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ORIGINAL PAPER

Dry ice blasting, a new tool for barrel regeneration treatment

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Abstract The aim of this work was to evaluate the effect on wine of dry ice blasting, used for the regeneration treatment of barriques, from a microbiological, chemical and sensory point of view. Microbiological analyses were performed on a laboratory model system and in small barrels to test the efficacy of dry ice blasting on the reduction in microbial loads. Subsequently, in order to study the chemical and sensory effects of dry ice blasting on wine, two barriques, used in the cellar, were used. One barrique was sulphited, as per common cellar practice, and one was cryosandblasted (*R*). After 6 months, the wines were compared. Data showed differences between the two wines concerning the volatile components. For instance, wine R had a statistically significant higher content of eugenol, *cis*-oak lactone and *trans*-oak lactone. These results were confirmed using sensory analysis, as wine *R* had an evident increase in intensity of boisé and vanilla notes. The advantages derived in using this method are the possibility of utilising a barrique, destined to be eliminated, for almost one more year, which is more sustainable from an economic and environmental point of view for the winemaker, and then the possibility to clean barrels reducing the use of SO_2.

Keywords Dry ice blasting · Microbiological control · Regenerated barrique · Wood aromas · Sensory analysis

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Introduction

During ageing in wood, wine and spirits undergo several physical–chemical modifications resulting in considerable evolution of their composition, colour, stability and sensory properties [1–5]. The quantity of volatile compounds that are potentially extractable is influenced by the rate of release of these compounds from the wood and the contact between wine and wood.

The use of oak wood in winemaking is a common practice for the maturation of wine, but it is an expensive technique and barrels are usually reused for several years. Prolonged use of the same barrique implies some drawbacks, such as that oak wood does not release new flavours, or that the risk of infections by microorganisms increases dramatically. Therefore, microbiological sanitation of the barrels is of great importance in the proper management of the wine conservation and ageing process. Among the microorganisms present in must and wine, some are considered detrimental to the organoleptic quality of the wine, especially yeasts belonging to the genus *Dekkera/Brettanomyces*. These yeasts can cause serious economic loss to the wine industry due to their ability to spoil wines [6]. In the cellar, *Brettanomyces* have been isolated from the equipment, walls, floors and principally oak barrels. From an organoleptic point of view, the development of these microorganisms generates off-odours in wine, described as horse sweat, burnt plastic or plastic [7], which mask the fruity and floral aromas of wine. These unpleasant notes are related to the degradation of the hydroxycinnamic acids in volatile compounds, such as 4-ethylphenol and 4-ethylguaiacol [7, 8]. Together to these phenolic volatile compounds, *Brettanomyces* also produce other undesirable compounds as acetic acid, ethyl acetate, fatty acids and pyridine derivatives [9–11]. In addition, some authors have also reported

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the ability of some *Brettanomyces* strains to produce biogenic amines [12]. Even more, there are also species of bacteria that, if not properly controlled, can live and multiply in wine during ageing and on the porous surface of the wood when the barrels are emptied, including acetic acid bacteria and some lactic acid bacteria [13–15]. Therefore, it is clear that, to obtain wine aged in wood with high-quality characteristics, a proper sanitation of the barrel before filling is an essential step.

Nowadays, the most applied methods for decontamination of barrels are based on the use of chemical additives and the application of physical processes [16]. Among the chemical additives, sulphur remains the most widely used method, both through the practice of "mechage", i.e. the burning of capsules of sulphur within the barrel, and through washing with solutions of liquid sulphur dioxide. Physical methods consist in classical washes with water at normal pressure, or with the use of high-pressure water, both hot and cold. However, these methods, which are generally used in cellars, are not able to deeply sanitize the wood. For these reasons, alternative methods have been proposed.

For instance, Falguera et al. [17] proved that UV irradiation was effective in partially inactivating polyphenol oxidase and in reducing volatile acidity (an indirect spoilage measure) in white wines, but important aromatic compounds of wines were affected. Gonzalez-Arenzana et al. [18] applied microwave technology to reduce the microbial contaminants of oak barrels and showed that the treatment eliminated about 36–38 % of the initial total yeast population; therefore, it could only be used as a complementary treatment. Schmid et al. [19] demonstrated that hot water can reduce microbial loads, but it does not sanitize the surfaces, and moreover, stronger treatments with high-pressure hot water require a great amount of water. The same authors also tested high-pressure ultrasounds combined with hot water, obtaining good results regarding microbial contamination, although the high-pressure equipment needed would bring extra cost for the winery [20]. On the other hand, the effectiveness of ozone and consequently its antimicrobial efficacy are influenced by several parameters such as pH, temperature and the presence of ozone-consuming compounds [21, 22]. In this line, Guzzon et al. [23] showed that an aqueous ozone solution was effective in eliminating viable cells present in solution, but its activity was strongly related to environmental variables, such as temperature and cell concentration.

All the cited methods have some disadvantages, e.g. chemical disinfectants may not be used at the correct concentration, or left for the appropriate contact time [24]; high-pressure water hoses cause aerosols, thus, can spread microbiological contamination and have adverse health effect for the operator [25]; steam can be dangerous and cause aerosols similarly to high-pressure cleaning; UV irradiation could not be very effective because of the porosity of wood [26]; gamma irradiation, although effective, is not popular among consumers; ozone gas is hazardous and can be dangerous to human health [27]; and finally, neither of these methods regenerate the oak wood.

For all these reasons, in this study, an alternative physical treatment of the barrels using dry ice blasting was investigated, and its effect on the rejuvenation of oak wood on wine was evaluated from a microbiological, chemical and sensory point of view. First, the study was conducted in the laboratory and focused on its microbiological aspect; then the study was scaled up to barriques (225 L), as those used in the cellar, to study the chemical and sensory aspects of wine. The efficacy and effect of dry ice blasting on wine were also compared with sulphiting treatment, a widely method used in the cellar.

Materials and methods

Dry ice blasting treatment

The dry ice blasting treatment was performed using Microblast CO_2 MB MONO (MEC s.r.l., Caltignaga, Italy), a microblast single-hose dry ice blast cleaning system that can operate up to a maximum air pressure of 12 bar, thus making it suitable for removing more of the difficult surface coverings that may be encountered. It is equipped with a pneumatic revolver device supplied with a "Mono" gun, 5-m hose and cylindrical barrel. The blaster is constructed from INOX 304 material with a 40-kg dry ice capacity operating up to a maximum of 1.2 kg/min. The MB/MONO single-hose unit uses dry ice pellets with a diameter of 3 mm. The applied parameters were: supersonic nozzle (40 × 3 mm), 7–7.5 bar operating pressure, with a treatment duration of 5 s × dm^2. The overall duration for a barrique was of about 25 min.

Microbiological control

Laboratory model: 1 dm^2 piece of barrel wood

For the preparation of contaminated red wine, *Brettanomyces bruxellensis* ISE371 belonging to the CREA-ENO (Centro di Ricerca per l'Enologia) collection was grown in YEPG broth and then inoculated in the wine at 1×10^6 cell/mL. *Lactobacillus brevis* ISE5033 (a spoilage strain able to produce tyramine [14]) was grown in MRS broth and inoculated in the wine at 2×10^6 cell/mL. The wine was a Barbera with the following characteristics: 13 % alcohol (v/v), pH 3.5, free SO_2 4.8 mg/L.

A laboratory model was created as follows: three barriques were cut into pieces of 1 dm^2 of surface area that were taken for the lateral side and from the staves. This model was similar to the one used by Schmid et al. [19]. The pieces of staves were introduced into tanks containing the contaminated wine, and they were stored at room temperature for 5 days. Three samples were introduced into wine containing *Brettanomyces*, and three samples were immerged in wine containing *L. brevis*.

Microbial counts were determined using contact plates. Plates were made after incubation in the spoiled wine, and subsequently, all the models were dry ice blasted and again plated in order to evaluate the microbial load abatement. For the yeast count, dichloran rose bengal chloramphenicol agar (VWR, Milan, Italy) was used. For the bacterial count, TSA plates were used (VWR). Plates were incubated at 25 °C for 10 days.

Laboratory model: Trials on 2-litre barrels

Barrels and wine contamination *Brettanomyces bruxellensis* ISE371 belonging to the CREA-ENO collection was grown in YEPG broth and then inoculated at 1×10^6 cell/mL into Barbera wine having the characteristics described above. Contaminated wine was put into new 2-L oak barrels, made by a barrel manufacturer, and stored at 15 °C. No SO_2 was added to the wine during ageing. Experiments were performed in duplicate.

Barrel treatments and microbiological control After 5 months of ageing, the barrels were completely emptied, and one of the lateral sides was removed from each of them. Four different parts were sampled: the two lateral sides, the bottom of the barrels and the top side near the bunghole. As control, before the treatment, the inner surfaces were sampled with contact plates using dichloran rose bengal chloramphenicol agar, a selective medium for yeasts associated with food spoilage.

Three different treatments were performed: (i) washing with hot water: the barrel was filled up with water at 100 °C and kept at room temperature for 1 day (indicated as *A*); (ii) washing with SO_2 (2 g/L): the barrel was filled up with this solution and kept at room temperature for 1 day (indicated as *S*); and (iii) dry ice blasting (indicated as *R*).

After the treatment, the surfaces of each of the barrels were sampled again with contact plates.

To check the presence of *Brettanomyces* after ageing, the wine contained in the barrels was plated onto YEPGA medium (yeast extract 1 %, peptone 1 %, glucose 2 %, agar 1.5 %) with the addition of 1 % cycloheximide and ampicillin. Plates were incubated at 25 °C for 10 days. Experiments were performed in duplicate.

Finally, to detect the presence of *B. bruxellensis* in wines after the treatment, the barrels were filled with new pasteurised Barbera wine and incubated at 15 °C (no SO_2 was added) for 1 month, before the wine was plated onto YEPGA medium with the addition of 1 % cycloheximide and ampicillin.

Colonies were identified by RFLP according to Esteve-Zarzoso [28].

Chemical and sensory impact on wine of regenerated barriques

Trials on 225-L barriques

To simulate real cellar conditions and to investigate the sensory impact of the dry ice blasting treatment on wine, an assay was performed on 225-L barriques. Barriques originating from the same manufacturer, which had been used for 4 years and purchased at the same time, were subjected to different treatments: one was cryosand-blasted as previously described (indicated as *R*) and one was treated with an SO_2 solution (1 kg $K_2S_2O_5$ in 225 L), a current widely method used in wine cellar, which remained in the barrique for 1 day before it was emptied (indicated as *S*).

After the treatment, the barriques were filled up with Barbera wine (Cantina Sociale of Fontanile, Italy), 13.3 % alcohol (v/v) and pH 3.39. The wine was aged for 6 months at 15 °C in the experimental cellar of CREA-ENO. The wine aged in the dry ice blasted barrique was designated wine *R*; the wine aged in the sulphited barrique was designated wine *S*.

The SO_2 content in wine was checked monthly and maintained at 25 mg/L.

After ageing, wines were subjected to chemical and sensory analysis to evaluate the organoleptic differences between the two wines. In addition, to investigate the presence of microorganisms, wines were also plated on YEPGA added with 1 % cycloheximide and ampicillin and on MRS with ampicillin.

Chemical analysis

Physiochemical composition Wines were analysed after ageing: total acidity was determined according to EEC methods (EEC regulation 2676/90 1990); polyphenolic composition was determined as described by Di Stefano et al. [29]. The colour of the wine was tested using CIELAB parameters; the colour intensity (E420 + E520 + E620) and the colour hue (E420/E520) were also determined. Colour differences between two colour points in the CIELAB space were calculated with ΔE (OIV-MA-AS2-11).

Free volatile compounds related to barrel ageing Volatile compounds (cyclotene, guaiacol, *trans*-oak lactone, *cis*-oak lactone, methylguaiacol, maltol, eugenol and vanillin) were extracted using solid-phase extraction with the method proposed by Mateo et al. [30], later modified by Bosso et al. [31]. Briefly, 30 millilitres of wine was diluted threefold in water, and then, 300 μL of 1-heptanol (51.43 mg/L) was added as the internal standard; the mixture was loaded into a reversed-phase C18 EC cartridge, 1 g (Biotage AB, Uppsala, Sweden), previously activated with 5 mL of methanol and 5 mL of water. After washing with water (5 mL), the free volatile substances were eluted with 6 mL of HPLC-grade dichloromethane; the organic phase was dried with the addition of anhydrous sodium sulphate, concentrated tenfold by evaporation and analysed using gas chromatography-mass spectrometry (GC–MS) using an Agilent 6890 Series gas chromatograph (Agilent Technologies, Inc., Santa Clara, Calif., USA) equipped with an Agilent 5973 N Mass Selective Detector (MSD). The concentrate (1 μL) was analysed on a Zebron ZBWAX column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, Torrance, Calif., USA).

Conditions were as described by Bosso et al. [31]: helium was used as the carrier gas, with a constant flow of 1 mL/min. The source and the transfer line were kept at 230 °C and the injector at 250 °C.

The compounds were identified by comparing their spectra and retention times with those of pure reference compounds injected in the same conditions.

The spectra were recorded in single ion monitoring (SIM) mode, and the selected ion fragments were: 70 m/z for 1-heptanol (as internal standard), 124 m/z for guaiacol, 151 m/z for vanillin, 138 m/z for methyl guaiacol, 137 m/z for 4-ethyl guaiacol, 164 m/z for eugenol, 107 m/z for 4-ethyl phenol, 164 m/z for isoeugenol and 99 m/z for oak lactone isomers. The calibration curves were obtained by adding increasing quantities (six levels) of pure reference compounds to a model wine.

The oven temperature was kept at 45 °C for 2 min, then increased to 60 °C at a rate of 30 °C/min, increased again from 60 to 230 °C at a rate of 2 °C/min and then kept at 230 °C for 20 min. Standards were purchased from Sigma-Aldrich (Milan, Italy) at the maximum available grade of purity.

Sensory analysis

All sessions were conducted at the Centro di Ricerca per l'Enologia (CREA-ENO). The panel was composed of 14–18 trained assessors (researchers and technicians at the CREA-ENO). ISO (3591-1977) approved glasses and an ISO (8589-2007) tasting room were employed for all of the sensory sessions. Each sample (30 mL) was coded by three random digits and covered with a Petri dish.

Duo-trio test Sensory evaluation of the wines was performed in duplicate using a discriminant test, the duo-trio test (UNI EN ISO 10399). Results were elaborated by calculating the total number of judges who provided a correct answer, which was compared with the theoretical value reported in a statistical table (UNI EN ISO 10399). On the basis of the level of significance chosen and the number of judges, there are a minimum number of correct answers which establishes a statistically significant difference between the two products. Thus, if the number of correct responses is equal or greater than the tabulated value, it can be concluded that there is a perceptible sensory difference between the products.

Wine profile analysis The wine sensory profiles were determined following the procedure derived from the ISO standards (11035-1994) according to Cravero et al. [32]: the descriptors were chosen from a predefined odour list according to Guinard and Noble [33], integrated with colour and flavour (taste and mouth-feel sensation) descriptors.

Firstly, terms describing colour, odour and flavour of the wines were collected after 4 and 6 months of ageing in the barriques. The descriptors were chosen when their frequency of identification was higher than "(number of assessors)/2″ for colour and flavour or "(number of assessors)/4″ for odour.

Subsequently for the wines aged for 6 months, the intensity of the chosen descriptors was measured on a structured scale ranging from 0 to 80 mm. Finally, the assessors gave their own wine quality perception using the same unstructured scale to evaluate the colour, odour, flavour and global pleasantness; the latter was also expressed with a structured scale (from 1 to 10).

Statistical analysis

Chemical and sensory data (sensory profiles) were statistically analysed: via the t test and one-way ANOVA (treatment effect, assessor effect) using SPSS (SPSS 15.0 for Windows 2004; SPSS, Chicago, IL, USA).

Results

Microbiological control

In the first trial, a laboratory model was constructed dividing the wood of three barrels into pieces of 1 dm^2 of surface area. These wood models were kept in wine, artificially contaminated with *Brettanomyces* or *Lactobacillus*

brevis, for 5 days, and then analysed with contact plates. As displayed in Table 1, before treatment, the surfaces of all the rose bengal plates, selective for yeasts, were completely covered by colonies, being them uncountable (>10,000 CFU/dm^2). After dry ice blasting, the number of colonies was drastically reduced with a maximum of 200 CFU/dm^2. Likewise, plates selective for bacteria gave similar results (Table 2). Therefore, this treatment allowed an abatement of 97.8–100 % of the microbial load.

In the second assay, 2-L barrels, which were filled up with contaminated wine, were used and different treatments were compared. The data showed that the population in the contaminated wine contained in barrels was about 5×10^5 CFU/mL, and RFLP analysis of the colonies revealed that they were *B. bruxellensis* (data not shown). Table 3 reports the results of the microbial reduction obtained after treatments of the artificially contaminated barrels and shows that dry ice blasting treatment (about 99 % reduction) was fully comparable to that of the other two treatments commonly used in cellars (hot water and sulphitation).

Moreover, after the treatments, these barrels were filled up with new pasteurised wine to also evaluate the effectiveness of the treatment on the new wines. Wines stored in these treated barrels were plated on selective medium to detect the presence of *B. bruxellensis*. Data showed that, after 1 month, no contaminating yeast was present in the wines stored in barrels *S* and *R*, while 270 CFU/mL were detected in the wine stored in barrel A (treated with hot water). Even though the most commonly used oak barrel in wine industry is of 225 L, here smaller barrels were used to firstly test and verify the effectiveness of the dry ice blasting method in the microbiological control, avoiding the contamination of hundreds litres of wine with *Brettanomyces.* However, we are quite confident that the method can be scaled up to larger oak barrels: When 6-month aged wines in the treated barriques were plated, no colony growth was detected.

Table 1 Total yeast population from contact plates in the laboratory model (CFU/100 cm^2) before and after dry ice blasting treatment

Samples	Before treatment	After treatment	Abatement %
1A (barrique 1, lateral side A)	>10,000	0	100.00
1B (barrique 1, lateral side B)	>10,000	0	100.00
2A (barrique 2, lateral side A)	>10,000	25	99.75
2B (barrique 2, lateral side B)	>10,000	21	99.79
3A (barrique 3, lateral side A)	>10,000	30	99.70
3B (barrique 3, lateral side B)	>10,000	38	99.62
4A (barrique 1, stave A)	>10,000	105	98.95
4B (barrique 1, stave B)	>10,000	42	99.58
5A (barrique 2, stave A)	>10,000	68	99.32
5B (barrique 2, stave B)	>10,000	114	98.86
6A (barrique 3, stave A)	>10,000	211	97.89
6B (barrique 3, stave B)	>10,000	127	98.73

Impact of dry ice blasting on chemical components of the wine

To analyse the impact of this technique on the sensory and chemical aspects, and to simulate real winemaking conditions, a third assay was performed using 225-L already used barriques.

Chemical analysis

Using the CIELAB parameters, the colour of wine can be precisely determined by measuring the wavelength in the visible spectrum; these parameters also allow the quantification of differences in colour among different wines. Thus, CIELAB colour measurements are used extensively by many industries and are gaining greater use as descriptors for colour by the wine industry.

Table 4 reports the colour analysis of wines *R* and *S* and show some significant differences only for some of the parameters analysed. In wine *R*, the saturation is lower than wine *S*. The parameter *b** is significantly lower in *R*, which means that wine *R* has a higher blue colour component. The h* indicates that the colour of *R* shifts towards blue hues. In addition, the overall colorimetric difference calculated as ΔE (OIV-MA-AS2-11: R2006) was also evaluated. Since it has been reported (Martinez et al. [34]) that chromatic changes can be perceived by the human eye if ΔE values are up to 2.7, and in our study the ΔE value between *R* and *S* was 0.37, it can be concluded that the differences determined by the instrument are not perceivable and hence that dry ice blasting treatment does not alter the colour of wine. Additional chemical analysis showed that the acidity and pH values were similar in both wines, as well as the polyphenolic content (Table 5); therefore, this treatment does neither influence these chemical components of wine.

Volatile aroma analysis related to ageing in barriques

No differences were found for aromatic compounds, such as esters and higher alcohols, present in wine at the end of

Table 2 Lactic acid bacteria population from the laboratory model (CFU/100 cm^2) before and after the dry ice blasting treatment

Samples	Before treatment	After treatment	Abatement %
1A (barrique 1, lateral side A)	>10,000	6	>99.94
1B (barrique 1, lateral side B)	>10,000	6	>99.94
2A (barrique 2, lateral side A)	>10,000	20	>99.80
2B (barrique 2, lateral side B)	>10,000	20	>99.80
3A (barrique 3, lateral side A)	>10,000	0	100.00
3B (barrique 3, lateral side B)	>10,000	0	100.00
4A (barrique 1, stave A)	>10,000	0	100.00
4B (barrique 1, stave B)	>10,000	0	100.00
5A (barrique 2, stave A)	>10,000	0	100.00
5B (barrique 2, stave B)	>10,000	0	100.00
6A (barrique 3, stave A)	>10,000	0	100.00
6B (barrique 3, stave B)	>10,000	0	100.00

Table 3 Microbiological analysis on the total yeast population in 2-L barrels (CFU/100 cm^2) three different treatments

Treatment	Sampled zone	Yeast before	Yeast after	Abatement %
A (hot water)	Lateral side 1	>10,000	60	99.4
	Lateral side 2	>10,000	29	99.7
	Bottom	>10,000	24	99.8
	Bunghole	>10,000	142	98.6
S (sulphited)	Lateral side 1	>10,000	4	100.0
	Lateral side 2	>10,000	10	99.9
	Bottom	>10,000	8	99.9
	Bunghole	>10,000	2	100.0
R (dry ice blasted)	Lateral side 1	>10,000	59	99.4
	Lateral side 2	>10,000	42	99.6
	Bottom	>10,000	106	98.9
	Bunghole	>10,000	93	99.1

the alcoholic fermentation (data not shown). Volatile compounds extracted during ageing are reported in Table 6. Some compounds have statistically significant differences between the two wines, so that wine *R* contains a higher quantity of eugenol, *cis*-oak lactone and *trans*-oak lactone than wine *S*, demonstrating that dry ice blasting treatment of the barriques influences positively the volatile composition of the final wines.

Sensory analysis

A duo-trio test (two repetitions), to determine whether there were sensory differences between the wines aged in the differently treated barriques, showed statistically significant result (Fig. 1). In the first session, 14/18 assessors recognised differences between the wine and answered correctly. In the second, the number of correct answers was 14/17. These results were statistically significant as, with 17 or 18 tasters, the minimum number of correct answers to achieve a significant result ($p < 0.05$) is 13.

Even though individual preference influences the evaluation of pleasantness, both wines were similarly appreciated by the panel (Fig. 2). Only the flavour and global pleasantness, especially softness, were classified as little higher for *R*, according to the mouth-feel characteristics. The global pleasantness expressed with a structured scale was also very similar.

The sensory profiles of wines were also analysed: firstly, terms describing colour, odour and flavour (taste and mouth-feel sensation) of the wines were collected after 4 months of ageing (data not shown). In wine *R* the cherry and vanilla descriptors were recognised with greater frequency, while in wine *S* fresh herbaceous and jam notes were perceived with a greater frequency. At the time of testing, boisé/oak wood was still not very noticeable (only two tasters detected it).

A second collection of descriptors of colour and fragrance was made after 6 months of ageing by the CREA-ENO *panel* (14 assessors). The data (Fig. 3) proved the evolution of wine: the vanilla and boisé/oak descriptors were recognised by a greater number of assessors in both wines, although they were more evident in wine *R*. Wine *S* had more frequencies of the floral-violet and red berries (raspberry and blackberry) descriptors, while fresh herbaceous notes were no longer perceived. In both wines, the frequencies increased for the jam odour.

Finally, the sensory profiles were defined (Fig. 4). Samples have a similar ruby red colour intensity and violet highlights, but wine *R* showed a more complex aroma, characterised by a higher intensity of oak wood/boisé (statistically different) and vanilla. Moreover, wine *R* resulted

Table 4 Colour analysis of wines from two differently treated barriques

	R	*S*	*t* test
Brightness (y%)	0.0253958 ± 0.00022	0.025084 ± 0.00005	
Saturation S%	98.045275 ± 0.05256	98.25172 ± 0.01998	0.03
Tone	611.95627 ± 0.00933	611.9507 ± 0.00075	
*L**	18.108219 ± 0.09741	17.96803 ± 0.02114	
*a**	52.11104 ± 0.10736	51.97708 ± 0.02501	
*b**	43.117457 ± 0.03457	43.58709 ± 0.03830	0.006
*h**	0.6912359 ± 0.00141	0.697828 ± 0.00067	0.02
*c**	67.636382 ± 0.06068	67.834 ± 0.00545	0.04
E420	3.1632725 ± 0.21705	3.072845 ± 0.00586	
E520	4.6828025 ± 0.02499	4.809038 ± 0.00988	
E620	1.046735 ± 0.00552	1.05047 ± 0.00132	
CT	0.6753944 ± 0.04275	0.638973 ± 0.00009	
Colour intensity	8.89281 ± 0.24756	8.932353 ± 0.01706	

$\Delta E_{(R-S)}$ 0.37

The last column contains the result of the *t* test

Table 5 Chemical data

	R	*S*
pH	3.39	3.39
Total acidity (g/L)	5.63	5.7
Volatile acidity (g/L)	0.445	0.478
Total polyphenols (mg/L)	1553.63	1519.84
Total antocians (mg/L)	204.79	201.96
Total flavonols (mg/L)	1705.68	1680.96

Table 6 Volatile composition (μg/L) of the wines aged in a sulphited barrique (S) and in a cryosandblasted barrique (R)

	S	SD	*R*	SD	*p* value
Cyclotene	275.37	±32.8	292.59	±2.83	
Guaiacol	3.95	±0.4	4.17	±0.01	
Trans-oak lactone	21.13	±1.0	29.20	±1.68	0.0283
Cis-oak lactone	19.71	±0.8	30.96	±1.14	0.0074
Methylguaiacol	0.26	±0.0	0.23	±0.01	
Maltol	168.65	±7.1	163.91	±7.92	
Eugenol	2.60	±0.22	4.37	±0.49	0.0427
Vanillin	274.05	±36.40	267.83	±16.34	
4-Ethylphenol	n.d			n.d	
4-Ethylguaiacol	n.d			n.d	

The *p* value resulting from the *t* test is reported

n.d not detected

in a slightly greater spicy note, along with a slightly higher intensity of cherry and jam notes. No significant differences were observed regarding the flavour, as bitter, astringency, structure, taste-olfactory persistence and taste balance were

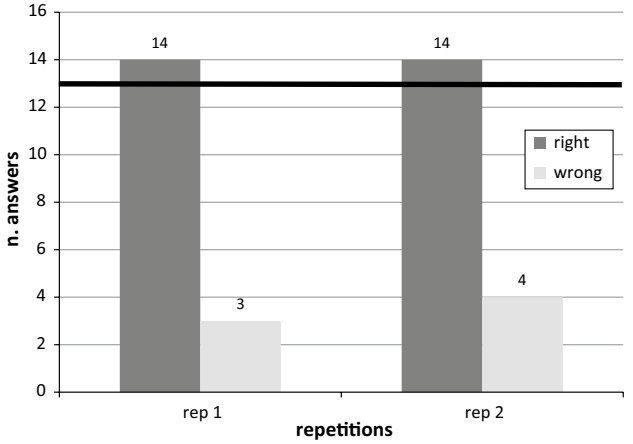

Fig. 1 Results of the duo-trio test two repetitions of the two wines obtained after ageing in the differently treated barriques. The *horizontal line* represents the limit of the right answer that defines the significance of the test for $p < 0.05$

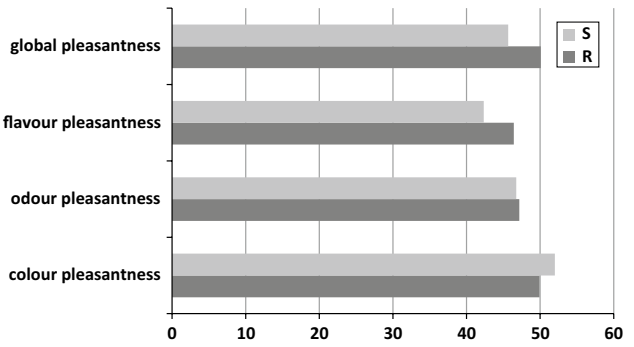

Fig. 2 Comparison of the pleasantness of the two wines expressed by the assessors regarding colour, odour and flavour

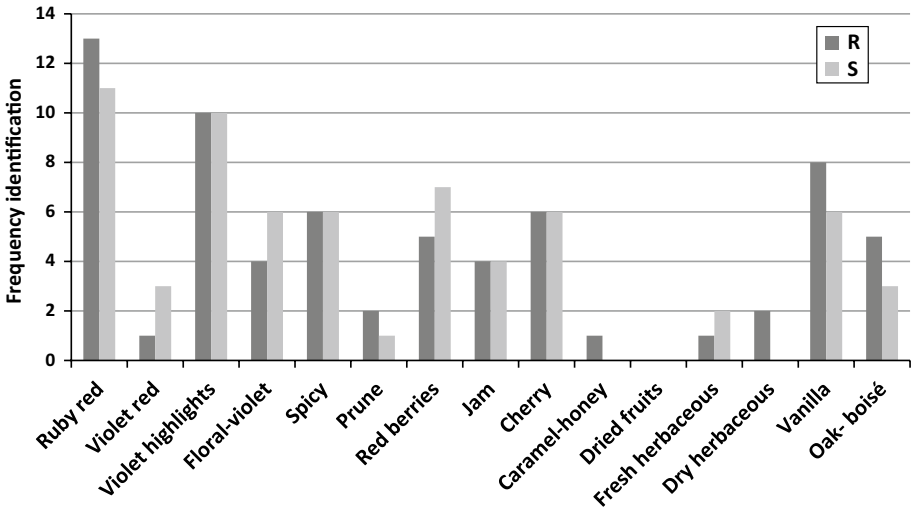

Fig. 3 Collection of descriptors related to the colour and odour of wine *R* and *S* after 6 months of ageing made by 14 tasters

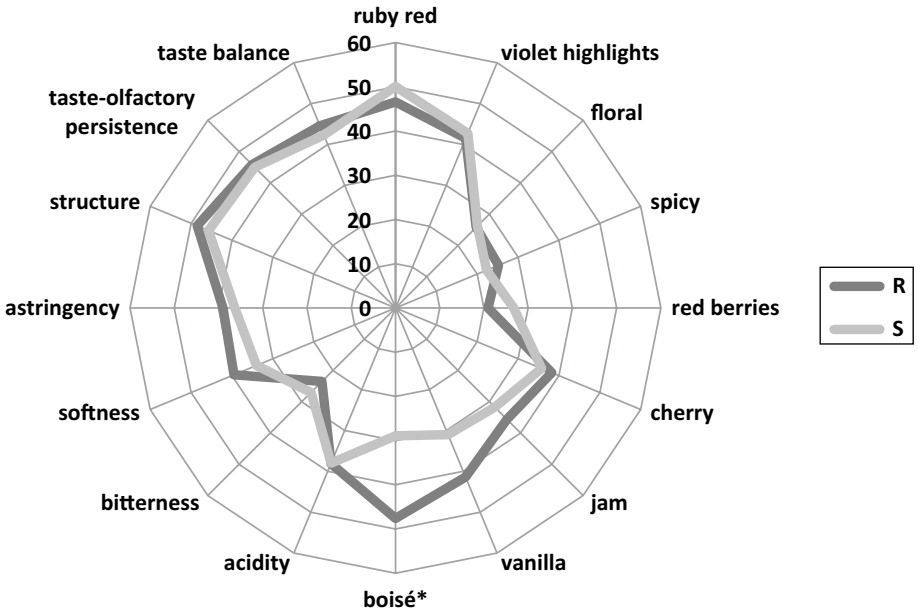

Fig. 4 Comparison of the sensory profiles of the two wines after 6 months of ageing showing the statistically significant differences (*asterisk*; one-way ANOVA)

very similar, and only softness intensity was found slightly more intense in the wine *R*.

These results were in agreement with the analytical results that showed levels of *cis*-oak lactone, eugenol and *trans*-oak lactone in greater concentration in wine R from the barrique treated with dry ice blasting.

Discussion

In this work, the microbiological, chemical and sensory effects of the treatment of barrels with dry ice blasting were studied.

Ageing in barrels represents a risk factor for the development of spoilage microorganisms, in particular, *Brettanomyces*. Chatonnet et al. [7] identified oak barrels as a niche for *Brettanomyces*, and it is known that repeated use of the same barrels increases the contamination risk; therefore, the sanitation of barrels is of primary importance to obtain wines of high quality.

In this study, the effect of dry ice blasting on the microbiological contamination of wood surfaces and in 2-L barrels showed a 98–100 % reduction in the microbiological contaminants.

Moreover, our results demonstrate that the dry ice blasting allows to deeply sanitize the wood, avoiding the contamination of the new wine, contrary to what it is observed with the widely used treatment with hot water. In fact, contaminated barrel treated with dry ice blasting did not spoil new wine.

Our results indicated that dry ice blasting has a quite good effectiveness in total yeast and bacterial population

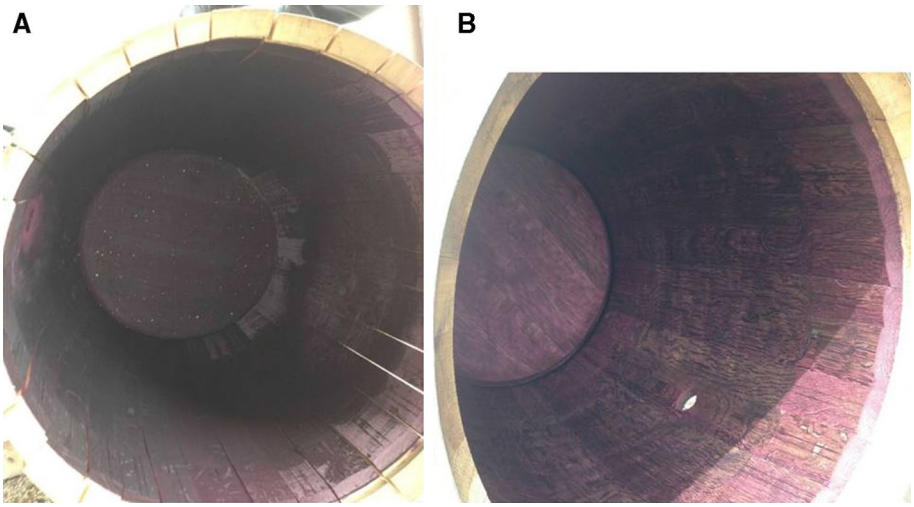

Fig. 5 Comparison of the inner surface of the barrique before (**a**) and after (**b**) the dry ice blasting treatment; this treatment removes deposits and tartrate crystals from wood

reduction (about 98 %). This effectiveness is better than that described previously [18] applying a microwave technology in staves obtained from two different types of barrels that eliminated 36–38 % of the total yeast population and 91–100 % of lactic acid bacteria. Likewise, this methodology also improved the results reported by Guzzon et al. [35] using UV treatment that resulted in 35 % of yeasts being eliminated, probably due to the roughness of wood which can shelter the microorganisms. In this line, thermal treatment has been described to eliminate about the 67 % of yeast population, and similar result was obtained with ozone. Guzzon et al. [23] analysed the effect of ozone in eliminating microorganisms using aqueous solution with different cell concentrations and reported that the mechanism of action was different among the species. Therefore, dry ice blasting treatment demonstrated a greater effectiveness in total microorganism population reduction than previously described methods.

Having assessed the microbiological effects, a third assay was performed to simulate real wine cellar conditions and to evaluate the effect of the rejuvenation of oak wood on wine; to this purpose, two barriques were used and dry ice blasting was compared with sulphur treatment. These differently treated barriques were used to age Barbera wine. After ageing, the aroma and the sensory profiles of the wines were analysed.

During the course of barrel ageing, red wine is exposed to air/oxygen and micro-oxygenation due to the porosity of wood. This oxygen exchange, the main process during wine ageing, is believed to be responsible for the improvement in wine organoleptic characteristics [36]. Oxygen can react with phenolic compounds in wine causing the formation of pigments that affect colour, flavour and the mouth feel of wine [37]. The main positive effects of ageing (controlled oxidation) include softening of tannins, development of complex aromas and improvement in the wine's body and mouth feel [38].

Likewise, the rejuvenation of barrels, achieved by dry ice blasting, also allows a better contact between wine and wood, which has a positive impact on wine characteristics. This was demonstrated in this study by analysing the aroma compounds, as the content of eugenol, *trans*-oak lactone and *cis*-oak lactone was statistically significantly higher in wine *R*. The oak lactones, responsible of oak/boisé aroma, are naturally present in wood, and their concentration varies according to the species and the geographical origin [39]. Our data demonstrate that dry ice blasting increases oak/boisé aroma in wine, as it enhances the extraction of the lactones and volatile aroma from the used wood by the elimination of residues on the inner wood surface (Fig. 5).

The wine sensory profiles presented here supported the chemical data. The *panel* firstly recognised that the two wines were different with the duo-trio test, and subsequently recognised in wine *R* a statistically significant increase in wood/boisé notes and a higher intensity of vanilla; in the mouth, wine *R* resulted softer than wine *S*. No differences related to the colour were detected according to ΔE analysis [34].

Previous studies [40, 41] have quantified the threshold values of some compounds in hydroalcoholic solutions: vanillin 0.065 mg/L, cis-oak lactone 0.025 mg/L, trans-oak lactone 0.11 mg/L and guaiacol 0.02 mg/L. The threshold values in wine showed that the "just-noticeable difference" for oak lactones is 10 µg/L, although it may be reduced 50-fold in the presence of vanillin levels similar to that in oak-aged barrels [42]. These findings are in accordance with the ones reported here, as the presence of significative differences in oak lactones, together with the presence of vanillin, was consistently linked to the respective sensory descriptors of wines.

During wine ageing in the barrel, volatile compounds extracted from oak wood contribute to aromatic notes of vanilla, smoke and spices [43]. Once extracted, the compounds from oak wood can undergo chemical or biochemical transformations in the wine [44], and as a consequence, they can modify their concentration [4, 45, 46]. The oak compounds which mainly contribute to wine aroma are: guaiacol (burnt overtones), oak lactone (woody and vanilla notes), eugenol (spices, cloves character) and vanillin (vanilla character). In this line, effectiveness similar to that described here, microorganism kill rate of >97 % and removing of tartrate deposits, has been described using high-power ultrasonics (HPU) to sanitize barrels [19]. However, dry ice blasting conferred an increased note of vanilla and boisé to wine, effects that were not observed by Schmid et al. [19] after duo-trio test, as no sensory differences were found comparing HPU with hot water cleaning.

Dry ice blasting uses small pellets of dry ice sprayed through a jet nozzle in combination with compressed air to remove paints, oil, grease, dirt, ink, adhesives and other materials. Thus, it can be applied in oenology for the treatment of used barrels, as it removes the sediments and tartrates and allows wine to be in contact with regenerated wood. Upon immediate impact, the dry ice sublimates into environmentally safe and innocuous CO_2 gas. This treatment does not use chemicals or generate waste effluents, neither does it produce any by-products, making it ecologically friendly. It can also improve chemical and toxicological safety and is more sustainable from an economic and environmental point of view. Dry ice blasting gives also advantage in terms of time and costs; in fact, the treatment for barriques takes about 25 min, a very short time when compared with other used methods for wood regeneration, with a cost of about 100 euros/barrique.

Even more, employment of dry ice blasting allows avoiding, or reducing, the use of SO_2 for barrel cleaning and, therefore, can be a valid alternative to SO_2 treatment. Control of the use of sulphites in food and beverages is important due to its effects on human health, as it can cause allergic reactions, headaches and asthma [47].

Conclusion

In this study, we analysed the effect of dry ice treatments on wine and showed that it is a technique that offers several advantages to currently used methodologies. It allows removal of deposits on wood, and therefore, it regenerates the barrique. In fact, wine aged in a dry ice blasted barrel increases its aromatic profile, enhancing the vanilla and boisé notes, due to the barrique being rejuvenated. This was demonstrated both by chemical and sensory analysis. Moreover, since oak barrels can be a source of contamination, their sanitization is fundamental to preserve wine quality and human health. Hence, as prevention of spoilage microorganisms plays a central role in the winemaking process, microbiological effects of the treatment were also investigated, showing that dry ice treatment allows a good sanitization of the wood, comparable to that of SO_2 treatment. Even more, since the current tendency is to replace SO_2 in winemaking, or reduce its use, this study demonstrated that dry ice blasting can be a valid alternative to SO_2 for the cleaning treatment of used barrels.

Finally, dry ice blasting also confers an economic advantage for the winemaker, because the barriques are usable for at least one more year, treatment for barriques takes about 25 min and cost is about 100 euros/barrique. Ultimately, the treatment does not leave residues, thus offering advantages for the environment.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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