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Effect of some winemaking factors on rotundone levels of *Pelaverga di Verduno* wines

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Abstract

Pelaverga di Verduno Protected Designation of Origin is a red wine obtained from *Pelaverga piccolo*, an autochthonous grape variety grown in Piedmont. This wine is characterized by a light colour and a unique, intense spicy aroma. The analysis of this wine detected a significant concentration of rotundone (approximately 40 ng L^{-1}), which is known to impart a distinctive pepper note and has a very low olfactory threshold (16 ng L^{-1} in wine). The measured concentrations, well above the perception threshold, could link rotundone directly to the spicy notes highlighted by the sensorial analysis of *Pelaverga* wines. The impact of certain oenological factors on the concentration of this compound in wine was therefore assessed, showing that moderate amounts of oxygen could lead to a significant increase in the concentration of rotundone in finished wines. Conversely, different doses of sulfur dioxide or storage temperature of the wines had no effect on this compound.

Keywords Rotundone · Sesquiterpenes · Oxygen · Wine aroma · Spicy · Pelaverga

Introduction

The aromatic compounds present in grapes play a fundamental role in the aromatic characterization of the wine. Some of them, such as monoterpenes, have been extensively studied [1] and their fundamental role in determining the characteristic aroma of wines obtained from certain varieties, such as Muscat, is well known [2]. Similarly, the varietal compounds, known as polyfunctional thiols, have a key role in defining the unique aroma of wines obtained from "semi-aromatic" varieties such as Sauvignon Blanc [3]. However, only recent research has investigated the sensory role in wine of a large group of natural grape-derived terpene compounds, sesquiterpenes. Most of them are polycyclic compounds characterized by different structures functionalized with oxygen. Bicyclic sesquiterpenes and, in particular, those characterized by the guaiane structure, are the most important from a sensorial point of view. They are related to generic spicy and balsamic notes while their biological role appears to be linked to that of repellents for herbivores or attractants for their predators [4]. α-guaiene is a molecule generated enzymatically from germacrene-A and is considered to be the precursor of a highly important odorant called rotundone [5]. Rotundone, which has a very low perception threshold (approximately 16 ng L^{-1} in wine), has been closely linked to an intense scent of black pepper that is characteristic of some wines [6]. It was first identified in Australian Shiraz wines [6], and subsequently found in other wines, namely Duras, Gamay and Graciano [7]. Moreover, recently, the quantitative analysis of 77 Spanish wines highlighted that its concentration was extremely variable among wines and above the perception threshold level in only 2 wines, from Camaru and Negral grapes [8]. With regards to Italian grapes and wines, recent research has highlighted high concentrations of this compound in Vespolina, Schioppettino, Grüner Veltliner and probably Groppello di Revò [9] with concentration up to 5.44 μg kg^{-1} in Vespolina grapes, but lower levels are retrieved in wines probably due to a low extraction yield during winemaking, ranging from 5 to 7% of the initial concentration. Moreover, preliminary

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olfactometric analyses also highlighted the possible presence of this compound in *Ruchè*, *Grignolino* and *Verduno Pelaverga* wines, but in this case no quantification was carried out [10].

Numerous research studies have revealed how both maturity and growing conditions affect its concentration in grape. In this regard, vineyard microclimate can play a pivotal role on its distribution both within the vineyard and within the bunch zone. Generally, cooler conditions improve its accumulation and, in particular, the air temperature of the bunch zone seems to be the major factor affecting the final concentration of rotundone in grapes [11–14].

On the other hand, until now, only a few studies have assessed the impact of oenological factors on the rotundone content of wines [9]. Recent research [15] has indicated that some winemaking techniques, such as the use of macerating enzymes, do not lead to significant changes of the rotundone concentration in wines. Sesquiterpenes can be found in the grape skin predominantly in the free form, and not as glycosides, and therefore, mainly physical factors, such as pressing intensity and maceration time, can presumably promote the extraction of rotundone from grapes and subsequently its final content in wine [4]. Finally, with regards to the hypothesis of any chemical transformation that may positively (generation from precursors) or negatively (conversion in odorless compounds) change the rotundone content in wine, there is a distinct lack of knowledge, to date, of the effect of sulfur dioxide (SO_2), oxygen and/or temperature control during storage on rotundone content in wines.

The *Pelaverga piccolo* is a minor variety cultivated in a small area near Barolo, in Piedmont. The PDO (Protected Designation of Origin) *Verduno Pelaverga* Wine is obtained from this cultivar in addition to a maximum 15% of non-aromatic red grape varieties cultivated in Piedmont. In 2018, 1.193 hL were produced by just 31 cellars [16]. The wine is characterized by a pale ruby colour with garnet hints, due to the low level of anthocyanidins and dominance of disubstituted anthocyanins [17, 18]. However, the peculiarity of *Verduno Pelaverga* wine is its unique aroma, which is characterized by very intense spicy, black and white pepper notes that tend to change into liquorice and cloves during bottle storage [19]. Key wine aroma compounds, including rotundone, are important from both varietal characterization and sensorial point of view and their concentration in wines can be modulated through appropriate agronomic and oenological techniques. This knowledge is essential for the development and implementation of specific winemaking protocols for certain grapes, aimed to highlight their qualities and uniqueness and represents a necessary step for the development of varietal oenology. Thus, the present study aimed not only to relate, for the first time, the typical spicy pepper notes of *Pelaverga* wine to the chemical composition of this wine, but also to verify how some important oenological parameters, such as storage temperature, oxygen and SO_2 content, could influence the concentration of rotundone in wines.

Materials and methods

Chemicals and reagents

The rotundone standard was provided by Eptes (Vevey, Switzerland). All other standards were purchased from Merck (Merck KGaA, Frankfurter Strasse 250, Darmstadt, 64293, Germany).

Wine samples and experimental plan

The *Verduno Pelaverga* PDO wine (27 L) was kindly provided by the winery *Azienda Fratelli Alessandria* (Via Beato Valfrè, 59, 12060 Verduno, Cuneo, Italy). It was obtained from 100% *Pelaverga piccolo* grapes during the 2018 vintage. Spontaneous fermentation was carried out in steel tanks (50 hL) for 6–8 days at a controlled temperature of 25 °C. After raking off, spontaneous malolactic fermentation took place in steel or tanks at 25 °C, usually the wine is bottled in the spring of the following year. The wine used for the experiment had the following general composition: ethanol 14.0% v/v, titratable acidity 5.44 g L^{-1} as tartaric acid, reducing sugars 1.4 g L^{-1}, pH 3.45, volatile acidity 0.54 g L^{-1} as acetic acid, malic acid 0.2 g L^{-1}, sugar-free extract 26.7 g L^{-1}, total polyphenols 1808 mg L^{-1} and total SO_2 was lower than 30 mg L^{-1}.

The effect of oxygen concentration, SO_2 content and temperature on *Pelaverga* rotundone levels was studied according to the experimental protocol reported in Table 1. Wine was bottled in clear Bordeaux style 750 mL glass wine bottles and sealed with natural cork. The bottles were stored under controlled temperature (4 and 20 °C), in a vertical position and in the darkness for the entire time of the test. A complete factorial plan with three factors (SO_2, oxygen and temperature) at two levels was followed for a total of eight trials. Each trial was performed in triplicate, for a total of 24 bottles. A micro-oxygenator Parsec S.A.En. 4000 (Parsec s.r.l. Via Tevere, 54, Sesto F.no, Firenze, Italy) set on macro-modality was used to increase the oxygen content of the sample bottles.

A luminescence-based technology was used to measure oxygen: NomaSense TM O_2 Trace (PreSens GmbH, Regensburg, Germany). The measurement of oxygen in the tank was performed using a specific dipping probe consisting of a polymer optical fibre with an oxygen-sensitive sensor foil attached to one end. To measure the oxygen in the bottle, two fibre-optic oxygen minisensors, based on a 2-mm polymer optical fibre, were used. The system detects oxygen (oxygen

Table 1 Experimental plan for studying the effect of SO_2, oxygen and temperature on rotundone levels in wine

	Oxygen (O_2)	Sulfur Dioxyde (SO_2)	Temperature (T)
A: O_2: +4 mg L^{-1}; SO_2: +90 mg L^{-1}; T = 20 °C	+	+	+
B: O_2: +4 mg L^{-1}; SO_2: +0 mg L^{-1}; T = 20 °C	+	–	+
C: O_2: +0 mg L^{-1}; SO_2: +90 mg L^{-1}; T = 20 °C	–	+	+
D: O_2: +0 mg L^{-1}; SO_2: +0 mg L^{-1}; T = 20 °C	–	–	+
E: O_2: +4 mg L^{-1}; SO_2: +90 mg L^{-1}; T = 4 °C	+	+	–
F: O_2: +4 mg L^{-1}; SO_2: +0 mg L^{-1}; T = 4 °C	+	–	–
G: O_2: +0 mg L^{-1}; SO_2: +90 mg L^{-1}; T = 4 °C	–	+	–
H: O_2: +0 mg L^{-1}; SO_2: +0 mg L^{-1}; T = 4 °C	–	–	–

partial pressure) in solution (dissolved oxygen, DO) and in the gaseous phase (headspace, HS) using separate sensors. The sensors (spots) are glued into the bottles before bottling: this technology allows the non-invasive measurement of both DO and HS. The bottles were only briefly exposed to light during the measurements.

The HS (hPa) and DO (mg L^{-1}) oxygen levels were measured every day during the first week and twice a week during the following weeks. SO_2 was added at a concentration of 90 mg L^{-1} (as potassium metabisulfite) in four out of eight samples following the experimental plan and just after oxygenation. Finally, four bottles were stored, as planned, at 4 °C and the other four at 20 °C for 3 months. Free and total SO_2, volatile acidity, total acidity and pH were determined according to CE methods [20].

Rotundone determination

The quantitative analysis of rotundone was carried out using an improved version of a previously validated and published method [7]. In accordance with this method, 30 µL of internal standard solution (benzyl benzoate 100 µg L^{-1} in ethanol) was added to 50 mL of wine, the wine was then loaded into a 200 mg Bond ELUT PPL cartridge. The cartridge bed was then washed with 5 mL of water followed by 20 mL of an aqueous solution containing methanol (70% v/v) and 1% $NaHCO_3$. After drying the cartridges, elution was achieved using 2 mL of hexane containing 25% (v/v) of ethyl ether. The extract was then concentrated to 200 µL using a gentle stream of nitrogen. Multidimensional gas chromatography mass spectrometry (GC-GC–MS) analysis was carried out using an Agilent 7890A gas chromatograph equipped with a Deans switch device (Agilent Technologies, USA) allowing the selective transfer of heart cuts from the first column to the second. The first column was a DB-5MS column (15 m length, 250 µm i.d., 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) combined with a flame ionization detector (FID) and the Deans switch. An uncoated, deactivated column (6.7 m length, 180 µm i.d.) from Agilent was used as a restrictor between the FID detector and the Deans switch. The carrier gas helium was delivered at a constant pressure of 36 psi. The second column was a SAPIENS-WAX MS (Teknokroma, Barcelona, Spain) (30 m length, 250 µm i.d., 1 µm film thickness) directly connected to an Agilent 5975C mass spectrometer. The pressure was kept constantly at 31 psi. A quadrupole mass detector was operated in selected ion monitoring mode (SIM) with electron ionization. Quantifier ion for rotundone was *m/z* 218 and qualifier ions were *m/z* 203 and *m/z* 161. Ten µL of the extract were injected in the large volume mode of the Programmable Temperature Vaporizing (PTV) injector. The peak area of rotundone was normalized by that of the internal standard. As detailed in [7], the concentration of rotundone was determined using an external calibration curve prepared by analyzing aliquots of a model wine (12% v/v water/ethanol solution containing 5 g L^{-1} tartaric acid with pH adjusted to 3.5) with known concentrations of rotundone ranging from 0.5 to 300 ng L^{-1}. The area of rotundone was normalized by that of the internal standard and then interpolated in the calibration curve. Analyses were performed in triplicate. Results were processed using a complete 3-factor ANOVA, and by Fischer's test (for mean comparison).

Sensory analysis

The panel was composed of 11 trained assessors: 5 researchers, 4 technicians of CREA and 2 wine experts, all of them with a long experience in wine sensory analysis and no anosmia for rotundone. The sensory sessions were carried out in the CREA-VE sensory analysis laboratory (ISO 8589-2007 tasting room) using ISO (3591-1977) approved glasses. All the samples, both commercial and experimental wines, were tasted blindly, identified with a three-digit code and in a randomized order.

Descriptor selection

A preliminary odour description of three commercial wines (two samples 2016 and one sample 2017) representative of the *Pelaverga Verduno* PDO was carried out following a procedure described in [21], similar to other methodologies [22, 23]. According to the procedure, during the first section the attributes are chosen and in a second phase the attributes are quantified. More specifically, the panel selected the attributes (descriptors) with the help of a list of odors derived from the Noble wine aroma wheel [24]. The list had three specificity levels: first level (generic), second level (medium-generic) and third level (specific), for example, respectively, Fruity, Berry: blackberry, strawberries, raspberries, black currant (cassis). The selection of the odor descriptors was based on a frequency threshold of identification. The second level attributes (dry herbaceous and resinous-balsamic, in this case) generally are chosen if their identification frequency is equal or greater than: (n° of wines × n° of assessors)/2, that is (3*11)/2 = 16, while the third level descriptors (rose, violet, nutmeg, cloves, pepper, raspberry, cherry, almond, plums and jam, in this case), if their identification frequency is equal or greater than: (n° of wines × n° of assessors)/4, that is (3*11)/4 = 8 [21]. The panel confirmed the selected attributes by comparison with suitable standards. For this purpose, pure standard compounds were suitably diluted: β-ionone for violet, benzaldehyde for almond, a commercial distilled rose water for rose, raspberry juice for raspberry, cherry syrup for cherry, dried plums for plums, a mixture of cherry and plum jam for jam, spices (nutmeg, cloves, pepper), some standards of the Aromaster kit (© 2010 A. Mirey) for blackberries, dry herbaceous and resinous-balsamic scents.

Sensory profile definition

For the definition of the odour sensory profile, the selected descriptors were quantified in duplicate using non-structured scales (scale from 0 to 100). To this purpose, five commercial Pelaverga Verduno PDO wines were used (including those employed in 2.4.1 and other two from vintage 2016) as well as eight experimental wines listed at (Table 1). The wines were served to the panel with a simultaneous multiple presentation (four or five samples served together, and attributes rated one at a time across samples). Both acquisition and processing of qualitative and quantitative sensory data were carried out using the FIZZ software (Biosystems, Couternon, France). The wine sensory profiles obtained (average of two panel repetitions), were represented with radar diagrams. The quantitative sensory results were processed using ANOVA and Tukey's test ($p = 95\%$) employing XLSTAT® software (Addinsoft, New York).

Results and discussion

Wine oxygen consumption

The oxygenation lasted approximately 2 h to achieve the theoretical oxygen saturation level (8.4 mg L^{-1}). The real average amount of dissolved oxygen detected in the samples at bottling was 6.1 mg L^{-1} in oxygenated wines and 3.5 mg L^{-1} in non-oxygenated wines. The oxygen consumption rate (OCR) monitored over 60 days, was quite rapid and after 2 months of storage the oxygen present in all the bottles was less than 1 mg L^{-1}. The OCR was significantly influenced by the temperature in line with Arrhenius law and according to Pascual [25] and by SO_2 levels according to the literature [26, 27]. In particular, with regards to the effect of temperature, the samples stored at low temperature exhibited a slower OCR, and at the end of the 2-month storage period the average value was approximately fourfold higher in wines stored at 4 °C (1.01 mg L^{-1}) than those stored at 20 °C (0.26 mg L^{-1}). The dissolved oxygen consumption in the samples stored at a lower temperature was more gradual than in wines stored at 20 °C, reaching a steady state after approximately 15 days (Fig. 1).

The oxygen measurements carried out in the HS of the bottles exhibited a similar trend confirming that temperature can significantly affect gas consumption under the experimental conditions. This fact is emphasized by the data related to total oxygen consumption expressed as the sum of DO and HS oxygen present in the bottles. Differing to DO consumption, total oxygen decreased more slowly, and an average value of 0.93 mg L^{-1} remained after 2 months of bottling (Fig. 2).

Sensory analysis

The descriptive sensory analysis of *Pelaverga* wine enabled selection of the following olfactory descriptors which were subsequently used for quantitative sensory analysis. The most specific olfactory descriptors (third level attributes) with a frequency higher than the limit threshold were: rose (14), violet (11), nutmeg (10), cloves (9), pepper (25), blackberries (8), raspberries (13), cherries (14), plums (8), jam (10) and almond (8). The less specific descriptors (second level attributes), with a frequency higher than the limit threshold, were dry herbaceous (16) and resinous-balsamic (16). The average odour sensory profile obtained from quantitative sensory analysis of the commercial wines analysed is reported in Fig. 3. *Pelaverga* wine is characterized by intense floral and fruity notes: rose, violet, raspberry, blackberry and cherry. Other

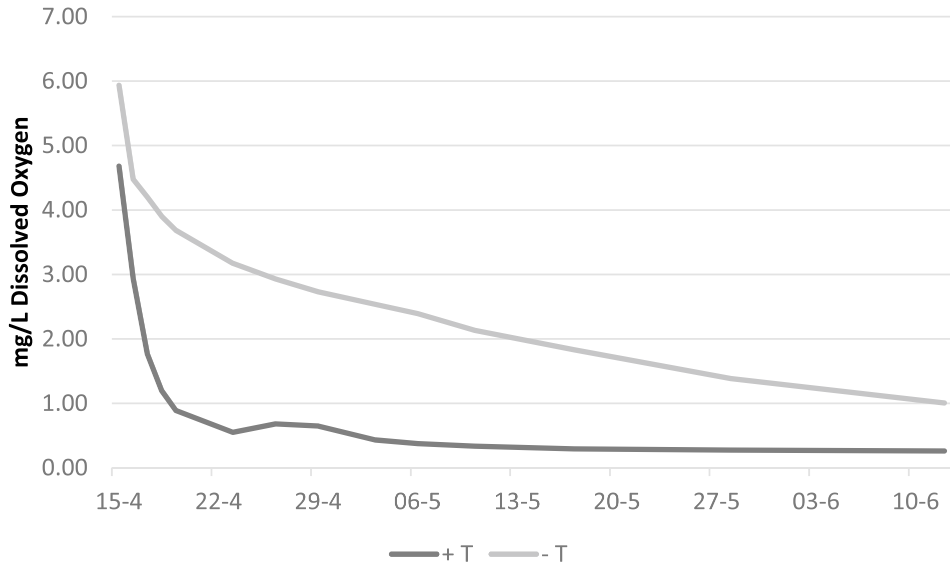

Fig. 1 Average dissolved oxygen (mg L^{-1}) trend in wines stored at 20° C (dark line, + T) and at 4 °C (light line, –T)

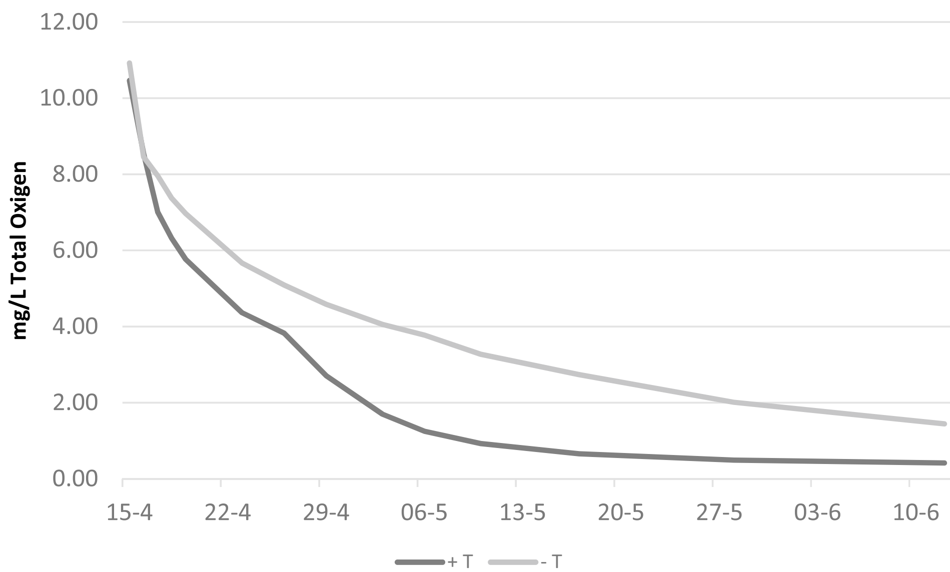

Fig. 2 Average total oxygen (mg L^{-1}) trend in wines stored at 20° C (dark line, T +) and at 4 °C (light line, T–)

relevant descriptors as well as dry herbaceous, almond and the distinctive note of black pepper are crucial for the *Pelaverga* olfactory characterization.

Furthermore, during this trial, the short-term effect of oxygen, SO_2 and temperature on the sensorial profile of *Pelaverga* wine was assessed and the possible relationships between these factors and the perception of the spicy notes was studied (Table 2). After 3 months of storage, the effect of oxygen was weak and no significant differences were highlighted between high and low oxygenated wines as regards spice intensity. On the contrary, the storage temperature had an important sensory effect on wines; almost all the selected descriptors have a higher average intensity in wines stored at a low temperature. Significant differences were found for the floral notes of violet, for the spicy notes and for berries (blackberry and raspberries). Conversely, the presence of SO_2 had a significant sensorial effect on the notes of cherry, blackberry and raspberry, reducing these perceptions in wines with greater quantities of SO_2. This effect could possibly be linked to a greater formation of sulfonates between SO_2 and carbonyl compounds such as diacetyl or β-damascenone, involved in the perception of blackfruit notes [28].

Rotundone analysis

Preliminary analysis was carried out on three different commercial *Pelaverga di Verduno* wine samples. An average rotundone content of 34 ng L^{-1} (minimum of 5.3 ± 0.2 ng L^{-1} and a maximum of 48.5 ± 0.4 ng L^{-1}) was observed.

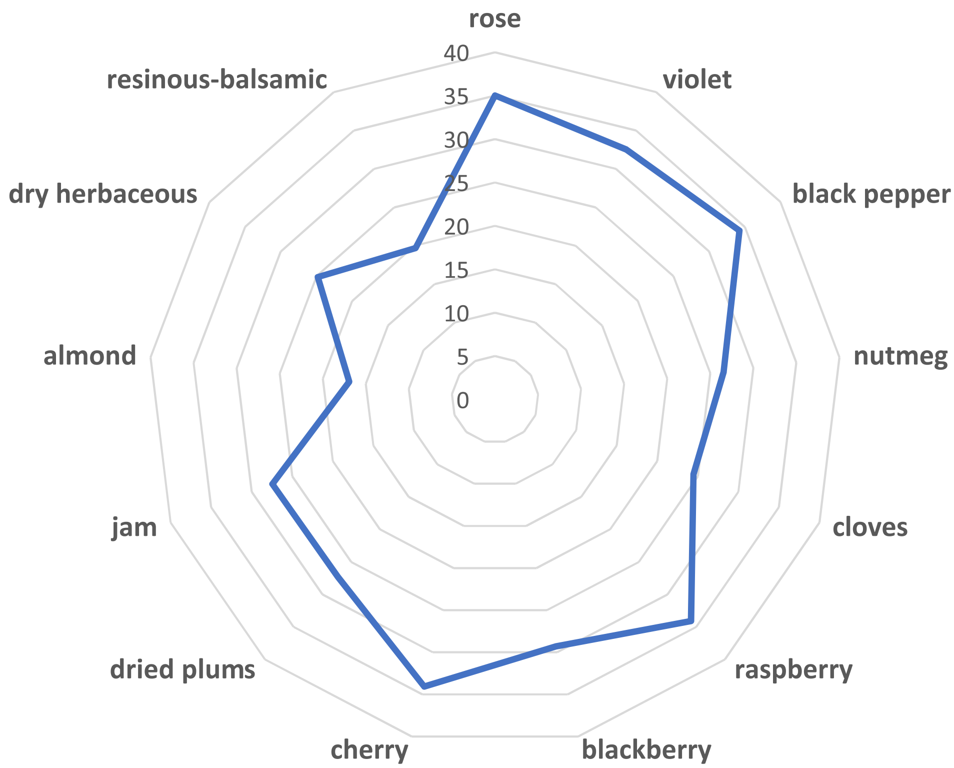

Fig. 3 Odour sensory profile of Pelaverga di Verduno PDO wine

Table 2 The effect of each factor (Oxigen, Sulfur dioxide and temperature) on wine sensory attributes after 3 months of bottling, ANOVA results. Different letters within each row denote significant differences between the wines at $p < 0.05$)

Attribute	Main effects											
	Oxygen (O_2)				Sulfur dioxyde (SO_2)				Temperature (T)			
	Low O_2	High O_2	F	Sig	Low SO_2	High SO_2	F	Sig	Low T	high T	F	Sig
Rose	36,7	33,3	1,452	ns	36,7	33,3	1,488	ns	36,3	33,8	0,785	ns
Violet	32,4	32,7	0,026	ns	32,8	32,3	0,040	ns	35,0a	30,1b	5,149	*
Black pepper	35,8	32,7	1,620	ns	33,8	34,7	0,131	ns	35,6	32,8	1,295	ns
Nutmeg	27,9	25,3	1,850	ns	26,3	26,8	0,086	ns	27,9	25,2	2,032	ns
Cloves	24,7	24,2	0,078	ns	24,8	24,1	0,143	ns	26,8a	22,1b	5,930	*
Raspberry	35,0	33,2	0,730	ns	38,3a	29,9b	15,957	**	37,9a	30,3b	13,011	**
Blackberry	29,2	29,4	0,003	ns	33,1a	25,5b	8,463	*	33,0a	25,6b	8,233	*
Cherry	34,8	33,3	0,587	ns	37,4a	30,8b	6,162	*	37,1	31,1	5,016	ns
Dried plums	28,6	26,0	1,560	ns	28,4	26,2	1,078	ns	29,1	25,5	2,891	ns
Jam	28,7	26,3	0,914	ns	29,0	25,9	1,694	ns	28,7	26,2	1,125	ns
Almond	17,3	16,6	0,128	ns	17,4	16,5	0,211	ns	18,0	15,9	1,273	ns
Dry herbaceous	26,5	23,2	2,587	ns	25,1	24,5	0,090	ns	25,9	23,7	1,146	ns
Balsamic	20,5	19,0	0,418	ns	20,4	19,0	0,341	ns	19,1	20,3	0,245	ns

*, **, *** and ns: significant at $p < 0.05$, 0.01 and 0.001 and not significant (Sig.), respectively

These differences in rotundone concentration probably depend on both viticultural and oenological factors, as previously described. According to a review by Geffroy et al. [29], analytical results on rotundone content of several monovarietal wines showed that the minimum concentration values of the rotundone in wines vary between 0.6 ng L^{-1} and 457 ng L^{-1} with a median of 28 ng L^{-1}, while the maximum values measured in the same wines vary between 8 and 1176 ng L^{-1} with a median of 69 ng L^{-1}. Many "spicy" wines such as Syrah reported in that work, had an average minimum concentration value of 46 ng L^{-1} and an average maximum concentration of 117 ng $L^{-1}$and a direct correlation between peppery notes and high levels of rotundone was proved. Moreover, as reported by Wood et al. [6], a level of about 20 ng L^{-1} of rotundone in Shiraz wine is perceived as moderate pepper aroma intensity by expert tasters, while 75 ng L^{-1} is considered as high intensity. Since literature reports that the perception threshold of rotundone in wine is of 16 ng L^{-1}, the definitely higher levels of rotundone detected in most of the analyzed Pelaverga wines during this experience, very likely may impact on enhancing the sensory perception of pepper and "spicy" notes in those wines.

The experimental wines were then analyzed to verify any effect of temperature, dissolved oxygen or SO_2 on the rotundone content of the wines after the first 3 months of storage. Table 3 shows how neither SO_2 nor temperature had a statistically significant effect on the concentration of rotundone during this short storage period. It was expected that the carbonyl function of rotundone could react with free SO_2 present in solution forming an SO_2-binding carbonyl compound and leading to a decrease of free sesquiterpene, in a similar way to that observed for norisoprenoids β-damascenone in a model wine [28]. Clearly, in real-wine conditions, given the relatively low concentration of rotundone and the high concentrations of many other carbonyl compounds naturally present in wine that may react with SO_2, this reaction is virtually inefficient. In reference to temperature, no significant effect was found, in accordance with the literature where rotundone is indicated as a rather stable molecule under normal wine storage conditions [30]. On the contrary, oxygen has a low but statistically significant effect on the concentration of rotundone in wines (Table 3). The effect exerted by oxygen is temperature dependent and the ANOVA analysis highlights significant interactions between these two factors ($O_2 \times T$, $p = 0.002$). At 20 degrees differences between oxygenated and non-oxygenated thesis are marked and statistically significant, while at 4° C these differences are inconsistent.

Table 3 ANOVA results regarding the main effects of the considered factors (oxygen, sulfur dioxide and temperature) on the rotundone concentration of wines

Sample	Oxygen (O_2)	Sulfur Dioxyde (SO_2)	Temperature (T)	Concentration (ng L^{-1})
Wine A	+	+	+	49.6 ± 3.2
Wine B	+	−	+	48.0 ± 2.5
Wine C	−	+	+	41.1 ± 2.1
Wine D	−	−	+	44.3 ± 1.3
Wine E	+	+	−	44.8 ± 2.6
Wine F	+	−	−	44.7 ± 2.6
Wine G	−	+	−	45.8 ± 1.9
Wine H	−	−	−	45.3 ± 0.6
F value	8,062	0.070	0.447	–
Sig.	**0.011**	0.795	0.513	–
High level	46.8	45.6	45.8	–
Low level	44.1	45.3	45.1	–

Bold p values are < 0.05; **all concentrations are expressed as ng L^{-1} (mean of 3 measures)

From a sensorial point of view (Table 2), the tasting panel noticed no differences as regard peppery notes between the wines that underwent greater oxygenation from those with less oxygen contact, but it is possible to assume that the difference in the detected concentration of rotundone is lower than its odor differential threshold.

This result, which needs further confirmation, allows us to hypothesize that rotundone could increase in presence of oxygen at 20 °C, probably due to the chemical oxidation of some aromatic precursor during wine storage. In *Vitis vinifera* grapes, the formation of rotundone has been assumed to originate from the precursor α-guaiene that, in turns, is enzymatically generated in grapes from germacrene A [31]. Subsequently, Cytochrome P450 CYP71BE5 in the grapevine (*Vitis vinifera*) can catalyse its transformation into the spicy aroma compound (-)-rotundone [32, 33]. However, it has also been hypothesized that the transformation of the precursor α-guaiene to rotundone can be achieved via a non-enzymatic route or by aerial autoxidation [5, 34]. Previous research has detected α-guaiene concentrations in grapes up to 0.26 μg kg^{-1} [35] so it could be easily transferred to must during the first stages of grape maceration and may undergo a transformation into rotundone by self-oxidation both during the first fermentative stages and storage.

Conclusions

The valorization of wines obtained from rare and native varieties is important in terms of biodiversity protection, culture and local tradition and also as an opportunity for winegrowing and enterprises to diversify their production. Wines produced in a specific territory may gain considerable market success because they are highly recognizable in other words, products of *terroir* expression. From a sensorial point of view, indeed, many of the peculiarities attributed to these "territorial" wines refer to specific aromatic characteristics, which is undoubtedly one of the main variables associated with the quality perceived by the wine consumer.

During this research the presence of rotundone, a sesquiterpene, at average concentration levels well above its perception threshold, was highlighted for the first time in *Pelaverga di Verduno* wines. This result suggests a relationship between the high levels of rotundone and the spicy note highlighted by the sensory analysis of the wines although further reaserch is necessary to verify this hypothesis.

Neither the storage temperature nor the different levels of SO_2 influenced the concentration of this compound over the period of a few months. On the contrary, a significant effect of oxygen led to an increase of the rotundone concentration in wines. This result allows us to hypothesize that the planned use of macro-oxygenation techniques during the prefermentative and fermentative phases could contribute to positively change the aromatic profile of *Pelaverga* wines.

Generally, identifying the genuinely important molecules from an olfactory point of view is fundamental to orient the cellar strategies and identify suitable tools useful for the quality of the final product. Finally, results can be used for the promotion and commercial valorization of the wines both in the Italian and international market.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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

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