Postharvest ozone fumigation of Petit Verdot grapes to prevent the use of sulfites and to increase anthocyanin in wine

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Abstract

Background and Aims: The request by the consumer for safer food is also pushing the wine sector to find alternative solutions to sulfur dioxide. The aim of this research has been to evaluate the application of ozone as a sanitising agent of grapes before vinification in order to avoid the use of sulfur dioxide.

Methods and Results: Postharvest ozone fumigation overnight of Petit Verdot grapes increased anthocyanin concentration by 19% versus 9% in the Control (untreated) during fermentation/maceration. Fermentation kinetics were faster in wine made from ozone-treated grapes (16 vs 21 days for the Control), and extraction of phenolic substances and anthocyanin was more rapid than that in the Control wines, Ozone treatment significantly reduced the count of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts but also significantly decreased the acetic acid bacteria. The final wine produced from ozone-treated grapes was characterised by low volatile acidity (similar to that of the Control wine) with a significant reduction in sulfur dioxide (17 and 8 mg/L, respectively, total and free sulfur dioxide). Sensory evaluation revealed a strong fruity aroma.

Conclusions: Ozone gas treatment of grapes reduced the microbial count significantly and increased the extraction of phenolic substances and the aroma of the final wine.

Significance of the Study: Postharvest ozone fumigation can be used to produce wine without sulfur dioxide.

Keywords: *anthocyanin, grape, microbial count, ozone, sensory evaluation, SO_2*

Introduction

Sulfur dioxide (SO_2) is the most commonly used preservative in the wine industry because of its antimicrobial and antioxidant properties. Unfortunately, SO_2 has some well-known drawbacks for human health. Moreover, the addition of SO_2 to wine can modify the sensory properties of the wine, by neutralising the aroma and by causing aroma defects (Raposo et al. 2016). Several technologies, such as pulsed electric field, ultrasound, high pressure and UV light have been proposed in order to prevent the use of SO_2. In addition several chemical additives have been tested, such as colloidal silver complex, dimethyl dicarbonate, ascorbic acid, hypophosphorous acid, thiodipropionic acid, Trolox C, stannous chloride, sodium hypochlorite, Sporix and hydroxytirosol, as well as natural products such as lysozyme and bacteriocins.

Postharvest ozone fumigation of grapes has been suggested as a means to produce wine without the addition of SO_2 (Purovino, PC Engineering, Uggiate Trevano, Italy). In addition, postharvest ozone treatment, which is generally used for sanitation purposes, may help to increase several fractions of phenolic substances in tablegrapes and winegrapes (Artés-Hernández et al. 2007, Mencarelli et al. 2011, Carbone and Mencarelli 2015).

Ozone can change the aromatic profile of winegrapes favouring glycosylation (De Sanctis et al. 2015) but can also induce the formation of small, but significant, amounts of compounds of sugar oxidation, such as furaldehyde (sweet, brown, woody, bready, caramellic and with a slight phenolic nuance), hydroxymethylfurfural (fatty, buttery, musty, waxy and caramellic) and 3-hydroxy-2,3-dihydromaltol (sweet, caramellic, cotton candy, jammy fruity and burnt with bready nuances), in white winegrapes treated with ozone at low temperature (Carbone 2015). These compounds are generally the result of sugar dehydration and are indicative of maderisation, heating of wine or heat concentrated must, that is, a strong oxidation process (Williams et al. 1983, Pereira et al. 2010). It is also well known that plant responses to ozone (0.15–0.30 mg/L in air) produce isoprene, monoterpenes and C6 compounds (Loreto and Schnitzler 2010). Ozonolysis of isoprene produces methylvinylketone and methacrolein, the first in reaction with phenol produces raspberry ketone, and the second has a wild hyacinth aromatic nuance (Vickers et al. 2009).

Petit Verdot is a common cultivar in the Médoc wine region of France but, in the last few years, because of the favourable climate conditions, it has been grown in central Italy, from Tuscany to Latium. Because of its particular aromatic characteristics, Petit Verdot has been nicknamed 'seasoning' or 'spice box' to highlight that this cultivar should be used only in small amounts in wine blends because it provides strong small red fruit flavours and highly intense colour. Depending on the particular terroir, the production of 100% Petit Verdot wine is becoming increasingly common.

Lima et al. (2011) compared the colour features and concentration of phenolic substances of three cultivars known for their colour intensity, Petit Verdot, Tempranillo and Shiraz, grown in Brazil. Petit Verdot wine had a colour intensity (IC) significantly higher than that of the wines made from the other two cultivars. Sivilotti et al. (2011) defined Petit Verdot grapes as a cultivar that reaches full ripening with difficulty, is sensitive to powdery mildew, with a concentration of phenolic substances, anthocyanin and proanthocyanidin of 1623, 970 and 2258 mg/kg, respectively. These values are much higher than

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those of Merlot but significantly lower than those of other colourant cultivars, such as Lancelotta, Lambrusco Maestri, Tintoria and Turchetta. The same authors also found a high concentration of proanthocyanidin in the berry seeds. Cejudo-Bastante et al. (2011) characterised the aromatic compounds of Petit Verdot and found that esters, such as ethyldecanoate and ethyloctanoate, which were the highest in concentration, decreased with microoxygenation while the nor-isoprenoids were increased. They reported notes of nut fruits, red plum and currants, which are the main aromatic attributes of Petit Verdot.

In this work, we tested the effect of postharvest ozone fumigation on Petit Verdot grapes to produce wine without the addition of SO_2. We monitored the microbial content of the grapes, the fermentation pattern, grape and wine composition and the sensory characteristics of the wine in comparison with those of a Petit Verdot wine made with the addition of SO_2.

Materials and methods

Material and postharvest treatment

Petit Verdot grapes were picked at the Falesco Winery (Montecchio, Italy) when ripe (235–240 g/L reducing sugars). The ozone fumigation was applied to 500 kg (treated) with a similar quantity used for the Control (untreated). Bunches were hand harvested and placed in perforated boxes (60 × 40 × 30 cm) that are normally used for grape dehydration. The boxes were taken to the postharvest laboratory and placed in a cold room overnight (12 h) at 4°C and 70% RH with normal ventilation from cooler fans. Grapes were fumigated with ozone (max 20 g/h with 6% w/w of ozone) with a flow rate at maximum 150 NL/h (NL= normal litre) rate in a 9 m^3 cold room (Ozone generator A series, PC Engineering, Uggiate Trevano, Italy). The Control grapes were handled under similar environmental conditions in another cold room; however, they were powdered superficially with potassium metabisulfite without any specific calculation, to cover lightly the surface of the top layer of bunches to avoid cross contamination. The next morning, the ozone-treated grapes were vinified with equipment that was treated with ozonated water before starting vinification.

Vinification

The following steps were undertaken: destemming and pressing, prefermentation maceration at 8 ± 1°C for 3 days in a stainless steel tank, heating to 22 ± 1°C and yeast inoculation (20 g/hL) with rehydrated commercial *Saccharomyces cerevisiae* (Premium Supertuscan, Vason, Verona, Italy). The must was fermented at 24 ± 1°C with the following steps: day 1, two pump-overs (no air), two pump-overs (air); day 2 and 3, one pump-over (no air), one pump-over (air); and day 4, one pump-over (air).

Between day 5 and the end of sugar fermentation (0.5–0.6 g/L), the fermentation was subjected to one pump-over per day, moving 10% of the whole mass. At the end of the fermentation, the headspace of the tank was saturated with carbon dioxide for an 8-day post-fermentation maceration. The treated and Control wines were racked directly into 225 L barrels that had been previously washed with ozonated water and saturated with ozone gas until filled with wine. In both wines, malolactic fermentation started spontaneously, without inoculation.

In the Control vinification, SO_2 (as potassium metabisulfite) was added at the beginning of fermentation (5 g/hL) and after malolactic fermentation (3 g/hL). No SO_2 was added to the Treated wine. The wines were aged in barrels with fine lees for 4 months.

Analyses

For the must and wine, five 750 mL bottles were set aside for analysis of ethanol, reducing sugars, pH, titratable acidity (TA), volatile acidity, total and free SO_2, phenolic substances, anthocyanin and colour index (Organisation Internationale de la Vigne et du Vin 2009).

The phenol extractability indices are usually assessed in accordance with the procedure proposed by Glories and Augustin (1993) and Saint-Cricq et al. (1998). In our case, only anthocyanin was measured. Ten replicates of 1 kg of grape berries were used for treated and Control grapes, which were macerated at two pH values (3.2 and 1.0) for 4 h. The extracts were filtered and centrifuged for 3 min at 1200 *g* before analysis. The concentration of anthocyanin at pH 3.2 and pH 1.0 was measured spectrophotometrically at 540 nm according to Ribéreau-Gayon and Stonestreet (1965) (Lambda 3B UV-vis spectrophotometer, Perkin-Elmer Instruments, Seer Green, Beaconsfield, England) and expressed as mg/L of the prevalent anthocyanin in grapes (malvidin-3-glucoside). The concentration of tannin, expressed as mg/L, in the grape skin and seed was measured according to Glories' method as reported in Kontoudakis et al. (2010).

After each postharvest treatment, the number of bacteria and yeasts on the treated and Control grapes was determined by media serial dilution plating. Ten grape bunches were sampled in different positions of the cold room, and individual grape berries were randomly and aseptically removed from each grape bunch to obtain samples of about 50 g, replicated threefold. Yeasts and bacteria on the grapes were detected and quantitatively estimated (as cells/mL) through the direct plating method (Fleet et al. 1984).

Treated and Control wines were assessed by a panel of mainly winemakers with experience in wine tasting. Because of this experience in wine tasting but not in sensory analysis for scientific purposes, the panel was trained in descriptive sensory analysis using discriminative tests. The wines were assessed in a standard sensory analysis laboratory at our department, following all the procedures for wine tasting (Italian Ministry of Agriculture, Food and Forest Policy 2011). After sniffing and tasting, judges used a 10 cm unstructured scale as described by Cejudo-Bastante et al. (2011).

Statistical analysis

The data were subjected to ANOVA and significance was evaluated for $P < 0.05$. Mean values were compared by Tukey's test ($P < 0.05$ or 0.01) using GRAPHPAD PRISM 3.05 (GraphPad Software, La Jolla, CA, USA).

Results and discussion

Ozone treatment did not affect the sugar concentration, TA and pH of the grapes. Neither did the cold overnight treatment without ozone affect these parameters. Ozone, however, significantly increased the concentration of anthocyanin and skin tannin (Table 1). The concentration of anthocyanin (pH 1) rose significantly, compared with that in the berry at harvest, after the ozone treatment (about 16%) but increased by only 6% in the untreated grapes. Anthocyanin at pH 3.2 increased even more (about 19%) in ozone-treated berries compared with that in the berries at harvest, while the increase was about 9% in the Control grapes. The values of the extractability index in the ozone-treated and Control samples were lower than at harvest; this indicates a greater extractability of anthocyanin,

Table 1. Composition of Petit Verdot musts at harvest and after ozone treatment of grapes compared with that of Control grapes.

Attribute	Harvest	Ozone-treated	Control
Total sugars (g/L)	238±10a	240±5a	235±8a
Titratable acidity (g/L)	6.4±0.4a	6.3±0.4a	6.2±0.2a
pH	3.4±0.1a	3.5±0.0a	3.5±0.1a
Anthocyanin pH 3.2 (mg/L)	589±11c	702±15a	643±18b
Anthocyanin pH 1.0 (mg/L)	708±18b	821±17a	754±16b
Extractability (%)	16.8a	14.5b	14.7b
Skin tannin (mg/L)	24.3±0.8b	28.1±0.8a	25.8±1.0b
Seed tannin (mg/L)	35.8±0.9a	35.2±1.0a	36.5±1.4a

Values are the means of ten analyses ± standard deviation of grape bunches sorted at winery arrival and in the cold room after treatment. Means in the same row followed by the same letter are not statistically different ($P < 0.05$).

because the lower the index value, the greater is the extractability. Skin tannin was also affected by the ozone treatment, increasing by 14%, while that in the Control sample remained unchanged. Seed tannin was not modified by ozone treatment and there was no significant difference among samples.

Ozone is considered as one of the most powerful oxidative stressors. In addition to microbial sanitation for which it has been used in the food industry (Khadre et al. 2001) and in particular with fresh fruits and vegetables (Horvitz and Cantalejo. 2014), interest in ozone treatment is related to its stress action, which stimulates the biosynthesis of phenolic substances in tablegrapes (Gonzáles-Barrio et al. 2006, Artés-Hernández et al. 2007, Yaseen et al. 2014).

Ozone induces an increase of different phenolic fractions such as flavonols and flavanols not only in tablegrapes but also in red (Mencarelli et al. 2011) and white cultivars (Carbone and Mencarelli 2015). Heath (2008) stated that 'when plants are observed under a low dose of ozone, some physiological and metabolic shifts occur'. He concluded that ozone concentration and the time of treatment can lead to different metabolic responses: a short pulse activates the ethylene/wounding pathway, while a longer treatment affects wall synthesis and secondary products. Phenolic substances are known as secondary metabolites, and they mainly play an antioxidant role. After ozone enters the leaf tissue, it reacts first with extracellular antioxidants (i.e. phenolic substances) that appear to stimulate ascorbate production and/or how ozone moves between compartments. The production of hydrogen peroxide (H_2O_2) next to the membrane appears to both trigger a pathogen-like response and generate more H_2O_2 (Heath 2008).

An oxidative burst can be the main cause of grape veraison with the stimulation of the colour pigments in red grapes, where H_2O_2 plays a key role (Pilati et al. 2014). Here, by speculation, the observed increase in anthocyanin and skin tannin might be the reaction of berry cells to a light to moderate stress (low ozone flow rate), which induces the antioxidant-protective response with synthesis of phenolic substances. In contrast, even at low concentration, long term ozone treatment can have a negative impact on the concentration of phenolic substances (Botondi et al. 2015). In our tests, extractability increased, however, the increase occurred also in the control grapes. Thus, the effect is more ascribable to the 10°C temperature of the treatment than to ozone. Indeed, Heredia et al. (2010) showed that holding grapes in cold-storage for 24 h prior to crushing leads to wines with a more intense and stable colour.

A significant difference in the alcoholic fermentation between the Control and treated samples was observed in the first 10 days (Figure 1). The curves are typical fermentation curves,

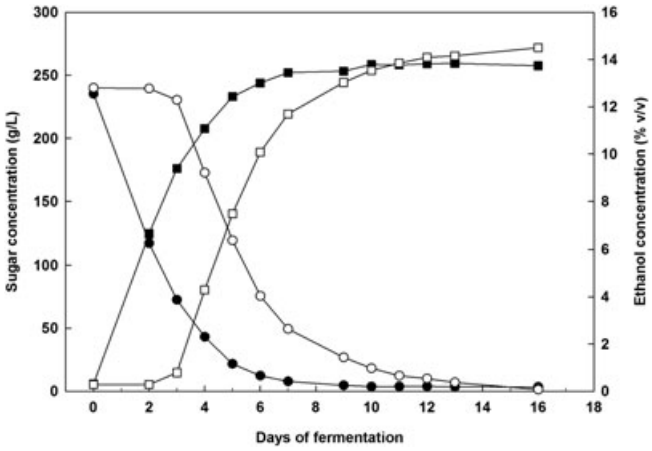

Figure 1. Effect of ozone fumigation (●,■) of Petit Verdot grapes on sugar consumption (●,○) and alcohol production (■,□) during fermentation of musts compared to that of untreated (○,□) grapes. Data are the means of five bottles. A significant difference ($P < 0.05$) between the two curves applies to the whole fermentation process, except from day 9 until the end.

but a difference between the Control and untreated samples was noted. After 10 days, sugars decreased from 240 to 1.3 g/L in the treated sample. In the Control, however, fermentation started after 3 days and had not finished completely after 10 days, requiring a further 6 days to fall below 2 g/L of residual sugars. The delay in the start of fermentation was due to the latent period as reported by Ribéreau-Gayon et al. (2006a), but this delay was not observed in must from treated grapes. The final concentration of ethanol was 14.5 and 13.9% v/v in the Control and treated wines, respectively, after 16 days.

In samples treated with ozone, a significant reduction was observed in acetic acid bacteria, different lactic bacteria, yeasts such as *Hanseniaspora uvarum* and *Pichia membranifaciens*, but above all in *S. cerevisiae* (Table 2). Cleaning of the surface of the berries by ozone gas led to the immediate start of fermentation after the yeast inoculation, without the yeast having to compete with other microorganisms.

The significant presence in our grapes of non-*Saccharomyces* yeasts, such as *Hanseniaspora* (*Kloeckera*), *Pichia* and *Zygosaccharomyces*, makes it difficult to monitor the fermentation process properly and to obtain a final product with a consistent composition, especially given the high number of bacteria. The massive inoculation of commercial yeast, *S. cerevisiae*, which places this microorganism in an improved position to dominate, usually ensures a stable wine composition. In our case, despite the inoculation of the Control must

Table 2. Effect of ozone treatment of Petit Verdot grapes on the microbial count of the berry surface compared to that of Control grapes.

	Cells/mL	
	Ozone-treated	**Control**
Acetic acid bacteria	46×10^3	29×10^4*
Lactobacillus brevis/hilgardii/fermentum	<40	40
Lactobacillus casei/paracasei/mali/nagelii	<40	110*
Lactobacillus kunkeei	<40	<40
Lactobacillus plantarum	<40	40
Oenococcus oeni	<40	<40
Pediococcus species	<40	40
Brettanomyces bruxellensis	<40	<40
Hanseniaspora uvarum	22×10^3	35×10^3*
Pichia membranifaciens	40	1.9×10^3*
Saccharomyces cerevisae	<40	860×10^3*
Zygosaccharomyces bailii	70	340*

Data values (cells/mL) are the means of ten microbial analyses from ten sets of berries from different bunches from each treatment group. Asterisk (superscript of the highest value) indicates a significant difference between the two sample values ($P < 0.01$).

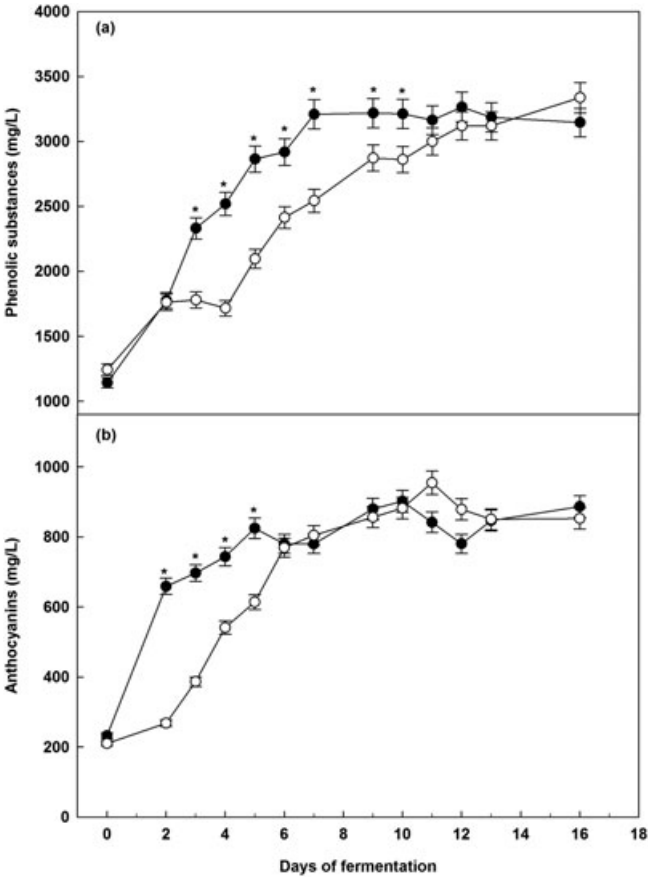

Figure 2. Effect of ozone treatment (●) of Petit Verdot grapes on (a) phenolic substances and (b) anthocyanins during the alcoholic fermentation compared to that of untreated (○) grapes. Data are the means of the analysis of five bottles. Bars refer to the (±) standard deviation; an asterisk indicates significant difference between the two sample values ($P < 0.05$).

with a commercial yeast, fermentation started slowly, and towards the end of the fermentation, the rate declined slowly. In contrast this did not happen in the treated must. Thus, the massive inoculation of commercial yeast is also insufficient to guarantee a regular fermentation pattern in Control must. It has been shown that non-*Saccharomyces* yeasts can develop to a significant extent during the early stages of juice fermentation (Fleet 2008, Ciani et al. 2010). The competition for nutrients has a significant effect on fermentation kinetics (Medina et al. 2012). Another factor that could be considered as the reason for the initial slow fermentation rate in the Control must is the presence of SO_2. Sulfur dioxide is reported to be important to control microbial development (it can be bacteriostatic and bactericide) in the must before the fermentation starts, and this is the reason why it is added to must before inoculation. It is also known that, at the beginning, SO_2 binds rapidly to sugars of the must and also to ketoacids and acetaldehyde, losing its antiseptic action. With respect to yeasts, addition of SO_2 is first and foremost to ensure a delay in the initiation of fermentation, allowing a limited cooling of the grapes (Ribéreau-Gayon et al. 2006b). The fermentation is also spread out over a longer period in this manner, avoiding an excessive rise in temperature. Sulfiting has also been considered to kill apiculate yeasts (*Kloeckera* and *Hanseniaspora*) developing before other yeasts (first few hours in the fermentor, coming from the grapes) and producing wines with lower alcohol concentration (Romano et al. 1992), but some doubts have been raised by Fleet and Heard (1993).

As a consequence of the rapid start of fermentation and the regular fermentation rate of treated must, the extraction of phenolic substances and anthocyanin was significantly greater than in the Control must (Figure 2). In the first 5 and 7 days, anthocyanin and phenolic substances, respectively, were extracted, while in the Control must, extraction required 11 and 16 days. Ozone treatment led to rapid and regular fermentation kinetics and increased the extractability of phenolic substances and anthocyanin.

This result is in contrast with that obtained on grapes. Indeed, in the extractability test performed on berries of the treated and Control samples (Table 1), the anthocyanin extractability index was similar, while during fermentation, the anthocyanin concentration (and also the concentration of phenolic substances) was significantly higher in the must from ozone-treated grapes. This discrepancy may be for two reasons.

First, the conditions of anthocyanin extractability from small samples of berries measured in the laboratory are completely different to that under commercial conditions. The results are thus only indicative. The homogenisation technique used in the laboratory is invasive; thus, it can mask small differences in resistance to extraction, which can be observed in a regular maceration/fermentation of a large mass in a tank where several factors play a role.

Second, in addition to the induction effect of ozone on the biosynthesis of phenolic substances as described earlier, ozone gas affects the cell wall texture and cell membrane composition (Vaultier and Jolivet 2015). Changes induced by ozone in cell wall components or by cell wall-produced H_2O_2 are potential initial signals of stress initiation. In tomato (10 mg/L applied for 10 min) and kiwifruit (0.3 mg/L applied over 2–4 months), ozone gas did not affect the cell wall disassembly, and inhibited cell wall degrading enzymes by reducing ethylene production and action (Rodoni et al. 2010, Minas et al. 2014). In grape, which is a non-climacteric fruit, ethylene does not play a major role in ripening, thus the cell reaction to ozone may be different and is strongly dependent on the concentration and exposure time. In tablegrape, ozone treatment at low concentration stimulated catalase activity but not lipoxygenase (Yaseen et al. 2014). In contrast in winegrape, shock ozone treatment

(1.5 g/h applied over 18 h) increased the activity of polygalacturonase and pectimethylesterase (Botondi et al. 2015). Using a high concentration of ozone (30 mg L applied over 24 h), Laureano et al. (2016) found an increase in berry skin resistance in winegrape. In unpublished research work done by our group in collaboration with California State University, Fresno and University of California, Davis (De Sanctis et al. 2014), we found that the cell wall disassembly in Cabernet Sauvignon treated with a variable concentration of ozone is strongly dependent on ripening stage and ozone concentration.

Our results appear to confirm the higher extractability of phenolic substances and anthocyanin from ozone-treated grapes, which represents a great advantage in terms of vinification time and thus cost. The greater extraction is also confirmed by the TA at the end of fermentation/maceration (Table 3), which was significantly higher in the treated sample than in the Control. The pH, as expected, was lower in the treated sample than that in the Control, while in grapes (Table 1), no difference was found before and after the treatment. Volatile acidity at the end of fermentation/maceration (Table 3) increased as expected but without any difference between the two samples.

After malolactic fermentation and wine stabilisation, the composition and sensory characteristics of the wines were analysed (Table 4). A significant difference was observed only for TA and anthocyanin with values higher in the treated wine. In contrast, as expected, total and free SO_2 were higher in the Control wine. Anthocyanin and TA values confirmed the findings observed at the end of fermentation, although the values were lower, as expected. The colour index confirmed the higher concentration of anthocyanin. While the TA was higher in the treated wine, the pH was not significantly different between the two samples, although it was slightly higher in the Control wine. The reason for this discrepancy regarding the pH and TA could be due to a buffer effect in the treated wine as consequence of acid buffering due to a greater extractability. Volatile acidity, which was the greatest concern for the wine without SO_2 protection, was similar in the two wine samples.

The treated wine showed a more intense red colour with purple nuances, which was emphasised by all the panellists. In fact, the panellists scored the treated wine higher than the Control for the olfactory sensations of fruity, small red fruits and particularly blackberry, which also received the highest score. Nuances of cherry liqueur, nail varnish and spices were perceived as significantly higher in the treated wine than in control (Table 5). No significant difference in the descriptions of the taste was noted. All the panellists scored the overall quality of the treated wine higher than that of the control, especially in terms of aroma.

The difference in fruity flavour has also been reported in other wines from grapes of different cultivars treated with ozone and appears to be a typical characteristic (http://www.wineresearchteam.it/en/; http://www.cellartracker.com/wine.asp?iWine=2025235). Only one paper on white wine from ozone-treated grapes has been published where grapes were characterised by a concentration of glycosylated volatiles higher than that of the untreated grapes (De Sanctis et al. 2015). Glycosylation is the chemical protection of cells from toxic defence compounds such as many volatile organic compounds (VOCs) (Maffei 2010); thus, it is possible that skin cells with increased VOCs due to the ozone effect tend to glycosylate these compounds.

Table 4. Effect of ozone treatment of Petit Verdot grapes on the composition of wines after malolactic fermentation and wine stabilisation compared with that of wines from Control grapes.

Attribute	Ozone-treated	Control
Alcohol (%)	13.9 ± 0.2	14.5 ± 0.2
Titratable acidity (g/L)	5.7 ± 0.1*	5.0 ± 0.1
Volatile acidity (g/L)	0.55 ± 0.04	0.53 ± 0.03
Free SO_2 (mg/L)	8 ± 1	36 ± 4*
Total SO_2 (mg/L)	17 ± 2	60 ± 5*
Reducing sugars (g/L)	0.5 ± 0.0	0.6 ± 0.0
pH	3.6 ± 0.0	3.8 ± 0.0*
Phenolic substances (mg/L)	3398 ± 32	3333 ± 36
Anthocyanin (mg/L)	620 ± 11*	542 ± 17
Colour index	16.8 ± 0.1*	16.1 ± 0.1

Data are the means of the analysis of five bottles (±standard deviation). Asterisk (superscript of the highest value) indicates a significant difference between the two sample values ($P < 0.05$). SO_2, sulfur dioxide.

Table 5. Effect of ozone treatment of Petit Verdot grapes on the main olfactory and gustative perceptions in a discrimination test of the wines compared to that of Control grapes.

Sensory perception	Ozone-treated	Control
Fruity	8*	6
Blackberry	8*	6
Red fruits	7*	5
Cherry liqueur	5*	3
Nail varnish	5*	3
Citrus	3	3
Flowery	4	5
Spicy	6*	4
Tobacco	3	3
Acidity	5	4
Astringency	5	6
Body	6	6
Global quality	8*	6

Data are the means of the tastings of three bottles. Asterisk (superscript of the highest value) indicates a significant difference between the two sample values ($P < 0.01$).

Table 3. Effect of ozone treatment of Petit Verdot grapes on the titratable acidity, volatile acidity and pH of wines compared with that of wines from Control grapes.

Attribute	Ozone-treated SF	Ozone-treated EF	Control SF	Control EF
Titratable acidity (g/L)	6.6 ± 0.1a	6.4 ± 0.1a	6.1 ± 0.1b	6.1 ± 0.1b
Volatile acidity (g/L)	0.01b	0.32 ± 0.04a	0.01b	0.27 ± 0.02a
pH	3.5 ± 0.0bc	3.5 ± 0.0c	3.6 ± 0.0ab	3.6 ± 0.0a

Data are the means (±SD) of five bottle analyses. Different letters in each row indicate a significant difference among samples ($P < 0.05$). EF, end of fermentation; SF, start of fermentation.

Ozone is a damaging pollutant for plant cells, and one of the first recognised ozone effect is the denaturation of the lipid in cellular membranes. Thus volatiles that are associated with lipid peroxidation are also emitted more in ozone-stressed leaves. Compounds such as C6, methanol and methylsalycilate are markers of ozone damage (Loreto and Schnitzler 2010), but also isoprene and monoterpenes are emitted in response to acute and heavy doses of ozone (150–300 µg/L) (Calfapietra et al. 2009). In addition, acyl-transferases that catalyse the transfer of an acetyl group from acetyl-CoA to an alcohol for the formation of esters are considered as modifying enzymes in the formation of volatile compounds emitted by plant cells under stress conditions (D'Auria 2006, Pichersky et al. 2006).

In wine, the perception of a fruity flavour is usually attributed to the formation of esters or nor-isoprenoids. Cejudo-Bastante et al. (2011) reported that these compounds are characteristic of Petit Verdot. Ester formation (ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl hexanoate and 2-phenylethyl acetate) with fruity nuances occurs especially during fermentation because of yeast activity (Swiegers et al. 2005), but the ozone may have affected the VOC metabolism of the skin cell as suggested earlier. Sulfur dioxide is known to neutralise aromas and to bind to sugars and ketoacids, both of which are involved in the formation aroma compounds. Roussis and Sergianitis (2008) have shown in model white wine that SO_2 does not protect esters during storage for 60 days, while good protection was possible by a mixture of caffeic and gallic acids. As a higher concentration of hydroxycinnamic acids, which include gallic and caffeic acids, has been found in ozone-treated grapes (Artes-Hernández et al. 2007, Carbone and Mencarelli 2015, DeSanctis et al. 2015), we can hypothesise that these compounds provide a protective effect against ester loss and thus give a greater fruity aroma to the wine.

Conclusions

The postharvest ozone treatment of grapes in a cold room at 10°C was for one night and at a low ozone concentration. Energy consumption for the ozone generator was less than 1 kWh. Thus, the treatment is affordable. In our case, Petit Verdot wines without SO_2 addition did not suffer from volatile acidity and showed a higher concentration of anthocyanin and a strong fruity flavour. It is imperative to take into account the condition of the grapes: bunches must be sound thus harvest must be manual. In conclusion, this treatment has good prospects for the production of wine without SO_2 addition but also for increasing the fruity flavour of wine. Finally, the aspect of ageing must be taken into account. This type of wine without addition of SO_2 requires a constant monitoring to ensure that no cross contamination occurs especially when they are stored in barrels. In our experience with different cultivars of red wines, if attention is paid to sanitation, no problem of contamination occurs.

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