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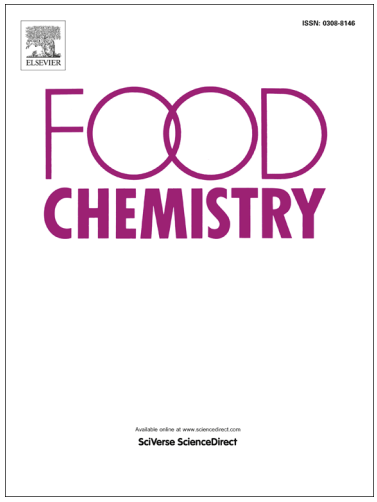

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WINEGRAPES DEHYDRATION UNDER OZONE-ENRICHED ATMOSPHERE: INFLUENCE ON BERRY SKIN PHENOLS RELEASE, CELL WALL COMPOSITION AND MECHANICAL PROPERTIES

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ABSTRACT

Gaseous ozone has been recently proposed as sanitizing agent to control mycobiota on grapes. The aim of this work was to evaluate the impact of ozone treatment during winegrapes dehydration (10 and 20% weight loss) on the content of phenolic compounds after treatment and their extractability during simulated maceration. The results showed that the ozone effect depends on the profile and content of anthocyanins and flavanols. For varieties characterized by prevalence of di-substituted anthocyanins and high flavanol contents, no significant differences were observed in phenolic compounds contents, but lower anthocyanin extractability was found. Instead, for varieties rich in anthocyanins and with a tri-substituted prevalent profile, lower anthocyanin contents were found at 20% WL, but their extractability was significantly increased. Using multivariate analysis, the extractability was correlated with skin cell wall composition and mechanical properties. Proteins, non-cellulosic glucose and total phenols contributed mainly to explain phenolic compounds extractability in withered grapes.

Keywords: Ozone, postharvest treatment, winegrapes withering, phenolic compounds, cell wall composition

Abbreviated title: Ozone modifies skin cell wall composition and phenols extractability

1. Introduction

Grape withering is a widespread technique used in wine industry to produce special wines with peculiar features, such as *passiti*, reinforced, *sfursat* and ice wines. Unlike the drying process, where the fast water removal avoids grapes over-ripening and senescence metabolism, dehydration during the withering process involves slow water loss and, as a consequence, grape berry composition changes depending on metabolic responses to water stress and on the susceptibility to fungal attack (Mencarelli & Tonutti, 2013). During "off-vine withering", grape dehydration takes place in detached bunches. "Natural off-vine withering" occurs under uncontrolled environmental conditions, whereas "forced off-vine withering", better defined as "controlled withering", is carried out in controlled thermohygrometric conditions using technology (Mencarelli & Tonutti, 2013).

The metabolism of berries during postharvest dehydration involves primary metabolites changes, such as sugars respiration/fermentation, gluconeogenesis and malate catabolism, and influences secondary metabolism, such as lignin pathway, cell wall composition, aroma and phenolic compounds, as responses to osmotic and oxidative stress (Bonghi, Rizzini, Gambuti, Moio, Chkaiban, & Tonutti, 2012). The direct consequence of water loss is metabolites concentration, in particular sugars, volatile compounds and polyphenols, although synthesis and loss can also occur (De Rosso et al., 2016).

Regarding red grape phenolic compounds, anthocyanins from skins, and monomeric, oligomeric and polymeric flavanols from both skins and seeds strongly influence the quality of final product depending on their contents and chemical features because they are responsible for colour, astringency and bitterness of the wine (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009). Grape dehydration leads to wines with increased mean degree of polymerization (mDP) of flavanols and reduced monomeric flavanol contents (Bonghi et al., 2012; Moreno, Cerpa-Calderón, Cohen, Fang, Qian, & Kennedy, 2008), whereas controversial results are reported for grape anthocyanins depending on the variety, withering conditions and anthocyanin substitution patterns (Bellincontro et al., 2009; Bonghi et al., 2012; Mikulic-Petkovsek, Jug, Rescic, & Rusjan, 2017; Toffali et al.,

2011). The extractability of phenolic compounds depends not only on the grape richness but also on the tendency to yield up them. In berry skins, anthocyanins are located inside cell vacuoles, whereas flavanols are mainly linked to the cell wall (Quijada-Morín, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2015). Therefore, skin cell wall constitutes the first barrier to phenolic compounds release even though the chemical and structural characteristics of phenolic compounds, such as stereochemistry, conformational flexibility, molecular weight and substitution pattern, together with cell wall composition and porosity can strongly influence their extractability (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014; Hernández-Hierro et al., 2014; Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006; Quijada-Morín et al., 2015).

Skin cell wall composition is variety-dependent, but postharvest dehydration can strongly influence the polysaccharides degradation because the higher the dehydration the higher the demethoxylation and depolymerization of pectins as a consequence of berry enzyme activities (Zoccatelli et al., 2013). This natural degradation of cell wall has a key role in berry skin softening (Yakushiji, Sakurai, & Morinaga, 2001; Rolle, Giacosa, Río Segade, Ferrarini, Torchio, & Gerbi, 2013). In particular, skin hardness parameters determined by instrumental texture analysis, such as berry skin break energy (W_{sk}) and berry skin break force (F_{sk}), have been largely investigated as predictors of the easiness of phenolic compounds to be released from skins to the wine (Río Segade et al., 2014).

An important aspect to take into account during dehydration is the microbiological control of grapes: the high relative humidity around berries together with cracks in the berry skin can bring to mould infection, which is a danger to the wine quality and can lead to a production loss. Moreover, fungi development can cause the formation of compounds dangerous for human health, in particular some fungal species belonging to *Aspergillus* genus are responsible for ochratoxin A (OTA) contamination (Valero, Marín, Ramos, & Sanchis, 2008). Nowadays, the control of environmental conditions and the use of sulphur bentonite are the possible solutions to reduce the pathogen attack on berries (Mencarelli & Tonutti, 2013). Sulphur bentonite causes blanching of red

grapes and could compromise secondary metabolites located in the skin. As an innovative alternative, ozone is a powerful tool to reduce fresh grapes microbiota, leading to satisfactorily healthy berries and resulting in faster and better controlled alcoholic fermentation (Bellincontro, Catelli, Cotarella, & Mencarelli, 2017; Cravero et al., 2016). Moreover, phenolic compounds extractability is not negatively affected or, in some case, is even enhanced in fresh grapes (Bellincontro et al., 2017; Paissoni et al., 2017), as well as phenolic compounds content in withered grapes (Botondi, De Sanctis, Moscatelli, Vettraino, Catelli, & Mencarelli, 2015), depending on the dose/time ratio of the ozone treatments and on the variety.

Nowadays, no studies on the impact of ozone treatments during the winegrape dehydration process on the extractability of the skin phenolic compounds have been made. Therefore, the aim of this work is to evaluate if the use of ozone as a sanitizing tool during grape dehydration affects the final content in withered grapes or the extractability of skin phenolic compounds during simulated maceration in a wine-like solution, as well as to try to justify those effects on the basis of skin cell wall composition and mechanical properties that are studied in withered ozone-exposed grapes also for the first time in this work.

2. Materials and methods

2.1. Grape samples and dehydration process

Whole bunches of *Vitis vinifera* L. cv. Nebbiolo and Barbera red winegrapes were harvested at technological maturity (about 24 ºBrix) in vineyards located in Piedmont region (Cuneo province, North-West Italy) in 2015. Once in the laboratory, for each grape variety a set of randomly selected grape berries (about 2 kg) was taken as fresh sample (fresh berries). The other bunches were cut in smaller clusters (5-6 berries each), visually inspected to remove unhealthy or damaged berries and randomly arranged in a single layer into twelve small perforated boxes (20 cm ×30 cm, about 1.5 kg of clusters each) for correct aeration. Six sample boxes were partially dehydrated into an ozone-enriched chamber and the other six boxes into an air chamber (control), taking three boxes at 10%

weight loss and three boxes at 20% weight loss for both ozone-treated and control grapes. Weight loss (WL) was monitored daily, and thermohygrometric parameters were continuously recorded using a data logger (HOBO H8 RH/Temp, Onset Computer Corporation, Bourne, MA, USA) to confirm that the environmental conditions were similar in the two withering chambers. Temperature and relative humidity (RH) were controlled at 20 °C and 70% RH (Ossola et al., 2017) using dehumidifiers and air conditioning systems. In the ozone-enriched chamber, the ozone was continuously 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 into the chamber was set at 30 µL/L (Paissoni et al., 2017) and constantly monitored with a BMT 964 UV-photometric ozone analyzer (BMT Messtechnik GmbH, Stahnsdorf, Germany) that controls the ozone generator output.

2.2. Standard chemical parameters

For each variety studied, a first set of three berry subsamples (100 g each) of fresh grapes, as well as of air-treated and ozone-treated grapes dehydrated at 10 and 20% WL, were randomly collected to determine standard technological parameters. For each subsample, grape must was obtained by manual crushing and centrifugation. Reducing sugars (glucose and fructose, g/L), organic acids (tartaric acid, malic acid, citric acid, g/L), ethanol (% v/v) and glycerol (g/L) were determined by high performance liquid chromatography (HPLC) (Agilent Technologies, Santa Clara, CA, USA) using a refractive index detector and a diode array detector (DAD) set to 210 nm (Ossola et al., 2017). Titratable acidity (g/L tartaric acid) was estimated according to the International Organization of Vine and Wine method (OIV, 2018). pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany).

2.3. Phenolic composition

2.3.1. Extraction of total phenolic compounds

Total content determination of phenolic compounds in fresh berries, as well as in air-treated and ozone-treated dehydrated berries, was performed as described by Río Segade et al. (2014). Briefly, for each grape variety and sample, a second set of three replicates of 10 berries were randomly selected and manually peeled with a laboratory spatula to separate skins from pulps. The berry skins were weighed and quickly immersed into 50 mL of a hydroalcoholic buffer solution at pH 3.2 containing 12% v/v ethanol, 5 g/L tartaric acid and 2 g/L sodium metabisulfite (solution B). The pulps were separately collected into tubes containing 100 mg sodium metabisulfite, weighed and diluted (9:1, m/m) with 5 mol/L sulphuric acid. Afterwards, an Ultraturrax high-speed homogenizer (IKA Labortechnik, Staufen, Germany) was used to homogenize the suspensions (Ultraturrax T25 at 8000 rpm for 1 min for skins, and Ultraturrax T10 at 9500 rpm for 30 s for pulps). Homogenized suspensions were subsequently centrifuged in a PK 131 centrifuge (ALC International, Milan, Italy) for 15 min at 3000×g at 20 °C. Phenolic compounds were determined in the resulting pulp and skin solutions.

2.3.2. Extractability assessment of skin phenolic compounds

A third set comprised three replicates of 20 berry skins for fresh grapes, as well as for air-treated and ozone-treated dehydrated grapes, which were used to study the phenolic compounds extractability during simulated maceration as previously reported by Río Segade et al. (2014). For each variety and replicate, the skins were carefully manually removed from the pulp, weighed and quickly immersed into 100 mL of a hydroalcoholic buffer solution at pH 3.2 containing 12% v/v ethanol, 5 g/L tartaric acid and 100 mg/L sodium metabisulfite (solution A). Extractability solutions were kept at 25 °C for 7 days, and samples were taken at 3, 6, 9, 12, 24, 48, 85 and 168 h for phenolic compounds determination. The extraction percentage was calculated as the ratio between phenolic compounds contents in each solution A and in the solution B.

2.3.3. Phenolic compounds determination

The spectrophotometric determination of total anthocyanins (TA), flavanols reactive to vanillin (FRV) and proanthocyanidins (PRO) was performed as reported by Río Segade et al.

(2014) using an UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Solutions A and B from the skins were directly analyzed, whereas the pulp extracts were submitted to reverse-phase solid-phase extraction (RP-SPE) using a 1 g Sep-Pak C-18 cartridge (Waters Corporation, Milford, MA, USA) with methanol as the eluent to remove sugars and organic acids that can interfere with the analysis. The contents for skins were calculated as both mg/kg grape (wet weight) and mg/g skin (lyophilized, dry weight) in order to consider overall changes (dehydration and ozone) in grapes phenolic composition and to underline differences imputable only to ozone treatment, respectively. The contents for pulps were calculated as mg/kg grape. The results were expressed as malvidin-3-glucoside chloride (Extrasynthèse, Genay, France) for TA, (+)-catechin (Sigma-Aldrich, Saint-Louis, MO, USA) for FRV and cyanidin chloride (Sigma-Aldrich) for PRO.

For the determination of the anthocyanin profile, berry skin extracts (solution B) and C-18 purified pulp extracts were diluted 1:1 with 0.3 mol/L hydrochloric acid, 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 (Río Segade et al., 2014). The amounts of individual anthocyanins were expressed as percentages.

2.4. Cell wall composition

For each variety, a fourth set of 300 berries for fresh grapes, as well as for ozone-treated and air-treated dehydrated grapes, were randomly taken to determine the skin cell wall composition. All berries were peeled using a laboratory spatula. The skins were carefully removed from the pulp, lyophilized and then manually ground to a fine powder with a mortar and pestle.

2.4.1. Isolation of cell wall material

The isolation of cell wall material was performed following the procedure proposed by De Vries, Voragen, Rombouts, and Pilnik (1981) and adapted by Apolinar-Valiente, Romero-Cascales, López-Roca, Gómez-Plaza, and Ros-García (2010). Briefly, 5 g of lyophilized berry

skins were suspended in boiling water for 5 min, homogenized for 1 min at 10,000 rpm and centrifuged for 15 min at 3000×g. The raw alcohol-insoluble solids were obtained after treating the residue several times with fresh 70% v/v ethanol for 30 min at 40 °C, until the Dubois test (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) indicated no sugars in the ethanol phase. After centrifugation, the alcohol-insoluble solids (AIS) was washed twice with 96% v/v ethanol and once with acetone, and finally dried overnight under an air stream at 20 °C. The recovered cell wall material was manually ground and quantified as mg/g fresh skin.

2.4.2. Determination of cell wall composition

A set of four AIS replicates (10 mg each) were treated with 72% v/v sulfuric acid for 1 h at 30 °C and subsequently with 1 M sulfuric acid for 3 h at 100 °C for acid hydrolysis. Uronic acids were determined in the resulting solution by the colorimetric 3,5-dimethylphenol assay using galacturonic acid from Sigma-Aldrich as a standard (Bautista-Ortín, Ben Abdallah, Castro-López, Jiménez-Martínez, & Gómez-Plaza, 2016). Neutral carbohydrates were also quantified in this solution as total glucose (Bautista-Ortín et al., 2016). Non-cellulosic glucose was determined performing directly acid hydrolysis with 1 mol/L sulfuric acid (Apolinar-Valiente et al., 2010) in other set of four replicates (10 mg each). Total glucose and non-cellulosic glucose were determined using an enzymatic kit from R-Biopharm (Darmstadt, Germany). Cellulosic glucose content was calculated as the difference between total glucose and non-cellulosic glucose contents. Klason lignin was determined gravimetrically after indirect acid hydrolysis (72% v/v sulfuric acid for 1 h at 30 °C and 1 mol/L sulfuric acid for 3 h at 100 °C) as described by Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, and Ros-García (2016).

In a third set of four AIS replicates (10 mg each), proteins and total phenols were extracted with 1 mol/L sodium hydroxide for 10 min at 100 °C and then quantified as reported by Apolinar-Valiente et al. (2010). Proteins were determined by the colorimetric Coomassie Brilliant Blue assay with Bovine Serum Albumin fraction V from J.T. Baker (Center Valley, PA, USA) as a standard. Total phenols were quantified spectrophotometrically by the Folin-Ciocalteu method

using gallic acid from Sigma-Aldrich as a standard. All results were expressed as mg/g AIS cell wall material (mg/g CW).

2.5. Mechanical properties

A TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with a HDP/90 platform and a 5 kg load cell, was used for skin texture analysis. For each variety and sample, a fifth set composed of three replicates of 20 randomly selected grape berries were manually peeled, and the skins were removed from the pulp using a laboratory spatula. Each skin was individually punctured using a P/2N needle probe (Stable Micro Systems) and a test speed of 1 mm/s (Rolle, Torchio, Zeppa, & Gerbi, 2008). The skin hardness was experimentally assessed by measuring two parameters: berry skin break force (N, as F_{sk}) and berry skin break energy (mJ, as W_{sk}).

2.6. Statistical analysis

Statistical analyses were performed using the SPSS statistics software package (IBM Corporation, Armonk, NY, USA). One-way analysis of variance (ANOVA) was carried out to establish significant differences between air and ozone treatments for grapes dehydrated at 10 and 20% WL. Pearson's correlation coefficients were calculated to determine significant relationships between phenolic compounds extractability and skin mechanical properties or cell wall components. Multivariate regression was used to propose a model that can explain better these relationships.

3. Results and discussion

3.1. Grape berries chemical composition

Grape analyses were performed before the treatment (fresh berries) to characterize the initial grape berries, and on dehydrated berries at 10 and 20% WL under both air and ozone-enriched atmosphere to assess the differences in the content of primary metabolites and phenolic compounds

imputable to the treatment during dehydration (ozone-treated and air-exposed grapes). Results are shown in Tables 1 and 2 for Barbera and Nebbiolo grapes, respectively. Regarding standard technological parameters, significantly higher contents of reducing sugars were found in ozone-treated samples with respect to air-exposed berries for Nebbiolo at 20% WL (p<0.05) and Barbera at both 10% (p<0.01) and 20% WL (p<0.05), ranging from +6.8% to +13.7%. Increased sugars contents in ozone-treated fruits, in particular fructose and glucose, were previously reported for the storage of tomato fruit and papaya (Ali, Ong, & Forney, 2014; Tzortzakis, Borland, Singleton, & Barnes, 2007). The other technological parameters were not significantly affected by the berries exposure to ozone, except for glycerol where the trend was not evident. In the case of long-term but intermittent ozone treatments of grapes (1.5 g/h continuous flow followed by 0.5 g/h for 4 h each day during dehydration), the malate catabolism, which is responsible for the decrease of malic acid content and titratable acidity value, could be due to a double stress response (gluconeogenesis and respiration by water stress and oxidation by ozone stress) as hypothesized by Botondi et al. (2015). However, titratable acidity did not decline during dehydration when the grapes were previously shock-ozone treated at 1.5 g/h continuous flow for 18 h (Botondi et al., 2015). Malic acid contents were unaffected by continuous ozone treatment also during tomato fruit storage for six days when compared with air-exposed fruits (Tzortzakis et al., 2007). According to Heath (2008), different metabolic pathways are stimulated by ozone exposure, depending on ozone dose or exposure time regimes.

Regarding phenolic composition, it is important to understand if the changes in partially dehydrated grapes are due to chemical reactivity, degradation phenomena or metabolic induction by ozone exposure. Barbera and Nebbiolo red winegrapes were chosen for this study to evaluate the effects of ozone during the partial dehydration of two varieties with distinctive content and profile of phenolic compounds (Río Segade et al., 2014). Taking into account that the diffusion of anthocyanins from the skin to the pulp occurs during grape dehydration due to the structural alterations in the skin (Marquez, Perez-Serratosa, Varo, & Merida, 2014), phenolic compounds

were determined in both berry skins and pulps (Tables 1 and 2). TA contents from skins and pulps were not influenced by the ozone treatment in Nebbiolo at both 10 and 20% WL, whereas significantly lower TA contents (-11.3%, $p<0.05$, and -49.7%, $p<0.001$, for skins expressed as dry weight and pulps, respectively) were found in ozone-treated Barbera winegrapes at 20% WL with respect to control samples. A small TA decrease was observed in the skin of air-treated Barbera winegrapes, but it was partially offset by the increased release of TA to the pulp during dehydration. Ozone-enriched atmosphere favoured this decreasing effect more than the water loss, although it was less balanced by releasing TA to the pulp during dehydration. It was previously demonstrated that postharvest physical treatments on whole berries can facilitate the anthocyanin release from the skin to pulp (Río Segade et al., 2014). On the other hand, Botondi et al. (2015) observed that dehydration contributed negatively to the TA content, which was compensated for ozone-exposed Pignola red winegrapes using both shock and long-term but intermittent treatments at 20% WL, but greater TA losses were reported at 35% WL for ozone-treated berries with respect to untreated samples. Tiwari, O'Donnell, Patras, Brunton, & Cullen (2009) showed that the degradation of free anthocyanin forms is due to the oxidizing potential of ozone, and it is favoured when long treatments are applied. However, the metabolic response to ozone stress depends on ozone dose, exposure time and treatment temperature (Heath, 2008) but also on grape variety as shown by our results. In our experimental conditions, ozone did not influence negatively TA content in whole grape berries, and a slight decrease was observed when the 20% WL was reached only for the Barbera variety (about -5% considering together skin and pulp), which is characterized by a high content of anthocyanins.

Regarding individual skin anthocyanins, in both varieties, a significant decrease in the percentage of di-substituted anthocyanins at 10% WL was observed: cyanidin-3-glucoside and peonidin-3-glucoside in ozone-treated Barbera grapes (for both compounds -0.7%, $p<0.05$) and peonidin-3-glucoside in ozone-treated Nebbiolo (-2.8%, $p<0.05$) when compared with control samples. In our case of study, differences in the skin anthocyanin composition between ozone-

treated and air-exposed samples could be better justified by chemical reactivity than by release from skins to pulp during the grape treatment. In fact, the anthocyanin profile of the pulp was not significantly different for ozone-treated and control berries dehydrated at 10% WL (Tables 1 and 2). This is a positive effect of ozone exposure because di-substituted anthocyanin forms are released faster from the skin during maceration, and therefore can undergo more easily oxidation, than tri-substituted anthocyanins, particularly cyanidin derivatives (González-Neves, Gil, & Barreiro, 2008). At 20% WL, a significant increase of skin tri-substituted anthocyanins was found due to ozone effect: delphinidin-3-glucoside (+0.8%, $p<0.05$) and malvidin-3-glucoside (+3.3%, $p<0.05$) for Barbera and Nebbiolo, respectively. The greater presence of malvidin derivatives can favour a more stable red pigmentation through interaction with flavanols and ethanal (Cheynier, Souquet, Kontek, & Moutounet, 1994), and it is particularly important for di-substituted prevalent varieties, such as Nebbiolo. Considering the pulp, Barbera grapes dehydrated at 20% WL under ozone-enriched atmosphere showed a significantly increased percentage of peonidin-3-glucoside (+3.4%, $p<0.05$) and decreased relative amounts of delphinidin-3-glucoside and petunidin-3-glucoside (-4.5 and -4.1%, respectively, both $p<0.001$). Tiwari et al. (2009) reported different degradation kinetics for each individual anthocyanin during ozone treatment of grape juice, where malvidin-3-glucoside, delphinidin-3-glucoside and cyanidin-3-glucoside decreased 99, 95 and 78%, respectively, after 10 min at an ozone concentration of 1.6% (w/w). However, in our study, malvidin-3-glucoside derivatives in the skin and pulp were not negatively affected by the ozone treatment.

Regarding flavanols, the response to the ozone treatment was quite similar to that observed for anthocyanins. At 10% WL, no significant differences were found in both skin and pulp monomeric and oligomeric (FRV) and polymeric (PRO) flavanols between ozone-treated and control samples for both the varieties analyzed (Tables 1 and 2). Instead, at 20% WL, FRV showed inverse trends in the two varieties studied: a significantly increased FRV content was observed for ozone-treated samples in Nebbiolo skins (+14.6%, $p<0.05$, for wet berry weight), but a decrease was found in Barbera skins (-21.8%, $p<0.05$, for dry skin weight) and pulps (-42.0%, $p<0.05$). For

PRO contents in Barbera winegrapes, a decrease was reported only in the skins with the ozone treatment (-21.4 and -14.0%, both $p<0.05$, for dry skin weight and wet berry weight, respectively). In Nebbiolo, no significant differences were found in PRO content. The different behaviour of the two varieties under the same ozone treatment could be associated with the dehydration effect for Nebbiolo and with a combined effect of dehydration and ozone for Barbera. The varietal differences in the flavanic profile could justify these results. Carbone and Mencarelli (2015) showed a great reduction of both total flavanols and total phenolics contents for ozone-treated Grechetto white winegrapes (1.5 g/h ozone for 12 h at 10 ºC) when compared to air-exposed fresh berries, whereas Bellincontro et al. (2017) observed a significant increase in flavanols for Petit Verdot red winegrapes fumigated at max 20 g/h with 6% (w/w) of ozone at 4 ºC (+8.9%). Botondi et al. (2015) reported no significant differences in total phenolics contents of Pignola red winegrapes just after shock-ozone treatment (1.5 g/h ozone for 18 h at 10 ºC), but they also showed a greater decrease when ozone-treated samples were then dehydrated at 20 and 35% WL under atmosphere enriched for 4 h/day with 0.5 g/h of ozone with respect to dehydration in air atmosphere.

3.2. Skin phenolic compounds extractability

In addition to the differences of phenolic compounds content between air-exposed and ozone-treated grapes, TA, FRV and PRO extractabilities were also assessed through simulated maceration of the skins. The results are shown in Figures 1 and 2 for Barbera and Nebbiolo, respectively. Regarding TA extractability, the two varieties showed different kinetics. For Barbera, a significantly higher TA extractability was found for ozone-treated grapes at 20% WL from the beginning up to 48 h of maceration (Figure 1b). For longer maceration times, the differences were not significant as also occurred for grapes dehydrated at 10% WL throughout the entire maceration process (Figure 1a). At the end of maceration, the TA extraction yield and extractable content for ozone-treated Barbera grapes were not significantly different from those for air-exposed samples (Table 3). On the contrary, in Nebbiolo at both 10 and 20% WL, ozone treatment leaded to a

significantly lower anthocyanin extraction throughout the maceration process with respect to the air-exposed grapes: the greater the %WL the lower the ozone unfavourable effect (Figure 2a,b). In particular, at the end of maceration, TA extraction yield was reduced by -9.1% (p<0.05) and -7.7% (p<0.001) for 10 and 20% WL, respectively, and therefore the TA extractable content (Table 3) for ozone-treated samples also decreased when compared to air-exposed grapes at both 10 and 20% WL (about -11.8%, p>0.05, and -12.9%, p<0.01, respectively, considering dry skin weight, and -13.0% and -13.9%, respectively, both p<0.05, considering wet berry weight). The same significant differences were observed by assessing together anthocyanins released in the pulp during dehydration and those extracted after 168 h of maceration: the TA extractability was 49.0% for Barbera air-treated grapes at 10 and 20% WL, 47.5 and 51.2% for Barbera ozone-exposed grapes at 10 and 20% WL, 67.3 and 56.8% for Nebbiolo air-treated grapes at 10 and 20% WL, and 58.3 and 49.0% for Nebbiolo ozone-exposed grapes at 10 and 20% WL, respectively.

In fresh grapes, Bellincontro et al. (2017) reported a higher anthocyanin extraction during Petit Verdot grapes industrial-scale fermentation after a shock ozone treatment (12 h, max 20 g/h with 6% w/w of ozone). During simulated maceration, Paissoni et al. (2017) found an increased anthocyanin extractability in Nebbiolo grapes after shock ozone treatment (24 and 48 h, 30 µL/L), whereas in the same conditions the anthocyanin extractability for Barbera was not significantly affected by the treatment. In the present study on partially dehydrated grapes, an inverse trend was observed for the Nebbiolo variety. This highlights that, in addition to the ozone effect on the TA extractability, the dehydration process can induce changes in the skin cell wall composition and texture as will be reported later.

Regarding the anthocyanin profile at the end of maceration (Table 3), no significant difference was observed for Barbera grapes dehydrated at 10 or 20% WL under ozone-enriched atmosphere and air exposure, as previously reported by Paissoni et al. (2017) in fresh grapes. However, Nebbiolo showed significantly lower di-substituted anthocyanin percentages (-2.4% for cyanidin-3-glucoside and -2.5% for peonidin-3-glucoside, both p<0.05) for ozone-exposed grapes

only at 20% WL in favour of higher malvidin-3-glucoside amounts (+5.9%, p<0.01). This may result in improved colour stability, since malvidin-3-glucoside structure is less prone to oxidation (Cheynier et al., 1994).

In Barbera, FRV extractability at the beginning of maceration was significantly higher in ozone-treated grapes than in air-exposed samples at both the dehydration levels (until 168 h for 10% WL and 48 h for 20% WL, as shown in Figure 1c,d). Although ozone treatment seems to facilitate the FRV extraction in this variety, the extractable content of flavanols at the end of maceration decreased significantly in Barbera grapes dehydrated at 20% WL under ozone-enriched atmosphere (-20.2 and -26.6%, both p<0.05, for FRV considering dry skin weight and wet berry weight, respectively, and -18.1%, p<0.05, and -25.0%, p<0.01, for PRO considering dry skin weight and wet berry weight, respectively; Table 3). PRO extraction kinetics was not modified in ozone-treated Barbera grapes (Figure 1e,f). In Nebbiolo, as it can be seen in Figure 2c,d, FRV extraction during maceration was not influenced by the treatment (ozone or air), and significant differences were found only at the end of maceration for 20% WL grapes when a lower extraction yield was observed for ozone-treated grapes (-4.6%, p<0.05). Nevertheless, Nebbiolo grapes dehydrated at 20% WL under ozone-enriched atmosphere showed increased PRO extractability until the end of maceration (Figure 2f), at which time no significant differences were observed in agreement with the extractable PRO contents (Table 3). Decreased flavanol contents were found in Nebbiolo grapes dehydrated at 10% WL in ozone-enriched atmosphere (-5.1%, p<0.001, and -6.0%, p<0.05, for FRV considering dry skin weight and wet berry weight, respectively, and -9.0 and -10.1%, both p<0.05, for PRO considering dry skin weight and wet berry weight, respectively; Table 3).

Considering together skin flavanols released in the pulp during dehydration and those extracted after 168 h of maceration, no change was found in the significance of the differences with respect to only extractable skin flavanols. On the one hand, the FRV extractability was 58.2 and 56.8% for Barbera air-treated grapes at 10 and 20% WL, 62.9 and 56.7% for Barbera ozone-exposed grapes at 10 and 20% WL, 87.6 and 68.8% for Nebbiolo air-treated grapes at 10 and 20%

WL, and 86.0 and 63.4% for Nebbiolo ozone-exposed grapes at 10 and 20% WL, respectively. On the other hand, the PRO extractability was 57.4 and 55.2% for Barbera air-treated grapes at 10 and 20% WL, 53.2 and 56.7% for Barbera ozone-exposed grapes at 10 and 20% WL, 75.0 and 54.0% for Nebbiolo air-treated grapes at 10 and 20% WL, and 73.5 and 56.0% for Nebbiolo ozone-exposed grapes at 10 and 20% WL, respectively.

The different effect of ozone exposure on the extractability of flavanols, particularly oligomeric forms (FRV), for partially dehydrated Barbera and Nebbiolo red winegrapes with relation to that previously published on fresh grapes (Paissoni et al., 2017) confirms the need to relate phenolic compounds extractability with skin cell wall composition and texture.

3.3. Skin cell wall composition and mechanical properties

Berry skin cell wall (CW) composition and mechanical properties were reported in Table 4. No significant differences were found when compared ozone-treated and air-exposed samples at the two dehydration levels in both Barbera and Nebbiolo varieties regarding CW total phenols contents, whereas a significantly higher proteins content (+9.3%, $p<0.01$) was observed only in ozone-treated Nebbiolo grapes at 10% WL.

For the two varieties studied, several changes were found in polysaccharides and lignin contents of CW between grapes partially dehydrated under ozone-enriched and air atmosphere. Neutral polysaccharides contents, expressed as total glucose, were significantly reduced in ozone-treated samples for Barbera at 20% WL (-11.6%, $p<0.001$), whereas increased for Nebbiolo at both 10 and 20% WL (+11.5%, $p<0.05$, and +7.2%, $p<0.01$, respectively). In particular, non-cellulosic glucose, which represents the hemicelluloses constituent of CW, was significantly reduced by the ozone treatment in Barbera at 10 and 20% WL (-35.8%, $p<0.01$, and -48.2%, $p<0.001$, respectively) and in Nebbiolo at 20% WL (-27.3%, $p<0.05$). In the two varieties, cellulosic glucose contents increased with the dehydration process. This increase was significantly higher in ozone-treated Nebbiolo samples at 10 and 20% WL (+10.1%, $p<0.05$, and +11.0%, $p<0.01$, respectively) when

compared with air-exposed grapes, whereas no significant differences were found between air-exposed and ozone-treated Barbera grapes.

Higher cellulosic glucose amount could justify a reduced TA extraction from ozone-treated Nebbiolo because a significant negative correlation between cellulosic glucose content and anthocyanin extraction was found (n= 10, considering average values for each of two varieties, three sampling points, and ozone and air grapes exposure during partial dehydration; R= -0.757, p<0.05). This agreed with the findings reported by other authors who highlighted that samples with the lowest TA extractability are characterized by high contents of cellulosic glucose (Ortega-Regules et al., 2006). In addition, a reduced non-cellulosic glucose content in ozone-treated samples might facilitate the TA and FRV extraction (n= 10, R= -0.661, p<0.05 and R= -0.735, p<0.05, respectively), particularly at the first maceration stages of Barbera, probably as a consequence of its higher non-cellulosic glucose contents in both fresh and partially dehydrated grapes in relation to Nebbiolo. Quijada-Morín et al. (2015) also reported a negative correlation between hemicellulosic constituents (i.e. non-cellulosic glucose) of skin CW and flavanol extraction in Tempranillo grapes. Anyway, in the present study, in partially dehydrated Nebbiolo grapes, the decrease of extraction yield for TA under ozone treatment was not observed for FRV and PRO. A higher cellulose presence in the skin CW is related to higher proanthocyanidin extractability (Quijada-Morín et al., 2015), and therefore the increased cellulosic glucose content in ozone-treated samples at 20% WL may facilitate the PRO release from skins. In our study, even though Nebbiolo grapes at 10 and 20% WL had similar cellulosic glucose contents, different PRO extraction kinetics were found and they will be justified later.

Although lignin contents increased in all partially dehydrated samples with respect to fresh berries, the lignification process seems to occur more slowly in Nebbiolo for ozone-treated grapes. These showed slightly lower lignin contents at 10% WL than air-exposed samples, but the content increased at 20% WL until achieving significantly higher values with the use of ozone (+17.8%, p<0.05). On the contrary, ozone-treated Barbera samples at 10% WL showed higher lignin contents

(+27.3%, p<0.05), but no significant differences were found at 20% WL between ozone-treated and air-exposed berries. Hernández-Hierro et al. (2014) have reported that lignin would prevent anthocyanin extraction from skins. Lignin together with cellulose combines to produce a very resistant material to chemical and biological degradation (Düsterhölt, Engels, & Voragen, 1993). This fact may justify the small differences in TA extractability among partially dehydrated Barbera grapes, for which the lowest TA extraction yields corresponded to the highest lignin contents. As well, it partially explains the lowest TA extraction yield obtained for Nebbiolo grapes dehydrated at 20% WL under ozone-enriched atmosphere (Figure 2b), given the higher content in both lignin and cellulosic glucose.

Pectic polysaccharides represent up to the 80% of grape skins polysaccharides, and their degradation strongly influences the phenolic compounds release (Apolinar-Valiente et al., 2016). In our study, pectic polysaccharides were evaluated as uronic acids, and a significantly higher content was found in both the two varieties at 10% WL (+29.8%, p<0.001, and +18.0%, p<0.05, for Barbera and Nebbiolo, respectively) and in Barbera at 20% WL (+48.0%, p<0.001) for ozone-treated samples. The dehydration and ozone effects were more evident in Barbera grapes, which also presented a higher quantity of uronic acids than Nebbiolo in fresh berries (Table 4). This increase could have contributed to facilitate the TA and FRV extraction for ozone-treated Barbera grapes at 20% WL, but only during the first 48 h of maceration, because TA extractability is positively related to the uronic acid content of skin CW (Hernández-Hierro et al., 2014; Ortega-Regules et al., 2006). Nevertheless, pectic polysaccharides fraction of skin CW has a high tendency to associate with proanthocyanidins, limiting their release (Quijada-Morín et al., 2015). This may explain the lower PRO extraction yield for ozone-treated Nebbiolo grapes at 10 % WL, particularly evident at 24 and 48 h of maceration.

In spite of the differences in CW composition between the dehydration treatments studied, no significant differences were found in the skin mechanical properties of Nebbiolo, whereas both F_{sk} and W_{sk} parameters were significantly higher in ozone-treated samples than in air-exposed ones

for Barbera dehydrated at 20% WL (+24.8%, p<0.01, and +23.5%, p<0.05, for F_{sk} and W_{sk}, respectively; Table 4). This increase is directly associated with skin hardening. According to the CW composition, this difference in the texture parameters might be linked to the significant changes in neutral carbohydrates, non-cellulosic glucose and uronic acids contents found in the skin CW of Barbera at 20% WL (Table 4). Previous studies performed on Corvina grape berries have highlighted that the skin mechanical properties are negatively correlated with the %WL during partial dehydration (Rolle et al., 2013), this correlation being significant for the F_{sk} parameter as observed in the present study for air-exposed Barbera grapes. Nevertheless, Laureano et al. (2016) reported an increased W_{sk} value in Barbera fresh grapes after post-harvest ozone treatments (30 µL/L, 24 h) in agreement with the results showed in Table 4 for Barbera dehydrated at 20% WL. Skin hardening has a direct impact on the extractability of phenolic compounds (Rolle et al., 2008), although the effect of pre-harvest grape berry treatments on the skin mechanical properties as well as the relationship between these texture parameters and the extraction yield are variety-dependent (Río Segade et al., 2014). In our conditions, despite the possible favourable effect of ozone exposure of Barbera grapes at 20% WL on the TA extractability, the W_{sk} parameter was negatively correlated with TA extraction (n= 10, R= -0.645, p<0.05), as well as with FRV extraction (n= 10, R= -0.656, p<0.05).

3.4. Multivariate analysis

Multivariate linear regression (MLR) was performed to better understand the relationship of skin cell wall (CW) composition and mechanical properties with phenolic compounds extractability (Table 4 and Figures 1 and 2). TA, FRV and PRO extraction percentages were chosen as dependent variable, and CW composition (proteins, total phenols, non-cellulosic glucose, cellulosic glucose, uronic acids and lignin) together with the texture parameter W_{sk} were independent variables. The obtained R^2 values (multiple determination coefficient), B (non-standardized regression coefficient) and β (standardized regression coefficient) were calculated. Furthermore, the MLR model was

obtained excluding W_{sk}, namely considering only CW composition, but it fitted better (higher R^2 value) taking into account both the skin CW composition and mechanical properties together for all the dependent variables (R^2= 0.948, 0.915 and 0.931 for TA, FRV and PRO models, respectively, considering CW composition alone, and R^2= 0.999, 0.986 and 0.993 for TA, FRV and PRO, respectively, considering CW composition and W_{sk} together).

For TA extractability, proteins, total phenols, non-cellulosic glucose, lignin and W_{sk} resulted to be statistically significant (p<0.001), and the final model is represented by the following equation (1):

Equation (1)

$$\textbf{TA extractability}\ (\%) = 255.262 - 2.558\,[\text{Proteins}] + 0.988\,[\text{Total phenols}] - 0.434\,[\text{Non} - \text{cellulosic glucose}] - 0.071\,[\text{Lignin}] - 28.925\,[W\text{sk}]$$

A negative relationship was found between TA extractability and proteins (β= -0.792), non-cellulosic glucose (β= -0.715), lignin (β= -0.533) and W_{sk} (β= -0.312), whereas CW total phenols were positively correlated (β= 0.665). Therefore, the variables that contribute most to the model are proteins and non-cellulosic glucose contents. This model is partially in accordance with that previously reported by Hernández-Hierro et al. (2014) where a negative correlation of TA extraction with lignin and glucose contents was also found but, in our study, no significant influence of pectic polysaccharides was observed. Ortega-Regules et al. (2006) showed an opposite influence of the CW composition on TA extractability, where higher non-cellulosic glucose and proteins contents facilitated TA extraction, whereas it was prevented by a higher total phenols quantity. Nevertheless, the contribution of these three parameters to the model was low compared to others such as fucose, galactose and mannose contents. Taking into account what was commented in the previous section (section 3.3) and the contribution of each variable to the model, we can hypothesize that lower non-cellulosic glucose and lignin contents in the skin CW after ozone treatment explain the higher TA extraction in the first maceration stages for Barbera grapes at 20%

WL. Moreover, lower TA extraction in Nebbiolo can be mainly explained by a higher amount of proteins in ozone-treated grapes at 10% WL. Conversely, at 20% WL, lignin contents became the most influent parameter on the decreased TA extractability in ozone-treated Nebbiolo samples.

For FRV extractability, proteins, total phenols, non-cellulosic glucose, cellulosic glucose, lignin and W_{sk} were statistically significant (p<0.01). The model obtained is defined by the following equation (2):

Equation (2)

$$\textbf{FRV extractability}\ (\%) = 273.744 - 3.800\,[\text{Proteins}] + 2.153\,[\text{Total phenols}] - 0.929\,[\text{Non} - \text{cellulosic glucose}] + 0.423\,[\text{Cellulosic glucose}] - 0.135\,[\text{Lignin}] - 68.228\,[W\text{sk}]$$

As for TA extractability, proteins (β= -0.750), non-cellulosic glucose (β= -0.976), lignin (β= -0.643) and W_{sk} (β= -0.469) were negatively correlated with the FRV extractability, whereas CW total phenols were positively correlated (β= 0.924). In addition, cellulosic glucose contents resulted positively correlated with FRV extractability (β= 0.424). In this case, the variables that contribute most to the model are non-cellulosic glucose, total phenols and proteins. Quijada-Morín et al. (2015) found a positive correlation between the cellulose content and monomeric and oligomeric flavanol extractabilities. Therefore, the higher the cellulose content in the CW, the higher the FRV extractabilities. On the contrary, non-cellulosic and pectic polysaccharides showed an opposition to the FRV release. In the present study, lower non-cellulosic glucose contents in the skin CW after ozone treatment explain well the higher FRV extraction in the first maceration stages for Barbera grapes at 10 and 20% WL. In our case, according to the models obtained, uronic acids influenced only polymeric flavanol (PRO) extractabilities, whose negative effect was particularly evident in Nebbiolo grapes partially dehydrated at 10% WL under ozone-enriched atmosphere after 24 and 48 h of maceration. As observed for FRV, the higher the cellulose content in the CW, the higher the PRO extractability but only in Nebbiolo at 20% WL. In fact, regarding PRO extractability, the same

parameters defining FRV model resulted to be also statistically significant (p<0.05) with the addition of uronic acids contribution; in detail, proteins (β= -0.864), non-cellulosic glucose (β= -1.235), lignin (β= -0.778), uronic acids (β= -0.396) and W_{sk} (β= -0.501) were negatively correlated with the PRO extractability, whereas CW total phenols (β= 0.787) and cellulosic glucose (β= 0.804) were positively correlated, as reported in the following equation (3):

Equation (3)

$$\textbf{PRO extractability}\ (\%) = 262.772 - 3.579\,[\text{Proteins}] + 1.499\,[\text{Total phenols}] - 0.961\,[\text{Non} - \text{cellulosic glucose}] + 0.656\,[\text{Cellulosic glucose}] - 0.133\,[\text{Uronic acids}] - 0.133\,[\text{Lignin}] - 59.584\,[W\text{sk}]$$

At the end of maceration, the FRV and PRO extractabilities for ozone-treated and air-exposed grapes were not statistically different (Figures 1c-f and 2c-f), probably due to the long contact time of skins with the hydroalcoholic solution, which facilitates flavanol extraction independently on the initial CW composition or mechanical properties (Bautista-Ortín et al., 2016).

Finally, it is important to point out that the varietal differences in the phenolic composition, namely chemical features and molecular mass of flavanols, influence their extractability because different adsorption and chemical interaction phenomena with skin CW are involved (Quijada-Morín et al., 2015; Ruiz-Garcia, Smith, & Bindon, 2014). In fact, the ozone treatment in Barbera grapes (richer in anthocyanins but poorer in flavanols; Table 1) strongly influenced FRV extractabilities (Figure 1c,d), but no significant changes were found in PRO extractabilities, even if both the skin CW composition and mechanical properties were strongly affected by the treatment (Table 4). The opposite phenomena were found in Nebbiolo (poorer in anthocyanins but richer in flavanols; Table 2) where PRO extractabilities were more affected (Figure 2e,f). A variety-dependence of the ozone influence on phenolic compounds extractability was also previously observed in fresh grape berries (Paissoni et al., 2017).

4. Conclusions

New technologies may aid to maintain the berries in good phytosanitary conditions during grape dehydration without negatively affecting the quality of grapes and to preserve the final wine quality. Ozone has been used to prevent moulds and microbiological contaminations, but to date no studies were performed on the influence of ozone sanitizing treatments during winegrape dehydration on the extractability of the skin phenolic compounds. In our findings, ozone has a variety-dependent effect, which can be strongly related to the phenolic profiles of grapes, in particular to anthocyanins. Nebbiolo, which is a di-substituted anthocyanins prevalent variety, reported no change in the content of total anthocyanins just after ozone-assisted dehydration, but their extraction yield was lower with respect to the control at 10 and 20% WL. On the contrary, although lower contents of anthocyanins were found in Barbera grapes (tri-substituted anthocyanins prevalent) just after dehydration at 20% WL under ozone-enriched atmosphere, their extractability was significantly increased during the first 48 h of maceration. Regarding oligomeric and polymeric flavanols, their extractability was less affected by the ozone treatment. Nevertheless, ozone caused changes in the extractability of flavanols in the first hours of maceration, particularly in oligomeric flavanols for Barbera and polymeric flavanols for Nebbiolo. In the case of Nebbiolo, lower extractable contents of polymeric flavanols were found in grapes partially dehydrated at 10% WL under ozone atmosphere, although no significant differences were observed in their content just after treatment. Therefore, the winemaking process should be adapted depending on the variety and on the target wine.

Several factors other than the chemical structure and content of phenolic compounds influenced their extractability, such as the amount and composition of skin cell wall material and skin hardness. In our study, the ozone-induced modification of skin cell wall composition together with skin hardness parameters fitted well in multivariate models to predict anthocyanins, oligomeric flavanols and polymeric flavanols. As a general trend, higher non-cellulosic glucose contents prevent the phenolic compounds release from skins.

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FIGURE CAPTIONS

Fig. 1. Effect of gaseous ozone exposure on the extractability of total anthocyanins (a, b), monomeric and oligomeric flavanols (c, d) and polymeric flavanols (e, f) during maceration for Barbera winegrapes partially dehydrated at 10% WL (a, c, e) and 20% WL (b, d, f). 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 between air (■) and ozone (○) treatments for each maceration time.

Fig. 2. Effect of gaseous ozone exposure on the extractability of total anthocyanins (a, b), monomeric and oligomeric flavanols (c, d) and polymeric flavanols (e, f) during maceration for Nebbiolo winegrapes partially dehydrated at 10% WL (a, c, e) and 20% WL (b, d, f). 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 between air (■) and ozone (○) treatments for each maceration time.

Table 1. Chemical composition of fresh berries and partially dehydrated berries under air and ozone atmosphere for Barbera winegrapes.

Compound	Units	Fresh berries	10% Average berry WL			20% Average berry WL		
			Air	Ozone	Sign	Air	Ozone	Sign
Grape must								
Reducing sugars	g/L	254±2	270±2	307±1	**	307±2	333±4	*
Titratable acidity	g/L tartaric acid	9.1±0.2	8.4±0.0	8.3±0.3	ns	8.6±0.4	8.5±0.2	ns
pH	-	3.06±0.03	3.10±0.01	3.05±0.01	*	3.09±0.01	3.10±0.02	ns
Tartaric acid	g/L	9.83±0.02	9.05±0.01	9.46±0.14	ns	9.97±0.04	10.01±0.11	ns
Malic acid	g/L	1.86±0.09	1.31±0.01	1.44±0.10	ns	1.79±0.82	1.28±0.01	ns
Citric acid	g/L	0.24±0.01	0.27±0.01	0.27±0.01	ns	0.27±0.01	0.31±0.02	ns
Ethanol	% v/v	0.00±0.01	0.14±0.01	0.14±0.02	ns	0.45±0.23	0.26±0.05	ns
Glycerol	g/L	0.05±0.01	0.85±0.09	0.51±0.03	*	1.47±0.01	2.28±0.09	**
Grape skin								
TA	mg malvidin-3-G chloride/g skin (dry weight)	33.0±1.4	26.3±1.3	25.4±0.6	ns	24.0±0.6	21.3±1.1	*
	mg malvidin-3-G chloride/kg grape (wet weight)	1534±95	1491±7	1558±58	ns	1487±170	1441±75	ns
Dp-3-G	%	12.6±0.6	13.2±0.4	13.1±0.5	ns	12.1±0.4	12.9±0.3	*
Cy-3-G	%	3.6±0.1	3.9±0.3	3.2±0.2	*	3.5±0.4	3.7±0.7	ns
Pt-3-G	%	13.4±0.5	13.7±0.3	13.9±0.4	ns	13.2±0.2	13.6±0.2	ns
Pn-3-G	%	4.6±0.4	4.7±0.4	4.0±0.2	*	4.5±0.6	4.6±0.7	ns
Mv-3-G	%	43.9±0.4	44.0±0.3	45.1±1.0	ns	45.9±0.3	45.4±0.9	ns
Σ Acetyl-G	%	10.3±0.3	9.6±0.4	9.6±0.7	ns	8.8±0.3	8.3±0.3	ns
Σ Cinnamoyl-G	%	11.5±0.2	10.9±0.1	11.1±0.2	ns	12.0±0.4	11.5±0.9	ns
FRV	mg (+)-catechin/g skin (dry weight)	8.54±0.96	8.29±0.67	7.39±1.17	ns	9.68±0.95	7.57±0.14	*
	mg (+)-catechin/kg grape (wet weight)	397±43	486±63	479±56	ns	603±107	512±20	ns
PRO	mg cyanidin chloride/g skin (dry weight)	32.2±2.9	29.1±3.9	28.2±3.6	ns	30.3±3.5	23.8±0.6	*
	mg cyanidin chloride/kg grape (wet weight)	1495±111	1696±165	1825±96	ns	1868±136	1607±64	*
Grape pulp								
TA	mg malvidin-3-G chloride/kg grape (wet weight)	21.2±3.4	31.0±9.9	22.1±1.1	ns	58.5±2.2	29.4±2.9	***
Dp-3-G	%	5.6±0.3	5.1±1.1	4.8±1.0	ns	8.7±0.6	4.2±0.4	***
Cy-3-G	%	12.4±1.7	9.0±1.6	9.5±2.5	ns	7.2±1.0	8.6±0.6	ns
Pt-3-G	%	7.8±0.2	8.2±1.5	7.9±0.6	ns	11.5±0.2	7.4±0.6	***
Pn-3-G	%	20.9±1.3	16.4±3.1	15.3±2.8	ns	11.5±0.8	14.9±1.4	*
Mv-3-G	%	44.7±2.6	52.8±3.4	54.5±5.1	ns	52.6±1.5	56.7±2.1	ns
Σ Acetyl-G	%	0.0±0.0	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0	-
Σ Cinnamoyl-G	%	8.5±0.4	8.5±0.3	7.9±0.3	ns	8.3±0.8	8.2±0.9	ns
FRV	mg (+)-catechin/kg grape (wet weight)	12.0±1.7	13.4±0.8	12.4±1.2	ns	22.4±1.5	13.0±3.8	*
PRO	mg cyanidin chloride/kg grape (wet weight)	48.0±6.3	48.0±5.7	47.2±7.4	ns	81.3±11.7	52.8±15.2	ns

All data are expressed as average value ± standard deviation (n = 3). TA: total anthocyanins, 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, FRV: flavanols reactive to vanillin, PRO: proanthocyanidins, WL: weight loss. Sign: *, **, *** and ns indicate significance at p<0.05, 0.01, 0.001 and not significant, respectively, for the differences between air and ozone treatments at the same dehydration level.

Table 2. Chemical composition of fresh berries and partially dehydrated berries under air and ozone atmosphere for Nebbiolo winegrapes.

Compound	Units	Fresh berries	10% Average berry WL			20% Average berry WL		
			Air	Ozone	Sign	Air	Ozone	Sign
Grape must								
Reducing sugars	g/L	258±19	270±2	274±2	ns	307±2	328±2	*
Titratable acidity	g/L tartaric acid	6.4±0.2	5.1±0.0	5.2±0.1	ns	5.3±0.2	5.3±0.1	ns
pH	-	3.16±0.04	3.32±0.02	3.27±0.01	ns	3.25±0.03	3.26±0.03	ns
Tartaric acid	g/L	7.51±0.50	7.05±0.27	6.41±0.20	ns	7.86±0.10	7.36±0.36	ns
Malic acid	g/L	1.40±0.26	1.00±0.06	1.07±0.05	ns	0.94±0.01	1.84±0.57	ns
Citric acid	g/L	0.19±0.07	0.13±0.01	0.14±0.01	ns	0.01±0.01	0.01±0.02	ns
Ethanol	% v/v	0.00±0.01	0.05±0.01	0.03±0.01	ns	0.14±0.07	0.33±0.30	ns
Glycerol	g/L	0.21±0.30	0.18±0.06	0.07±0.03	ns	1.29±0.13	0.83±0.18	ns
Grape skin								
TA	mg malvidin-3-G chloride/g skin (dry weight)	13.3±1.1	13.0±1.2	13.3±0.2	ns	13.4±0.1	13.5±0.4	ns
	mg malvidin-3-G chloride/kg grape (wet weight)	612±36	701±40	710±12	ns	760±25	796±29	ns
Dp-3-G	%	7.1±0.0	7.3±0.6	7.9±0.2	ns	7.2±0.5	6.8±0.4	ns
Cy-3-G	%	8.9±0.7	13.9±0.6	13.1±0.8	ns	12.5±0.6	11.0±1.6	ns
Pt-3-G	%	5.7±0.1	5.4±0.4	5.6±0.2	ns	5.4±0.5	5.3±0.3	ns
Pn-3-G	%	32.8±1.5	36.5±0.5	33.7±0.9	*	35.9±1.3	34.1±0.7	ns
Mv-3-G	%	31.5±1.0	23.9±0.8	25.8±1.5	ns	25.3±1.1	28.6±1.4	*
Σ Acetyl-G	%	4.4±0.4	3.8±0.2	4.0±0.2	ns	3.5±0.1	3.6±0.2	ns
Σ Cinnamoyl-G	%	9.5±1.0	9.3±0.1	9.9±1.0	ns	10.2±1.0	10.6±1.3	ns
FRV	mg (+)-catechin/g skin (dry weight)	36.0±3.0	35.3±4.1	33.9±2.4	ns	36.7±3.5	40.3±0.7	ns
	mg (+)-catechin/kg grape (wet weight)	1658±99	1907±178	1809±129	ns	2084±200	2389±33	*
PRO	mg cyanidin chloride/g skin (dry weight)	78.3±6.4	81.6±0.9	75.5±5.6	ns	90.2±7.6	84.4±7.6	ns
	mg cyanidin chloride/kg grape (wet weight)	3607±225	4419±138	4026±320	ns	5123±453	5003±456	ns
Grape pulp								
TA	mg malvidin-3-G chloride/kg grape (wet weight)	10.4±1.9	11.0±0.6	9.4±2.2	ns	13.3±0.6	13.1±0.1	ns
Dp-3-G	%	3.7±0.6	4.4±0.5	5.2±0.8	ns	5.3±1.0	4.7±0.5	ns
Cy-3-G	%	33.9±2.0	37.5±5.0	36.9±3.5	ns	31.0±1.7	30.9±3.2	ns
Pt-3-G	%	2.8±0.4	2.9±0.3	3.2±0.5	ns	3.4±0.5	3.1±0.1	ns
Pn-3-G	%	41.6±0.6	38.9±2.5	38.3±1.9	ns	42.0±0.8	41.7±2.3	ns
Mv-3-G	%	15.4±0.5	13.7±2.0	13.4±1.3	ns	14.9±1.4	16.6±1.3	ns
Σ Acetyl-G	%	0.0±0.0	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0	-
Σ Cinnamoyl-G	%	2.5±0.3	2.7±0.2	2.9±0.5	ns	3.5±0.3	3.0±0.3	ns
FRV	mg (+)-catechin/kg grape (wet weight)	73.4±12.2	92.7±8.0	76.7±21.4	ns	103.4±10.0	98.6±12.7	ns
PRO	mg cyanidin chloride/kg grape (wet weight)	176±23	188±24	161±38	ns	190±5	183±14	ns

All data are expressed as average value ± standard deviation (n = 3). TA: total anthocyanins, 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, FRV: flavanols reactive to vanillin, PRO: proanthocyanidins, WL: weight loss. Sign: * and ns indicate significance at p<0.05 and not significant, respectively, for the differences between air and ozone treatments at the same dehydration level.

Table 3. Extractable content of phenolic compounds in Barbera and Nebbiolo skins, evaluated after a 7-day maceration, for fresh berries and partially dehydrated berries under air and ozone atmosphere.

Compound	Units	Fresh berries	10% Average berry WL			20% Average berry WL		
			Air	Ozone	Sign	Air	Ozone	Sign
BARBERA								
TA	mg malvidin-3-G chloride/g skin (dry weight)	18.8±0.5	12.4±1.7	11.7±0.1	ns	10.8±0.5	11.2±1.4	ns
	mg malvidin-3-G chloride/kg grape (wet weight)	721±28	660±63	605±15	ns	733±13	692±32	ns
Dp-3-G	%	7.2±0.6	6.7±1.1	5.3±0.2	ns	7.1±0.8	6.4±0.7	ns
Cy-3-G	%	2.9±0.1	3.0±0.6	2.5±0.3	ns	2.9±0.6	3.2±0.5	ns
Pt-3-G	%	10.9±0.3	10.9±0.6	10.2±0.2	ns	10.9±0.4	10.5±0.5	ns
Pn-3-G	%	4.4±0.2	4.9±0.6	4.5±0.4	ns	4.6±0.6	4.9±0.3	ns
Mv-3-G	%	54.6±0.7	57.0±2.4	59.1±0.4	ns	56.5±1.5	58.0±1.6	ns
Σ Acetyl-G	%	12.1±0.4	10.5±0.3	10.9±0.2	ns	10.1±0.3	9.9±0.3	ns
Σ Cinnamoyl-G	%	7.8±0.2	7.0±0.2	7.5±0.5	ns	8.0±0.5	7.1±0.2	ns
FRV	mg (+)-catechin/g skin (dry weight)	6.20±0.82	4.60±0.41	4.46±0.38	ns	5.14±0.31	4.10±0.44	*
	mg (+)-catechin/kg grape (wet weight)	237±33	245±19	231±22	ns	349±18	256±44	*
PRO	mg cyanidin chloride/g skin (dry weight)	20.5±0.5	15.9±1.1	14.3±0.3	ns	15.5±1.1	12.7±1.3	*
	mg cyanidin chloride/kg grape (wet weight)	786±26	847±67	739±26	ns	1047±38	785±39	**
NEBBIOLO								
TA	mg malvidin-3-G chloride/g skin (dry weight)	9.03±0.21	8.53±0.34	7.52±0.62	ns	7.37±0.11	6.42±0.17	**
	mg malvidin-3-G chloride/kg grape (wet weight)	304±9	346±11	301±18	*	366±17	315±11	*
Dp-3-G	%	5.5±0.2	3.8±0.8	3.9±0.5	ns	4.3±0.7	3.5±0.1	ns
Cy-3-G	%	9.0±1.0	9.2±0.7	7.9±1.1	ns	10.8±0.8	8.4±0.4	*
Pt-3-G	%	5.4±0.2	4.2±0.5	4.4±0.3	ns	4.3±0.3	4.0±0.1	ns
Pn-3-G	%	34.7±2.2	38.3±2.0	35.1±2.2	ns	38.1±1.0	35.6±0.6	*
Mv-3-G	%	34.5±2.8	32.8±0.9	36.8±2.9	ns	30.0±1.3	35.9±1.4	**
Σ Acetyl-G	%	4.2±0.1	4.4±0.3	4.6±0.1	ns	4.2±0.1	4.3±0.2	ns
Σ Cinnamoyl-G	%	6.7±0.1	7.2±0.4	7.4±0.4	ns	8.3±0.4	8.3±1.1	ns
FRV	mg (+)-catechin/g skin (dry weight)	31.1±2.2	29.3±0.3	27.8±0.1	***	23.5±0.3	24.0±0.7	ns
	mg (+)-catechin/kg grape (wet weight)	1049±74	1185±13	1114±30	*	1166±51	1176±32	ns
PRO	mg cyanidin chloride/g skin (dry weight)	62.2±2.2	57.8±2.7	52.6±1.4	*	45.5±1.9	44.3±1.7	ns
	mg cyanidin chloride/kg grape (wet weight)	2096±78	2344±97	2108±25	*	2258±189	2174±43	ns

All data are expressed as average value ± standard deviation (n = 3). TA: total anthocyanins, 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, FRV: flavanols reactive to vanillin, PRO: proanthocyanidins, WL: weight loss. Sign: *, **, *** and ns indicate significance at p<0.05, 0.01, 0.001 and not significant, respectively, for the differences between air and ozone treatments at the same dehydration level.

Table 4. Skin mechanical properties and cell wall composition of fresh berries and partially dehydrated berries under air and ozone atmosphere for Barbera and Nebbiolo winegrapes.

Parameter	Units	Fresh berries	10% Average berry WL			20% Average berry WL		
			Air	Ozone	Sign	Air	Ozone	Sign
BARBERA								
Mechanical properties[a]								
F_{sk}	N	0.987±0.041	0.931±0.144	0.985±0.033	ns	0.824±0.058	1.028±0.011	**
W_{sk}	mJ	0.544±0.056	0.574±0.120	0.618±0.007	ns	0.520±0.056	0.642±0.044	*
Cell wall composition[b]								
Skin CW	mg/g fresh skin	70.5	59.0	58.7	-	62.4	60.9	-
Proteins	mg BSA/g CW	83.1±3.2	84.2±2.5	87.5±1.6	ns	83.3±4.2	83.5±4.3	ns
Total phenols	mg gallic acid/g CW	53.1±2.9	52.9±2.3	62.8±4.7	ns	63.9±2.6	58.4±4.5	ns
Neutral carbohydrates	mg glucose/g CW	204±8	212±7	210±10	ns	250±6	221±6	***
Non-cellulosic glucose	mg glucose/g CW	13±1	23±3	15±1	**	51±2	26±3	***
Cellulosic glucose	mg glucose/g CW	191±8	189±7	196±10	ns	199±4	195±4	ns
Uronic acids	mg galacturonic acid/g CW	229±21	151±11	196±6	***	127±11	188±7	***
Lignin (Klason)	mg/g CW	235±8	253±15	322±28	*	323±28	291±22	ns
NEBBIOLO								
Mechanical properties[a]								
F_{sk}	N	0.747±0.031	0.853±0.054	0.824±0.054	ns	0.825±0.047	0.839±0.021	ns
W_{sk}	mJ	0.361±0.020	0.475±0.033	0.452±0.033	ns	0.438±0.042	0.447±0.018	ns
Cell wall composition[b]								
Skin CW	mg/g fresh skin	55.5	44.5	49.8	-	50.6	51.7	-
Proteins	mg BSA/g CW	83.0±5.3	80.4±3.0	87.9±0.8	**	85.7±2.3	88.1±4.3	ns
Total phenols	mg gallic acid/g CW	61.1±4.0	64.5±2.8	69.8±3.2	ns	65.1±4.4	66.1±5.8	ns
Neutral carbohydrates	mg glucose/g CW	169±11	174±5	194±8	*	181±5	194±1	**
Non-cellulosic glucose	mg glucose/g CW	2±1	10±1	9±1	ns	16±1	12±3	*
Cellulosic glucose	mg glucose/g CW	167±10	168±6	185±7	*	164±4	182±3	**
Uronic acids	mg galacturonic acid/g CW	139±13	139±7	164±14	*	160±15	151±6	ns
Lignin (Klason)	mg/g CW	336±18	414±39	361±19	ns	359±5	423±16	*

All data are expressed as average value ± standard deviation. [a]Three replicates of 20 berry skins (n = 3). [b](n = 4). CW: cell wall, BSA: bovine serum albumin, F_{sk}: berry skin break force, W_{sk}: berry skin break energy, WL: weight loss. Sign: *, **, *** and ns indicate significance at p<0.05, 0.01, 0.001 and not significant, respectively, for the differences between air and ozone treatments at the same dehydration level.

Highlights

- Impact of gaseous ozone during dehydration of two winegrape varieties was studied
- Ozone effects strongly depended on the profile and content of phenolic compounds
- Anthocyanin extraction was higher in treated Barbera but lower in Nebbiolo
- Oligomeric and polymeric flavanol extraction was poorly affected by ozone treatment
- Phenolic extraction, cell wall composition and texture traits were strongly related

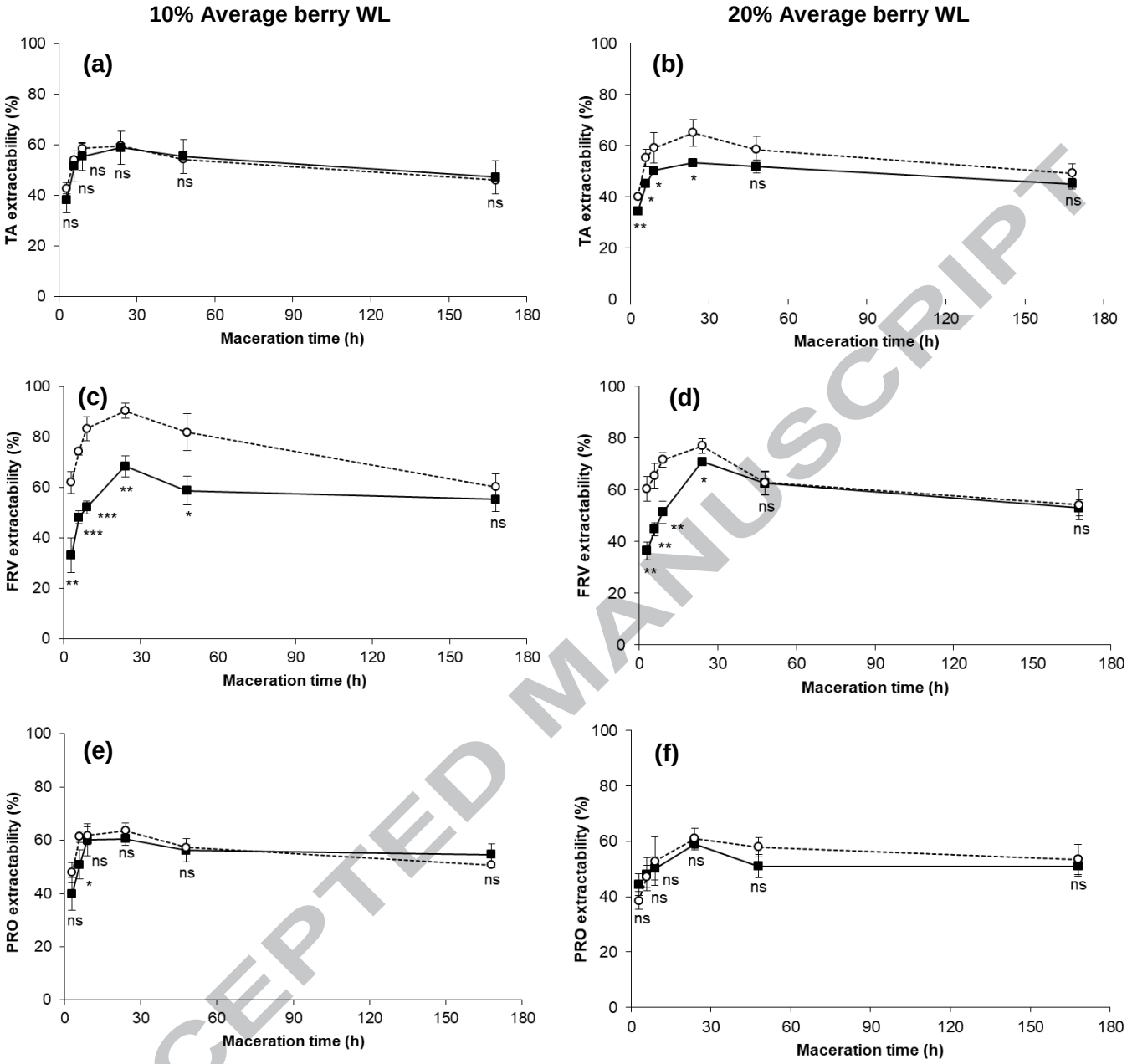

Figure 1

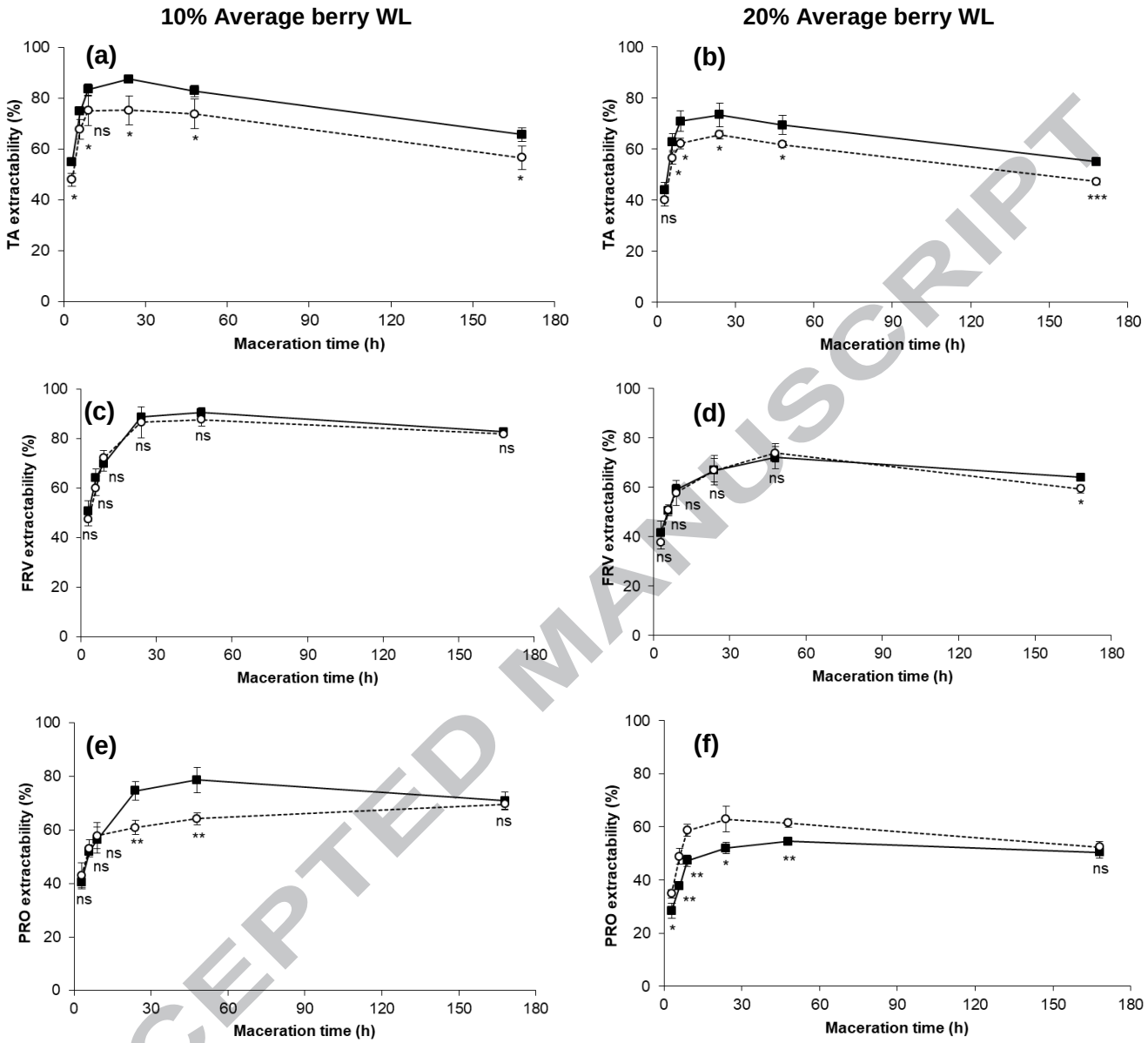

Figure 2