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Microbial stabilization of grape musts and wines using coiled UV-C reactor

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ABSTRACT

UV-C light is well known for its germicidal properties and is widely used for water disinfection. However, its low penetration into absorbing liquids, such as wines and musts, reduces drastically the microbial inactivation effectiveness. Additionally, wines require UV-C doses to be as low as possible to avoid any possible light-struck flavors. In order to add to the technologies that allow the reduction of SO_2 use, a coiled UV-C reactor was designed to inactivate microorganisms in wines and musts. Due to its unique hydrodynamic characteristics, this design could improve the exposure probabilities of the microorganisms to the UV-C light in absorbing liquids. In a first step, theoretical and measured fluid dynamics parameters such as Dean number were employed to improve the operating conditions of the reactor. The higher the Dean number, the higher the UV-C dose delivery efficiency in this reactor, and thus the lower the dose required to inactivate a given load of microorganisms. The second step investigated the impact of different wines on microbial inactivation efficiency and the UV-C doses required to inactivate microorganisms frequently found in wines. White and rosé wines, with low absorbances at 254 nm, required lower doses (≈ 600 J/L) than red wine (≈ 5000 J/L) because their absorption coefficient is ten times lower. The tolerance of microbial strains to UV-C treatments was variable, with higher resistance observed for yeast than for bacteria. In the third step, treatments conducted at semi-industrial scale showed that physicochemical and sensorial properties of wines and musts were not altered, highlighting the possible relevance of such a reactor on an industrial scale.

KEYWORDS

UV-C light, Dean vortices, wine, microbial inactivation, coiled reactor, process

HIGHLIGHTS

- Design of a coiled UV-C reactor for microbial stabilization of wines and musts
- Focus on inactivation efficiency in multiple strains and wine varieties
- Chemical and sensorial analyses to ensure treatment does not affect the organoleptic properties of the product

INTRODUCTION

Overall microbial activity plays a major role in winemaking, from alcoholic fermentation through to malolactic fermentation. However, certain species can cause defects, such as *Brettanomyces bruxellensis* yeast associated with volatile phenol production (Chatonnet *et al.*, 1992; Du Toit and Pretorius, 2000), or the *Acetobacter aceti* bacteria associated with acid acetic production (Du Toit and Pretorius, 2000). Microbial population control is therefore imperative. Sulfur dioxide (SO_2) is currently one of the most widely used additives in winemaking, for its antimicrobial and antioxidant properties (Ribéreau-Gayon *et al.*, 2006), but the limitation of chemical inputs in the winemaking process is one of the major concerns of consumers and winemakers (Regulation EC 606/2009). Among the non-thermal food decontamination processes, UV-C light (from 100 to 280 nm) demonstrates interesting perspectives, especially for its low energy consumption compared to classic thermal processes. Because of high DNA absorption at 260 nm, the high energy of UV-C at 254 nm released by low mercury vapor lamps is known to induce formation of dimers in the DNA of microorganisms. Once the DNA is damaged, the microorganisms can no longer reproduce (Bintsis *et al.*, 2000).

The efficiency of treatment depends on the ability of the light to reach the microorganisms. This process has therefore been limited to clear liquids (due to the lack of penetration in absorbing) and/or turbid liquids (Koutchma, 2009). Absorbance has been reported as the major parameter affecting UV inactivation efficiency (Koutchma *et al.*, 2004), with absorption coefficients at 254 nm varying from 0.01 cm^{-1} for water to 290 cm^{-1} for milk (Koutchma, 2009). Using different pilot designs to improve UV-C light penetration in absorbing liquids, authors have studied the possibility of treating a range of products, such as orange juice (Keyser *et al.*, 2008), apple juice (Keyser *et al.*, 2008; Franz *et al.*, 2009), grape, cranberry and grapefruit juices (Guerrero-Beltran and Barbosa-Canovas, 2005; Guerrero-Beltran *et al.*, 2009), apple cider (Unluturk *et al.*, 2004), liquid eggs (de Souza *et al.*, 2014), and grape juice and wine (Fredericks *et al.*, 2011).

However, high UV-C intensity can induce light-struck flavors in wines, described as 'cabbage flavors' (Fracassetti *et al.,* 2017). Photosensitized riboflavin induces the formation of superoxide anion radicals and singlet oxygen (Pathak and Joshi, 1984), which are highly reactive. Riboflavin is mainly produced by yeast during alcoholic fermentation and presents absorption peaks at 222 and 266 nm (Orlowska *et al.*, 2013). Limiting UV-C doses and using a monochromatic lamp emitting at 254 nm may help to avoid this defect.

The flow pattern inside the reactor strongly influences the summed UV-C dose, as the position and residence time of the microorganisms can vary significantly. In a curved pipe, the main streamline is accompanied by secondary motion in the plane of the cross-section (Dean and Hurst, 1959; Mishra and Gupta, 1979). These secondary flows, also called Dean vortices, are known to improve the mixing of fluids in pipes, allowing the UV-C light to encounter larger volumes of the liquid (Schmidt and Kauling, 2007; Franz *et al.*, 2009; Müller *et al.*, 2014) and ideal plug flow reactor efficiency to be approached. For our purpose, an ideal mixing close to the UV-C lamp could allow a homogeneous treatment of the wine and thus maximize the probability for an undesirable microorganism to be targeted by UV light. The greater the mixing capabilities of the process, the lower the required UV-C doses. Therefore, a coiled UV-C reactor was built in this study to improve flow pattern and, as a consequence, reduce the UV-C doses required to inactivate undesired microorganisms in must and wine. Flow parameters such as Reynolds number were studied in order to select the best operating conditions on the pilot plant. In the next step, inactivation capabilities of the process were investigated at lab scale on multiple wine and strains varieties and the required UV-C doses for inactivation were identified. In the last step of the work, musts and wine were treated in semi-industrial conditions. Microbial, physicochemical and sensorial analyses were performed over time to quantify the impact of the treatments on organoleptic properties of the wine.

MATERIALS AND METHODS

1. The coiled tube reactor

1.1 Presentation

The UV-C module consisted of a mercury low pressure amalgam germicidal lamp, 77 cm long

with maximum peak radiation at 254 nm, surrounded by a quartz sleeve (Suzhou Xicheng Water Treatment Equipment, China). A food-grade fluorinated ethylene propylene (FEP) tube (Serto S.A.R.L, France), diameter 4/6 mm (in/out), chosen for its physical properties (flexibility, good UV transmittance and low moisture absorption), was wound around the quartz sleeve (figure 1). An aluminum foil was wrapped around the tubes in order to improve the reflection of UV-c light. Up to three modules could be used in a serial set-up. For safety reasons, the modules were inserted into opaque PVC tubes.

1.2. Treatment of musts and wines

The wine was pumped from a stainless steel tank and through the reactor using a diaphragm pump (Axflow, France) at flow rates of between 50 and 250 L/h. UV-C dose delivery was adjusted by varying the number of modules (from one to three) and the number of passages through the whole reactor. After each passage, a sterile stainless steel tank was used to collect the treated wine and the tubes were rinsed with water and sanitized with technical ethanol.

1.3. Spectra measurements

Spectra measurements were carried out at the Centre des Lasers Intenses et Applications (CELIA) in Bordeaux, with two Ocean Optics HR2000+ UV spectrophotometers. An optical fiber was connected to a beam concentrator placed close to the lamp in a dark room. One spectrophotometer collected data from 190 to 340 nm and the second one from 290 to 390 nm. The data presented in this work is relative intensity versus wavelength. The reference for 100% was the highest peak. A blank was subtracted from the measurements.

1.4. Flow conditions

In curved pipes, secondary flows generated by centrifugal forces appear in a plane perpendicular to the pipe axis. Due to the curvature of the pipe, pressure is higher near the wall furthest from the center of curvature than close to the nearest wall. As a result, the flow is more stable and the transition from laminar to turbulent flow occurs at a higher critical Reynolds number than for straight pipes. According to Mishra and Gupta (1979), this critical number Re_{cr} is (1) depending on the internal diameter of the tube (d_i) and the total diameter of the coil (d_c):

$$Re_{cr} = 2.10^4 (d_i/d_c)^{0.32} \quad (1)$$

It is also common to use the dimensionless modified Dean number De' (2), based on Reynolds number Re, to characterize the flow in coiled pipes:

$$De' = Re \sqrt{\frac{d_i}{d_{c'}}} \quad (2)$$

Where dc' (3) is the effective coil diameter including pitch b (distance between coils):

$$d_c' = d_c \left[1 + \left(\frac{b}{\pi d_c}\right)^2\right] \quad (3)$$

These dimensionless numbers will be used to describe the flow conditions in the following experiments.

1.5. Dosimetry

The theoretical UV-C dose per liter of treated liquid (D_{th} in J/L) was calculated (4) as the UV-C output of the lamp (P_{lamp} in W) per flow rate Q (L/s). This represents the UV-C dose delivered to the liquid for an ideal and perfectly homogenous treatment (depending only on lamp power and retention time). UV absorbance by

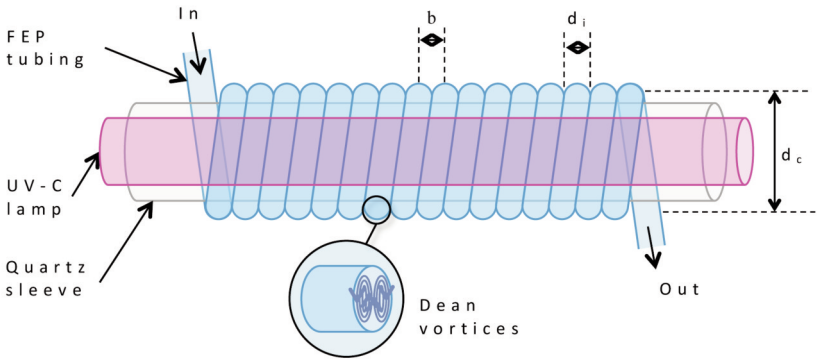

FIGURE 1. Schematic representation of a single module in the coiled UV-C reactor.

FEP tubing and other losses (reflected and scattered light) were taken into account in a factor of general transmittance T (%):

$$D_{th} = \frac{P_{lamp}}{Q} * T \quad (4)$$

In order to measure the real UV-C dose received by an absorbing liquid, a chemical iodide/iodate actinometer (Rahn, 1997) has been used. This solution is optically opaque to light below 290 nm and absorbs all the germicidal wavelengths, as a highly absorbent wine would act. The method was validated in coiled reactors by Müller *et al.* (2014). A solution of 0.6 M potassium iodide and 0.1 M potassium iodate in 0.01 M borate buffer was irradiated and the linear formation of triiodide was monitored by spectrophotometry at 352 nm in a Lambda 25 spectrophotometer from Perkin Elmer in 1 cm quartz cuvettes:

The UV-C dose (D_m in J/L) could be calculated using the following equation (5):

$$8KI + KIO_3 + 3H_2O + hv \rightarrow 3I_3^- + 6HO^- + 9K^+$$

Where $A_{352\ nm}$ is the measured absorbance, $P_{254\ nm}$ the number of joules per Einstein of 254 nm photons (4.716×10^5 J/einst), pl the path length of the cuvette, φ the quantum yield (effects per photon in mol/einst) and $\varepsilon_{352\ nm}$ the molar absorption coefficient of triiodide at 352 nm (27600 L/mol/cm). The quantum yield was calculated as follows:

$$D_m = \frac{A_{352\ nm} * P_{254\ nm}}{pl * \varphi * \varepsilon_{352\ nm}} \quad (6)$$

Where c_i is the concentration (in mol/L) of the iodide measured by spectrophotometer ($A_{300\ nm}$ x 1.061^{-1}) and T_i the temperature in °C. In order to cover the whole range of flow rates and because of the high sensibility of the chemical reaction, the UV-C lamp was 93 % covered with aluminum sheet. The final results were extrapolated to the full length of the lamp.

2. Musts and wines

In the preliminary investigations, four different wines were studied: AOP Bordeaux Blanc, a Sauvignon white wine; AOC Côtes de Gascogne, a Gros Manseng sweet white wine; AOP Bordeaux Rosé, a Cabernet Franc rosé wine; and AOP Bordeaux Rouge, a Merlot red wine. All the wines were sterile filtered and the turbidity was low (< 1 NTU). In order to study the influence of absorbance and strain resistance in the coiled reactor, free sulfur dioxide (SO_2) was removed from the wines by addition of a small quantity of hydrogen peroxide and the wines were inoculated prior to the experiments.

In the second part of the work, UV-C treatments were performed at two different stages of the vinification process: before bottling on the AOP Bordeaux Rouge Merlot red wine and replacing the mutage step on the AOC Pacherenc-du-Vic-Bilh Gros Manseng sweet must. Microorganisms were naturally present in the wines. For each condition, 50 L of wine was treated and protected from oxygen by inerting the tanks with CO_2. After treatments, wines were bottled and stored at 12 °C for further analyses.

2. Microbiological studies

2.1. Strain culture

Dry active *S. cerevisiae* FX10 yeast (Laffort, France) and strains of *B. bruxellensis* CBS2499 and AWRI1608 from our lab collection were cultured in YPD broth (2 % glucose, 1 % yeast extract and 1 % peptone w/v) at +28 °C for 4 and 10 days, respectively. YPD broth was autoclaved at 120 °C for 15 min. *A. aceti* 08ba01 bacteria from our lab collection were cultured in grape juice medium (25 % red grape juice v/v, 1 % yeast extract w/v and 0.1 % Tween 80 v/v, pH of 4.5) for 10 days.

2.2. Microbial enumeration

Microbial counts were determined by plating serial 10-fold dilutions of the samples on the corresponding solid medium. A volume of 100 µL was plated in 9-cm diameter petri dishes and all the analyses were repeated three times. Yeast was enumerated on YPD (+2 % agar w/v) with chloramphenicol (0.1 mg/mL) and biphenyl (0.15 mg/mL) after 2–3 days of incubation at 27 °C. Bacteria were enumerated in a grape juice medium (+2 % agar w/v) with pimaricin (0.1 mg/mL) after 10–12 days of incubation at 28 °C. All incubations were performed in the dark, as DNA repair mechanisms are known to be less effective in dark conditions (Salcedo *et al.*, 2007). The number of colonies counted was expressed as a logarithmic concentration (CFU/mL) and the limit of detection was 10 CFU/mL.

4. Analyses

4.1. Esters quantification

Sample preparation for ester analysis was conducted using solid-phase microextraction

(SPME). The solution of sample and internal standards was homogenized and loaded in an autosampling device. Gas chromatography analyses were carried out using an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Hewlett Packard). The mass spectrometer was operated in the electron ionization mode at 70 eV in the selected-ion-monitoring (SIM) mode. The protocol was established and widely described by Antalick *et al.* (2010). In the results below, the groups of esters are as follows: ethyl esters – ethyl propanoate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate; higher alcohol esters – propyl acetate, isobutyl acetate, isoamyl acetate, hexyl acetate and octyl acetate; aliphatic acid esters – ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl isovalerate; aromatic esters – ethyl phenylacetate, acetate phenylethyl, ethyl dihydrocinnamate and ethyl cinnamate.

4.2. Standard physicochemical analyses

Absorbance of wines at 254 nm was measured with a Lambda 25 spectrophotometer from Perkin Elmer in a quartz cuvette under 1 cm of optical path length with appropriate dilutions. The absorption coefficients (in cm^{-1}) of the samples were determined from the ratio between absorbance at 254 nm and path length.

The total polyphenols index (TPI) was measured at 280 nm under 1 cm of optical path in a quartz cuvette. The red wine was diluted to 1/100 before measurement. The result was expressed as the product of the measured absorbance by the dilution.

Color change in the L*a*b* color space was measured with a Perkin Elmer Lambda 25 spectrophotometer under 1 cm of optical path in a quartz cuvette. The L*a*b* color space describes all perceivable colors mathematically in the three dimensions: L* for lightness, from 0 (black) to 100 (transparent), and a* and b* for the color opponents green–red and blue–yellow, from -300 to 299. Differences between samples are expressed as a delta, where ΔEab* is the color difference between the control and the sample.

Other standard analyses, such as volatile and total acidity, alcohol, pH or sulfur dioxide were carried out on OenoFoss (FOSS France). This analysis method is based on the principle of Fourier-transform infrared spectroscopy (FTIR) technology. It consists in analyzing the spectrum of a sample of must or wine in the medium infrared. The light is absorbed in the sample according to the constituents of the wine such as sugars and acids. The absorption is converted by a mathematical model in a prediction of the concentration of the different constituents.

4.3. Sensorial analyses

Sensorial analyses were conducted to evaluate the impact of UV-C treatment on wines. A triangle test was performed to see whether panelists could detect a difference between the controls and treated wines. Three coded samples in black ISO glasses were presented to 21 panelists from the ISVV, Bordeaux University, who were moderate-to-expert level wine tasters. The samples were evaluated at a controlled room temperature of 20°C in individual booths (NF EN ISO 8589:2007). Two samples were identical and one was different. The panelists were asked to identify the different samples after olfactory examination. The presentation order was randomized among the panelists.

4.4. Statistical analyses

All the experiments were triplicated. Means and standard deviations were calculated using Microsoft Excel. For the triangle tests, statistical analysis was performed on the basis of the binomial law corresponding to the distribution of answers in this type of test, with a 5% level of confidence.

RESULTS

1. Characteristics of the coiled UV-C reactor

The emission spectrum of the UV-C lamps used to build the pilot was measured to confirm that low pressure mercury vapor lamps emit mainly at germicidal wavelengths. As expected, the main peak was observed at 253.7 nm (Figure 2). There is also a peak at 365 nm at 20% relative intensity. However, the range of the measurement was not exhaustive, and it was possible to see a visible blue light by the naked eye when the lamps were turned on. This emission spectrum does not show critical peaks at 222 and 266 nm that can photosensitize riboflavin. In future work, it could be interesting to eliminate undesired wavelengths.

Improving flow conditions in order to maximize homogeneity of treatment in the UV-C reactor is a major concern when treating wines: higher

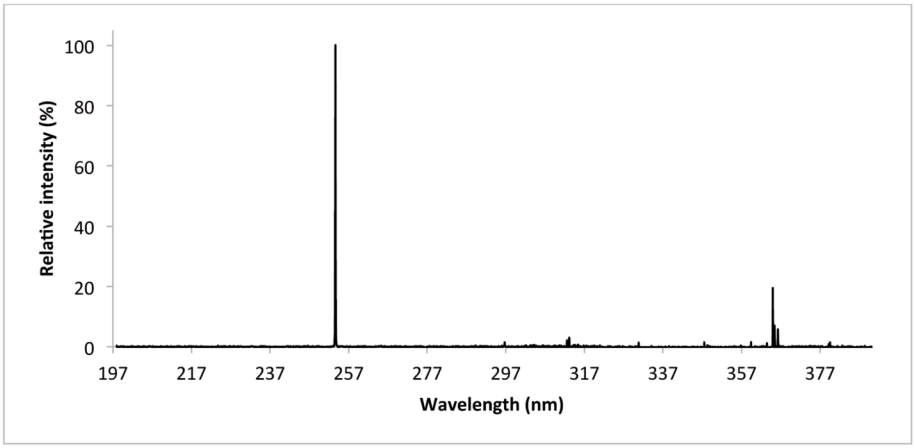

FIGURE 2. Spectrum of low pressure mercury vapor amalgam UV-C lamps.

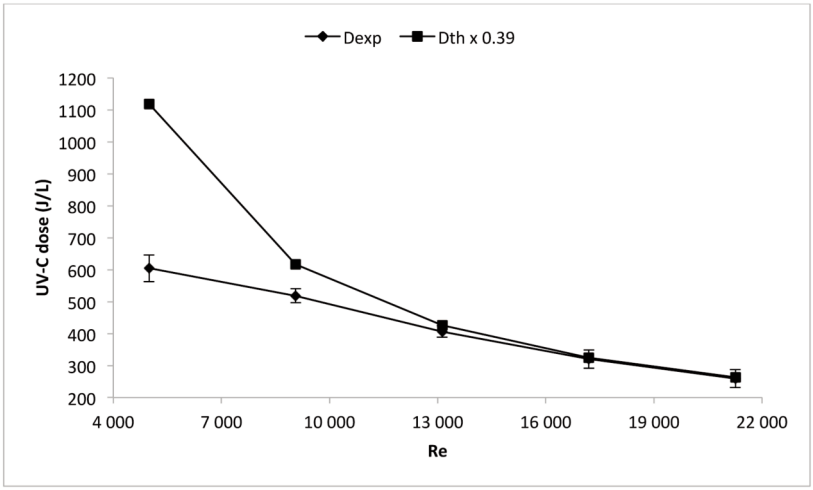

FIGURE 3. Actinometer-measured and calculated theoretical UV-C doses delivered by the coiled reactor in a single module.

homogeneity leads to maximizing microbial inactivation efficiency, and the higher the inactivation efficiency, the lower the required UV-C dose and thus, the lower the lower the formation of undesired flavors under excessive UV-C doses. Electrical energy consumption could also be positively impacted by improving this inactivation efficiency. The UV-C doses delivered by the coiled reactor into an absorbing liquid (i.e. the actinometer solution) at Reynolds numbers between 7500 and 21,000 in a single UV-C module measured by actinometer were compared to theoretical doses. This experiment has two objectives. First, measure the real UV-C dose delivered to an absorbing liquid (such as red wine). Second, improve the homogeneity of the treatment by selecting the best Reynolds number.

The results (Figure 3) show that at a low Reynolds number (< 10,000), the measured doses were lower than the theoretical doses. At a high Reynolds number (> 10,000), the measured and calculated doses were very similar if we consider a transmittance factor T of 39 %. Note this transmittance factor was not measured in this study, it is estimated from the difference between the theoretical and measured doses to obtain a fitting at high Reynolds numbers. However, Galante *et al.* (2010) measured a transmittance of approximately 35 % through 250-µm-thick FEP films, which tend to confirm our approximation. Increased radial mixing at a high Reynolds number can explain the improved dose delivery. The same tendencies were observed by Müller *et al.* (2014) in a coiled UV-C reactor. The critical Reynolds number (Re_{cr} = 10,000) may indicate that turbulence plays a role in this enhancement. For further experiments, a

Reynolds number of 19,000 was applied, corresponding to a modified Dean number De' = 6500 (flow rate of 200 L.h^{-1} in the pilot plant).

2. Treatment of wine: preliminary investigations

2.1. Influence of absorbance

The absorption coefficients of the four wines were measured and listed in Table 1. These values were used to calculate the penetration lengths beyond which 90 % of the UV-C light was absorbed. The results showed that penetration is extremely low in wines compared to water, especially for red wine, at 0.02 cm. Improved UV-C pilot design is therefore required to treat such absorbing liquids.

To understand the impact of absorbance on microbial inactivation, a standard load (10^6 CFU/mL) of *S. cerevisiae* was inoculated into the four different wines and treated with the coiled UV-C reactor at different UV-C doses. The results as log N (CFU/mL) versus UV-C dose (J/L) are presented in Figure 4. White and rosé wines, with an absorption coefficient of 5 and 8 cm^{-1}, respectively, required a UV-C dose of 570 J/L for total inactivation (< 10 CFU/mL). Red wine, with an absorption coefficient of 47 cm^{-1}, required a UV-C dose of 5100 J/L to achieve the same result, which is ten times higher than for white or rosé wine. These results indicate that absorbance is a major parameter that influences the efficiency of UV-C treatment, despite the improved dose delivery occurring in the coiled UV-C reactor. It is also possible to observe that the required dose was proportional to the absorption coefficient for white or rosé and red wines. However, this approximation seems not to be valid for sweet white wine, which required 2300 J/L with a quite low absorption coefficient of 10 cm^{-1}. The temperature difference between input and output never exceeded 2 °C.

TABLE 1. Absorbance and transmittance measurements for different wines before inoculation.

	Absorption coefficient (cm^{-1})	Penetration for 90 % absorption (cm)	Turbidity (NTU)
Water	0.01	100	0
White wine	5	0.2	< 1
Sweet white wine	10	0.1	< 1
Rosé wine	8	0.13	< 1
Red wine	47	0.02	< 1

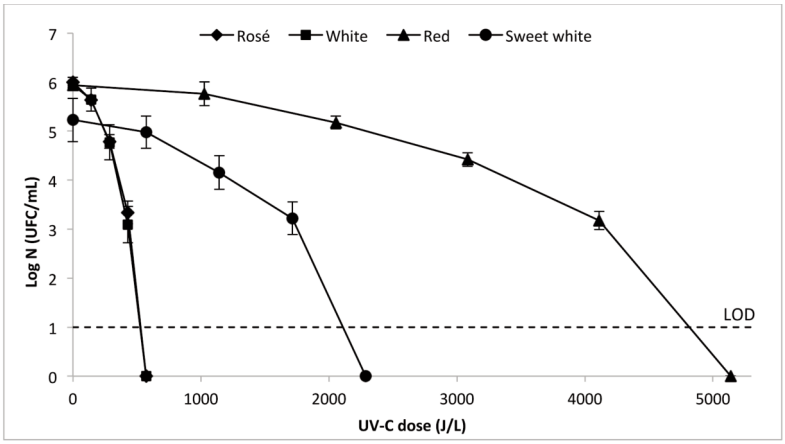

FIGURE 4. Inactivation kinetics of *S. cerevisiae* in different wines (white, rosé, red and sweet white).

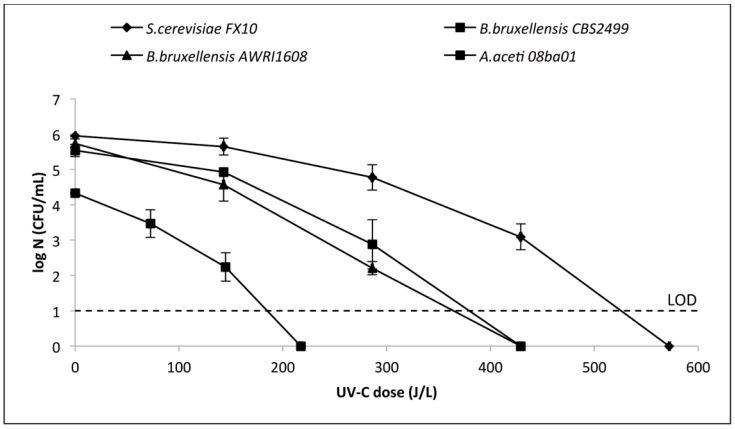

FIGURE 5. Inactivation kinetics of different strains in a white wine.

and 8 cm^{-1}, respectively, required a UV-C dose of 570 J/L for total inactivation (< 10 CFU/mL). Red wine, with an absorption coefficient of 47 cm^{-1}, required a UV-C dose of 5100 J/L to achieve the same result, which is ten times higher than for white or rosé wine. These results indicate that absorbance is a major parameter that influences the efficiency of UV-C treatment, despite the improved dose delivery occurring in the coiled UV-C reactor. It is also possible to observe that the required dose was proportional to the absorption coefficient for white or rosé and red wines. However, this approximation seems not to be valid for sweet white wine, which required 2300 J/L with a quite low absorption coefficient of 10 cm^{-1}. The temperature difference between input and output never exceeded 2 °C.

2.2. Influence of strains

In order to acquire more information about the efficiency of the coiled UV-C reactor on different strains found frequently in wines, *S. cerevisiae* FX10*, B. bruxellensis* CBS2499 and AWRI1608 and *A. aceti* 08ba01 were inoculated separately into batches of Sauvignon white wine at 10^6 CFU/mL. These strains are not necessarily all representative of the microbial population in such a white wine, but for the purpose of the experiment (i.e. to get a standard matrix for all experiments), white wine was chosen. Yeast showed higher tolerance to UV-C treatment than bacteria. The UV-C dose required to inactivate *A. aceti* (< 10 CFU/mL) was three times lower than that for *S. cerevisiae* (Figure 5). This result is in agreement with the literature (Franz *et al.*, 2009 and Guerrero-Beltran and Barbosa-Canovas, 2005). In comparison with yeast, bacterial DNA contains more thymine. As UV-C radiation targets primarily these pyrimidines, bacteria should be more vulnerable to this treatment (Fredericks *et al.*, 2011). It can also be observed that *S. cerevisiae* FX10 is more tolerant to UV-C treatment than *B. bruxellensis*. However, more information is necessary to draw definitive conclusions about strain resistance, especially when considering DNA reparation mechanisms (Sinha and Häder, 2002).

3. Integration into the winemaking process

After validating the operating conditions at lab scale, the efficiency of the coiled UV-C reactor was studied at semi-industrial scale at two stages of the vinification process: before bottling on Merlot red wine and as a mutage operation on Gros Manseng sweet white must. A total of three different treatments and one control were performed in both experiments: 870, 1740 and 2610 J/L for the red wine, 580, 1160 and 1740 J/L for the sweet must.

3.1. Microbial inactivation efficiency

Preliminary microbial counts showed that the Merlot red (37 NTU) contained a relatively high concentration of bacteria (10^6 CFU/mL) and a low concentration of yeast (3.10^2 CFU/mL). The sweet white must was in its last stage of alcoholic fermentation before mutage, thus had high turbidity (2000 NTU) and a high concentration of yeast (3.10^7 CFU/mL).

Microbial counts were performed immediately after treatment (Figures 6 and 7). The sweet white must was treated successfully and total inactivation was achieved at 1740 J/L. The total yeast count was still equal to zero 7 days later,

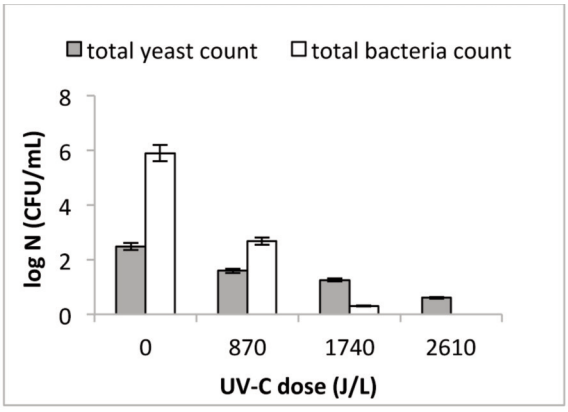

FIGURE 6. Total yeast and bacteria counts after UV-C treatments of Merlot red wine.

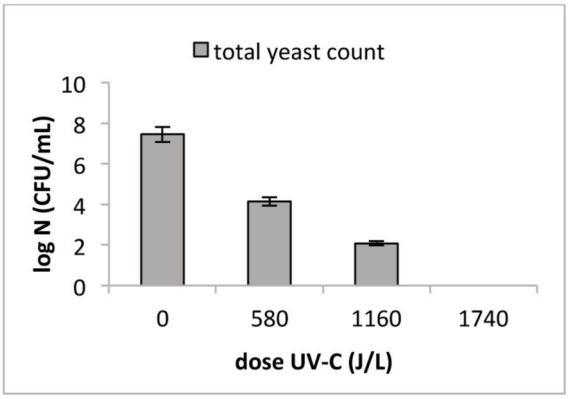

FIGURE 7. Total yeast counts after UV-C treatments of Gros Manseng sweet white must.

TABLE 2. Standard chemical analyses on sweet white must after UV-C treatments of 1160 and 1740 J/L.

	Control		UV-C 1160 J/L		UV-C 1740 J/L	
	D+11	D+90	D+11	D+90	D+11	D+90
Alcohol % vol	12.25	12.25	12.30	12.25	12.30	12.25
Glucose + fructose g/L	82		83		83	
Total acidity g/L H_2SO_4	4.9	4.7	5.0	4.8	5.0	4.7
Volatil acidity g/L H_2SO_4	0.62	0.58	0.59	0.59	0.60	0.6
pH	3.11	3.19	3.10	3.14	3.09	3.14
Free sulfur dioxyde mg/L	37	31	40	31	39	31
Total sulfur dioxide mg/L	157	146	161	146	161	148
Turbidity (NTU)	53	-	29	-	46	-
OD (280 nm) x 10	11	-	11	-	11	-
OD (420 nm)	0.158	0.106	0.142	0.097	0.141	0.096
OD (320 nm)	9.15	-	9.07	-	9.03	-

without addition of SO_2, which means that fermentation did not resume (data not shown). Turbidity seems to not play an imperative role in the treatment of sweet wines, as the UV-C dose required to inactivate yeast in this very turbid must was even lower than that previously observed in the preliminary investigations (2290 J/L with low turbidity). Bacteria in red wine were inactivated at 2610 J/L, yeasts were still present below the detection limit of 100 CFU/mL. According to previous results obtained at lab scale, the inactivation performance of the coiled UV-C reactor at semi-industrial scale showed great efficiency and a large scale of application during the winemaking process.

3.2. Chemical and sensorial analyses

Chemical and sensorial analyses were carried out over time to quantify the impact of the previous UV-C treatments on wines. Firstly, standard oenological analyses were performed after 11 and 90 days on the sweet white must and after 8 and 60 days on the red wine (Tables 2 and 3). These results did not show significant differences between the treated and untreated samples.

Six months after treatment, the color changes of the treated samples (relative to control values) were measured (Table 4) and did not show any significant differences for red or sweet white wine, if we consider the human perception threshold to be close to $\Delta E_{ab} = 2$ (Mokrzycki and Tatol, 2011).

The TPI and anthocyanin content were measured over time up to six months after treatment on red wine (Table 5). No significant differences were observed between treated and untreated samples. According to Volf *et al.* (2014), polyphenols can be degraded by UV-C treatments performed in batch mode for a few hours. The authors also

TABLE 3. Standard chemical analyses on red wine after UV-C treatments of 1740 and 2610 J/L.

	Control		UV-C 1740 J/L		UV-C 2610 J/L	
	D+8	D+60	D+8	D+60	D+8	D+60
Alcohol % vol	13.6	13.6	13.6	13.6	13.6	13.6
pH	3.84	3.85	3.84	3.91	3.84	3.92
Total acidity g/L H_2SO_4	2.70	2.90	2.70	2.70	2.70	2.70
Volatil acidity g/L H_2SO_4	0.47	0.48	0.47	0.47	0.47	0.46
Free sulfur dioxyde mg/L	27	22	25	22	24	22
Total sulfur dioxide mg/L	59	55	57	51	55	58
Turbidity (NTU)	25	59	26	80	24	40

TABLE 4. L*a*b* color delta measurements of wines 6 months after treatment (vs untreated wines).

	ΔL*	**Δa***	**Δb***	**ΔE_{ab}**
Sweet – 1160 J/L	0.27	0.46	-0.92	1.06
Sweet – 1740 J/L	0.26	0.55	-1.18	1.33
Red – 1740 J/L	-0.58	-0.59	-0.99	1.29
Red – 2610 J/L	-0.95	-1.14	-1.61	2.19

TABLE 5. Total polyphenols index (TPI) and anthocyanin content measurements in red wine up to 6 months after treatment.

	Treatment + 1 day		Treatment + 2 months		Treatment + 6 months	
	TPI	Anthocyanins (mg/L)	TPI	Anthocyanins (mg/L)	TPI	Anthocyanins (mg/L)
Control	56 ± 3	413 ± 21	52 ± 3	435 ± 22	53 ± 3	346 ± 17
Red – 1740 J/L	56 ± 3	408 ± 20	55 ± 3	416 ± 21	55 ± 3	371 ± 19
Red – 2610 J/L	57 ± 3	404 ± 20	55 ± 3	420 ± 21	54 ± 3	352 ± 18

TABLE 6. Triangle test results for UV-C treated wines vs control 20 months after treatments.

Wine	Correct answers	Result at 5 %
Sweet – 1160 J/L vs control	8/21	NS
Sweet – 1740 J/L vs control	7/21	NS
Red – 1740 J/L vs control	6/21	NS
Red – 2610 J/L vs control	11/21	NS

mentioned the higher stability of polyphenols when irradiated in a complex matrix. As wine is a complex matrix and the UV-C treatments performed in this study lasted from 2 to 15 seconds, it is normal that no differences should be observed. Six months after treatment, ethyl ester, higher alcohol acetate, aliphatic ester and aromatic ester concentrations were quantified by gas chromatography (figure 8). The UV-C treatments performed in the improved coiled UV-C reactor did not affect polyphenol or sensitive aromatic contents up to six months after treatment.

As sensorial analyses, triangle tests were performed to find whether the panelists could perceive a difference between the treated and untreated wines. Sweet white wines treated at 1160 and 1740 J/L and red wines treated at 1740 and 2610 J/L were compared to untreated controls. With 5 % tolerance, the results of these tests were negative for all the conditions mentioned above, as tested by 21 panelists of our institute, mainly researchers in oenology (Table 6). For the time being, the panelists cannot perceive a difference between UV-C treated and untreated wines up to 20 months after UV-C treatment for red wine and sweet white wine. Sensorial analysis should be continued over time.

3.3. Energy cost

The energy balance is calculated by taking into account the number of lamps and the number of passages in the reactor necessary for the total inactivation of the *S. cerevisiae* yeasts (the most resistant in this study). The electrical power absorbed was 135 W per lamp and the pump absorbs a maximum power of 1 kW. Table 7 summarizes the energy consumption calculated

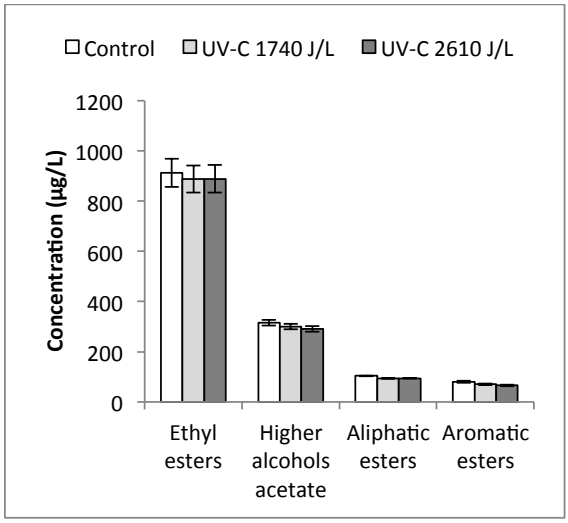

FIGURE 8. Esters quantification in red wine by GC-MS six months after UV-C treatment.

for the total microbiological stabilization of different wines using the helical UV-C reactor in comparison with flash pasteurization (industry.gov.au) and tangential filtration (vignevin.com). The microbiological stabilization using this type of reactor is very interesting because of its energy balance. Energy consumption is at least as low for red wine (0.016 kWh/L), as with other processes, and much lower for low absorbing wines such as whites and rosés with 0.007 kWh/L, which represents only 35 % of the energy consumption of a flash pasteurization. Studies are currently in progress in order to upscale the process and improve energy consumption by reducing pressure drop.

CONCLUSION

In order to reduce chemical inputs in winemaking, especially sulfur dioxide, the treatment of wines and musts by UV-C technology was investigated. Preliminary measurements confirmed that matrix absorbance plays an imperative role in the inactivation of microorganisms by UV-C light. As excessive UV-C light intensities may induce off flavors in wines containing high concentrations of riboflavin, it was necessary to optimize the UV-C doses delivered to the treated wines. In the coiled UV-C reactor, actinometric measurements showed that a high Dean number and probably turbulences increase the efficiency of mixing and therefore UV-C dose homogeneity in an absorbing liquid. Optimal operating flow parameters were identified and used later to observe a relationship of proportionality between absorbance and the dose required for total inactivation in the case of white/rosé and red wines. However, this relationship does not apply for sweet wines, probably due to the presence of residual sugars, and more investigations should be performed. As mentioned in the literature, different strains show different tolerance to UV-C treatment. For example, *A. aceti* bacteria required a UV-C dose three times smaller than that for *S. cerevisiae* and more investigations are currently being conducted. Treatments conducted at semi-industrial scale showed that chemical and sensorial analysis performed on UV-C-treated Merlot red wine (up to 2610 J/L) and Gros Manseng sweet white wine (up to 1740 J/L) over time did not show any significant differences. In conclusion, the UV-C light treatment could be successfully applied on wines and musts thanks to the coiled reactor with optimized flow parameters. Because of the small diameter of the tubes used in this small pilot, pressure drop was relatively high (meaning higher energy consumption for pumping) and improvements on the physical design of the reactor are currently in progress.

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TABLE 7. Energy cost in kWh/L for UV-C, flash pasteurization and tangential filtration stabilization processes.

	White and rosé	Sweet white	Red
UV-C pilot plant	0.007	0.01	0.016
Flash pasteurization		0.02	
Tangential flow filtration		0.01 to 0.04	

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