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Plasma Activated Water for wine barrels disinfection

Ana Sainz-García [a], Ana González-Marcos [a], Ignacio Muro-Fraguas [a], Rodolfo Múgica-Vidal [a], Félix Gallarta-González [b], Lucía González-Arenzana [c], Isabel López-Alfaro [c], Pilar Santamaría [c], Rocío Escribano-Viana [c], Fernando Alba-Elías [a,*], Elisa Sainz-García [a]

[a] Department of Mechanical Engineering. University of La Rioja, C/ San José de Calasanz 31, 26004, Logroño, La Rioja, Spain
[b] Department of Chemistry. University of La Rioja, C/ Madre de Dios 51, 26006, Logroño, La Rioja, Spain
[c] Institute of Grapevine and Wine Sciences (Government of La Rioja, University of La Rioja, CSIC), Finca La Grajera, Ctra. De Burgos, LO-20, Km. 6, Salida 13, 26007, Logroño, Spain

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ABSTRACT

Barrel aging is crucial for the production of high-quality wines, with barrel reuse playing a key role in this process. Therefore, disinfecting and cleaning barrels are vital to prevent health and safety issues, being *B. bruxellensis* one of the most extended problems. In this study, naturally contaminated oak barrels with *B. bruxellensis* were immersed during 3 h in four Plasma Activated Water (PAW) generated for 1.5 min, 5 min, 15 min and 30 min. The presence of secondary radicals (OH•, NO•, NO_2•) was observed after HPLC and spectrometry analysis. The results suggested that those reactive species played an important role in the inactivation of *B. bruxellensis*. PAW_5 (generated during 5 min), which achieved a reduction of 3.49 ± 0.83 logarithmic units in *B. bruxellensis* population, was chosen as the best one in terms of economic and time consumption. Thus, an ecofriendly, sustainable and inexpensive solution was presented to inactivate *B. bruxellensis* from wine barrels.

1. Introduction

According to experts, the Spanish wine market has increased by almost 5% during the past years (Español, 2022). This fact has caused the demand for quality wines to increase, thus motivating the improvement of manufacturing conditions, food security and safety in the industry.

Aging is one of the most important steps when producing high quality wines. The use of oak wood barrels gives personality to the specific wine. Broadly speaking, during aging different processes and reactions take place giving physical and chemical stability to wines. Furthermore, since the complexity and delicacy of wines are reached, their personal aromas and taste are enriched (González-Arenzana et al., 2019). Regarding aging barrels, a differentiation between new and used ones should be done. Although, in the first case, malolactic fermentation and changes in tannins, pigments and polysaccharides are promoted, used barrels develop the volatile acidity of wines (Tomás, 2016, pp. 2013–2014). It is also worth mentioning some aspects derived from the aging of wine, such as the economic costs involved in the investment in new barrels, their limited lifespan or their cleaning (García-Alcaraz et al., 2020).

Despite the fact that barrel reusing is a recommendable practice, it is essential to be vigilant about the microbiological contamination that could arise in wooden barrels. The porous structure of wood facilitates the penetration of wine up to 8 mm, thus causing microbial contamination in crevices and cracks of the staves by different types of microorganisms such as lactic acid bacteria (LAB), acetic acid bacteria (AAB) and *Brettanomyces bruxellensis* (*B. bruxellensis*) (Bartowsky & Henschke, 2008; Costantini, Cersosimo, Del Prete, & Garcia-Moruno, 2006; Suárez, Suárez-Lepe, Morata, & Calderón, 2007). Furthermore, the latter is identified as the major cause of yeast contamination in wines and has increased in the last years because of changes in winemaking practices including the increment of sugar in wines, the decrease of sulfiting and filtering processes and the long-time aging inside barrels (Alston, Fuller, Lapsley, & Soleas, 2018; Mira de Orduña, 2010). During the International Wine Challenge of 2008, it was determined in 16% the total wine

* Corresponding author.
E-mail addresses: ana.sainz@unirioja.es (A. Sainz-García), ana.gonzalez@unirioja.es (A. González-Marcos), ignacio.muro@unirioja.es (I. Muro-Fraguas), rodolfo.mugica@unirioja.es (R. Múgica-Vidal), felix.gallarta@unirioja.es (F. Gallarta-González), lucia.gonzalez@unirioja.es (L. González-Arenzana), isabel.lopez@unirioja.es (I. López-Alfaro), psantamaria@larioja.org (P. Santamaría), roescrv@unirioja.es (R. Escribano-Viana), fernando.alba@unirioja.es (F. Alba-Elías), elisa.sainzg@unirioja.es (E. Sainz-García).

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faults. However, it is difficult to estimate the total litters of discarded wine due to *B. bruxellensis* spoilage (Compounds, 2008).

One of the most prominent outcomes associated with that yeast is the so-called "Brett" character, including off-aromas such as wet wool, burn plastic, horse sweat, medicinal and mousy (Licker, Acree, & Henick-Kling, 1998). This yeast is difficult to eliminate due to its ability of resisting the characteristics of wine such as low pH and oxygen, assimilable nitrogen concentrations and high ethanol concentrations (Curtin, Varela, & Borneman, 2015).

Regarding the most commonly used methods for cleaning and disinfecting wine barrels, a review carried out by Velasco (2012) encompasses some of them. They can be categorized in two groups: chemical and physical. Within the first one, sulfur dioxide (known as sulfuring) is the most widespread method and it is available in liquid, gas or solid pills. Since there is a controversy over the use of sulfuring, the European Commission proposed the prohibition of this technique (Europea, 1998); however, it is still legal. Nonetheless, in order to prevent negative sensorial characteristics in wines (residual sulfites, undesirable compounds generation or unfavorable interactions with wood barrels) and health problems among workers (allergic, skin and respiratory diseases), there is a need of looking for alternatives. They include the use of oxidizing agents (chlorinate oxidants), hydrogen peroxide, sodium percarbonate, permanganate and ozone. Among the physical treatments, hot water (80–90 °C) is the most popular technique. Additionally, water vapor at 105 °C, electromagnetic treatment by microwaves, ultrasounds, dry ice and negative oxygen are employed (Velasco, 2012). Despite the fact that these methods are widely used in wineries, they have some disadvantages: [1] potential alteration of wood composition; [2] low thermal conductivity of wood necessitating long treatment times; [3] high cost and time consumption; [4] final products that may be toxic to consumers and [5] inadequate penetration through the wood resulting in treatments that are only superficially effective for cleaning and disinfecting barrels (Costantini et al., 2006; Velasco, 2012).

Plasma Activated Water (PAW) is generated when Atmospheric Pressure Cold Plasma (APCP) transfers energy and some chemical reactivity from gas to water (Zhou et al., 2020). This technology has been recognized as one of the most sustainable and economic technologies, since PAW is generated just with water and electricity and any waste is produced. PAW has been proven to be useful, obtaining good results in several applications like the inactivation of microorganisms, the improvement of seed germination or the treatment of cancer cells, among others (Zhou et al., 2020). Despite the presence of reactive species (e.g., positive and negative electrons, molecules and ultraviolet photons or neutral and excited atoms) the potential applications of PAW are attributed to the activity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Iuchi et al., 2018; Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015).

Considering all the above, PAW could be a solution for wine barrels disinfection, enabling their reuse without sanitary problems. Therefore, the aim of the present study was to verify the effectiveness of PAW technology to inactivate *Brettanomyces bruxellensis* in naturally contaminated wine barrels and to propose this technology as a sustainable and efficient alternative to the technologies that are currently employed for cleaning and disinfecting wine barrels.

2. Materials and methods

2.1. Staves samples

Staves were obtained from oak wood barrels employed in the production of aged wine that were naturally contaminated with *B. bruxellensis*. The samples of this study consisted in portions of 5 × 5 cm that were cut from the staves. In order to determine the level of *B. bruxellensis* viable population in the wood, three portions of staves were analyzed following the protocols described in section 2.4.

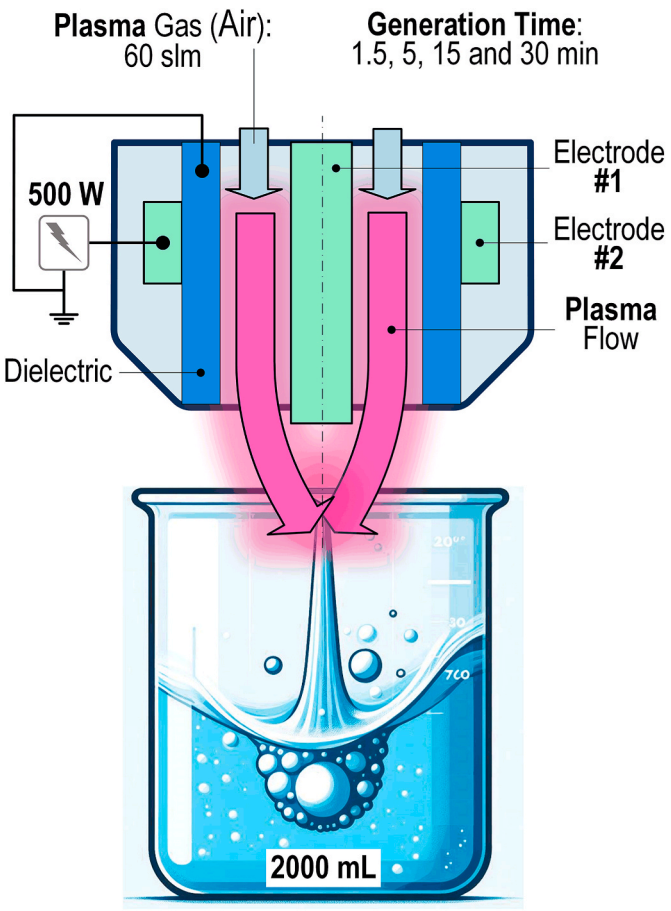

Fig. 1. PAW generation system and detail of the plasma-water interaction.

2.2. Preparation of PAW solutions

The APCP equipment employed in this study was the PlasmaSpot500 (Molecular Plasma Group, Foetz, Luxemburg). It comprises two cylindrical electrodes with an aluminum oxide dielectric tube between them. The internal electrode is grounded and the external one is connected to a high voltage source.

PAW was produced by exposing 2000 mL of purified water (PW) to the plasma jet, as shown in Fig. 1. Four different PAW were generated varying the generation time: 1.5, 5, 15 and 30 min, resulting in samples labeled as PAW_1.5, PAW_5, PAW_15 and PAW_30, respectively. For all treatments, air at 60 slm was used as plasma gas and the plasma power was set at 500 W. The distance between the PW surface and the end of the plasma nozzle remained consistently at 30 mm.

The four PAW used in this study were previously investigated in Sainz-García et al. (2023) for chemical decontamination, specifically to decompose TCA from corks. On the other hand, the present work examines the effectiveness of those PAW to decontaminate oak barrels from *B. bruxellensis*.

2.3. Characterization of physicochemical properties of PAW

The physicochemical parameters of PAW at room temperature were measured after PAW generation using different methods, as described in Sainz-García et al. (2023). A portable multimeter sensION MM150 DL with a 50 48 probe (Hach Company, United States of America) was used for oxidation-reduction potential (ORP), electrical conductivity (EC) and pH measurements. For nitrate (NO_3^-) quantification a portable Imacimus® MultiIon analyser (NTsensors S.L., Spain) was required. Nitrite (NO_2^-) concentrations were measured through colorimetric

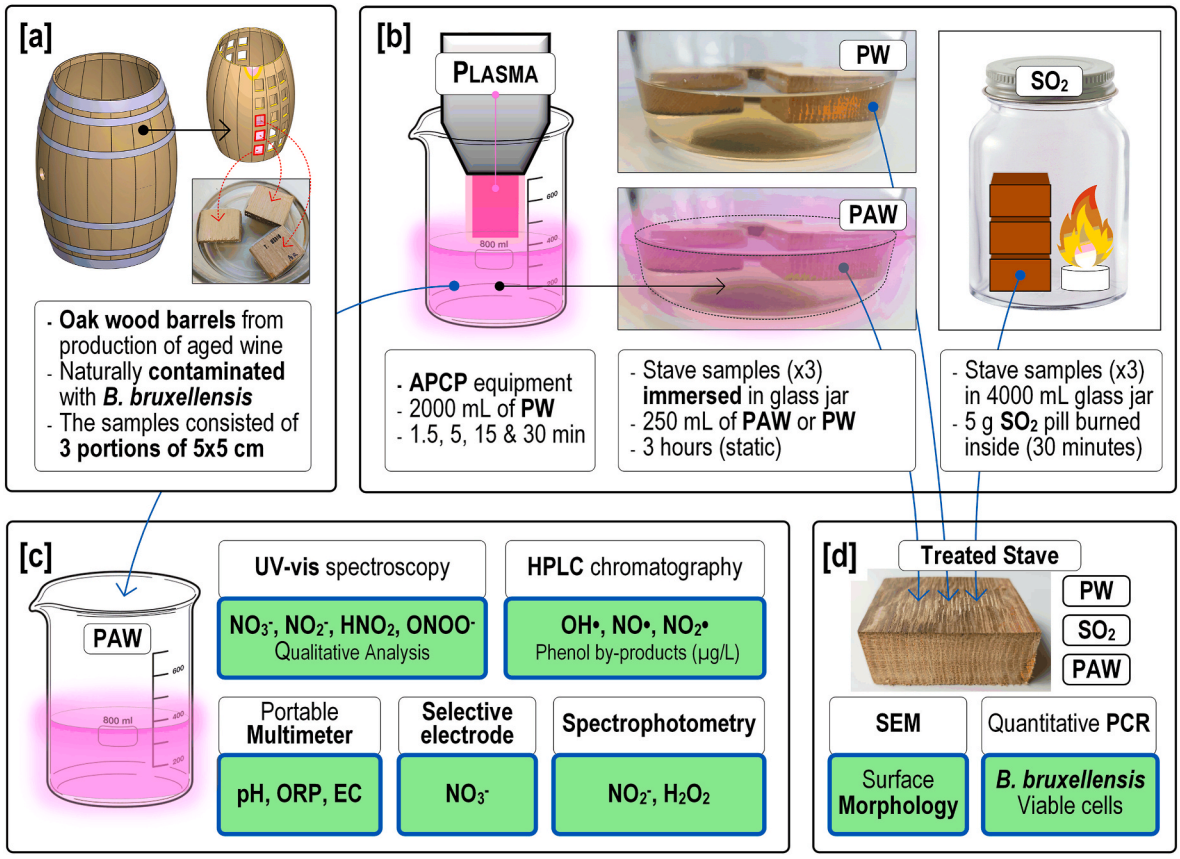

Fig. 2. Scheme of the samples treatment process: [a] Wood samples from oak barrels, [b] PAW generation and samples decontamination with PW, PAW and SO_2 and [c] and [d] PAW and sample characterization.

Griess assay by spectrophotometry. This technique is based on determining nitrites after their reaction with sulfanilic acid in low pH forming diazonium ion. This ion combines with α-naphthylamine to form a magenta dye which can be identified and quantified at 548 nm (Jablonowski & von Woedtke, 2015). Hydrogen peroxide (H_2O_2) concentration was determined by spectrophotometry at 407 nm measuring the absorbance of titanium peroxide. For all photometric measurements, an Onda V-11 SCAN spectrophotometer (Giorgio Bormac s.r.l., Italy) was used. For these tests, Titanium (IV) oxysulfate and Griess reagent were purchased from Sigma Aldrich (USA).

In order to confirm the presence of secondary reactive oxygen and nitrogen species (RONS), HPLC analyses were performed. This method was based on the reaction between OH•, NO• and NO_2• with phenol (C_6H_5-OH) (Lukes, Dolezalova, Sisrova, & Clupek, 2014). Specifically, OH• reacts with phenol to produce benzoquinone, NO• with phenol generates 4-nitrosophenol, and the reaction of NO_2• with phenol yields 2-nitrophenol. The concentrations of the phenol by-products were determined using a HPLC system with UV detection (Agilent 1100 Series, Agilent Technologies, Spain). The analysis was performed following the procedure explained in Sainz-García et al. (2023). Phenol degradation by-products were quantified using calibration curves prepared from known concentrations of benzoquinone (Sigma Aldrich, USA) ($2.05{\cdot}10^{-4}$ M), 4-nitrosophenol (TCI Chemicals, Japan) ($1.52{\cdot}10^{-3}$ M) and 2-nitrophenol (Sigma Aldrich, USA) ($2.11{\cdot}10^{-3}$ M). Typical calibration curves are: benzoquinone: A (mAU) = $0.09 + 6.99{\cdot}10^{5}$ c (M) (from $2{\cdot}10^{-6}$ to $4{\cdot}10^{-5}$ M); 2-nitrophenol: A (mAU) = $-0.24 + 1.50{\cdot}10^{5}$ c (M) (from $2{\cdot}10^{-5}$ to $4{\cdot}10^{-4}$ M); 4-nitrosophenol: A (mAU) = $-1.00 + 3.78{\cdot}10^{5}$ c (M) (from $1{\cdot}10^{-5}$ to $3{\cdot}10^{-4}$ M).

Moreover, UV–vis spectroscopy (Agilent 8453 UV–visible Spectrophotometer) was used to identify the presence of other reactive species in PAW. Particularly, the range from 280 nm to 400 nm was studied. Within this wavelength range, it is possible to identify species such as nitrates, nitrites, nitrous acid or peroxynitrites.

2.4. Plasma activated water and control treatments

For PAW and PW treatments, three contaminated samples were immersed in 500 mL of each liquid during 3 h (Fig. 2[b]). Regarding sulfur dioxide (SO_2) treatment, a 4000 mL glass jar was filled with three contaminated samples. Then, a 5 g sulfur pill was burned inside and finally, the jar was closed during 30 min (Fig. 2[b]). SO_2 and PW treatments were used as controls.

2.5. Quantification of the viable B. bruxellensis population

The initial average contamination level of *B. bruxellensis* for the naturally contaminated barrels was 4.35 ± 0.26 logarithmic units of viable cells per gram of wood.

Once the treatments with PAW, PW and SO_2 were carried out, the samples were brushed with an automatic wood planer reaching a depth of 1 cm. The chips from each treatment were collected in sterile plastic bags and their weight was recorded. Subsequently, 600 mL of sterile Trypticasein Soy Broth (TSB, Conda, Madrid, Spain) recovering medium was added and the sealed bags were incubated at 28 °C in an orbital shaker at 100 rpm for 24 h. After incubation, the liquid was recovered and centrifuged (4 °C, 30 min, 10000 g). The pellet obtained was resuspended in Ringer's solution making up to a volume of 15 ml in sterile plastic tubes.

In order to quantify the viable population of *B. bruxellensis* present in wood, the samples obtained were sent for analysis to the Excell Ibérica laboratories (Logroño, Spain). Viable *B. bruxellensis* cells were analyzed by quantitative PCR with Eva Green®. Previously, samples were treated

Table 1
Physicochemical properties of PAW and PW.

Sample	PW	PAW_1.5	PAW_5	PAW_15	PAW_30
pH	7.00 ± 0.05	4.46 ± 0.15	4.01 ± 0.17	3.58 ± 0.09	3.10 ± 0.12
Electrical Conductivity (μS/cm)	2 ± 0.5	25 ± 5	52 ± 7	103 ± 13	190 ± 15
Oxidation Reduction Potential (mV)	250 ± 10	315 ± 13	355 ± 10	383 ± 9	408 ± 21
Nitrates (mg/L)	0.00	3.67 ± 0.31	4.13 ± 0.15	9.90 ± 1.30	17.90 ± 2.05
Nitrites (mg/L)	0.00	0.74 ± 0.05	2.58 ± 0.27	3.47 ± 0.13	5.01 ± 0.87

with propidium monoazide (PMA™, Biotium, Fremont, CA) and subjected to a protocol for DNA extraction.

2.6. Morphological characterization

A COXEM EM-30N Scanning Electron Microscope (SEM) operating at 10 kV was used to analyze the surface morphology of the treated and untreated samples. For this purpose, portions of 0.5 × 0.5 cm were obtained from the 5 × 5 cm wood samples. Sample surfaces were coated with a thin layer of gold using a plasma sputtering apparatus before SEM examination to make them conductive.

2.7. Statistical analysis

Experiments were conducted in triplicate. An ANOVA was performed to determine if the reduction of *B. bruxellensis* among the various treatments and the control exhibited significant differences. If the results of this test indicated statistically significant differences, post hoc comparisons were conducted using Fisher's LSD test between each pair of groups to identify specific treatment variations. The significance level (alpha) was set at 0.05. All analyses were executed using Statgraphics Centurion software (v19, The Plains, USA).

3. Results and discussion

3.1. PAW characterization

The physicochemical attributes of the different PAW are shown in Table 1.

Regarding pH, after 1.5 min of plasma exposure, the pH of PAW_1.5 decreased from 7.00 to 4.46 ± 0.15 and further declined to 3.10 ± 0.12 after 30 min of treatment (PAW_30). On the other hand, a progressive linear rise in EC was noted when increasing the generation time. Thereby, EC rose from 5 μS/cm to 25 ± 5 μS/cm after 1.5 min of treatment and further increased to 190 ± 15 μS/cm after 30 min. The same trend was observed for ORP measurements, increasing from 315 ± 13 mV to 408 ± 21 mV for 1.5 and 30 min of PAW generation time, respectively. Regarding the chemical analysis of nitrates and nitrites, there was a significant rise in the concentration of both species with the plasma activation time. Consequently, the concentration values increased achieving 17.90 ± 2.05 mg/L (NO_3-) and 5.01 ± 0.87 mg/L (NO_2-) for PAW_30. H_2O_2 was also measured, however, it was not detected regardless the PAW studied. It is hypothesized that when an excess of NO_2- is present, H_2O_2 undergoes complete reaction to generate peroxynitrite.

The changes in the values of all of those parameters are mostly due to the formation of active ions and oxidizing species (e.g. NO_2-, H+, NO_3-) during the generation of PAW.

All of these facts are in good agreement with the work done by Rathore, Patel, Butani, & Nema (2021) where significant raises in EC and ORP, as well as a pH reduction, were observed as the plasma activation time increased (Rathore et al., 2021). Furthermore, similar findings have been reached by other researchers (El Shaer et al., 2020; Pan et al., 2017; Zhang et al., 2016).

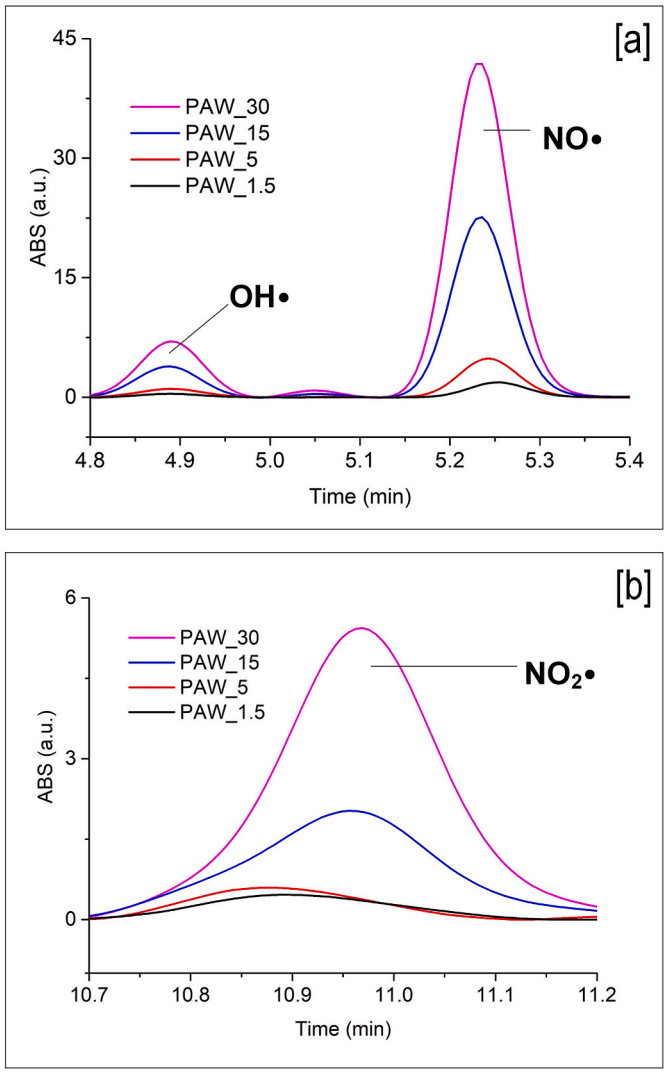

Fig. 3. HPLC chromatogram for each PAW: [a] OH• and NO• peaks; [b] NO_2• peak.

Table 2
Quantification of phenol by-products.

Phenol by-product concentration (μg/L)	PAW			
	PAW_1.5	PAW_5	PAW_15	PAW_30
OH• (benzoquinone)	8.2	23.1	91.7	168.1
NO• (4-nitrosophenol)	227.0	465.1	1873.0	3404.8
NO_2• (2-nitrophenol)	209.5	253.9	696.1	1729.6

In order to ascertain if PAW contained other secondary reactive species, HPLC analyses were conducted. The presence of OH•, NO• and NO_2• was evaluated through the detection of the phenol degradation by-products. Fig. 3 shows the HPLC chromatograms for each PAW.

Table 2 presents the phenol by-products concentrations for each PAW analyzed. These concentrations give an idea about the values of the reactive species that react with phenol to generate each phenol by-product (OH•, NO• and NO_2•). Regarding benzoquinone, it was reached 8.2 μg/L in PAW_1.5, increasing up to 168.1 μg/L in PAW_30. On the other hand, the concentration of 4-nitrosophenol was 227.0 μg/L in PAW_1.5 in comparison to the 3404.8 μg/L in PAW_30. Finally, the 2-

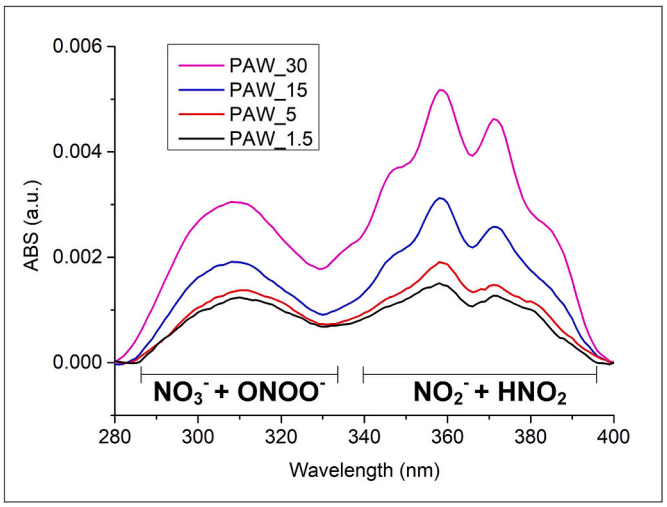

Fig. 4. UV–vis spectrum of each PAW.

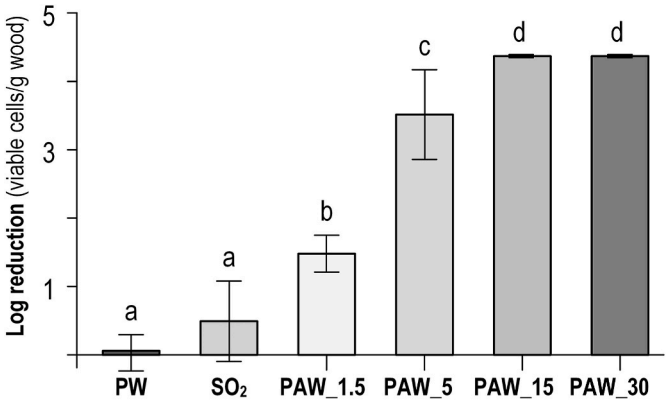

Fig. 5. Reduction of *B. bruxellensis* in wood samples after 3 h of contact with PW, SO_2 and PAW treatments. Different letters indicate statistically significant differences ($p < 0.05$).

nitrophenol concentrations ranged from 209.5 μg/L to 1729.6 μg/L when the plasma activation time increased from 1.5 to 30 min. In this respect, some authors argue that these radicals may play a key role in bacteria inactivation, but very few researchers have worked on the detection and quantification of these reactive species in PAW. For instance, Tarabová et al. (2018) found phenol by-products of these three radicals after HPLC analysis suggesting an evidence of the presence of OH•, NO• and NO_2•(Tarabová et al., 2018). Moreover, Akiyama and Heller (2017) and Lukes et al. (2014) suggested some cyclic reactions through which these three radicals are generated:

$$NO_2^- + H^+ \leftrightarrow HNO_2 \quad [a]$$

$$2\,HNO_2 \rightarrow \mathbf{NO}\bullet + \mathbf{NO_2}\bullet + H_2O \quad [b]$$

$$2\,\mathbf{NO_2}\bullet + H_2O \rightarrow NO_3^- + NO_2^- + 2\,H^+ \quad [c]$$

$$4\,\mathbf{NO}\bullet + O_2 + 2\,H_2O \rightarrow 4\,NO_2^- + 4\,H^+ \quad [d]$$

$$4\,\mathbf{NO_2}\bullet + O_2 + 2\,H_2O \rightarrow 4\,NO_3^- + 4\,H^+ \quad [e]$$

$$NO_2^- + H_2O_2 + H^+ \rightarrow O{=}NOOH + H_2O \quad [f]$$

$$O{=}NOOH \rightarrow \mathbf{OH}\bullet + \mathbf{NO_2}\bullet \quad [g]$$

Thus, NO_2- are unstable at low pH and react with HNO_2 via reaction [b] generating nitric oxide radical (NO•) and nitrogen dioxide radical (NO_2•). A hydrolysis of NO_2• takes part to produce NO_2- and NO_3- (reaction [c]). Moreover, the secondary radicals NO• and NO_2•, which are known as "acidified nitrites" (Machala et al., 2013), can react with dissolved oxygen to form NO_2^- and NO_3^-, respectively (reactions [d] and [e]). Finally, via reaction [g] peroxynitrite is transformed into OH• and NO_2•.

Moreover, UV–vis spectroscopy was used to identify the presence of other reactive species in PAW. Specifically, the range from 280 nm to 400 nm was studied. Fig. 4 shows the spectrum of each PAW analyzed. Thus, the spectrum of PAW_30 showed the highest values of absorbance, followed by PAW_15, PAW_5 and PAW_1.5. It is worth mentioning the characteristic group of five peaks between 330 nm and 395 nm that several researchers have associated with an overlapping that is caused by HNO_2 and NO_2^- (Jung et al., 2015; Ki et al., 2020; K. Liu, Liu, & Ran, 2020; Methods, 2009; Yost & Joshi, 2015). These peaks could be an indication of the presence of "acidified nitrites" as reactions [a] and [b] show. The additional overlap occurring at around 302 nm is also noteworthy and involves the presence of NO_3^- and peroxynitrite (Brisset & Pawlat, 2016; K. Liu et al., 2020).

3.2. *Brettanomyces bruxellensis* inactivation by PAW

Fig. 5 illustrates the reduction of the *B. bruxellensis* population after 3 h immersed in each PAW and control (PW and SO_2). Almost no reduction was observed in any of the controls used in this study (PW or SO_2 treatment). However, the population of *B. bruxellensis* was significantly reduced in the samples treated with PAW (statistically significant differences were found between each PAW treatment and each control). Specifically, a reduction of 1.46 ± 0.27 logarithmic units was achieved with PAW_1.5, whereas 3.49 ± 0.83 log reductions were achieved with PAW_5, and total inactivation (4.35 ± 0.00 log) was accomplished with PAW generated during 15 and 30 min. Regarding the PAW treatments, statistically significant differences were found between them, except in the case of PAW_15 and PAW_30. This fact is in good agreement with Guo et al. (2017) who observed higher *S. cerevisiae* inactivation from grapes when using PAW generated during the longest times of their study (30 and 60 min). Thus, 0.38 ± 0.17 and 0.53 ± 0.07 log CFU/mL reductions were achieved after 30 min of PAW/yeast contact. Tian et al. (2017) also obtained higher yeast reductions after their longest treatment time suggesting that the efficacy of PAW for microbial inactivation could be dependent on the PAW generation time.

Therefore, the physicochemical characteristics of each PAW played a key role to understand why the response of microorganisms was not the same with all PAW. As mentioned above, the longer the treatment time, the higher the OH•, NO• and NO_2• concentrations. Those reactive species, as well as peroxynitrite, are known to have strong antimicrobial activity (Akiyama & Heller, 2017; Bao, Lu, He, & Liu, 2016; Machala et al., 2013). They are capable of triggering oxidation and nitration reactions in biological cells such as peroxidation of lipids, proteins and DNA damage (Lukes et al., 2014; Shen et al., 2016; Thirumdas et al., 2018; Van Gils, Hofmann, Boekema, Brandenburg, & Bruggeman, 2013; Yost & Joshi, 2015). In this sense, other researchers suggested yeast death as a consequence of RONS affecting the oxidation-reduction state of antioxidants and causing membrane damage and cell structure disruption (Guo et al., 2017; Tian et al., 2017). Moreover, intrinsic ROS have been shown to increase after PAW treatment inside yeast cells provoking an over-accumulation that can cause oxidative stress and cell death (X. Liu et al., 2021; R. Zhang et al., 2020). Taken together, a possible pathway which ends up in cell death was suggested. The mechanism could be started by a lipid peroxidation in the cell membrane, followed by a rupture and damage of the cell membrane resulting in a leakage; then RONS could easily enter the cell and accumulate provoking a potential membrane shock and finally resulting in cell death (X. Liu et al., 2021).

There are other authors who studied the use of PAW for killing yeasts. Ryu et al. (2013), compared PAW with saline solution and observed their highest cell damage when treating *S. cerevisiae* with PAW

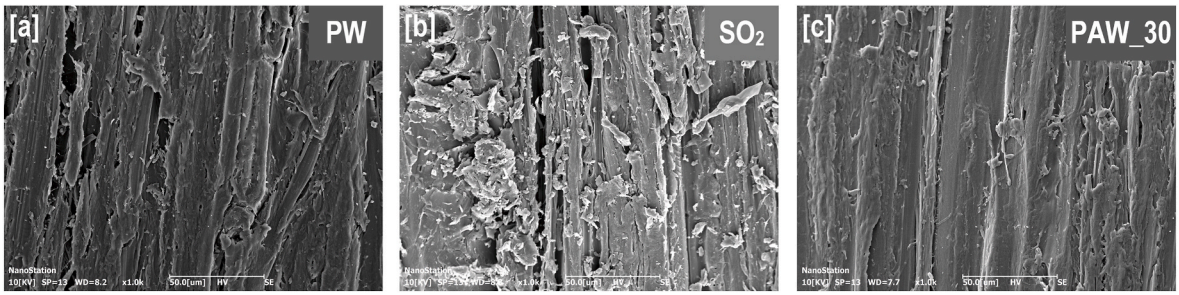

Fig. 6. SEM images of samples after: [a] PW treatment, [b] SO_2 treatment and [c] PAW_30 treatment.

during 120 s, achieving an inactivation of $2{\cdot}10^7$ cells/mL. Since PAW was the one with the top levels of reactive species, those authors suggested a crucial role of OH•. Moreover, 5.85 log CFU/g of *S. cerevisiae* inactivation in fresh grapes were reported (Xiang et al., 2020). Finally, *C. albicans* was also studied by Julák, Scholtz, Kotúčová, and Janoušková (2012) obtaining a complete inactivation ($1{\cdot}10^7$ CFU/mL) after 24 h incubation with PAW.

3.3. Morphological characterization

The morphology of the wooden surface was analyzed by SEM. Fig. 6 shows SEM images that were taken after treatment with PW, SO_2 and PAW_30.

On the one hand, comparing PAW_30 treatment and PW treatment no morphological differences were found. Thus, a smooth surface with well-defined fibers and wood vessels was observed after both treatments. On the other hand, a different structure was observed after SO_2 treatment. In this case, these features are characterized by an increase in wood roughness and by the presence of wood agglomerates, which are suggested to result from the chemical damage that sulfuring provokes in the structure of wood. Thereby, a breakage of wood occurs after SO_2 treatment, generating vessels and agglomerates of wooden debris.

To the best of our knowledge, there is no research where the wooden surface morphology has been studied after PAW treatment. However, there are several works focused on the topography of wood after direct plasma treatment. Sainz-García et al. (2021, p. 139) observed no changes in oak morphology after 12-passes treatment with 500 W and three different plasma gases (air, nitrogen and argon). Moreover, Asandulesa, Topala, and Dumitrascu (2010) showed no alterations in the appearance of either oak or beech following 5 s helium plasma treatment. Similar findings were found by Novák et al. (2015) who treated beech wood during 120 s with air plasma and observed no significant differences in size and shape of vessel, fibrils and holes between the pristine sample and the treated sample.

4. Conclusions

We have investigated PAW as a sustainable and inexpensive technology to reuse wine wooden barrels during wine aging, preventing health issues and economic waste. It was demonstrated that the longer the PAW generation time, the higher the *B. bruxellensis* inactivation achieved. Furthermore, the implication of specific reactive species when inactivating this yeast was studied. OH•, NO•, NO_2• were proposed as the reactive species that play the main role when attacking microorganisms. Then, several pathways for their formation were shown. Thus, an increase in the concentration of RONS was observed as the PAW generation time increased. Moreover, no negative morphological effects in the surface of wood samples were observed after PAW treatment, which suggested no reduction of the functional and bulk properties of the oak wood.

Finally, PAW generated during 5 min (PAW_5) and 3 h of PAW/wood contact was selected as the best treatment in terms of economic and time costs since it achieved 3.23 log reductions of *B. bruxellensis*. In conclusion, inactivating *B. bruxellensis* of oak wine barrels with PAW could be a real solution to reuse aging barrels while preventing health problems.

Within the frame of this work, the authors have found some limitations to solve in future researches. We are making effort to generate an effective RONS concentration in PAW in the shortest treatment time and to test different PAW generation configurations that improve the diffusion of RONS leading to a more effective PAW. Finally, a work regarding how this process would affect wine quality is being carried out.

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CRediT authorship contribution statement

Ana Sainz-García: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Ana González-Marcos:** Validation, Supervision, Project administration, Investigation, Funding acquisition, Data curation. **Ignacio Muro-Fraguas:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Rodolfo Múgica-Vidal:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Félix Gallarta-González:** Validation, Resources, Data curation. **Lucía González-Arenzana:** Writing – review & editing, Investigation, Data curation. **Isabel López-Alfaro:** Writing – review & editing, Resources, Investigation, Data curation. **Pilar Santamaría:** Resources, Data curation. **Rocío Escribano-Viana:** Writing – review & editing, Investigation, Data curation. **Fernando Alba-Elías:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elisa Sainz-García:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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