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Wine quality implications of the treatment of oak wood with plasma activated water (PAW): A preliminary study

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ABSTRACT

Microbiological and chemical contamination of oak barrels poses significant challenges in the wine ageing process. This study explores the Plasma Activated Water (PAW) as an innovative probable solution for cleaning oak wood of various origins. Although data demonstrated reductions in the culturable microbial populations of three wine spoilage microorganisms from the tested wood (American, French and Spanish with medium and plus toastings), this initial study was focused on physicochemical and sensorial parameters of wines. Importantly, wines aged with PAW-treated wood showed physicochemical parameters like those of traditional oak barrel ageing, maintaining colour and avoiding oxidation. Sensory analysis revealed high quality red wines with harmonious balance of fruity and spicy notes, comparable to those treated with sulphur, without significant differences between PAW generation methods. The results of this study highlight PAW as a promising and sustainable method that might be tested in the wine industry under industrial conditions. PAW could offer an effective approach to disinfecting oak wood, ensuring consistent wine quality without compromising sensory or physicochemical attributes. This research provides a valuable preliminary contribution to the scientific community and the wine industry, paving the way for the adoption of PAW as a cutting-edge technology in wine production.

1. Introduction

The use of oak wood barrels in the elaboration and aging of wines is a practice that is considered as a very favourable element in the organoleptic evolution of wines. During the aging in barrels, the wine-wood exchanges enrich the product in aromas and taste sensations. In addition, it favours the micro-oxygenation that causes a physical and chemical stability in the product and gives it the delicacy, balance and aromatic complexity so appreciated by the consumer (Jackson, 2017). The used barrels do not have the same potential as the new ones, but they still have an excellent value for numerous wines and alcoholic products at a lower cost, so its good maintenance is essential for this purpose (García-Alcaraz et al., 2020).

The problems that arise during the aging of the wine are essentially associated with microbiological or chemical contamination (Agnolucci, Tirelli, Cocolin, & Toffanin, 2017)since the specific characteristics of oak wood make it difficult to disinfect and clean it. In fact, microorganisms take refuge in the natural pores of the wood sometimes causing organoleptic alterations in the wine, such as the synthesis of the volatile phenols (stable aroma, leather, etc.), the increase of volatile acidity (vinegar aroma) or the biogenic amines formation (Delso, Berzosa, Sanz, Álvarez, & Raso, 2023). Traditionally, the wood barrels have been cleaned and maintained with hot and pressurized water and with the burning of sulphur discs. This practice generates a toxic gas so that it is going to be limited by the European Commission (European Commission, 2010). Pinto, Baruzzi, Cocolin, and Malfeito-Ferreira (2020) gathers some important details about the high percentages of wines with a phenolated organoleptic profile, affected by *Brettanomyces,* in France, Italy, Portugal and Australia and about the economic losses that it would be causing. Data about the impact of the microbial spoilage of wines on

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the wine sector from an economical point of view are not easily found, and sometimes are not actualized, but some authors estimated some years ago the economic loss at about 1.4 million $ all over the world (Fugelsang & Edwards, 2007).

The Atmospheric Pressure Cold Plasma (APCP) is a source of UV photons, charged particles (positive and negative ions), free radicals, and excited atoms and molecules with a high antimicrobial capacity. The specific mechanism of microbial inactivation achieved with plasma is not precisely known. In contrast, it is well known that UV radiation can inhibit the bacteria multiplication by inducing the formation of thymine dimers in DNA, and its great lethal effect has been exploited for years to the treatment of surfaces (Liu, Chen, Yang, & Zhou, 2008). The results obtained so far show that APCP is an adequate technique to improve the microbiological quality of a wide range of foods, both of vegetable and animal origin (Asl et al., 2022; Bourke, Ziuzina, Boehm, Cullen, & Keener, 2018; Pan, Cheng, & Sun, 2019). Most of these studies have achieved successful inactivation rates of undesirable microorganisms (Lee et al., 2011; Song et al., 2009; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014). In contrast, in most of these studies the effect caused by these treatments on the nutritional and sensory characteristics of the food has not been evaluated, even though reactive species interacting with some food components could cause certain chemical reactions and thus changes in the specific characteristics of food.

APCP technology only consumes compressed air and electricity to generate the plasma in many cases. It is generated under atmospheric pressure and at room temperature. In addition, it does not require filters or other consumable materials, so no auxiliary facilities are required in this regard. The only experience related to the use of APCP technology for sanitizing oak wood barrels was direct APCP treatments applied to the surface of oak wood barrel fragments that were artificially contaminated with three spoilage microorganisms (Sainz-García et al., 2021).

Despite the promising results obtained with the direct APCP treatments, they should be shortened, automatized and even the barrels should be disassembled for its industrial application. Furthermore, in the specific case of wood, APCP must reach the deepest pore what makes the direct application even more difficult. To overcome these problems, plasma activated water (PAW) (Schnabel et al., 2016), containing mainly reactive species, could be an alternative method for the sanitization of foods and food contact surfaces. Some of the studies have been focused on the utilization of PAW for bacterial inactivation and efficiently controlling bacteria growth because its easy application replacing the traditional sanitizing solutions applied for disinfection (Han, Park, & Kang, 2023; Wang, Han, Liao, & Ding, 2021; Xiang et al., 2019; Zhai, Liu, Xiang, Lyu, & Shen, 2019). The generated PAW can be applied with the current barrel washing systems, so they can be cleaned and sanitized at the same time.

For all the above, it would be very interesting to assess the effects of the application of PAW to improve the process of cleaning and disinfection of the oak wood barrels. In addition, to corroborate its action against microbial contamination, as well as their possible effects on the quality of the wines during their conservation. The results obtained are of great interest for the worldwide wine sector, since the process of cleaning and sanitization of barrels is a key point of quality control during the aging of the wine, and currently raises important problems.

Therefore, the objective of this research was to determine the impact of disinfecting the oak wood barrels with PAW on the physicochemical and sensorial quality of the red wine with a view to begin the adaptation of the technology to an industrial environment of wineries in a future.

2. Materials and methods

2.1. PAW generation

An atmospheric pressure plasma jet system (PlasmaSpot500, Molecular Plasma Group, Foetz, Luxemburg) was used to generate PAW. This system consists of a plasma torch that operates at atmospheric pressure, with two cylindrical electrodes in coaxial arrangement that are separated by a dielectric barrier of Al_2O_3. Each PAW was generated independently in triplicate (n = 3).

Two different experimental setups of PAW generation were performed: the direct PAW configuration (Fig. 1a) and the recirculated PAW configuration (Fig. 1b). The main difference between both configurations is the way the plasma interacts with deionized water (DW) during the PAW generation. On the one hand, in the direct PAW configuration (PAW-D), the plasma jet affects the surface of the water volume, generating different reactive oxygen and nitrogen species (RONS) which are diffused into the body of water. On the other hand, in the recirculated PAW configuration (PAW-R), the plasma jet acts on the water recirculated flow when it leaves the central electrode through its interior after being pumped from the container where the body of water is located.

After filling a 3.000 mL beaker with 2.000 mL of DW, plasma was turned on for 5 min. For both configurations, compressed air at 60 slm was used as plasma gas and plasma power was set to 500 W. The distance between the end of the plasma nozzle and the DW surface was constant at 30 mm to optimize the transport of RONS from plasma to water while lowering the water losses.

Each type of PAW was used for an oak wood treatment explained in the next section. PAW-D for Treatment 1 (T1) and PAW-R for Treatment 2 (T2).

2.2. PAW treatments and wine sampling

Wood from three origins (American, French and Spanish) and with two toasting degrees (medium and plus) were analysed. For this, three replicates were prepared for each treatment: T1, T2, SO_2; and DW (Control). The oak wood treatments consisted of treating three oak stumps (70 mm × 50 mm) floating for 30 min in the waters described above and of smoking with sulphur dioxide until oxygen was exhausted, reproducing the treatments usually carried out in the barrels of the cellars and maintaining the relation of wood surface regarding liquid contained.

After treatments, they were put into glass jars with 3 l of young red wine with a nitrogen atmosphere and covered. The Tempranillo grapes were harvested at their optimum point of maturity, grapes were destemmed and crushed and introduced into stainless steel tanks. Wine was elaborated by the traditional method at the ICVV experimental winery, inoculating commercial starter cultures for the development of the alcoholic and malolactic fermentations. Wine was sampled after their MLF and samples were kept for 8 wk in a chamber at 35 °C simulating an accelerated ageing of wine.

2.3. Analysis of physicochemical parameters

The wines were characterized by measuring the alcoholic strength, pH, total acidity, volatile acidity, colour intensity and tonality according to the International Organization of Vine and Wine (OIV, 2022b). The tartaric acid was determined according Rebelein method (Lipka & Tanner, 1974). The total phenolics were determined as the total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after the dilution of the samples. The polymerization index was calculated according to Ruiz (Ruiz, 1999) and the Ionization index was determined according to Glories (1978). Acetaldehyde was analysed by enzymatic methodology with an enzymatic automatic analyser (Y200, Biosystems S.A., Barcelona, Spain).

2.4. Study of microbial inactivation

This study was carried out with three wine spoilage microorganisms, using strains acquired from the Spanish Type Culture Collection (CECT). They were one Lactic Acid Bacteria (LAB) (*Pediococcus (P.) pentosaceus*

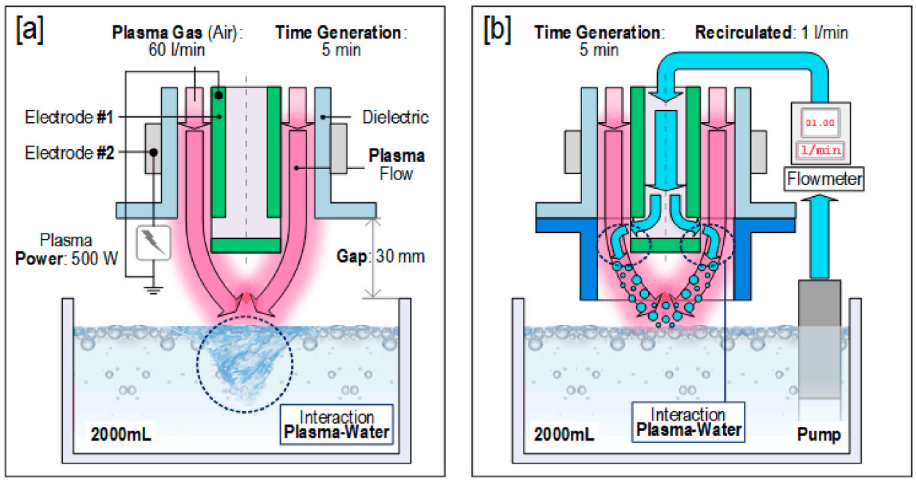

Fig. 1. Schemes of the setups to generate PAW: [a] Direct PAW configuration and [b] Recirculated PAW configuration.

CECT 923), one Acetic Acid Bacteria (AAB) (*Acetobacter (A.) pasteurianus* CECT 824) and one yeast (*Brettanomyces (B.) bruxellensis* CECT 11045). *P. pentosaceus* was grown in Man Rogosa Shape (MRS) (De Man, Rogosa, & Sharpe, 1960) broth in an incubator at 28 °C for 48 h. *A. pasteurianus* was cultured in Mann broth (25 g/L D-mannitol, 3 g/L peptone, 5 g/L yeast extract) at 25 °C for 48 h and. *B. bruxellensis* was grown in Glucose Yeast Peptone (GYP) broth (20 g/L glucose, 5 g/L yeast extract, 5 g/L peptone) at 25 °C for 48 h. After incubation, when the cultures reached stationary phase (10^8 -10^9 Colony Forming Units/mL), cells of each strain were collected by centrifugation at 10.000×*g* at 4 °C for 30 min. The pellet obtained was resuspended in 50 mL of saline solution (0.9 g/100 mL NaCl) and inoculated in 450 mL of sterile synthetic wine (yeast extract 4 g/L, glycerol 2 g/L, DL-Malic 6 g/L, ethanol 100 mL/L). Fragments of 50 mm of American oak staves with a medium toasting were used as samples. The staves had been previously sterilized at 121 °C and 1 bar for 20 min prior to contamination with the cultures prepared in synthetic wine. Samples per triplicate were contaminated by immersion in each of the cultures for 48 h in an orbital shaker (80 rpm) at 27 °C. After that, the same treatment for the oak wood fragments described in section 2.2 was carried out to perform the microbial inactivation study. When treatments were completed, each treated and untreated oak wood stave was brushed with an automatic wood planer to a deep of 1 cm. The chips gathered in sterile plastic bags were weighted and then 300 mL of sterile Trypticasein Soy Broth (TSB, Conda, Madrid, Spain) recovering medium was added. Sealed bags were incubated at 25 °C in an orbital shaker at 100 rpm for 24 h. After incubation, the liquid was recovered and centrifuged (10.000 g; 30 min; 4 °C).

The obtained samples were serially diluted and spread on the different culture media plates previously specified. After the incubation period of 48 h, colony forming units (CFU) were counted in plates with growth between 30 and 300 CFU and expressed in each sample as the average CFU per gram of wood. All microbiological analyses were conducted in triplicate for each experiment.

2.5. Sensory analysis

The sensory analysis was carried out by a panel of 10 semi-trained tasters using approved wine black glasses to mask the colour of the wine, thus studying the olfactory and gustatory phases.

Firstly, to evaluate the existence of significant differences between the samples, a discriminative test was carried out: multiple difference test (Meilgaard, Civille, & Carr, 1987), in which the magnitude of the difference between samples with respect to the control was asked at the level of olfactory and gustatory phases, using an ordinal qualitative variable of 6 points: None (0), Very Slight (1), Slight (2), Moderate (3), Much (4) and Very Much (5).

After this, a descriptive test was carried out when significant differences were found using two tasting cards, one with attributes for the olfactory phase and the other one with attributes for the taste phase, using a 7-point structured scale in both cases. The instructions given to the tasters for the olfactory phases were: "*smell the samples from left to right and rate the intensity and quality of the smell (the greater the intensity, the greater the perception of the stimulus, be it positive or negative). Score only the perceived series and attributes: series (floral, fruity, fermentative, balsamic mineral vegetable, spicy, wood, empyreumatic, chemical, animal, others*"). In the case of the gustatory phase, the instructions were: "*taste the samples from left to right and rate the intensity and quality of flavour (the greater the intensity, the greater the perception of the stimulus, whether positive or negative). Score only the perceived attributes: sweet, acid, bitter, salty, metallic, umami, astringent, drying, fresh, burning, pungent, alcoholic, aromas (aftertaste), defects, others. Rate the attributes: body, unctuous, persistence, harmony (of the olfactory and gustatory phases as a whole)*".

2.6. Statistical analysis

The analytical and microbial parameters observed for each of the samples were analysed with R software (v4.2.2; R Core Team, 2022) and analyses of the variance (ANOVA) were assessed. The significant differences between mean values were determined by Tukey's HSD test and differences were considered as significant when the *p* value was below 0.05.

The statistical study of the sensory analysis was determined with the results of a discriminative and a descriptive test. In both cases, the Kruskal-Wallis test was used to determine whether or not there were statistically significant differences between the medians of the magnitudes of the difference of the treatments regarding the control. When the results of the Kruskal-Wallis test were statistically significant, a post hoc Mann-Whitney's multiple comparisons test was conducted to determine exactly which groups were different. The considered level of significance (alpha) was 0.10. All analyses were performed using R software (v4.2.2; R Core Team, 2022).

3. Results and discussion

3.1. Inactivation of microorganisms

In Fig. 2 the microbial viable and cultivable (VC) population (log CFU) achieved after each treatment for each microorganism analysed are shown. In the case of *B. bruxellensis* the results showed a significant reduction of 3 log CFU/g wood for the T2 treatment. No significant

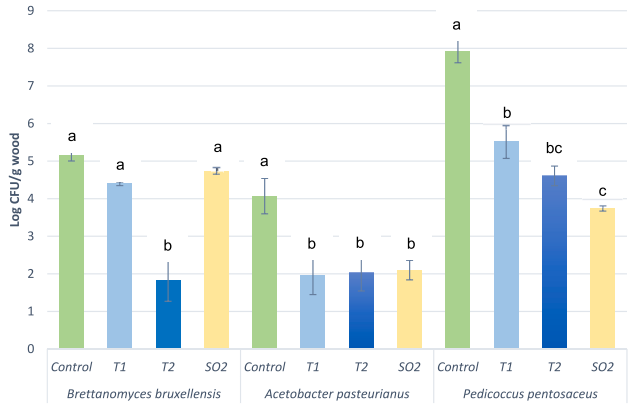

Fig. 2. Microbial viable and cultivable population (log CFU/g wood) after wood treatments (T1, T2 and SO_2) and DW (Control) contaminated with different microorganisms. [a-c] Different letters indicate statistically significant differences ($p < 0.05$) between treatments for each microorganism and error bars expressed as standard deviation of the data.

differences were found for the rest of the treatments. Regarding *A. pasteurianus*, similar inactivation results were achieved for all the developed treatments, including T1, T2 and sulphur burning (around 4 log CFU/g wood in the control to 2 log CFU/g wood whichever the treatment used). Finally, significant differences in inactivation between treatments with PAW and SO_2 were found for *P. pentosaceus*. A decrease of about 3 log CFU/g was obtained for the PAW treatments, without significant differences between the method of PAW generation, and a decrease of 4 log CFU/g for the SO_2 treatment. Thus, it could be said that the sensitivity depended on the treatment and on the species, being the most effective treatment for one microorganism the least effective for another one. Also comparing the inactivation results for the two methods of PAW generation, it could be established that the effectiveness for both PAW was similar for the studied bacteria, but it was higher with T2 for *B. bruxellensis*.

These results agree with some studies performed in other food or beverages, with APCP and with other emerging technologies. In this way, differences in the inactivation degree have been described depending on the species on the initial concentration of the microorganism and on the plasma characteristics (Dubrovin, Emanuel, Lazra, & Cahan, 2023; Liu et al., 2008; Marina et al., 2019; Miao & Jierong, 2009; Sedghizadeh, Chen, Schaudinn, Gorur, & Jiang, 2012; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014; Wiegand et al., 2013). Similar results have been observed with other non-thermal technologies (González-Arenzana et al., 2013, 2015, 2016), even at strain level. Therefore, studies like these are necessary to establish the best strategy to achieve the greatest sanitization.

Besides this, it should be considered that the microbial inactivation was performed with inoculated populations while natural populations long established in wood depth are likely more resistant. Therefore, these promising results are still preliminary.

3.2. Impact of PAW and SO_2 wood treatment on wine physicochemical parameters

The initial wine had an alcoholic strength of 12.6% (v/v), a pH of 3.60, a total acidity of 5.38 g/L of tartaric acid, a volatile acidity of 0.67 g/L of acetic acid, a colour intensity of 7.74, an anthocyanin content of 460.61 mg/L, a percentage of anthocyanins in their coloured forms of 16.29%, an anthocyanin polymerization index of 1.76 and a TPI of 40.94 (Tables 1 and 2).

To illustrate the differences due to the wood origins and toasting degree, Tables 1 and 2 show the conventional oenological parameters of wines in contact with the six different types of untreated woods after forced aging (8 weeks (wk) at 35 °C) compared to initial wine without aging. It was possible to observe that the stabilization of the wines under the indicated conditions for 8 wk served to characterize the wines after a period of contact with wood to resemble the results of traditional aging. Thereby, the pH decreased slightly (significantly in all samples except for American medium toasting -AM- and French plus toasting -FP-), increasing the total acidity in American plus toasting (AP) and Spanish plus toasting (SP). The volatile acidity increased with respect to the initial wine in the wines in contact with American and Spanish oak wood with plus toasting, while it hardly changed during the aging in French oak. In the same way, the differences observed in the tartaric acid

Table 1
Chemical parameters for the wines in contact with untreated woods from different origins and different toasting degrees (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) after forced aging and initial wine without wood contact (No wood).

Samples	pH	TA (g/l)	VA (g/l)	Tar.A (g/l)	FSO_2 (mg/l)
No Wood	3.60 ± 0.01a	5.38 ± 0.13bc	0.67 ± 0.01cd	2.75 ± 0.03 ab	40.55 ± 4.71a
AM	3.57 ± 0.05 ab	5.36 ± 0.02bc	0.75 ± 0.01bc	2.72 ± 0.07abc	15.61 ± 1.91c
AP	3.54 ± 0.01bc	5.68 ± 0.14a	0.85 ± 0.04b	2.77 ± 0.03a	24.24 ± 3.14b
FM	3.54 ± 0.01b	5.47 ± 0.06abc	0.63 ± 0.05d	2.64 ± 0.03bcd	18.52 ± 2.60bc
FP	3.57 ± 0.01 ab	5.30 ± 0.06c	0.60 ± 0.03d	2.61 ± 0.03cd	21.84 ± 0.72bc
SM	3.49 ± 0.01c	5.42 ± 0.02bc	0.74 ± 0.03bc	2.55 ± 0.02d	0.0 ± 0.0d
SP	3.53 ± 0.01bc	5.54 ± 0.06 ab	1.03 ± 0.07a	2.65 ± 0.07bcd	0.0 ± 0.0d

Data expressed as means ± SD. Means followed by different letters in the same column differ by Tukey ($p < 0,05$). TA: total acidity; VA: volatile acidity; Tar. A: tartaric acid; FSO_2: free SO_2.

Table 2
Colour parameters for the wines in contact with untreated woods from different origins and different toasting degrees (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) after forced aging and initial wine without wood contact (No wood).

Samples	ANT (mg/l)	CI	T	TPI	II	PI
No Wood	460.61 ± 5.56a	7.74 ± 0.51c	0.65 ± 0.03c	40.94 ± 0.22bc	16.29 ± 1.63c	1.76 ± 0.03d
AM	154.55 ± 30.17b	10.51 ± 0.47 ab	0.70 ± 0.04bc	39.12 ± 0.53c	34.17 ± 1.47 ab	3.58 ± 0.38 ab
AP	166.42 ± 16.74b	9.94 ± 0.22 ab	0.77 ± 0.03 ab	43.73 ± 3.02bc	31.96 ± 0.81b	2.81 ± 0.01c
FM	129.06 ± 37.81bc	10.46 ± 0.14 ab	0.77 ± 0.05 ab	66.62 ± 5.64a	34.11 ± 0.46 ab	3.52 ± 0.51abc
FP	165.26 ± 43.07b	10.02 ± 0.33 ab	0.81 ± 0.05a	51.01 ± 10.41abc	31.76 ± 1.02b	2.87 ± 0.24bc
SM	154.92 ± 4.56b	10.66 ± 0.23a	0.76 ± 0.00 ab	62.20 ± 0.42a	35.11 ± 0.31a	3.38 ± 0.01abc
SP	74.47 ± 6.07c	9.71 ± 0.31b	0.79 ± 0.02 ab	55.07 ± 8.67 ab	31.77 ± 0.92 ab	3.69 ± 0.18a

Data expressed as means ± SD. Means followed by different letters in the same column differ by Tukey ($p < 0.05$).
ANT: anthocyanin; CI: colour intensity; T: tonality; TPI: total polyphenolic index; II: ionization index; PI: polymerization index.

content were very low, with a small decrease in FP and Spanish medium toasting (SM). Logically, the free SO_2 content decreased.

Overall, the anthocyanin content decreased significantly, while the tonality and the colour intensity increased considerably (Table 2). The percentage of anthocyanins in their coloured forms (ionization index) increased significantly in all cases, as well as their polymerization. Regarding the total polyphenol content, it also increased during forced aging significantly in French medium toasting (FM) and SM, an increase that was less pronounced in American oak. Thus, these variations in the physicochemical parameters of the wines during the accelerated ageing to which they were subjected resembled the evolution experienced during ageing in traditional oak barrels (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2005).

The analysis of the wines after two months of contact with the treated and untreated wood fragments at 35 °C are shown in Tables 3 and 4. In Table 3, the chemical parameters of the wines with significant differences between samples are shown. These data allowed us to observe some significant differences in the pH, although these differences cannot be considered unfavourable for the samples not treated with sulphur burning, since they were of the order of hundreds. Similarly, the variations observed in total acidity, although significant in some cases, were not relevant. Volatile acidity, a parameter that may be related to the sanitary state of the wine due to the presence of acetic bacteria, unfavourable for quality (Reynolds, 2021), was not changed in any case, except in the case of the wines in contact with treated wood fragments (SO_2, T1, and T2) from AM wood, where it was slightly higher. If we consider the concentration of acetaldehyde (A), a compound related to wine oxidation and negative for quality when it is found in high quantity, the wood treatments with PAW were equivalent to sulphiting, since no significant differences were found between the wines in contact with those fragments in all types of wood. On the other hand, it should be considered that both the PAW and the SO_2 treatments prevented oxidation of the wines in contact with the fragments of medium toasted American oak since the control wines in contact with untreated wood presented significantly higher concentrations of acetaldehyde, in amounts that can already be considered negative for quality (Avramescu, Noguer, Avramescu, & Marty, 2002; Khalafyan et al., 2023), and also in medium toasting French oak wood decreased the variability between samples, in terms of standard deviations.

Regarding the parameters related to colour (Table 4), no major differences in colour intensity and tonality were found between treatments with any of the types of wood. These parameters characterize well the visual perception of the wine Ribéreau-Gayon, Glories, Maujean, and Dubourdieu (2006), so it can be concluded that the colour of the wines was not negatively affected by the treatments carried out on the wood. In the rest of the colour parameters (anthocyanins, percentage of anthocyanins in their coloured forms, polymerization index and total polyphenols) differences were not observed. In any case, the small variations detected in some of the cases cannot be considered negative for quality.

Table 3
Chemical parameters for the wines in contact with different origins and different toasting degrees (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) after treatments with PAW (T1 and T2) with burning sulphur disc (SO_2) and with distilled water (C).

Oak type	Treatment	pH	TA (g/l)	VA (g/l)	FSO_2 (mg/l)	A (mg/l)
AM	C	3.57 ± 0.05a	5.36 ± 0.02c	0.75 ± 0.01c	15.61 ± 1.91a	41.40 ± 13.67a
	SO_2	3.51 ± 0.01a	5.63 ± 0.02a	0.81 ± 0.01b	16.86 ± 0.72a	13.40 ± 1.39b
	T1	3.55 ± 0.01a	5.47 ± 0.06b	0.85 ± 0.01a	16.44 ± 3.74a	10.55 ± 3.55b
	T2	3.56 ± 0.02a	5.48 ± 0.04b	0.85 ± 0.01a	15.61 ± 0.73a	11.03 ± 1.65b
AP	C	3.54 ± 0.01a	5.68 ± 0.14a	0.85 ± 0.04a	24.24 ± 3.14a	9.36 ± 2.66a
	SO_2	3.50 ± 0.01b	5.61 ± 0.06a	0.80 ± 0.01a	23.09 ± 2.60a	12.46 ± 0.80a
	T1	3.54 ± 0.01a	5.59 ± 0.25a	0.76 ± 0.07a	23.50 ± 4.38a	7.31 ± 1.93a
	T2	3.54 ± 0.01a	5.41 ± 0.06a	0.79 ± 0.01a	22.67 ± 4.49a	11.55 ± 4.05a
FM	C	3.54 ± 0.01a	5.47 ± 0.06b	0.63 ± 0.05a	18.52 ± 2.60b	38.73 ± 47.94a
	SO_2	3.50 ± 0.01b	5.62 ± 0.04a	0.67 ± 0.04a	23.50 ± 0.072a	13.55 ± 2.86a
	T1	3.54 ± 0.0a	5.45 ± 0.06b	0.61 ± 0.02a	17.69 ± 1.25b	14.25 ± 1.76a
	T2	3.54 ± 0.01a	5.42 ± 0.02b	0.62 ± 0.01a	17.27 ± 0.72b	15.04 ± 1.24a
FP	C	3.57 ± 0.01a	5.30 ± 0.06b	0.60 ± 0.03a	21.84 ± 0.72a	15.52 ± 3.30a
	SO_2	3.53 ± 0.01b	5.54 ± 0.04a	0.63 ± 0.05a	22.67 ± 3.30a	9.51 ± 1.35a
	T1	3.56 ± 0.01a	5.37 ± 0.03b	0.62 ± 0.03a	21.84 ± 1.44a	15.47 ± 2.03a
	T2	3.57 ± 0.01a	5.34 ± 0.07b	0.60 ± 0.01a	20.18 ± 2.49a	18.32 ± 6.46a
SM	C	3.49 ± 0.01 ab	5.42 ± 0.02a	0.74 ± 0.03a	0.0 ± 0.0b	19.48 ± 0.45a
	SO_2	3.47 ± 0.02b	5.42 ± 0.01a	0.78 ± 0.07a	1.33 ± 0.58a	19.52 ± 6.57a
	T1	3.50 ± 0.01a	5.41 ± 0.03a	0.79 ± 0.02a	0.33 ± 0.58 ab	20.84 ± 3.35a
	T2	3.49 ± 0.01 ab	5.37 ± 0.03a	0.79 ± 0.01a	0.33 ± 0.58 ab	19.35 ± 2.71a
SP	C	3.53 ± 0.01a	5.54 ± 0.06a	1.03 ± 0.07a	0.0 ± 0.0a	13.51 ± 17.04a
	SO_2	3.47 ± 0.01b	5.37 ± 0.03b	0.87 ± 0.04a	0.0 ± 0.0a	24.22 ± 1.46a
	T1	3.51 ± 0.01a	5.47 ± 0.05 ab	0.90 ± 0.09a	0.0 ± 0.0a	24.28 ± 6.70a
	T2	3.52 ± 0.01a	5.53 ± 0.06a	0.95 ± 0.04a	0.0 ± 0.0a	23.39 ± 2.29a

Data expressed as means ± SD. Means followed by different letters in the same column differ by Tukey ($p < 0.05$). TA: total acidity; VA: volatile acidity; TA: tartaric acid; FSO_2: free SO_2. Acetaldehyde: A.

3.3. Impact of PAW and SO_2 wood treatment on wine sensory characteristics

The discriminative sensorial analysis carried out made possible to establish significant differences according to the treatment in all types of wood tested except for the Spanish medium toasting (SM) oak wood (Table 5).

The magnitude of the differences between the four samples tasted (C, SO_2, T1 and T2) regarding a known control were significant for most of the wines except for the aged with Spanish oak with medium toasting. Wines with wood treated with the burning of sulphur discs were described as moderately and much different in three samples at the olfactory phase and as moderately different in four out of the six wine samples at the gustatory phase. In the case of AM oak wood, differences were observed between the control and the wood treated with sulphur and with T2 at the olfactory phase; and between the control and the wood treated with sulphur and with T1 at the gustatory phase. In the case of wines from the ageing with FP wood treated with the two PAWs (T1 and T2) both phases were found as moderately different to the control and the same was observed with the T2 of the SP wood at the olfactory phase. The rest of the differences with the control sample were described by panellists as very slight or slight.

The post hoc Mann-Witney's multiple comparison test was developed for every sample except for wines in contact with SM and results where significant differences were found are shown in Table 6. Regarding the results for wines with AM wood, significant differences were found between the SO_2 treatment and the control wine at both

Table 4
Colour parameters for the wines in contact with different origins and different toasting degrees (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) after treatments with PAW (T1 and T2). with burning sulphur disc (SO_2) and with distilled water (C).

Oak type	Treatment	ANT (mg/l)	CI	T	TPI	II	PI
AM	C	154.55 ± 30.17a	10.51 ± 0.47a	0.70 ± 0.04b	39.12 ± 0.53b	34.17 ± 1.47a	3.58 ± 0.38a
	SO_2	235.01 ± 80.68a	9.93 ± 0.16 ab	0.76 ± 0.04 ab	44.45 ± 2.07a	33.59 ± 0.29 ab	2.56 ± 0.10b
	T1	191.90 ± 9.95a	10.03 ± 0.21 ab	0.76 ± 0.03 ab	41.49 ± 0.90 ab	31.70 ± 0.92b	2.87 ± 0.06b
	T2	157.42 ± 19.43a	9.71 ± 0.29b	0.80 ± 0.03a	44.01 ± 1.26a	34.22 ± 0.13a	2.92 ± 0.07b
AP	C	166.42 ± 16.74 ab	9.94 ± 0.22a	0.77 ± 0.03a	43.73 ± 3.02a	31.96 ± 0.81a	2.81 ± 0.01a
	SO_2	196.88 ± 19.83a	9.92 ± 0.07a	0.74 ± 0.02a	45.28 ± 1.30a	33.52 ± 1.63a	2.54 ± 0.18b
	T1	156.65 ± 12.07 ab	10.01 ± 0.16a	0.75 ± 0.04a	42.53 ± 1.50a	31.54 ± 0.16a	2.84 ±0 .01a
	T2	135.38 ± 21.28b	10.13 ± 0.47a	0.77 ± 0.02a	41.52 ± 1.60a	33.24 ± 0.42a	2.99 ± 0.08a
FM	C	129.06 ± 37.81a	10.46 ± 0.14a	0.77 ± 0.05a	66.62 ± 5.64a	34.11 ± 0.46b	3.52 ± 0.51a
	SO_2	172.74 ± 23.28a	10.34 ± 0.19a	0.83 ± 0.05a	73.51 ± 5.26a	36.31 ± 0.55a	3.04 ± 0.16a
	T1	146.30 ± 22.01a	10.32 ± 0.20a	0.80 ± 0.06a	61.76 ± 6.79a	33.86 ± 0.60b	3.19 ± 0.03a
	T2	149.18 ± 25.01a	10.24 ± 0.19a	0.80 ± 0.02a	62.30 ± 5.91a	34.29 ± 0.31b	3.14 ± 0.07a
FP	C	165.26 ± 43.07a	10.02 ± 0.33a	0.81 ± 0.05a	51.01 ± 10.41b	31.76 ± 1.02a	2.87 ± 0.24a
	SO_2	191.70 ± 6.97a	10.30 ± 0.06a	0.75 ± 0.03a	78.92 ± 5.27a	31.90 ± 1.36a	2.53 ± 0.23a
	T1	165.84 ± 42.73a	10.25 ± 0.10a	0.81 ± 0.05a	66.29 ± 13.09 ab	32.25 ± 0.63a	3.01 ± 0.24a
	T2	150.33 ± 27.66a	10.01 ± 0.52a	0.82 ± 0.04a	56.82 ± 7.48 ab	32.22 ± 0.35a	2.96 ± 0.16a
SM	C	154.92 ± 4.56a	10.66 ± 0.23a	0.76 ± 0.00a	62.20 ± 0.42a	35.11 ± 0.31a	3.38 ± 0.01a
	SO_2	186.53 ± 29.07a	10.19 ± 0.72a	0.74 ± 0.04a	64.02 ± 12.76a	33.21 ± 4.24a	2.41 ± 1.13a
	T1	179.06 ± 30.51a	10.63 ± 0.34a	0.74 ± 0.03a	67.28 ± 4.16a	34.08 ± 2.50a	3.16 ± 0.38a
	T2	177.34 ± 19.20a	10.73 ± 0.22a	0.75 ± 0.02a	65.07 ± 8.82a	34.50 ± 1.60a	3.17 ± 0.21a
SP	C	74.47 ± 6.07b	9.71 ± 0.31b	0.79 ± 0.02a	55.07 ± 8.67a	31.77 ± 0.92b	3.69 ± 0.18 ab
	SO_2	120.43 ± 13.70a	10.77 ± 0.55a	0.78 ± 0.03a	59.97 ± 7.56a	35.28 ± 1.07a	3.57 ± 0.07b
	T1	85.40 ± 14.36b	10.56 ± 0.42 ab	0.79 ± 0.01a	58.11 ± 4.80a	32.49 ± 1.31b	3.78 ± 0.13 ab
	T2	101.50 ± 6.23 ab	11.23 ± 0.25a	0.75 ± 0.01a	63.07 ± 9.49a	32.07 ± 0.88b	3.93 ± 0.08a

Data expressed as means ± SD. Means followed by different letters in the same column differ by Tukey ($p < 0.05$).
ANT: anthocyanin; CI: colour intensity; T: tonality; TPI: total polyphenolic index; II: ionization index; PI: polymerization index.

Table 5
Magnitude of the difference (median) of the olfactory and gustatory phases according to the wood (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) and PAW treatment (T1 and T2) or sulphur burning (SO_2) and untreated (Control)) for each phase as results of the Kruskal-Wallis test.

Phase	Wine	Treatment				Kruskal-Wallis test		
		C	SO_2	T1	T2	X^2	df	*p*
Olfactory	AM	0	1.5	1.5	2.5	10.695	3	0.013[a]
	AP	0.5	4	2	2	17.573	3	<0.001[c]
	FM	1	4	1	2	15.002	3	0.002[b]
	FP	0	1	3	3	8.314	3	0.040[a]
	SM	1.5	2	1.5	1	2.679	3	0.444
	SP	1	3	1	3	12.209	3	0.007[b]
Gustatory	AM	0.5	3	2	1	15.197	3	0.002[b]
	AP	1.5	3.5	2.5	2.5	8.711	3	0.033[a]
	FM	1	3.5	2	2	9.803	3	0.020[a]
	FP	0.5	1.5	3	3	11.052	3	0.011[a]
	SM	1	2	2	0	2.905	3	0.406
	SP	1	3	2	2	10.015	3	0.018[a]

df: degrees of freedom.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

phases, and also at the olfactory description of T2 and control, and at the gustatory description of the T1 and control. In the case of wines with AP oak wood, differences between the wine control and the wine with the wood treated with sulphur were observed again at the olfactory and gustatory phase, and also differences were reported for the wines with wood treated with sulphur and with the wood treated with both PAW. Once again, differences between the wine control and the wine with wood treated with sulphur were observed at the olfactory and gustatory phase in the case of wines with FM oak wood, and between the wine with the wood treated with sulphur and with T1 at the olfactory phase. Finally, differences between the wine control and the wines with the wood treated with T2 at the gustatory phase were established for wines with FP oak wood, and between the two types of PAWs at olfactory phase for wines with SP oak wood, and between the control and the wood treated with sulphur at the gustatory phase for the same wines.

In summary, the discriminative test, which was based on the multiple difference test and involved a total of 12 tests (three origins of oak wood and two different toasting degrees for each origin, evaluated at both the

Table 6
Post hoc Mann-Whitney's multiple comparisons test for the discriminative test results of the olfactory and gustatory phases according to the wood (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) and PAW treatment (T1 and T2) or sulphur burning (SO2) and untreated (Control)).

Phase	Wine	Pairs of treatments					
		C–SO_2	C-T1	C-T2	SO_2-T1	SO_2-T2	T1-T2
Olfactory	AM	**0.026**[a]	ns	**0.028**[a]	ns	ns	ns
	AP	**0.004**[b]	ns	ns	**0.029**[a]	**0.007**[b]	ns
	FM	**0.006**[b]	ns	ns	**0.032**[a]	ns	ns
	SP	ns	ns	ns	ns	ns	**0.026**[b]
Gustatory	AM	**0.013**[a]	**0.012**[a]	ns	ns	ns	ns
	AP	**0.036**[a]	ns	ns	ns	ns	ns
	FM	**0.027**[a]	ns	ns	ns	ns	ns
	FP	ns	ns	**0.032**[a]	ns	ns	ns
	SP	**0.010**[a]	ns	ns	ns	ns	ns

df: degrees of freedom. ns: no significant differences.
***$p < 0.001$.
[a] $p < 0.05$.
[b] $p < 0.01$.

olfactory and gustatory levels), showed significant differences between samples in 10 out of 12 total tests (83% of the cases studied). In most cases, (58%, 25% on the olfactory level and 33% on the taste level) the sample treated with sulphur produced wines that were significantly different from the control sample; and in 8% of the cases (at the olfactory level), the sample treated with sulphur produced wines that were significantly different from the rest of the samples in the study. Only in one case, representing 8% of the study (Spanish oak wood with plus toasting in the olfactory phase), differences were established between the treatments of the wood with PAW generated by different methods (direct and recirculated). For its part, the sample treated with T1 was not different from the control in 92% of the cases; nor did it differ significantly from the sample treated with sulphur in 83% of the cases, all at the olfactory level. Similarly, the T2-treated sample was not different from the control in 83% of cases; nor did it differ significantly from the sample treated with sulphur in 92% of the cases.

The descriptive test with a 7-point structure scale for olfactory and gustatory phase was performed when significant differences were found in the discriminative test and the descriptors that determined these differences at olfactory and gustatory level are shown in Table 7. Thus, the sulphur-treated sample showed significant differences with the control in the score obtained for the woody notes at the olfactory phase, being 2,5 points higher for the sulphur-treated samples, and 3 points higher in astringency with respect to the control at the taste level, for AM oak wood. Moreover, the sample treated with sulphur was described as lower quality, less spicy and with more chemical notes on the olfactory level than the control sample, and as more drying on the taste level for AP oak wood. The sulphur-treated sample also presented lower olfactory quality than the both PAW-treated samples for AP oak wood and was also described as having less spicy character and less woody notes than the T1-treated sample for AP oak wood; it was also described as less fruity than the T1-treated sample for FM oak wood.

The minor differences between the sample treated with T1 and control were described as 2,5 points higher in astringency for the PAW (in AM oak wood). In the case of the sample treated with T2, it was described as less fruity than the control at olfactory level for AM oak wood, and less astringent than control for FP oak wood. Only one difference was described between the samples treated with T1 and T2, which pertained to the wood notes in SP. These notes were found to be higher for T1.

In a nutshell, the descriptive test showed that the wines resulting from the treatments of the wood with PAW (both direct and

Table 7
Median calculated on significant sensory attributes punctuation (1–7 points) as results of the Kruskal-Wallis test and the post hoc Mann-Whitney's multiple comparisons test in the descriptive test according to the wood (AM: American medium toasting; AP: American plus toasting; FM: French medium toasting; FP: French plus toasting; SM: Spanish medium toasting; SP: Spanish plus toasting) and PAW treatment (T1 and T2) or sulphur burning (SO2) and untreated (Control)) for each phase.

Phase	Wine	Attribute	*Treatment*				*Kruskal-Wallis test*		
			C	SO_2	T1	T2	X^2	df	*p*
Olfactory	AM	Fruity	4 a	3.5 ab	3.5 ab	2 b	8.214	3	0.042[a]
		Wood	0.5 b	3 a	1.5 ab	2 ab	8.614	3	0.035[a]
	AP	Chemistry	0 b	1.5 a	0 ab	0 ab	10.267	3	0.016[a]
		Quality	4.5 a	2 b	4.5 a	4 a	11.952	3	0.007[b]
		Spiced	2.5 a	0 c	3 ab	1 ab	11.972	3	0.007[b]
		Wood	1.5 ab	0 b	3.5 a	1 ab	8.411	3	0.038[a]
	SP	Wood	3 ab	2.5 ab	3.5 a	0 b	9.826	3	0.020[a]
	FM	Fruity	2 ab	1 b	3 a	2.5 ab	9.500	3	0.023[a]
		Spiced	0 b	2.5 a	3 ab	3 ab	10.977	3	0.012[a]
Gustatory	AM	Astringent	0 c	3 ab	2.5 b	1 abc	13.494	3	0.004[b]
	AP	Drying	0.5 b	3 a	0 ab	0 ab	12.080	3	0.007[b]
	SP	Drying	4 a	1.5 b	2 ab	3 ab	8.065	3	0.045[a]
	FM	Quality	4.5 a	3 b	4 ab	4 ab	11.619	3	0.009[b]
	FP	Astringent	2.5 b	3.5 ab	3 ab	1 a	7.495	3	0.058 ·

df: degrees of freedom.
$p < 0.10$.
[a] $p < 0.05$.
[b] $p < 0.01$.

recirculated), corresponded in some cases to higher quality wines, especially in terms of smell, being fruitier on the nose, spicier and with more pleasant notes of wood than the sulphur treatment traditionally carried out in wineries. The only descriptor related to defective quality was established at the olfactory level for a sample treated with sulphur, which was described with chemical notes, a sensation that was not perceived in the rest of the study samples. In this way, the greatest differences were established between the control and the sulphur-treated samples, being the quality of the latter generally lower.

To our knowledge, this study was the first one that investigating the sensory implications on the quality of wines in contact with PAW-treated oak wood. Other works have reported an effect on the characteristics of the wines treated directly with APCP that has sometimes been favourable and sometimes not for the quality (Huzum & Nastuta, 2021; Marina et al., 2019; Niedźwiedź, Simeonov, Waśko, & Polak-Berecka, 2022; Pankaj, Wan, Colonna, & Keener, 2017; E. Sainz-García et al., 2019) but in our case the results are very promising to use this technology at industrial level as neither the sensory nor the physicochemical characteristics of the treated wines were not negatively affected in any case.

4. Conclusions

The treatment with PAW of the oak wood of different origins and different toasting degrees gave rise in all the cases studied to quality red wines, both at the olfactory and gustatory levels, with a balance between fruity and spicy notes, without finding major differences between the wines in contact with PAW-treated woods generated by different methods. The wines that were treated with burning sulphur discs that differenced significatively, were described as less spicy, more chemical, astringent, and drying when the wood was from France or America. The results of the study are promising for the wine industry, as they could offer a sustainable probable alternative for disinfecting oak wood without compromising wine quality. In the future, it would be interesting to extend the study to a wider number of oenological microorganisms, to naturally contaminated barrels and to further investigate the long-term effects and scalability in industrial settings. Therefore, continued research and development is essential to fully integrate this innovative method into winemaking practices, ensuring consistent quality and meeting industry standards.

CRediT authorship contribution statement

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

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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