Influence of winemaking techniques with low sulphur dioxide on wine varieties Chardonnay, Pinot and Montepulciano

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Summary

Due to health concerns, techniques to substitute sulfite (SO_2) in winemaking, without compromising high quality wines production, have been encouraged in the last years. In this work, two white wines, Chardonnay and Pinot, and one red wine, Montepulciano, were produced by using ascorbic acid, lysozyme and oenological tannins in the winemaking process instead of SO_2. Oenological parameters, volatile profile and sensory quality were evaluated. The results showed that replacement of SO_2 with ascorbic acid, lysozyme and tannins influenced the chemical composition of wines by altering volatile composition, the sensorial quality and the concentration of non-volatile compounds such as tartaric, malic, lactic and shikimic acids. Wines fermented with SO_2 showed higher total volatile alcohol concentrations, while the presence of ascorbic acid, lysozyme and oenological tannins increased the level of volatile esters. Finally, on the basis of descriptive sensory analysis, Chardonnay showed the most significant increment in qualitative parameters followed by Pinot and Montepulciano. Overall, from gathered findings it can be concluded that replacing sulfites with lysozyme, ascorbic acid and tannins, during the winemaking, might positively contribute to production of good quality wine.

Keywords

additives; sensory analysis; sulphur compounds; grapes; flavour

Aroma is a key organoleptic attribute for consumers of wine. It can depend on grape variety and viticulture practice, winemaking technology and storage conditions [1–4]. Volatile compounds such as higher alcohols, aldehydes, ethyl esters of fatty acids, ketones, monoterpenes, volatile phenols and norisoprenoids are mainly responsible for the aroma of wine [3, 5, 6]. Besides, non-volatile components such as procyanidins and tannins were reported to contribute to flavour and sensory properties of wine [1].

Sulphur dioxide (SO_2) is widely used as additive during the vinification process (from must pressing to wine bottling) in order to protect wines (in particular white wine) due to its antimicrobial and antioxidant activities. Indeed, it inhibits growth of yeasts and bacteria and it also inhibits enzymatic and non-enzymatic browning during production and storage. Additionally, it can favour the solubilization of pigments (namely anthocyanins) during grape maceration and the clarification of wine by precipitating colloidal matter [7]. Generally, SO_2 is added to wine in the form of sulfite salts (such as sodium sulfite or sodium metabisulphite). However, low amounts of SO_2 are also naturally produced by yeasts during wine fermentation [7].

Sulfites are included in the list of allergens of Regulation (EU) No 1169/2011 [8] on the basis of their association with triggering of asthmatic responses in certain individuals, although studies addressing this issue in wine were inconclusive as wine-induced asthmatic responses may be complex and may involve several cofactors [9, 10]. However, the opinion that sulfites may cause negative health effects, such as migraine and headache, appears to be common [11].

For this reason, recently, there has been

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a growing interest in the replacement of this agent during the winemaking process, which is becoming increasingly feasible due to technological improvement [12]. In particular, ascorbic acid, lysozyme and tannins were investigated to replace SO_2, especially to protect wine aroma during storage [13–16]. Ascorbic acid has been used as antioxidant because it is able to avoid auto-oxidation of phenolic compounds and reduce the generated ortho-quinones back to their original phenolic form [1, 17, 18]. However, ascorbic acid oxidation leads to the formation of hydrogen peroxide, which can cause spoilage reactions interfering with wine quality during storage [18]. Lysozyme was proposed as an antimicrobial agent during alcoholic fermentation to prevent growth of spoilage lactic and acid bacteria [19]. Tannins are used to facilitate the clarification of musts and wines, contribute to wine structure, stabilize the colouring compounds in red wines, improve the sensory impact of the final product and to prevent the oxidative phenomena of musts and wines that are likely a consequence of a dual mechanism involving inhibition of enzymes and radical-scavenging activity [13, 20, 21].

It is well known that a reduced amount of SO_2 in wine, i.e. only that which is produced by yeast, evidently changes the wine chemistry during storage [22]. Therefore, the aim of this work was to evaluate wines of three grape varieties (Chardonnay, Pinot, and Montepulciano) produced with the addition of ascorbic acid, lysozyme and tannins in the winemaking process, by sensory analysis, determination of volatile compounds and determination of oenological parameters. The obtained results were compared with those for wines produced by the traditional winemaking process using SO_2.

MATERIALS AND METHODS

All chemicals, flocculants, starter culture and enzymes for winemaking were purchased from Lafood, Conegliano, Italy.

Samples

Two batches of 25 kg grapes from each variety (Chardonnay, Pinot, and Montepulciano) were manually harvested from August to September 2011 in area of Silvi Marina (Abruzzo, Italy) and cooled overnight at 8 °C in refrigerated cells. Then, each batch was vinified as described in the following sub-section and the obtained wines of the former batch were treated by using potassium metabisulfite (10 $g{\cdot}hl^{-1}$). Wines of the latter batch were treated by adding gallic tannin (5 $g{\cdot}hl^{-1}$) and ascorbic acid (20 $g{\cdot}hl^{-1}$) without any sulfite supplementation.

Winemaking

Regarding white wines, after de-stemming, grapes were crushed under inert atmosphere and the free-run juice was quickly clarified via nitrogen flotation (for 1 h at 400 kPa) using pectolytic enzyme (lysozyme, 1.5 $g{\cdot}hl^{-1}$), polyvinylpolypyrrolidone (PVPP, 10 $g{\cdot}hl^{-1}$) decolorizing carbon (20 $g{\cdot}hl^{-1}$ and 100 $g{\cdot}hl^{-1}$ for Chardonnay and Pinot, respectively), and gelatin (10 $g{\cdot}hl^{-1}$ for Chardonnay and 3 $g{\cdot}hl^{-1}$ for Pinot), as flocculants. Clear juice was then moved to a fermentation tank and was added after ammonium sulphate (20 $g{\cdot}hl^{-1}$), ammonium phosphate (5 $g{\cdot}hl^{-1}$), thiamin (25 $g{\cdot}hl^{-1}$), malic acid (10 $g{\cdot}hl^{-1}$) and tartaric acid (50 $g{\cdot}hl^{-1}$). Then, alcoholic fermentation was quickly induced via inoculation of activated (for 12 h) *Saccharomyces cerevisiae* starter (25 $g{\cdot}hl^{-1}$). Fermentation lasted 9 days with a nearly complete consumption of reducing sugars (5 $g{\cdot}l^{-1}$), being carried out in a temperature-controlled stainless steel tank with temperature starting from 18 °C and then gradually being lowered to 14.5 °C, after the first day, and finally at 10 °C.

One week after the end of alcoholic fermentation, the wines were separated from the yeast and grape lees, and racked to clean tanks to be further settled using β-glucanase (EC 3.2.1.6; 3 $g{\cdot}hl^{-1}$), β-glucosidase (EC 3.2.1.21; 2 $g{\cdot}hl^{-1}$), metatartaric acid (7 $g{\cdot}hl^{-1}$), PVPP (5 $g{\cdot}hl^{-1}$), gelatine (2 $g{\cdot}hl^{-1}$), and granular sodium bentonite (40 $g{\cdot}hl^{-1}$). Then, they were left to age for 3 months with two bâtonnages.

Regarding red wines, after de-stemming, grapes were crushed under inert atmosphere and macerated (for 24 h at 25 °C) with wood powder (100 $g{\cdot}hl^{-1}$), chestnut tannin (10 $g{\cdot}hl^{-1}$), and lysozyme (3 $g{\cdot}hl^{-1}$) for the extraction of colour. Subsequently, the must was quickly clarified via nitrogen flotation (for 1 h at 400 kPa) using pectolytic enzyme (lysozyme, 1.5 $g{\cdot}hl^{-1}$), PVPP (10 $g{\cdot}hl^{-1}$) and decolorizing carbon (20 $g{\cdot}hl^{-1}$), as flocculants. Clear juice was then moved to fermentation tank and ammonium sulphate (20 $g{\cdot}hl^{-1}$), ammonium phosphate (5 $g{\cdot}hl^{-1}$) and thiamin (5 $g{\cdot}hl^{-1}$) were added. The alcoholic fermentation was quickly induced via inoculation of the activated (for 12 h) *S. cerevisiae* starter (20 $g{\cdot}hl^{-1}$). Fermentation lasted 9 days with a nearly complete consumption of the reducing sugars (3 $g{\cdot}l^{-1}$), being carried out in a temperature-controlled stainless steel tank at 18 °C. Finally, the wines were separated from the yeast and grape lees, and racked to clean tanks.

Then, β-glucanase (3 g·hl^{-1}) and β-glucosidase (2 g·hl^{-1}) were added and the wines were left to age for 3 months with two bâtonnages.

Determination of oenological parameters

Oenological analyses were carried out according to International Organization of Vine and Wine (OIV) methods [23], described in the following sections according to each parameter: ethanol (AS312-01B), reducing sugars (AS311-01C), total acidity (AS313-01), volatile acidity (AS313-02), pH (AS313-15), tartaric acid (AS313-05B), malic acid (AS313-10), lactic acid (AS313-06), shikimic acid (AS313-17), gluconic acid (D1-01), dry extract (AS2-03A), glycerol (AS312-04), potassium (AS322-02A), ash (AS2-04), free and total sulphur dioxide (AS323-04A), density (AS2-01B), carbon dioxide (AS314-01), colour intensity (AS2-07A), hue (AS2-07A), total anthocyanins and flavonoids (AS315-11).

Analysis of volatile compounds

Free volatiles were extracted from the wine samples headspace by solid phase microextration (SPME) using a triple-phase fibre (divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm; Sigma-Aldrich, St. Louis, Missouri, USA). Their profiles were investigated by means of gas chromatography - mass spectrometry (GC-MS) [24]. An Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (Agilent, Santa Clara, California, USA) and a DB-Wax column (60 m × 0.25 mm × 0.5 μm) from J&W Scientific (Folsom, California, USA) were used. The chromatographic conditions were: injection mode splitless, injection temperature 250 °C; temperature setting 40 °C (5 min) to 200 °C (15 min) at 2 °C·min^{-1}, to 250 °C·min^{-1} at 1 °C·min^{-1}, mass analyser quadrupole, interface temperature 280 °C, carrier gas helium, flow 1.0 ml·min^{-1}. The MS electron inozation was at 70 eV. The mass spectral data acquisition scan interval was 1.0 s, and data were collected over a mass range of m/z from 28 to 300.

NIST-2004 spectral library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) was used for peak identification, while the peak area was used to represent the amount of each volatile for its semi-quantification [4]. Relative quantification (in percent) was performed based on the use an internal standard (2-octanol, Sigma-Aldrich).

Sensory analysis

The panel of judges consisted of four oenology researchers from CREA-Council for Agricultural Research and Economics (Turi, Italy) and of six professional tasters from the National Organization of Wine Tasters (ONAV, Asti, Italy). The judges were asked to assign a score for different parameters of the wines such as colour (lightness, intensity and gradient colour), aroma (exotic fruit, citrus fruit, pome fruit, floral, herbaceous, dry fruit and caramel for white wines; fruit, floral, spicy, caramel, herbaceous, phenolic and sweet for red wines), taste (sweet, bitter, alcohol, acidic, astringent, mineral, intensive, persistent and taste structure), and final consideration (evolution stage and quality) using a sensory analysis tasting sheet with a scale ranging from 0 (absence of perception) to 10 (maximum perception) [2, 25]. The mean scores of attributes were submitted to quantitative descriptive analysis (QDA) in order to generate the sensory profile of wines, and plotted on a radar graph.

Statistical analysis

The data, obtained from three replicates of each wine, were statistically analysed using ANOVA and Fisher's least significant difference (LSD) post-hoc test for comparing the means, where appropriate, using Statistica, version 8.0 software (StatSoft, Tulsa, Oklahoma, USA). $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Oenological parameters

It has been well ascertained that chemical composition of wines can be deeply influenced by varieties but also by viticultural practices and winemaking technologies [2, 3, 22, 26, 27]. Chemical composition of wines made from distinct varieties is often different [26], additionally, vinification practices have also an impact on the oenochemical composition. Tab. 1 shows the oenological parameters of Chardonnay, Pinot and Montepulciano wines, produced with or without added SO_2. Considering Chardonnay, chemical composition was similar to that published previously [27]. Ethanol concentration was above 87 g·l^{-1} in all wines, without significant difference ($P < 0.05$) independently from the use of SO_2, like previously reported in a recent study [22]. The exception was Pinot, which contained less alcohol in wines produced without added sulfite. Consequently, different levels of reducing sugars in wines with or without added SO_2 were found only in Pinot samples (Tab. 1). This was in contrast with data previously published for other Italian wines, in which total acidity was unaffected by sulfites [22]. As regards acidity, no differences between samples were ob-

Tab. 1. Oenological parameters of wines produced with and without addition of sulphite.

Parameter	Chardonnay		Pinot		Montepulciano		*S*
	With SO_2	Without SO_2	With SO_2	Without SO_2	With SO_2	Without SO_2	
Ethanol [$g\cdot l^{-1}$]	107.3^{a}	108.9^{a}	109.7^{a}	86.8^{b}	105.8^{a}	105.0^{a}	*
Reducing sugars [$g\cdot l^{-1}$]	0.95^{c}	0.91^{c}	0.85^{c}	1.53^{b}	2.97^{a}	3.11^{a}	*
Total acidity [$g\cdot l^{-1}$]	6.70^{a}	6.27^{b}	6.34^{ab}	5.67^{c}	6.92^{a}	6.36^{ab}	*
Volatile acidity [$g\cdot l^{-1}$]	0.44	0.43	0.32	0.27	0.35	0.37	ns
pH	3.50	3.49	3.30	3.35	3.38	3.50	ns
Malic acid [$g\cdot l^{-1}$]	2.87^{a}	2.58^{a}	1.89^{b}	1.66^{b}	1.66^{b}	0.82^{c}	*
Lactic acid [$g\cdot l^{-1}$]	0.20	0.21	0.19	0.19	0.27	0.27	ns
Tartaric acid [$g\cdot l^{-1}$]	3.21^{b}	2.91^{c}	3.35^{b}	3.10^{bc}	4.29^{a}	4.10^{a}	*
Shikimic acid [$mg\cdot l^{-1}$]	42.7^{a}	35.9^{b}	5.5^{d}	4.2^{d}	17.3^{c}	15.6^{c}	*
Gluconic acid [$g\cdot l^{-1}$]	0.22^{c}	0.30^{bc}	0.50^{a}	0.39^{b}	0.39^{b}	0.32^{bc}	*
Dry extract [$g\cdot l^{-1}$]	24.48	24.53	21.53	19.36	33.56	33.22	ns
Glycerol [$g\cdot l^{-1}$]	6.27^{b}	8.21^{ab}	6.34^{b}	4.52^{c}	9.10^{a}	9.03^{a}	*
Potassium [$g\cdot l^{-1}$]	1.19	1.11	0.85	0.88	1.20	1.25	ns
Ash [$g\cdot l^{-1}$]	2.83	2.61	2.13	2.19	2.57	2.77	ns
SO_2 free [$mg\cdot l^{-1}$]	12.8^{b}	nd	22.4^{a}	nd	4.0^{c}	nd	*
SO_2 combined [$mg\cdot l^{-1}$]	57.0^{a}	9.6^{c}	57.0^{a}	6.4^{c}	17.0^{b}	8.0^{c}	*
SO_2 total [$mg\cdot l^{-1}$]	69.8^{a}	9.6^{c}	79.4^{a}	6.4^{c}	20.0^{b}	8.0^{c}	*
Sulphates [$g\cdot l^{-1}$]	0.54^{a}	0.44^{b}	0.52^{a}	0.43^{b}	0.49^{ab}	0.42^{b}	*
Density [$kg\cdot l^{-1}$]	0.992	0.992	0.990	0.993	0.996	0.996	ns
CO_2 [$mg\cdot l^{-1}$]	381^{c}	467^{b}	500^{ab}	565^{a}	209^{e}	251^{d}	*
Glucose [$g\cdot l^{-1}$]	0.91^{d}	1.30^{c}	2.40^{b}	2.31^{b}	4.54^{a}	4.49^{a}	*
Fructose [$g\cdot l^{-1}$]	0.36^{d}	0.42^{d}	0.81^{c}	0.76^{c}	1.52^{a}	1.35^{b}	*
Colour intensity					13.67	14.38	ns
Colour hue	–	–	–	–	0.512	0.538	ns
Total anthocyanins [$mg\cdot l^{-1}$]	–	–	–	–	336	333	ns
Total flavonoids [$mg\cdot l^{-1}$]	–	–	–	–	1957	1957	ns
Flavonoids less anthocyanins [$mg\cdot l^{-1}$]	–	–	–	–	1467	1472	ns

Values represent the mean of three repetitions. Values with the same superscript letters within lines do not differ significantly at $P < 0.05$. Total acidity is expressed as grams of tartaric acid per litre of sample. Volatile acidity is expressed as grams of acetic acid per litre of sample.
S – significance level of one-way ANOVA, * – significant at $P < 0.05$, ns – not significant at $P < 0.05$, nd – not detected.

served neither concerning volatile acidity nor pH. The use of SO_2 appeared to influence total acidity ($P < 0.05$), with lower values being determined in wines without added sulfites, as particularly evident in the case of Pinot (Tab. 1). Total acidity is mainly dependent on organic acids already present in the must (such as tartaric acid, malic acid and shikimic acid) or produced during the fermentation (such as lactic acid) and positively related to wine structure [7]. The concentrations of tartaric acid (2.91–4.29 $g\cdot l^{-1}$), malic acid (0.82–2.89 $g\cdot l^{-1}$) and shikimic acid (4.20–42.70 $mg\cdot l^{-1}$) seemed to follow the trend of total acidity in wines with or without SO_2. Conversely, no difference among the lactic acid levels were determined in the analysed samples. It is known that many acids are derived from oxidative metabolism of saccharides of must or wine [17] but, in our case, oxidation of saccharides can be considered minimal, since consistent level of gluconic acid (product of glucose oxidation) were only found in Pinot and Montepulciano with SO_2, which, however, had no effect on pH of the samples (Tab. 1).

Determination of the level of glycerol is very important because the compound contributes to viscosity and softness of wine, with a positive effect on its taste [26]. Significant difference in glycerol concentrations between samples ($P < 0.05$) was determined, even though a homogeneous pattern was not observed. Indeed, Chardonnay presented a higher concentration of glycerol (8.21 $g\cdot l^{-1}$) in samples produced without SO_2 compared to the wine with added sulfite (6.27 $g\cdot l^{-1}$). On the contrary, in Pinot, higher concentrations of glycerol

($6.34\ g\cdot l^{-1}$) were in wines produced using SO_2 compared to those produced without SO_2 ($4.52\ g\cdot l^{-1}$). Finally, in the case of glycerol concentration in Montepulciano, no influence by sulfite was registered.

Combined and free SO_2 were also analysed. As expected, the free SO_2 concentration in wines without added sulfite was zero, whilst a certain amount of combined SO_2 was revealed (Tab. 1), owing to the natural production of SO_2 by yeasts during wine fermentation and to sulphur compounds present in the grapes [7, 22, 28]. SO_2 is well known for its prickly characteristic sensation, perceived between 20 $mg\cdot l^{-1}$ and 80 $mg\cdot l^{-1}$.

Tab. 2. Relative quantity of volatile compounds in wines produced with and without addition of sulphite.

RT [min]	Compound	Chardonnay		Pinot		Montipulciano		*S*
		With SO_2	Without SO_2	With SO_2	Without SO_2	With SO_2	Without SO_2	
Esters								
8.3	3-Methylbutyl acetate	0.097^{c}	0.170^{b}	0.341^{a}	0.308^{a}	0.026^{d}	0.009^{e}	*
14.1	Ethyl hexanoate	0.072^{b}	0.099^{b}	0.381^{a}	0.442^{a}	0.073^{b}	0.011^{c}	*
17.0	Hexyl acetate	0.018^{c}	0.041^{bc}	0.075^{b}	0.155^{a}	nd	nd	*
22.7	Ethyl heptanoate	nd	nd	nd	nd	0.001	nd	ns
33.5	Ethyl octanoate	0.654^{cd}	0.804^{c}	2.605^{b}	3.474^{a}	0.425^{d}	0.094^{e}	*
34.8	Isopentyl hexanoate	0.001	nd	0.005	0.005	nd	nd	ns
35.7	Isopentyl heptanoate	nd	nd	nd	nd	0.001	nd	ns
39.1	Propyl octanoate	nd	0.001	0.001	0.002	nd	nd	ns
40.2	Ethyl nonanoate	nd	0.0003	0.004	0.003	0.001	0.0003	ns
41.1	Isobutyl *n*-caprylate	nd	nd	0.001	0.001	nd	nd	ns
43.3	Methyl decanoate	0.001	nd	0.001	0.002	nd	nd	ns
45.7	Ethyl decanoate	0.469^{b}	0.406^{b}	1.641^{a}	1.892^{a}	0.105^{c}	0.043^{d}	*
46.4	3-Methylbutyl octanoate	0.004	0.005	0.014	0.018	0.004	0.001	ns
47.4	Diethylbutanedioate (diethyl succinate)	nd	nd	0.001	0.001	0.001	nd	ns
47.9	Ethyl 9-decenoate	0.007	0.003	0.013	0.014	0.002	0.001	ns
52.8	2-Phenylethyl acetoate	0.008	0.008	0.016	0.020	0.002	0.001	ns
54.5	Ethyl dodecanoate	0.048^{b}	0.031^{bc}	0.179^{a}	0.201^{a}	0.012^{cd}	0.007^{d}	*
54.7	3-Methylbutyl-pentadecanoate	0.002	0.002	0.006	0.001	0.001	nd	ns
Alcohols								
7.8	2-Methyl-1-propanol	nd	nd	0.008	nd	nd	nd	ns
13.7	3-Methylbutanol	0.096^{d}	0.103^{cd}	0.263^{b}	0.140^{c}	0.356^{a}	0.144^{c}	*
25.2	1-Hexanol	0.002	0.002	0.007	0.010	0.004	0.001	ns
35.4	1-Heptanol	nd	nd	nd	nd	0.003	0.002	ns
41.9	1-Octanol	nd	nd	nd	nd	0.002	nd	ns
43.0	2,3-Butanediol	nd	0.001	nd	nd	nd	0.001	ns
56.3	Phenylethyl alcohol	0.008^{c}	0.006^{c}	0.009^{c}	0.007^{c}	0.055^{a}	0.021^{b}	*
Others								
19.3	2-Octanone	0.003	0.003	0.002	0.005	0.003	0.003	ns
41.2	3,7-Dimethyl-1,6-octadien-3-ol	nd	nd	nd	nd	0.001	nd	ns
64.1	Octanoic acid	0.003	0.001	0.008	0.014	nd	nd	ns
Total contents for classes								
Esters		1.382^{d}	1.570^{c}	5.285^{b}	6.538^{a}	0.655^{e}	0.167^{f}	*
Alcohols		0.106^{d}	0.111^{d}	0.287^{b}	0.157^{c}	0.421^{a}	0.169^{c}	*
Others		0.006	0.004	0.010	0.019	0.04	0.003	ns
Total contents (29 compounds)		1.494^{d}	1.685^{c}	5.582^{b}	6.714^{a}	1.108^{e}	0.339^{f}	*

The values represent the mean of three repetitions. Values with the same superscript letters within lines do not differ significantly at $P < 0.05$. *RT* – retention time, *S* – significance level of one-way ANOVA, * – significant at $P < 0.05$, ns – not significant at $P < 0.05$, nd – not detected.

In our case, because of total SO_2 was very close to 80 mg·l^{-1}, it was able to influence the sensory analysis of Chardonnay (69.8 mg·l^{-1}) and Pinot (79.4 mg·l^{-1}) wines (Tab. 1). Of course, concentration of sulphates, which derived from the oxidation of sulfites, was higher in all samples produced with SO_2, too (Tab. 1).

Carbon dioxide (CO_2) as well as C6 monosaccharides were influenced by the presence of SO_2 in wine. Indeed, higher values of CO_2 were determined in all samples produced without SO_2 ($P < 0.05$), whereas glucose and fructose were found to be more concentrated in Pinot and Montepulciano with added sulfites or in Chardonnay without SO_2 (Tab. 1). The parameters of colour intensity and hue, total anthocyanins and total flavonoids were measured only in the Montepulciano red wines (Tab. 1). Oxidative processes could have affected these parameters due to the absence of sulfites [28], however, no statistically relevant differences were found in our case. These results suggest the efficacy of ascorbic acid and lysozyme as antioxidant agents during winemaking, although off-flavour development was previously described on the basis of evaluation of volatiles and sensory assessment, and was attributed to microbial spoilage [26].

Volatile compounds

Studying the aroma of neutral wine grape varieties, such as Chardonnay, Pinot and Montepulciano, the analysis is focused towards the identification of the by-products of alcoholic fermentation, amino acid metabolism and utilization of unsaturated fatty acids [29]. Twenty-nine compounds were identified in the volatile fraction from the three wines (Tab. 2). These compounds were grouped into different classes, such as esters, the most abundant class with 19 compounds, followed by alcohols (with 7 compounds) and others 3 compounds (Tab. 2).

Higher levels of esters were found in white wines without SO_2 [13]. In particular, in our case, the concentration of ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate, which are enzymatically derived from fatty acids during yeast fermentation and from ethanolysis of acylCoA formed during fatty acids synthesis or degradation [30], as well as the concentration of hexyl acetate and 3-methylbutyl acetate, were more abundant in Chardonnay and Pinot without SO_2 ($P < 0.05$). On the other hand, considering Montepulciano, the presence of the same esters (even though in lower concentrations) was prominent in samples with added sulphites (Tab. 2). These compounds make a positive contribution to the general quality of wine being responsible for the typical “fruity” aroma of young wines [30, 31].

Since the protective effect of SO_2 can be excluded due to its absence, the tannins used in vinification were probably the most positive influencing factor on ester formation and preservation of white wines Pinot and Chardonnay. As previously suggested, this may be due to the ability of tannins added before fermentation to affect the levels of oxygen in musts and wines, as a consequence of a double mechanism of enzyme inhibition of radical-scavenging activity. Moreover, tannins can quickly reduce the oxygen availability, contributing to preserve the amounts of esters in wines [20, 21].

Fusel alcohols, consisting of C6 *n*-alcohols of, which are related to the lipoxygenase activity of the grape, as well as branched and aromatic alcohols (such as benzyl alcohol and 2-phenylethanol) derived from amino acids, can be synthesized by yeasts [30]. These compounds, in particular branched and *n*-alcohols, are believed to negatively influence the aromatic properties of wines [3], so that the levels of 2-methyl-1-propanol and 3-methyl-1-butanol are currently used as a criterion of quality for wines and spirits [29]. Sulphite had a positive influence on the production of alcohols and, indeed, higher abundance of fusel alcohols was found in Pinot and Montepulciano wines with added sulphites ($P < 0.05$). It was reported that, during fermentation, sulphites can promote the synthesis of some alcohols by influencing the Ehrlich pathway [7]. 3-Methyl-1-butanol and 2-phenylethanol mainly contributed to this class of compounds in both samples ($P < 0.05$). It is worth noting that a relationship between volatile composition (i.e. esters and fusel alcohols) and wine quality is not simple. Indeed, wine aroma is not just influenced by those components present at levels higher than its perception threshold, but also by their overall synergistic effect [29].

Sensory analysis

Fig. 1–3 display multiple-attribute comparisons of sensory analysis of Chardonnay, Pinot and Montepulciano with and without SO_2. Overall, radar graphs clearly show that the three samples differed in each sensory attribute. The flavour of a wine depends on its chemical composition relating to non-volatile components, responsible for perception of astringency, bitterness and acidity, and to the type and concentration of volatile compounds contributing to wine aroma [32].

The Chardonnay wine (Fig. 1) without SO_2 showed the highest sensory quality, comprising aroma, taste and colour attributes, probably due to the antioxidant properties of the gallic tannin and

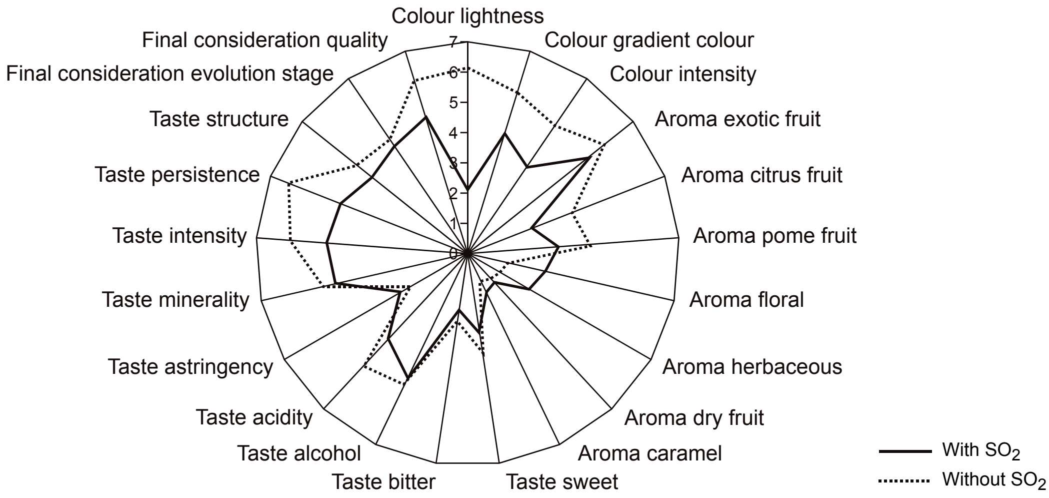

Fig. 1. Radar graph showing sensory profile of Chardonnay with and without SO_2.

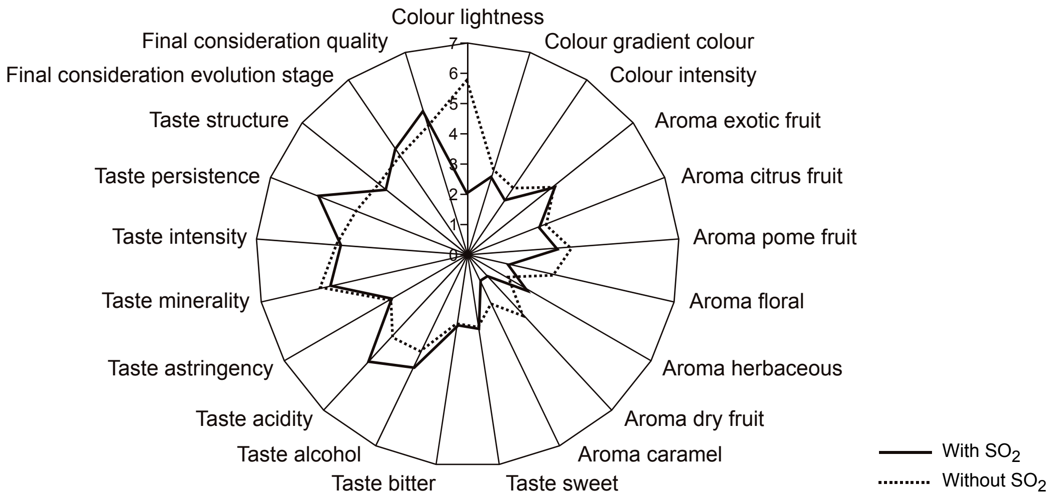

Fig. 2. Radar graph showing sensory profile of Pinot with and without SO_2.

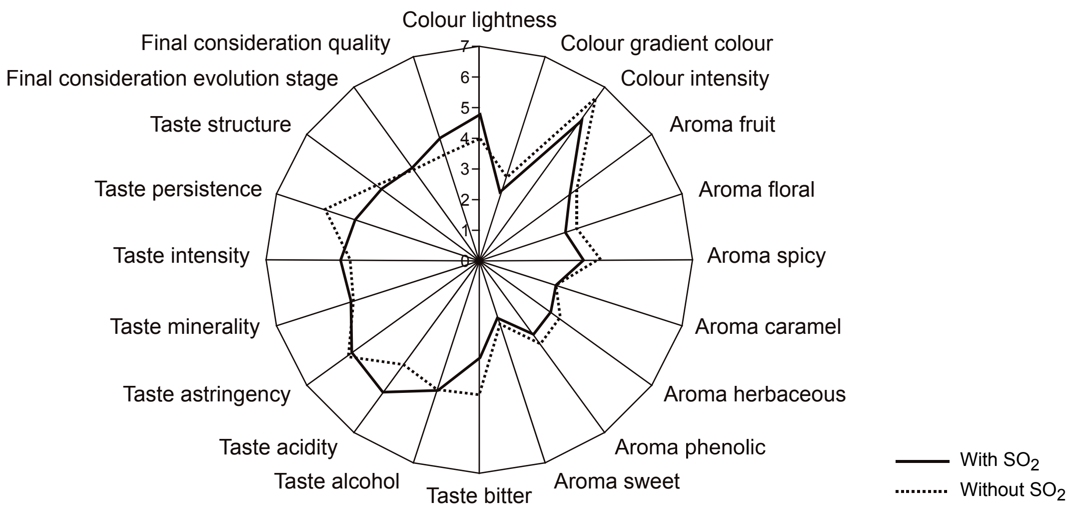

Fig. 3. Radar graph showing sensory profile of Montepulciano with and without SO_2.

ascorbic acid added during the winemaking [14]. Its aroma attributes of exotic, citrus and pome fruits might be related to the presence of ethyl decanoate, ethyl dodecanoate, ethyl octanoate and/or ethyl hexanoate, esters of straight-chain fatty acids. These are considered as more important contributors to wine aroma than ethyl esters of branched-chain fatty acids [26, 33].

Pinot wine without SO_2 showed the highest values in almost all aroma attributes evaluated, together with the lightness (Fig. 2), which proved the effectiveness of tannins in the clarification process. Montepulciano wine without SO_2 showed the highest values of aroma attributes, the persistent taste and, of course, the colour intensity (Fig. 3). This might reflect the fact that SO_2 can cause bleaching of anthocyanins resulting in a loss of colour in red wines [34].

CONCLUSION

The results showed that the replacement of SO_2 with ascorbic acid, lysozyme and oenological tannins influenced the volatile composition, the sensorial quality and the concentration of tartaric, malic and shikimic acids of Chardonnay, Pinot and Montepulciano wines. The lack of sulphite stabilization did not impact wine quality. Wines fermented with SO_2 showed higher total volatile alcohol amounts, while the use of ascorbic acid, lysozyme and oenological tannins increased the level of volatile esters. Chardonnay wine showed the highest increment in positive sensorial attributes, followed by Pinot and Montepulciano wines, showing that the oenological additives improved the quality of wines, in particular of the white ones. Although adding SO_2 is still a widespread practice in winemaking process, gathered results are major arguments in favour of the hypothesis that wines of good sensorial quality can be obtained without using hazardous chemical additives.

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