# Suitability of UV-C irradiation for white grape must

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**Short title**: UV-C treatment of white grape must

## Abstract

**Background and Aims**: Ultraviolet (UV)-C irradiation is used for the reduction of microbial spoilage in food. Its effectiveness for the treatment of white grape must has been investigated considering different microbial species and modification of must composition.

**Methods and Results**: Static and dynamic laboratory-scale systems emitting at 254 nm were used for the treatment of *Vitis vinifera* cv. Chardonnay and cv. Crimson grape juices of variable turbidity. The must samples were singularly inoculated with a pure culture of wine spoilage strains belonging to three species, *Dekkera bruxellensis*, *Acetobacter aceti* and *Lactobacillus brevis*, and treated with UV-C ranging from 300 to 1800 J/L. Cell counts and microbial reduction (derived from α values, according to the Weibull model), colour change, polyphenol oxidase (PPO) inactivation and formation of α-dicarbonyl compounds were evaluated. After treatment at the maximum UV-C dosage under static conditions, yeast counts of 4.91–5.99 $\log_{10}$ CFU/mL and bacterial counts of 4.12–5.05 $\log_{10}$ CFU/mL were achieved, depending on the strain being tested. Polyphenol oxidase (PPO) activity decreased by up to 7.8 ± 1.4% with variation attributed to must turbidity, which indicated that clarification of must before UV-C treatment can lead to PPO inactivation. No significant variation in the colour or the concentration of α-dicarbonyl compounds was detected. Results were confirmed for grape juice with the continuous flow apparatus.

**Conclusion**: The UV-C systems achieved microbial reduction without formation of oxidative compounds, suggesting that the experimental conditions employed did not cause any noticeable oxidative phenomena.

**Significance of the Study**: The UV-C treatment of grape juice can be considered a potential alternative to sulfur dioxide addition for processing of white must. Improved efficacy of treatment can be achieved by increasing the flow rate and by clarification of must, making the system potentially applicable under industrial conditions.

***Keywords:*** *chemical oxidative markers, microbial reduction, polyphenol oxidase activity, UV-C radiation, white grape must*

## Introduction

Winemaking is a well-known process where the fermentative capabilities of yeast and bacteria influence the quality of the end product (Ribéreau-Gayon et al. 2006). Grapes and must are contaminated naturally by different microbial populations that can be responsible for undesirable secondary transformations; in particular, non-*Saccharomyces* yeasts, acetic acid bacteria (AAB), lactic acid bacteria (LAB) and moulds. The metabolic activities of these microorganisms can negatively affect wine quality as a consequence of increased volatile acidity, the release of off-flavours, changes to viscosity or colour, and/or the formation of haze or precipitation (Du Toit et al. 2006, Fugelsang and Edwards 2007, Bartowsky and Henschke 2008). Beside the growth of undesirable microorganisms, white must can undergo oxidation due to the activity of grape-derived polyphenol oxidase (PPO). This enzyme can oxidise hydroxycinnamoyl-tartaric acids to *o*-quinones, which are highly reactive compounds and known precursors of the polymers responsible for browning of must (Cheynier and Ricardo da Silva 1991, Macheix et al. 1991).

To counteract the proliferation of spoilage species, the addition of sulfur dioxide ($SO_2$) is frequently carried out to inhibit microbial growth (Boulton et al. 1996, Vigentini et al. 2013). Moreover, this routine winemaking practice also prevents enzymatic and chemical oxidation (Fugelsang and Edwards 2007). Sulfur dioxide can, however, be detrimental to human health (Vally and Thompson 2001, Pozo-Bayón et al. 2012). As such, $SO_2$ up to a concentration of 200 mg/L is permitted in white wines, but it is mandatory for 'contains sulfites' to be specified on labelling when the concentration exceeds 10 mg/L (European Commission 2009). In recent years, there has been increasing interest from wine producers to find alternative strategies for managing both microbial spoilage and oxidation, so as to limit the use of $SO_2$. Ultraviolet (UV) radiation is widely used in food processing for reducing microbial contaminants (Bintsis et al. 2000). Irradiation wavelengths in the range of 100–280 nm (UV-C) have been proven to have microbicide power when applied to surfaces or clear liquid foods (Guerrero-Beltrán and Barbosa-Cánovas 2006, Hadjock et al. 2008, Keyser et al. 2008, Koutchma et al. 2016). Dispersed solids can limit the efficacy of UV-C treatment because

they act as a shielding material. Microbial reduction is achieved by photolysis, resulting in DNA modification being induced by UV-C exposure, which prevents cell duplication (Thompson 2003). Irradiation with UV-C has also been used during processing of coloured and turbid liquids, such as apple juice (Falguera et al. 2011), grape must (Fredericks et al. 2011, Falguera et al. 2013) and wine (Matias et al. 2016, Mijowska et al. 2017). The impact of UV treatment, however, needs further investigation, especially the resistance of different microbial strains and the potential for activation of oxidative reactions. Nevertheless, Rizzotti and co-authors (2015) reported that UV-C irradiation was effective at reducing the microbial counts present in wine by up to five log CFU/mL.

This study aimed to evaluate laboratory-scale irradiation systems allowing the treatment of grape must through measured UV-C dosage in order to investigate the inactivation rate of different wine spoilage microorganisms present in the wine environment. In addition, the influence of UV-C irradiation on chemical oxidative markers known to affect wine quality (Müller et al. 2014), were evaluated.

## Materials and methods

*Samples*

Grape juice samples were obtained from *Vitis vinifera* cv. Chardonnay and cv. Crimson, both harvested in 2013 and 2015 vintages. Chardonnay must (50 L), free from treatment with $SO_2$, was obtained from a private company located in Franciacorta area (Lombardia, Italy) using industrial grape pressing conditions. The must was allowed to settle for 12 h at 4°C before being stored at -18 ± 1°C until usage.

A laboratory prepared Crimson grape juice (20 L) was obtained by manual pressing of grape under $N_2$ flow and by clarification under $N_2$ flow by storage overnight at 4°C . The clarified must was then stored at -18 ± 1°C until usage. Grape lees were frozen and later used to adjust the turbidity of must samples. The chemical composition of the must samples was determined in an accredited

laboratory (Enoconsulting, Erbusco, Brescia, Italy) using official methods of the Organisation Internationale de la Vigne et du Vin (OIV) (Table 1) (Organisation Internationale de la Vigne et du Vin 2010, 2015).

*Microbial strains and culture media*

Two yeast strains belonging to *Dekkera bruxellensis* (CBS2499, CBS74$^T$) and four bacterial strains ascribed to *Acetobacter aceti* (DSM3508$^T$ and DSM2002) and *Lactobacillus brevis* (DSM20054$^T$, UMB7308) were selected for investigation, with the aim being to calculate their survival rate under different UV-C irradiation conditions. Yeast cells were cultured in YPD medium according to previous methodology (Vigentini et al. 2016); while AAB were grown in YPM medium (5 g/L yeast extract, 3 g/L peptone, 25 g/L mannitol, pH 6.2) and LAB were grown in Lactobacilli MRS broth (Difco, Becton Dickinson, Franklin Lakes, NJ, USA). All strains were maintained as pure concentrated cultures and were stored at -80°C in their corresponding medium with the addition of 20 % (v/v) glycerol.

*Preparation of the inoculum and microbiological analyses*

Fresh cells were prepared for the controlled contamination of must samples. They were revitalised from -80°C glycerol stocks by inoculating at 1% (v/v) in a suitable medium dependent on the strain being tested. Briefly, *D. bruxellensis* and *A. aceti* strains were grown under aerobic conditions at 25°C for 72 and 96 h, respectively. *Lactobacillus brevis* strains were cultivated under anaerobic conditions (GasPak System, BBL, Becton Dickinson) at 30°C for 48 h. The must samples were inoculated at a starting concentration of approximately 1 x $10^7$ CFU/mL, with cells in late exponential phase. The optical density of fresh cell cultures was measured at 600 nm with a spectrophotometer (Jenway, UV-visible spectrophotometer, model 7315, Bibby Scientific, Stone, England). Plate counts were carried out in the aforementioned media, with the addition of 18 g/L (w/v) agar (Merck, Darmstadt, Germany). Aliquots (100 µL) of appropriate decimal dilutions were prepared in sterile

123 Peptone Water (peptone 10 g/L, pH 6.5) and spread on agar plates; colonies were counted following
124 incubation as outlined in OIV-MA-AS4-01 (Organisation Internationale de la Vigne et du Vin 2010).
125
126 *Equipment used for UV-C irradiation*
127 Grape must was treated with two UV-C systems: one involving static conditions and one employing
128 dynamic conditions. The first apparatus comprised a 20 W low-pressure mercury vapour lamp (HER
129 357 model, ADP, Peschiera Borromeo, Milan, Italy) emitting at 254 nm. The emission intensity of
130 the applied radiation was constantly and accurately monitored with an HD2021T.4 sensor (DeltaOhm
131 Caselle, Selvazzano, Padova, Italy) to . The sensor had a detection range from 2 to 200 $W/m^2$, with a
132 linear output of 4 to 20 mA, recorded by means of an HP 34970A data logger (Agilent Technologies,
133 Santa Clara, CA, USA), interfaced with software that recorded the energy emitted during treatment
134 (Benchlink data logger, Agilent). The apparatus also comprised a sanitising chamber (200 mm in
135 length, with a 20 mm radius) positioned between the cylindrical quartz sleeve (5 mm radius) of the
136 lamp and an external coaxial cylinder made from polymethylmethacrylate (PMMA), with an internal
137 volume of 80 mL and a thickness of 5 mm; the must was loaded by means of a duct (Figure 1). Part
138 of the lamp remained external to the chamber for the continuous monitoring of UV-C dosage by the
139 sensor connected to the data acquisition device. Since the UV-C energy used during treatment was
140 known and the current intensity that penetrated through the section profile could be measured, it was
141 possible to set the UV-C irradiation according to the volume of the medium, instead of by area, as
142 previously reported by other authors (Fredericks et al. 2011, Rizzotti et al. 2015, Matias et al. 2016).
143 The output current was transformed into the relative UV-C power (W) and then divided by the volume
144 of sample being treated (W/L). The dosage was calculated in real-time considering the applied time
145 for the treatment (1 W*s/L = 1 J/L).
146 The second apparatus consisted of a sanitising chamber positioned between a quartz glass (10
147 mm radius) which contained a 70 W cylindrical quartz lamp (HER 668 model, ADP, Peschiera
148 Borromeo, Milan, Italy) emitting at 254 nm and with an external coaxial stainless steel cylinder of

14.5 mm radius; the internal thickness was of 4.5 mm. The flow of liquid inside the chamber was achieved with a peristatic pump (MasterFlex L/S, Cole-Parmer, Vernon Hills, IL, USA) so as to avoid contact with air.

*Microbial inactivation by UV-C treatment*

For the treatment of grape juice under static conditions, 500 mL samples of must were first heat treated in a water bath at 85°C for 1 min, to inactivate any residual microbial populations. Samples were immediately chilled in ice and maintained at 4°C until inoculation. Microorganisms were inoculated, one at a time, immediately before the UV-C irradiation. Microbial suspensions were then subjected to UV-C irradiation at 0, 300, 600, 900, 1200, 1500 or 1800 J/L; with each treatment in triplicate. Microbial counts were also made on untreated must. Microbiological data were analysed with SPSS software (Win 12.0 program, SPSS, Chicago, IL, USA) to perform linear and non-linear regression analysis and modelling. The mathematical model of inactivation that gave the best interpolation of experimental data points was evaluated using the adjusted determination coefficient ($R^2$ adj) and mean square error (MSE) values, according to the approach outlined by Taze et al. (2015). Plate count values (CFU/mL) were transformed in decimal logarithm to match the normal distribution of data. The UV-C dosage required to attain a decimal reduction in viable count was calculated as the reciprocal of the slope of regression curve in case of the linear model (D value), and as the α value in the case of the Weibull model.

For UV-C treatment under dynamic conditions, must samples were first centrifuged at 5000 *g* for 30 min at 4°C, to settle grape solids and most microorganisms. The level of residual microbial contamination in the supernatants was assessed by plate counts. The efficacy of microbial inactivation achieved using the prototype operating under dynamic conditions was evaluated as follows. Must samples were co-inoculated with three microbial strains (one for each species), with the strains chosen having shown the highest resistance to UV-C radiation in the experiments carried out with the

apparatus operating under static conditions. The inoculated musts were then irradiated at 1800 J/L; with each treatment in triplicate. To determine the effect of must turbidity on microbial inactivation, the turbidity of samples was increased from 17 ± 1 to 212 ± 4 NTU, by adding the grape lees collected after settling, prior to inoculation.

*Evaluation of colour changes in must due to UV-C treatment*

The colour of must samples was evaluated using the CIE L*a*b* system by measuring reflectance with a Lambda 650 spectrophotometer (PerkinElmer, Waltham, MA, USA), with white used as the reference colour. The L*, a* and b* values were determined by the software Color (PerkinElmer), setting the observer at 10° and the light source D65. The values of the Chroma (1), hue angle (2), and ΔE (difference between two colours) (3) were also calculated. Colour measurements were carried out in triplicate on both untreated and UV-C (1800 J/L) treated must samples .

$$Chroma = \sqrt{a^{*2} \times b^{*2}} \quad (1)$$

$$Hue\ angle = arctg\ \frac{a^*}{b^*} \quad (2)$$

$$\Delta E = \sqrt{(L^*_{UM} - L^*_{TM})^2 + (a^*_{UM} - a^*_{TM})^2 + (b^*_{UM} - b^*_{TM})^2} \quad (3)$$

Where *UM* and *TM* denote untreated and UV-C treated must, respectively.

*Assessment of PPO activity in must*

The inactivation of the PPO enzyme was evaluated by measuring the increase in absorbance at 400 nm, using catechol (Sigma-Aldrich, St Louis, MO, USA) as a substrate. One unit (U) of PPO was defined as the amount of enzyme leading to an increase of one absorbance unit (AU) at 400 nm in 10 min (Rapeanu et al. 2005, Ülker-Yerlitürk et al. 2008, Falguera et al. 2011). The determination of PPO was carried out in triplicate on both untreated and UV-C (1800 J/L) treated must samples, prepared from 2013 Chardonnay grapes. To determine the effect of UV-C treatment on PPO

inactivation, the laboratory-must prepared in the laboratory from 2013 Crimson grapes was subjected to UV-C irradiation at 0, 300, 600, 900, 1200, 1500 or 1800 J/L, and PPO activity determined, with each treatment in triplicate.

The inactivation of PPO was also determined after treatment of must prepared from 2015 Chardonny and Crimson grapes under dynamic conditions. Must samples were also treated with UV-C irradiation after turbidity was increased to 252 ± 6 NTU following addition of grape lees, in a manner similar to that outlined above for the microbial reduction assays.

*Quantification α-dicarbonyl compounds in must*

Chemicals and reagents, such as 2,3-pentanedione, 2,3-diaminobenzene, sodium hydroxide, barium chloride and methanol, were purchased from Sigma-Aldrich. The concentration of α-dicarbonyl compounds was determined spectrophotometrically (Lambda 25, PerkinElmer) after their reaction with 2,3-diaminobenzene under basic conditions, as described by the OIV-MA-AS315-20:R2010 method (Organisation Internationale de la Vigne et du Vin 2010), with some modifications. Barium chloride was added to 100 mL grape must to a final concentration 3.2 g/L which was then centrifuged at 5000 *g* for 10 min (Sorvall centrifuge, Thermo Fisher Scientific, Waltham, MA, USA). A 20 mL aliquot of the supernatant was then eluted through a solid phase extraction (SPE) cartridge packed with 1 g of C18 (Strata C18-T, Phenomenex, Torrance, CA, USA). The eluted must (10 mL) was placed in a tube, to which 2,3-diaminobenzene (5 mg) was added, after pH adjustment to 8.0 by addition of 1 mol sodium hydroxide. The tube was closed using a screw-cap fitted with a teflon-faced seal and mixed until complete dissolution of the reagent was achieved. The mixture was then stored at 60°C for 3 h, before being cooled in an ice bath. The cooled mixture was filtered (0.22 µm PVDF, Millipore, Bedford, MA, USA) and the total amount of α-dicarbonyl compounds was determined spectrophotometrically by measuring the absorbance at 313 nm (Lambda 25, PerkinElmer). The spectrophotometric data were acquired and processed with a UV-Winlab data processor and viewer (PerkinElmer). The total amount of α-dicarbonyl compounds was quantified using an external

standard method at 313 nm. The calibration curve was obtained by spiking grape must with 2,3-pentanedione at 5.0–30.0 mg/L. The method showed a linear response and gave a high correlation coefficient (r = 0.959). The limit of detection andquantification was 0.75 and 2.5 mg/L, respectively. Recovery was assessed in triplicate in grape must spiked with 15 mg/L 2,3-pentanedione and was found to be 99%.

Grape must was not heat-treated prior to quantification of α-dicarbonyl compounds. The determinations were carried out in triplicate on both untreated and UV-C (1800 J/L) treated must samples prepared in triplicate.

*Statistical analysis*

The SPSS software was used for all statistical analysis. Statistical differences between untreated and UV-C treated musts in terms of colour change, PPO inactivation and of the concentration of α-dicarbonyl compound in both static and dynamic prototypes, and the microbial reductions obtained with the static vs dynamic prototypes were determined by the F-test, and were considered significant at $P < 0.05$. The same software was used to perform linear and non-linear regression analysis and modelling of microbiological data, in particular, to determine values of α and β parameters from the Weibull model.

## Results and discussion

*Development of laboratory-scale prototypes for the UV-C treatment of grape must*

First, an apparatus operating under static conditions for treatment of liquid was developed by optimising size, lamp power and the minimum thickness allowing penetration of UV-C radiation through the entire must sample. The penetration profile of UV rays in Chardonnay grape must (76 ±4 NTU) was determined using quartz cuvettes, with an optical path length of 1, 5 and 10 mm (Snowball and Hornsey 1988, Lodi et al. 1996) and the relevant attenuation of current was monitored with the HD2021T.4 sensor connected to the data acquisition device. As expected, the signal decreased as the optical length increased, and no UV-C radiation could be recorded when the optical length exceeded 5 mm. For this reason, a maximum thickness of 5 mm was established. Furthermore, as shown in Figure 2, no variation in lamp emission was found throughout five replicated cycles of irradiation, except during the first test, when the lamp was cold. The lamp emission can be affected by its temperature, which can increase during usage; consequently, the UV-C radiation cannot be accurately predicted (Lau et al. 2009). A rise in temperature might influence the efficacy of UV-C treatment. As such, temperature was measured, to identify any effect related to variation in temperature during UV-C treatment. Temperature changes were negligible (+2°C) and, consequently, temperature was not expected to influence any of the parameters investigated, that is microbial reduction, inactivation of PPO, change in colour or the formation of α-dicarbonyl compounds.

*Microbicide effectiveness of the UV-C treatments*

The fungal and bacterial counts found in the 2013 Chardonnay grape must following heat treatment were both <1 $\log_{10}$ CFU/mL, demonstrating that preliminary processing operations were effective.

Must samples with a turbidity level of 76 ± 4 NTU were inoculated with one of two yeast strains or four bacterial strains, and subjected to UV-C treatment using the static prototype, at six dosage values, ranging from 300 to 1800 J/L. A non-linear, inverse relationship was observed between the supplied energy and the number of surviving cells. Convex curves were shaped in the

inactivation diagrams obtained by interpolation of experimental points expressed as $\log_{10}$ CFU/mL plate counts (Figure 3) The values of adjusted determination coefficients and mean square errors were higher when the Weibull model was used in comparison to the linear model, indicating that the former better described the data trends (Table 2). Consequently, α values obtained with the Weibull model were considered to estimate the log microbial reduction. Figure 3 also shows the fit of the Weibull and linear first-order models.

As expected, the highest decrease in viability was observed at the dosage of 1800 J/L, where the calculated reduction in microbial counts reached 4.91–5.99 $\log_{10}$ CFU/mL and 4.12–5.05 $\log_{10}$ CFU/mL for yeast and bacteria, respectively (Table 3). Taking into account that the microbial count in must after grape crushing is usually 3–4 $\log_{10}$ CFU/mL (Fugelsang and Edwards 2007, Vigentini et al. 2016), these results indicate that the species studied can be successfully inactivated by UV-C treatment at 1800 J/L, under real conditions. As deduced by the calculated D values, the yeast strain that was most sensitive to UV-C irradiation was *D. bruxellensis* CBS2499, while *A. aceti* DSM3508$^T$ strain proved to be less resistant to UV-C treatment amongst the bacterial species (Table 2). These results confirm that the detrimental effect of UV-C exposure is species-related, as previously reported (Fredericks et al. 2011, Mijowska et al. 2017).

In order to compare the microbial reduction achieved in the current study with that observed by Fredericks et al. (2011), the $\log_{10}$ CFU/mL reduction of some microorganisms was calculated for the same UV-C dosages reported byFredericks et al., using data obtained from the Weibull model (α values) we obtained. For treatments on yeast : UV-C irradiation of *B. bruxellensis* ISA 1649 at 459 J/L gave 0.47 $\log_{10}$CFU/mL in unclarified Chenin Blanc (Fredericks et al. 2011), whereas in the current study, irradiation of *D. bruxellensis* CBS2499 and CBS74$^T$ at the same UV-C dosage, gave 1.10 and 1.03 $\log_{10}$ CFU/mL, respectively. When UV-C irradiation was applied at 918 J/L microbial reduction for *B. bruxellensis* ISA 1649 was 1.24 $\log_{10}$ CFU/mL (Fredericks et al. 2011), compared with 2.59 and 2.27 $\log_{10}$ CFU/mL for *D. bruxellensis* CBS2499 and CBS74$^T$ in the current study. The highest UV-C treatment of *B. bruxellensis* ISA 1649, being 1377 J/L, employed by Fredericks et al.

295 (2011), achieved 1.83 $\log_{10}$ CFU/mL reduction, whereas that calculated for *D. bruxellensis* CBS2499
296 and CBS74$^T$ in the current study was 4.29 and 3.61 $\log_{10}$ CFU/mL, respectively. These outcomes and
297 the relevant α values indicate a strain-specific resistance to UV-C exposure by yeast cells (Table 2).
298 Indeed, a significantly different dosage of UV-C radiation was required to induce a 90% reduction in
299 the populations of *D. bruxellensis* CBS2499 (α = 427 ± 50 J/L) and CBS74$^T$ (α = 449 ± 41 J/L) strains
300 ($P$ = 0.030). Despite differences in experimental conditions, the variation in resistance to UV-C
301 irradiation observed by *D. bruxellensis* strains may be associated with the recognised genetic and
302 physiological intraspecific polymorphism found in this species (Agnolucci et al. 2009, Hellborg and
303 Piškur 2009, Vigentini et al. 2012).

304 Similar findings were observed for treatments on bacteria; *L. brevis* species showed $\log_{10}$
305 CFU/mL reduction comparable to that reported for irradiation of *L. plantarum* 130 inoculated into
306 Shiraz juice by Fredericks et al. (2011). A UV-C dosage of *L. plantarum* at 918 J/L resulted in a $\log_{10}$
307 CFU/mL reduction of 1.86, while the estimated reduction for *L. brevis* DSM20054$^T$ and DSM7308
308 was 1.82 and 1.93 $\log_{10}$ CFU/mL, respectively. After UV-C irradiation at 1377 J/L, however, *L.*
309 *plantarum* 130 gave a 3.38 $\log_{10}$ CFU/mL reduction, which was significantly different ($P$ = 0.005)
310 from the reduction calculated for *L. brevis* DSM20054$^T$ and DSM7308, being 3.12 and 3.05 $\log_{10}$
311 CFU/mL, respectively.

312 The AAB and LAB species showed similar strain-related behaviour, with significant
313 variations in α values being detected ($P$ = 0.001 for *A. aceti* species and $P$ = 0.015 for *L. brevis*
314 species) (Table 2). In this study and according to data published in the literature, Gram positive
315 bacterial cells exhibit greater resistance to UV-C radiation than Gram negative bacteria (Thompson
316 et al. 2003, Guerrero-Beltrán and Barbosa-Cánovas 2004). *Lactobacillus brevis* DSM20054$^T$ proved
317 to be the least sensitive to UV-C treatment amongst the microorganisms studied, and required a UV-
318 C dosage of 588 ± 31 J/L to reduce cell concentration by one log cycle.

319 The mould, yeast, LAB and AAB counts for the 2015 Chardonnay grape must used in trials
320 carried our with the dynamic protoype, before inoculation with test strains, were respectively < 1,

3.18 ±0.22, 2.30 ±1.22 and < 1 $log_{10}$ CFU/mL, respectively. These levels of contamination were considered negligible if compared to the cell concentration of the inoculum. The results obtained with the UV-C irradiation system working in a continuous flow, confirmed the microbial inactivation levels found with the static one. Indeed, in the case of more turbid must sample (212 ± 4 NTU), mean values of $log_{10}$ CFU reduction were 4.80 ±0.86, 4.60 ±0.63 and 3.97 ±1.29 for *D. bruxellensis* CBS74$^T$, *A. aceti* DSM2002 and *L. brevis* DSM20054$^T$, respectively. Even if the microbial count reductions were slightly lower for the must with higher turbidity (212 ± 4 NTU), however they were not significantly different from those obtained with the less turbid must (17 ± 1 NTU), as well as with the static prototype (Table 4). The only significant difference observed was for treatment of the *D. bruxellensis* CBS74$^T$ strain, which gave a higher $log_{10}$ reduction in experiments involving more clarified must (17 ± 1 NTU) under dynamic conditions. These data suggest the efficacy of UV-C treatment is affected by both the strain and the must turbidity, but increased flow rates can achieve more effective microbial inactivation.

Collectively, these findings confirm the effectiveness of UV-C treatment in killing fungal and bacterial cells in an oenological matrix, in agreement with results reported previously (Fredericks et al. 2011; Falguera et al. 2013, Rizzotti et al. 2015).

*Effect of UV treatment on PPO inactivation and must composition*

The effect of UV-C irradiation on white must was evaluated in terms of inactivation of the PPO enzyme, changes in colour and the formation of α-dicarbonyl compounds.

Although the UV-C treatment was found to have suitable microbicide activity, to provide an effective alternative to $SO_2$ for treatment of grape must, UV-C treatment should also impede PPO activity. Irradiation with UV-C can break the disulfide bonds present in cystine and induce either direct or indirect oxidation of sulfur-containing amino acids and aromatic amino acids (Augenstein and Riley 1964, Davies and Truscott 2001). The UV-induced PPO inactivation in grape must, as well as in other fruit, has been reported in the literature, but the treatment conditions employed were either

not clearly described or not comparable to one other (Guerrero-Beltrán and Barbera-Cánovas 2006, Falguera et al. 2013, Müller et al. 2014). Moreover, it is not clear whether the treatment conditions that yield microbicide effects also achieve enzyme degradation. Therefore, the UV-C treatment conditions applied previously were evaluated for PPO inactivation. Grape must (prepared from 2015 Crimson grapes under laboratory conditions) was clarified (21 ± 2 NTU) before UV-C irradiation (at up to 1800 J/L) under static conditions. The starting PPO activity (1.04 U PPO/L) linearly decreased as the irradiation energy increased (Figure 4), with PPO activity reduced by 30% (0.7 U PPO/L) following irradiation at 1800 J/L. This level of inactivation is useful for protecting grape must from oxidation, but is not comparable to the inactivation level achieved with $SO_2$ addition (Valero et al. 1992). A high level of irradiation energy can also induce either direct or indirect oxidation of many flavour-related grape precursors, such as carotenoids and cysteinylated compounds (Davies and Truscott 2001), therefore low energy treatments are preferable. The efficacy of dynamic UV-C treatment (at 1800 J/L) on PPO activity was also assessed. Clarified grape must was spiked with grape lees to achieve a turbidity level comparable to vinification conditions (252 ± 6 NTU). Under these conditions, the loss of PPO activity was considerably lower (7.8 ± 1.4%) than that achieved under static conditions. This was likely due to the turbidity level being 12-fold higher than that for the grape must used in UV-C treatment under static conditions, as well as an optical density value at 254 nm being twofold lower in the must used in the latter experiments (Table 1). The results obtained were comparable to data reported by Muller et al. (2014), despite a higher turbidity value being reported in treated grape juice. The significant shielding effect exerted by the suspended grape solids, expressed as absorbance at 254 nm, is well known. To confirm the influence of flow rate on PPO inactivation, experiments were repeated with the flow rate being lowered from 0.44 to 0.072 L/min, using dynamic treatment conditions (laminar flow) but maintaining UV-C irradiation at 1800 J/L. Under these conditions, negligible variation in PPO activity was observed after the treatment. The low inactivation of the PPO enzyme can be responsible for must oxidation if no antioxidant is added. Even though PPO inactivation was lower using the dynamic apparatus, these results suggest that

373 increasing flow rate and/or decreasing turbidity, could improve PPO inactivation. In this way, the
374 oxidation of must could be limited, preventing browning and preserving the desired yellow colour.

375 No significant difference ($P > 0.05$) was detected between any of the colour parameters
376 measured for untreated and treated must samples, under either static or dynamic conditions (Table 5).
377 A decrease in a* and b*, however, was observed for must samples treated with UV-C irradition under
378 static vs dynamic conditions; although the turbidity of the must samples treated using the static and
379 dynamic apparatus (being 17 ± 1 vs 252 ± 6 NTU, respectively) may explain the variation of a* and
380 b* values. The lightness (L*) and hue (a*, b*) values did not significantly change following UV-C
381 treatment at increasing intensity, up to 1800 J/L. Similar results were obtained by Falguera et al.
382 (2013) and Unluturk and Atilgan (2015) when white grape must was considered. Further confirmation
383 that colour was not affected by UV-C treatment was obtained by calculating ΔE; the ΔE values of
384 0.66 and 0.35 calculated for must treated under static conditions, respectively, reflect small and very
385 small differences in colour (Ford and Roberts 1998). This was in contrast to the major degradative
386 effects of UV-C irradiation on the free anthocyanins observed in red grape musts reported by Falguera
387 et al. (2013).

388 α-Dicarbonyl compounds are normal by-products of chemical oxidation in wine (Elias et al.
389 2008) and their concentration is often higher in sweet wines (da Silva Ferreira et al. 2007). This is
390 because the Maillard reaction can occur, even at room temperature, and it can be facilitated by the
391 presence of metal ions, especially iron and copper, in both wine and grape must (Jackson 2000,
392 Danilewicz 2003). The most abundant constituents of must are represented by aldo and keto-sugars,
393 compounds which are easily oxidised. The potential for radicals formed during UV-C irradiation to
394 oxidise sugars to generate α-dicarbonyl compounds should be taken into account (Danilewicz 2003).
395 The formation of α-dicarbonyl compounds after UV-C treatment of must was therefore considered in
396 the current study. The concentration of α-dicarbonyl compounds present in must samples before UV-
397 C treatment at 1800 J/L was 123.0 ± 22.4 mg/L, whereas after treatment, the concentration, 124.4 ±
398 22.5 mg/L, was not significantly different. Must samples treated with UV-C did not show the

399 formation of oxidative molecules that could negatively impact wine quality. Similar results were
400 obtained following compositional analysis of the laboratory-prepared must; no significant difference
401 in the concentration of α-dicarbonyl compounds was observed before and after UV-C treatment,
402 being 192.5 ± 27.0 and 212.0 ± 28.2 mg/L, respectively.
403
404 **Conclusions**
405 This study suggests that UV-C irradiation shows promise as an alternative to $SO_2$ in winemaking. A
406 relationship between UV-C exposure (J/L) and decimal reduction values (α) was established for
407 several microbial strains involved in wine spoilage. Despite increasing interest from the wine
408 industry, there are few studies in the literature concerning the inactivation of the species investigated
409 here, by UV-C treatment, and so this study addresses a key knowledge gap. The UV-C irradiation
410 applied to white must effectively reduced microbial counts, without promoting the formation of
411 chemical compounds associated with oxidation and therefore, negatively affecting must composition.
412 Ultra violet irradiation can lead to the inactivation of PPO if a suitable flow rate can be applied to
413 prevent any shielding effect imparted by suspended materials. The application of UV-C radiation
414 might therefore offer winemakers an alternative to the $SO_2$ additions currently employed at different
415 stages of must processing, thereby responding to the consumer demand for high quality, $SO_2$-free
416 wines.
417
418 **Acknowledgements**
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545

## Figure captions

**Figure 1**. Ultraviolet-C irradiation apparatus developed for laboratory scale treatment of wine under static conditions.

**Figure 2**. Preliminary tests, Test 1 (——), Test 2 (——), Test 3 (——), Test 4 (——) and Test 5 (——), showing the kinetics of the lamp radiation intensity over time; the average value, which excluded Test 1 (i.e. when the lamp was cool), is also shown (——).

**Figure 3**. Microbial inactivation of (a) *Brettanomyces bruxellensis* CBS2499, (b) *Brettanomyces bruxellensis* CBS74$^{T}$, (c) *Acetobacter aceti* DSM3508$^{T}$, (d) *Acetobacter aceti* DSM2002, (e) *Lactobacillus brevis* DSM20054$^{T}$, and (f) *Lactobacillus brevis* DSM7308, in white must treated with UV-C irradiation at variable dosage (0–1800 J/L). The black line was plotted by the Weibull model (——); the red line was plotted by linear model (——).

**Figure 4**. Residual polyphenol oxidase activity determined in laboratory-prepared must treated with UV-C irradiation at variable dosage (0–1800 J/L). y= -0.0001x + 1.0155, $R^2$ = 0.9844.

564 **Table 1:** Chemical parameters of the musts treated with UV-C irradiation. The must batches produced in
565 vintage 2013 were used for the static UV-C system, while those prepared in vintage 2015 were treated in the
566 dynamic UV-C system.

567

| Grape cultivar | Vintage | Sugars (g/L) | pH | Total acidity (g/L tartaric acid) | Turbidity (NTU) | $AU_{256nm}$ |
|---|---|---|---|---|---|---|
| Chardonnay | 2013 | 187±19 | 3.20±0.05 | 6.8±0.4 | 76±4 | 1.28±0.08 |
| Chardonnay | 2015 | 193±19 | 2.80±0.05 | 7.5±0.3 | 17±1 | 1.67±0.1 |
| Crimson | 2013 | 180±9 | 3.60±0.05 | 2.1±0.2 | 21±2 | 2.75±0.15 |
| Crimson | 2015 | 207±12 | 4.10±0.05 | 1.7±0.1 | 101±4 | 4.18±0.18 |

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576 **Table 2** Estimated parameters of the Weibull and linear models ± standard deviation.

577 Different letters as superscript of numbers in the same column mean significant differences among the strains

578 ($p < 0.05$).

| Species | Strain | Weibull model | | | | Linear model | | |
|---|---|---|---|---|---|---|---|---|
| | | D (J/L) | Shape | $R^2$ adj[#] | MSE[§] | D (J/L) | $R^2$ adj | MSE |
| *D. bruxellensis* | CBS2499 | 427 ± 50[a] | 1.25 ± 0.12 | 0.986 | 0.297 | 294 ± 7[a] | 0.973 | 0.370 |
| | CBS74[T] | 449 ± 41[b] | 1.15 ± 0.09 | 0.988 | 0.196 | 357 ± 22[b] | 0.983 | 0.233 |
| *A. aceti* | DSM3508[T] | 412 ± 30[a] | 1.06 ± 0.06 | 0.993 | 0.145 | 345 ± 20[c] | 0.993 | 0.145 |
| | DSM2002 | 462 ± 34[c] | 1.19 ± 0.07 | 0.992 | 0.169 | 370 ± 12[be] | 0.989 | 0.201 |
| *L. brevis* | DSM20054[T] | 588 ± 31[d] | 1.34 ± 0.07 | 0.994 | 0.130 | 435 ± 21[d] | 0.977 | 0.253 |
| | DSM7308 | 511 ± 55[e] | 1.13 ± 0.11 | 0.978 | 0.218 | 385 ± 30[e] | 0.976 | 0.238 |

579

580 [#]: Adjusted determination coefficient

581 [§]: Mean square error

582

583 **Table 3** Mean of reduction of microbial counts (expressed as $log_{10}$ CFU reduction) obtained with tested strains

584 using the static prototype UV-C system. Reported data are mean values (n=3) ± standard deviation.

585

| Species | Strain | Initial counts ($Log_{10}$ CFU/mL) | $Log_{10}$ reduction (300 J/L) | $Log_{10}$ reduction (600 J/L) | $Log_{10}$ reduction (900 J/L) | $Log_{10}$ reduction (1200 J/L) | $Log_{10}$ reduction (1500 J/L) | $Log_{10}$ reduction (1800 J/L) |
|---|---|---|---|---|---|---|---|---|
| *D. bruxellensis* | CBS2499 | 7.27 ± 0.07 | 0.65 ± 0.12 | 1.53 ± 0.16 | 2.53 ± 0.15 | 3.61 ±0.10 | 4.77 ± 0.02 | 5.99 ± 0.12 |
| | CBS74$^T$ | 7.29 ± 0.05 | 0.63 ± 0.09 | 1.40 ± 0.11 | 2.22 ± 0.10 | 3.08 ± 0.06 | 3.98 ± 0.01 | 4.91 ± 0.08 |
| *A. aceti* | DSM3508$^T$ | 7.25 ± 0.15 | 0.72 ± 0.07 | 1.49 ± 0.08 | 2.29 ± 0.07 | 3.10 ± 0.04 | 3.92 ± 0.00 | 4.76 ± 0.06 |
| | DSM2002 | 7.19 ± 0.05 | 0.60 ± 0.07 | 1.37 ± 0.09 | 2.21 ± 0.09 | 3.12 ± 0.05 | 4.07 ± 0.01 | 5.05 ± 0.07 |
| *L. brevis* | DSM20054$^T$ | 6.97 ± 0.11 | 0.41 ± 0.05 | 1.03 ± 0.07 | 1.77 ± 0.07 | 2.60 ± 0.05 | 3.50 ± 0.01 | 4.47 ± 0.05 |
| | DSM7308 | 7.15 ± 0.11 | 0.55 ± 0.10 | 1.20 ± 0.12 | 1.89 ± 0.11 | 2.61 ± 0.07 | 3.36 ± 0.01 | 4.12 ± 0.08 |

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588 **Table 4** Comparison of microbial count reductions (expressed as $\log_{10}$ CFU reduction) obtained by using static
589 and dynamic prototype UV-C irradiation system at 1800 J/L dosage. Data are reported as mean values (n=3)
590 ± standard deviation.

591 Different letters as superscript of numbers in the same row mean significant differences among the UV-C
592 treatment applied ($p < 0.05$).

593

| **Flow condition** | Static | Dynamic | Dynamic |
|---|---|---|---|
| **Turbidity** | 76 ±4 NTU | 17 ± 1 NTU | 212 ± 4 NTU |
| *D. bruxellensis* CBS74$^T$ | 4.91 ± 0.08$^a$ | 6.30 ± 0.00$^b$ | 4.80 ± 0.86$^a$ |
| *A. aceti* DSM2002 | 5.05 ± 0.07$^a$ | 5.19 ± 0.51$^a$ | 4.60 ± 0.63$^a$ |
| *L. brevis* DSM20054$^T$ | 4.47 ± 0.05$^a$ | 4.42 ± 0.21$^a$ | 3.97 ± 1.29$^a$ |

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603 **Table 5** Colour indexes determined by the CIE L*a*b* on must with different turbidity treated with the (*)
604 static and (#) dynamic prototypes at a UV-C dosage of 1800 J/L. Data are reported as mean values (n=3 for the
605 untreated must; n=9 for the treated must) ± standard deviation.

606

| Must sample | Turbidity (NTU) | L* | a* | b* | Chroma | Hue angle | ΔE |
|---|---|---|---|---|---|---|---|
| Untreated | 17 ±1 | 34.95 ± 0.09 | 0.35 ± 0.08 | 1.10 ± 0.18 | 0.30 ± 0.05 | 1.16 ± 0.15 | |
| Treated* | | 34.99 ± 0.19 | 0.19 ± 0.07 | 0.73 ± 0.11 | 0.22 ± 0.05 | 1.34 ± 0.18 | 0.66 ± 0.09 |
| Untreated | 252 ±6 | 30.86 ± 0.10 | 1.23 ± 0.03 | 1.81 ± 0.08 | 2.23 ± 0.05 | 0.97 ± 0.15 | |
| Treated# | | 31.10 ± 0.16 | 1.17 ± 0.03 | 1.75 ± 0.07 | 2.04 ± 0.09 | 0.98 ± 0.02 | 0.35 ± 0.09 |

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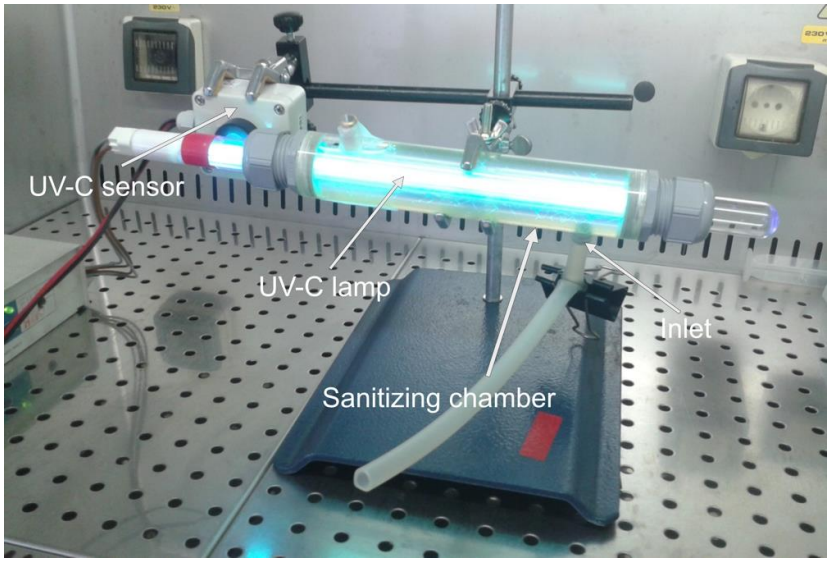
610 Figure 1

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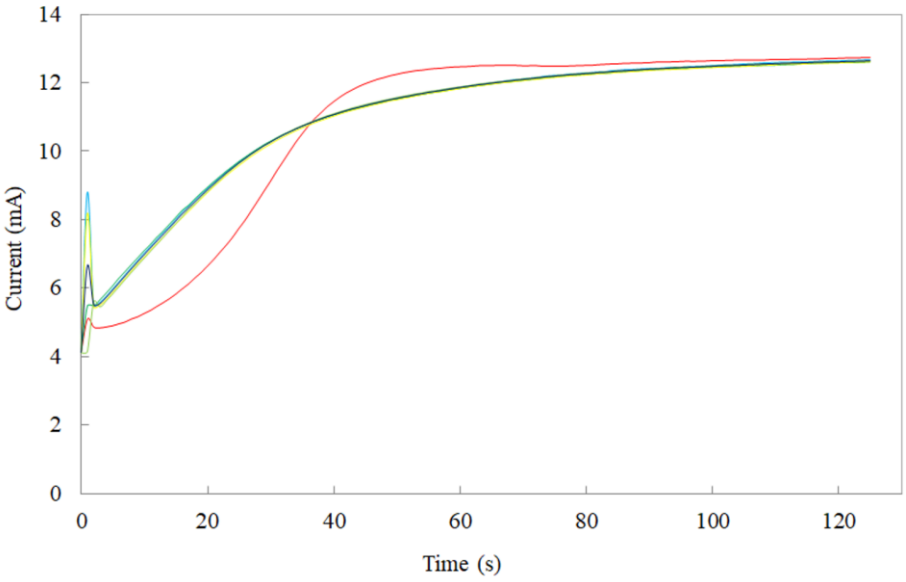
613 Figure 2

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617 Figure 3

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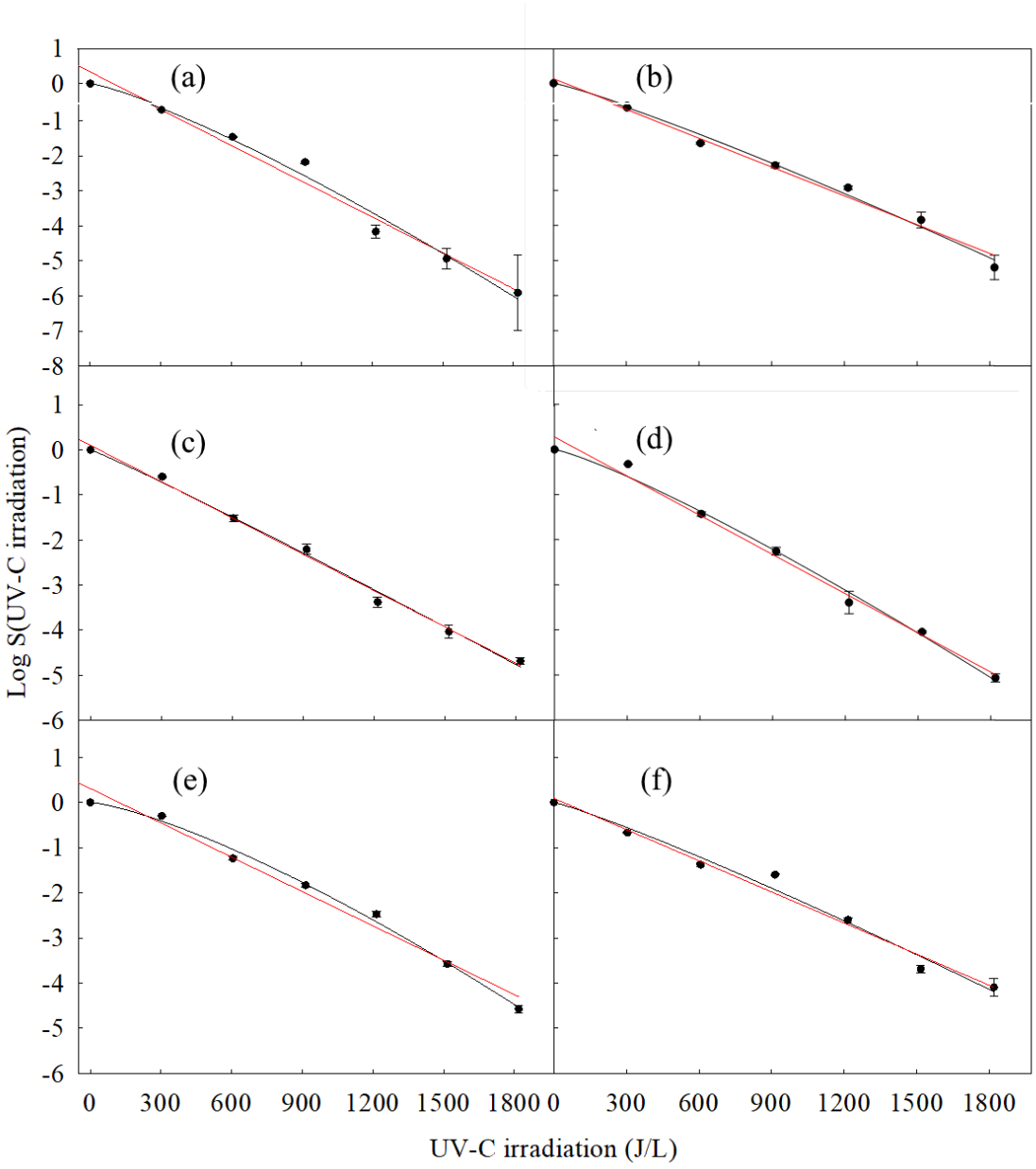


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622 Figure 4

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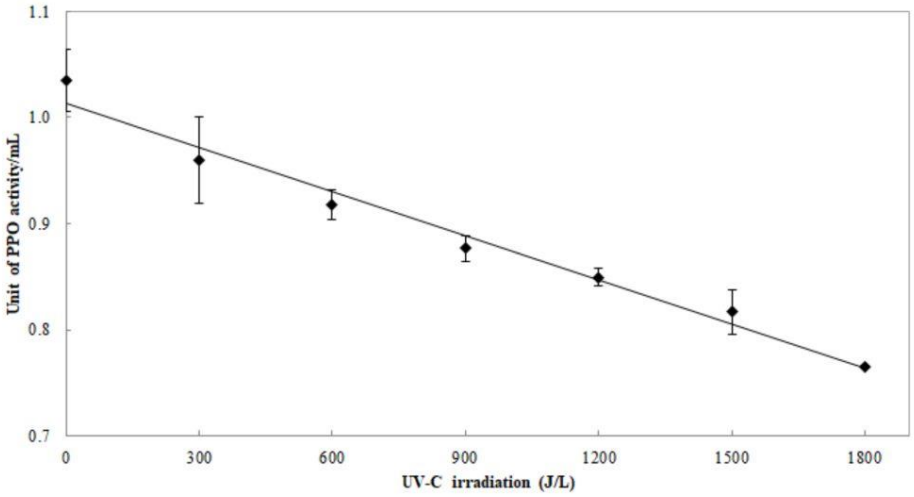


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