



Sanitisation of wine-ageing barrels using near Ultraviolet radiation

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Currently, the application of good disinfection practices can adequately control the proliferation of microorganisms in barrels, and thus prevent the alteration of wine. One of the most widely used methods is the burning of sulfur wicks, which releases sulfur dioxide (SO₂). However, its use is limited by a European Union directive³ due to the repercussions it may have on consumer health. As a result, there is a tendency for winemakers to adopt new disinfection methods that represent lower expenditure in terms of resources and greater security for both the operators and consumers⁴. Near ultraviolet radiation (UV-C) is a technique currently applied for activities such as water disinfection or the sterilisation of workspaces that have smooth surfaces. Empirical experiments conducted on the disinfection of barrels have also shown that after racking and cleaning the interior of the barrel with pressurised hot water (using equipment by Kärcher Spain) at 80 °C for 20 min, a UV-C transmitter can be used for 15 minutes to disinfect a 225 L barrel⁵. The aforementioned study lacks scientific information regarding the effects of UV-C radiation on the population of microorganisms. In the present study, the germicidal potential of UV-C radiation applied to the interior of barrels used for wine-ageing was evaluated; for this purpose, a prototype was designed by the companies E. VILA PROJECTS & SUPPLIES S.L. and AUTOMATISMOS ARAPIASA, S.L.

Material & Methods

The experiments were performed using 9 oak barrels (225 L) that were 5 to 6 years old and from different manufacturers (3 Cadus, 2 Billon, 2 Groupe Vicard, 1 Torner y 1 Damy). The barrels were randomly numbered from 1 to 9 and put into three groups of three barrels each. Six of the oak barrels were inoculated with *Brettanomyces bruxellensis* using 20 L of water with a final concentration of 1x10⁶ CFU/mL (previously grown on liquid YPD up to 1x10⁸ CFU/mL). This water was distributed within the barrel by periodical shaking and rotating for the next 7 days. The other 3 barrels were kept as uninoculated controls. Then, the barrels were emptied and sampled for microbiological analysis. The sampling of culturable yeasts and bacteria in the barrels inner surface was carried out by introducing 20 L of water at each of the 9 barrels. After thoroughly shaking and rotating the barrels, a sample of 1,5 L of water was taken using a vacuum pump, sterile tubes and kitasato flasks. Finally, dilutions of these samples were plated by triplicate on different culture media (YPD agar medium for total yeasts, lysin agar medium for non-*Saccharomyces* yeasts and GYC agar medium for acetic acid bacteria⁶). In each experiment,

The ageing of wine in oak barrels represents a critical stage in winemaking at which product alteration can occur due to bacteria, yeasts or fungal growth. The control of microorganisms, such as yeasts of the *Brettanomyces* genus¹ and acetic acid bacteria², is of great importance, since the alterations that they can cause in wine can be very serious and cause great economic losses.

the sampling was carried out before and after each of the following treatments:

➔Treatment 1: Application of pressurised hot water (150 °C, 75 bars, 1 min, Kärcher HDS)

➔Treatment 2: Application of UV-C radiation using an ozone-free UV-C lamp with amalgam emission, (253,7 nm, 130W). Prototype designed by the companies E. Vila Projects & Supplies S.L. and Automatismos Araipiasa, S.L.

➔Combination of Treatments 1 & 2.

Results

Effect of the treatment on the reduction of the total yeast population in barrels

The treatments applied to each group of barrels were the following: application of pressurised hot water for 1 min (Treatment 1), application of UV-C radiation for 10 min (Treatment 2), a combination of Treatments 1 & 2, hot water at pressure for 1 min and subsequent treatment with UV-C radiation for 10 min (Figure 1). In Figure 2 the effects of separate or combined treatments are shown. All treatments induced a significant reduction (an average of 1.46 log₁₀ CFU/mL) in the total yeast populations before and after treatment. However, when a second UV-C treatment of 10 min was applied after the water treatment, the total population in yeast decreased and was significantly different from the first result (p < 0.05 according to the Fisher LSD Test) (Figure 2). The results for CFU in the LYS medium were not significantly different from those in YPD, revealing that the detected yeasts were probably non-*Saccharomyces* yeasts.

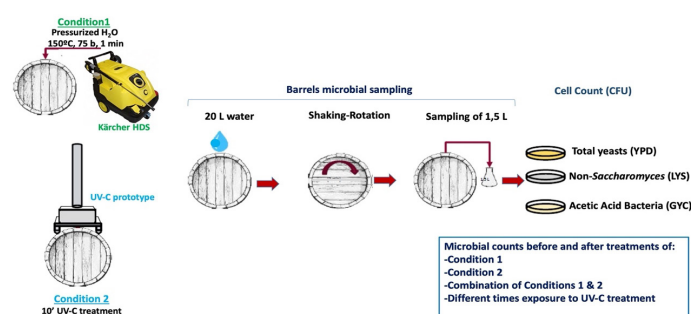


FIGURE 1. Experimental design.

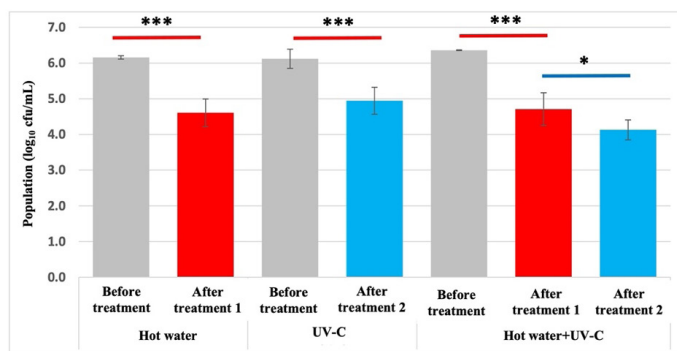


FIGURE 2. Effect of treatment on the reduction of total yeast populations. Treatment 1, Pressurized hot water, 1 min; Treatment 2, UV-C 10 min. *, $p < 0.05$; ***, $p < 0.0001$ (Fisher, LSD).

In order to confirm these results, a replicate of this experiment was carried out two weeks later, in which the treatment was applied randomly to the barrels (Table 1). Once again, the results confirmed the effectiveness of the pressurized hot water treatment and of the usefulness of UV-C radiation treatment as a disinfection complement.

TABLE 1. Effect of treatments on the reduction of total yeast populations ($\log_{10}$ CFU/mL).

	Pressurised hot water treatment	UV-C (10 min) treatment	Combined treatments
Before treatment	5.78	6.05	6.34
After treatment 1	3.36 ¹	4.98 ¹	4.17 ¹
After treatment 2	-	-	3.56 ²
Significant differences	$p^1 < 0.0001$	$p^1 < 0.0001$	$p^1 < 0.0001$ $p^2 = 0.064$

¹ Significant difference between the first treatment and the initial value before treatment;

² Significant difference between the second and first treatment.

The results obtained for the plates containing GYC medium for the enumeration of acetic acid bacteria (Figure 3), show a decrease of $2.49 \pm 1.04 \log_{10}$ CFU/mL in the treatment with pressurized hot water, $1.17 \pm 0.18 \log_{10}$ CFU/mL in the UV-C radiation treatment and $3.69 \pm 0.21 \log_{10}$ CFU/mL in the combined treatments. This confirms the ability of the combined treatment to control the proliferation of these bacteria.

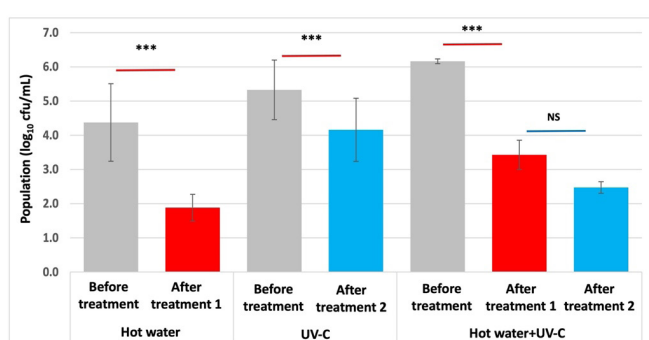


FIGURE 3. Effect of the treatment on the reduction of the populations of acetic acid bacteria. Treatment 1, pressurized hot water for 1 min; Treatment 2, UV-C for 10 min. NS, Not significant; ***, $p < 0.001$ (Fisher, LSD).

Effect of duration of UV-C radiation on the reduction of the microorganism population

In this experiment, the effect of duration of exposure to UV-C radiation was studied as a complement to the previous treatment of pressurized hot water. After receiving the latter treatment for 1 min all the barrels were treated with UV-C radiation for 2.5, 5 and 10 min in triplicate.

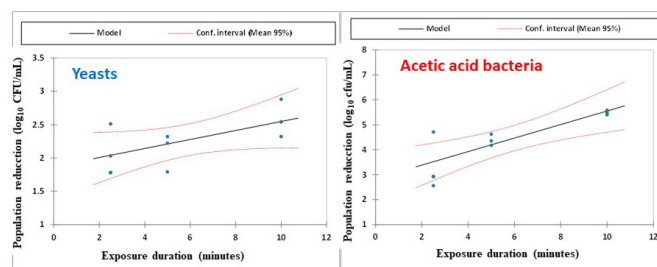


FIGURE 4. Effects of treatment duration on the reduction of populations of yeasts and acetic bacteria. Pressurized hot water treatment, 1 min and UV-C, 2.5, 5 and 10 min. Yeasts: no significant effect of treatment duration ($p = 0.075$); Acetic bacteria: there is a significant effect of treatment duration ($p = 0.006$).

The data of the counts in yeasts and acetic acid bacteria are shown in Figure 4. It can be seen that similar results were obtained for the exposure durations of 2.5 and 5 min, with approximately a $2.11 \log_{10}$ decrease in the yeast population. On the other hand, a higher exposure duration (10 min) resulted in an increase in the effectiveness of the treatment, with a $2.58 \log_{10}$ decrease in the population. This represents a yeast population reduction of approximately 50 % in logarithmic units and more than 99 % of the initial population in CFU/mL. However, the differences in the effects produced by the different treatment durations are not statistically significant.

Regarding the populations of acetic acid bacteria, we were able to observe a significantly positive effect of UV-C treatment time on the decrease in viable cells: from $3.40 \log_{10}$ CFU/mL to $5.49 \log_{10}$ CFU/mL for durations of between 2.5 and 10 min respectively (Figure 4).

Conclusions

Our results show that it is possible to use UV-C radiation to disinfect barrels for a maximum of 10 min after a 1 min treatment with pressurized hot water. This combined treatment allows an effective level of disinfection to be obtained within a short period of time, providing benefits such as a reduction in energy consumption, water consumption and exposure to work-related risks (compared to previous literature describing similar population reduction for 30 min of each individual treatment¹). Our study also shows that the populations of yeasts or of acetic acid bacteria are strongly reduced by the combined treatment. ■

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