Impact of post-harvest ozone treatments on the skin phenolic extractability of red winegrapes cv Barbera and Nebbiolo (Vitis vinifera L.)

Impact of post-harvest ozone treatments on the skin phenolic extractability of red winegrapes cv Barbera and Nebbiolo (*Vitis vinifera* L.)

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Abstract

Recently the use of ozone as sanitizing agent has been proposed on winegrapes in order to control mycobiota after harvest. The aim of this work was to investigate possible indirect physico-chemical effects of ozone treatment on berry skin phenolic composition and extractability. *Vitis vinifera* L. cv Nebbiolo and Barbera, chosen for their different anthocyanin profiles, were post-harvest treated for 24 and 72 hours with gaseous ozone (30 µL/L). Skin anthocyanin and flavanol extractability was assessed during maceration (6, 24, 48, 96, 168 and 240 hours) using a wine-like solution. In our experimental conditions, ozone did not affect significantly the final extraction yield of anthocyanins (TA), proanthocyanidins (PRO), and flavanols reactive to vanillin (FRV) in Barbera, although TA and FRV extractabilities were higher in control samples than in ozone-treated samples during the first stages of maceration. In Nebbiolo, the final TA extraction yield was positively influenced by the ozone treatment (68.6, 64.2, and 59.9% for 24 hours ozone-treated berries, 72 hours ozone-treated berries and control samples, respectively). Final PRO and FRV extractability also increased in both ozone-treated samples compared to the control (+8.6-9.1% for PRO and +7.3-11.7% for FRV). No significant differences were found among treatments for individual anthocyanins in both cultivars at the end of maceration. Therefore, the use of ozone as sanitizing agent in red varieties prior to winemaking process can be considered because it did not negatively affect the extractability of skin anthocyanins and flavanols.

Keywords: ozone, anthocyanins, flavanols, extractability, grape post-harvest treatment

1. Introduction

Wine industry is looking forward for innovative, safe for human health and environment, antimicrobial products allowing chemical treatment reduction in the winemaking process and not negatively affecting the quality of the final product. Ozone has been tested in food industry, as used in both ozonized water and gaseous form, giving good results in preventing fungi and bacteria growth on a wide spectrum of vegetables and fruits, due to its oxidant activity, and leaving no chemical residues on foods decomposing itself rapidly into oxygen (Glowacz, Colgan, & Rees 2015; Khadre, Yousef, & Kim, 2001; Sengun, 2014). Gaseous ozone has been already tested for table grapes storage in order to contain fungi responsible for berry decay (i.e. *Botrytis cinerea*, *Aspergillus* spp., *Fusarium* spp), to maintain the product's visual, sensory, textural and nutritional quality, and to reduce pesticide residues (Artés-Hernández, Artés, & Tomás-Barberán, 2003; Cayuela, Vazquez, Perez, & Garcia, 2009; Feliziani, Romanazzi, & Smilanick, 2014; Gabler, Smilanick, Mansour, & Karaca, 2010). As well, ozone fumigation has been used on winegrapes during the withering process, as an alternative to sulphur derivates in order to both prevent moulds development and to reduce indigenous yeast population (Botondi, De Sanctis, Moscatelli, Vettraino, Catelli, & Mencarelli, 2015; Carbone & Mencarelli, 2015). In particular, the viability reduction of *Brettanomyces bruxellensis*, which is related with off-flavours production in wine (Kheir, Salameh, Strehaiano, Brandam, & Lteif, 2013), would be advantageous.

In addition to improve fresh product quality, ozone has been confirmed as phenolic compounds elicitor, stimulating chemical defence responses such as the synthesis of polyphenols, in particular increasing up to 4-fold resveratrol content, and keeping stable anthocyanin content during the storage of red table grapes cv Napoleon (Artés-Hernández et al., 2003). Nevertheless, ozone applied in post-harvest can permeate inside fruits through lenticels, and in damaged grapes through cuts or cracks in the cuticle (Forney, 2003), and reacts with grape compounds. In fact, ozone has a high oxidant potential acting both directly and indirectly, attaching itself to the double bound of organic compounds and by its intermediate radicals, which can react with a wide range of grape molecules (Criegee, 1975; Cullen, Tiwari, O'Donnell, & Muthukumarappan, 2009). Among them, flavonoids can be susceptible to both degradation reactions, depending on the electrochemical stability of the B ring substituent. In particular up to 99% anthocyanin degradation has been reported in less than 10 minutes in grape juice treated with ozone, to different extents according to individual anthocyanin reactivity (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a).

Phenolic compounds are strictly associated with red wine quality; among them, anthocyanins extracted from skins are responsible for young wine colour. The grapevine genome determines the anthocyanin profile, but several factors such as vineyard practices, climate, soil features, and seasonal conditions can influence anthocyanin accumulation during grape ripening (Ortega-Regules, Romero-Cascales, López-Roca, Ros-García, & Gómez-Plaza, 2006a). Monomeric, oligomeric and polymeric flavan-3-ols from skins and seeds contribute to astringency and bitterness, and together with anthocyanins are involved in aged wine colour. Their contribution on the organoleptic properties of wine depends on their content and structural features, such as stereochemistry, hydroxylation pattern, position of the linkage, and in particular the degree of polymerization (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2008; Kennedy & Jones, 2001; Mattivi, Vrhovsek, Masuero, & Trainotti, 2009; Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003).

Phenolic compounds extraction depends on grape composition, extraction technique and cell wall degradation. During ripening and post-harvest treatment, differences in cell wall composition could be responsible for different anthocyanin extractability, and together with cell porosity, for flavanol extractability (Bindon, Bacic, & Kennedy, 2012; Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006b; Quijada-Morín, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2015). Moreover, phenolic compounds have different propensity to be retained

by the cell wall depending on their structure. The mechanical resistance of cell walls to phenols release has permitted to predict phenolic compound extractability from berry physical properties. In fact, texture analysis has been proved to be a reliable tool to relate extractability and skin mechanical properties. In particular, a significant correlation has been found between skin hardness and the extraction of anthocyanins and flavanols with low and high molecular mass (Rolle, Torchio, Zeppa, & Gerbi, 2008; Río Segade et al., 2014). Recently, Laureano et al. (2016) demonstrated increased berry skin hardness for table and wine grapes after post-harvest gaseous ozone exposure (30 µL/L) for 24 hours, evidencing a role of the ozone exposure on the berry skin mechanical features. Therefore, it may affect the extraction of phenolic compounds from the skins.

The impact of post-harvest ozone treatments on the phenolic compounds extractability of winegrapes has not been studied nowadays. Therefore, in this work skin phenolic compounds extractabilities were evaluated in red grape berries exposed to continuous ozone treatment for 24 and 72 hours, and then compared to berries exposed to atmospheric air. Extraction kinetics of anthocyanins, low and high molecular mass flavanols were tested through simulated maceration using a wine-like solution in order to understand ozone related effects. Highly cultivated varieties of North-West Italy producing renowned worldwide wines, *Vitis vinifera* L. Nebbiolo and Barbera, were chosen for their different phenolic profiles. Nebbiolo grapes have a profile composed mainly by di-substituted anthocyanins and high flavanol content, whereas Barbera is characterized by tri-substituted anthocyanin prevalence and low flavanol concentration (Lambri et al., 2015; Río Segade et al., 2014).

2. Materials and Methods

2.1 Grape samples

Whole bunches of *Vitis vinifera* L. cv Nebbiolo and Barbera were harvested once reached 24°Brix at experimental vineyards located in North-West Italy, Piedmont Region, in 2014. Once in the laboratory, for each variety a subsample of berries with short attached pedicels was taken from different bunch parts (shoulders, middle, and bottom). Berries were sorted by flotation as described by Rolle et al. (2012) using different saline solutions with sodium chloride contents ranging from 130 to 170 g/L, with the aim to increase intersample homogeneity. The most representative density class (1107 kg/m^3) was chosen for both varieties, which represented about 33% and 57% (w/w) of total pre-sorting berry weight for Nebbiolo and Barbera, respectively. Sorted berries were then washed with water, visually inspected, and those with damages on the skin were discarded prior to be disposed in boxes (30 × 20 cm) in a single layer for experimental treatments.

Three sample boxes for each variety were exposed for 72 hours to atmospheric air at 20°C (control). Other three boxes were introduced into a sealed chamber, where they were exposed to a continuous 30 µL/L ozone concentration for 72 hours (OZ72) at 20°C. Other three boxes were exposed for 24 hours to ozone at 20°C and for 48 hours to atmospheric air condition (OZ24). In all cases, the average relative humidity was 70%. The ozone was supplied by an ozone generator (C32-AG, Industrie De Nora Spa, Milan, Italy) with a nominal production capacity of 32 g O_3/h. Ozone concentration in the chamber was continuously monitored by recirculation of the ozone-enriched air (120 m^3/h flow) from the chamber with a BMT 964 UV-photometric ozone analyzer (BMT Messtechnik Gmbh, DE) that controlled the ozone generator output. The relative humidity in the chamber was controlled by dehumidifiers, and the thermohygrometric conditions were constantly monitored and recorded using a data logger (HOBO H8 RH/Temp, Onset Computer Corporation, Bourne, MA).

2.2 Assessment of phenolic compound extractability

Three replicates of 40 berry skins for each treatment and for the control were used to study the phenolic compounds extractability as previously reported by Río Segade et al. (2014). The skins were carefully manually removed from the pulp using a laboratory spatula, weighed, and quickly immersed in 100 mL of a hydroalcoholic buffer solution at pH 3.2 containing 12% ethanol, 5 g/L tartaric acid and 100 mg/L sodium metabisulfite (solution A). Extractability solutions were kept at 25°C for 10 days and solution A samples were taken at 6, 24, 48, 96, 168 and 240 hours for phenolic compounds determination. After 240 hours the skins were removed from the solution A and quickly immersed in 100 mL of a hydroalcoholic buffer with higher sodium metabisulfite content, i.e. 2 g/L (solution B). Afterwards, the skins were homogenized using an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) for 1 min at 8000 rpm, and subsequently centrifuged for 15 min at 3000 × *g* at 20°C using a PK 131 centrifuge (ALC International, MI, Italy). The supernatant was collected and used to determine non-extracted phenolic compounds (Río Segade et al., 2014).

To calculate the extraction percentage, phenolic compounds were determined in the skins from three sets of 10 fresh grapes berries (3 replicates) following the extraction protocol described for non-extracted phenolic compounds but the skins were directly immersed in the solution B.

2.3 Chemical analysis

2.3.1 Reagents and standards

Solvents of HPLC-gradient grade and all other chemicals of analytical reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, UK). About standards for calibration curves, malvidin-3-glucoside chloride was supplied by Extrasynthèse (Genay, France), whereas cyanidin chloride and (+)-catechin were purchased from Sigma. For identification purposes, anthocyanin standards (delphinidin-3-glucoside chloride, malvidin-3-glucoside chloride, petunidin chloride, peonidin-3-glucoside chloride, and cyanidin-3-glucoside chloride) were purchased from Extrasynthèse.

2.3.2 Technological parameters determination

At harvest, three replicates of 100 fresh berries were manually crushed, and the standard physicochemical parameters were determined in the grape juice obtained by centrifugation. Organic acids (citric, tartaric, and malic acids, g/L) and reducing sugars (glucose and fructose, g/L) were quantified using an HPLC system equipped with a DAD set to 210 nm and a refractive index detector, respectively, as described by Giordano, Rolle, Zeppa and Gerbi (2009). Chromatographic separation was performed using a 300 mm × 7.8 mm i.d. Aminex HPX-87H cation exchange column and a cation H^+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA) at 65°C. The mobile phase was 0.0065 mol/L H_2SO_4 at 0.8 mL/min flow-rate. Titratable acidity was estimated as g/L of tartaric acid following the OIV method (OIV, 2008), and pH was determined by potentiometry using an InoLab 730 pHmeter (WTW, Weilheim, Germany).

2.3.3 Phenolic compounds determination

Phenolic compounds were determined by spectrophotometric methods (Rigo et al., 2000; Torchio, Cagnasso, Gerbi, & Rolle, 2010) using an UV-1800 spectrophotomer (Shimadzu Corporation, Kyoto, Japan). Total anthocyanins (TA) were expressed as malvidin-3-glucoside chloride, flavanols reactive to vanillin (FRV) as (+)-catechin, and proanthocyanidins (PRO) as

cyanidin chloride. Proanthocyanidins were transformed into cyanidin by acid hydrolysis at 100°C with a ferrous salt ($FeSO_4$) as catalyst (Bate-Smith reaction). Extracted phenolic compounds for each sampling point (6, 24, 48, 96, 168, 240 hours) (solution A), non-extracted phenolic compounds (solution B), and total phenolic compounds (fresh berry skins) were calculated as mg/g of skins, allowing to minimize the effect of berry weight, and then expressed as extraction yield. For each type of phenolic compounds, the extraction yield was estimated as the content in the solution A at each sampling point divided by the content in fresh berry skins, whereas the percentage of non-extracted phenolic compounds from skins was estimated as the content in the solution B divided by the content in fresh berry skins.

For the determination of the anthocyanin profile, berry skin extracts were filtered through 0.45 µm PTFE membrane filters (Pall Corporation, Port Washington, NY, USA) and injected (50 µL) in the HPLC-DAD system. The HPLC-DAD system and chromatographic conditions were previously reported in the literature (Río Segade et al., 2014). Briefly, a LiChroCART column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany) and packed with LiChrospher 100 RP-18 (5 µm) particles supplied by Alltech (Deerfield, IL, USA) was used. The mobile phases were A=formic acid/water (10:90, v/v), and B=formic acid/methanol/water (10:50:40, v/v), working at 1 mL/min flow-rate. The free forms of anthocyanins were identified by comparing the retention time of each compound with that of pure standard, whereas the tentative identification of the acylated forms was done by comparing the DAD spectrum and retention time of each chromatographic peak with those available in the literature (Pomar, Novo, & Masa, 2005). The amounts of individual anthocyanins were expressed as percentages.

2.4 Statistical Analysis

Statistical analyses were performed using the SPSS statistics software package (version 19.0; IBM Corporation, Armonk, NY, USA). One-way analysis of variance (ANOVA) was carried out and Tukey-b ($p < 0.05$) test was used to establish significant differences.

3. Results and discussion

3.1 Grape composition at harvest

The most represented density class was 1107 kg/m^3, corresponding to a reducing sugar content of about 250 g/L in both cultivars, and therefore it was chosen. Complete technological ripeness data, skin phenolic composition and anthocyanin profiles of grapes at harvest are reported in Table 1. Anthocyanin profiles of Nebbiolo and Barbera berries were in accordance with those reported in literature for these varieties (Lambri et al., 2015; Mattivi, Guzzon, Vrhovsek, Stefanini, & Velasco, 2006; Torchio et al., 2010). Barbera is characterized by a high tri-substituted anthocyanins percentage with a malvidin-3-glucoside prevalence, whereas Nebbiolo is rich in di-substituted anthocyanins with a predominance of peonidin-3-glucoside. Barbera grapes presented lower FRV and PRO contents, whereas they were more abundant in TA compared to Nebbiolo in accordance with previous results (Río Segade et al., 2014; Rolle et al., 2012; Torchio et al., 2010).

3.2 Anthocyanin extraction kinetics

Anthocyanin extraction kinetics, expressed as extraction yield, is reported in Figure 1. Ozone treatments of Barbera grapes did not show significant effects on final extraction yield, although some differences were found at the beginning of maceration. In fact, the anthocyanin extractability of the control sample was higher than that of ozone-treated grapes: control sample

showed a significantly different extraction yield (p<0.05) from OZ24 grapes only at 6 hours of maceration (+2.68%), whereas significant differences (p<0.05) were found compared to OZ72 grapes at 6, 24 and 48 hours of maceration (+4.03%, +8.93%, +9.48%, respectively). At 48 hours of maceration, for both control and OZ24 grapes, the maximum extraction was reached (71.67% and 66.17%, respectively), whereas for OZ72 grapes it was achieved at 96 hours of maceration (63.04%). Probably, these differences are due to a slowest anthocyanin extraction in long ozone-treated samples. After reaching the maximum extraction yield, it decreased progressively for all the trials as maceration progressed because released anthocyanin compounds can suffer chemical reactions and also be fixed again onto the skins. Nevertheless, this decrease was lower in OZ72 grapes, and so that the differences were shortened. No significant differences were found after 48 hours among the different treatments, and at the end of maceration the final yield was 63.44%, 59.87%, and 59.69% in control, OZ24 and OZ72 samples, respectively.

The ozone treatment effect was more remarkable in Nebbiolo grapes, where the anthocyanin extraction occurred faster than in Barbera grapes. The highest extraction yield was reached at 24 hours of maceration with values of 90.16%, 86.88%, and 78.65% in OZ24, OZ72, and control grapes, respectively. From early stages of maceration (6 hours), significant differences were found between ozone-treated and control samples ($p < 0.01$), but not between the two ozone treatments. Nevertheless, at the end of maceration, when the extraction yield for control, OZ24 and OZ72 samples was 59.91%, 68.62%, and 64.23%, respectively, significant differences among all the samples were found ($p < 0.01$). At any maceration time, OZ24 sample gave the higher anthocyanin extraction yield, followed by OZ72. Ozone treatments facilitated the anthocyanin release from the skins into the wine-like solution without increasing the loss of released anthocyanins.

In Barbera grapes, longer maceration times seemed to reduce the initial differences in anthocyanin extractability among treatments, on the contrary in Nebbiolo the differences among treatments increased towards the end of the maceration period. Ozone can interact with the cell wall through disassembly phenomena leading to a decrease in pectin solubilization (Rodoni, Casadei, Concellón, Chaves Alicia, & Vicente, 2010). Even if pectin solubilization is a required process to allow anthocyanin extraction, harder berry skins could be connected with a greater cell wall fragility allowing an easier phenolic compounds release in the medium (Río Segade et al., 2014). Laureano et al. (2016) found an increase in skin hardness in different table and wine grape varieties after ozone treatment (probably as occurred for Nebbiolo in this work). However, the skin hardening grade was variety dependent. In particular, in Barbera grapes with densities lower than 1119 kg/m^3, no significant increase of skin break energy (W_{sk}) was found, justifying the absence of significant differences except for the early maceration stages. Studies on cell wall composition showed some differences in the contents of uronic acids, cellulosic glucose, proteins, lignin and polyphenols among varieties, which can strongly influence the anthocyanin extractability (Hernández-Hierro et al., 2014; Ortega-Regules et al., 2006b).

3.3. Anthocyanin profiles

Barbera and Nebbiolo anthocyanin profiles are shown in Tables 2 and 3, respectively. In all Barbera samples, malvidin-3-glucoside was the most abundant compound, reaching the maximum relative abundance at the end of maceration (48.27%, 49.20%, and 47.27% for control, OZ24, and OZ72, respectively). However, at the same maceration time, no significant differences in the anthocyanin profiles were found among the treatments, except for the non-extracted peonidin-3-glucoside fraction between control and OZ72 samples, showing a significantly higher concentration in control (+ 1.03%) than in OZ72 samples. No significant effect of ozone treatments was found on Nebbiolo anthocyanin profile, which is characterized by a high content of di-substituted anthocyanins: peonidin-3-glucoside was the main compound along maceration in all samples with a

relative abundance of 50.59%, 51.46%, and 50.48% for control, OZ24, and OZ72 samples, respectively, at the end of maceration.

In both varieties higher differences in the anthocyanin profile were given by the maceration time. The extraction kinetics of individual anthocyanins was constant for all the treatments, confirming that it is dependent on each individual anthocyanin form (González-Neves, Gil, & Barreiro, 2008). Generally, for Barbera grapes in all the treatments, di-substituted anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) were extracted first, reaching the highest percentage at 6 hours of maceration and then decreased progressively. Cyanidin-3-glucoside was also released at the beginning of maceration in Nebbiolo grapes, decreasing afterwards along maceration. Cyanidin-3-glucoside is extracted early during vinification (González-Neves et al., 2008), but the higher contribution of this form to the anthocyanin profile (and therefore to the content) for Nebbiolo than for Barbera explains the faster extraction of total anthocyanins in Nebbiolo samples. In fact, cyanidin is considered as the easiest anthocyanin to be extracted but the fastest form to decrease in grape juice. This is due to its oxidation during the early stages of winemaking when oxidative enzymes are more active and more oxygen is dissolved, and to the higher oxidability rate of the catechol substituent respect to the other anthocyanin substituents (Sarni, Fulcrand, Souillol, Souquet, & Cheynier, 1995). In the present study, simultaneously to the significant decrease of cyanidin-3-glucoside, as maceration progressed a higher contribution of malvidin-3-glucoside on the total anthocyanins was observed for Nebbiolo and Barbera grapes in all samples.

In Barbera, petunidin-3-glucoside and delphinidin-3-glucoside reached the highest extraction percentage at 48 hours, although it was only significantly different for petunidin-3-glucoside in OZ72 samples. However, their relative abundances decreased afterwards in all samples, achieving the lowest percentages at the end of maceration. On the other hand, malvidin-3-glucoside increased continuously until the end of maceration. Conversely, in Nebbiolo, delphinidin-3-glucoside contribution was stable during maceration and petunidin-3-glucoside increased slightly at the end of maceration in control and OZ72 samples. Malvidin-3-glucoside also increased continuously during maceration representing the second most abundant anthocyanin form after 96 hours of maceration. The different kinetics of malvidin-3-glucoside and peonidin-3-glucoside can explain the differences among the two varieties at the point of highest extractability for total anthocyanins, where the peonidin prevalent-variety reached the highest extraction percentage before the malvidin-prevalent variety, as a consequence of the different affinity of anthocyanins to be released in the medium (Di Stefano, Borsa, & Gentilini, 1994). At the end of maceration, the Barbera and Nebbiolo extracts showed the highest percentages of mono-hydroxylated B-ring forms (malvidin and peonidin), which are less prone to oxidation leading to greater colour stability. Delphinidin, cyanidin, and petunidin are more oxidable and their concentration decreases more rapidly (Cheynier, Souquet, Kontek, & Moutounet, 1994).

In both varieties, acetyl derivatives were not influenced by neither the treatment nor the maceration time, whereas cinnamoyl derivatives seem to be affected by the maceration time. In fact, in Barbera the higher percentage of cinnamoyl derivatives was reached at 24, 48, and 96 hours for control, OZ24, and OZ72 samples, respectively, whereas in Nebbiolo the maximum contribution was observed at 24 hours.

The post-harvest ozone treatments tested did not modify or negatively influence the anthocyanin profiles of grapes. A previous work reported that physical treatments applied on fresh grapes, such as microwave, freezing, and steam blanching, can affect individual anthocyanin extractability (Río Segade et al., 2014), but this did not occur with ozone. In accordance with a previous study, the extraction kinetics of individual anthocyanins highlighted that their release during maceration depends on different solubility and structure of individual compound, and their

content is influenced by the reactivity in the medium (Cheynier et al., 1994). The different affinity of individual anthocyanins for cell wall components conditions their extractability, and once solubilized in the medium, they can undergo reactions leading to losses or adducts neo-formation (Gonzales-Neves et al., 2008; Ortega-Regules et al. 2006b; Sarni et al., 1995). In general, the ratio tri-substituted/di-substituted anthocyanins increased with maceration in both varieties. In fact, an initial peonidin-3-glucoside and cyanidin-3-glucoside diffusion is followed by a higher tri-substituted anthocyanin extraction, in particular malvidin-3-gucoside (Di Stefano et al., 1994). This can result in an improvement of wine colour stability, since malvidin-3-glucoside is the most stable form of free anthocyanins.

3.4. Oligomeric and polymeric flavanol extraction kinetics

The determination of proanthocyanidins (PRO) using Bate-Smith reaction can estimate high molecular mass flavanols (i.e. ≥ 5 units, polymeric flavanols), whereas flavanols reactive to vanillin (FRV) account for flavanols of 2-4 units and monomers (oligomeric flavanols) (Vrhovsek, Mattivi, & Waterhouse, 2001).

Figure 2 shows the extraction kinetics of oligomeric flavanols (FRV), expressed as extraction yield. Barbera grapes showed, in general, a lower FRV extraction yield than Nebbiolo, particularly in the ozone-treated grapes. In Barbera, as occurred for anthocyanin extraction, in the early maceration stage (6 hours), the two ozone-treated samples achieved significantly lower FRV extraction percentages than the control samples (-7.59% and -9.46% for OZ24 and OZ72, respectively; $p<0.01$). Then, no significant differences between the two ozone-treated samples were found, whereas some differences between OZ72 and control samples were found during maceration. After 6 hours of maceration, the FRV extractability agreed for OZ24 and control samples, but significantly lower extraction percentages were observed during maceration (i.e. at 96 and 168 hours) for OZ72 samples compared to control samples ($p<0.05$). Ozone treatments resulted in a slower FRV extraction: the maximum yields of 72.94%, 62.27%, and 50.05% were recorded at 96, 168, and 240 hours for control, OZ24, and OZ72, respectively. However, the final FRV extraction yield was not significantly different among treatments (66.10%, 59.38%, and 50.05% for control, OZ24, and OZ72, respectively) because the extraction percentage decreased for control and OZ24 samples after achieving the maximum value whereas it continued to increase in OZ72 samples until 240 hours of maceration.

Regarding Nebbiolo, higher FRV extraction yields were reached at 168 hours for all the trials. Contrarily to ozone-treated Barbera samples, Nebbiolo grapes treated with gaseous ozone had significantly higher FRV yields than the control sample ($p<0.05$) at 24, 96 and 168 hours of maceration for OZ24 and at 24, 96, 168 and 240 hours for OZ72. The final FRV extraction yield was 78.82%, 86.13%, and 90.55% for control, OZ24, and OZ72, respectively. In general, the longer the maceration the smoother the differences among samples, probably due to cell wall degradation phenomena and the ethanol enriched medium which may facilitate the compounds extraction (Canals, Llaudy, Valls, Canals, & Zamora, 2005).

Polymeric flavanols (PRO) extraction kinetics for both Nebbiolo and Barbera winegrape varieties is shown in Figure 3. In Barbera, PRO extraction was not significantly influenced by the ozone treatment at any maceration time, probably due to high standard deviations among replicates as well as to low values of extraction yield. As occurred for anthocyanins and oligomeric flavanols, the two ozone-treated samples showed lower PRO extraction percentages than the control samples, particularly OZ72 at maceration times lower than 168 hours. As seen also for oligomeric flavanols, ozone treatments slowed down the extraction kinetics: the highest PRO yield of 45.14%, 34.12%, and 32.53% was reached at 48, 96, and 168 hours for control, OZ24, and OZ72 samples, respectively. On the contrary, Nebbiolo showed significantly different PRO extraction kinetics

among treatments. In the early stages of maceration (i.e. between 24 and 48 hours), significantly different PRO extraction yields were found among all three treatments themselves ($p<0.001$), in particular reaching higher extraction percentages in OZ24 samples followed by OZ72. In both ozone-treated samples, similar PRO extraction yields were observed at 96 hours of maceration (95.62% and 97.56% for OZ24 and OZ72 samples, respectively), whereas the control reached significantly lower values of 83.54% ($p<0.01$). These differences were kept along maceration and ozone-treated grapes had significantly higher extraction yields than control samples at 240 hours (80.51%, 89.08%, and 89.59% for control, OZ24, and OZ72 samples, respectively).

In Nebbiolo, ozone-treated grapes showed increased flavanol extraction yield, which was more evident in polymeric flavanols than in oligomers from the early stages of maceration. The oligomeric fraction is more easily extracted than the polymeric, because flavanol extraction becomes more difficult as the polymerization degree increases (Quijada-Morín et al., 2015). Polymeric flavanols strongly interact with the components of the skin cell wall, but its porosity also influences the extractability of these compounds. Ozone treatments decrease pectin solubilization and can lead to changes in the affinity degree between the cell wall and high molecular mass flavanols (Quijada-Morin et al., 2015; Rodoni et al., 2010). Changes in the skin cell wall composition facilitate the adsorption of high molecular mass fractions in relation with enhanced cell wall porosity (Bindon, et al., 2012). As in grape ripening, the increase in the cell wall porosity can result in a greater adsorption of highly polymerized flavanols in the pores, leading to a slower or decreased extractability (Bindon, et al., 2012; Quijada-Morín et al., 2015). Indeed, as the flavanols concentration increases, the selectivity of cell walls for the adsorption of high molecular mass flavanols decreases due to a concentration-dependent effect (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014). It partially explains the differences in extraction kinetics between the two varieties. A reduced and slow extraction of polymeric flavanols can be common in varieties with low flavanol contents, as it happened in Barbera. In Nebbiolo, higher skin flavanol concentrations could decrease the membrane selectivity for high molecular mass flavanols, resulting in an easier polymeric flavanols extraction accordingly to the concentration-dependent effect described by Bindon et al. (2014). Increased skin hardness after ozone treatment probably also facilitates the release of flavanols during maceration of Nebbiolo grapes (Laureano et al., 2016; Río Segade et al., 2014). Considering that the amount and structure of extracted flavanols are related to the grape variety (Mattivi et al., 2009), further studies should be done taking into account flavanols profiles and interactions with cell walls during ozone treatment to better understand these variations.

3.5. Ozone effects on phenolic compounds extractability

Ozone treatment showed different tendencies in the two varieties, leading to an increased skin phenolic compounds extraction in Nebbiolo grapes, while it did not influence the final extraction yield of Barbera grapes. Therefore, the ozone influence on phenolic compounds extractability was variety-dependent. Skin cell wall composition, thickness and hardness, berry weight as well as phenolic composition have a great effect on the extraction kinetics and extraction yield of phenolic compounds. Laureano et al. (2016) reported that post-harvest gaseous ozone treatments lead to an increase in skin hardness in all the grape varieties studied, but the hardening degree is variety-dependent. In detail, higher skin break energy (Wsk) values were observed in ozone-treated Barbera only on berries with high level of ripeness (i.e 1,119 kg/m3), while at 1,107 kg/m3, Barbera grape density of this study, no difference were found.

Moreover, Río Segade et al. (2014), studying correlations between W_{sk} and phenolic compounds extractability, found an inverse relationship in the varieties studied: W_{sk} is positively correlated with phenolic compounds extractability in Nebbiolo, whereas in Barbera lower PRO, FRV and TA extraction yields were achieved for higher values of W_{sk} in berries belonging to the same density class (1107 kg/m^3). Mechanical properties, such as skin break energy, depend mainly

on skin cell wall composition, which varies according to the maturity and to the grape variety (Hernández-Hierro et al., 2014; Ortega-Regules et al., 2006b). During grape ripening, berry firmness loss involves complex phenomena associated with the disassembly of the pectin network at the primary cell wall and middle lamella (Ortega-Regules et al., 2006b). This degradation is derived from the action of hydrolytic enzymes. Among them, pectinmethylesterase (PME) catalyzes the demethylesterification of pectin residues, releasing sites accessible to polygalacturonase (PG) (Roe & Bruemmer, 1981). Botondi et al. (2015) studied PME and PG activities in shock ozone treatments (18 hours, 1.5 g/h) and long treatments (4 hours each day, 0.5 g/h) prior to or during withering, respectively, of wine grapes. They reported that those enzymes are unaffected by the ozone immediately after the treatment, but they showed a decline of PME activity in all samples and of PG activity in untreated berries after dehydration. In other horticultural products like tomatoes ozone fumigated at 10 µL/L for ten minutes, no differences were found in PG and PME activities immediately after the treatment, whereas after 9 days of storage PME showed a 50% decrease in its activity compared to the untreated sample (Rodoni et al., 2010).

D'Haese, Horemans, De Coen, & Guisez (2006) highlighted that ozone-stress responses in *Arabidopsis thaliana* exposed to 150 ng/L ozone for 8 hours a day during two days include up-regulating genes involved in cell wall stiffening and repressing those related to cell elongation processes. In our experimental conditions, probably there was not enough treatment time and/or maceration time to appreciate this effect, considering that the berries were processed after three days of treatment. Nevertheless, a possible induction of cell wall stiffening could have contributed to skin hardening of Nebbiolo grapes after ozone treatment promoting increased extractability of phenolic compounds.

Other cell defense response to ozone stress is the synthesis of antioxidants, such as flavanols (Artés-Hernández et al., 2003; Carbone & Mencarelli, 2015). In particular, a study on white winegrapes cv. Grechetto showed a significant increase in (+)-catechin concentration after 12 hours of 1.5 g/h gaseous ozone treatment followed by one day of storage, showing a fast response of cells to ozone stress (Carbone & Mencarelli, 2015). However, other studies found no significant differences in total polyphenol and anthocyanin content in red winegrape cv. Pignola (Botondi et al., 2015), enforcing the supposition that grapes response to ozone stress could depend on the variety, as well as on the exposure time and ozone concentration.

Regarding the treatment time effect, in our findings OZ72 samples gave lower extractability confronted to the OZ24 samples in Nebbiolo for TA and PRO, whereas no significant differences were found for Barbera. Farther the hypothesis mentioned above, we cannot exclude an oxidation of phenolic compounds in samples treated with longer ozone exposure. Ozone oxidant activity is known, as it is decomposing itself either spontaneously or in contact with oxidable substrates such as phenolic compounds. Through direct reaction, ozone attaches itself to a double bound of organic compounds forming an unstable primary ozonide, which cleaves to form carbonyl compounds. In anthocyanins, the ring-opening is responsible for their degradation, leading to chalcone formation (Criegee, 1975; Tiwari, O'donnell, Patras, Brunton, & Cullen, 2009b). Tiwari et al. (2009a) found that gaseous ozone treatment (1.6 % w/w) for 10 minutes in processing grape juice causes losses of 78%, 95%, and 99% of cyanidin-3-glucoside, delphinidin-3-glucoside, and malvidin-3-glucoside, respectively. Although even small quantities of ozone can strongly compromise the anthocyanin content of juices, no change was observed after ozone shock treatment of grapes (Artés-Hernández et al., 2003; Botondi et al., 2015). Moreover, ozone plays an important role in the formation of ozone derivative species with high reactivity, such as $^{\bullet}O_2^-$, $HO_2^{\bullet}$, $^{\bullet}OH$, and $^{\bullet}O_3^-$, which facilitates phenolic compounds degradation in a greater extent as their attitude to release electrons increases (based on the B-ring substituent). As a consequence, variety differences in the concentration of anthocyanins and flavanols, and their chemical patterns and degree of polymerization, can influence the extent of ozone effect on phenolic compounds extractability and final content.

4. Conclusions

The use of ozone as sanitizing agent has been largely discussed in table grapes storage. Nevertheless, ozone treatment of winegrapes is an innovative technology, which deserves further research. Our study was focused on the post-harvest treatment of winegrapes with short ozone treatments (maximum three days to allow the next production phases) prior to their processing in order to avoid mycobiota spoilage and to limit the use of sulphur dioxide.

Ozone influenced the early stages of skin maceration for both Nebbiolo and Barbera grapes, leading to a higher anthocyanin extraction yield in Nebbiolo grapes and lower in Barbera. This can be due to the faster extraction of di-substituted anthocyanins, hence an improved extraction of total anthocyanins in the peonidin-prevalent variety was observed. The final anthocyanin content was not influenced for Barbera, while it increased for Nebbiolo after treatment. Moreover, ozone did not influence the final individual anthocyanin extractability, respecting the varietal anthocyanin fingerprint. For Nebbiolo, a higher flavanol extraction in ozone-treated grapes, in particular high molecular mass flavanols, can improve wine colour stability during ageing through combinations with anthocyanins. Oligomeric and polymeric flavanol extraction was slowed in both varieties after the ozone treatment, in higher extent as long as the treatment exposure time increased.

Considering these results, the use of gaseous ozone on winegrapes should be considered as a possible tool in winemaking because phenolic compounds extractability is not affected or is enhanced in ozone-treated grapes, mainly depending on the variety and, to a lesser extent, on the exposure time.

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References

Artés-Hernández, F., Artés, F., & Tomás-Barberán, F.A. (2003). Quality and enhancement of bioactive phenolics in cv. Napoleon table grapes exposed to different postharvest gaseous treatments. *Journal of Agricultural and Food Chemistry, 51(18),* 5290-5295.

Bindon, K.A., Bacic, A., & Kennedy, J.A. (2012). Tissue-specific and developmental modifications of grape cell walls influence the adsorption of proanthocyanidins. *Journal of Agricultural and Food Chemistry, 60(36),* 9249-9260.

Bindon, K.A., Madani, S. H., Pendleton, P., Smith, P.A., & Kennedy, J.A. (2014). Factors affecting skin tannin extractability in ripening grapes. *Journal of Agricultural and Food Chemistry, 62*(5), 1130-1141.

Bordiga, M., Travaglia, F., Locatelli, M., Coïsson, J.D., & Arlorio, M. (2011). Characterisation of polymeric skin and seed proanthocyanidins during ripening in six Vitis vinifera L. cv. *Food Chemistry, 127(1),* 180-187.

Botondi, R., De Sanctis, F., Moscatelli, N., Vettraino, A.M., Catelli, C., & Mencarelli, F. (2015). Ozone fumigation for safety and quality of wine grapes in postharvest dehydration. *Food Chemistry, 188,* 641-647.

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry, 53(10),* 4019-4025.

Carbone, K., & Mencarelli, F. (2015). Influence of short-term postharvest ozone treatments in nitrogen or air atmosphere on the metabolic response of white wine grapes. *Food and Bioprocess Technology, 8(8),* 1739-1749.

Cayuela, J.A., Vazquez, A., Perez, A.G., & Garcia, J.M. (2009). Control of table grapes postharvest decay by ozone treatment and resveratrol induction. *Food Science and Technology International, 15(5),* 495-502.

Cheynier, V., Souquet, J.M., Kontek, A., & Moutounet, M. (1994). Anthocyanin degradation in oxidising grape musts. Journal *of the Science of Food and Agriculture, 66(3),* 283-288.

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P.L. (2008). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry, 57(2),* 545-553.

Criegee, R. (1975). Mechanism of ozonolysis. Angewandte *Chemie International Edition in English, 14(11),* 745-752.

Cullen, P.J., Tiwari, B. K., O'Donnell, C.P., & Muthukumarappan, K. (2009). Modelling approaches to ozone processing of liquid foods. *Trends in Food Science & Technology, 20(3),* 125-136.

D'Haese, D., Horemans, N., De Coen, W., & Guisez, Y. (2006). Identification of late O3-responsive genes in Arabidopsis thaliana by cDNA microarray analysis. *Physiologia Plantarum, 128(1),* 70-79.

Di Stefano, R., Borsa, D., & Gentilini, N. (1994) Estrazione degli antociani dalle bucce dell´uva durante la fermentazione [Anthocyanins extraction from grape skins during fermentation]. *L´Enotecnico 5,* 76–83

Feliziani, E., Romanazzi, G., & Smilanick, J.L. (2014). Application of low concentrations of ozone during the cold storage of table grapes. *Postharvest Biology* and *Technology, 93,* 38-48.

Forney, C. F. (2003). Postharvest response of horticultural products to ozone. In D. M. Hodges (Ed.), Postharvest Oxidative Stress in Horticultural Crops (pp. 13–43). New York: The Haworth Press Inc.

Gabler, F.M., Smilanick, J.L., Mansour, M.F., & Karaca, H. (2010). Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. *Postharvest Biology and Technology, 55(2),* 85-90.

Giordano, M., Rolle, L., Zeppa, G., & Gerbi, V. (2009). Chemical and volatile composition of three Italian sweet white passito wines. *Journal International des Sciences de la Vigne et du Vin, 43,* 159–170.

Glowacz, M., Colgan, R., & Rees, D. (2015). The use of ozone to extend the shelf-life and maintain quality of fresh produce. *Journal of the Science of Food and Agriculture, 95(4),* 662-671.

González-Neves, G., Gil, G., & Barreiro, L. (2008). Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. *European Food Research and Technology, 226(6),* 1349-1355.

Hernández-Hierro, J.M., Quijada-Morín, N., Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., Rivas-Gonzalo, J.C., & Escribano-Bailón, M.T. (2014). Relationship between skin cell wall composition and anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo at different grape ripeness degree. *Food Chemistry, 146,* 41-47.

Kennedy, J.A., & Jones, G.P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry, 49(4),* 1740-1746.

Khadre, M.A., Yousef, A.E., & Kim, J.G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science, 66(9),* 1242-1252.

Kheir, J., Salameh, D., Strehaiano, P., Brandam, C., & Lteif, R. (2013). Impact of volatile phenols and their precursors on wine quality and control measures of Brettanomyces/Dekkera yeasts. *European Food Research and Technology, 237(5),* 655-671.

Lambri, M., Torchio, F., Colangelo, D., Río.Segade, S., Giacosa, S., De Faveri, D.M., Gerbi, V., & Rolle, L. (2015). Influence of different berry thermal treatment conditions, grape anthocyanin profile, and skin hardness on the extraction of anthocyanin compounds in the colored grape juice production. *Food Research International, 77,* 584-590.

Laureano, J., Giacosa, S., Río Segade, S., Torchio, F., Cravero, F., Gerbi, V., Englezos, V., Carboni, C., Cocolin, L., Rantsiou, K., Faroni, L.R.D., & Rolle, L. (2016). Effects of continuous exposure to ozone gas and electrolyzed water on the skin hardness of table and wine grape varieties. *Journal of Texture Studies, 47,* 40-48.

Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., & Velasco, R. (2006). Metabolite profiling of grape: flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry, 54*(20), 7692-7702.

Mattivi, F., Vrhovsek, U., Masuero, D., & Trainotti, D. (2009). Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Australian Journal of Grape and Wine Research, 15(1),* 27-35.

OIV. (2008). *Compendium of international methods of wine and must analysis*. Paris, France : Organisation Internationale de la Vigne et du Vin.

Ortega-Regules, A., Romero-Cascales, I., López-Roca, J.M., Ros-García, J.M., & Gómez-Plaza, E. (2006a). Anthocyanin fingerprint of grapes: environmental and genetic variations. *Journal of the Science of Food and Agriculture, 86(10),* 1460-1467.

Ortega-Regules, A., Romero-Cascales, I., Ros-García, J.M., López-Roca, J.M., & Gómez-Plaza, E. (2006b). A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. *Analytica Chimica Acta, 563(1),* 26-32.

Peleg, H., Gacon, K., Schlich, P., & Noble, A.C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture, 79(8),* 1123-1128.

Pomar, F., Novo, M., & Masa, A. (2005). Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography. *Journal of Chromatography A, 1094,* 34–41.

Quijada-Morín, N., Hernández-Hierro, J.M., Rivas-Gonzalo, J.C., & Escribano-Bailón, M.T. (2015). Extractability of low molecular mass flavanols and flavonols from red grape skins. relationship to cell wall composition at different ripeness stages. *Journal of Agricultural and Food Chemistry, 63(35),* 7654-7662.

Rigo, A., Vianello, F., Clementi, G., Rossetto, M., Scarpa, M., Vrhovšek, U., & Mattivi, F. (2000). Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *Journal of Agricultural and Food Chemistry, 48(6),* 1996-2002.

Río Segade, S., Torchio, F., Giacosa, S., Ricauda Aimonino, D., Gay, P., Lambri, M., Dordoni, R., Gerbi, V., & Rolle, L. (2014). Impact of several pre-treatments on the extraction of

phenolic compounds in winegrape varieties with different anthocyanin profiles and skin mechanical properties. *Journal of Agricultural and Food Chemistry, 62(33),* 8437-8451.

Rodoni, L., Casadei, N., Concellón, A., Chaves Alicia, A. R., & Vicente, A. R. (2010). Effect of short-term ozone treatments on tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. *Journal of Agricultural and Food Chemistry, 58(1),* 594-599.

Roe, B., & Bruemmer, J.H. (1981). Changes in pectic substances and enzymes during ripening and storage of "Keitt" mangos. *Journal of Food Science*, *46*(1), 186-189.

Rolle, L., Torchio, F., Zeppa, G., & Gerbi, V. (2008). Anthocyanin extractability assessment of grape skins by texture analysis. *Journal International des Sciences de la Vigne et du Vin, 42(3),* 157-162.

Rolle, L., Torchio, F., Giacosa, S., Río Segade, S., Cagnasso, E., & Gerbi, V. (2012). Assessment of physicochemical differences in Nebbiolo grape berries from different production areas and sorted by flotation. *American Journal of Enology and Viticulture, 63,* 195–204.

Sarni, P., Fulcrand, H., Souillol, V., Souquet, J. M., & Cheynier, V. (1995). Mechanisms of anthocyanin degradation in grape must-like model solutions. *Journal of the Science of Food and Agriculture, 69(3),* 385-391.

Sengun, I. Y. (2014). Influence of ozonated water on microbial load and shelf life of shredded carrots. *Italian Journal Food Science,*26, 383-389.

Tiwari, B. K., O'Donnell, C. P., Patras, A., Brunton, N., & Cullen, P. J. (2009a). Anthocyanins and color degradation in ozonated grape juice. *Food and Chemical Toxicology, 47(11),* 2824-2829.

Tiwari, B.K., O'donnell, C.P., Patras, A., Brunton, N., & Cullen, P.J. (2009b). Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. *Food Chemistry, 113(4),* 1119-1126.

Torchio, F., Cagnasso, E., Gerbi, V., & Rolle, L. (2010). Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Analytica Chimica Acta, 660,* 183−189.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture, 83(6),* 564-573.

Vrhovsek, U., Mattivi, F., & Waterhouse, A.L. (2001). Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. *VITIS-Journal of Grapevine Research, 40 (2),* 87-91.

Table 1. Chemical composition of Barbera and Nebbiolo winegrapes at harvest before ozone treatments (fresh grapes).

	Barbera	Nebbiolo
Reducing sugars (g/L)	249 ±1	248±1
Total acidity (g/L tartaric acid)	9.71±0.69	7.13±0.11
pH	3.21±0.01	3.18±0.01
Tartaric acid (g/L)	8.14±0.06	8.20±0.11
Malic acid (g/L)	3.17±0.11	2.38±0.02
Citric acid (g/L)	0.42±0.05	0.31±0.05
FRV (mg (+)-catechin/g skin)	1.94±0.17	6.27±0.31
PRO (mg cyanidin chloride/g skin)	10.22±1.09	14.82±0.36
TA (mg malvidin-3-glucoside chloride/g skin)	12.13±1.33	4.85±0.33
Dp-3-G (%)	14.77±0.31	4.50±0.26
Cy-3-G (%)	8.27±0.80	17.95±0.40
Pt-3-G (%)	12.99±0.23	3.44±0.11
Pn-3-G (%)	8.49±0.41	51.04±0.42
Mv-3-G (%)	35.50±0.88	14.52±0.30
∑Acetyl-G (%)	11.72±0.77	2.82±0.15
∑Cinnamoyl-G (%)	8.25±0.12	5.73±0.07

All data are expressed as average value ± standard deviation (*n*= 3). FRV= flavanols reactive to vanillin, PRO= proanthocyanidins, TA= total anthocyanin, Dp-3-G= delphinidin-3-glucoside, Cy-3-G= cyanidin-3-glucoside, Pt-3-G= petunidin-3-glucoside, Pn-3-G= peonidin-3-glucoside, Mv-3-G= malvidin-3-glucoside, G= glucoside.

Table 2. Anthocyanin profile of berry skins during maceration for untreated and postharvest ozone treated Barbera winegrapes.

Treatment	Maceration time (h)	Di-substituted B-ring		Tri-substituted B-ring			∑Acetyl-G (%)	∑Cinnamoyl-G (%)
		Cy-3-G (%)	Pn-3-G (%)	Dp-3-G (%)	Pt-3-G (%)	Mv-3-G (%)		
Control	6	8.97±0.16c	10.15±0.34b	10.86±0.25c	11.18±0.37ab	40.46±0.39a	11.92±0.26	6.46±0.08b
	24	8.04±0.25bc	9.14±0.25a	11.23±0.39c	11.22±0.29ab	41.18±0.43a	12.20±0.29	6.98±0.04c
	48	7.83±0.29b	9.08±0.23a	11.62±0.45c	11.88±0.21b	40.73±0.76a	11.98±0.35	6.88±0.38bc
	96	7.37±0.45ab	8.91±0.16a	10.87±0.44c	11.57±0.29b	42.34±0.84a	12.02±0.35	6.91±0.04bc
	168	6.66±0.57a	8.73±0.18a	9.88±0.42b	11.29±0.29ab	44.74±0.94b	12.11±0.33	6.59±0.09bc
	240	6.40±0.62a	8.90±0.27a	8.26±0.41a	10.73±0.30a	48.27±1.32c	11.46±0.70	5.98±0.18a
	Sign[a]	***	***	***	**	***	ns	***
	Non-extracted	7.12±1.22	9.30±0.33β	6.29±1.16	11.03±1.14	43.56±2.07	11.09±0.67	11.60±0.51
OZ24	6	8.29±0.92d	9.78±0.55b	10.09±0.81bc	10.88±0.41ab	42.21±1.40a	12.18±0.78	6.57±0.46ab
	24	7.46±0.53cd	8.66±0.23a	11.06±0.39c	11.19±0.16ab	41.87±0.97a	12.64±0.25	7.13±0.30b
	48	7.14±0.51bcd	8.54±0.26a	11.27±0.31c	11.64±0.14b	41.67±0.83a	12.48±0.23	7.26±0.28b
	96	6.67±0.50abc	8.37±0.33a	10.40±0.37bc	11.35±0.14b	43.62±0.93ab	12.57±0.13	7.03±0.23b
	168	5.84±0.47ab	8.07±0.30a	9.54±0.55b	11.11±0.25ab	46.03±1.11b	12.63±0.22	6.78±0.14b
	240	5.49±0.45a	8.14±0.32a	8.02±0.75a	10.54±0.48a	49.20±1.64c	12.53±0.22	6.08±0.05a
	Sign[a]	***	***	***	*	***	ns	**
	Non-extracted	5.99±0.52	8.84±0.09αβ	5.60±0.78	10.34±1.03	45.34±1.52	11.71±0.35	12.18±0.59
OZ72	6	8.83±0.38c	9.95±0.34b	10.30±0.33b	11.18±0.19ab	41.82±0.55ab	11.63±0.45	6.28±0.20a
	24	7.85±0.50bc	8.94±0.39ab	11.21±0.26c	11.40±0.15bc	41.61±0.60ab	12.07±0.26	6.94±0.22b
	48	7.73±0.41bc	8.92±0.27ab	11.51±0.27c	11.84±0.22d	41.06±0.20a	11.96±0.16	6.97±0.21b
	96	7.14±0.59ab	8.54±0.46a	11.10±0.15c	11.77±0.13cd	42.48±0.49b	11.93±0.31	7.03±0.18b
	168	6.50±0.65ab	8.38±0.52a	10.18±0.10b	11.41±0.07bc	44.65±0.54c	12.09±0.39	6.79±0.21ab
	240	6.18±0.70a	8.42±0.61a	8.84±0.37a	10.86±0.16a	47.27±0.13d	12.13±0.40	6.30±0.31a
	Sign[a]	***	**	***	***	***	ns	**
	Non-extracted	6.34±0.65	8.27±0.50α	7.21±0.64	12.31±0.69	43.41±1.05	10.59±0.54	11.87±0.86
	Sign[b]	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns, ns,ns,ns,*	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns

All data are expressed as average value ± standard deviation (n= 3). [a,b]Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters ([a]) within the same column indicate significant differences among maceration times for each treatment according to the Tukey-b test ($p < 0.05$). Different Greek letters ([b]) within the same column indicate significant differences among treatments for each maceration time according to the Tukey-b test ($p < 0.05$). OZ24= ozone treatment during 24 h, OZ72= ozone treatment during 72 h. Dp-3-G= delphinidin-3-glucoside, Cy-3-G= cyanidin-3-glucoside, Pt-3-G= petunidin-3-glucoside, Pn-3-G= peonidin-3-glucoside, Mv-3-G= malvidin-3-glucoside, G= glucoside.

Table 3. Anthocyanin profile of berry skins during maceration for untreated and postharvest ozone treated Nebbiolo winegrapes.

Treatment	Maceration time (h)	Di-substituted B-ring		Tri-substituted B-ring			∑Acetyl-G (%)	∑Cinnamoyl-G (%)
		Cy-3-G (%)	Pn-3-G (%)	Dp-3-G (%)	Pt-3-G (%)	Mv-3-G (%)		
Control	6	18.48±1.41c	50.42±0.99	4.40±0.29	3.15±0.05a	16.11±0.53a	3.01±0.21	4.42±0.17a
	24	17.41±0.99bc	49.64±1.13	4.75±0.26	3.69±0.19b	16.37±0.56a	3.00±0.17	5.14±0.17d
	48	17.00±0.99abc	49.66±0.88	4.78±0.28	3.90±0.05b	16.70±0.45a	2.94±0.15	5.03±0.13cd
	96	16.25±0.98abc	50.09±1.07	4.65±0.27	3.87±0.19b	17.38±0.58a	3.00±0.11	4.77±0.16bc
	168	15.08±1.03ab	50.44±0.98	4.46±0.30	3.71±0.10b	18.74±0.68b	3.06±0.12	4.51±0.07ab
	240	14.51±0.98a	50.59±1.04	4.25±0.28	3.97±0.14b	19.52±0.69b	2.95±0.09	4.22±0.10a
	Sign[a]	**	ns	ns	***	***	ns	***
	Non-extracted	10.50±0.87	53.12±0.87	2.01±0.25	2.77±0.11	17.99±0.56	3.02±0.11	10.59±0.12
OZ24	6	18.18±1.04c	50.84±1.54	4.26±0.31	3.19±0.36	16.06±1.42a	3.12±0.10	4.36±0.21ab
	24	17.50±0.91c	50.42±0.83	4.53±0.24	3.61±0.10	15.92±0.99a	3.00±0.13	5.03±0.16d
	48	17.09±0.87bc	50.54±0.95	4.53±0.23	3.74±0.20	16.22±1.04a	2.98±0.10	4.90±0.23cd
	96	16.31±0.85abc	50.91±0.90	4.40±0.23	3.74±0.18	16.95±1.08ab	3.01±0.05	4.68±0.20bcd
	168	15.17±0.90ab	51.12±1.07	4.19±0.23	3.71±0.31	18.29±1.31ab	3.04±0.06	4.48±0.25abc
	240	14.53±0.75a	51.46±1.16	4.03±0.18	3.79±0.32	19.09±1.16b	2.98±0.10	4.12±0.19a
	Sign[a]	**	ns	ns	ns	*	ns	**
	Non-extracted	10.92±1.27	53.57±1.83	1.79±0.17	2.32±0.59	17.94±1.37	3.07±0.05	10.39±0.59
OZ72	6	17.44±0.30e	51.02±1.29	4.24±0.19	3.18±0.22a	16.54±0.91a	3.11±0.10	4.47±0.18b
	24	16.76±0.20d	49.53±0.49	4.68±0.18	3.80±0.05b	17.00±0.46a	3.00±0.07	5.23±0.14e
	48	16.40±0.25d	49.72±0.35	4.70±0.22	3.84±0.04b	17.14±0.52a	3.02±0.06	5.17±0.14de
	96	15.50±0.23c	49.89±0.43	4.59±0.23	3.98±0.05b	18.10±0.52ab	3.07±0.04	4.87±0.10cd
	168	14.38±0.19b	50.04±0.53	4.35±0.26	4.00±0.10b	19.42±0.57bc	3.15±0.02	4.65±0.09bc
	240	13.54±0.11a	50.48±0.39	4.15±0.20	4.04±0.13b	20.59±0.57c	3.05±0.06	4.16±0.16a
	Sign[a]	***	ns	ns	***	***	ns	***
	Non-extracted	9.99±0.20	52.99±0.72	1.62±0.45	2.13±0.03	19.67±0.32	3.11±0.04	10.50±0.14
	Sign[b]	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns	ns,ns,ns,ns, ns,ns,ns

All data are expressed as average value ± standard deviation (n= 3). [a,b]Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters ([a]) within the same column indicate significant differences among maceration times for each treatment according to the Tukey-b test ($p < 0.05$). OZ24= ozone treatment during 24 h, OZ72= ozone treatment during 72 h. Dp-3-G= delphinidin-3-glucoside, Cy-3-G= cyanidin-3-glucoside, Pt-3-G= petunidin-3-glucoside, Pn-3-G= peonidin-3-glucoside, Mv-3-G= malvidin-3-glucoside, G= glucoside.

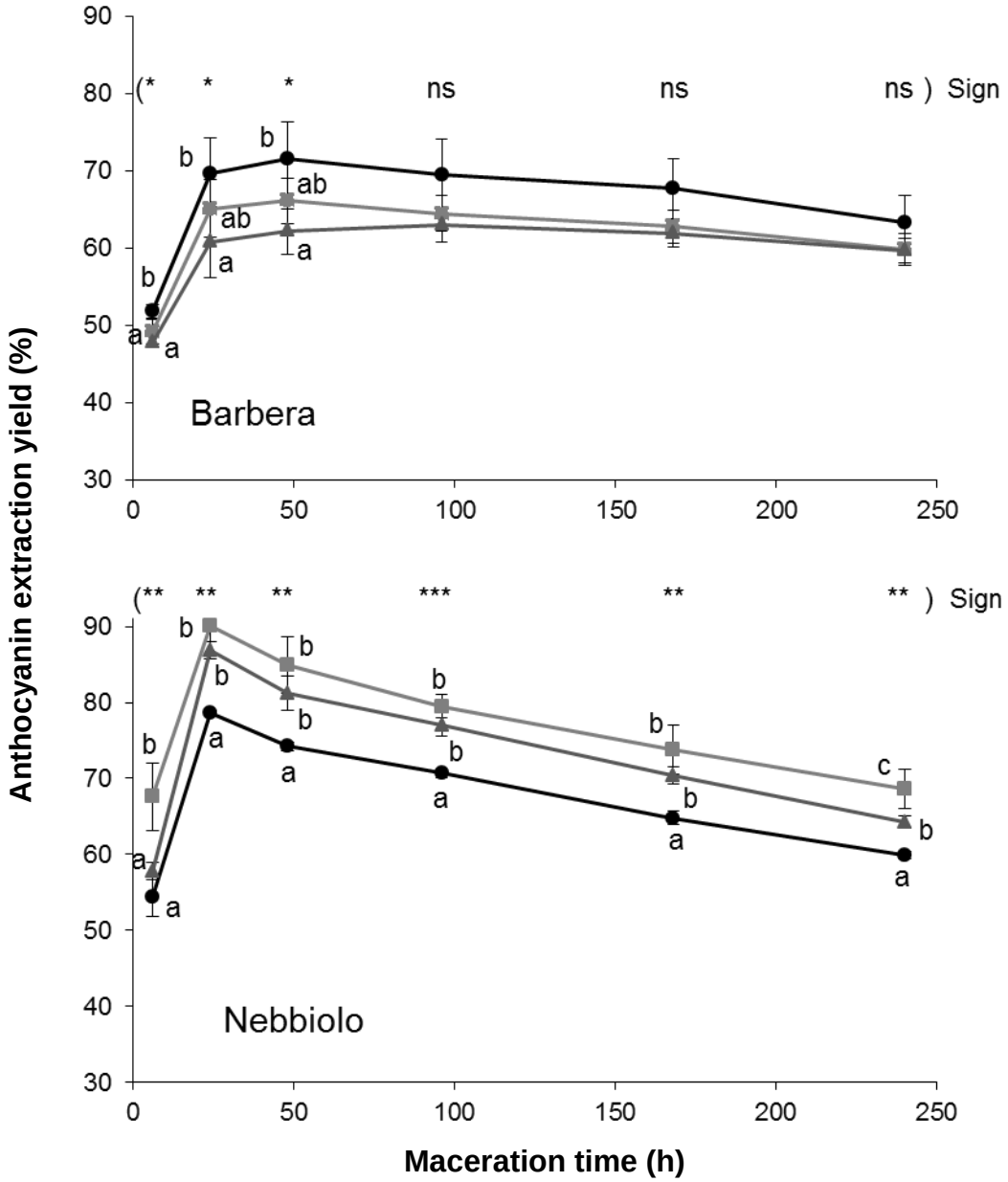

Figure 1. Effect of ozone treatment on the anthocyanin extraction during maceration for Barbera and Nebbiolo winegrapes. All data are expressed as average value ± standard deviation (n= 3). Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively, for the differences among treatments (●, control; ■, ozone treatment during 24 h; ▲, ozone treatment during 72 h) for each maceration time. Different letters indicate significant differences according to the Tukey-b test ($p < 0.05$).

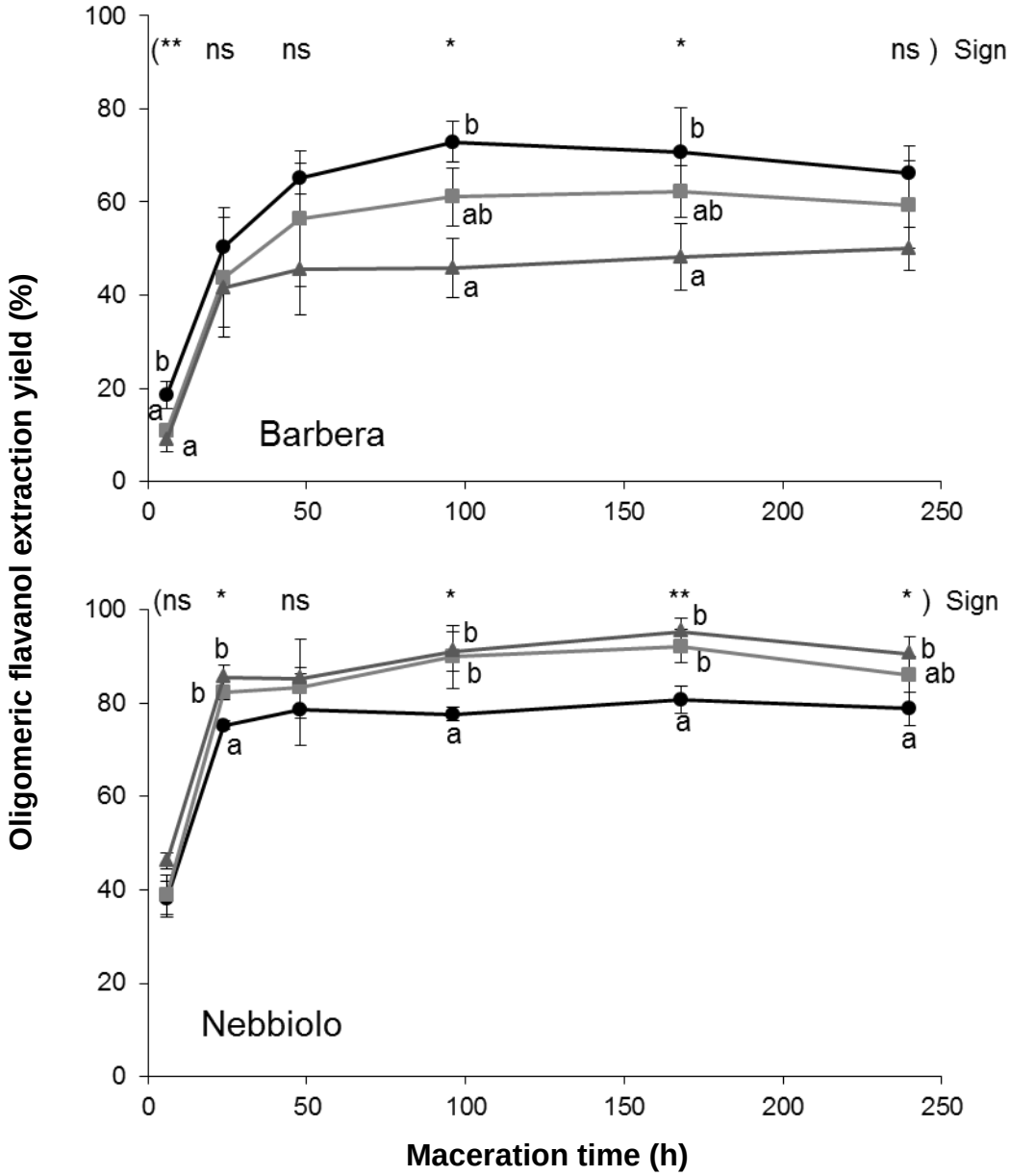

Figure 2. Effect of ozone treatment on the oligomeric flavanol extraction during maceration for Barbera and Nebbiolo winegrapes. All data are expressed as average value ± standard deviation (n= 3). Sign: *, ** and ns indicate significance at $p < 0.05$, 0.01 and not significant, respectively, for the differences among treatments (●, control; ■, ozone treatment during 24 h; ▲, ozone treatment during 72 h) for each maceration time. Different letters indicate significant differences according to the Tukey-b test ($p < 0.05$).

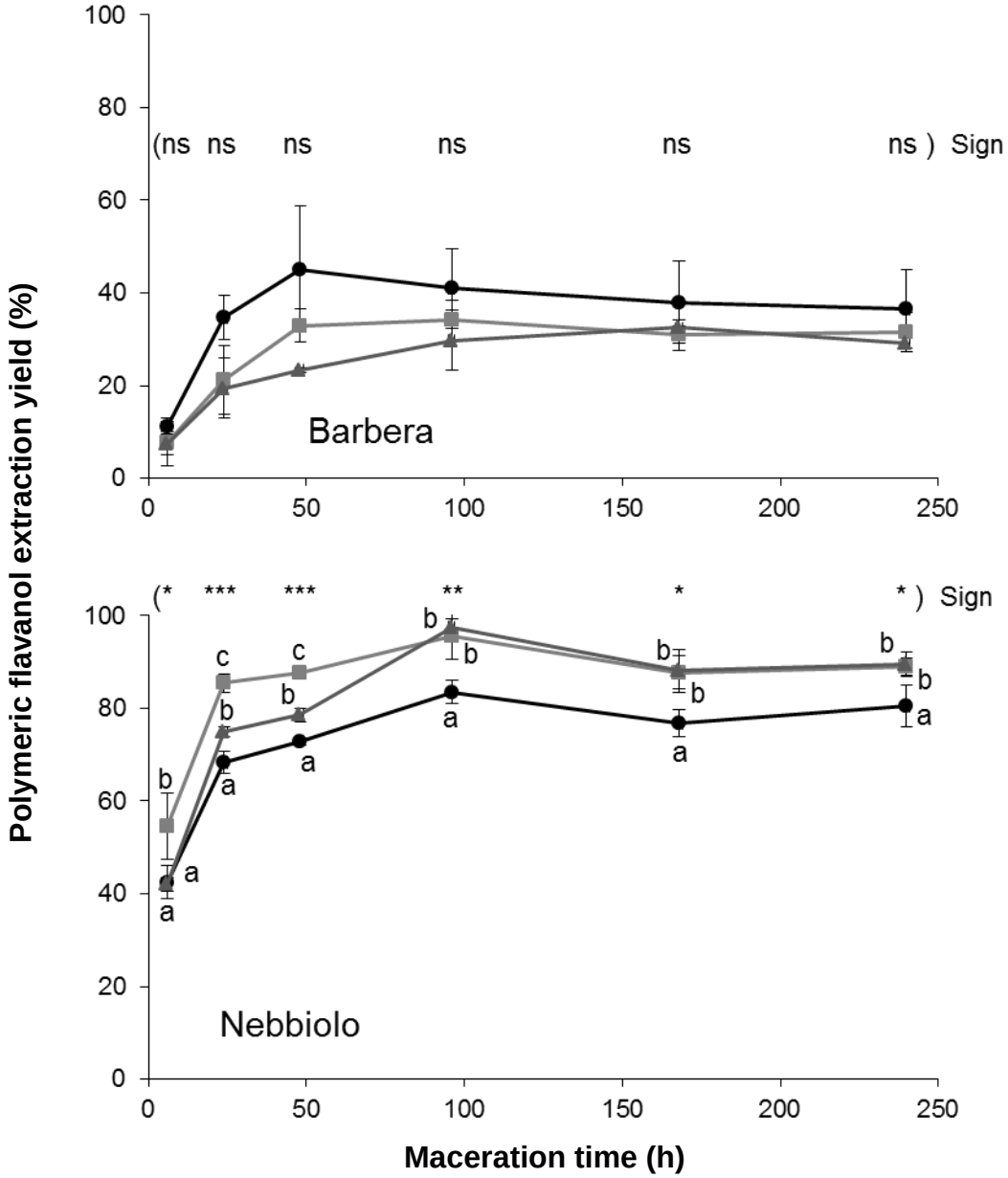

Figure 3. Effect of ozone treatment on the polymeric flavanol extraction during maceration for Barbera and Nebbiolo winegrapes. All data are expressed as average value ± standard deviation (n= 3). Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively, for the differences among treatments (●, control; ■, ozone treatment during 24 h; ▲, ozone treatment during 72 h) for each maceration time. Different letters indicate significant differences according to the Tukey-b test ($p < 0.05$).