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 redaction 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].

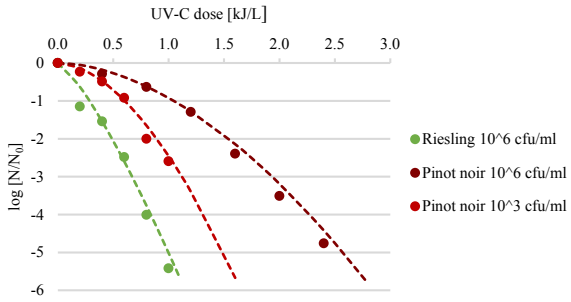

Figure 1. Inactivation kinetics of *D. bruxellensis* with increasing UV-C doses for different inoculation numbers in Riesling and Pinot noir wine. The dotted curves show the inactivation kinetics as determined by the Weibull model.

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.

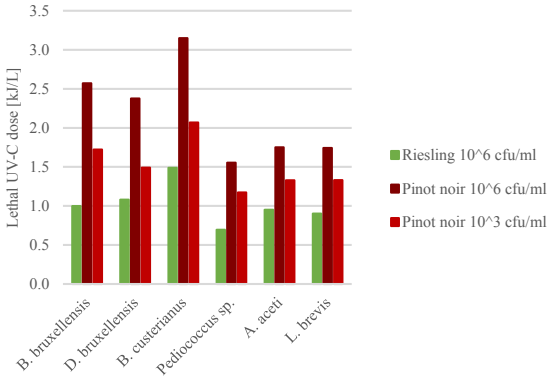

Figure 2. Lethal inactivation doses for different microorganisms inoculated as single cultures with inoculation number 10^6 cfu/ml in Riesling wine and inoculation number 10^6 cfu/ml and 10^3 cfu/ml in Pinot noir wine.

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.

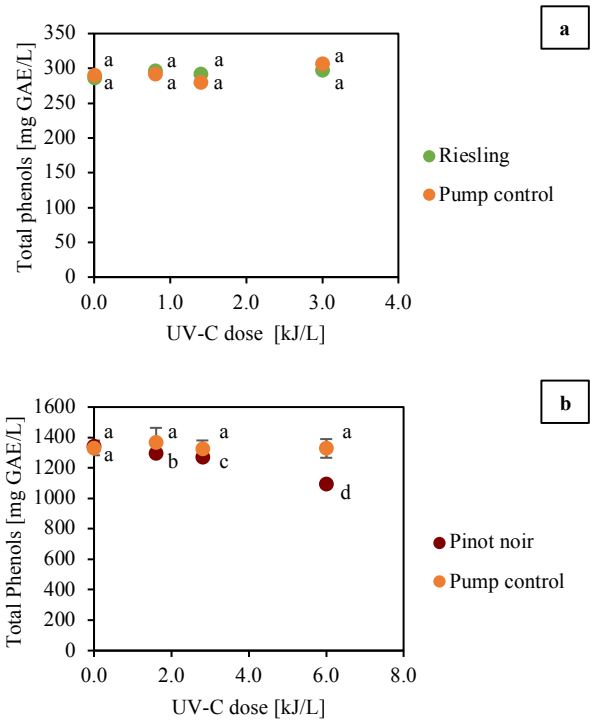

Figure 3. Total phenols of UV-C treated and untreated in Riesling (a) and Pinot noir (b) wine. Different letters represent significant differences ($p \leq 0.05$) between the samples.

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].

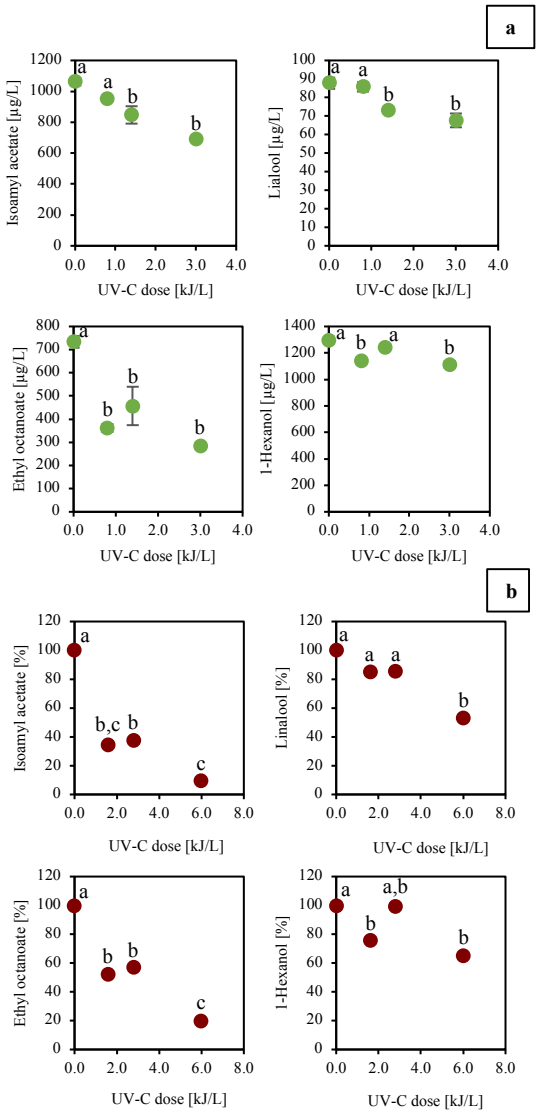

Figure 4. Isoamyl acetate, ethyl octanoate, linalool and 1-hexanol of UV-C treated and untreated Riesling (a) and Pinot noir (b) wine. The value of Pinot noir measurements was related to untreated samples. Different letters represent significant differences ($p \leq 0.05$) between the samples.

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).

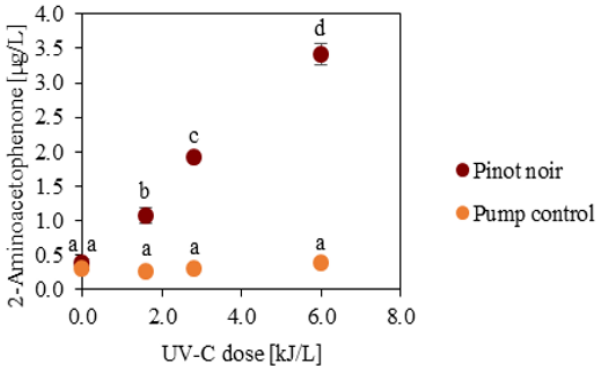

Figure 5. 2-AAP formation in Pinot noir wine during UV-C treatment. Different letters represent significant differences ($p \leq 0.05$) between the samples.

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.

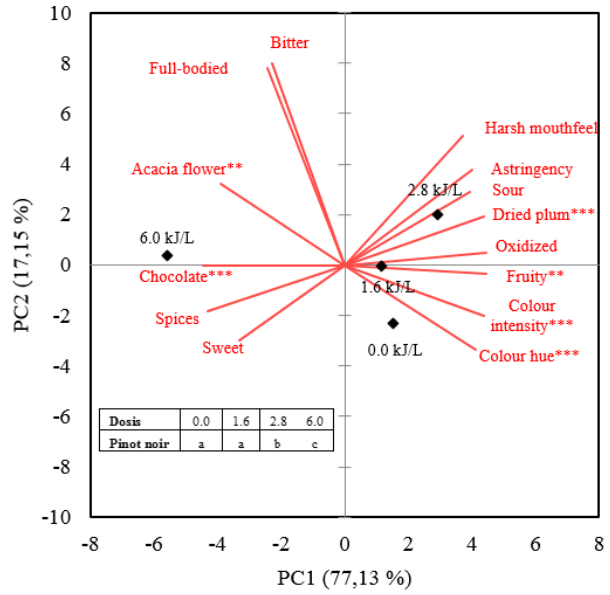

Figure 6. Principal component analysis of treated and untreated Pinot noir wine. UV-C doses were 1.6, 2.8 and 6.0 kJ/L. PCA space was calculated for fourteen sensory attributes.

** 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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