the number of possible orientations of S (Atkins et al., 2022; Balzani et al., 2024).

$$M=2S+1 \qquad \textit{Equation 13}$$

During the transition from S_0 to T_n, the spin orientation of the excited electron is changed to the same spin orientation so that the two electrons have a parallel spin state (Figure 11). In this state S corresponds to a value of 1 with a multiplicity value of 3. However, due to the selection rule, the direct transition between S_0 and T_n is unlikely. It can only occur in rare cases, such as in the cases of spin-orbit coupling during phosphorescence. It is referred to as "spin-forbidden" transitions or by the non-radiative transitions. The selection rule is an important rule for observing spectral transitions. This rule specifies that the transitions between states with different total spin quantum numbers are forbidden (Persico & Granucci, 2018; Wöhrle et al., 2012).

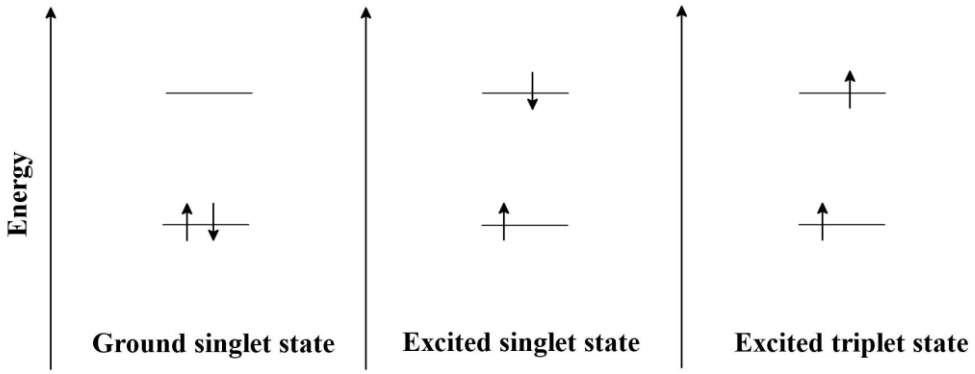


***Figure 11**:Shema of the spin orientation of electrons in singlet ground state and excited singlet and triplet states (Latscha et al., 2015).*

Not only does the selection rule influence the transition probability, but also the vibrational states of the molecule. During the electron transition, there is an instantaneous reorientation of the electrons in the system and a delayed geometry relaxation (oscillation period) of the nuclear framework. The electron excitation to a higher energy level occurs in the femtosecond range, but the oscillation period is in the picosecond range. This means that the nuclear distance does not change during the transition (Wöhrle et al., 2012).

Thus, the total energy (E(e,n)) of the energy level can be approximately described as the energy of an electron state (E_e) and the potential energy of the nucleus (vibrational state) (E_υ) (Persico & Granucci, 2018) (Equation 14).

$$E(e,n) \approx E_e + E_\upsilon \qquad \textit{Equation 14}$$

The harmonic oscillator describes the vibration states, and their energy is described in Equation 15. Each energy level has a series of quantized vibrational states (Diem, 2021).

$$E_v = \left(n + \frac{1}{2}\right) hv \qquad \textit{Equation 15}$$

The Franck-Condon principle (Figure 12) shows that the transition probability between the single electronic states is influenced by the overlap of the vibrational wave functions, which determines the intensity of the transitions.

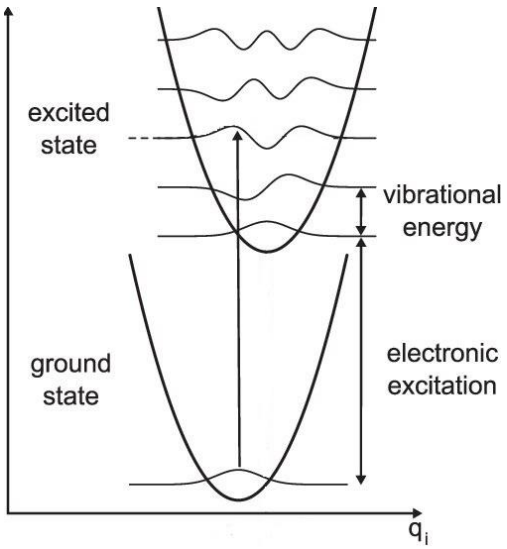


***Figure 12**: Franck-Condon-Prinzip (modified) (Pittner et al., 2013).*

The corresponding energy for the electron transition can come either from light absorption or collisions of the molecule with other electrons or other molecules. If the transition of the electron takes place between two vibratory states, as described above, this is called a vibratory transition. The Franck-Condon principle can be used to calculate the possible intensity of the corresponding transition, and with this knowledge, the bands that appear in the spectrum can be assigned to corresponding functional groups (Atkins et al., 2022; Wöhrle et al., 2012).

There are a variety of photophysical processes in which the electronic state changes due to light absorption, which can be represented in the Jablonski diagram (Figure 14). The radiating processes are either fluorescence or phosphorescence. Fluorescence is a process from an S_1 state to S_0 with the retention of multiplicity by emitting a photon. Phosphorescence is an electron transition from a T_1 state in S_0 while changing the multiplicity by emitting a photon.

In cases where the electron is transferred into a second excited singlet state (S_2), the electron cannot directly reach to S_0 through the fluorescence process (Figure 13). Deactivation takes place via non-radiation processes such as internal conversion (IC) or intersystem crossing (ISC) (Diem, 2021; Wöhrle et al., 2012). IC is a non-radiative process between states with equal multiplicity (Turro et al., 2009; Coyle, 1991). The energy difference between the transition from S_2 to S_1 is there converted into vibrational energy or heat released into the system. This can promote the thermal reaction in the system which can lead to the formation of radicals (Diem, 2021; Wöhrle et al., 2012).

Another non-radiative transition is the ISC (Figure 13), where the electron performs a spinflip and follows the transfer from the S_1 into the T_1. In the T_1 state, the electron is usually metastable. Due to the effort to reach the energy minimum in the system, the corresponding electron from T_1 tries to transfer to the S_0. The light emitted during this process is phosphorescence. Phosphorescence usually has a lower energy (longer wavelength) than the absorbed radiation. The energy difference thus released in the system can trigger, influence, or stabilize many possible reactions. However, the molecules in the T_1 state can also initiate photocatalytic reactions under the cleavage of a photon (Diem, 2021; Latscha et al., 2008). This molecule can be considered as a radical (Turro et al., 2009; Coyle, 1991).

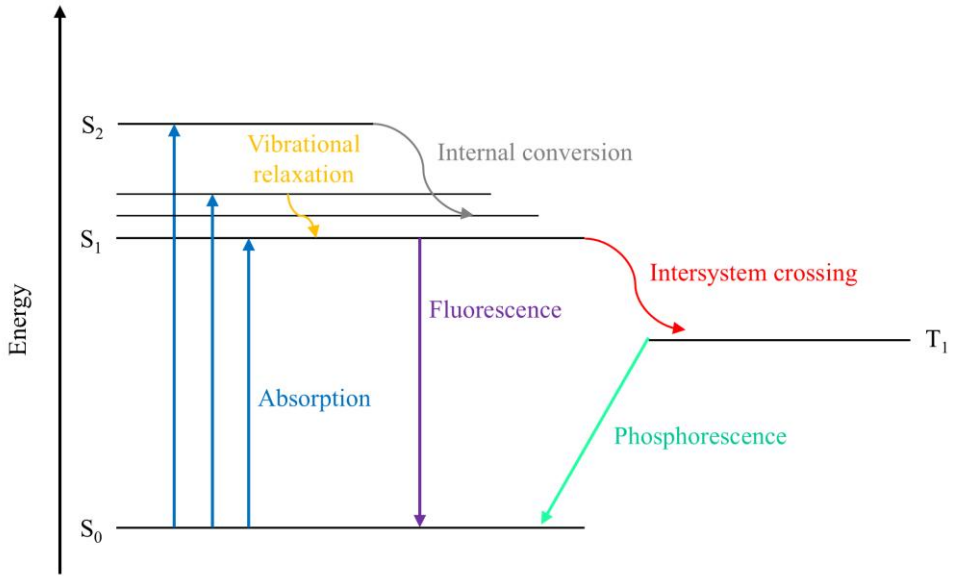


***Figure 13**: Jablonski diagram (Balzani et al., 2024).*

2.3.2 Light-induced reactions in wine

The interaction of the wine with light can significantly change its sensory properties and stability. Photochemical reactions can promote undesirable changes in wine, such as the reduction of aroma-active compounds, color changes (Benítez et al., 2005; Dias et al., 2012), formation of off-flavors (Fracassetti et al., 2021), and changes in the phenolic profile (Oliveira et al., 2011).

One of the best-known reactions associated with the negative influence of light on wine is the formation of the off-aroma "light-struck taste" or "Goût de Lumiere" (Dozon & Noble, 1989). The light-struck taste is promoted by exposure to light between 370 and 450 nm and is responsible for the formation of undesired flavors in wine, such as cooked cabbage, onion, and rotten eggs (D'Auria et al., 2009). This involves the formation of methionine through a photo-oxidation reaction sensitized by RF (Figure 14). Previous studies reported that the formation of a light-struck taste in wine significantly increases with the concentration of RF in wine over 50 - 80 µg/L (Mattivi et al., 2000). Photo-oxidation can occur via two reaction mechanisms: type I and type II. In type I, there is a reaction between RF in T_1 and methionine. In a type II reaction, O_2 is converted from its ground state to an excited singlet state ($^1O_2^*$) and RF from T_1 to S_0. $^1O_2^*$ reacts directly via non-radical reactions with electron-rich compounds such as methionine. Methionine contains a sulfur atom with four non-bonding electrons that are rapidly attracted to $^1O_2^*$ (Fracassetti et al., 2021; Remucal & McNeill, 2011). RF-catalyzed photooxidation of methionine leads to the formation of methional, which further reacts to methanethiol via the retro-Michael reaction and is subsequently degraded to dimethyl disulfide. The odor threshold of methanethiol in white wine is between 0.3 - 0.5 µg/L (Siebert et al., 2010), methional - between 1 - 10 µg/L (Siebert et al., 2010), and dimethyl disulfide - between 30 - 40 µg/L (Fracassetti, Limbo, et al., 2021).

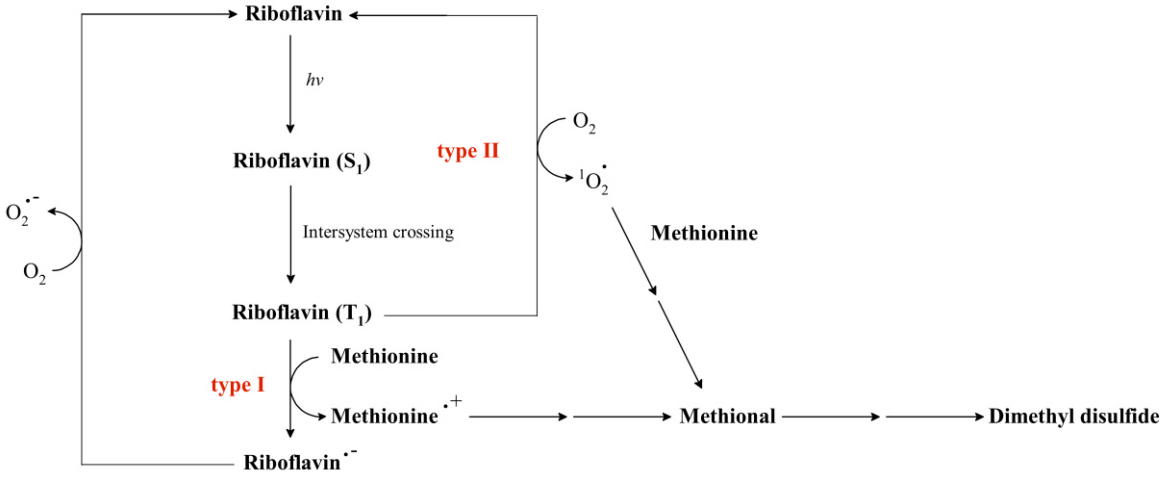


***Figure 14**: The photo-oxidation of methionine catalyzed by riboflavin (Filipe-Ribeiro et al., 2021).*

RF plays a significant role as a photosensitizer by the formation of methional in wine as well as in other photochemical reactions. RF is formed during alcoholic fermentation and serves as a precursor for the coenzymes flavin mononucleotide and flavin dinucleotide, which are significant for cell redox reactions. The concentration of RF in wine varies between 200 and 320 µg/L depending on the used strain of *Saccharomyces cerevisiae* (Di Canito et al., 2023). It has an absorption maximum at 225, 275, 370 and 450 nm and exists in cationic form at low pH (< 4.0) (Orlowska et al., 2012; Drössler et al., 2002).

In its cationic form, RF is non-fluorescent. The photosensitizing property of RF is well-known. Light exposure promotes the transition of RF from the ground singlet state to a short-lived excited singlet state ($^{1}RF^{*\cdot}$). Through the isoenergetic, radiationless intersystem crossing (ISC) process, with a quantum yield of 0.67, a transition to the excited triplet state of RF ($^{3}RF^{*}$) is achieved. $^{3}RF^{*}$ is a biradical that is a powerful oxidant with a standard electrode potential of +1.7 V vs. NHE (Lu et al., 2000). From this step, a distinction is made between type I and type II RF-photosensitized reactions (Figure 15). In type I, the direct interaction between $^{3}RF^{*}$ and a molecule takes place, which leads to the formation of $^{2}RF^{\cdot-}$ and a molecule radical. After that, $^{2}RF^{\cdot-}$ returns to its ground singlet state by being oxidized by O_2, forming $O_2^{\cdot-}$. Subsequently $O_2^{\cdot-}$ may form H_2O_2 or $OH^{\cdot}$. The deactivation of $^{3}RF^{*}$ by reducing the substance at type I reaction can be achieved through electron transfer, hydrogen atom transfer, and step-wise proton-coupled electron transfer processes dependent of thermodynamic properties of $^{3}RF^{*}$ and substrate properties as standard electrode potential, pK_a and bond dissociation enthalpy. In the type II reaction, deactivation of $^{3}RF^{*}$ can be achieved through energy transfer from O_2, leading to the formation of singlet-excited oxygen $^{1}O_2^{*}$. Due to the electrophilic properties of $^{1}O_2^{*}$, it additionally reacts with substances by adding to electron-rich sites on the substance, such as double bonds (Cardoso et al., 2012).

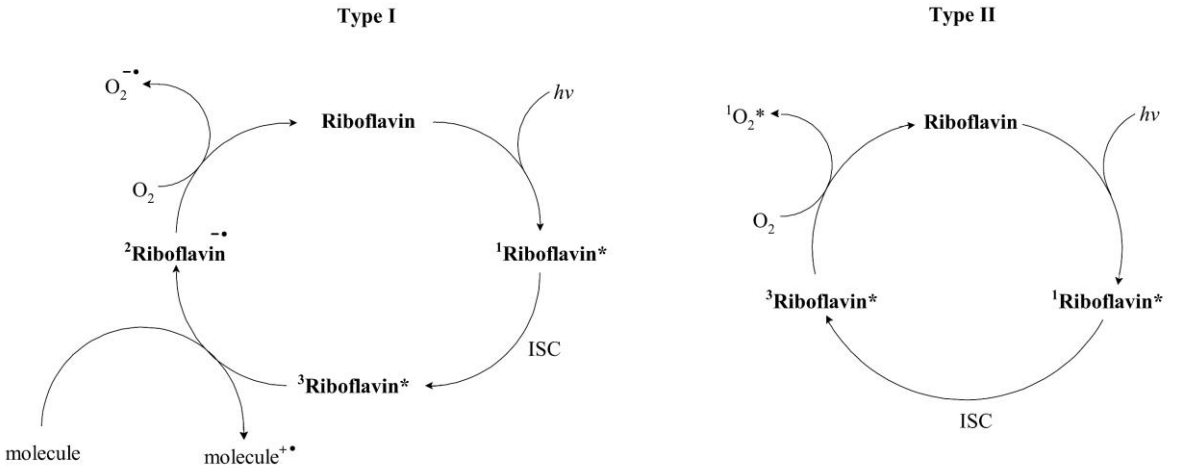


***Figure 15**: Photosensitized riboflavin reactions type I and type II (Cardoso et al., 2012).*

Despite its property as a photosensitizer, RF, under light treatment, can degrade via intramolecular photoreduction, photoaddition, or photodealkylation into various degradation products such as lumichrome, lumiflavine, carbocymethylflavin, or cyclodehydroriboflavin (Figure 16). $OH^{\cdot}$, $O_2^{\cdot-}$ or $^1O_2^*$ formed by photosensitized process contribute to the degradation of RF. Parameters such as pH, the presence of transition metals, temperature, light sources and oxygen concentration can affect the degradation rate and resulting degradation products (Sheraz et al., 2014). Depending on the pH of the solution, the major degradation product of RF in acidic conditions is lumichrome. In contrast in alkaline conditions, it degrades to lumiflavine via formylmethylflavin as an intermediate. The rate of photolysis of RF depends on its ionization states and susceptibility to excitation (Ahmad et al., 2004). Substances that favor the type I RF-photosensitized reaction under low oxygen conditions protect RF against degradation by $O_2^{\cdot-}$ (Jung et al., 2007; Cardoso et al., 2012).

In addition to the formation of off-flavors, light can promote the degradation of aroma-active substances in wine. Kim et al. (2021), Golombek et al. (2019) and Grant-Preece et al. (2017) have shown that UV-VIS treatment can lead to the degradation of the following classes of volatiles, such as esters, high alcohols, terpenes, and C13-norisoprenoids. Cellamare et al. (2008) have shown that the presence of RF induces the photodegradation of esters in model wines. The exact photochemical degradation of the ester in wine is not yet fully understood, but it could be acidic ester hydrolysis, where carboxylic acid and alcohol are formed (Ramey & Ough, 1980). Light-induced degradation of other classes of substances could affect various photochemical and oxidative reactions triggered by light. Light treatment can promote the formation of the ROS, which in turn can react with the double bond of molecules and for example, to the formation of ketone. Golombek et al. (2019) have shown the formation of unknown degradation products of linalool and ß-damascenone in model wine in the presence of RF after UV-C treatments.

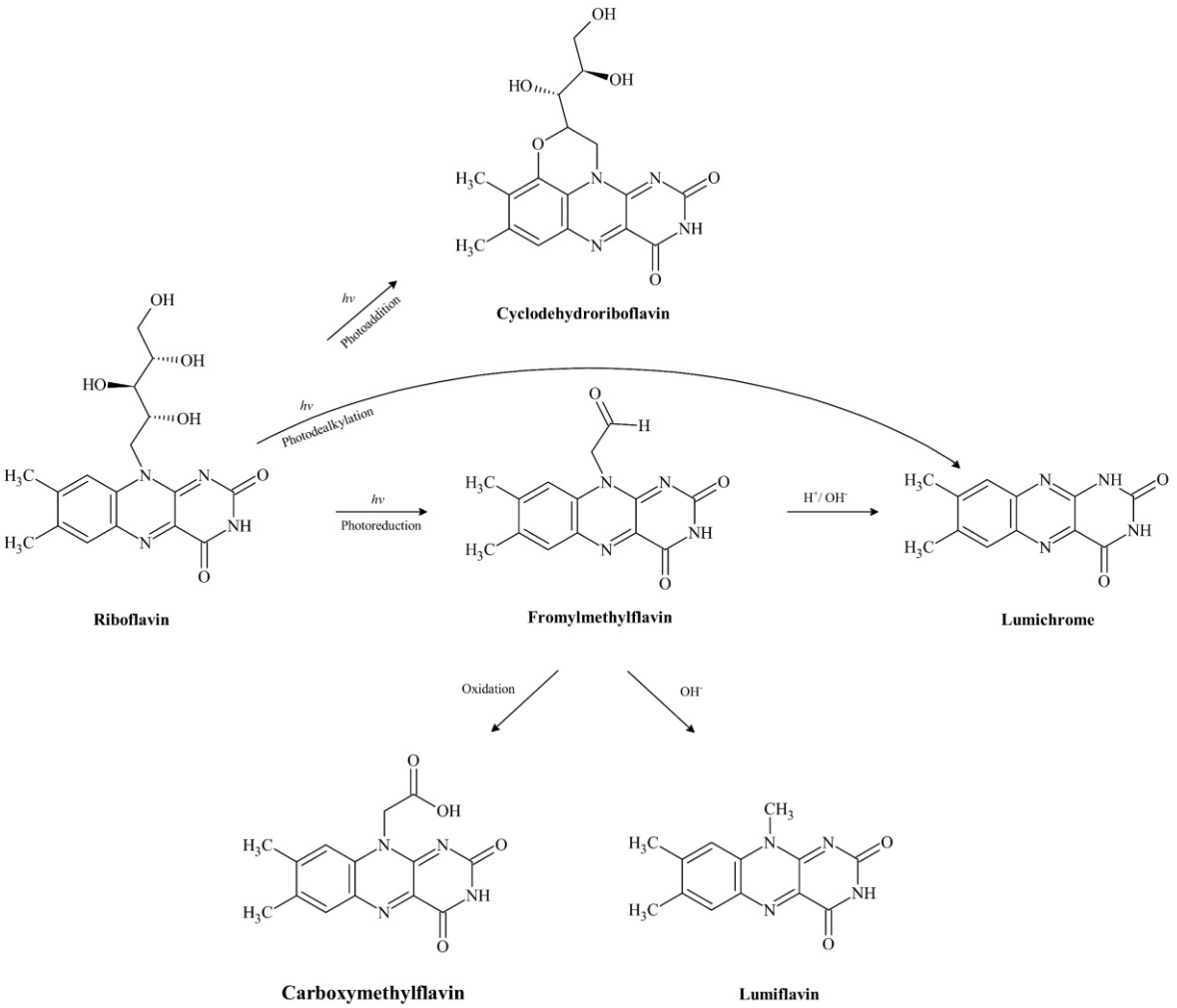


***Figure 16**: Schema of the photodegradation of riboflavin in aqueous solution (Sheraz et al., 2014).*

Furthermore, light can promote significant color changes in wine. Light treatment can promote the formation of yellow xanthylium cation pigment in wine (Figure 17) (Dias et al., 2013). The formation of xanthylium cation pigment occurs through a reaction between catechin and glyoxylic acid, which was previously formed by the oxidative degradation of tartaric acid by the interaction with light (Grant-Preece et al., 2017). Furthermore, it has been shown that transition metals, especially iron, can promote the formation of the xanthylium cation pigment due to their oxidative potential (George et al., 2006).

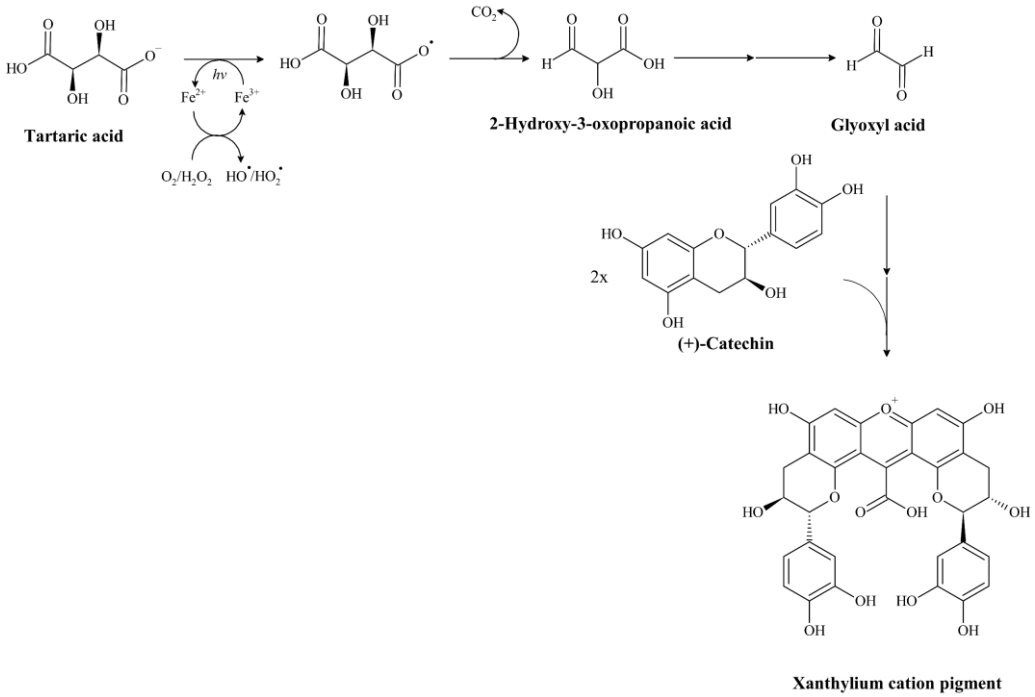


Figure 17*: Light-induced formation of xanthylium cation pigment in the presence of transition metals (George et al., 2006; Es-Safi, 2001; Grant-Preece et al., 2017).*

The ROS formed by a light reaction can promote a variety of other reactions, including the formation of quinone through the oxidation of phenols. Quinones are reactive substances that can react with various nucleophilic substances such as other phenols, thiols, or amino acids (Figure 18) (Oliveira et al., 2011; Waterhouse & Nikolantonaki, 2015). The reaction rate of phenolic compounds with reactive oxygen species depends on their ability to form a stable product radical. Oligomeric and polymeric phenolic compounds (procyanidins and condensed tannins) can react with ROS in a similar way to monomeric phenols (Waterhouse & Laurie, 2006). These reactions can lead to significant changes in the olfactory perception as well as in the color of the wine (Fulcrand et al., 2006).

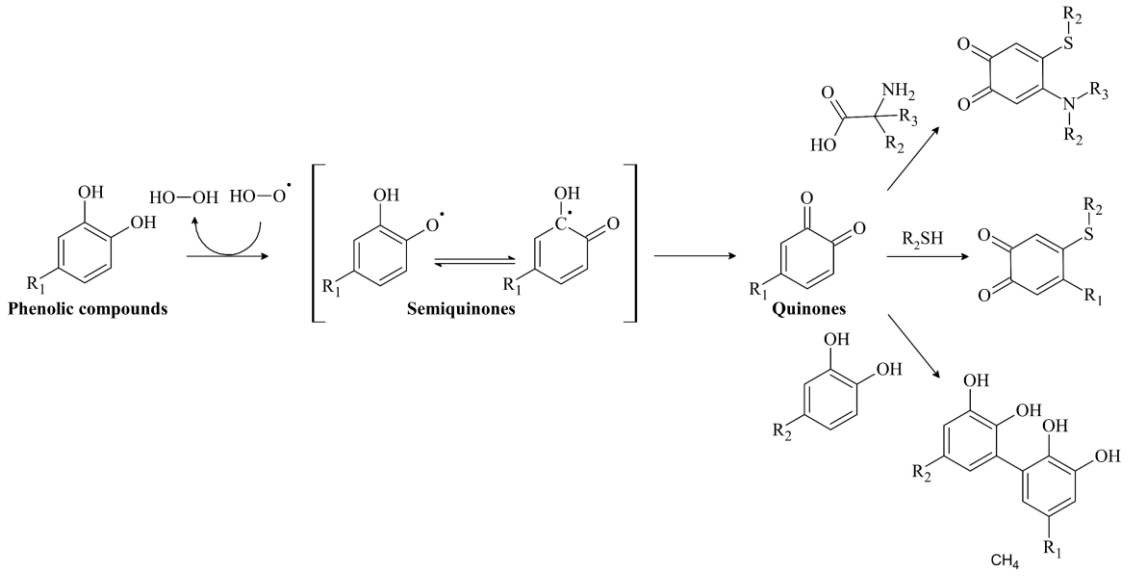


Figure 18*: Formation of quinones and subsequent reaction with phenols, thiols, and amino acids (Waterhouse & Nikolantonaki, 2015).*

3. Scope and aim

The wine industry currently faces challenges due to climate change and has to deal with safety concerns regarding the consumption of problematic ingredients such as alcohol and sulfites. UV-C technology is a chemical-free, non-thermal method of microbial stabilization with a wide range of applications, from water treatment to the food industry (Koutchma et al., 2009). Various studies demonstrated the potential of UV-C technology for microbial stabilization of wine (Fredericks et al., 2010; Junqua et al., 2020). This could avoid or reduce the use of SO_2 in wine production. Numerous aspects, such as product composition, technological parameters, and the resistance of harmful microorganisms, influence the effectiveness of the technology (Atilgan et al., 2020). Investigations on the chemical and sensory properties of UV-C treated wine due to its high-energetic electromagnetic irradiation must reveal the boundaries of this technology. A deterioration in wine quality after applying the technique could significantly limit the use of UV-C technology. In particular, the study by Golombek et al. (2020) showed that an overdose of UV-C in grape musts could cause the off-flavor 2-AAP in the produced wine. Most studies are limited to the examination of the basic parameters of the wine after UV-C treatment. No studies were currently available on the use of UV-C technology in terms of determining influencing factors that impact the effectiveness of UV-C. Also, a valid evaluation of the impact of different UV-C doses with regard to the impact on the chemical and sensory properties of wine was missing. Therefore, the first part of this dissertation was dedicated to the question of whether UV-C treatment can efficiently inactivate wine-relevant harmful microorganisms, what parameters can influence the effectiveness of the process, and how the different UV-C doses can affect the chemical and sensory properties of wine.

A major negative effect of light on wine was photo-induced oxidation. Oxidation is one of the main phenomena that can affect the quality of wine. This process facilitates interactions with oxygen and transition metals, such as iron, promoted by light and temperature (Macías et al., 2001). Studies showed the effectiveness of adding antioxidants in wine, such as glutathione, ascorbic acid, tannins, and SO_2, which can scavenge radicals and accordingly prevent oxidation processes. Fracassetti et al. (2019) emphasized the effectiveness of hydrolyzable tannins and SO_2 in preventing light-induced oxidation reactions, such as the formation of methional, known by the term "light-struck taste". SO_2 plays a crucial role in preventing oxidative processes in wine due to its ability to scavenge hydrogen peroxide and react with saturated aldehydes like acetaldehyde (Elias & Waterhouse, 2010; Grant-Preece et al., 2017). Furthermore, hydrolyzable tannins have a high antioxidant potential considering their ability to provide hydrogen or electrons or form chelation complexes with metals (Magalhães et al., 2014; Vignault et al., 2018). The use of enological tannins is recommended by the International Organization of Vine and Wine (OIV) to stabilize wine (Resolution OIV OENO 624-2022). The second part of the dissertation focused on investigating different antioxidants to mitigate the oxidative effects possibly enhanced by UV-C treatment.

The term "atypical aging" is often referred to the substance 2-AAP. It is an off-flavor associated with sensory attributes such as naphthalene or mothballs. Its sensory threshold is between 0.5 µg/L and 1.5 µg/L (Christoph et al., 1995). The best-known reaction pathway is the radical co-oxidation of sulfites after fermentation and during wine storage, in particular through the degradation of indole-3-acetic acid (Christoph et al., 1998; Dollmann et al., 2015; Hoenicke,

Borchert, et al., 2002). A less known formation pathway of 2-AAP is through the light-induced degradation of TRP promoted in the presence of RF (Horlacher & Schwack, 2014). The concentration of TRP and RF in wine can be significantly varied by such parameters as grape variety and strain of *Saccharomyces cerevisiae* used during alcoholic fermentation (Di Canito et al., 2023; Hoenicke et al., 2002). Based on this, UV-C treatment needs to be investigated in relation to the formation of 2-AAP as a critical factor. A limited number of publications that describe the formation of 2-AAP via RF-photosensitized reaction were available, and restrictive factors of the reaction are widely unknown. Morozova et al. (2013) suggested, based on the sensory evaluation, that transition metals have an impact on the formation of 2-AAP in wine. Transition metals are strongly associated with the Fenton reaction, in which acetaldehyde is formed as a consequence of ethanol oxidation promoted by transition metals. Oxygen concentration plays a major role in the Fenton reaction (Elias & Waterhouse, 2010). Based on this, the impact of oxygen and transition metals on the formation of 2-AAP via RF-photosensitized reaction was investigated in the fourth part of the dissertation in order to elaborate a concept for mitigating the formation of 2-AAP during UV-C treatment.

A further focus of this dissertation was the investigation of the long-term microbial stability of UV-C-treated wine during a 12-week storage period. Many wine bottles are stored for months or even years to gain in flavor, texture, and complexity. This makes it essential that a microbial stabilization method can guarantee long-term protection. A re-increasing cell count of microorganisms in the wine during storage may result from the complex mechanisms of bacteria or yeast cells, which can repair damage caused by UV radiation (Goosen & Moolenaar, 2007; Terleth et al., 1989). Therefore, it was examined whether UV-C doses that inactivate microorganisms also lead to sufficient irreparable damage of the DNA in order to guarantee the long-term preservation of wine.

The wavelength of 254 nm is the standard wavelength in UV-C technology. This wavelength is close to the absorption maximum of nucleic acids at ~260 nm. The light energy promotes the formation of pyrimidine dimers of DNA and RNA. This damage to the DNA or RNA structure prevents the replication process of microorganisms. Early technological developments have focused on mercury-vapor lamps that provide high-energetic monochromatic light at 254 nm. The availability of this technology has contributed to the fact that this wavelength has become the standard wavelength in UV-C technology (Koutchma et al., 2009). Nevertheless, some studies revealed that not only 254 nm have high germicidal efficacy against harmful microorganisms. For example, some research showed the potential of using a wavelength of 280 nm instead of 254 nm, allowing LEDs to be used as light sources instead of mercury-vapor lamps. They are environmentally friendly, mainly since the use of mercury has been severely limited since 2017, in accordance with Regulation (EU) 2017/852 based on the Minamata Convention. Accordingly, a further aim of this dissertation was to investigate if an application at 280 nm could be used as an alternative method for microbial inactivation.

4. Cumulative part of the dissertation

4.1 UV-C treatment: A non-thermal inactivation method for microbiological stabilization of must and wine

To evaluate the possible applications of UV-C technology for the microbial stabilization of wine, a study was carried out in which increasing UV-C doses were applied to inactivate common harmful microorganisms in red and white wine of the grape varieties Riesling and Pinot Noir. After the UV-C treatment, the wines were subjected to microbial, chemical, and sensory analyses.

The results showed that UV-C treatments could successfully inactivate all evaluated harmful microorganisms in wine. The comparison of the 5-log inactivation doses for each harmful microorganism showed that the microbial effectiveness of UV-C treatment is influenced by factors such as wine absorption, cell count, and the type of harmful microorganism. Chemical investigations showed that the increasing UV-C dose led to a change in wine color and a decrease in aroma-active substances in Riesling and Pinot Noir wines. It is essential to point out that the chemical changes in white wine occurred at significantly lower UV-C doses than in red wine. This indicates a higher sensitivity of the white-grape wine to UV-C treatment, which can probably be explained by the lower antioxidant capacity of white grapes. In addition, UV-C treatment has been found to promote the formation of 2-AAP and the decrease in total phenolic content in Pinot Noir wine. However, the sensory investigations showed that only from a UV-C dose, which is twice as high as that for a microbially relevant 5-log inactivation dose, a significant influence of the off-flavors was sensory noticeable. The effect of UV-C treatment has also been observed at microbial-relevant doses, mainly due to the increasing formation of aging aromas, indicating the acceleration of the oxidative process in wine.

The application of UV-C technology for the microbial stabilization of wine showed promising results. Due to detectable changes in the aroma profile at microbial-relevant doses, further investigations should be carried out to decrease wine sensitivity. Adding antioxidants could represent the possibility of mitigating the oxidative effect of UV-C treatment by increasing the wine's antioxidative capacity.

UV-C treatment: A non-thermal inactivation method for microbiological stabilisation of must and wine

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Disclaimer:

The presented work was exclusively performed at the DLR Rheinpfalz (Svetlana Cvetkova and master thesis Nadezda Shirokova).

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43rd World Congress of Vine and Wine

UV-C treatment: A non-thermal inactivation method for microbiological stabilisation of must and wine

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Abstract. UV-C treatment is discussed as an effective and efficient method to inactivate harmful microorganisms in wine and other viticultural products. In comparison to other stabilisation techniques, the application of UV-C is thought to be beneficial to reduce energy costs and to minimize SO_2 addition. The object of this work was to determine the lethal UV-C dose for harmful microorganisms such as *Brettanomyces bruxellensis* and *Acetobacter aceti*. The concept of 5-log inactivation was applied and the Weibull model was used to compare different microbial and wine parameters. Microbial relevant UV-C doses and 2-fold overdose treatments and how they affected chemical and sensory changes of wine were investigated. Riesling and Pinot noir wine, which have different absorbance at 254 nm, were individually inoculated with microorganisms at different inoculation numbers. The results showed that the Weibull model is appropriate to predict the lethal UV-C dose. Already at microbially relevant doses, UV-C treatment can lead to significant changes in the colour and concentration of aroma compounds in white wine. Higher concentrations of 2-aminoacetophenone were found with increasing UV-C doses. Hence, UV-C overdosing can cause the "atypical ageing" off-flavour in wine. However, microbially relevant UV-C doses change the sensory properties of wine more towards a typical ageing character.

1 Introduction

UV-C treatment is a modern method to inactivate microorganisms in drinking water, on surfaces, in the air and in different foods. Nevertheless, it is not yet used in the wine industry. The main principle of microbiological stabilisation by UV-C is based on Grotthus Draper's law [13]. During treatment, UV-C induces a series of photochemical reactions that cause the breakage of DNA, consequently suppressing cell reproduction [27]. In previous research, it was found that UV-C treatment can be used to inactivate microorganisms in grape must without significantly changing its olfactory and gustatory properties [6]. Another field of application is the inactivation of microorganisms in wine. The predominant methods for microbiological stabilisation in winemaking are the use of sulfur dioxide (SO_2) and high-temperature short-time pasteurization, both of which have their disadvantages. SO_2 is a suspected allergen and is thus increasingly the subject of public scrutiny. Pasteurisation is associated with high energy costs and can change wine's aroma. A possible alternative for the microbiological stabilisation of wine can be the use of UV-C.

Different microorganisms have different UV-C sensitivity [6] and therefore require different lethal UV-C doses. According to 21 CFR 179 [28] the lethal UV-C dose corresponds to a 5-log inactivation of the microorganisms based on the US FDA's 5-log requirement for pathogenic microorganisms that can grow in fruit juices. Factors, such as cell wall thickness, structure, and variation in nucleic acid configuration affect the susceptibility of microorganisms to UV-C treatment [26]. In addition, process parameters such as reactor type, treatment time and temperature [2], as well as the wine properties, such as absorbance and composition have an influence on the inactivation efficacy [11]. Hence, it is important to use a mathematical model that can be comprehensively applied for different microorganisms, process parameters, and wine properties. Atiglan et al. [1] discussed the kinetics of UV-C irradiation in foods and recommended different mathematical models to be used for different foodstuff. Based on the recommendations of Atiglan et al. [1], Hirt et al. [11] carried out a series of experiments with *Saccharomyces cerevisiae* in model wines and real wines considering different conditions and found that the best mathematical model describing the inactivation kinetics in wine was the Weibull model (Eq. (1)).

$$log\left(\frac{N}{N_0}\right) = -\left(\frac{D}{\delta}\right)^p \quad (1)$$

The Weibull model is a non-linear model. This model has two kinetic parameters: p – is shape parameter and describes the concavity ($p > 1$ downward concavity, $p < 1$ upward concavity and $p = 1$ curve is linear) of the inactivation curve, δ – is the scale parameter which describes the first decimal reduction doses of surviving cells, D – is the lethal dose of microorganisms, N – is the number of viable microorganisms in wine and N_0 – is the

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inoculation number of microorganisms in wine. It is thought, that the Weibull model is also applicable for other microorganisms in wine apart from *Saccharomyces cerevisiae*.

Wine is characterized by a complex matrix which contains a high number of substances. A good portion of these substances is able to absorb UV and/or visible light, Hence, UV- treatment can potentially lead to a large number of reactions in the wine such isomerization, rearrangement or couplings reactions which in turn can change the sensory, gustatory as well as olfactory characteristics of the wine. In general, UV light is known to promote polymerization reactions of polyphenols which can cause no-enzymatic browning of wine [14]. Furthermore, UV light can degrade aroma active substances such as monoterpenes through a photo-oxidative reaction [21]. However, UV-C treatment promotes not only the degradation of substances, but also the formation of aroma active substances such as 2-Aminoacetophenone (2-AAP) through a photosensitized reaction by riboflavin [9]. Odour of the 2-aminoacetophenone associated with so-called "untypical ageing" off-flavour in wine. [12]

The objective of this study was to determine the lethal UV-C doses (5-log inactivation) for different harmful microorganisms in wine. Riesling and Pinot noir wines were used considering different wine properties in respect to their absorbance. Microbial contamination was regarded in a worst case and realistic scenario by means of different inoculation numbers. For a commercial application of UV-C in the wine industry, it is necessary to define sufficient UV-C doses for the inactivation of harmful microbes, while not changing the sensory properties of wine. Therefore, this study also investigated UV-C induced changes of chemical and sensory properties in Riesling and Pinot noir in the UV-C range that is relevant for the inactivation of harmful microbes. Colour characteristics, total phenols, aroma active compounds, and sensory characteristics were examined and discussed in relation to the required UV-C dose.

2 Material and method

Riesling wine with an absorbance of 12.5 at 254 nm and Pinot noir wine with an absorbance of 30.0 at 254 nm, produced in 2019 at the DLR Rheinpfalz (Neustadt an der Weinstraße, Germany), were used for this study. The wines were processed after fermentation without the use of SO_2. Experiments were carried out using a pilot-scale thin-film reactor with flow control elements developed at the Max Rubner-Institut (Karlsruhe, Germany). The reactor consists of a 20 W low-pressure mercury lamp surrounded by quartz glass with maximum peak radiation at 254 nm (UV-Pro N 36-2, otca GmbH, Kürten, Germany), placed in the centre of a steel tube. Flow guide elements with a gap width of 0.3 mm are located between the lamp and the steel tube. Wine was pumped through the device with a peristaltic pump (Pump drive Pd 5206, Heidolph, Schwabach, Germany) at a flow rate of 100 L/h. Increasing UV-C doses (in steps of 0.2 kJ/L) were obtained by recirculating wine through the reactor multiple times. To exclude an influence of oxygen ingress during the process, control runs without irradiation (pump controls) were conducted and compared to UV-C treated wines at respective UV-C doses. The UV-C doses were determined using a previously described chemical actinometry method based on potassium iodide/iodate [18].

The following microorganisms were used for the experiments: *Brettanomyces bruxellensis, Dekkera bruxellensis, Brettanomyces custerianus, Pediococcus sp., Acetobacter aceti*, and *Lactobacillus brevis.* The microorganisms were inoculated separately. The inoculation number was 10^6 cfu/ml or 10^3 cfu/ml. All experiments were carried out in triplicate. Microbiological analysis was performed in 10^{-5} dilutions (in 0.9% NaCl solution) using YPD medium with chloramphenicol and kanamycin for yeasts, and MRS and YPM medium with cycloheximide for bacteria. The agar plates were incubated at 25°C for 7 days, after which colonies were counted.

For chemical and sensory tests, wines were treated with three different UV-C doses: Riesling with 0.8, 1.4, and 3.0 kJ/L and Pinot noir with 1.6, 2.8, and 6.0 kJ/L. Analysis of aroma compounds in the Riesling wine was done by a headspace solid phase microextraction (HS-SPME) GC-MS method, using a 30 m × 0.25 mm i.d. fused silica capillary column ZB-Wax [9]. Analysis of aroma compounds in the Pinot noir wine was done by a HS-SPME-GC×GC-qMS method [19]. The GC×GC-qMS data were evaluated using an image-based method [22]. 2-AAP was measured by HS-SPME MDGC-MS/MS using a 30 m × 0.25 mm i.d. fused silica capillary column StabilWaxMS [9]. Photometric analysis was performed according to OIV-MA-AS2-11 [17] using a Varian Cary100 spectrometer. Total phenolic content was determined using the Folin-Ciocalteu method, as described by OIV-MA-AS2-10 [16], using an Arena Konelab 20i (Thermo Fisher Scientific, Watham, USA). Descriptive sensory analysis was carried out by 15 judges according to DIN 10969. All statistical analyses were performed using XLSTAT (Version 19.02, Addinsoft, France). Statistical calculations were performed using analysis of variance and Tukey-Kramer post-hoc test ($\alpha = 0.05$).

3 Result and Discussion

3.1 UV-C inactivation kinetics of yeast and bacteria in wine

Figure 1 shows the inactivation of *Dekkera bruxellensis* with increasing UV-C doses for different inoculation numbers in Riesling and Pinot noir. *Dekkera bruxellensis* is yeast and was chosen as an example for a deteriorative microorganism producing a common off-flavour in wine known as 'horse sweat'. This yeast is globally considered to be one of the main causes of wine spoilage [21].

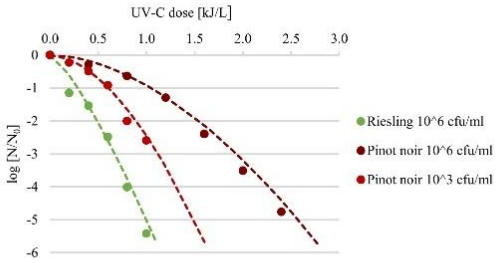

Figure 1. Inactivation kinetics of *D. bruxellensis* with increasing UV-C doses for different inoculation numbers in Riesling and Pinot noir wine. The dotted curves show the inactivation kinetics as determined by the Weibull model.

The results show that the inactivation curves are not linear and have a downward concavity. This model was used in this study for the determination of the lethal dose for all inoculated deteriorative microorganisms.

3.2 UV-C efficacy of yeast and bacteria inactivation in wine

Figure 2 shows the lethal UV-C dose for six different, frequently occurring, deteriorative microorganisms in Riesling and Pinot noir wine. The inoculation number in Pinot noir was 10^6 cfu/ml and 10^3 cfu/ml.

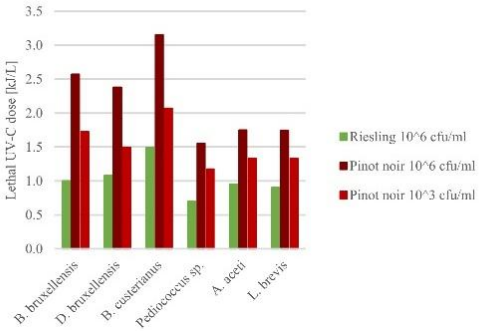

Figure 2. Lethal inactivation doses for different microorganisms inoculated as single cultures with inoculation number 10^6 cfu/ml in Riesling wine and inoculation number 10^6 cfu/ml and 10^3 cfu/ml in Pinot noir wine.

The results show that all inoculated microorganisms required lower lethal doses in Riesling than Pinot noir at the same inoculation number. This difference can be explained by the different absorbance values in wine. The increasing absorption of the wine is linked to the more complex matrix in Pinot noir. This in turn leads to the fact that the increasing concentration of the substance at the absorption of 254 nm leads to the increasing absorption of the UV-C and thus the decrease of inactivation efficacy of UV-C treatment [10]. Furthermore, it could be observed that yeasts are more UV-C resistant than bacteria. Yeasts have less pyrimidine base and different cell membranes and thicknesses which causes less UV-C sensibility [26]. In addition, results indicate that the same microorganisms in the same wine but with different inoculation numbers required different lethal doses. The reason for this observation is that the increasing number of microorganisms in the medium leads to an increase in the shielding/shadowing effects [1]. However, not only these factors have an effect on inactivation efficacy. Gayan et al. [8] pointed out that also the growth state and the tendency of agglutination are important factors influencing the microbial resistance.

3.3 Influence of UV-C treatment on colour in Riesling and Pinot noir

Tables 1 and 2 show the influence of UV-C treatment on CIE L*a*b* coordinates of the treated and untreated Riesling and Pinot noir wine.

Table 1. L*, a*, and b* values of UV-C treated and untreated Riesling wine. Different letters represent significant differences ($p \leq 0.05$) between the samples.

Riesling			
UV-C dose [kJ/L]	L*	a*	b*
0.0	98.1 ±0.06 c	-1.3±0.02 c	11.3±0.02 a
0.8	98.7±0.02 a	-0.9±0.02 b	9.6±0.02 b
1.4	98.4±0.04 b	-1.0±0.01 b	9.3±0.04 d
3.0	98.0±0.05 c	-0.8±0.01 a	9.5±0.02 c

Table 2. L*, a*, and b* values of UV-C treated and untreated Pinot noir wine. Different letters represent significant differences ($p \leq 0.05$) between the sampls.

Pinot noir			
UV-C dose [kJ/L]	**L***	**a***	**b***
0.0	28.7±0.01 d	9.8±0.02 a	1.5±0.01 c
1.6	29.4±0.01 b	9.0±0.01 b	1.7±0.01 b
2.8	30.1±0.01 a	8.2±0.02 d	1.7±0.01 b
6.0	29.0±0.01 c	8.4±0.01 c	2.8±0.02 a

The results show that the UV-C treatment leads to significant changes in the colour coordinates L*, a*, and b* of both wines. Colour decreases were observed for the yellow and green hues in the Riesling wine and for the red and yellow hues in the Pinot noir wine. The decrease of yellow and green hue in Riesling wine can be explained by a possible UV induced degradation of carotenoids and chlorophyll [23]. Overall, UV-C treatment of Riesling caused a colour loss. The effect of colour changes in Pinot noir wine can be explained by non-enzymatic browning reactions through the photo-induced oxidation of polyphenols and the following formation of pigments, for example the production of xanthylium cations by the reaction between flavan-3-ols and glyoxylic acid [14]. Overall, UV-C treatment of Pinot noir caused browning of the wine. Colour differences became significant at 0.8 kJ/L in Riesling and at 1.6 kJ/L in Pinot noir. The Riesling colour changes correspond to one year of dark bottle storage at a temperature of 12°C [5].

3.4 Influence of UV-C treatment on polyphenolic compounds in Riesling and Pinot noir

Figure 3a and 3b show the influence of UV-C treatment on total phenols of the treated and untreated Riesling and Pinot noir wine. ANOVA revealed no significant decrease in total phenols concentration during UV-C treatment in Riesling. In Pinot noir, a dose of 1.6 kJ/L caused a significant decrease of polyphenol concentrations. The possible explanation of this observation could be the higher concentration of total phenols. Studies by Tahmaz and Söylemezoglu [25] revealed UV-C light as a promoter for the decrease of catechin and gallic acid and trans-resveratrol in red wines.

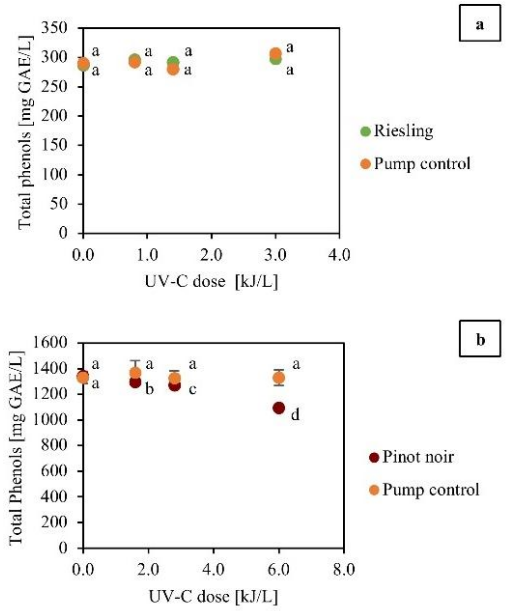

Figure 3. Total phenols of UV-C treated and untreated in Riesling (a) and Pinot noir (b) wine. Different letters represent significant differences ($p \leq 0.05$) between the samples.

3.5 Influence of UV-C treatment on selected aroma active compounds in Riesling and Pinot noir

Figure 4a and 4b depicts the concentration of aroma active substances, in detail an acetate and an ethyl ester, a monoterpene and a C6-alcohol in UV-C treated and untreated Pinot noir and Riesling wine. The results show decreasing aroma active substances in Riesling and Pinot noir wine during UV-C treatment. A significant decrease in ethyl octanoate and hexanol was observed in both grape varieties from the first treatment dose. From the dose of 3 kJ/L in Riesling and 6 kJ/L in Pinot noir wine, the measurements showed a significant decrease for all four substances. The decrease of aroma active substances can be explained by photo-induced reactions catalysed by riboflavin [4]. These changes correspond to one year of dark bottle storage of white wine at a temperature of 12°C [5].

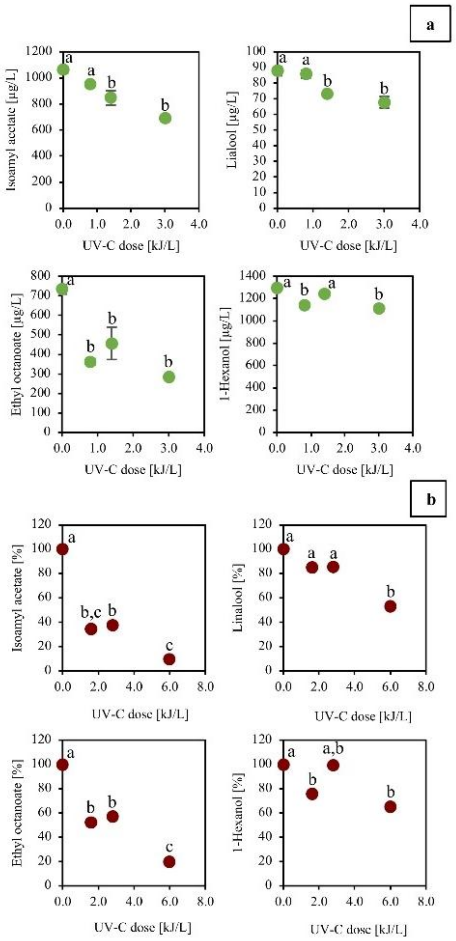

Figure 4. Isoamyl acetate, ethyl octanoate, linalool and 1-hexanol of UV-C treated and untreated Riesling (a) and Pinot noir (b) wine. The value of Pinot noir measurements was related to untreated samples. Different letters represent significant differences ($p \leq 0.05$) between the samples.

3.6 Risk of Off-flavour formation in Pinot noir

Figure 5 shows the influence of UV-C treatment on the production of 2-AAP in the treated and untreated Pinot noir wine. It is evident from that the increasing UV-C dose leads to an increasing concentration of 2-AAP. UV excites riboflavin to a singlet excited state. The photosensitized riboflavin generates singlet oxygen which further reacts with tryptophan and leads to formation of 2-AAP [12]. The published sensory threshold of 1.5 µg/L 2-AAP was exceeded with the dose of 2.8 kJ/L [3]. These tendencies could be confirmed on the basis of sensory analysis (see chapter 3.7).

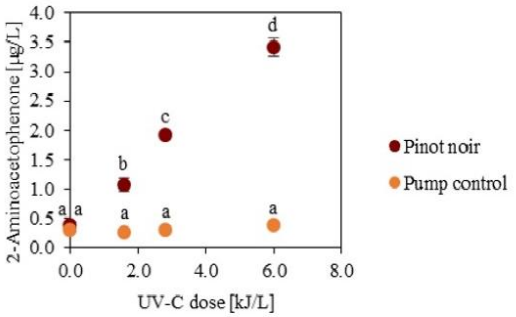

Figure 5. 2-AAP formation in Pinot noir wine during UV-C treatment. Different letters represent significant differences ($p \leq 0.05$) between the samples.

3.7 Influence of UV-C treatment on sensory properties in Pinot noir

Figure 6 shows the principal component analysis of descriptive sensory data of treated and untreated Pinot noir wine with different UV-C doses.

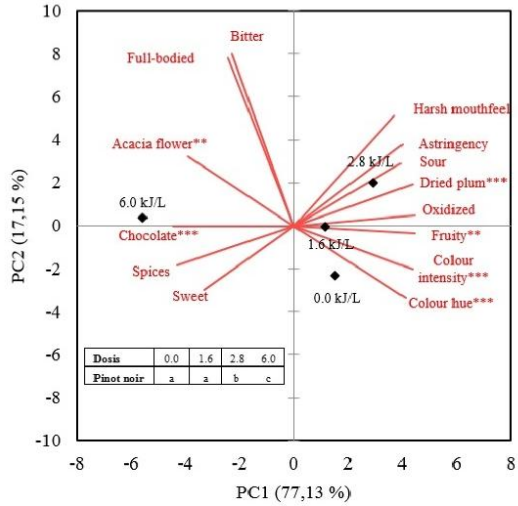

Figure 6. Principal component analysis of treated and untreated Pinot noir wine. UV-C doses were 1.6, 2.8 and 6.0 kJ/L. PCA space was calculated for fourteen sensory attributes.

** significant at $p < 0.01$, *** significant at $p < 0.001$.

UV-C treatment doses of 1.6 and 2.8 kJ/L changed the sensory profile towards 'dried plum' indicating a typical ageing effect of the wine. With 6 kJ/L, the wine's aroma was described by 'acacia flower' and less 'fruity' indicating an typical ageing effect of the wine. These findings are in agreement with the observations made earlier with increasing 2-AAP concentrations (chapter 3.6) and decreasing concentrations of esters, monoterpenes and C6-alcohols (chapter 3.5). Besides 'dried plum' and 'acacia flower', the sensory attributes 'colour intensity', 'colour hue', and 'chocolate' were significantly changed upon UV-C treatment with 6 kJ/L.

4 Conclusion and Outlook

The Weibull model was found to be appropriate to determine the lethal UV-C dose in realistic conditions. The principle to determine the lethal UV-C dose was successfully applied for different harmful microorganisms with different microbial contamination in different wines. It was observed that the lethal UV-C dose was influenced by the species of microorganism, inoculation number and absorption of the wine. The chemical and sensory studies have shown that even lower UV-C doses can lead to significant changes in the wine. Especially for the colour and the aroma compounds in white wine, UV-C treatment seems to cause changes already at doses lower than microbially relevant. Riboflavin in wine was found to have a high impact for the formation of 2-AAP during UV-C treatment. Sensory studies have shown that UV-C treatment can lead to changes in the sensory profile of the wine. However, a significant UV-C influence on off-flavours was only observed for higher UV-C doses than microbially relevant. For microbially relevant UV-C doses, the sensory profile of wine was shifted in the direction of wine ageing.

Further studies could apply the Weibull model for different wines and different conditions to validate the 5-log inactivation principle as proposed. Other harmful microorganisms that occur in wine due to climate change could be regarded. Further studies should also consider parameters like the growth stage of microorganisms and the wine pH since it is thought that these parameters have an influence for the determination of the lethal UV-C dose. The chemical and sensory studies have shown that wine is sensitive to UV-C treatment and even lower UV-C doses can lead to significant changes. For this reason, investigations should be carried out to determine whether the addition of protective substances such as ascorbic acid and tannins or oxygen and riboflavin reduction can minimize the influence of UV-C treatment on the wine.

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4.2 UV-C induced changes in a white wine: Evaluating the protective power of hydrolyzable tannins and SO_2

The publication in Section 4.1 has shown that the application of UV-C treatment led to the entire inactivation of all examined harmful microorganisms in wine. However, an increasing UV-C dose promotes oxidative processes in wine. These effects were already noticeable at microbial-relevant doses during the sensory analysis. The following part of this dissertation focused on the detailed evaluation of the impact of increasing UV-C dose on the Chardonnay wine in combination with the evaluation of the effectiveness of SO_2 and hydrolyzable tannins as antioxidants against the advancing oxidative process. The sensory evaluation has shown that an increasing UV-C dose led to an increase of oxidative processes in white wine. That was mainly reflected in a change in color intensity towards golden-yellow, as well as an increase in the olfactory attributes "burnt" and "oxidative." The chemical analyses have shown that UV-C treatment also causes an increase of 2-AAP and acetaldehyde concentration in white wine, as well as a decrease in the aroma-active substances of the higher alcohols, C13-norisoprenoids, monoterpenes, and esters. The increasing chemical effect of UV-C treatment was equivalent to the increasing UV-C dose.

Unexpectedly, adding SO_2 did not lead to the expected protective effect. Increased concentration of SO_2 significantly accelerated the odor attributes "burnt" and "oxidative" in wine. Hydrolyzable tannins have shown effective antioxidant protection due to their properties as strong proton donors. The presence of hydrolyzable tannins causes a significant decrease in the formation of acetaldehyde and 2-AAP in the white wine. The study has shown the possibility of using hydrolyzable tannins as a preventive agent against the oxidative effect of UV-C treatment, especially in cases where a higher UV-C dose than the 5-log inactivation dose is required or the wine already contains a certain amount of SO_2. A high concentration of SO_2 in the wine should be avoided to ensure wine quality.

UV-C induced changes in a white wine: Evaluating the protective power of hydrolysable tannins and SO_2

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Disclaimer:

The presented work was exclusively performed by the DLR Rheinpfalz (Svetlana Cvetkova and the gas chromatographic measurements were performed by chemical technicians Michael Wacker and Jutta Keiser). For more information, refer to the author contribution section.

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ORIGINAL RESEARCH ARTICLE

UV-C-induced changes in a white wine: Evaluating the protective power of hydrolysable tannins and SO_2

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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_2) 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_2 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_2, hydrolysable tannins, tryptophan, acetaldehyde

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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 stabilty, 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-oxidisation 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 xanthylium 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 xanthylium cation pigments in white wine under light exposure. Xanthylium 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_2 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-sensitised degradation (Cellamare *et al*., 2009), and that it can also degrade monoterpenes and C13-norisoprenoides (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 photosensitiser, 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-sensitised 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_2, 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_2. As well as scavenging hydrogen peroxide, it reacts with molecules such as acetaldehyde or methional (Danilewicz, 2016; Fracassetti *et al*., 2019). However, SO_3^{2-} - a dissociated form of SO_2 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_{aq}^-) and sulfite radicals ($SO_3^{\cdot-}$) (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_{aq}^- is highly reactive towards unsaturated bonds, as well as O_2 (Buxton *et al*., 1988; Cao *et al*., 2021). This can lead to the formation of further radicals like the superoxide ($O_2^{\cdot-}$) and the hydroperoxyl radical ($HO_2^{\cdot-}$)(Equations 3, 4 and 5). $SO_3^{\cdot-}$ is an oxidant that can promote a range of reactions, leading to, for example, the formation of the peroxymonosulfate radical ($SO_5^{\cdot-}$) and the sulfate radical ($SO_4^{\cdot-}$) (Equations 6, 7, 8 and 9) (Cao *et al*., 2021). $SO_4^{\cdot-}$ 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).

(Equation 1) $SO_3^{2-} + hv \rightarrow SO_3^{\cdot-} + e_{aq}^-$

(Equation 2) $HSO_3^- + hv \rightarrow SO_3^{\cdot-} + H^\cdot$

(Equation 3) $e_{aq}^- + O_2 \rightarrow O_2^{\cdot-}$

(Equation 4) $O_2^{\cdot-} + H^+ \rightarrow HO_2^\cdot$

(Equation 5) $O_2^{\cdot-} + HO_2^\cdot \rightarrow HO_2^- + O_2$

(Equation 6) $SO_3^{\cdot-} + O_2 \rightarrow SO_5^{\cdot-}$

(Equation 7) $SO_5^{\cdot-} + HSO_3^- \rightarrow HSO_5^- + SO_3^{\cdot-}$

(Equation 8) $SO_5^{\cdot-} + HSO_3^- \rightarrow H^+ + SO_4^{\cdot-} + SO_4^{2-}$

(Equation 9) $SO_5^{\cdot-} + SO_3^{2-} \rightarrow SO_3^{\cdot-} + SO_5^{2-}$

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 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 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 SO_2, 0 g/hl hydrolysable tannin), SO_2 (55 mg/l SO_2, 0 g/hl hydrolysable tannin) and hydrolysable tannins (20 mg/l 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 ±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_2 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_2 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 xanthylium 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_2 concentration caused an increase in the *citrus* and *burnt* attributes and a decrease in the *colour intensity, oxidised* and *honey* attributes. SO_2 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

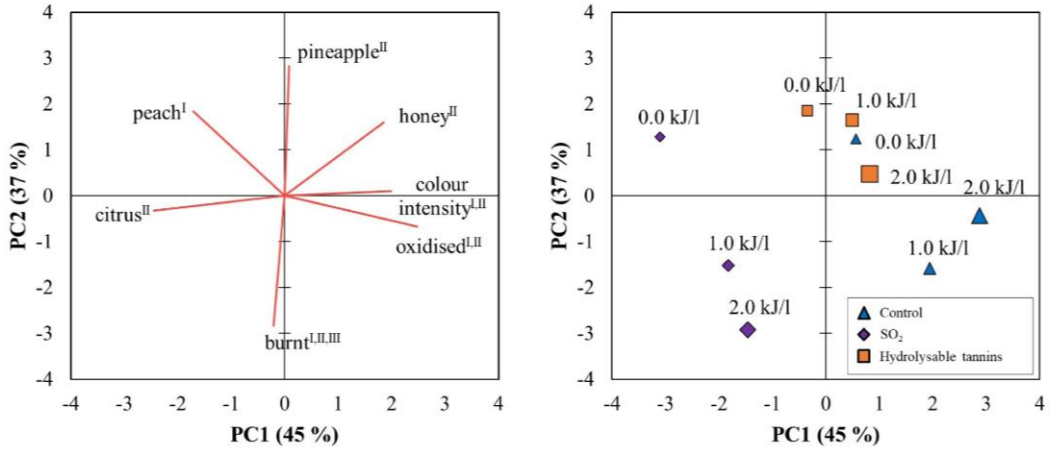

FIGURE 1. Principal component analysis of descriptive sensory data of Chardonnay wine treated with different UV-C doses and supplemented with antioxidants: control (20 mg/l free SO_2, 0 g/hl hydrolysable tannins), SO_2 (55 mg/l SO_2, 0 g/hl hydrolysable tannins), hydrolysable tannins (20 mg/l SO_2, 20 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_2 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_2 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_2 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_2 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_2 during the UV-C treatment is the ability of to absorb UV-C light at 254 nm and produce a series of SO_3^{2-} reactive oxidative species (Cao *et al*., 2021). These findings suggest that UV-C causes SO_2 to lose its ability to act as an antioxidant. These results are in accordance with those of Fracassetti *et al*. (2020), who found SO_2 to have a pro-oxidative effect on methionine degradation during light exposure. UV-C might also be responsible for SO_2 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_2, 0 g/hl hydrolysable tannins), SO_2 (55 mg/l SO_2, 0 g/hl hydrolysable tannins), hydrolysable tannins (20 mg/l SO_2, 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 $p \leq 0.05$.

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^{bc}	3.0^{abc}	4.4^{bc}	1.9^{d}
	1.0	4.2^{ab}	2.4^{bc}	4.9^{ab}	4.0^{ab}
	2.0	4.5^{a}	2.0^{c}	6.0^{a}	2.5^{cd}
SO_2	0.0	3.0^{c}	3.3^{ab}	2.3^{d}	1.9^{d}
	1.0	3.3^{bc}	2.8^{abc}	3.6^{c}	3.6^{bc}
	2.0	3.8^{abc}	2.8^{abc}	4.0^{bc}	4.9^{a}
Hydrolysable tannins	0.0	4.0^{abc}	3.5^{a}	4.1^{bc}	2.0^{d}
	1.0	4.5^{a}	3.4^{ab}	3.8^{bc}	2.2^{d}
	2.0	4.7^{a}	2.7^{abc}	4.5^{bc}	2.2^{d}
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

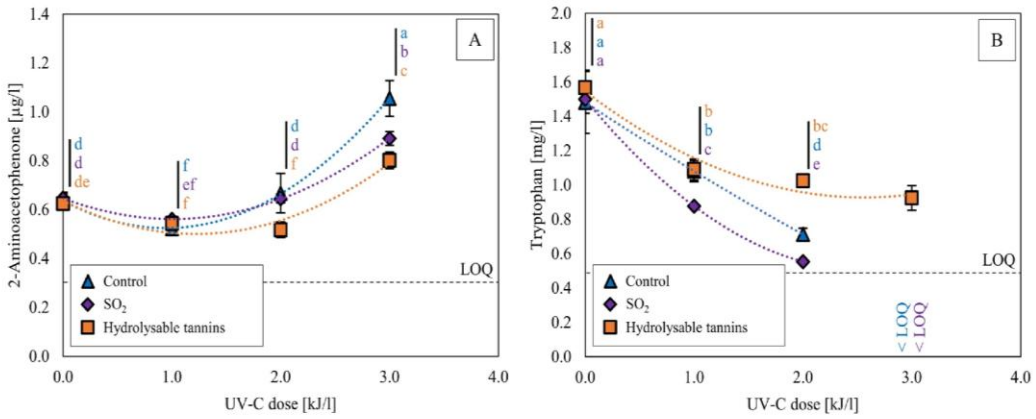

FIGURE 2. Effects of UV-C treatment on 2-aminoacetophenone (A) and tryptophan (B) concentrations in Chardonnay wine in its original form (control), with increased SO_2 content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C treatment according to Fisher's test with $p \leq 0.05$.

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_2 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

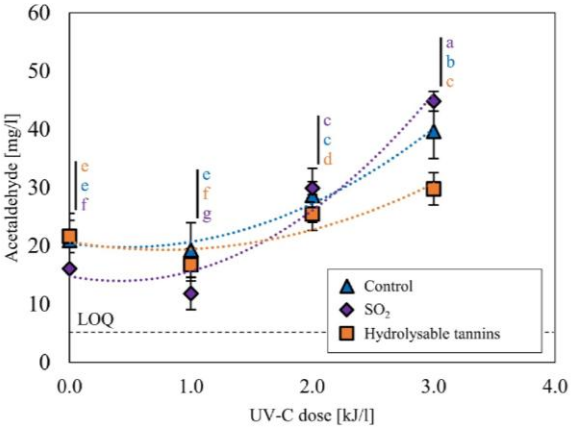

FIGURE 3. Effect of UV-C treatment on the concentration of acetaldehyde in Chardonnay wine in its original form (control), with increased SO_2 content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C treatment 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 riboflavinmediated 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 β-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_2 concentrations, despite 2-AAP formation being diminished. It is possible that radicals produced by SO_2 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_2, 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_2 on acetaldehyde formation became significant, while hydrolysable tannins mitigated acetaldehyde formation and increased SO_2 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_2 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_2 before UV-C treatment is due to the ability of SO_2 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

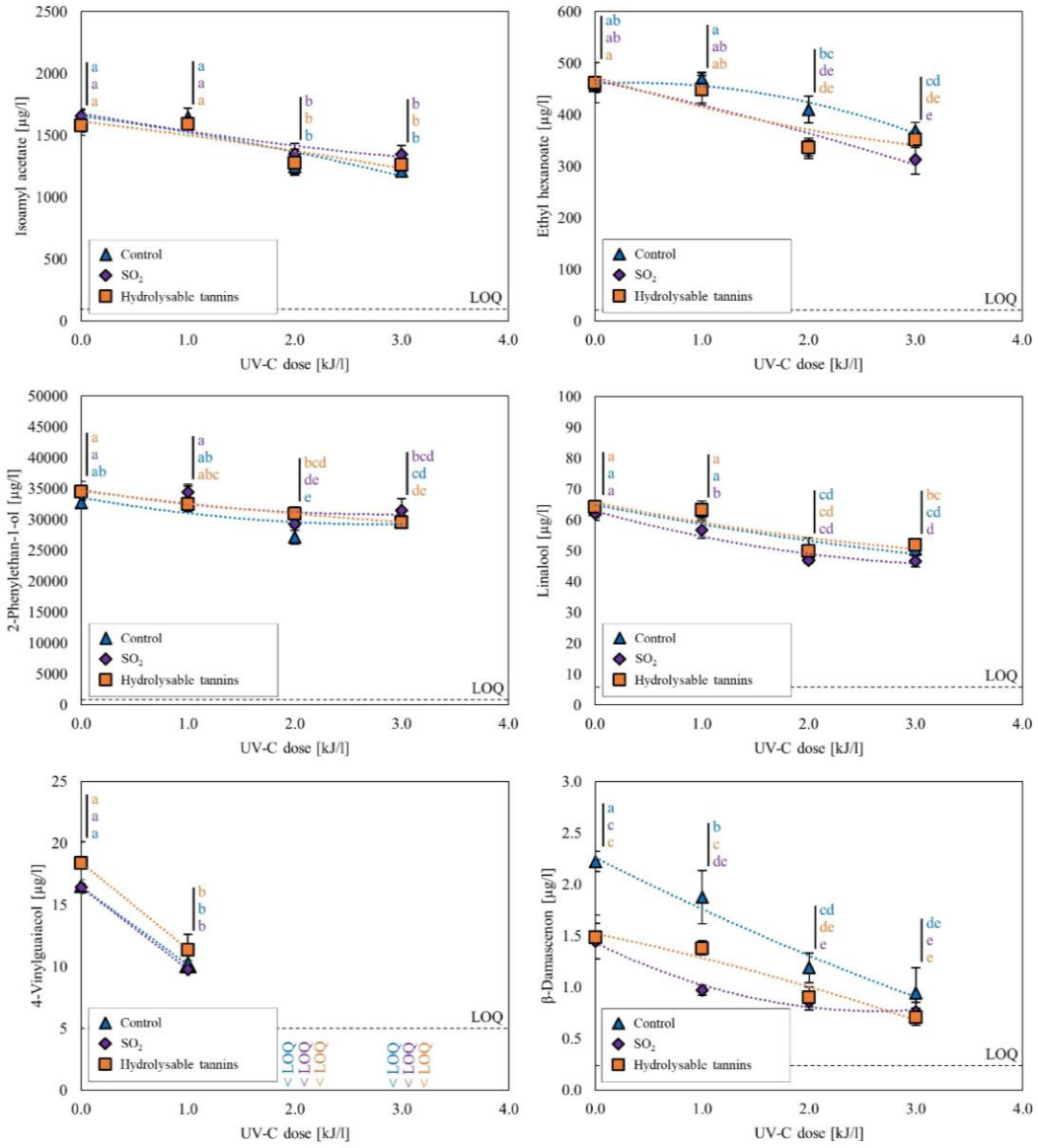

FIGURE 4. Effect of UV-C treatment on the concentration of volatile compounds in Chardonnay wine in its original form (control), with increased SO_2 content and supplemented with hydrolysable tannins. The data were processed by one-way ANOVA. Different letters show significant differences due to UV-C 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 β-damascenone, linalool and 4-vinylguiacol 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 β-damascenone (C13-norisoprenoids) and linalool (monoterpenes) via riboflavin in model wine.

TABLE 3. Basic chemical parameters of UV-C treated Chardonnay wine in its original form (control), with increased SO_2 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 $p \leq 0.05$.

PART 1/2

UV-C dose [kJ/l]	Alcohol [% vol.]			pH			Residual sugar [g/l]		
	Control	SO_2	Hydrol. tannins	Control	Control	Hydrol. tannins	Control	SO_2	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	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 ± 0.3 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	0.8 ± 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	0.8 ± 0.2 a	0.7 ± 0.2 a
p-value	0.639	0.526	0.843	0.812	0.513	0.697	0.977	0.864	0.901

PART 2/2

UV-C dose [kJ/l]	Free SO_2 [mg/l]			Antioxidant capacity [mmol Trolox eq./l]			Gallic acid [mg/l]			Total phenols [mg/l GAE]		
	Control	SO_2	Hydrol. tannins	Control	SO_2	Hydrol. tannins	Control	SO_2	Hydrol. tannins	Control	SO_2	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-Cinduced 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_2 and hydrolysable tannins decreased the concentration of ß-damascenone regardless of the UV-C treatment; this may be due to reactions between ß-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_2, gallic acid, total phenols and antioxidant capacity decreased with UV-C treatment. Free SO_2 decreased with UV-C treatment in the wines with high SO_2 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_2 is light sensitive. The SO_2 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_2 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_2 is regarded as a falsepositive 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_2 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_2 mitigated the formation of 2-AAP but accelerated the degradation of tryptophan, suggesting that SO_2 has a major impact on reaction pathways leading to unknown reaction products. In contrast to 2-AAP, acetaldehyde formation was accelerated by SO_2 supplementation, thus indicating that it promoted the photo-Fenton reaction. Overall, in combination with UV-C, SO_2, 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_2 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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4.3 2-Aminoacetophenone formation through UV-C induced degradation of tryptophan in the presence of riboflavin in model wine: Role of oxygen and transition metals

The photo-induced formation of 2-AAP based on the low sensory threshold and high variability of the concentration of educt in wine remained the restrictive factor that limits the use of UV-C technology for microbial stabilization of wine. A limited number of literatures was available on this topic, making it relevant for research. The topics such as reaction mechanism, reaction kinetics, and influencing factors of the photo-induced formation of 2-AAP in wine have been hardly researched, which makes the specific optimization of UV-C technology difficult. Morozova et al. 2013 mentioned in their work, based on the sensory studies, the possible influence of the transition metals on the formation of the 2-AAP in Riesling. In enology, the transition metals are associated with the Fenton reaction, in which oxygen plays a significant role. According to this, the study investigates the formation of 2-AAP under UV-C induced degradation of TRP in model wines in the presence of the transition metals Fe^{2+} and Cu^{2+} and a variation in oxygen content.

The results showed that regardless of the parameters chosen, less than 25% of the TRP was degraded through the first-order kinetic under the formation of the 2-AAP. With increasing UV-C exposure time and depending on the oxygen concentration in the wine, a significant formation of 2-AAP was observed, indicating the critical role of oxygen in the photoinduced reaction. The presence of transition metals decreases the formation of 2-AAP while acetaldehyde is formed. The photo-Fenton reaction catalyzed by transition metals competes directly with 2-AAP formation. RF acted as a photo-sensitizer in this reaction. The degradation of RF via first-order kinetics was determined during the experiment. The lowering of oxygen content significantly increased the degradation rate of RF. The same effect was observed in the presence of the transition metal, especially iron, and was further enhanced in combination with low oxygen content. This was due to the oxygen uptake during the photo-Fenton reaction, which prevented the regeneration of RF and can thus be understood as another limiting factor of the reaction of the 2-AAP.

Based on the study, it can be concluded that the preventive reduction of oxygen in the wine before UV-C treatment could be an effective resource against the formation of 2-AAP and possibly other photo-induced oxidation processes. In future studies, this hypothesis should be investigated on real wines.

2-Aminoacetophenone formation through UV-C induced degradation of tryptophan in the presence of riboflavin in model wine: Role of oxygen and transition metals

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Disclaimer:

The presented work was exclusively performed by the DLR Rheinpfalz (Svetlana Cvetkova and master thesis Sarah Edinger). For more information, refer to the author contribution section.

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2-Aminoacetophenone formation through UV-C induced degradation of tryptophan in the presence of riboflavin in model wine: Role of oxygen and transition metals

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ABSTRACT

2-Aminoacetophenone is an off-flavor that can result from tryptophan degradation via riboflavin-photosensitized reaction. This study investigates the impact of light exposure, provided by a UV-C source, oxygen concentrations and transition metals on the formation of 2-aminoacetophenone in model wine containing tryptophan and riboflavin. Irrespective of oxygen and transition metals, >85% of tryptophan were degraded via first-order kinetics to unknown product(s). However, longer light exposure and more oxygen caused 2-aminoacetophenone concentrations to increase. Transition metals decelerated the 2-aminoacetophenone formation and acetaldehyde was formed suggesting photo-Fenton reaction occurred as a competitive reaction. The degradation rate of riboflavin inclined with less oxygen and in the presence of transition metals due to the depletion of oxygen by photo-Fenton reaction. Oxygen plays an important role in the regeneration of riboflavin and therefore must be seen as an intensifier for light-induced 2-aminoacetophenone formation. This paper provides new insights into riboflavin-photosensitized reactions.

1. Introduction

Since 1988, a new off-flavor in white wine has been observed in France and Germany. The off-flavor was described as wet wool, naphthalene, mothball, and acacia blossom. It was named "atypical aging" (ATA). Rapp et al. (1993) postulated 2-aminoacetophenone (2-AAP) being the main compound responsible for ATA. Since then, 2-AAP has not only been detected in wine, but also in foodstuff such as milk, cheese, milk powder, tortilla chips, and beer (Hoenicke, Borchert, et al., 2002). The sensory threshold of 2-AAP in white wine is between 0.5 and 1.5 µg/l (Christoph et al., 1995). Stress situations in viticulture, such as water deficit and insufficient nitrogen supply, are considered to be the reason for ATA (Hoenicke, Simat, et al., 2002; Schwab et al., 1999). 2-AAP is formed in wine through the degradation of indole-3-acetic acid (IAA) by radical co-oxidation of sulfites after fermentation and during wine storage (Christoph et al., 1998; Dollmann et al., 2015; Hoenicke, Borchert, et al., 2002). Morozova et al. (2013) and Vukajlović et al. (2023) suggested that transition metals have an impact on the off-flavor ATA and the formation of 2-AAP in wine through the IAA degradation pathway. The typical concentration of IAA in wine is in the range of 3–90 µg/l (Hoenicke et al., 2001).

Another formation mechanism of 2-AAP is the light-induced degradation of tryptophan (TRP) through N-formylkynurenine and kynurenine as intermediates (Horlacher & Schwack, 2014). The typical concentration of TRP in wine is between 0.2 and 24 mg/l (Hoenicke et al., 2001). The light-induced degradation of TRP requires riboflavin (RF) as a photosensitizer. The literature describes two possible mechanisms of RF-photosensitized reactions: type I and type II (Cardoso et al., 2012; Insińska-Rak & Sikorski, 2014). Fig. 1 shows the formation of 2-AAP through RF-photosensitized degradation of TRP via type I and type II reaction mechanisms. Both mechanisms start with the absorption of visible and/or UV light. RF transitions from ground state to singlet excited state RF* (S_1). In the next step, transition from S_1 occurs through intersystem crossing (ISC) to triplet excited state RF* (T_1). In type I mechanism, hydrogen is

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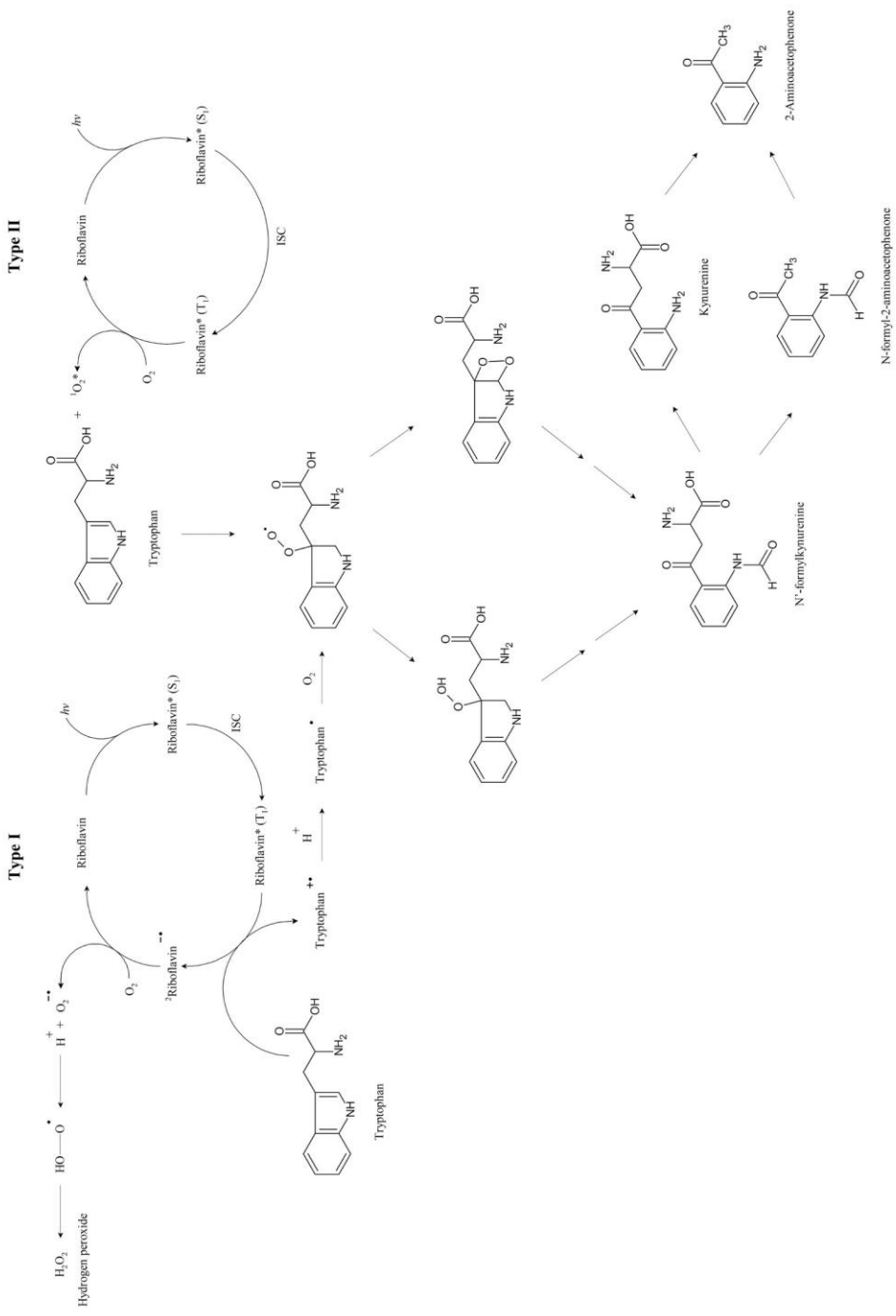

Fig. 1. Formation of 2-aminoacetophenone through riboflavin-photosensitized degradation of tryptophan via type I and type II reaction mechanism. Abbreviations: S_1: singlet excited state of riboflavin; T_1: triplet excited state of riboflavin; ISC: intersystem crossing; ^{2}riboflavin$^{\bullet-}$: riboflavin radical anion; $O_2^{\bullet-}$: superoxide anion radical; $^1O_2$: excited singlet electronic state of oxygen. The postulated reaction schemes were modified and adapted from Insińska-Rak and Sikorski (2014) and Horlacher and Schwack (2014).

split off from TRP; RF radical anion ($^2RF^{-\bullet}$) and TRP radical ($TRP^{+\bullet}$) are formed. Then, $TRP^{+\bullet}$ and $^2RF^{-\bullet}$ react with oxygen, RF returns to its ground state and produces superoxide anion radical ($O_2^{-\bullet}$). The superoxide anion radical converts to hydroperoxyl radical or hydrogen peroxide in acidic conditions. TRP reacts with RF in triplet excited state mostly via type I mechanism (Cardoso et al., 2012). In type II mechanism, the energy transfer takes place between a donor, in this case RF in T_1 state, and an acceptor, here the oxygen molecule. RF in T_1 state returns to its ground state and shifts the oxygen from its ground state to its excited singlet electronic state ($^1O_2^*$). Then, $^1O_2^*$ reacts with TRP, which is one of the amino acids that can readily react with electrophilic $^1O_2^*$ (Min & Boff, 2002), via the above mentioned intermediates to 2-AAP (Cardoso et al., 2012; Horlacher & Schwack, 2014; Insińska-Rak & Sikorski, 2014).

The typical concentration range of RF in wine is 150–200 μg/l (Fracassetti et al., 2017; Mattivi et al., 2000). RF acts not only as a photosensitizer, but is also susceptible to light-induced degradation. RF degradation can occur in any food containing RF, for example in green leafy vegetables, eggs, milk, and wine (Cardoso et al., 2012). The known photodegradation products of RF are lumiflavin, lumichrome, formylmethylflavin, carboxymethylflavin, and cyclodehydroflavin. Parameters such as the light intensity, oxygen concentration, and the presence of transition metals have an influence on RF degradation and eventually on the degradation products formed (Ahmad et al., 2017; Sheraz, Kazi, Ahmed, Anwar, & Ahmad, 2014).

Hydrogen peroxide, which can result from RF-photosensitized degradation of TRP, is a substrate for the Fenton reaction. The Fenton reaction, catalyzed by ferrous iron, results in hydroxyl radical. In more recent times, researchers used the term "Fenton reaction" or "Fenton-like reaction" in a broader context, including other low-valent transition metals, such as copper (Wang, 2008), and also including the coupled oxidation of vicinal phenols and consecutive oxidation reactions, such as the oxidation of ethanol to acetaldehyde in wine by consumption of oxygen (Clark et al., 2007; Danilewicz, 2003; Elias & Waterhouse, 2010). Fig. 2 shows the reaction mechanism of the ferrous iron-catalyzed formation of acetaldehyde in wine (Elias & Waterhouse, 2010).

The photo-Fenton reaction is an extended version of the Fenton reaction. A photo-reduction of ferric iron and cupric copper takes places during light exposure (Ameta et al., 2018), therefore serving a similar function as phenols in the context of the Fenton reaction. The photo-Fenton reaction occurs in wine when exposed to light (Clark et al., 2007; Espejo, 2016; Grant-Preece et al., 2015). The light-induced degradation of TRP, resulting in 2-AAP, as well as the photo-Fenton reaction, resulting in acetaldehyde, requires oxygen or reactive oxygen species (ROS). The photo-Fenton reaction is based on the presence of transition metals; the RF-photosensitized formation of 2-AAP is independent form transition metals.

The aim of this study was to determine the effect of light exposure with different exposure times through UV-C on the RF-photosensitized 2-AAP formation from TRP under the influence of oxygen and transition metals. Photo-Fenton reaction, which is triggered by light and transition metals, could be a competitive reaction to the RF-photosensitized formation of 2-AAP resulting in acetaldehyde which was analyzed in this study. It is known that oxygen plays an important role in type I and type II RF-photosensitized reaction as well as in photo-Fenton reaction. Accordingly, different oxygen contents were investigated possibly affecting the formation of 2-AAP. The study also addressed the degradation of RF, which is influenced by light intensity, oxygen concentration, and presence of transition metals, and potentially has an effect on 2-AAP formation. A possible degradation product of RF is discussed. To reveal the relation between the different reactions, kinetics assessment was conducted utilizing increasing light exposure times. The formation rates of 2-AAP were investigated in context with degradation kinetics of TRP revealing the reaction yield of 2-AAP under the influence of light, oxygen, and transition metals.

2. Materials and methods

2.1. Materials

L-(+)-tartaric acid (≥99.7%), acetaldehyde (≥99.5%), acetonitrile (≥99.5%) and sodium sulfate (≥99%) were purchased from Carl Roth (Karlsruhe, Germany); 2-aminoacetophenone (≥98%), L-tryptophan (≥98%), (−)-riboflavin (≥98%), lumichrome (≥98%), sodium hydroxide (≥98%), copper(II) sulfate pentahydrate (≥98%), and iron(II) sulfate heptahydrate (≥99.5%) were purchased from Merck (Darmstadt, Germany); d_5–2-aminoacetophenone (≥98%) was purchased from Eptes Sàrl (Vevey, Switzerland), ethanol (≥96%) was purchased from Berkel AHK (Ludwigshafen, Germany); potassium hydrogenphosphate (≥99.5%) and phosphoric acid (≥85%) were purchased from ORG Laborchemie GmbH (Bunde, Germany). All chemicals were of analytical reagent grade as minimum. HPLC grade water was obtained from a Milli-Q system (Purelab flex 4, Veolia Water Technology GmbH, Celle, Germany).

2.2. Experimental design

The effect of light exposure through increasing UV-C exposure times (0, 130, 260, 390, 520, 650, 780 s), different oxygen concentrations, and

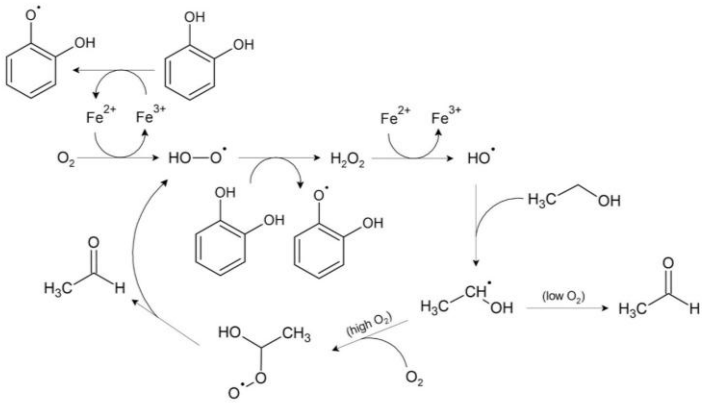

Fig. 2. Ferrous iron-catalyzed formation of acetaldehyde in wine under high and low dissolved oxygen concentration (adapted from Elias & Waterhouse, 2010).

the addition of different transition metals Cu^{2+} and Fe^{2+} were investigated in a model wine at 20 °C. A full factorial experimental design was applied. The model wine consisted of 12% (*v*/v) aqueous ethanol, 0.53 µmol/l RF, 290 µmol/l TRP, 33.3 mmol/l tartaric acid and the pH was adjusted to 3.3 with 10 mol/l sodium hydroxide solution. Cupric copper (90 µmol/l Cu^{2+}) or ferrous iron (90 µmol/l Fe^{2+}) were added depending on the experiment by freshly prepared stock solutions. The concentration of ferrous iron was chosen based on the work of Danilewicz (2007). An equivalent concentration was used for cupric copper in order to compare the reaction kinetics for both transition metals. After the preparation of the model wine, the oxygen concentrations were adjusted to 6, 125 or 250 µmol/l by sparging the model wine with nitrogen. Dissolved oxygen was non-invasively determined with oxygen-sensitive-spots (PSt3, PreSens Precision Sensing GmbH, Regensburg, Germany). Light exposure was applied immediately after preparation of model wine at a wavelength of 254 nm. The UV-C exposure time corresponds to UV-C doses as determined by chemical actinometry using an iodide/iodate solution according to the method of Rahn (1997) (Supplementary table 1). Five connected annular thin-film reactors, developed by the Max Rubner-Institut (Karlsruhe, Germany), were used for the UV-C exposure. Each reactor was equipped with a 20 W low-pressure mercury lamp and with fluid-guiding elements (Hirt et al., 2022). The reactors were connected to each other with Tygon® standard thermoplastic soft polyvinyl chloride 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 pump the model wine through the reactors. A F30-VC/3 cooling system (Julabo Labortechnik, Seelbach, Germany) was included to avoid a heat effect of UV-C lamps. The temperature fluctuations during the experiments were ± 1.0 °C. To prevent oxygen ingress during the experiments, pipes and 2 l Schott bottles were sparged with argon before UV-C exposure. The oxygen fluctuation during the experiments was ±0.2 mg/l. The samples were protected from ambient light during model wine preparation, before and after UV-C exposure by covering the 2 l Schott bottles with aluminum foil. All experiments were conducted in triplicates.

2.3. HS-SPME-GC–MS/MS analysis of 2-AAP

The analysis of 2-AAP was carried out according to a published method of Schmarr et al. (2016) with some modifications. The samples were analyzed by headspace solid-phase microextraction coupled with gas chromatography coupled with a triple quadrupole mass spectrometry (HS-SPME-GC–MS/MS). It was composed of three main modules: autosampler with SPME option (TriPlus RSH, Thermo Fisher Scientific, Waltham, USA), gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, Waltham, USA), and the mass spectrometer (MS TSQ Quantum XLS Ultra, Thermo Fisher Scientific, Waltham, USA). 1 g sodium sulfate was added to 2 ml of sample. 10 µl of d_5–2-AAP were added as internal standard (2 µg/l in model wine). HS-SPME was carried out with 10 mm Carbon WR fiber (95 µm; CTC Analytics, Zwingen, Switzerland). Fiber conditioning was performed before and after the measurement for 10 min at 270 °C under nitrogen. After sample incubation for 5 min at 70 °C, the extraction was carried out at 70 °C for 40 min with an agitation speed of 500 rpm. The second step was a thermal desorption at 220 °C for 2 min (splitless mode) with a flow rate of 50 ml/min. The system was equipped with the column Zebron ZB-WAXplus™ (30 m × 0.25 mm i.D. × 0.25 µm; Phenomenex, Torrance, USA). Helium was used as carrier gas at a constant flow of 1.2 ml/min. The oven temperature was programmed from 40 °C (3 min hold) at 5 °C/min to 180 °C and at 20 °C/min to 200 °C (5 min hold). Electron impact ionization was performed at 70 eV in positive mode. The temperatures of the MS-transfer line and ion source were 230 °C. Argon was used as a collision gas with a collision cell pressure of 1.1 mTorr. The mass spectrometer was operated in full scan mode with a range of *m/z* 40 to *m/z* 250. Quantitative analysis was done by seven-point external calibration in model wine. The calibration range for 2-AAP was from 1.5 nmol/l to 59 nmol/l.

2.4. Enzymatic analysis of acetaldehyde

Acetaldehyde was quantified using a commercially available enzymatic assay (REF 984347, Thermo Fischer Scientific, Waltham, USA) according to Närkki et al. (2015) on an automated Konelab 20i (Typ 954; Thermo Fisher Scientific, Waltham, USA). Sample preparation and measurement were done according to the official guideline of enzymatic assay. Quantitative analysis was done by five-point external calibration in model wine. The calibration range was from 20 µmol/l to 453 µmol/l.

2.5. Absorption spectra measurement of RF degradation product

For qualitative analyses of RF degradation product, absorption spectra from samples were taken before and after UV-C exposure time of 780 s. Spectra were recorded using a double-beam UV/VIS spectrometer Jasco V750 (Jasco, Tokyo, Japan) from 200 nm to 800 nm with a wavelength interval of 0.2 nm using the software Spectra Manager Version 2 (Jasco, Tokyo, Japan). Samples were measured in 10 mm UV cuvettes (Brand, Wertheim, Germany). As a possible RF degradation product, lumichrome was used as a reference. The spectrum of lumichrome was compared to the spectrum of RF in 12% (*v*/v) aqueous ethanol with 33.3 mmol/l tartaric acid at pH 3.3, both at a concentration of 5 µmol/l.

2.6. HPLC-DAD analysis of TRP and RF

The quantification of TRP and RF was carried out according to Golombek et al. (2021) with some modifications. The samples were analyzed by high-performance liquid chromatography (HPLC) (Jasco, Tokyo, Japan) with diode array detection (DAD) (MD-4010, Jasco, Tokyo, Japan). Detection wavelengths were 268 nm for RF and 280 nm for TRP. The injection volume was 10 µl at a flow of 1 ml/min and the column temperature was 25 °C. A Gemini NX-C18 column (150 mm × 4.5 mm, 3 µm particle size; Phenomenex, Torrance, USA) was used. The analysis was carried out by gradient elution. Mobile phase A consisted of 5/95 (v/v) acetonitrile/phosphate buffer and mobile phase B consisted of 50/50 (v/v) acetonitrile/phosphate buffer. Phosphate buffer consisted of 10 mmol/l KH_2PO_4 adjusted to pH 1.5 with 85% H_3PO_4. The following gradient was used: from 10 to 100% mobile phase B in 22 min, afterwards the gradient was kept for 5 min. The duration of column re-equilibration was 5 min under starting conditions (10% mobile phase B). Quantitative analysis was done by six-point external calibration with RF or TRP in model wine described earlier. The calibration range for RF was from 0.26 µmol/l to 0.58 µmol/l. The calibration range for TRP was from 39 µmol/l to 100 µmol/l.

2.7. Data analysis and kinetic calculations

Statistical analysis was performed using XLSTAT (Version 2021.2.2.1147, Addinsoft Sàrl, Paris, France). Normality of data was tested by the Shapiro-Wilk method ($p \leq 0.05$). Analytical data was analyzed for significant differences between exposure times using analysis of variance (ANOVA). Different letters indicate significant differences at $p \leq 0.05$ based on Fisher's least significant difference (LSD) post-hoc test. The first-order rate constant k[c] was calculated with c as the measured concentration and c_0 as the start concentration for TRP and RF degradation during UV-C exposure with t as the increasing UV-C exposure time (Eq. 1).

$$ln\frac{c}{c_0} = -k[c] \times t \quad (1)$$

The rate constant for the TRP degradation product (k_1[unknown product(s)]) as well as the rate constant for 2-AAP ($k_2[2-AAP]$) were calculated with k[c] for TRP, c and t as defined earlier (Eq. 2).

$$-\frac{dc}{dt} = k_1[\text{unknown product(s)}] + k_2[2-AAP] = k[c] \quad (2)$$

3. Results and discussion

3.1. Impact of oxygen and transition metals on formation of 2-AAP

Fig. 3 shows the influence of light exposure through increasing UV-C exposure time and increasing oxygen on the 2-AAP formation in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). The 2-AAP concentration inclined with higher UV-C exposure time. Light activates RF from its ground state to T_1 which catalyzes the formation of 2-AAP through TRP degradation (Horlacher & Schwack, 2014). It is suggested that increasing UV-C exposure accelerated the formation of 2-AAP. Higher oxygen content accelerated the formation of 2-AAP showing that oxygen is involved in the formation of 2-AAP through type I and type II reaction mechanisms (Horlacher & Schwack, 2014; Insińska-Rak & Sikorski, 2014). Vice versa, no 2-AAP could be detected in the model wine without the influence of UV-C irrespective of the oxygen present. The presence of transition metals reduced the concentration of 2-AAP with increasing light exposure in comparison to the model wine without transition metals (Fig. 3B and C). Vukajlović et al. (2023) observed that cupric copper limits and ferrous iron promotes the 2-AAP formation during wine aging at 40 °C without light exposure. If light is regarded as an impact factor, a possible explanation for transition metals limiting the formation of 2-AAP is that photo-Fenton reaction is initiated as a competitive reaction to the RF-photosensitized 2-AAP formation through TRP degradation. Fig. 3A-C shows that the deceleration effect on 2-AAP formation was much more pronounced with ferrous iron (Fig. 3C) than with cupric copper (Fig. 3B). This can be explained by the lower redox potential of ferrous iron compared to cupric copper (Atkins et al., 2021), therefore exerting greater competition by the photo-Fenton reaction. However, the impact of oxygen on the RF-photosensitized 2-AAP formation in the model wine was similar irrespective of transition metals indicating that oxygen has an impact on the RF-photosensitized 2-AAP formation and on the photo-Fenton reaction.

3.2. Impact of oxygen and transition metals on formation of acetaldehyde

The data suggests that photo-Fenton reaction is a competitive reaction to the RF-photosensitized 2-AAP formation through TRP degradation. Accordingly, light exposure and oxygen can result in both 2-AAP and acetaldehyde. Fig. 4 shows the influence of light exposure through increasing UV-C exposure time and increasing oxygen content on the acetaldehyde formation in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). In model wine without transition metals (Fig. 4A), acetaldehyde was below limit of quantification (LOQ) over the whole range of investigated UV-C exposure. However, when oxygen concentration was high (250 µmol/l), acetaldehyde could be detected for an UV-C exposure time of >650 s. The increase of acetaldehyde as a result of the oxidation of ethanol without transition metals was probably caused by high concentrations of UV-C-induced ROS resulting from RF-photosensitized TRP degradation reaction. Model wine with cupric copper or ferrous iron showed steadily increasing acetaldehyde concentrations with exposure times >260 s or > 130 s, respectively (Fig. 4B and C). This confirms the assumption made earlier that photo-Fenton reaction occurred in the system with transition metals. The results suggest that photo-Fenton reaction consumed the hydrogen peroxide resulting from RF-photosensitized TRP degradation and acted as a competitive reaction to the formation of 2-AAP through fast consumption of oxygen during oxidation reaction of ethanol to acetaldehyde. Ferrous iron caused a greater increase in acetaldehyde than cupric copper which is in agreement with the findings shown earlier observing a deceleration effect on 2-AAP formation. The effect of the different redox potentials of ferrous iron and cupric copper is also reflected here in the formation of acetaldehyde. The oxygen accelerated acetaldehyde formation in the presence of ferrous iron (Fig. 4C). However, this effect was not observed with cupric copper (Fig. 4B). This discrepancy between the two transition metals can be explained by the strong photoreactivity characteristics of ferrous iron and therefore the efficient generation of hydroxyl radicals (Machulek et al., 2012). Although cupric copper is capable of photochemical reactions (Iboukhoulef et al., 2014), its relatively low photoreactivity compared to ferrous iron explains the slow formation of acetaldehyde, which in turn weakens the reinforcing effect of an increased oxygen concentration.

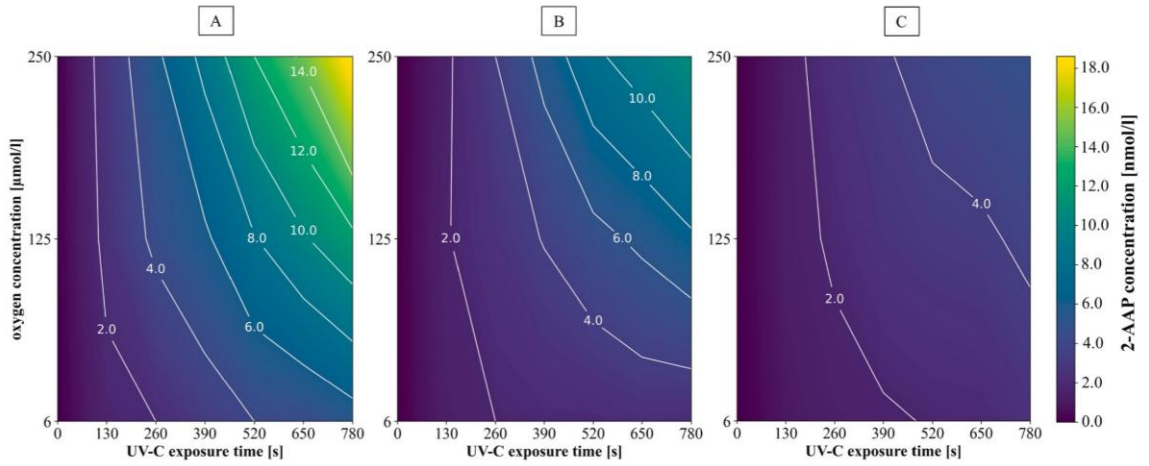

Fig. 3. Influence of light exposure through increasing UV-C exposure time and increasing oxygen content on the 2-AAP formation in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). Shown are the mean values of experimental replicates ($n = 3$).

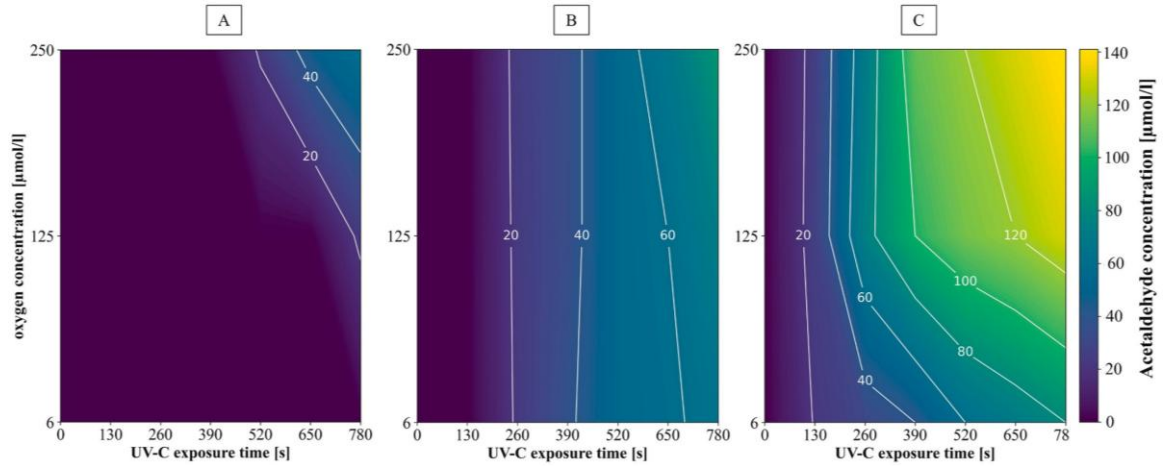

Fig. 4. Influence of light exposure through increasing UV-C exposure time and increasing oxygen content on the acetaldehyde formation in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). Shown are the mean values of experimental replicates ($n = 3$).

3.3. Impact of oxygen and transition metals on degradation of TRP

Fig. 5 shows the influence of light exposure through increasing UV-C exposure time on TRP degradation for three different oxygen concentrations in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). The first-order kinetics of TRP degradation was determined regardless of the oxygen concentration and the addition of transition metals (Fig. 5). Oxygen accelerated the degradation of TRP which is in agreement with the increased formation of 2-AAP (Fig. 3A). Transition metals decelerated the degradation of TRP which holds true with decreased formation of 2-AAP (Figs. 3B and C). These observations underline the role of oxygen and hence the influence of photo-Fenton reaction as a competitive reaction on the formation of 2-AAP. Table 1 shows the first-order rate constant of TRP degradation (k_{TRP}) and 2-AAP formation ($k_{2\text{-}AAP}$) for three different oxygen concentrations without transition metals, with cupric copper, and with ferrous iron in UV-C treated model wine. The comparison of k_{TRP} with $k_{2\text{-}AAP}$ reveals that TRP degradation outweighed 2-AAP formation suggesting that part of the TRP is also degraded to unknown product(s). The absorption maximum of TRP in the UV range is at 280 nm, the molecule absorbs the UV-C light and this causes the photolysis of TRP. The study of Bellmaine et al. (2020) has shown that TRP under UV-C light can degrade to a number of different products, such as 3-hydroroxytryptophan or 2,3-dihydro-4-quinolone. Overall, it can be proposed that the degradation of TRP results in 2-AAP and in unknown product(s) as shown in Eq. 3.

$$\text{unknown product(s)} \overset{k_{\text{unknown product(s)}}}{\leftarrow} \text{TRP} \overset{k_{2\_AAP}}{\rightarrow} 2-\text{AAP} \quad (3)$$

The ratio of rate constants $k_{2\text{-}AAP}/k_{\text{unknown product(s)}}$ in Table 1 reveal that between 1 and 15% of TRP were degraded to 2-AAP for all experimental conditions. Accordingly, >85% of TRP were degraded to unknown product(s) under the influence of UV-C, independent of the oxygen content and the presence of transition metals (Table 1). Oxygen had a significant impact on k_{TRP}, on $k_{2\text{-}AAP}$ as well as on $k_{\text{unknown product (s)}}$. With increasing oxygen content, 2-AAP formation was more accelerated than TRP degradation. Hence, the ratio $k_{2\text{-}AAP}/k_{\text{unknown product(s)}}$ increased with increasing oxygen content. In fact, oxygen shifted the reaction yield towards 2-AAP.

The addition of transition metals promoted the formation of unknown product(s). It is assumed that the photo-Fenton reaction acted as a competition reaction and inhibited the formation of 2-AAP through the depletion of oxygen. Hence transition metals shifted the reaction yield towards unknown product(s). The photo-Fenton reaction can result in hydroxyl radicals which can directly react with TRP. The indole ring of TRP carries a surplus of electrons. Because of high electron density, the indole ring is susceptible to be attacked particularly from oxidative agents (Van Wickern et al., 1997).

3.4. Impact of oxygen and transition metals on degradation of RF

Fig. 6 shows the influence of light exposure through increasing UV-C exposure time on RF degradation for three different oxygen concentrations in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). The illustration shows that the light-induced degradation of RF follows a first order kinetic which is in line with the findings of Fracassetti et al. (2019) and Ahmad et al. (2004). The first-order kinetics of RF degradation was determined regardless of the oxygen concentration and the addition of transition metals (Fig. 6). Oxygen decelerated the RF degradation suggesting quenching of RF states S_1 and T_1 (Fig. 6A) (Insińska-Rak & Sikorski, 2014). These results underline the importance of oxygen in the regeneration of RF, which in turn supports the formation of the 2-AAP. Transition metals accelerated the degradation of RF (Fig. 6B and C) indicating that oxygen is depleted by competitive photo-Fenton reaction and therefore oxygen is no longer available for the regeneration of RF. The strong acceleration of RF degradation in the presence of ferrous iron (Fig. 6C) suggests an additional influence such as complex formation between RF and ferrous iron (Ahmad et al., 2017). Ahmad et al., 2017 showed that metal-RF complexes can be formed in phosphate buffer under light exposure that inhibit the photolysis of RF. Table 2 shows the first-order rate constant of RF degradation (k_{RF}) for three different oxygen concentrations without transition metals, with cupric copper, and with ferrous iron in UV-C treated model wine. The results show that oxygen and transition metals have a significant influence on k_{RF}. In the model wine without transition metals, k_{RF} was 3.4-fold slower when the oxygen concentration increased from 6 to 250 µmol/l. Ferrous iron had the strongest influence on k_{RF}; it was almost 4-fold increased under aerobic conditions (250 µmol/l). The influence of oxygen on k_{RF} was less pronounced in the presence of transition metals which is an indication for the competitive photo-Fenton reaction. Following this hypothesis, oxygen was no longer available for the regeneration of RF.

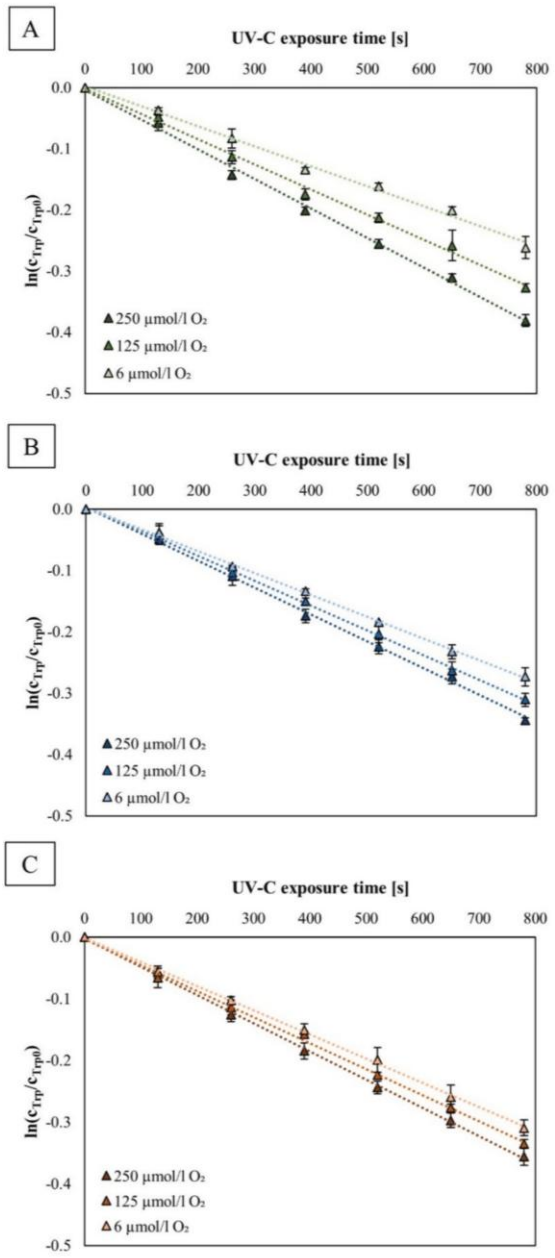

Fig. 5. Influence of light exposure through increasing UV-C exposure time on TRP first-order degradation for three different oxygen concentrations in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). Shown are the mean values of experimental replicates (n = 3) including the standard deviation.

A study of Sheraz, Kazi, Ahmed, Qadeer, et al. (2014) showed that RF can be degraded to lumichrome through photolysis in acidic conditions. Dias et al. (2012) reported RF degradation products change the absorbance of white wine in the visible region. RF is a yellow substance with two absorption maxima in the visible spectrum at 375 and 445 nm and it has a molar extinction coefficient of 12,530 mol cm^{-1} at 444 nm in an

Table 1
First-order rate constant of TRP degradation (k_{TRP}) and 2-AAP formation ($k_{2\text{-}AAP}$) for three different oxygen concentrations without transition metals, with cupric copper, and with ferrous iron in UV-C treated model wine. The rate constant of the formation of unknown product(s) ($k_{unknown\ product(s)}$) was calculated according to Eq. 2. Shown are the mean values of experimental replicates (n = 3) including the standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$).

Concentration of O_2 [µmol/l]	Transition metals	k_{TRP} [s^{-1}] ± s.d.	$k_{2\text{-}AAP}$ [s^{-1}] ± s.d.	$k_{unknown\ product(s)}$ [s^{-1}] ± s.d.	$k_{2\text{-}AAP}$/ $k_{unknown\ product(s)}$
6	–	$(3.3 \pm 0.1) \times 10^{-4}$ g	$(11.8 \pm 5) \times 10^{-6}$ e	$(3.2 \pm 0.2) \times 10^{-4}$ g	0.04
6	Cupric copper	$(3.6 \pm 0.1) \times 10^{-4}$ f	$(4.0 \pm 1) \times 10^{-6}$ g	$(3.5 \pm 0.1) \times 10^{-4}$ f	0.01
6	Ferrous iron	$(3.9 \pm 0.1) \times 10^{-4}$ e	$(4.1 \pm 0.5) \times 10^{-6}$ g	$(3.8 \pm 0.2) \times 10^{-4}$ de	0.01
125	–	$(4.1 \pm 0.1) \times 10^{-4}$ cd	$(41.0 \pm 6) \times 10^{-6}$ c	$(3.7 \pm 0.2) \times 10^{-4}$ ef	0.11
125	Cupric copper	$(4.0 \pm 0.1) \times 10^{-4}$ de	$(17.0 \pm 2) \times 10^{-6}$ d	$(3.8 \pm 0.1) \times 10^{-4}$ de	0.05
125	Ferrous iron	$(4.2 \pm 0.1) \times 10^{-4}$ c	$(9.7 \pm 0.7) \times 10^{-6}$ f	$(4.1 \pm 0.1) \times 10^{-4}$ bc	0.02
250	–	$(4.8 \pm 0.1) \times 10^{-4}$ a	$(61.8 \pm 2) \times 10^{-6}$ a	$(4.2 \pm 0.1) \times 10^{-4}$ b	0.15
250	Cupric copper	$(4.4 \pm 0.1) \times 10^{-4}$ b	$(46.1 \pm 3) \times 10^{-6}$ b	$(3.9 \pm 0.1) \times 10^{-4}$ cd	0.12
250	Ferrous iron	$(4.6 \pm 0.1) \times 10^{-4}$ b	$(14.8 \pm 0.3) \times 10^{-6}$ de	$(4.5 \pm 0.2) \times 10^{-4}$ a	0.03

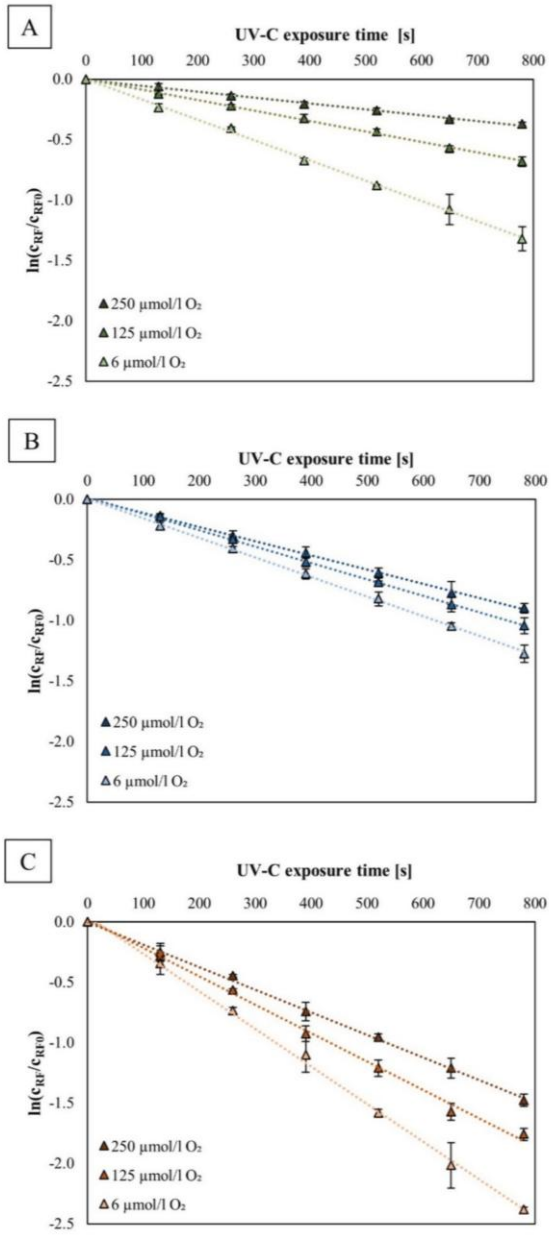

Fig. 6. Influence of light exposure through increasing UV-C exposure time on RF first-order degradation for three different oxygen concentrations in model wine without transition metals (A), with cupric copper (B), and with ferrous iron (C). Shown are the mean values of experimental replicates (n = 3) including the standard deviation.

acidic environment (Sheraz, Kazi, Ahmed, Qadeer, et al., 2014). Lumichrome is also a yellow substance with an absorption maximum in the visible spectrum at 356 nm and a molar extinction coefficient of 10,800 mol cm^{-1} at 356 nm in an acidic environment (Sheraz et al., 2014b). Vaid et al. (2019) investigated the photodegradation of RF, provided the

Table 2
First-order rate constant of RF degradation (k_{RF}) for three different oxygen concentrations without transition metals, with cupric copper, and with ferrous iron in UV-C treated model wine. Shown are the mean values of experimental replicates (n = 3) including the standard deviation. Different letters indicate significant differences ($p \leq 0.05$).

Concentration of O_2 [µmol/l]	Transition metals	k_{RF} [s^1] ± s.d.
6	–	$(16.8 \pm 0.3) \times 10^{-4}$ d
6	Cupric copper	$(16.2 \pm 0.2) \times 10^{-4}$ d
6	Ferrous iron	$(31.2 \pm 0.6) \times 10^{-4}$ a
125	–	$(8.7 \pm 0.2) \times 10^{-4}$ g
125	Cupric copper	$(13.6 \pm 0.2) \times 10^{-4}$ e
125	Ferrous iron	$(23.5 \pm 0.7) \times 10^{-4}$ b
250	–	$(4.9 \pm 0.2) \times 10^{-4}$ h
250	Cupric copper	$(11.7 \pm 0.2) \times 10^{-4}$ f
250	Ferrous iron	$(18.7 \pm 0.4) \times 10^{-4}$ c

absorption spectra of degradation products, and compared them with the absorption spectra of RF. They described the spectral changes of their RF-containing model system upon light exposure by a decrease of the absorption value at 445 nm and an increase of the absorption value between 350 and 360 nm indicating the formation of lumichrome. Fig. 7 shows the absorption spectra of RF (A) and lumichrome (B) at an equimolar concentration of 5 µmol/l in 12% (v/v) aqueous ethanol with 33.3 mmol/l tartaric acid at pH 3.3. The comparison of the two spectra reveals that hypochromic and hypsochromic effects in the spectra occurred due to photolysis from RF to lumichrome. Fig. 8 shows the absorption spectra of model wine with an oxygen concentration of 250 µmol/l and without transition metals for an UV-C exposure time of 0 s (A) and for an UV-C exposure time of 780 s (B). The absorbance values between 320 and 520 nm were 10 times lower in the experimental treatments (Fig. 8 A and B) since the model wine was spiked with RF at a concentration of 0.53 µmol/l which is a typical concentration for wine (Mattivi et al., 2000). However, the comparison of the two spectra in Fig. 8 reveals a similar effect as shown in Fig. 7 with the RF and lumichrome as reference substances. Light exposure through UV-C caused a hypochromic and hypsochromic effect of the spectra. This becomes apparent through the disappearance of the two peaks of riboflavin at 375 and 445 nm and the appearance of new peak at 356 nm. It is thought that lumichrome was formed as a consequence of RF photolysis. The photolysis occurs through the removal of the ribityl group and reconfiguration of a double bond in the ring structure (Insińska-Rak & Sikorski, 2014). It was proposed that the degradation of RF to lumichrome takes place through formylmethylflavin as an intermediate (Insińska-Rak et al., 2014; Sheraz et al., 2014b). Remucal and McNeill (2011) mentioned that the oxidation potential of lumichrome is inferior than RF based on their redox potential, which could have an impact on the formation rate of 2-AAP. Nevertheless, RF can also degrade to other degradation products such as carboxymethylflavin and cyclodehydroflavin (Sheraz et al., 2014a).

4. Conclusion

Higher oxygen content accelerated the formation of 2-AAP via type I and/or type II RF-photosensitized reactions. The presence of transition metals promoted the photo-Fenton reaction forming acetaldehyde, hence decelerating 2-AAP formation. The photo-Fenton reaction can be seen as a competitive reaction to the RF-photosensitized formation of 2-AAP. Based on its strong photoreactivity and its low reduction potential, ferrous iron mitigated 2-AAP formation more significantly than cupric copper. RF itself does not only act as a photosensitizer but also is degraded via first-order kinetics. The results suggest that lumichrome is a possible degradation product of RF. High oxygen mitigated the degradation of RF resulting in better regeneration of RF what, in turn, increases the reaction yield of 2-AAP. Transition metals accelerated the

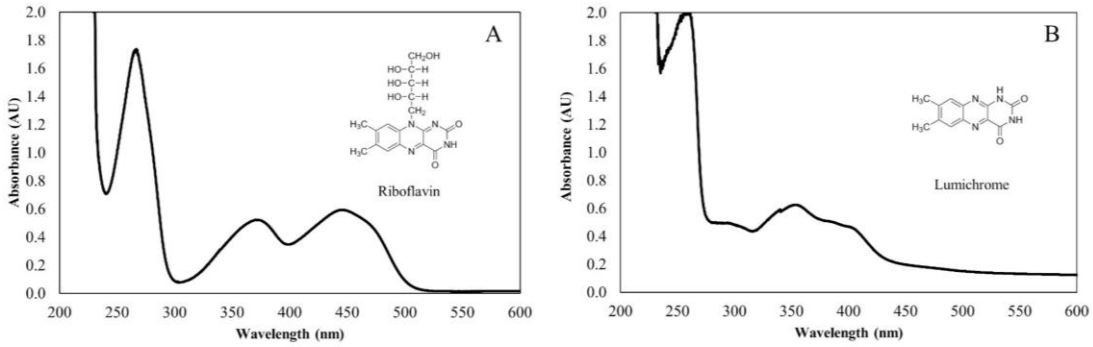

Fig. 7. Absorption spectra from 200 to 600 nm of RF (A) and lumichrome (B) at an equimolar concentration of 5 μmol/l in 12% (v/v) aqueous ethanol with 33.3 mmol/l tartaric acid at pH 3.3.

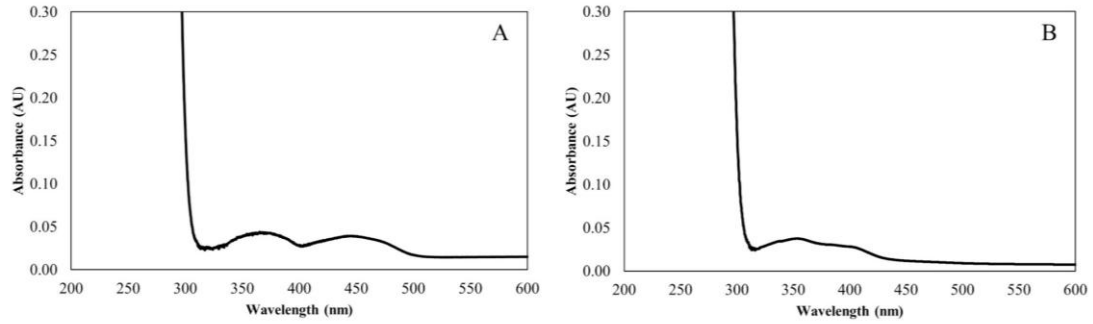

Fig. 8. Absorption spectra from 200 to 600 nm of the model wine with an oxygen concentration of 250 μmol/l without transition metals for an UV-C exposure time of 0 s (A) and for an UV-C exposure time of 780 s (B).

degradation of RF through oxygen being consumed via photo-Fenton reaction resulting in a decreased reaction yield of 2-AAP. >85% of TRP were degraded via first-order kinetics to unknown degradation product(s) during RF-photosensitized degradation reaction. In future studies, the formation of 2-AAP should be investigated in real wine under controlled light exposure, TRP and RF content. For a better understanding of RF-photosensitized degradation of TRP, it is necessary to elucidate the unknown degradation product(s) of TRP. Also, the role of lumichrome or other degradation products should be clarified. Overall, the results indicate that minor food constituents, such as oxygen and transition metals, need to be assessed in complex environments. Their reaction pathways can interfere with each other and reaction products can have notable effects on the characteristics of the final products.

CRediT authorship contribution statement

Svetlana Cvetkova: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sarah Edinger:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Daniel Zimmermann:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Benedikt Woll:** Writing – review & editing, Resources, Conceptualization. **Mario Stahl:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Maren Scharfenberger-Schmeer:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Elke Richling:** Writing – review & editing, Supervision. **Dominik Durner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140259.

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Supplementary material

***Supplementary Table 1**: UV-C exposure times and corresponding UV-C doses as determined by chemical actinometry using an iodide/iodate solution according to the method of Rahn (1997) applied in the experiment.*

UV-C exposure time [s]	UV-C dose [kJ/L]
130	1
260	2
390	3
520	4
650	5
780	6

***Supplementary Table 2**: Influence of light exposure through increasing UV-C exposure time on the concentration of TRP, RF, and 2-AAP in model wine with an oxygen concentration of 250 µmol/L without or with transition metals. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

UV-C exposure time [s]	Transition metals	TRP [µmol/L] ± s.d.	RF [µmol/L] ± s.d.	2-AAP [nmol/L] ± s.d.
	-	290 ± 3	0.53 ± 0.01	≤ LOQ
0	Cupric copper	290 ± 2	0.53 ± 0.02	≤ LOQ
	Ferrous iron	290 ± 5	0.53 ± 0.01	≤ LOQ
	-	274 ± 2	0.50 ± 0.01	2.7 ± 0.1
130	Cupric copper	277 ± 2	0.43 ± 0.01	≤ LOQ
	Ferrous iron	274 ± 4	0.41 ± 0.04	≤ LOQ
	-	252 ± 1	0.46 ± 0.01	7.6 ± 0.6
260	Cupric copper	260 ± 2	0.35 ± 0.02	4.0 ± 0.1
	Ferrous iron	256 ± 3	0.36 ± 0.01	2.9 ± 0.1
	-	238 ± 1	0.43 ± 0.01	8.9 ± 0.8
390	Cupric copper	244 ± 4	0.28 ± 0.01	4.0 ± 0.6
	Ferrous iron	241 ± 2	0.28 ± 0.02	3.8 ± 0.1
	-	224 ± 2	0.41 ± 0.02	12.4 ± 0.1
520	Cupric copper	232 ± 2	0.23 ± 0.02	9.3 ± 0.1
	Ferrous iron	227 ± 2	0.23 ± 0.01	4.7 ± 0.3
	-	213 ± 2	0.38 ± 0.01	14.4 ± 0.6
650	Cupric copper	221 ± 3	0.18 ± 0.02	11.3 ± 0.7
	Ferrous iron	215 ± 2	0.19 ± 0.01	5.3 ± 0.3
780	-	198 ± 1	0.36 ± 0.01	18.6 ± 0.3

	Cupric copper	205 ± 1	0.14 ± 0.01	12.9 ± 0.1
	Ferrous iron	203 ± 1	0.16 ± 0.01	5.7 ± 0.1

***Supplementary Table 3**: Influence of light exposure through increasing UV-C exposure time on the concentration of TRP, RF, and 2-AAP in model wine with an oxygen concentration of 125 µmol/L without or with transition metals. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

UV-C exposure time [s]	Transition metals	TRP [µmol/L] ± s.d.	RF [µmol/L] ± s.d.	2-AAP [nmol/L] ± s.d.
	-	289 ± 4	0.53 ± 0.01	≤ LOQ
0	Cupric copper	290 ± 3	0.53 ± 0.01	≤ LOQ
	Ferrous iron	290 ± 1	0.53 ± 0.03	≤ LOQ
	-	276 ± 2	0.46 ± 0.01	24 ± 0.1
130	Cupric copper	277 ± 2	0.46 ± 0.01	≤ LOQ
	Ferrous iron	271 ± 4	0.41 ± 0.01	≤ LOQ
	-	258±2	0.42±0.02	4.4±0.1
260	Cupric copper	261 ± 1	0.38 ± 0.01	4.3 ± 0.6
	Ferrous iron	258 ± 3	0.30 ± 0.02	2.2 ± 0.1
	-	243 ± 3	0.37 ± 0.01	5.7 ± 0.3
390	Cupric copper	249 ± 2	0.31 ± 0.01	4.8 ± 0.1
	Ferrous iron	246 ± 1	0.21 ± 0.01	3.3 ± 0.2
	-	234 ± 3	0.32 ± 0.02	7.6 ± 0.7
520	Cupric copper	235 ± 1	0.27 ± 0.01	6.4 ± 0.3
	Ferrous iron	231 ± 1	0.16 ± 0.01	3.5 ± 0.2
	-	223 ± 4	0.28 ± 0.01	10.3 ± 1.1
650	Cupric copper	222 ± 1	0.22 ± 0.01	7.0 ± 0.2
	Ferrous iron	218 ± 2	0.11 ± 0.01	3.7 ± 0.3
	-	208 ± 2	0.24 ± 0.01	11.7 ± 0.9
780	Cupric copper	211 ± 2	0.19 ± 0.01	7.8 ± 0.6
	Ferrous iron	205 ± 1	0.09 ± 0.01	4.4 ± 0.1

***Supplementary Table 4**: Influence of light exposure through increasing UV-C exposure time on the concentration of TRP, RF, and 2-AAP in model wine with an oxygen concentration of 6 µmol/L without or with transition metals. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

UV-C exposure time [s]	Transition metals	TRP [µmol/L] ± s.d.	RF [µmol/L] ± s.d.	2-AAP [nmol/L] ± s.d.
	-	290 ± 2	0.53 ± 0.02	≤ LOQ
0	Cupric copper	290 ± 3	0.53 ± 0.02	≤ LOQ
	Ferrous iron	290 ± 3	0.53 ± 0.02	≤ LOQ
	-	280 ± 2	0.43 ± 0.02	≤ LOQ
130	Cupric copper	279 ± 2	0.42 ± 0.02	≤ LOQ
	Ferrous iron	274 ± 2	0.42 ± 0.01	≤ LOQ
	-	267 ± 2	0.36 ± 0.01	2.2 ± 0.5
260	Cupric copper	263 ± 2	0.34 ± 0.01	2.0 ± 0.1
	Ferrous iron	262 ± 1	0.33 ± 0.01	≤ LOQ
	-	253 ± 1	0.28 ± 0.02	3.0 ± 0.9
390	Cupric copper	253 ± 42	0.35 ± 0.02	2.3 ± 0.1
	Ferrous iron	249 ± 3	0.27 ± 0.02	1.9 ± 0.1
	-	246 ± 2	0.22 ± 0.01	3.0 ± 0.3
520	Cupric copper	240 ± 2	0.22 ± 0.02	2.3 ± 0.1
	Ferrous iron	237 ± 4	0.23±0.01	2.1 ± 0.2
	-	236 ± 1	0.18 ± 0.01	3.9 ± 0.1
650	Cupric copper	229 ± 2	0.17 ± 0.02	2.4 ± 0.4
	Ferrous iron	223 ± 3	0.20 ± 0.01	2.2 ± 0.2
	-	222 ± 3	0.14 ± 0.01	4.4 ± 0.6
780	Cupric copper	219 ± 1	0.13 ± 0.01	2.7 ± 0.1
	Ferrous iron	212 ± 3	0.19 ± 0.01	2.9 ± 0.1

4.4 Comparing the effect of UV treatment at wavelengths 254 nm and 280 nm: inactivation of *Brettanomyces bruxellensis* and impact on chemical and sensory properties of white wine

The germicidal effect of UV wavelength extends over a wavelength of 254 nm. Some studies showed that the 280 nm wavelength has a significantly higher or similar germicidal effect as well as the 254 nm wavelength. This wavelength allows the implementation of LED technology to produce light instead of polluting mercury-vapor lamps, which have been severely restricted due to Regulation (EU) 2017/852 based on the Minamata Convention. The aim of this study was to investigate and compare the efficiency of UV treatment of white wine at a wavelength of 254 nm versus 280 nm for inactivating harmful microorganisms. Furthermore, the effect of both wavelengths on the chemical and sensory properties of wine was evaluated. The 20 W low-pressure mercury-vapor lamps were used to generate the wavelength of 254 nm, and LED modules comprised 36 LED lamps for 280 nm.

Wine treated with 280 nm showed a significantly increased germicidal effect against the harmful microorganism compared to 254 nm. Nevertheless, the chemical analysis has shown that the concentration of phenolics was significantly strongly affected via pseudo-first order at the wavelength 280 nm through the higher absorption at 280 nm. That led to a higher impact on the sensory properties of wine at 280 nm. The chemical analysis of the volatile compounds showed that they were similarly affected under both wavelengths. The sensory examination, in the form of the triangle test, showed that treatment of wine with a wavelength of 280 nm significantly affected the gustatory and olfactory properties of the wine compared to treatment at a wavelength of 254 nm.

The study showed that 280 nm, despite its better inactivation effectiveness, is unsuitable for microbial stabilization of wine, as it leads to significantly strong sensory changes at the same UV-C dose due to 254 nm. Further studies should investigate the synergetic effect of several wavelengths and the use of pulsed UV light.

Comparing the effect of UV treatment at wavelengths 254 nm and 280 nm: inactivation of *Brettanomyces bruxellensis* and impact on chemical and sensory properties of white wine

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Disclaimer:

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Comparing the effect of UV treatment at wavelengths 254 nm and 280 nm: inactivation of *Brettanomyces bruxellensis* and impact on chemical and sensory properties of white wine

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Abstract

Ultraviolet (UV) light at 254 nm is well known for its germicidal properties and widely used in food and beverage preservation, though UV light-emitting diodes (UV-LEDs) at 280 nm have advantages over traditional UV mercury-vapour lamps and are thus discussed to replace them. This study investigates the efficiency of 280 nm for inactivation of harmful yeast *Brettanomyces bruxellensis* in white wine as compared to 254 nm. Increasing UV doses were applied in a Riesling wine. Since another treatment wavelength potentially affects reaction kinetics of wine compounds, the chemical and sensory properties were investigated. The microbial analysis revealed greater efficiency of 280 nm compared to 254 nm through comparison by 5-log inactivation dose. It was shown that the Weibull model is suitable to describe the inactivation kinetics of *Brettanomyces bruxellensis* at 254 and 280 nm. Chemical analyses showed significant differences between UV treatments at 254 and 280 nm for the colour properties and phenol concentrations but not for the investigated volatile compounds. Phenol degradation was more pronounced with increasing UV doses at 280 nm as described by pseudo first-order kinetics. Sensory evaluation of the wine revealed that UV treatment at 280 nm changed odour and taste stronger than at 254 nm. Despite better microbial efficiency, the 280 nm approach seems less suitable for the UV treatment of white wine.

Keywords: UV-C, UV-LED, 280 nm, wine, *Brettanomyces bruxellensis*, phenols, volatile compounds

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1 Introduction

UV-C is discussed as a non-thermal and non-chemical technology for preservation of grape must and wine against harmful microorganisms (Fredericks et al., 2011; Diesler et al., 2019; Junqua et al., 2020). The method is already approved as a processing technology to inactivate microorganisms in some fluid products, such as fruit juice by the US FDA (Food and Drug Administration, 2012), for apple juice and cider by Health Canada (Health Canada, 2004). The European Union authorised UV-treated milk as a novel food to increase its Vitamin D content (European Union, 2017). The use of traditional mercury-vapor lamps in UV technology reveals some problems; the disadvantages are a high energy consumption, limited lifetime, emitting a broad spectrum, high operating temperatures, containing harmful mercury, which all affects their applicability with regard to safety and sustainability (Vilhunen et al., 2009). A future innovation could be UV-LEDs (ultra-violet light-emitting diodes) technology replacing mercury-vapor lamps. UV-LEDs are mercury-free two-terminal p-n junction semiconductor devices, which emit light in a narrow spectrum (Hinds et al., 2019). The new technology promises further important advantages such as their small size, low power consumption, fast time to reach operating time, as well as durability and efficiency (Popović et al., 2021).

The typical wavelength used for microbial inactivation is 254 nm. This wavelength is close to the absorption maximum at about 260 nm of the DNA of microorganisms (Bolton & Cotton, 2011). The structure of the DNA of microorganisms is modified when absorbing the UV light. Two adjacent thymine bases form a thymine dimer preventing microbial replication and leading to cell death (Rastogi et al., 2010; Ravanat et al., 2001). Oxidative stress is also crucial with regard to UV-induced cell inactivation. When UV light is absorbed by cellular chromophores, the photoproducts are various ROS (reactive oxygen species) such as hydroxyl radicals and hydrogen peroxide, which can cause oxidative damage to cellular macromolecules such as proteins and DNA (Desai and Kowshik, 2009). Recent studies have shown that wavelengths between 260 and 280 nm have a stronger germicidal effect than at 254 nm. Bowker et al. (2011), Popović and Koutchma (2020), Rattanakul and Oguma (2018) and Li et al. (2017) indicated more effective microbial inactivation and efficiency for UV light at 280 nm. In addition, longer wavelengths potentially have an increased penetration depth into the liquid media and therefore could are more attractive for technology purposes.

Atilgan et al. (2021) reported that different factors can affect the efficiency of UV-C treatment of food. Physicochemical and optical properties of food as well as the type of microorganisms and their cell count have an impact on UV-C efficiency. Microorganisms are known to exhibit different sensitivity against UV-C. Their sensitivity is categorized in the following order: bacteria > yeasts > bacterial spores > molds > viruses (Barbosa-Cánovas et al., 2005). Wine spoilage can occur through both bacteria and yeast. A widespread problem in wine are infections with *Brettanomyces bruxellensis* (Wedral et al., 2010), which belong to the domain of yeasts and are more resistant against UV-C than bacteria. Hirt et al. (2022) investigated and described the inactivation kinetics of another yeast, *Saccharomyces cerevisiae* with UV-C treatment at 254 nm in wine. The Weibull model was found to be the most suitable model to predict the inactivation of microorganisms in wine.

UV light in general is not only absorbed from the DNA of microorganisms, but also interacts with wine compounds through the formation of radicals, photo-oxidation and photosensitised reactions. This can result in colour changes (Li et al., 2008), degradation of

compounds relevant for wine quality (Golombek et al., 2021) and formation of off-flavors (Fracassetti et al., 2019). It was reported that the photodegradation of pigments can cause brightening (Falguera et al., 2011) or browning due to the photo-oxidation of phenols (Müller et al., 2014). Light can promote the formation of the yellow xanthylium cation through degradation of tartaric acid in the presence of catechin which affects the colour of wine (Dias et al., 2013). Islam et al. (2016) and Cvetkova et al. (2024a) reported a decrease of phenols which could be caused through photo-oxidation or photo-induced reaction in apple juice and wine. Golombek et al., (2021) and Cvetkova et al. (2024 a,b) showed that an overdose of UV-C, in fact a dose higher than what is needed for microbial inactivation, can produce 2-aminoacetophenone (2-AAP) and acetaldehyde and degrade volatile compounds such as aliphatic esters and C13-norisoprenoids in must and wine. Overdosing UV-C, through a complex interplay of chemical reactions, can also influence the sensory properties of wine. Especially white wine, because of its minor content of compounds with antioxidative effect, is susceptible to light-induced changes (Grant-Preece et al., 2015).

The influence and the comparison of different wavelengths for UV treatment have not been investigated in wine yet. In general, UV- or light studies set their focus on either microbial inactivation or the chemical effects of UV or light, but barely discuss both aspects in the same context. The objective of this study was to evaluate and compare the effectiveness of using UV-LEDs at 280 nm as an alternative light source for the traditional wavelength for UV-C technology at 254 nm to inactivate *Brettanomyces bruxellensis* in white wine. However, another treatment wavelength potentially changes reaction kinetics of wine compounds. In fact, it was shown before that the kinetics of light-induced reactions are influenced by the wavelength altering light-induced reactions (Albini 2015). In order to consider the effect of different wavelengths, chemical and sensory changes, related to some relevant volatile and phenol compounds, were analysed in addition to the microbial inactivation.

2 Materials and methods

2.1 Materials

Catechin, tartaric acid, acetaldehyde (≥99.5%), acetonitrile (≥99.5%), kanamycin sulfate, chloramphenicol, yeast extract peptone dextrose and sodium sulfate (≥99%) were purchased from Carl Roth (Karlsruhe, Germany); gallic acid, caftaric acid, caffeic acid, 2-aminoacetophenone (≥98%), sodium hydroxide (≥98%) were purchased from Merck (Darmstadt, Germany); 2-aminoacetophenone-d5 (≥98%) was purchased from Eptes Sàrl (Vevey, Switzerland); ethanol (≥96%) was purchased from Berkel AHK (Ludwigshafen, Germany); potassium hydrogen phosphate (≥99.5%) and phosphoric acid (≥85%) were purchased from ORG Laborchemie GmbH (Bunde, Germany). All chemicals were of analytical reagent grade as minimum. HPLC grade water was obtained from a Milli-Q system (Purelab flex 4, Veolia Water Technology GmbH, Celle, Germany).

2.2 Wine

The wine was produced from Riesling grapes (Vitis vinifera L. cv.), which were harvested from the experimental vineyards of the Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz (Neustadt an der Weinstraße, Germany) in the 2022 vintage. After harvest, grapes were destemmed, crushed and pressed off. The obtained grape must was clarified by

sedimentation, then transferred into a 100 L stainless-steel fermenter, inoculated with Saccharomyces cerevisiae (Lalvin CY 3079 YSEO; Lallemand, Montreal, Canada) and fermented at constant 18°C until < 1 g/L residual sugar. The wine was racked off the yeast lees, filtered with a sheet filter (BECO-COMPACT PLATE A400 SF/ASF; Eaton, Dublin, Ireland) using 0.4 µm filter sheets (BECO sterile 40; Eaton, Dublin, Ireland) and stabilized with 25 mg/L free SO2 (solution sulfureuse P15; Erbslöh, Geisenheim, Germany). The wine was stored at 12 °C in a 100 L sterile stainless-steel keg pressurized at ~1.4 bar with liquid nitrogen for two months until the start of experiments.

2.3 Inoculation with *Brettanomyces bruxellensis*

Two different UV reactors were utilized for the experiments. For both wavelengths, 5 L wine were treated with increasing UV doses: 0, 200, 400, 600, 800, 1000, and 1200 J/L to allow for determination of inactivation kinetics (chapter 2.6) and chemical reaction kinetics (chapter 2.13). For 254 and 280 nm, time-dose relationships were determined by chemical actinometry using an iodide/iodate solution according to the method of Rahn (1997), extended by Bolton et al. (2011) and Goldstein and Rabani (2008). The control treatment (0 J/L) was carried out separately with lamps turned off in both reactors. Each UV treatment was conducted in triplicate. For each wavelength and each UV dose, 50 mL treated wine were sampled and stored in a dark, cooled room at 14 °C for microbial and chemical analyses. For sensory evaluation, four 0.75 L Alsace-style bottles were samples at 0 and 1000 J/L for each wavelength. The bottles were sealed with screw caps and stored in a dark, cooled room at 14 °C until the sensory evaluation. Microbial analysis took place immediately after UV treatment. Chemical and sensory analysis took place one week after UV treatment.

2.4 Experimental setup for UV treatment

Two different UV reactors were used for the experiments. The UV reactors and the UV treatment of the wine are described in chapters 2.4.1 and 2.4.2. One week before start of UV experiments, the wine was filtered again using a bottle cap sheet filter (pore size 0.45 µm, SFCA-membrane; Thermo Scientific, Waltham, USA). The wine was then separated in two batches for UV treatments at wavelengths 254 and 280 nm. The wine had an absorption of 12.5 AU at 254 nm and 8.6 AU at 280 nm. For both wavelengths, 5 L of wine was treated with increasing UV doses: 0, 200, 400, 600, 800, 1000, and 1200 J/L to allow for the determination of inactivation kinetics (chapter 2.5) and chemical reaction kinetics (chapter 2.12). The UV doses were chosen based on the study of Hirt et al. (2022). For 254 and 280 nm, time-dose relationships were determined by chemical actinometry using an iodide/iodate solution according to the method of Rahn (1997), extended by Bolton et al. (2011) and Goldstein and Rabani (2008). The control treatment (0 J/L) was carried out separately with lamps turned off in both reactors. Each UV treatment was conducted in triplicate. For each wavelength and each UV dose, 50 mL of treated wine was sampled for microbial and chemical analyses. Microbial analysis took place immediately after UV treatment. The rest of the samples was frozen at -20 °C for chemical analyses. For sensory evaluation, four 0.75 L Alsace-style bottles were samples at 0 and 1000 J/L for each wavelength. The bottles were sealed with screw caps and stored in a dark room at 14 °C until sensory evaluation. Chemical and sensory analysis took place one week after UV treatment.

2.4.1 UV reactor for treatment at wavelength 254 nm

For the treatment of wine at 254 nm, a thin-film UV reactor, built and put into operation by the Institute for Food and Bioprocess Engineering of the Max Rubner Institute (Karlsruhe, Germany), was used. The reactor was equipped with five 20 W low-pressure mercury lamps (UVPro FMD Series; Bioclimatic B.V., Netherlands) and 35 fluid guiding elements (FGE) for flow control. The length of each FGE was 60 mm. The reactor was connected between each other with standard thermoplastic soft polyvinyl chloride (PVC) tubes (4.8 mm I.D., 9.8 mm O.D.; Heidolph, Schwabach, Germany). A peristaltic pump (Hei-FLOW Precision 06; Heidolph, Schwabach, Germany) was used to pass the wine through the reactor at a flow rate of 100L/h ± 1 L/h. A cooling system (Julabo F30VC/3; Seelbach, Germany) was included to keep the temperature in the reactor constant at 20°C ± 1.0 °C.

The processing of UV treatment was based on the standard operating procedure (SOP) described by Hirt et al. (2022). The 20 W low-pressure mercury lamps were turned on 45 minutes prior to the experiments to achieve the mercury evaporation temperature and uniform test conditions. To equalize the temperature of the reactor while the source is brought to operating temperature, water at 20 °C was pumped through the reactor. Before the start of each experiment, the tubes of the feeding peristaltic pump were sterilized with Bacillol® and then flushed with 2 L distilled water. Upon completion of each experiment, 2 L of demineralized water was pumped through the reactor. Then, a 70 % ethanol solution was circulated for 10 min, after which the reactor was flushed with 2 L demineralized water and finally blown dry with sterilized air. The lamps were kept on during the sanitizing process.

2.4.2 UV reactor for treatment at wavelength 280 nm

For the treatment of wine at 280 nm, a modified straight tube reactor based on a UV test chamber BS-04 (Opsytec Dr. Gröbel, Mannheim, Germany) was developed by the Institute for Food and Bioprocess Engineering of the Max Rubner Institute (Karlsruhe, Germany). Three 280 nm LED modules (each consisting of 12 LEDs, 297.3 x 44.6 mm, 1050mA, 630 mW; Lumitronix LED-Technik GmbH, Hechingen, Germany) were equipped in the test chamber. The reactor consisted of 24 straight UV-transparent fluorinated ethylene propylene (FEP) tubes (each 6 mm I.D., 6.6 mm O.D.; Heidolph, Schwabach, Germany) connected by UV-transparent U-turns. A peristaltic pump (Hei-FLOW Precision 06; Heidolph, Schwabach, Germany) was used to pass the wine through the reactor at a flow rate of 100 L/h. The temperature during the experiment was 20 °C ± 1.0 °C; active cooling was not necessary. The LED lamps do not require any preconditioning time and were switched on shortly before the start of experiments. The sanitizing steps at the start of the experiment and at the end of the experiment are identical to the steps described for the UV reactor at wavelength 254 nm (section 2.4.1).

2.5 Determination of microbial inactivation

After sampling of each UV dose including control (0 J/L), a tenfold dilution series was prepared in 0.9% NaCl. All dilutions were plated within one hour after the experiments on YPD agar with antibiotics (25 µg/mL kanamycin sulphate and 30 µg/mL chloramphenicol) in triplicate. The YPD agar plates were incubated at 30 °C for 48 h. After incubation, colonies were counted.

The Weibull model (Equation 1) was used to determine the inactivation kinetics of *Brettanomyces bruxellensis* in white wine at 254 nm and 280 nm. The Weibull model is shown in Equation 1, with N being the cell count reached at a certain UV dose, with N_0 being the initial cell count, with D_{UV} being the applied UV dose, with δ being the UV dose required for 1-log inactivation, and with p being the shape parameter describing concavity (Peleg and Cole, 1998; Weibull, 1951).

$$log\left(\frac{N}{N_0}\right) = -\left(\frac{D_{UV}}{\delta}\right)^p \qquad \textit{Equation (1)}$$

2.6 Photometric measurement and determination of chromatic characteristics of wine according to CIEL*a*b*

The absorption of wine at 254 nm and 280 nm and chromatic characterisation of samples were measured via a UV-VIS double-beam photometer (V-740; Jasco, Tokyo, Japan). The samples were measured in 10-mm pathlength micro-UV cuvette (Brand, Wertheim, Germany). Wine colour was analysed according to the method OIV-MA-AS2-11 (Resolution Oeno 1/2006) based on spectral measurements from 360 nm to 830 nm with a 1 nm interval. The trichromatic components L* (lightness), a* (green-red component), and b* (blue-yellow component) were calculated and provided according to the Commission Internationale de l´Eclairage (CIE, 1976). The colour difference (ΔE_{00}) was calculated based on the CIEDE2000 according to DIN EN ISO 11664-6:2016-12.

2.7 HPLC analysis of phenols

The quantification of catechin, gallic, caftaric and caffeic acid was carried out according to Golombek (2019) with some modifications. The samples were analysed by high performance liquid chromatography (HPLC) (Jasco, Tokyo, Japan). Diode array detection (DAD) (MD-4010; Jasco, Tokyo, Japan) was used for detection. Catechin and gallic acid was detected at wavelengths 280 nm, caftaric and caffeic acid at 320 nm. The injection volume was 10 µL at a flow of 1 mL/min. The. A Gemini NX C18 column (150 mm × 4.5 mm, 3 µm particle size; Phenomenex, Torrance, USA) was used for separation. The column temperature during measurement was 25°C. The analysis was carried out by gradient elution. Mobile phase A consisted of 5/95 (v/v) acetonitrile/phosphate buffer and mobile phase B consisted of 50/50 (v/v) acetonitrile/phosphate buffer. Phosphate buffer consisted of 10 mmol/L KH_2PO_4 adjusted to pH 1.5 with 85% H_3PO_4. Calibration was performed using an external calibration method.

2.8 HS-SPME-GC-qMS analysis of volatile compounds

The analysis of esters was carried out according to a published method by Golombek et al. (2021). The samples were analysed by headspace solid-phase microextraction coupled with gas chromatography coupled with quadrupole mass spectrometry (HS-SPME-GC-qMS). The system components were: autosampler (Combi PAL; CTC Analytics, Zwingen, Switzerland), gas chromatograph (Trace GC Ultra; Thermo Fisher Scientific, Waltham, USA), and a quadrupole mass spectrometer (Trace DSQ; Thermo Fisher Scientific, Waltham, USA). HS-SPME was carried out with 10 mm PDMS SPME fiber (fiber thickness: 95 µm, CTC Analytics; Zwingen, Switzerland). The system was equipped with the column Zebron ZB-5MS (30 m × 0.25 mm ID × 0.5 µm; Phenomenex, Torrance, USA). Helium was used as carrier gas at a constant flow of 1 mL/min. The oven temperature was programmed from 40 °C (3 min hold) at

4 °C/min to 150 °C and at 50 °C/min to 300 °C (3 min hold). Electron impact ionization was performed at 70 eV in positive mode. The temperatures of the transfer line and ion source were 250 °C. Argon was used as a collision gas with a collision cell pressure of 1.1 mTorr. The mass spectrometer was operated in full scan mode with a range from *m/z* 29 to *m/z* 300. Calibration was performed for the analysed compounds using an external calibration method as described by Golombek et al. (2021).

2.9 HS-SPME-GC-MS/MS analysis of 2-AAP

The analysis of 2-AAP was carried out according to a published method of Schmarr et al. (2016) with some modifications (Cvetkova et al., 2024b). The samples were analysed by headspace solid phase microextraction coupled with gas chromatography coupled with a triple quadrupole mass spectrometry (HS-SPME-GC-MS/MS). It was composed of three main modules: autosampler with SPME option (TriPlus RSH; Thermo Fisher Scientific, Waltham, USA), gas chromatography (Trace GC Ultra; Thermo Fisher Scientific, Waltham, USA), and the mass spectrometer (MS TSQ Quantum XLS Ultra; Thermo Fisher Scientific, Waltham, USA). HS-SPME was carried out with 10 mm Carbon WR fiber (95 µm, CTC Analytics; Zwingen, Switzerland). The system was equipped with the column Zebron ZB WAXplus™ (30 m × 0.25 mm i.D. × 0.25 µm; Phenomenex, Torrance, USA). Helium was used as carrier gas at a constant flow of 1.2 mL/min. The oven temperature was programmed from 40 C (3 min hold) at 5 °C/min to 180 °C and at 20 °C/min to 200 °C (5 min hold). Electron impact ionization was performed at 70 eV in positive mode. The temperatures of the MS-transfer line and ion source were 230 °C. Argon was used as a collision gas with a collision cell pressure of 1.1 mTorr. The mass spectrometer was operated in full scan mode with a range of *m/z* 40 to *m/z* 250. Calibration was performed using an external calibration method.

2.10 Enzymatic analysis of acetaldehyde

Acetaldehyde was quantified using a commercially available enzymatic assay (REF 984347; Thermo Fischer Scientific, Waltham, USA) on an automated Konelab 20i (Typ 954; Thermo Fisher Scientific, Waltham, USA) according to Närkki et al. (2015). Sample preparation and measurement were done according to the official guideline of enzymatic assay. Calibration was performed using an external calibration method.

2.11 Sensory evaluation

The wines treated with 1000 J/L at 254 nm and 280 nm were compared against the control wine and each other via triangle test to investigate the impact of UV treatment on potential colour, odour, and taste changes. The triangle test was carried out according to DIN EN ISO 4120:2021-06. 20 mL samples were presented in black DIN 10960 wine glasses (Schott Zwiesel, Germany) for odour and taste evaluation, and clear DIN 10960 wine glasses (Schott Zwiesel, Germany) for colour evaluation to 30 panellists (14 male/ 16 female) from Max Rubner-Institute. Each glass was covered with a plastic lid and coded with a three-digit number. The wine temperature during the evaluation was 18 °C. The samples were evaluated at room temperature in individual booths (NF EN ISO 8589:2007). The panellists were asked to identify the different samples for each parameter – colour, odour, and taste. The sample order was randomized for each panellist.

2.12 Statistical data analysis and kinetics calculation

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 ($p \leq 0.05$). Tukey least significant difference (HSD) post-hoc test ($p \leq 0.05$) was applied. Chemical data was evaluated for significant differences between treatments using two-way analysis of variance including interactions (ANOVA, $p \leq 0.05$). The triangle test data was examined for significant changes across replicates using the Clopper-Pearson method and the Thurstonian model ($p \leq 0.05$).

The calculation of coefficient of determination (R^2) (Equation 2) and the root mean squared error (RMSE) (Equation 3), using the calculated (cal) and experimentally determined (ex) logarithmic reduction, were calculated using the following formula:

$$R^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2} \qquad \textit{Equation (2)}$$

$$RMSE = \sqrt{\frac{1}{n}\sum\left[\left(log\frac{N}{N_0}\right)_{cal} - \left(log\frac{N}{N_0}\right)_{exp}\right]^2} \qquad \textit{Equation (3)}$$

For determination of kinetic rate, calculated using the following formula (Equation 4). c_0 is the start concentration of compound in wine, c is the concentration of compound during UV treatment, k is the kinetic rate of compound, D_{UV} is UV dose.

$$ln\frac{c}{c_0} = -k[c] \times D_{UV} \qquad \textit{Equation (4)}$$

3 Results and discussion

3.1 Microbial inactivation efficiency of UV treatment at 254 nm and 280 nm on *Brettanomyces bruxellensis*

The UV-C inactivation of yeast in wine can be described by the Weibull model (Hirt et al., 2022). The study considered the standard wavelength for the UV-C treatment at 254 nm. To extend the Weibull model to another wavelength in the UV region, it was necessary to investigate the model performance at 280 nm. Table 1 compares the model performance for *Brettanomyces bruxellensis* inactivation in Riesling at 254 nm and 280 nm wavelengths. Both R^2 and RMSE indicated slightly lower model performance for 280 nm compared to 254 nm. Nevertheless, microbial studies are often associated with a large bias and compared with the study by Soro et al. (2021), the obtained values are in an appropriate range for models of microbial inactivation. The Weibull function proved to be a suitable mathematical model for describing microbial inactivation at 254 nm and 280 nm.

***Table 1**: Weibull model performance for Brettanomyces bruxellensis inactivation in Riesling at 254 and 280 nm wavelengths.*

Wavelength [nm]	Regression coefficient R^2	Root mean square error RMSE
254	0.9906	0.32

Wavelength [nm]	Regression coefficient R^2	Root mean square error RMSE
280	0.9861	0.45

Figure 1A shows the influence of increasing UV doses on the inactivation of *Brettanomyces bruxellensis* in Riesling wine treated at 254 nm and 280 nm. UV inactivation was more efficient at a wavelength of 280 nm compared to 254 nm. To reach 5-log inactivation, ensuring the safety of food products (US FDA, 2004), a UV dose of 998 J/L was necessary at 254 nm. The same 5-log inactivation was obtained with a UV dose of 570 J/L at 280 nm. The inactivation efficiency at 280 nm was increased by 43% compared to 254 nm. Amino acids such as tryptophan and tyrosine have an absorption maximum at 280 nm (Pace et al., 1995). Accordingly, the UV light at 280 nm is better absorbed by proteins, ultimately resulting in greater damage of membrane proteins than shorter wavelengths such as the conventional germicidal wavelength of 254 nm (Li et al., 2017; Kim et al., 2017). It was also reported that light at 280 nm can damage important microbial components through reactive oxygen species (ROS), including peroxidation of membrane lipids and respiratory enzyme activity, physical membrane disruption and membrane potential loss (Kim et al., 2017). Previous studies have also shown that light at 280 nm suppressed the photo-reactivation of *E. coli.* (Nyangaresi et al., 2018; Li et al., 2017). These processes lead to a disruption of microorganisms and reduce the recovery of essential cellular functions explaining higher inactivation effectiveness at 280 nm compared to 254 nm. Nevertheless, the higher efficiency at 280 nm may also result from the different wine absorptions at 254 and 280 nm. The absorbance of the wine at 280 nm was 8.6 AU and therefore 30% lower than the absorbance of the wine at 254 nm (12.5 AU). It is known that the absorbance is inversely proportional to the indentation depth of UV (Atılgan et al., 2021). With an increasing wine absorption, an increasing UV dose is required to achieve the same inactivation.

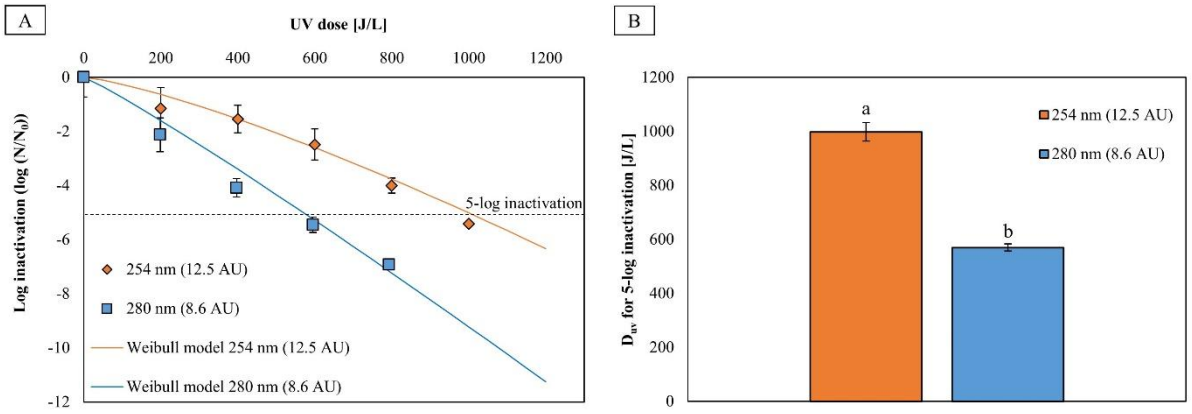

Figure 1*: A: Influence of increasing UV dose on the inactivation of Brettanomyces bruxellensis in Riesling wine treated at 254 nm (wine absorption: 12.5 AU) and at 280 nm (wine absorption: 8.6 AU). The solid lines show the inactivation progress using Weibull fits for the experimentally determined inactivation of Brettanomyces bruxellensis at 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation. B: UV dose for 5-log inactivation of Brettanomyces bruxellensis in Riesling wine treated at 254 nm (wine absorption: 12.5 AU) and at 280 nm (wine absorption: 8.6 AU). Shown are the mean values of experimental replicates including the standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$).*

3.2 Effect of UV treatment at 254 nm and 280 nm on colour

Table 2 shows the influence of increasing UV doses on the chromatic characteristics using the CIEL*a*b* colour space and the total colour difference in relation to the control in Riesling wine treated at 254 nm and 280 nm using the CIEDE2000 formula (ΔE_{00}). The L* value significantly decreased with increasing UV doses for both wavelengths. The negative colour coordinates a* (green) and the positive colour coordinate b* (yellow) significantly increased with higher UV doses for both wavelengths. Higher UV doses caused the ΔE_{00} to change significantly for both wavelengths. The decrease of L* as well as the increase of b* indicate browning of the wine with an increasing UV dose. Browning processes can occur during light exposure, and they are explained by photo-sensitized oxidation of phenols such as gallic acid, catechin and hydroxycinamic acids (Fernández-Recamales et al., 2006; Li et al., 2008; Simpson, 2016). Also, the light could have induced the formation of more complex structures such as xanthylium cations, which are known as yellow pigments (Dias et al., 2013; George et al. 2006; Maury et al. 2010). However, even for a UV dose of 1200 J/L, the ΔE_{00} value did not exceed the visible colour threshold of white wine, which was recently postulated with ΔE_{00}=0.64 (Hensel et al., 2023). A differential influence of the wavelength was observed for the b* value, but not for the L* and a* values (Table 2). The L* and a* values changed similar upon UV exposure for both wavelengths. Renner et al. (2022) showed a relation between increasing b* value and a visual browning process in wine. The b* value increased more upon UV treatment at 280 nm than at 254 nm indicating browning of the Riesling wine.

***Table 2**: Influence of increasing UV doses on the chromatic characteristics using the CIEL*a*b* colour space and the total colour difference in relation to the control in Riesling wine treated at 254 nm and 280 nm using the CIEDE2000 formula (ΔE_{00}). Shown are the mean values of experimental replicates including the standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$).*

UV dose [J/L]	Wavelength [nm]	L*	a*	b*	ΔE_{00}
0 (control)		99.10 ± 0.04 a	-1.58 ± 0.01 c	7.09 ± 0.01 g	-
200		98.87 ± 0.13 b	-1.49 ± 0.04 ab	7.17 ± 0.03 f	0.19 ± 0.04 bcd
400		98.90 ± 0.02 ab	-1.47 ± 0.01 a	7.22 ± 0.04 ef	0.22 ± 0.03 bcd
600	254	98.95 ± 0.06 ab	-1.54 ± 0.02 abc	7.27 ± 0.01 de	0.18 ± 0.01 cd
800		98.82 ± 0.04 b	-1.53 ± 0.02 abc	7.32 ± 0.01 cd	0.25 ± 0.01 abcd
1000		98.84 ± 0.05 ab	-1.52 ± 0.03 abc	7.35 ± 0.02 c	0.27 ± 0.03 abcd
1200		98.85 ± 0.05 ab	-1.56 ± 0.03 bc	7.43 ± 0.01 b	0.31 ± 0.01 ab
0 (control)		99.10 ± 0.04 a	-1.58 ± 0.01 c	7.09 ± 0.01 g	-
200	280	98.87 ± 0.06 b	-1.52 ± 0.03 abc	7.16 ± 0.05 fg	0.17 ± 0.04 d
400		98.90 ± 0.02 ab	-1.49 ± 0.01 ab	7.23 ± 0.01 ef	0.20 ± 0.01 bcd

UV dose [J/L]	Wavelength [nm]	L*	a*	b*	ΔE_{00}
600	280 nm	98.79 ± 0.14 b	-1.57 ± 0.02 c	7.33 ± 0.01 cd	0.26 ± 0.05 abcd
800		98.83 ± 0.05 b	-1.54 ± 0.02 abc	7.38 ± 0.01 bc	0.28 ± 0.01 abcd
1000		98.92 ± 0.05 ab	-1.55 ± 0.02 bc	7.44 ± 0.03 ab	0.30 ± 0.01 abc
1200		98.95 ± 0.02 ab	-1.55 ± 0.04 bc	7.52 ± 0.01 a	0.35 ± 0.01 a
p-value dose		0.0001	0.0001	0.0001	0.0001
p-value wavelength		0.835	0.093	0.0001	0.097
*p-value dose*wavelength*		0.061	0.674	0.004	0.362

3.3 Effect of UV treatment at 254 nm and 280 nm on phenols

Figure 2 shows the decrease of gallic acid (A), catechin (B), caffeic acid (C), and caftaric acid (D) concentrations in Riesling wine treated with increasing UV doses at 254 nm and 280 nm. Benítez et al. (2005) and Li et al. (2012) showed that the degradation of catechin, and gallic acid in aqueous solution under UV exposure corresponds to pseudo-first-order degradation kinetics. Applying the pseudo-first-order kinetics formula to the degradation of gallic acid, catechin, caffeic acid, and caftaric acid in Riesling wine, which was UV-treated at 254 nm and at 280 nm, showed that R^2 values were between 0.9812 and 0.9975 proving a high goodness of fit for pseudo-first-order degradation kinetics (Figure 2). The possible degradation reactions are photo-sensitized isomerization (Golombek, 2019), phenol oxidation and polymerization (Waterhouse et al., 2016). Each of the investigated phenols carries a catechol group. Due to its ortho-substitution, the energy of the OH-bonds is lowered while increasing the rate of abstraction of the proton, making it a perfect hydrogen donor (Waterhouse, 2002a). Phenols with ortho-substituted hydroxyl groups can react with ROS to form semiquinones and quinones. Those can further react with nucleophilic functional groups such as thiols or other phenols, in particular the nucleophilic flavan-3-ol (Danilewicz, 2003; Oliveira et al, 2011; Singleton, 1987; Waterhouse & Laurie, 2006). Catechin and its diastereomer epicatechin can react and form the yellow xanthylium cation pigment under light exposure (Es-Safi et al., 1999). All these reactions can eventually lead to the colour changes of wine (Cáceres-Mella et al, 2014; Dias et al, 2013; Li et al, 2008; George et al, 2006) which agrees with the results shown earlier. Also, an influence on astringency and bitterness of the wine is discussed with regards to the phenolic reactions described (Li et al., 2008; Robichaud and Noble, 1990).

Table 3 shows the pseudo first-order rate constant for the degradation of gallic acid, catechin, caffeic acid, and caftaric acid in Riesling wine treated at 254 and 280 nm. UV treatment had a stronger impact on the gallic acid degradation than on the degradation of the other investigated polyphenols. Fracassetti et al. (2021) and Cvetkova et al. (2024a) showed that hydrolysable tannins, containing high content of gallic acid, are strong radical scavengers reacting with ROS such as hydroxyl radicals, therefore attenuating oxidative processes initiated by light. This in turn causes a decrease of gallic acid concentration. The impact of wavelength

revealed that 280 nm significantly increased the degradation rate of all investigated polyphenols as compared to 254 nm (Table 3). Phenolic compounds have an absorption maximum at 280 nm (Waterhouse, 2002b) which can lead to efficient stimulation and consequently to faster degradation. An UV treatment of wine at 280 nm can lead to a greater change in the colour and taste properties of the wine than at 254 nm.

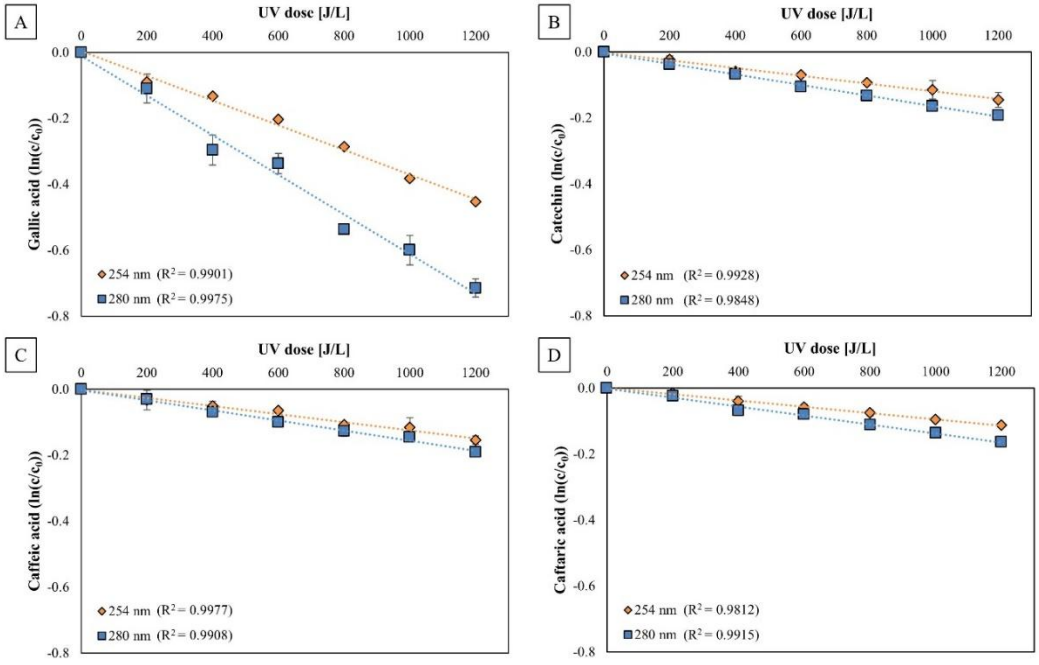

***Figure 2**: Pseudo-first-order degradation kinetics of gallic acid (A), catechin (B), caffeic acid (C) and caftaric acid (D) in Riesling wine treated at 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation.*

***Table 3**: Pseudo first-order rate constant with coefficients of determination of gallic acid, catechin, caffeic acid and caftaric acid in Riesling wine treated at 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$).*

Phenol	Wavelength [nm]	k [1/J]
Gallic acid	254	$(3.7 \pm 0.1) \times 10^{-4}$ b
	280	$(6.0 \pm 0.1) \times 10^{-4}$ a
Catechin	254	$(1.2 \pm 0.2) \times 10^{-4}$ de
	280	$(1.6 \pm 0.1) \times 10^{-4}$ c
Caffeic acid	254	$(1.2 \pm 0.1) \times 10^{-4}$ de
	280	$(1.5 \pm 0.1) \times 10^{-4}$ c
Caftaric acid	254	$(9.5 \pm 0.1) \times 10^{-5}$ e
	280	$(1.4 \pm 0.1) \times 10^{-4}$ cd
p-value phenol		0.0001

p-value wavelength	0.0001
*p-value phenol*wavelength*	0.0001

3.4 Effect of UV treatment at 254 nm and 280 nm on volatile compounds

Figure 3 shows the decreases of 3-methylbutyl acetate (A), ethyl hexanoate (B), ß-damascenone (C), and 4-vinylguaiacol (D) concentrations in Riesling wine treated with increasing UV doses at 254 and 280 nm. Pseudo-first-order degradation kinetics were applied resulting in R^2 values between 0.9629 and 0.9927 which prove a high goodness of fit. Golombek et al. (2021) and Cvetkova et al. (2024a) reported that UV-C treatment at 254 nm can lead to degradation of different volatile compound classes in must and wine through light-induced degradation reactions. For β-damascenone and aliphatic esters it was shown that photodegradation reactions, initiated through UV-C and VIS exposure, are mediated by riboflavin (Golombek et al., 2021; Cellamare et al., 2009).

Table 4 shows the pseudo first-order rate constant for the degradation of 3-methylbutyl acetate, ethyl hexanoate, ß-damascenone, and 4-vinylguaiacol in Riesling wine treated at 254 nm and 280 nm. UV degradation was strongest for 3-methylbutyl acetate and weakest for 4-vinylguaiacol. Ethyl hexanoate and ß-damascenone showed similar degradation kinetics in between those of 3-methylbutyl acetate and 4-vinylguaiacol. The differences in degradation kinetics of the investigated volatile compounds may be explained by the different bond types, substituents, and functional groups of the molecules which can either enforce or reduce non-radiative transitions (Mortimer, 2008). The different wavelengths showed no significant impact on the degradation rates of all four investigated volatile compounds (Table 4). It is assumed that the observed photodegradation reactions of all investigated volatile compounds are mediated by riboflavin. The absorbance of riboflavin is similar at 254 and 280 nm (Cvetkova et al. 2024b), therefore applying different wavelengths results in similar degradation rates.

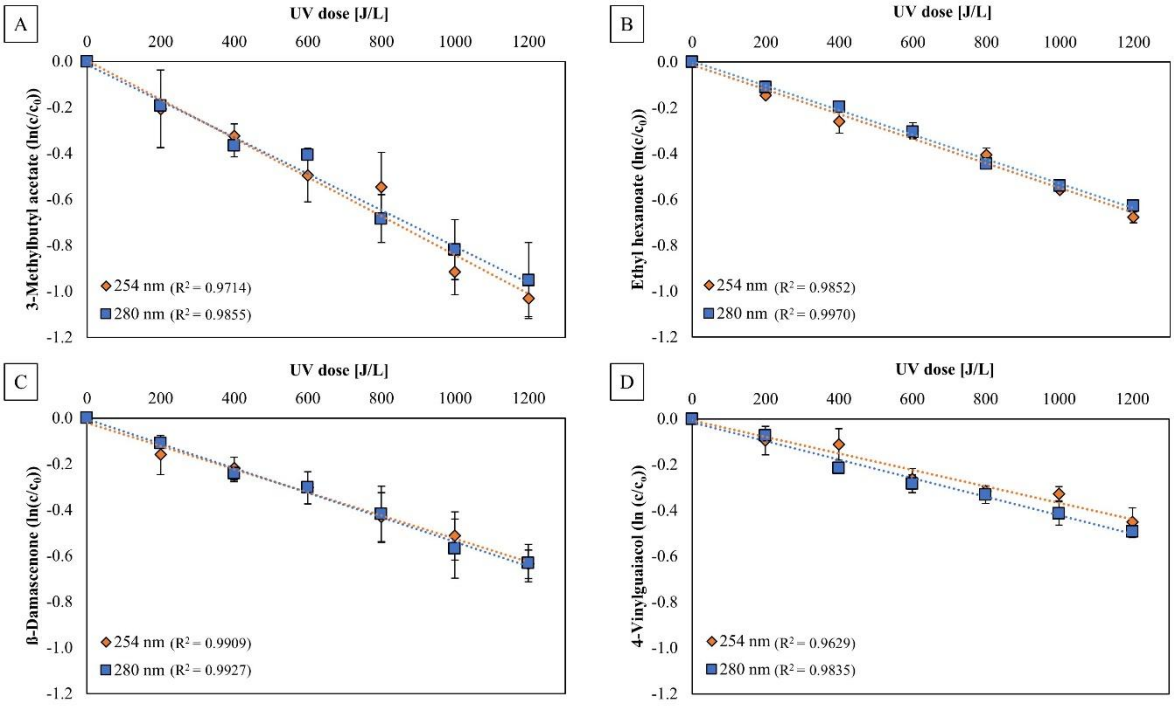

***Figure 3**: Pseudo-first-order degradation kinetics of 3-methylbutyl acetate (A), ethyl hexanoate (B), ß-damascenone (C) and 4-vinylguaiacol (D) in Riesling wine treated at 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation.*

***Table 4**: Pseudo first-order rate constant (k) with coefficients of determination (R^2) for volatile compounds 3-methylbutyl acetate, ethyl hexanoate, ß-damascenone and 4-vinylguaiacol in Riesling wine treated at wavelengths 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$).*

Volatile compounds	Wavelength [nm]	k [1/J]
3-Methylbutyl acetate	254	$(8.4 \pm 0.3) \times 10^{-4}$ a
	280	$(8.0 \pm 0.2) \times 10^{-4}$ a
Ethyl hexanoate	254	$(5.3 \pm 0.1) \times 10^{-4}$ b
	280	$(5.3 \pm 0.1) \times 10^{-4}$ b
ß-Damascenone	254	$(5.0 \pm 0.3) \times 10^{-4}$ b
	280	$(5.3 \pm 0.3) \times 10^{-4}$ b
4-Vinylguaiacol	254	$(3.6 \pm 0.4) \times 10^{-4}$ c
	280	$(4.1 \pm 0.3) \times 10^{-4}$ c
p-value volatile compounds		0.0001
p-value wavelength		0.376
*p-value volatile compounds *wavelength*		0.053

UV-C light at 254 nm can also promote the formation of 2-AAP through riboflavin-photosensitized reaction as well as acetaldehyde through photo-Fenton reaction (Cvetkova et al., 2024a, 2024b). Figure 4 shows the influence of increasing UV doses on the concentration of 2-AAP (Figure 4A) and acetaldehyde (Figure 4B) in Riesling wine treated at 254 nm and 280 nm. Neither 2-AAP nor acetaldehyde showed significant changes in concentration with increasing UV doses at 254 nm and 280 nm. Cvetkova et al. (2024a) reported no changes of 2-AAP in white wine at microbial relevant UV-C doses up to 1000 J/L which agrees with the results shown here. Also, no changes were observed in the acetaldehyde concentration in model wine for UV-C treatment above 2000 J/L (Cvetkova et al. 2024b).

An impact of different wavelengths on 2-AAP and acetaldehyde concentrations was not observed in the presented study (Figure 4A and B). The formation of 2-AAP requires riboflavin (Horlacher & Schwack, 2014) and the absorbance of riboflavin is similar at 254 and 280 nm (Sheraz et al., 2014). Accordingly, applying different wavelengths makes no significant difference. Cvetkova et al. (2024b) showed that riboflavin-photosensitized reaction and photo-Fenton reaction are linked to each other by hydrogen peroxide, which is produced while riboflavin transits from triplet to its ground state and serves as an educt for photo-Fenton reaction. This link can explain why no impact of different wavelengths was observed on acetaldehyde concentration.

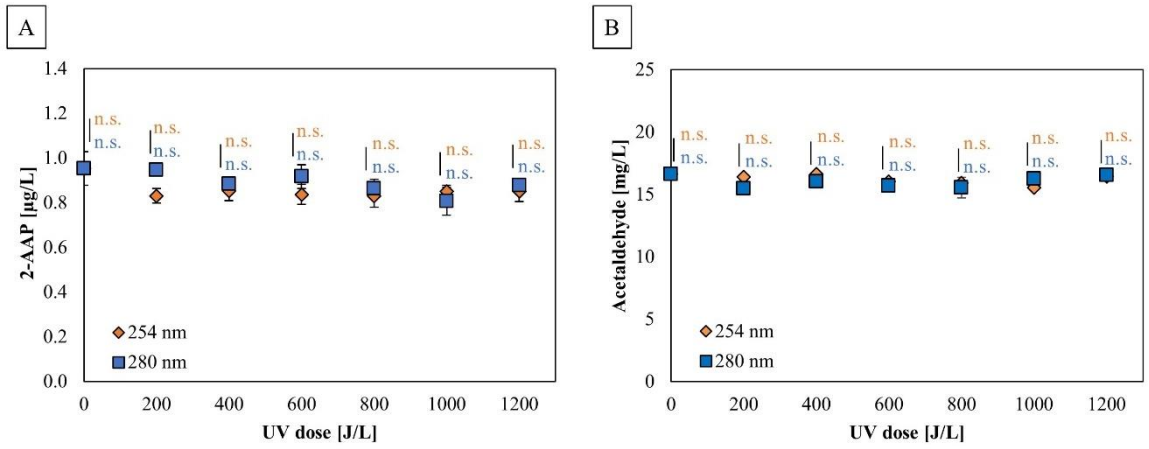

***Figure 4**: Influence of increasing UV doses on the concentration of 2-aminoacetophenone (A) and acetaldehyde (B) in Riesling wine treated at 254 nm and 280 nm. Shown are the mean values of experimental replicates including the standard deviation. n.s.-not significant ($p \leq 0.05$).*

3.5 Sensory differences due to UV treatment at 254 nm and 280 nm

Table 5 shows the triangle test results for UV treated Riesling with 1000 J/L at 254 and 280 nm. Each wavelength was compared with the control wine and both wavelengths were compared with each other. No significant differences in wine colour were observed due to UV treatment at 254 and 280 nm. This observation is consistent with the results of chromatic characteristics shown in section 3.2. The visible colour threshold of white wine, as provided by a ΔE_{00} value of 0.64 (Hensel et al., 2023), was not exceeded upon UV treatment.

Triangle test results showed significant changes in odour perception of UV treated Riesling at 254 and 280 nm (Table 5). The chemical analysis of the effect of UV treatment at 254 and 280 nm on volatile compounds in section 3.4 showed that an increasing UV treatment degraded volatile compounds. This could explain the significant different sensory perception between UV treated wines and control wine (0 J/L). Golombek et al. (2021) and Cvetkova et al. (2024a) reported that UV treatment at 254 nm can promote sensory changes in must and white wine. However, the ANOVA revealed higher significance for 280 nm than for 254 nm. Also, the wavelengths were tested significantly different. Accordingly, the Riesling was differently affected by UV treatment at 280 nm as compared to 254 nm. Volatile compounds (section 3.4) and off-flavors (section 3.5) did not show significant differences between wavelengths. Most likely the treatment at 280 nm activated the degradation and/or formation of unconsidered volatile compounds through the absorption of this wavelength and therefore promoted photosensitized reactions at 280 nm differently as compared to 254 nm.

The assessment of the wine taste revealed significant changes in Riesling which was UV treated at 280 nm but not at 254 nm. These results are consistent with the changes observed with phenolics in the UV-treated wine (section 3.2). It was found that UV treatment at 280 nm promoted stronger degradation of phenols, especially gallic acid. Since the perception of astringency and bitterness is correlated with the concentration of gallic acid in white wine (Robichaud and Noble, 1990), the stronger degradation at 280 nm might explain the influence on the taste of the wine treated at 280 nm.

***Table 5**: Triangle test results for UV treated Riesling with 1000 J/L at 254 and 280 nm. Each wavelength was compared with the control wine and the different wavelengths were compared with each other. Asterisks indicate statistically significant differences (*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, n.s.: not significant).*

Test pair	Colour		Odour		Taste	
	Correct answers (max. 30)	Signifi-cance	Correct answers (max. 30)	Signifi-cance	Correct answers (max. 30)	Signifi-cance
1000 J/L at 254 nm vs. 0 J/L (control)	12	n.s.	16	*	12	n.s.
1000 J/L at 280 nm vs. 0 J/L (control)	8	n.s.	21	***	21	***
1000 J/L at 254 nm vs. 1000 J/L at 280 nm	13	n.s.	17	**	19	***

4 Conclusion

The study showed that UV inactivation of *Brettanomyces bruxellensis* in white wine was more efficient at 280 nm than at 254 nm. The Weibull model was sufficiently utilized to describe the inactivation kinetics of *Brettanomyces bruxellensis* not only at 254 nm but also at 280 nm. For both wavelengths, the CIELab colour coordinates were affected with increasing UV doses. As shown by the b*coordinate, the treatment at 280 nm caused more browning than 254 nm suggesting more intense photo-oxidation at 280 nm. In fact, phenol degradation was more

pronounced at 280 nm as described by pseudo first-order kinetics. Four out of six investigated volatile compounds were degraded upon UV treatment, but treatment wavelength did not influence degradation rates. Acetaldehyde and 2-AAP concentrations were not affected by the investigated UV doses applied at 280 and 254 nm. Sensory evaluation of the wine showed that treatment at 280 nm changed odour and taste stronger than 254 nm. In summary, 280 nm had better microbial efficiency but also caused wine phenols to degrade faster. Hence, the 280 nm approach seems less suitable for treatment of with wine. In further studies on UV treatment of wine, the influence of wine absorption at the respective treatment wavelength has to be considered. Future research about UV treatment of wine should focus on wines with different phenolic contents.

5 Acknowledgements

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5. Additional relevant unpublished results

Investigation of the storage stability of UV-C treated wines

Abstract

The potential of UV-C technology as a possible method for non-thermal microbiological stabilization in wine was demonstrated. However, the long-term antimicrobial effect is yet to be shown. Some studies suggest that microorganisms have repair mechanisms to counter UV-C radiation, such as photo-reactivation or dark repair (Chan & Killick, 1995; Lindenauer & Darby, 1994; Jungfer et al., 2006). This can mitigate the efficacy of UV-C treatment. The presented work investigates the stability of UV-C treated Pinot Noir wine during a 12-week storage compared to control wine and untreated Pinot Noir. A microbial evaluation showed no significant increase of *Brettanomyces bruxellensis* cell growth in UV-C treated wine during the storage period. The chemical evaluation showed no significant differences of volatile compound class of esters between control and UV-C treated wine. Additionally, an increase of 4-ethylguaiacol concentration was detected in UV-C treated wine compared to control wine. However, the concentration of 4-ethylguaiacol was 17 times under the sensory threshold and 23 times lower than in untreated wine. The formation of 4-ethylguaiacol under UV-C treatment could be caused by photoinduced degradation of ferulic acid. The study has shown that microbial-relevant doses are strong enough to cause irreparable damage to nucleobases and prevent repeated increases of cell growth in wine during storage.

Material and Methods

Wine

The wine was made from the grape variety Pinot Noir (*Vitis vinifera L. cv*.). The grapes were harvested from the experimental vineyard at the Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz (Neustadt an der Weinstraße, Germany) in 2021. The must was inoculated with *Saccharomyces cerevisiae* (Lalvin CY 3079 YSEO; Lallemand, Montreal, Canada), fermented at a constant temperature of 18 °C up to a residual sugar content of < 1 g/L in a 100-litre-stainless-steel-fermenter. Before the start of the experiment, the wine was filtered using a bottle cap leaf filter (pore size 0.45 µm, SFCA membrane; Thermo Scientific, Waltham, USA). The wine had an absorption of 24 AU. The wine was then divided into three variants according to the experimental setup. The experiment used the *Brettanomyces bruxellensis* strain (internal strain numbers: Y191), provided by DLR Rheinpfalz (Neustadt an der Weinstraße, Germany). The strain was cultivated for 72 hours in a yeast extract peptone dextrose (YPD) nutrient broth at a pH of 6.5 in a shaking incubator (ZWY-1102C; Labwit Scientific, Melbourne, Australia) at 90 rpm.

Experimental setup

For the experiments, the Pinot Noir wine was divided into three batches as follows: wine without inoculation with *Brettanomyces bruxellensis* and UV-C treatment (control), wine inoculated with *Brettanomyces bruxellensis* (cell count: 10^5 CFU/mL) without UV-C treatment (untreated) and inoculated with *Brettanomyces bruxellensis* (cell count: 10^5 CFU/mL) and

subsequent treated with a UV-C dose of 2.4 kJ/L (treated). The microbial count was applied based on FDA Regulation 21 CFR 179 of 5-log inactivation. The UV-C dose was selected based on the absorption of the wine and the selected microbial. The determination of UV-C dose was made on the basis of Weibull model. Subsequently, all variants were subjected to 12-weeks of storage at a room temperature of 20 °C. The experiment was carried out in triplicate. The sampling for microbial analysis was taken every two weeks for all batches. In the end of the storage all batches were chemically analyzed and subjected to microbiological tests.

UV-C treatment of wine

For the experiment was used a thin-film UV reactor built by the Institute for Food and Bioprocess Engineering of the Max Rubner Institute (Karlsruhe, Germany). The reactor was equipped with five 20 W low-pressure mercury-vapor lamps (UVPro FMD Series; Bioclimatic B.V., Netherlands), fluid-guiding elements and connected through Tygon® thermoplastic soft polyvinyl chloride (PVC) tubes (wall thickness 2.5 mm, Heidolph, Schwabach, Germany). The reactor was equipped with a peristaltic pump (Pumpdrive 5206, Heidolph, Schwabach, Germany) and a Julabo F30VC/3 cooling system (Julabo Labortechnik, Seelbach, Germany). The flow rate during the treatment was 100 L/h and the temperature fluctuation ± 1.0 °C. The UV-C dose was controlled via chemical actinometry method of Rahn (1997).

Chemical analysis

All chemical analysis was conducted using the methods described and represented in previous studies.

Data analysis

XLSTAT (Version 2021.2.2.1147 (32-bit), Addinsoft SARL, Paris, France) was used for the statistical analysis. All data was tested on the normality of discrete data based on the Shapiro-Wilk method ($p \leq 0.05$). As well as Fisher's least significant difference (LSD) post-hoc test ($p \leq 0.05$) was used for all data.

Results and discussion

Microbiological stability of wine

Table 1 shows the cell count of *Brettanomyces bruxellensis* in the three wine variants of Pinot Noir (control, untreated, and treated) over the storage period of 12 weeks. Neither of the control and treated wine variants show any significant microbial growth of *Brettanomyces bruxellensis* during the storage period. However, this does not apply to the wine variant untreated. During the storage period, the increase of cell counts of *Brettanomyces bruxellensis* up to a 1-log level in the wine variant untreated was determined. These results are consistent with the studies by Kaya et al. (2015), Pala and Toklucu (2012), and Tran and Farid (2004), which did not demonstrate significant microbial growth during a storage study of UV-C treated orange and lemon-melon juice. The risk of recurrence of *Brettanomyces bruxellensis* due to possible dark repair could not be confirmed.

***Table 1**: Cell count of Brettanomyces bruxellensis in the three wine variants of Pinot Noir (control, untreated and treated) over the storage period of 12-weeks. The data was processed by one-way ANOVA. Different letters show differences due to according to Fischer`s test with $p \leq 0.05$; LOQ – limit of quantification. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

Week	Control [cfu/mL]	Untreated [cfu/mL]	Treated [cfu/mL]
0	<LOQ	$3.6 \cdot 10^5 \pm 4.0 \cdot 10^4$ d	< LOQ
2	<LOQ	$3.7 \cdot 10^5 \pm 4.0 \cdot 10^4$ d	< LOQ
4	<LOQ	$9.0 \cdot 10^5 \pm 3.0 \cdot 10^4$ c	< LOQ
6	<LOQ	$9.3 \cdot 10^5 \pm 5.0 \cdot 10^4$ c	< LOQ
8	<LOQ	$1.4 \cdot 10^6 \pm 4.0 \cdot 10^4$ a	< LOQ
10	<LOQ	$1.2 \cdot 10^6 \pm 8.0 \cdot 10^4$ b	< LOQ
12	<LOQ	$9.4 \cdot 10^5 \pm 7.0 \cdot 10^4$ c	< LOQ
p-value	-	< 0.0001	-

Volatile compounds

Table 2 shows the concentrations of ethylacetate, ethylbutanoate, isoamylacetate, ethylhexanoate, ethyloctanoate, and ethyldecanoate in the three wine variants of Pinot Noir (control, untreated and treated) after a storage period of 12-weeks. Esters are secondary aromatic compounds produced during yeast activity by alcoholic fermentation and are one of the most important aromas that give a fresh and fruity note to the wine (Ribereau-Gayon et al., 2021). A reduction of esters can lead to a significant reduction in wine quality. These compounds are formed during alcoholic fermentation by condensation of an organic acid and an alcohol catalyzed by Acetyl-Coenzyme A (Acetyl-CoA) and esterase (Moreno-Arribas & Polo, 2008). The untreated wine variant shows a significant increase in the concentration of esters which indicates that the metabolism of *Brettanomyces bruxellensis* during storage is the reason for the formation of esters. These results are consistent with the results of the microbial examination and show, at the chemical level, the microbial activity of the harmful organism during the storage period of 12 weeks. Additionally, no significant differences could be found between the ester in wine variants control and treated. This indicates no microbial activity in the wine variant UV-C during the storage. The UV-C treatment also showed no significant effect on the profile of ester. These results agree with the study by Junqua et al. (2020), which showed no significant decrease in the aroma-active substances of UV-C treated red wine after six months of storage.

***Table 2**: The concentration of volatile compounds of the aroma class ester in the three wine variants of Pinot Noir control, untreated and treated after a storage period of 12-weeks. The data was processed by one-way ANOVA. Different letters show differences due to wine variants according to Fisher's test with $p \leq 0.05$; LOD – limit of detection. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

Variants	Ethyl acetate [mg/L]	Ethyl butanoate [µg/L]	Isoamyl acetate [µg/L]	Ethyl hexanoate [µg/L]	Ethyl octanoate [µg/L]	Ethyl decanoate [µg/L]
Control	35 ± 4 a	151 ± 17 a	54 ± 8 a	155 ± 20 a	117 ± 18 a	< LOD
Untreated	57 ± 7 b	213 ± 23 b	594 ± 57 b	231 ± 38 b	242 ± 39 b	36±10

Variants	Ethyl acetate [mg/L]	Ethyl butanoate [µg/L]	Isoamyl acetate [µg/L]	Ethyl hexanoate [µg/L]	Ethyl octanoate [µg/L]	Ethyl decanoate [µg/L]
UV-C	33 ± 3 a	142 ± 16 a	58 ± 4 a	154 ± 16 a	118 ± 19 a	< LOD
p-value	< 0.0001	0.0002	< 0.0001	0.0002	< 0.0001	-

The metabolism of *Brettanomyces bruxellensis* causes the formation off-flavors in wine. It promotes the formation of 4-ethylguaiacol, 4-ethylcatechol and 4-ethylphenol, which are associated with the following off-notes, such as "animal" and "horse sweat", in wine (Chatonnet et al., 1992). Figure 1 shows the concentration of 4-ethylguaiacol in the wine variants (control, untreated, and treated) after the storage period of 12-weeks. The untreated wine variant showed a significant increased concentration of 4-ethylguaiacol compared to the control wine variant. Furthermore, the concentration of 4-ethylguaiacol in the untreated wine variant was above the sensory threshold of the red wine (Milheiro et al., 2017). The yeast *Brettanomyces bruxellensis* contains enzymes that are able to break down ferulic acid. This degradation leads to the formation of 4-vinylguaiacol, which in turn leads to the formation of 4-ethylguaiacol in an anaerobic environment and in the presence of ethanol (Waterhouse et al., 2016). These results are consistent with the results of the microbial examination and show the microbial activity of the harmful microorganisms in the untreated wine variant during the 12-week storage period.

The treated wine variant also shows a significant increase in the concentration of 4-ethylguaiacol compared to the control wine variant. However, the concentration was 23-times lower than the wine variant untreated. Furthermore, the concentration of 4-ethylguaiacol was 17-times under the sensory threshold for 4-ethylguaiacol in red wine (Milheiro et al., 2017). This increase in 4-ethylguaiacol could be due to the residual activity of *Brettanomyces bruxellensis* after UV treatment. UV-C radiation causes the formation of pyrimidine dimers in the DNA, which leads to DNA damage. In turn, this inhibits the reproduction processes of microorganisms, which ultimately leads to inactivation of the microorganism (Koutchma et al., 2009; Kowalski, 2009). The detected concentration of 4-ethylguaiacol could have been caused by the metabolic process of the harmful organism until cell death. This could also explain why the plate counting did not detect the yeast during the cell count determination. Nevertheless, it cannot be excluded that the formation of 4-ethylguaiacol was caused through light-induced degradation reaction of ferulic acid. Ferulic acid shows an absorption maximum at 254 nm (Holser, 2014). This absorption of UV-C radiation could lead to a light-induced degradation of ferulic acid and the formation of 4-ethylguaiacol.

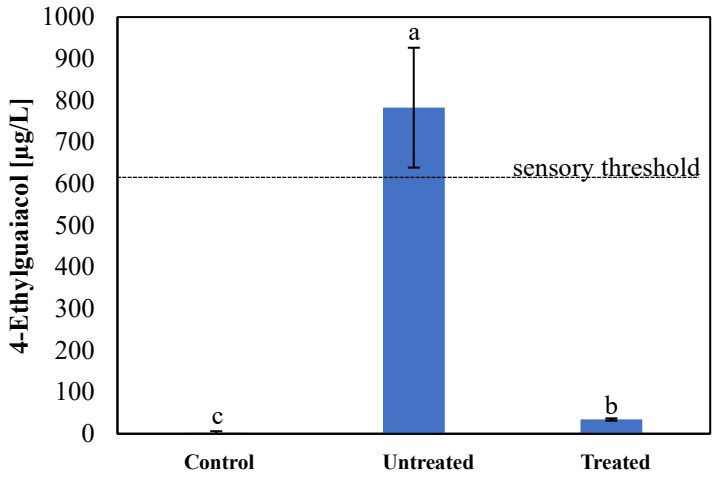

***Figure 1**: The concentration of 4-ethylguaiacol in the wine varieties of Pinot Noir (control, untreated and treated) after a storage period of 12-weeks. The data was processed by one-way ANOVA. Different letters show differences due to wine variants according to Fisher's test with $p \leq 0.05$. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

Phenolic profile

Table 3 shows the concentrations of catechin, gallic acid, caftaric acid and coumaric acid in the three wine variants of Pinot Noir (control, untreated, and treated) after a storage period of 12-weeks. No significant difference between all wine variants was found in the catechin and gallic acid concentrations. A comparison of the concentration of caftaric acid in the control and untreated wine variants also revealed no differences. However, coumaric acid shows a significant decrease in the untreated wine variant compared to the control wine variant. This can be applied to the microbial activity of *Brettanomyces bruxellensis* in wine. As the chapter 'Volatile compounds' describes, the yeast enzymatically degrades coumaric acid and ferulic acid in the wine, producing 4-ethylguaiacol and 4-ethylphenol. Furthermore, a significant decrease in the concentration of caftaric acid and coumaric acid in the treated wine variant compared to the control wine variant could be detected after the 12-week storage period. The possible reason for the decrease of coumaric acid after UV-C treatment may be explained by the metabolic processes of the harmful organism until its cell death (Chatonnet et al., 1992). The cause of the decrease in caftaric acid could be the photochemical reaction of the tartaric esters of hydroxycinnamic acids (Waterhouse & Nikolantonaki, 2015). The absorption maxima of caftaric acid are in the UV-C area (Makris et al., 2003). It follows that these compounds can be excited under the influence of UV-C radiation and can be degraded to quinone by oxidation. Nevertheless, it remains unclear why only caftaric acid was degraded after the UV-C treatment and gallic acid and catechin were not. One possible explanation could be the different sensitivity of caftaric acid, catechin and gallic acid to UV-C radiation. Disregardful, the degradation of phenolic compounds during UV-C treatment at the microbiologically relevant doses should not have a significant impact on the taste of the wine, based on previous studies.

***Table 3**: The concentrations of catechin, gallic acid, caftaric acid, and coumaric acid in the three wine variants of Pinot Noir control, untreated and treated after a storage time of 12-weeks. The data was processed by one way ANOVA. Different letters show differences due to wine variants according to Fischer`s´test with $p \leq 0.05$. Shown are the mean values of experimental replicates (n=3) including the standard deviation.*

Variants	Catechin [mg/L]	Gallic acid [mg/L]	Caftaric acid [mg/L]	Coumaric acid [mg/L]
Control	20 ± 3 a	16 ± 2 a	50 ± 1 a	1.3 ± 0.1 a
Untreated	19 ± 1 a	17 ± 1 a	52 ± 2 a	0.8 ± 0.1 c
Treated	19 ± 2 a	15 ± 1 a	44 ± 2 b	1.0 ± 0.1 b
p-value	0.813	0.296	0.003	0.0025

Conclusion

This study shows that the use of UV-C technology for microbial stability can have a microbial long-term stability of wine for more than 12-weeks. During the storage period after UV-C treatment, no increase in the cell count of *Brettanomyces bruxellensis* was observed. The risk of renewed contamination of *Brettanomyces bruxellensis* due to possible dark repairs mechanism could not be confirmed.

The chemical analysis could confirm the results of the microbiological tests. No significant differences in the concentration of volatile compounds between the wine variant control and treated, except for a marginal increase of 4-ethylguaiacol could be determinate. The increase of 4-ethylguaiacol concentration in wine variant treated could have been caused by the metabolic process of the harmful microorganism up to cell death after UV-C treatment. A significant increase in the concentration of ester compounds and 4-ethylguaiacol was found in the wine variant untreated, which indicates the microbial process during storage in this wine. Furthermore, a significant decrease in the concentration of caftaric acid was observed in the wine variant treated. The degradation of caftaric acid could be caused by the possible oxidative processes caused by the UV-C treatment.

Ongoing studies should investigate the exact reason for the formation of 4-ethylguaiacol in wine after UV-C treatment. Future studies should investigate the influence of radiation with wavelengths in the range of 310 - 480 nm during storage due to possible photoreactivation of harmful microorganism

6. Concluding remarks

In the first part of this dissertation, the effectiveness of UV-C technology against wine-typical harmful microorganisms in different wines was evaluated. A thin-film reactor with a low-pressure mercury-vapor lamp and flow guiding elements were used for all experiments. The inactivation of the harmful microorganisms was carried out with the wavelength 254 nm. The Weibull model was used to investigate inactivation kinetics. It was shown that the wine absorption, type of the harmful microorganisms, and the cell count of the harmful microorganisms significantly influenced the inactivation effectiveness of UV-C. Accordingly, these factors should be regarded to determine the UV-C dose. Chromatographic, photometric, and sensory analyses were used to investigate the influence of increasing UV-C doses at 254 nm on the wine. It was shown that an increased UV-C dose caused several photochemical reactions and, furthermore, the formation of off-flavors. The increasing UV-C dose changed the sensory characteristics in the direction of an aged wine. The main products of these reactions were 2-AAP and acetaldehyde. Aroma-active substances in the classes of C13-norisoprenoids, monoterpenes, and esters were degraded under UV-C treatment. Furthermore, it was found that an increased UV-C dose accelerated the degradation of phenolic compounds in wine. Nevertheless, the degradation of phenols had no significant influence on the gustatory profile of wine. Overall, the study showed the potential of UV-C technology for microbial stabilization of wine, with increasing sensory changes by raised UV-C doses.

Another study was carried out to investigate the influence of antioxidants SO_2 and hydrolyzable tannins on the irradiation of wine with UV-C light to reduce the oxidative processes in wine. Against expectations, it was found that SO_2, in combination with UV-C wavelength at 254 nm, had an oxidizing effect rather than an antioxidative effect. This is due to the ability of SO_2 to absorb light at 254 nm and to form sulfite radicals. Stabilized by the mesomeric effect, these radicals can undergo a series of reactions that promote the formation of 2-AAP and acetaldehyde. UV-C dose and SO_2 concentration were proportionally related to increasing acetaldehyde and 2-AAP concentrations. This property of SO_2 is used in water treatment in the so-called ARP. In addition, it was found that as the SO_2 concentration increased, the odor attribute "burnt" was significantly promoted during UV-C treatment. This indicates the possibility of forming new odor-active compounds, such as thiophenols, via sulfite radicals promoted reaction. Due to the ability to act as strong proton donors, hydrolyzable tannins showed a powerful antioxidant effect. A reduction of 2-AAP and acetaldehyde concentration in wine was observed. This can be attributed to decreased oxidative effects. The results show that during the intended application of UV-C technology with a wavelength of 254 nm, the SO_2 concentration should be as low as possible or dispensed with altogether. In special cases where a high UV-C dose at 254 nm is required above the microbial-relevant 5-log inactivation dose, regarding FDA HACCP regulation of food products safety (US FDA, 2004), hydrolyzable tannins should be added to provide the wine with strong oxidative protection against the increasing UV-C dose.

Due to the low sensory threshold of 0.5 to 1.5 µg/L (Christoph et al., 1995) and strong variation in the concentration of the educts, the formation of 2-AAP remains a restrictive factor that limits the use of UV-C technology for microbial stabilization of wine. Previous studies have shown a correlation between the RF-promoted formation of 2-AAP in the presence of transition metals, especially iron. In the field of enology, it is known that transition metals, such as iron

and copper, act as catalysts in the Fenton reaction. In earlier studies by Elias and Waterhouse (2010), it was shown that the Fenton reaction significantly influences oxygen concentration. Accordingly, the formation of 2-AAP due to the oxygen concentration in the presence of transition metals was investigated to find strategies to reduce the 2-AAP concentration in the treated wine. The results showed that a reduction in oxygen content in model wine led to a significant reduction in 2-AAP formation. In addition, oxygen concentration reduction inhibited RF regeneration which further mitigated the formation of 2-AAP. The degradation of RF to lumichrome was indicated via photometric analyses. The presence of transition metals during UV-C treatment at 254 nm promoted a photo-Fenton reaction. This reaction acted as a competing reaction to RF induced 2 AAP formation. Iron had greater effect on the formation of 2-AAP than copper due to its high photoreactivity and low reduction potential. It was shown that a total of less than 25% of the TRP was degraded to 2-AAP during UV-C irradiation. To reduce the photooxidative processes during UV-C treatment in wine, it is therefore advisable to minimize oxygen beforehand, for example, by membrane degassing. This could prevent the formation of 2-AAP in wine and the formation of reactive oxygen species produced during the photosensitizing reactions of RF.

Microbial stabilization should provide long-term protection. To assess the long-term effectiveness of UV-C technology for the microbial stabilization of wine, the Pinot Noir wine was subjected to a 12-week storage experiment. After 12-weeks of storage, no significant increase in *Brettanomyces bruxellensis* could be detected. The risk of renewed microbial contamination due to possible dark repair mechanisms could not be confirmed. The selected inactivation dose was strong enough to cause irreparable damage to the nucleobases. Chemical tests also showed no significant difference with the untreated control wine, except for an increase of 4-ethylguajacol, which was well below the sensory threshold.

The antimicrobial effect of UV-C radiation is not limited to the wavelength of 254 nm. The use of an alternative wavelength of 280 nm proved promising, which enabled the use of LEDs as an alternative to conventional low-pressure mercury-vapor lamps. Especially since the use of mercury is severely restricted according to Regulation (EU) 2017/852. As a result, investigations were carried out on the possible use of the wavelength of 280 nm, and its inactivation effectiveness was compared with that of 254 nm. The microbial investigations showed that the wavelength of 280 nm was more effective than 254 nm and required a lower dose to achieve 5-log microbial stabilization. However, it could not be concluded that this was due to the different absorption of the wine at the respective wavelengths. Nevertheless, the chemical and sensory analyses proved that using 280 nm is less suitable for microbial stabilization of wine. The wine contains a large number of phenolic compounds that have an absorption maximum of 280 nm. This led to increased photochemical reactions of the phenols, which had a significant impact on the sensory properties of the wine. Based on the study, it can be concluded that the wavelength of 280 nm is less suitable for the microbial stabilization of wine.

In summary, UV-C technology is a suitable method for microbial stabilization of wine, provided that the required dose is precisely determined to minimize the risk of promoting photooxidative processes in wine. An UV-C overdose can lead to a deterioration of the wine quality. When implementing this technology, the wavelength of 254 nm should be preferred over 280 nm. In addition, the factors of wine absorption at 254 nm, the type of harmful microorganisms, and the cell count of harmful microorganisms should be considered when

determining the required UV-C dose. The Weibull model proved to be a suitable means of determining the required UV-C dose. To ensure wine quality, the concentration of SO_2 in the wine should not exceed 30 mg/L. Vice versa, hydrolyzable tannins are preferable to be added before UV-C application.

The presented studies show that RF as a photosensitizer is significant in many light-induced reactions that can negatively affect wine quality during increasing UV-C treatment. For this reason, further research should address the following questions: On the one hand, to what extent does the concentration of the present RF influence the light-induced reaction under UV-C treatment in the wine? On the other hand, up to what concentration of RFs in wine are uncritical for forming off-flavors? It should examine the possible prevention method to reduce the final concentration of the RF in the wine. This can be achieved, for example, by the specific selection of experimental conditions, including strains of *Saccharomyces cerevisiae* on the one hand and the addition of fining agents such as bentonites and charcoal on the other hand.

Further studies presented in this thesis have some limitations. In future studies, other antioxidants such as ascorbic acid and glutathione were to be evaluated individually and in combination to reduce the oxidative effect of UV-C treatment. In addition, the effectiveness of reducing the oxygen content compared to real wine should be carried out in combination with other antioxidants. An alternative wavelength (280 nm) to the standard wavelength of 254 nm was tested. Further investigations should be evaluated with other LEDs from the 220 to 260 nm wavelength range for their germicidal effect. The current studies show that a synergistic effect of the different wavelengths can promote an additional increase in the inactivation effectiveness of UV-C technology, which should be tested in further experiments.

UV-C technology for microbiological stabilization has already been successfully applied and evaluated in several studies in different fields of wine production. The combined use of UV-C technology is a promising method for more ecological winemaking. It would be possible to reduce the use of chemicals to minimize harmful microorganisms during winemaking or, ideally, even to avoid them altogether.

However, due to the current legal situation, the use of UV-C technology in winemaking in the EU is not yet permitted. For this reason, efforts should be stepped up to authorize this new process, which is already being used in other areas of food preservation. The first steps for a corresponding approval of the process for microbiological stabilization of the wine are positive so that the next important step would be scaling up. The previous experiments on microbiological stabilization of the wines have been carried out on pilot plants on a laboratory scale and have not yet been carried out on an industrial scale. This system could collect important data and further optimize the corresponding technology.

7. References

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