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Study of High Power Ultrasound for Oak Wood Barrel Regeneration: Impact on Wood Properties and Sanitation Effect

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Abstract: This study aims to investigate the ability of high power ultrasound (HPU) to ensure oak barrel sterilization and wood structure preservation. Optimization was performed in terms of temperature and time and the impact of the HPU process on the porous material was also characterized. In this research, several wood characteristics were considered, such as the specific surface area, hydrophobicity, oxygen desorption and spoilage microorganisms after treatment. The study showed that the microbial stabilization could be obtained with HPU 60 °C/6 min. The results obtained show that microorganisms are impacted up to a depth of 9 mm, with a *Brettanomyces bruxellensis* population < 1 log CFU/g. The operating parameters used during the HPU treatment can also impact on wood exchange surface and oxygen desorption kinetics indicating that tartrate is removed. Indeed, the total oxygen desorption rate was recovered after HPU treatment, close to a new oak barrel, and thus may indicate that there is no impact on the ultrastructure (vessel, pore size or rays). Finally, wood wettability can also be impacted, depending on the temperature and the duration of exposure.

Keywords: high power ultrasound; wine aging; regeneration; sanitation; *brettanomyces*; oak wood barrel

1. Introduction

Aging red wines can be carried out in barrels to allow olfactory and gustatory modifications [1–4]. However, when aging takes place in wooden barrels, some organoleptic deviations may appear referred to as "brett flavor", referring to "stable", "leather", "manure" or "horse sweat". Bacteria and yeasts can penetrate into the wood to the same depth as the wine (close to 8 mm) [5] and then contaminate other wines if the oak barrels are not sterilized properly [6], even if polyphenols have a negative impact on bacteria viability [7]. *Brettanomyces* can provide organoleptic deviations due to production of undesirable compounds, such as 4-ethylphenol and 4-ethylgaiacol [8]. These molecules can deteriorate the wine quality [9]. To avoid such deviations, several treatments can be employed in wineries. However, Yap et al. [10] have argued that hot water and chemical treatments (the two treatments most commonly used) may be ineffective against the *Brettanomyces* spoilage yeast [11,12]. Several types of treatments are used in wineries to sanitize barrels, such as chemical agents (sulfur dioxide, ozone) or physical agents, with UV radiation, hot water, microwaves and ultrasounds showing varying levels of efficiency [5,11,13]. For example, microwave treatment enables only a 35% reduction in the *Brettanomyces* spp. population [14]. Guzzon et al. [13] studied the efficacy of aqueous steam, UV irradiation, gaseous, and aqueous O_3 for sterilizing oak barrels. Steam and O_3 were demonstrated to be the most effective treatments, eliminating as much as 90% of yeasts. Nevertheless, in this case, total sanitation is not completely ensured because the treatment is only carried out on the stave surface. Due to the porous nature of wood and the limited transmittance of UV radiation, this process appears to be ineffective. With regards to the steam treatment, some authors have shown that the microorganism inhibition is linked to the wood depth. A reduction of 3 log can be observed for depths of less than 3 mm, and 2 log if the depth is between 3 and 6 mm [15]. None of these processes are therefore completely effective and sulfur dioxide in wines should be managed carefully to avoid contamination.

Studies of the use of high power ultrasound (HPU) in industrial processes has recently been published [16,17]. In this process, electrical energy can be converted into ultrasound (20 kHz–10 MHz) at frequencies higher than those audible by the human ear (16–20 kHz). High power ultrasound is characterized by intensities in excess of 1 W/cm^2 and frequencies between 20 and 100 kHz [18–20]. When they are emitted in a liquid, this process forms high-energy micro bubbles (acoustic cavitation phenomenon) [21–23]. These cavitation bubbles generate very high temperatures locally (close to 80 °C) and pressures greater than 50 MPa [19]. These micro cavitation bubbles (diameters around 1 µm) act homogeneously throughout the fluid (Pascal's law) and can penetrate deeply into the pores of the wood. Piyasena et al. [24] showed that the cavitation phenomenon generated by HPU can kill microbial cells by cell disintegration in many cases [24]. The effect of ultrasound on the growth and viability of pathogenic bacteria such as *Escherichia coli, Pseudomonas fluorescens*, several yeast like *Saccharomyces cerevisiae*, *Brettanomyces,* as well as various fungi, algae and protozoa, has been summarized by Jiranek et al. [25]. These authors consider that the rate of inactivation of microorganisms varies with the power [26–28] and frequency [29] of the ultrasound applied. Thus, according to Tsukamoto et al. [28], the wave amplitude has a large influence on the inactivation rate for *S. cerevisiae* cells. According to Borthwick et al. [29], cell disruption in this species is greater at high frequencies (267 kHz compared to 20 kHz for the same exposure time). In addition, HPU and thermal treatments (45–60 °C) have synergistic effects on the inactivation of microorganisms [30].

The potential application of HPU technology in the wine industry has also been assessed by Yap et al. [10] and Jiranek et al. [25]. These authors argued that this process could be used for the management of microorganisms at different stages of winemaking. Yap et al. [31] studied the effect of HPU on wood barrels and noticed that HPU removes *Brettanomyces* spp. on the barrel surface and in the stave up to a depth of 4 mm.

Validation tests carried out in 2007 and 2008 by the Australian Wine Research Institute (AWRI) demonstrated that the cleaning and disinfection of barrels (American oak) using HPU technology was more efficient than with steam in conventional conditions of use (1000 psi/6900 kPa at 60 °C for 5 min). In this research, the HPU not only removed all the deposited tartrate, but also inhibited 100% of *Brettanomyces* cells on the surface and up to 4 mm deep in the wood [31]. However, microorganisms can be located deeper in the oak wood (close to 8 mm) and the impact of this treatment on the oak wood structure was not apprehended. Moreover, there were no optimization parameters for the HPU treatment in this study.

Recently, Porter et al. [32] studied the effect of HPU treatment on porous cleaning efficacy in American oak wine barrels using X-ray tomography. It was demonstrated that HPU can significantly remove tartrate deposits from the first two millimeters of oak surfaces, but this was not reproducible at a depth of 2–8 mm. An average of 89% total tartrate volume was removed from the surface layer in the first treatment, but this was further increased to 98% by increasing temperature and time treatment. A highly significant removal of stave surface tartrate crystals was also demonstrated with this cleaning technique at the temperatures studied. Only a few studies considered the oak wood characteristics after HPU treatment, even if oak is a fragile matrix. Oak wood consists of macromolecules such as cellulose, hemicellulose and extractive compounds like ellagitannins, lignin and aromatic precursors. Cellulose and hemicellulose are complex components in the cell wall of wood and make up a large part of oak wood composition (more than 50%). Hemicellulose is formed from covalent bonds with lignin (close to 30% of the total composition) and ester linkage with acetyl units and hydroxycinamic acids.

These bonds are important to ensure the mechanical stability of the structure. These bonds limit the extraction of hemicellulose from the cell wall matrix. The application of ultrasound in extraction and refining processes has drawn increasing attention recently for several applications, and these studies prove that HPU could have a significant impact on cell wall structure [33]. Furthermore, ultrasonic treatment is well established in the processing of plant raw materials, in particular, in extracting low molecular weight substances and pharmaceutically active compounds [34,35]. The authors also consider that HPU could be used to extract cellulose from several plant materials and this aspect is essential for our work. The mechanochemical effect of ultrasound is believed to accelerate the extraction of organic compounds from plant materials due to the disruption of cell walls.

The innovative point of this study is the characterization of the porous material by evaluating the specific surface, oxygen desorption rate, and hydrophobicity of the oak wood after treatment. These parameters are very important as a modification of the oak wood structure could induce several modifications (wettability, oxygen desorption). Indeed, wine might penetrate deeper if the specific surface area and hydrophobicity are changed. Thus, microorganisms could also penetrate further into the depth of the wood and oxygen desorption could be more important. These measurements could ensure that no modifications of the oak wood structure (on the surface and at a depth) are induced by HPU.

The aim of this work was to study the effect of different operating conditions (temperature and time) for HPU treatment on wood properties and sanitation effect. The study investigated the impact of high power ultrasound (HPU) on (i) the specific surface (B.E.T method); (ii) the oxygen desorption kinetics contained in the wood; (iii) the oak wood hydrophobicity (contact angle) and (iv) spoilage microorganism (*Brettanomyces bruxellensis*) removal. All these indications could provide insights into oak wood structure and sanitation possibilities up to 9 mm. Operating parameter optimization was performed in the first part and the microbial stabilization was carried out on with the optimized HPU parameters and the classical barrel treatment (steam) in the second part.

2. Materials and Methods

2.1. Barrel Treatment

2.1.1. High Power Ultrasound (HPU)

The HPU treatment process consisted in filling the barrel with water (heated to 40, 60 or 80 °C by an autonomous system) and then to inserting the sonotrode (Dyogéna, Blanquefort, France) (part that emits the ultrasound waves) into the bung hole (Figure 1), thereby allowing pressurization of the water inside the barrel (0.3 bar). HPU was emitted inside the barrel (frequency 20 kHz, 3.8 kW). The experimental barrel used for the HPU treatment was made in order to place and maintain the experimental staves inside the barrel (Figure 2). The hatch of the experimental barrel was closed for water filling.

HPU experiments were carried out to characterize the influence of operating conditions: temperature and time.

Figure 1. High power ultrasound (HPU) apparatus.

Figure 2. Experimental barrel for HPU treatment.

2.1.2. Aqueous Steam Treatment

The treatment by aqueous steam was carried out with an autonomous boiler Barriclean® (Bouyoud Distribution, Brive-la-gaillarde, France) supplying pressurized hot water during 10 min (1.1 bar, 110 °C) inside the barrel. Steam modalities were only used to compare results for microbiological aspects.

2.2. Lab Experimental Setup and Operating Conditions

The operating conditions investigated for HPU were processing time (4, 6 or 8 min) and temperature (40, 60 or 80 °C). The staves for testing were extracted from French oak barrels with a medium toast used during two years. The study was carried out on 10 types of stave in triplicate, after undergoing various HPU treatments. The control staves were untreated. All the operating conditions are summarized in Table 1. The staves were then cut in different ways to obtain the appropriate sample size for each analysis. All the experiments were carried out in triplicate.

Table 1. Operating condition testing and experimental design.

Treatment Temperature (°C)	Treatment Time (min)	Liquid Used for Treatment	HPU Power (kW)
40	4	water	3.8
	6		
	8		
60	4		
	6		
	8		
80	4		
	6		
	8		
No treatment			

2.3. Oak Wood Characterization

2.3.1. Determination of Specific Surface

Knowledge of the specific surface of a wood sample (from a stave) is an important parameter to appreciate the exchange surface between the wood and wine. The specific surface refers to the real area of an object, as opposed to its apparent surface area. This is estimated from the amount of nitrogen adsorbed in relation to its pressure at the boiling point of liquid nitrogen and at normal atmospheric pressure.

The SA 3100 BET (Beckman Coulter, Brea, CA, USA) measures the specific surface of granulated samples via gas adsorption using the Brunauer-Emmett-Teller (BET) method. In this so-called discrete method, the data points obtained and the gas pressures are balanced before the reading is recorded. The volume of adsorbed gas retained by the sample is calculated from the pressures recorded at each measuring point.

2.3.2. Oxygen Desorption Staves

Several 500 mL flasks (Schott®) were connected to a vacuum system generating a negative pressure of 0.2 bars. This negative pressure is comparable to that observed in the barrel. In the barrel, the 225 L of wine are in contact with the 2 m^2 of the internal surface of the barrel. In order to simulate the real conditions, the volume surface ratio was respected by introducing an exchange surface of 36.3 cm^2 of wood into each bottle filled with the model solution (12% ethanol v/v, 5 g/L tartaric acid and pH 3.5), made inert beforehand with nitrogen. The external surfaces of the two oak pieces (7.25 cm $\times$ 2.5 cm) were covered with silicone gel (Elastosil E43), with the exception of the inner side (toasted side) to reproduce the real conditions in an oak barrel. The inert model solution was changed every 6 days. This methodology was used to avoid the oxygen consumption of the released oak wood polyphenols [36]. Preliminary tests were realized to ensure that no oxygen transfer could occur through the silicone gel. The oxygen desorption kinetics of the wood pieces were monitored every day for 1 month. The oxygen concentration in the liquid flask was detected using a mobile optical fiber coupled with a sensor device. The luminescent system was of the Oxy-trace type (PreSens GmbH, Germany), coupled with a PSt3 type oxygen sensor (detection limit = 15 µg/L, 0–100% oxygen). The spot sensors were placed inside the liquid flask allowing detection of the oxygen concentration.

2.3.3. Contact Angle Measurement

The sessile drop contact angle measurement technique seeks to determine the wettability of the staves studied by characterizing the ease of a liquid drop spreading over a solid surface. We characterized here the hydrophobic/hydrophilic nature of the material. The experimental device used was a Digidrop Contact Angle Meter (GBX Scientific Instruments, Ireland). A 20 µL water droplet was placed on the stave and the contact angle was measured after stabilization for around 200 s. The contact angle is defined as the angle between the solid surface and a tangent, drawn on the drop surface, passing through the atmosphere–liquid–solid triple point (Figure 3) [37]. An image system analysis integrates the angle as a function of time and makes it possible to determine whether the drop enters the material.

For each modality studied, the contact angles were calculated from measurements on three pieces of stave from three different parts of each stave. The drop was systematically in a place where there were no apparent residues of tartrate, to avoid any experimental artefacts. For each experimental modality, the average contact angle was thus obtained from 9 individual measurements on three different samples.

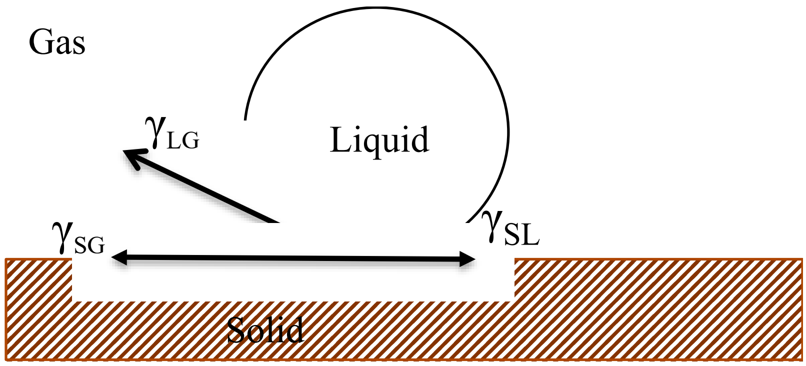

Figure 3. Schematic example of contact angle measurement.

2.4. Impact on Microorganisms

The impact of HPU on spoilage microorganisms, especially *Brettanomyces bruxellensis*, was investigated according to their depth in the wood.

The staves of French oak wood barrels of one or two years (medium toast) were incubated in a liquid culture of YPG (Yeast extract 10 g/L; bactopeptone 10 g/L; glucose 20 g/L; adjusted to pH 5), supplemented with antibiotics in order to limit the growth of bacteria, molds and yeast of the *Saccharomyces* genus (0.1 g/L chloramphenicol; 0.15 g/L biphenyl; 0.5 g/L cycloheximide), and containing *B. bruxellensis* L0539 (available through the "Centre de Ressources Biologiques Œnologiques" of Bordeaux University (CRBO)) in mid-exponential phase during 4 days at room temperature. The population was determined before and after treatment by drilling staves to different depths (0–2 mm; 2–5 mm; 5–9 mm) with a small drill bit and permits recovering 0.2 g of wood.

The wood samples recovered at different depths were incubated in 2 mL of sterile saline solution (9 g/L sodium chloride) during 48 h at room temperature under agitation. Serial dilutions of these samples were plated on solid YPG (Yeast extract 10 g/L; bactopeptone 10 g/L; glucose 20 g/L; agar 20 g/L; adjusted to pH 5) supplemented with antibiotics (0.1 g/L chloramphenicol; 0.15 g/L biphenyl; 0.5 g/L cycloheximide). Yeast populations in the primary dilution were monitored by fluorescence microscopy.

Colonies were counted after 7 days of incubation at 30 °C. All assays were performed in triplicate.

2.5. Statistical Analyses

Statistical data were analyzed using the Kruskal–Wallis non-parametric test (RStudio software, v1.0.143, RStudio Inc., Boston, USA, http://www.rstudio.com/) to identify the means that were significantly different. The statistically significant level was 5% ($p < 0.05$).

3. Results and Discussion

3.1. Specific Surface Area

The objective of this measurement is to characterize the specific surface area, which could have an impact on the liquid/solid exchange surface. The specific surface area of a wood sample (from a stave) could be defined as the total surface area of a material per unit of mass and has a particular importance for adsorption phenomena.

The specific surface area measured by the BET method for the different test modalities tested is presented in Figure 4. The specific surface area increases regardless of the HPU treatment modalities compared to the control (from 900% to 1400%). There is no effect of the time treatment on the specific surface contrarily to temperature. Indeed, at high temperature (80 °C), we notice that the specific surface area was around 2 m^2/g, which is significantly lower than 40 °C and 60 °C. These low values compared to other temperatures should indicate a wood modification of HPU treatment above 60 °C. This deterioration of wood integrity could involve a degradation of lignin, cellulose and hemicellulose [38]. Moreover the increase in the specific area could also indicate that tartrate is effectively removed from the wood structure during HPU treatment, especially for 40 °C and 60 °C, while remaining present in the untreated controlled staves. Figure 5 illustrates an example of tartrate removal from HPU treatment at 60 °C. However, the surface tartrate is more efficiently removed at 60 °C and 80 °C, instead of 40 °C. These results corroborate those of Porter et al. [32], which showed 98% of total tartrate volume removed with HPU treatment (4 KW, 12 min at 40–60 °C).

Ultrasound treatment clearly modifies the physiochemical structure of wood because we notice significant differences between all the HPU modalities and the control. These considerations were also observed by He et al. [38]. These authors considered that HPU could decrease the alkali metals in the resulting material, and significantly increase its crystallinity. It has been observed in the case of eucalyptus, and our results indicate that the same trend is obtained for oak wood. The authors considered that a rupture appears between the methyl/methylene groups in cellulose and contributes to removing cellulose, hemicellulose and lignin.

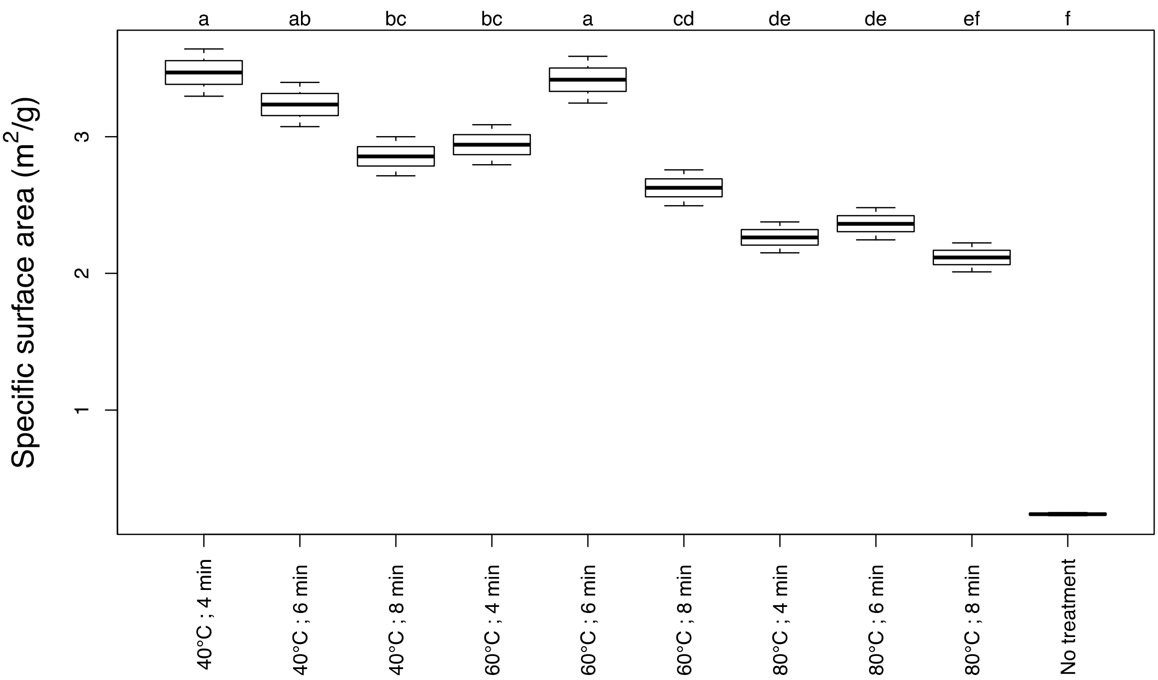

Figure 4. Specific surface of wood treated by HPU.

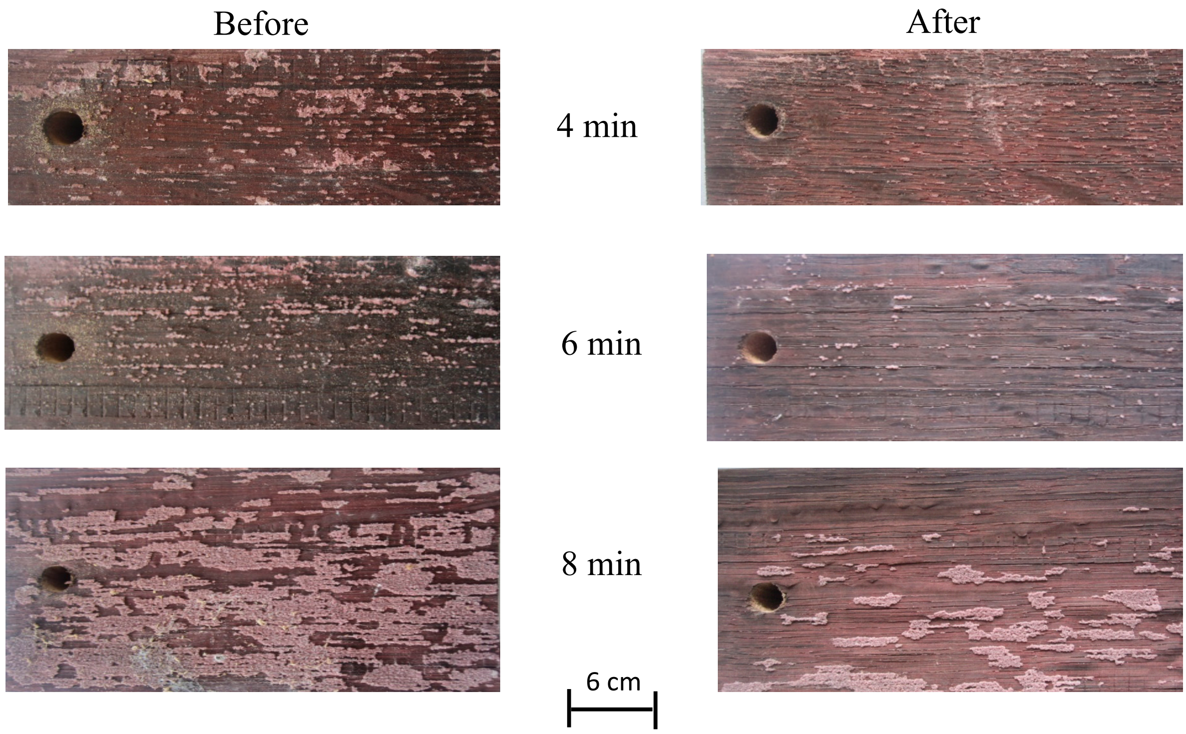

Figure 5. Examples of tartrate removal before and after HPU treatment (60 °C).

HPU also apparently increases the exposure of the material to the treatment solution and enhances its accessibility, as well as breaking down pits, which could generate collapses and micro channels, and removing attachments on the wood tissue. This aspect will have an impact on oxygen desorption and should be considered to validate our results. Finally, further investigation should be made on the specific surface deeper in the wood stave.

3.2. Oxygen Desorption of Staves

The influence of the HPU treatment on the quantity of oxygen desorbed and its kinetics was investigated with a specific vacuum system. The O_2 desorption monitoring was carried out over 26 days for each treatment considered in triplicate. The results for HPU treatment at 60 °C are presented in Figure 6.

We noticed that untreated staves desorbed less oxygen over the first 6 days. We could consider in this case that tartrate is still present in the oak wood vessels and thus could limit the oxygen desorption kinetic. For HPU treatment, in the first 6 days we noticed that O_2 desorption is higher for 8 min than for 6 min at 60 °C. Then, over the next 6 days, we observed that desorption of HPU 6 min was slightly higher than HPU 8 min, with a variation close to 0.5 mg/L. We can see in Table 2 that there was no difference in total oxygen transfer over 26 days for HPU treatment between 6 and 8 min. On the other hand, the total oxygen concentration desorbed was 5.57 ± 1.25 mg/L for untreated stave, which was significantly lower than HPU. In comparison to results obtained by Qiu et al. [39], the O_2 desorption rate for wood barrel during the first month was lower in this case (close to 10 mg/L for a new oak wood [36]). Considering other HPU temperatures (40 °C and 80 °C), the same trend could be observed (results not shown).

Our results indicate that oxygen desorption is highly impacted by HPU treatment. In the case of untreated oak wood, the oxygen desorption rate was two times lower, as was kinetics, especially for the first week. After HPU treatment, the sum was close to 10 mg/L indicating a significant variation. These values are still similar to an unused oak wood, which indicates that the oak structure is not impacted. The hypothesis of the appearance of micro channels was not verified here for the HPU treatment at 60 °C. Oak wood ultrastructure seems to be conserved.

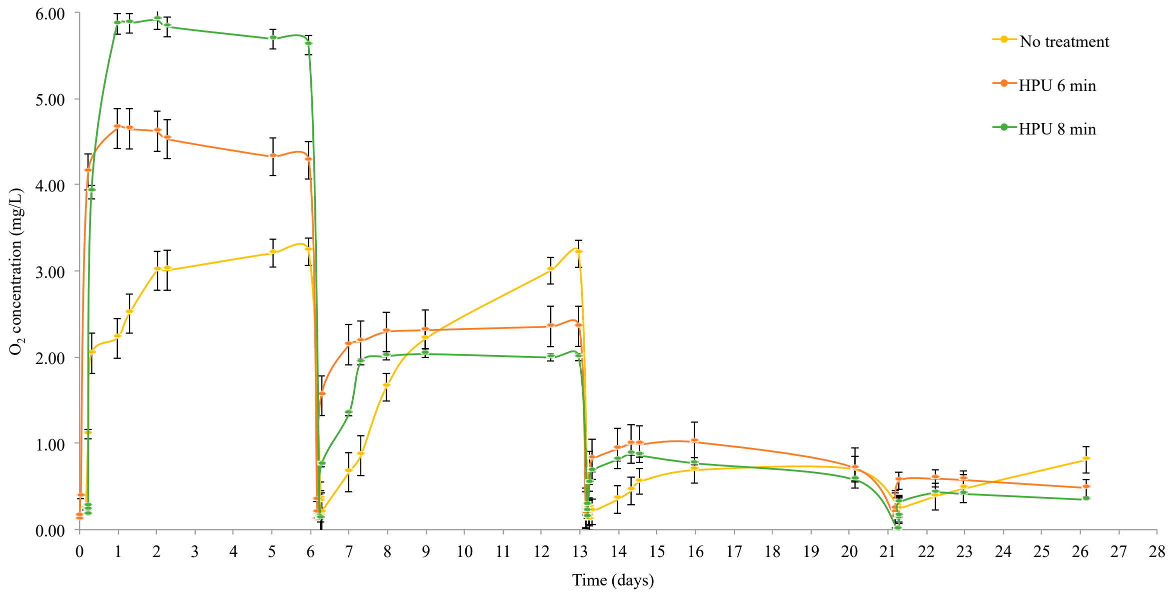

Figure 6. Influence of HPU treatment on O_2 desorption.

Table 2. Total oxygen desorption for different type of treatment over 26 days. The total oxygen concentration is the sum of each mean oxygen value during each 6-day period previously shown in Figure 6.

Type of Treatment	Total Oxygen Concentration (mg/L)
No treatment	5.57 ± 1.05 [a]
HPU 60 °C 6 min	8.11 ± 1.15 [b]
HPU 60 °C 8 min	8.63 ± 0.43 [b]

Subscript letter refers to the significant differences between the types of treatment (p = 0.05).

3.3. Contact Angle

The purpose of this analysis was to characterize the hydrophobic/hydrophilic nature of the contact surface. The contact angle could indicate the modifications that may possibly occur at the structural level of the staves after HPU treatment. By definition, the smaller the contact angle, the higher the hydrophobicity, which could have an impact on the absorption rate.

The contact angle measurements for HPU treatments are presented in Figure 7. We could consider that the duration of treatment does not seem to have a decisive influence on the hydrophobic nature of the wood surface. However, we noticed a trend in the HPU effect with the global increase in contact angle values. In the other types of treatment, no significant difference was observed compared to the control, although we did notice an upward trend in the contact angle. These results suggest that wood samples are more hydrophobic and significant differences are observed for some cases (80 °C and 40 °C/6 min). These differences are possibly related to lignin removal, the presence of hemicelluloses or other carbohydrate material and extractives at the fiber surface. These results are similar to those obtained by [33]. In our case, our results suggest that, HPU treatments and, more especially, high temperature (80 °C), could induce some modification because the contact angle is significantly different (higher than 50° for each case). The results obtained are in good agreement with those observed for the main wood components, reported by Young [40]. The authors proved the wettability of wood pulp fibers where hardwood lignin (kraft) has a contact angle of 60° and cellulose 33–34°. The authors consider that surfaces rich in lignin and extractives have higher contact angles, and the values obtained in this case were close to 60°. These values therefore indicate a greater proportion of extractives and lignin in the surface of oak wood, which is a more hydrophobic surface. The potential of ultrasound to extract polysaccharide components has been widely studied in different plants and plant tissues and this phenomenon is confirmed in our study. Ultrasounds are known to be a powerful tool for accelerating polysaccharide extraction. In our case, the extraction seems to be effective because the hydrophobic characteristics are increased in the case of HPU treatment at high temperature. This could indicate that polysaccharide content is also increased on the surface and their desorption will be higher. This parameter is essential because it will impact the oak wood wettability. In our case, the use of HPU generally led to an increase in the hydrophobicity of the wood (from 125% to 350%). Even if the link between wettability and O_2 desorption kinetics exists from a theoretical point of view, it is difficult to extrapolate in our case and additional experiments should be conducted to validate this hypothesis.

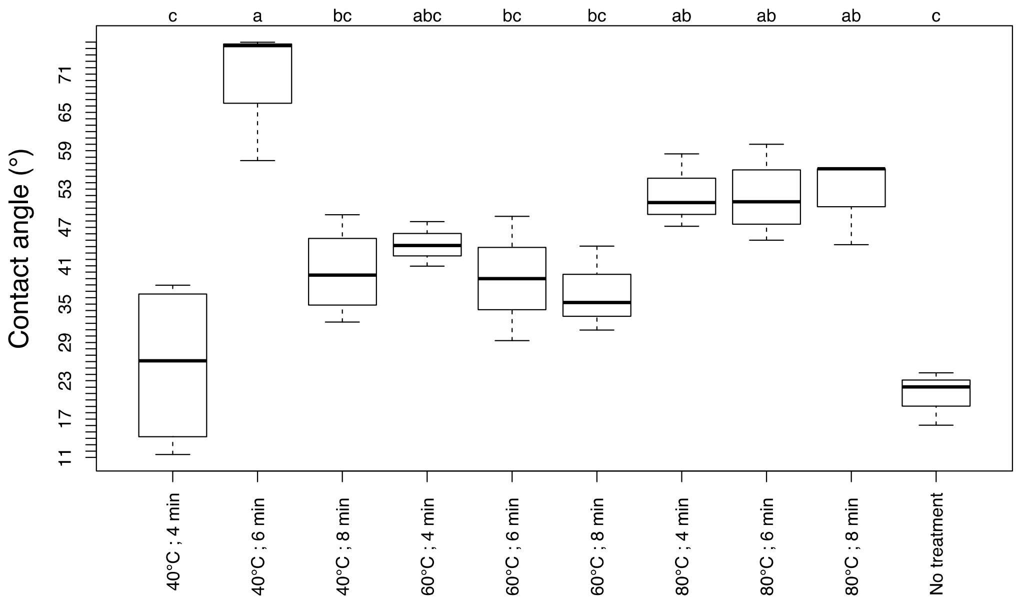

Figure 7. Influence of HPU treatments on wood hydrophobicity by contact angle measurements.

3.4. Sanitation Effect on Spoilage Microorganism B. Bruxellensis

Sanitation effects of two types of treatment (HPU 6 min/60 °C and steam 10 min/110 °C) were investigated for the removal of the spoilage microorganism *B. bruxellensis.* Staves inoculated with *B. bruxellensis* were treated with HPU and different depth samples were recovered in order to estimate the effectiveness of the in-depth treatment as we can see in Table 3.

If we focus on HPU treatment (6 min/60 °C), we notice that the post-treatment population of *B. bruxellensis* is lower than the detection limit (1 log CFU/g) to a depth close to 9 mm. This length represents the maximum depth reached by the wine by passive diffusion in the wood during aging and therefore corresponds to the maximum depth reached by *Brettanomyces* yeast. This efficiency is probably due to the synergistic effect between the HPU treatment, especially cavitation bubbles, and the thermal effect of the water at 60 °C brought into contact with yeast nested in the wood. On the other hand, we consider that steam treatment is less efficient because the post-treatment population of *B. bruxellensis* is unchanged from 2 mm to 9 mm. The depth efficiency for steam treatment is 2 mm with a population lower than the detection limit. We could consider that these results are due to the thermal inertia of the wood. During steam treatment, the first millimeters of the wood reach a temperature close to 100 °C, allowing elimination of *Brettanomyces* yeasts, whereas the temperature only reaches 45 °C at 5 mm. We can also see that there is no difference in the treatment response (HPU or steam) between staves of 1 or 2 years. Yap et al. [31] have studied the effect of HPU on wood barrels and noticed that HPU removed *Brettanomyces* spp. on the barrel surface and in the stave up to

4 mm, but they did not investigate to further depths. In comparison to another innovative treatment, González-Arenzana et al. [14] studied the microwaves capacity to sterilize French oak wood barrels and they were able to remove 35% of the population for *Brettanomyces* spp., 36% of total yeast, 90% of lactic acid bacteria and 100% of acetic bacteria up to a depth 8 mm [14]. In our case with optimized operating parameters, the HPU sanitation effect reaches a depth of 9 mm in the wood, which is also the depth to which the wine and therefore *Brettanomyces* yeast can penetrate.

According to these results, the barrels contaminated by *B. bruxellensis* can be reused if they are treated with HPU (3.8 kW, 6 min at 60 °C), unlike steam treatment that leaves viable *B. bruxellensis* cells in the depth of the wood, which can quickly become sources of recontamination. The HPU treatment is expected to target cells that are located deep within the pores of the staves, which would otherwise be untreated by classical barrel treatments.

Table 3. *B. bruxellensis* population before and after HPU or steam treatment on staves of one and two years at different sampling depth.

Stave Age (year)	Type of Treatment	Sampling Depth (mm)	*B. bruxellensis* Population before Treatment (log CFU/g)	*B. bruxellensis* Population Post Treatment (log CFU/g)
1	HPU 6 min 60 °C	0–2	7.73 ± 0.02	<DL
		2–5	5.89 ± 0.04	<DL
		5–9	4.23 ± 0.01	<DL
	Steam 10 min 110 °C	0–2	7.79 ± 0.05	<DL
		2–5	5.71 ± 0.03	4.92 ± 0.04
		5–9	4.63 ± 0.02	4.59 ± 0.03
2	HPU 6 min 60 °C	0–2	8.11 ± 0.04	<DL
		2–5	6.08 ± 0.02	<DL
		5–9	5.61 ± 0.01	<DL
	Steam 10 min 110 °C	0–2	7.91 ± 0.05	<DL
		2–5	6.82 ± 0.03	5.96 ± 0.02
		5–9	5.71 ± 0.02	5.63 ± 0.03

<DL: Detection limit (1 log CFU/g).

4. Conclusions

This study has shown that the combined effect of HPU and heat treatment may have an impact on wood sanitation, the wettability of wood, its specific surface and oxygen transfer kinetics. The operating parameters used during the HPU treatment are essential.

Specific surface measurement seems to be a relevant method for determining the tartrate removal efficiency of wood. This method has good repeatability and is not influenced by the heterogeneity of the wood surface. Concerning the hydrophobicity of the wood, we have shown that HPU could increase the contact angle, especially at high temperatures (80 °C). Thus, the HPU treatment enables the initial oxygen transfer capacity of the wood to be partially recovered particularly because of the surface tartrate removal. Nevertheless, we could go further in the experiments by extending to different wood origin and types of barrels (age, toast, wine, wood grain) or by investigating the ultrastructure of the wood.

Finally, the sanitation effect of HPU was investigated and permits removal of all viable *B. bruxellensis* cells up to a depth 9 mm with processing parameters set at 60 °C/6 min with 3.8 kW. These parameters (60 °C; 6 min) are the most efficient in regards to all of these issues.

Author Contributions: P.R. and R.G. conceived the experimental design; F.M. performed the contact angle, specific surface and oxygen desorption experiment; P.R. carried out the HPU and steam treatment, as well as the microbiology test; P.R. and R.G. supervised the experiments; P.R. and M.B. processed the results; M.B. wrote the paper: R.G., P.R. and F.M. reviewed the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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