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Impact of specific inactive dry yeast application on grape skin mechanical properties, phenolic compounds extractability, and wine composition

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**ABSTRACT**

Foliar treatments using two products based on *Saccharomyces cerevisiae* inactive dry yeast derivatives with specific formulations for white and red varieties were tested in two consecutive vintages on *Vitis vinifera* L. cv. Chardonnay, Cortese, and Nebbiolo grown in Piedmont (north-west Italy). The possible elicitor effect of the foliar treatment was assessed at harvest on the chemical composition and mechanical properties of grape berries. The accumulation and extractability of phenolic compounds in Nebbiolo grape skins were also studied. Wines were produced and analysed in terms of technological parameters, color characteristics, free volatile composition, and phenolic compounds. The treatments induced an +16 μm average increase in berry skin thickness, which makes the grapes more resistant to physical damages and pathogenous attacks. In Nebbiolo, this treatment enhanced the accumulation of anthocyanins (+33 mg/kg on average). However, the obtained results pointed out a vintage effect. In 2015, few significant differences between wines made from control and treated grapes were found. Instead, in 2016, Nebbiolo treated wines had a slightly worse chromatic quality as a consequence of lower contents of phenolic compounds, but they were richer in relative amounts of malvidin-3-glucoside.

**Keywords**: inactive dry yeasts; grapes; wines; texture analysis; phenolic composition; volatile compounds.

## 1. INTRODUCTION

For winemakers and oenologists, the starting point to get high quality wines is to harvest grapes with optimal compositional parameters. To reach this goal, vineyard management is of primary importance together with the choice of harvest time. Afterwards, the accumulation of secondary metabolites (i.e. phenolic and aroma compounds) achieved in the vineyard should be exploited in cellar using appropriate oenological practices to improve their extraction from grape tissues into the must/wine during maceration/fermentation. In fact, the perceived quality of a wine is strongly related to the presence of phenolic and aroma compounds (Quijada-Morín et al., 2012).

In the vine plant, secondary metabolites are produced and accumulated in grape berries (Coombe & McCarthy, 2000). The accumulation of berry compounds is influenced by the grapevine response to growing conditions and particular treatments, as a result of the interaction among genetic characteristics, environmental conditions, and cultural practices (Bell & Henschke, 2005). In particular, abiotic and biotic elicitors may induce the accumulation of defence compounds in the tissues, a response similar to that exerted under attack by microbial pathogens (Ferrari, 2010). Among biological elicitors, yeast extracts (YE) are known to induce secondary biosynthetic pathways as a result of plant defence responses stimulated by their content of several components, including chitin, N-acetylglucosamine oligomers, β-glucan, glycopeptides, and ergosterol (Ferrari, 2010; Granado, Felix, & Boller, 1995). In grape berries, the skin constitutes the fundamental protective barrier against physical damage and pathogens attack, and it is involved in the synthesis of important metabolites, such as phenolic and volatile compounds (Fournand et al., 2006; González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015).

Even if this is well-known by the viticultural-oenological sector, still few studies have been published nowadays on the elicitor effect of YE application on grapevine under field conditions. Recently, Šuklje et al. (2016) showed that the inactive dry yeast application on

Sauvignon blanc cv. grapes growing in South Africa increased the production of volatile compounds in the resulting wines and/or preserved better their aromatic composition. As well as for volatile compounds, changes in the phenolic composition of red grapes have been also reported, particularly for anthocyanins and stilbenes. Thereby, Portu, López, Baroja, Santamaría, & Garde-Cerdán (2016) reported that YE foliar treatment increased the anthocyanin content in grapes and resulting wines for Tempranillo cv. growing in Spain. Similar results were found by Villangó et al. (2015) where yeast foliar applications on Syrah cv. in two different vintages (warm and cool) in Hungary enhanced the ripening process in both years with a higher accumulation of anthocyanins in treated grapes, and this resulted in more balanced, more flavoured and complex wines. Nevertheless, other factors can also influence secondary metabolites accumulation, such as vintage, cultivation practices, and variety, leading to contradictory results. A study performed by Kogkou et al. (2017) in Greece on Agiorgitiko cv. showed no effects on the grape composition as induced by the inactivated yeast foliar treatment alone, but an increase in the phenolic content of the wines was observed when the foliar treatment was combined with irrigation. Regarding stilbenes, Gil-Muñoz, Fernández-Fernández, Crespo-Villegas, & Garde-Cerdán (2017) and Portu et al. (2018) evidenced an elicitor effect of cell wall yeasts on their synthesis in Tempranillo, Monastrell, Grenache, and Graciano cv. grapes from different zones of Spain, and in the wines even though variety and vintage effects were observed. In fact, climatological conditions of abiotic stress favoured the increased content of stilbenes.

The impact of YE application on berry skin mechanical properties can be of great importance to increase the grape resistance against fungal diseases and physical injuries (Gabler, Smilanick, Mansour, Ramming, & Mackey, 2003). Furthermore, the skin mechanical properties are considered valuable parameters to estimate the skin cell wall degradability and, therefore, the extractability of anthocyanins (Rolle, Torchio, Zeppa, & Gerbi, 2008). An increase of berry skin thickness was observed in treated grapes probably as a defence

mechanism against the presence of YE (Villangó et al., 2015), and this change could influence the anthocyanin release during the maceration process (Río Segade, Giacosa, Gerbi, & Rolle, 2011). Nevertheless, inside each variety berry texture traits are vintage dependent, particularly skin hardness parameters are related to seasonal climatic indices (Rolle, Gerbi, Schneider, Spanna, & Río Segade, 2011a).

The main aim of this study was to investigate the impact of a specific inactive dry yeast foliar spray treatment on the international Chardonnay winegrape variety, and on two Italian Cortese and Nebbiolo ones known worldwide. For the first time, the effect of two products, specifically developed for white and red varieties each, was evaluated on berry skin mechanical properties of the three varieties at three different ripeness levels defined by densimetric sorting. In Nebbiolo, the changes induced by YE treatment on skin phenolic compounds and their extractability during simulated maceration using a wine-like solution were assessed. Standard chemical parameters, chromatic characteristics, phenolic compounds, and volatile composition of Cortese and Nebbiolo wines made from control and treated berries were also determined.

## 2. MATERIALS AND METHODS

### 2.1 Vineyard and field trials

The experiment was carried out in Piedmont (Italy) in two consecutive vintages (2015 and 2016). The commercial vineyard of *Vitis vinifera* L. cv. Chardonnay used for this trial was located in Chieri (North-West Piedmont, N 45.016, E 7.788) at 380 m above sea level on silty-calcareous soil. Chardonnay vines were planted in 2005 at a spacing of 2.4 m × 0.9 m. *Vitis vinifera* L. cv. Cortese commercial vineyard was located in Novi Ligure (North-West Piedmont, N 44.723, E 8.799) at 200 m above sea level on a mildly calcareous soil with a moderate slope hill. Cortese vines were planted in 1975 with spacing of 2.4 m × 1.2 m. *Vitis*

*vinifera* L. cv. Nebbiolo grapes were produced in a commercial vineyard located in Acqui Terme (North-West Piedmont, N 44.700, E 8.420) at 156 m above sea level on clay-calcareous soil with a relevant slope hill. Nebbiolo vines were planted in 2004 at a spacing of 2.4 m × 0.9 m. All vines presented a lateral cordon trellis system and Guyot-type pruning. Soil fertilization was not conducted in 2015-2016 season for Chardonnay and Nebbiolo, whereas an organo-mineral fertilizer (250 kg/ha of Emonatural NPK 8.5.15, Fertben, MN, Italy) was applied to the soil for Cortese. For each variety, homogeneous blocks of 60 vines with buffer space between them were delimited and used for the trial.

The treated vines were sprayed with an inactive dry *Saccharomyces cerevisiae* yeast formulation (LalVigne® Aroma and LalVigne® Mature, Lallemand Inc., Montreal, Canada) specifically designed to be used with the patent foliar application technology WO/2014/024039. The first product (LalVigne® Aroma) was used on Chardonnay and Cortese white cultivars at a dose of 3 kg/ha for each treatment, while the latter product (LalVigne® Mature) on Nebbiolo red cv. at a dose of 1 kg/ha for each treatment. In brief, two applications of the water-suspended product at 700 L/ha each were carried out, at 5 % veraison and 10 days later. The resulting solution was then homogenously sprayed on the whole canopy using a manual spray irrigator, without dripping. No rain occurred in the 48 h subsequent to the treatments.

## 2.2 *Grape samples and density classes selection*

For each vintage and variety, the grapes were hand harvested at optimal ripeness (Table 1) separately from control (untreated) and treated vine blocks but at the same time. For each sample, about 20 kg of grapes were randomly selected and transported to the laboratory. In addition, only for Cortese and Nebbiolo grapes, the remaining clusters (200 kg) were transported in plastic boxes (maximum capacity of 20 kg to avoid grape crushing during transport) to the experimental cellar of the University of Turin for winemaking.

Once in the laboratory, the berries were manually separated from the stalk with harvest shears and then placed on paper trays. Two replicates of 100 berries were randomly taken for standard chemical determinations, while all the other berries were sorted according to their density by flotation as described by Fournand et al. (2006) and Rolle et al. (2011b) in twelve saline solutions ranging from 80 to 190 g/L sodium chloride, with densities between 1054 and 1125 kg/m$^3$.

After flotation, all berries were washed with water, dried using adsorbent paper, and weighed to obtain density distribution curves. Three density classes were chosen as follows: the most represented density class (by weight) was taken, the immediate above and below classes were discarded (to increase the berry heterogeneity among density classes), and the subsequent above and below classes were then taken. The damaged berries were rejected. For each density class considered, thirty berries were randomly selected for the determination of skin mechanical properties and, only for Nebbiolo cv., three replicates of 20 berries were used for the study of skin phenolic compounds extractability, as previously reported by Río Segade et al. (2014).

### 2.2.1 Phenolic compounds extraction from Nebbiolo grape skins

For each replicate of 20 berries, the skins were manually removed from the pulp using a laboratory spatula, weighed, and quickly immersed into 40 mL of a hydroalcoholic buffer solution at pH 3.20 containing 5 g/L tartaric acid, 50 mg/L $Na_2S_2O_5$, and 12% v/v ethanol (solution A; Río Segade et al., 2014). The skin maceration occurred at 25 °C, and samples were taken after 3, 8, 24, 48, and 168 h to determine the phenolic compounds released from skins (Río Segade et al., 2014). At the end of simulated maceration (168 h), the skins were removed and immersed into 40 mL of a second extracting solution, prepared as the previous solution (A) but containing 2 g/L $Na_2S_2O_5$ (solution B), and then homogenized at 8000 rpm for 1 min with an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik, Staufen,

Germany). The resulting skin suspension was 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 the phenolic fraction not released (non-extracted) from skins during the first maceration in a wine-like solution (Río Segade et al., 2014).

### 2.3 Cortese and Nebbiolo wines production

For each batch, wines were produced in duplicate (100 kg of grapes per replicate), and the same winemaking procedure was applied for control and treated grapes. Cortese white grapes were placed in a thermo-controlled room at 6 °C temperature overnight, and then pressed (PMA 4 pneumatic press, Velo SpA, Altivole, Italy) with a maximum pressure of 1.0 bar in presence of sulfur dioxide (20 mg/L) and of a pectolytic enzyme preparation (20 mg/L). The mash was then kept at 6 °C for two days for settling. Afterwards, the must obtained was brought to 17 °C temperature. At the same time, 30 g/hL GoFerm Protect (Lallemand Inc.) were dissolved in water at 40 °C, cooled down to 37 °C, and then 20 g/hL of selected yeasts (Lalvin QA23 Yseo, Lallemand Inc.) were added and rehydrated. After 30 min, the stirred yeast suspension was acclimated and inoculated in the must. Alcoholic fermentation was carried out at controlled temperature (20±2 °C).

Nebbiolo red grapes were destemmed and crushed (TEMA destemmer-crusher, Enoveneta, Piazzola sul Brenta, Italy), and the mash was then placed into a $CO_2$ saturated tank, where 25 mg/L sulfur dioxide were added. After three hours, the same rehydration and inoculation procedure previously conducted for Cortese was done using 30 g/hL GoFerm Protect (Lallemand Inc.) and 20 g/hL selected yeasts (Lalvin ICV D254 Yseo, Lallemand Inc.). Alcoholic fermentation was carried out at controlled temperature (28±2 °C). After 24 hours from the yeast inoculum, a bacteria sequential co-inoculum using VP41 MBR ML bacteria (Lallemand Inc.) at a dose of 1 g/hL was prepared and added in the fermenting must. Two punch-down per day were carried out in the first three days, then two pumping-over per

day (each one using one third of the total volume) until the end of maceration, which lasted ten days and was followed by the gentle pressing of the pomace cap using the aforementioned pneumatic press with a maximum pressure of 1.2 bar. A small aliquot of the press wine was joined to the free-run wine.

For both Cortese and Nebbiolo musts, two additions of 20 g/hL of nutrients (Fermaid E, Lallemand Inc.) corresponding to a total increase of 56 mg/L yeast assimilable nitrogen (YAN) were done during fermentation: the first at the beginning and the second at 1/3 of fermentation process. At the end of fermentations (less than 2 g/L of reducing sugars for the alcoholic fermentation, absence of malic acid for the malolactic fermentation), 45 mg/L sulfur dioxide were added. Wines were stored at 0 ℃ for 2 weeks for cold stabilization, filtered (Seitz K300 grade filter sheets, Pall Corporation, Port Washington, NY, USA), and bottled.

### 2.4 Grapes and wines analysis

#### 2.4.1 Reagents and standards

Solvents of HPLC-gradient grade and all other chemicals of analytical-reagent grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solutions were prepared in deionized water produced by a Milli-Q system (Merck Millipore, Darmstadt, Germany). Chemical standards of delphinidin-3-O-glucoside chloride, petunidin chloride, peonidin-3-O-glucoside chloride, cyanidin-3-O-glucoside chloride, malvidin-3-O-glucoside chloride and cyanidin chloride were supplied by Extrasynthèse (Genay, France), whereas (+)-catechin and 1-heptanol were purchased from Sigma-Aldrich.

#### 2.4.2 Standard chemical parameters of grapes and wines

In the musts resulting from manual grape crushing and centrifugation, and in the wines obtained after one month from bottling, reducing sugars (as sum of glucose and fructose, g/L) and organic acids (such as tartaric, malic, lactic, and acetic acids, g/L) were determined by

high performance liquid chromatography (HPLC) according to Giordano, Rolle, Zeppa, and Gerbi (2009). Ethanol (% v/v) and glycerol (g/L) in wines were determined following the same HPLC methodology. pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany), and titratable acidity (g/L as tartaric acid) was estimated according to the OIV-MA-F1-05:R2011 method (OIV, 2015).

### 2.4.3 Berry skin mechanical properties

Berry skin break force (N, as $F_{sk}$), skin break energy (mJ, as $W_{sk}$), and skin resistance to the axial deformation (N/mm, as $E_{sk}$) were instrumentally evaluated in the lateral face of each berry by a puncture test using a SMS P/2N needle probe (Rolle et al., 2008). Berry skin thickness (μm, as $Sp_{sk}$) was determined on a piece of skin (about 0.25 cm$^2$) from the lateral side of each berry by a compression test using a 2 mm SMS P/2 flat cylindrical probe (Río Segade et al., 2011). All measurements were carried out using a TA.XTplus texture analyser (Stable Micro Systems, Godalming, UK) equipped with a HDP/90 platform and a 5 kg load cell.

### 2.4.4 Phenolic compounds determination in Nebbiolo grapes and wines

Spectrophotometric methods were used on berry skin extracts and wine samples to determine the total anthocyanins index (mg malvidin-3-O-glucoside chloride/kg grape or L wine, as TA), flavanols vanillin assay [mg (+)-catechin/kg grape or L wine, as FRV], and proanthocyanidins assay (mg cyanidin chloride/kg grape or L wine, as PRO) (Di Stefano & Cravero, 1991; Torchio, Cagnasso, Gerbi, & Rolle, 2010). Furthermore, total flavonoids index [mg (+)-catechin/L wine, as TF] and monomeric anthocyanins index (mg malvidin-3-O-glucoside chloride/L wine, as MA) were determined in wines. The monomeric anthocyanins index was evaluated previous isolation on polyvinylpolypyrrolidone (PVPP) and elution with

an ethanol:water:HCl 37% (70:30:1) solution (Bosso et al., 2011). A UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used.

The wine anthocyanin profile was determined by HPLC-DAD previous purification on a 1-g Sep-Pak C18 SPE cartridge (Waters Corporation, Milford, MA, USA) according to the protocol described by Río Segade et al. (2014). The chromatographic separation was performed on a LiChroCART analytical column (250 mm × 4 mm i.d.) purchased from Merck (Darmstadt, Germany) and packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA), using formic acid/water (10:90, v/v) and formic acid/methanol/water (10:50:40, v/v) as mobile phases. The identification of anthocyanins was performed according to Río Segade et al. (2014). The different individual anthocyanin forms were expressed as area percentages (Rolle & Guidoni, 2007).

### 2.4.5 Color parameters of wines

CIEL*a*b* coordinates, including lightness (L*), red/green color coordinate (a*), and yellow/blue color coordinate (b*), color intensity and hue were evaluated using the OIV-MA-AS2-11:R2006 method (OIV, 2015). A UV-1800 spectrophotometer (Shimazdu Corporation) was used.

### 2.4.6 Free volatile compounds extraction and determination in wines

Free volatile composition was determined as described by Di Stefano (1991) and Torchio et al. (2012), using 100 mL of wine diluted 1:4 in deionized water and 1-heptanol as internal standard (1 mL of a 31.38 mg/L solution in 15 % v/v ethanol). The sample was loaded onto a 5-g Sep-Pak C18 SPE cartridge (Waters Corporation), and free volatile compounds were eluted with 30 mL of $CH_2Cl_2$. The free fraction was then dried with anhydrous $Na_2SO_4$ and concentrated to about 200 μL under a stream of nitrogen for the direct injection (1 μL in splitless mode) in the gas-chromatography/mass spectrometry (GC/MS)

system (Model 7890C and 5975, respectively; Agilent Technologies, Santa Clara, CA, USA). A DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific Inc., Folsom, California) was used, and the carrier gas was helium at a flow of 1 mL/min. The acquisition range was 35-350 *m/z*. Peak identification and data elaboration were carried out as described by Torchio et al. (2012).

### 2.4.7 Sensory analysis of wines

Sensory analyses were carried out by eighteen tasters (experienced consumers) who judged differences between wines made from control and treated grapes using a Duo-trio test (ISO 10399, 2004). Given a central reference sample, tasters were provided with an additional reference sample, either at the left or right side of the central sample, and with another different sample in the other side. The taster must identify the sample (left or right glass) that is the same as the reference (central) sample.

### 2.4.8 Statistical analysis

Statistical analyses were carried out using R Statistics software version 3.4.0 (R Core Team, 2017). Levene's and Shapiro-Wilk's tests were used for assessing the homogeneity of variance and analysis of variance (ANOVA) residuals normality, respectively. In case of heteroscedasticity, the ANOVA with Welch's correction was used, followed by Tamhane's T2 post-hoc test when null hypothesis was rejected. In the case of homoscedasticity, one-way ANOVA was used, and the HSD Tukey's test for $p < 0.05$ was applied to assess significant differences between groups. The significance of the correct number of responses in duo-trio sensory analysis was obtained by comparing the data with the tabulated critical number of correct responses provided by Meilgaard, Carr, & Civille (2006).

## 3. RESULTS AND DISCUSSION

### 3.1 Grape chemical composition at harvest

With the subdivision of berries in density classes by flotation, it was possible to build density distribution curves (Figure 1), which permit to compare the heterogeneity of berry ripeness for the control and treated samples. For white varieties, the treatment seems to have induced in both years a more heterogeneous ripening of berries, as demonstrated by a less narrow Gaussian bell-shaped distribution. Particularly concerning Chardonnay (second year) and Cortese (first year), there was a broader berry distribution with non-negligible percentage of treated berries belonging to the lower density classes (lower ripeness level). For red Nebbiolo grapes, no ripeness differences between control and treated samples were found in the first year, whereas in the second year a shift of the whole distribution curve for the treated sample towards higher density classes was reported.

In Chardonnay and Cortese white grapes, the difference of berry ripeness observed in the density distribution curves between control and treated samples was not confirmed by the berry sugar content because no significant differences induced by the in-field treatment were found (Table 1). However, a lower sugars/acids ratio was evidenced for Cortese juices from treated grapes only in the 2015 vintage, since both pH and titratable acidity shifted significantly towards more acid values. The minor discrepancy between density distribution curves and sugars content could be attributable to differences in the acid composition and berry size because they influence grape classification based on density, even though in a limited contribution with respect to reducing sugars (Rolle et al., 2012).

Similarly, in Nebbiolo grapes, the differences in the standard chemical parameters seem not to agree with what was expected according to the density distribution curves. In fact, treated grapes showed a significantly increased sugar content in the juices of 2015 vintage, as well as higher pH and lower titratable acidity values, in relation to control samples although there was a clear overlapping of the two distribution curves (Figure 1). This treatment effect

was not confirmed in the 2016 vintage when a higher percentage of denser berries was found in the treated sample (Figure 1), and no significant differences were found in reducing sugars and acidity, although a higher content of tartaric acid was observed in treated berries (Table 1).

Villangó et al. (2015) found that the foliar treatment done by spraying inactive dry yeasts increased the sugar content and reduced significantly the acidity of Syrah juices at commercial harvest date, leading to a higher ripeness in YE treated berries. Nevertheless, there were vintage effects in agreement with the results of the present study. Other researchers showed no variation with the yeast treatment in the standard chemical parameters of white or red winegrape varieties (Kogkou et al., 2017; Portu et al., 2016; Šuklje et al., 2016). Therefore, a variety effect could also be hypothesized on these parameters.

### 3.2 Berry skin mechanical properties

Table 2 shows skin texture parameters of berries belonging to the three different density classes selected for each of the three varieties studied in two consecutive years. This permits to assess if the effect of the inactive dry yeast treatment on skin mechanical properties depends mainly on ripeness stage. Chardonnay was the variety most influenced by the treatment as showed by the changes in all skin texture parameters, even if not significant for all density classes. The berry skin hardness was assessed through the measure of three mechanical parameters: skin break force ($F_{sk}$), skin break energy ($W_{sk}$), and skin resistance to the axial deformation ($E_{sk}$). However, very few variations in these parameters were induced by the foliar treatment. For Chardonnay, the skins from treated berries resulted to be harder and stiffer than those of control berries in the 2016 vintage only for berries with densities between 1075 and 1081 kg/m$^3$ or between 1100 and 1107 kg/m$^3$. This aspect could represent a possible advantage since harder skins increase the grape resistance against fungal diseases and physical injuries (Jiang, Shi, & Zhu, 2013). For intermediate values of density, these three

skin mechanical parameters also were found higher in treated Chardonnay samples but the differences were not significant. Apart from the treatment effect, in most cases berry skin mechanical parameters increased with the increase of berry density.

The berry skin thickness ($Sp_{sk}$) was the mechanical parameter most affected by the inactive yeast treatment, and the three varieties studied showed a significant increase at least for the berries belonging to one density class. A vintage effect was observed with some common trends in all the varieties. Although $Sp_{sk}$ values were almost always higher in treated samples with respect to the control, significant differences were found for the less dense berries (A for Chardonnay, and B for Cortese and Nebbiolo) in the 2015 vintage and for the denser berries (B and C for Chardonnay, and D for Cortese and Nebbiolo) in the 2016 vintage. Furthermore, in the 2015 vintage Cortese berries showed significantly thicker skins in the treated samples for all the density classes selected (B, C, and D) in relation to control samples.

Villangó et al. (2015) also reported that foliar spraying with inactive dry YE resulted in an increase in $Sp_{sk}$ in all sampling dates but in a decreased $F_{sk}$ in some harvest dates. In the present work, there was no relationship between the changes occurred in $Sp_{sk}$ and $F_{sk}$ values.

The study of berry skin texture parameters is useful for at least two main reasons. First of all, the skin is the berry part more susceptible to physical damages and pathogens attack, and must be a functional protective barrier. Some works reported a positive correlation of skin thickness with a higher resistance of grapes to pests (Gabler et al., 2003). Therefore, the increase of $Sp_{sk}$ induced by the treatment may also be a beneficial aspect for the grape preservation during on-vine and postharvest withering process. Furthermore, the synthesis of several components, such as phenolic (i.e. anthocyanins) and aroma compounds, takes place in the skin (González-Barreiro et al., 2015). For red grape varieties, the study of skin mechanical properties may give important information about phenolic ripeness, and particularly about anthocyanins extractability, which is related to the degradation of the skin

cell wall (Río Segade, Rolle, Gerbi, & Orriols, 2008). In past studies on Mencía grapes, a mathematical relationship between $Sp_{sk}$ values and anthocyanin extractability was found for different grape ripening stages (Río Segade et al., 2011), where an increase in $Sp_{sk}$ of +66 μm resulted in a decrease of -8.3% for the anthocyanin extractability in wine-like solutions. Therefore, it is important to evaluate YE treatment effect on the phenolic composition and extractability of Nebbiolo skins.

### 3.3 Phenolic composition and extractability of Nebbiolo grape skins

For the red variety Nebbiolo, the extractability study for skin phenolic compounds during seven days of simulated maceration in a wine-like solution (Figure 2) and the total phenolic composition at the end of maceration (as sum of maximum extracted and non-extracted contents; Table 3) were evaluated. The trial was done on the most representative density class in the first year (2015C) and it was expanded on all the three density classes selected in the second year (2016B, 2016C, and 2016D). This sampling scheme permits the comparison between control and treated grape berries belonging to the same density class.

The treated samples showed a higher total content and extraction of anthocyanins in 2015C and 2016B when compared to control berries, whereas no significant differences were observed in 2016C and 2016D. An increase in the content of total and extractable anthocyanins induced by the inactive dry yeast treatment was also previously found by Villangó et al. (2015) on Syrah grapes. In the present study, faster extraction kinetics were observed on grapes of year 2016 experiment when the maximum amount of extracted anthocyanins was achieved at 24 h of maceration, instead of 48 h needed on grapes of year 2015.

Although Río Segade et al. (2011) evidenced that thicker skins are characterized by a lower release of anthocyanins, in the present study an important advantage of this foliar treatment is the higher accumulation of anthocyanins in the skins, but without reducing their

ease to be released during simulated maceration when the skin thickness increases. In 2015C and 2016B samples, significant variations between control and treated berries were not observed in the $Sp_{sk}$ values, and the extracted content of anthocyanins was even higher in treated samples. In fact, anthocyanins are extracted during the maceration/fermentation step as a function of their content in berry skins as well as of the chemical composition and physical characteristics of skin cell wall (Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006). Therefore, the total content of anthocyanins could contribute strongly to their release together with skin mechanical properties.

Another aspect to consider is that the two vintages studied evidenced different climatic conditions. In summer 2015, the weather station of Acqui Terme (44.6787 N, 8.4612 E; Arpa Piemonte, 2017), the closest available to the Nebbiolo growing field, registered 3 days above 40 °C and 33 days above 35 °C (Figure S1), while the optimum berry temperature for the synthesis of anthocyanins is around 30 °C (Spayd, Tarara, Mee, & Ferguson, 2002) and temperatures above 35 °C hinder their accumulation or may even start the degradation (Mori, Goto-Yamamoto, Kitayama, & Hashizume, 2007). In year 2015, the treatment induced a higher accumulation of anthocyanins with respect to control grapes (+21%). In summer 2016, temperature did not exceed 40 °C, while the number of days with temperatures above 35 °C was 22 (Figure S2), and the total content of anthocyanins was satisfactory in riper grapes. Nevertheless, the less ripe berries (density class B, 1088−1094 kg/m$^3$) had a low total content of anthocyanins, which was significantly improved by the treatment (+17%). A similar behaviour was observed for extracted contents of anthocyanins. Therefore, the foliar application of inactive dry YE seems to better promote the synthesis of anthocyanins or to reduce the degradation increasing their accumulation when it is relatively low.

Regarding skin flavanols, the treatment effect depended on the vintage. The total content of proanthocyanidins and flavanols reactive to vanillin increased in treated berries in 2015 but decreased in 2016, although the differences between control and treated grapes were

significant only for the berries belonging to the density class C (Table 3). For these same berries, the vintage effect was also evident for extraction kinetics with significantly higher extracted contents in treated samples in 2015C from 24 h of maceration (with exception of 168 h for proanthocyanidins) but lower in 2016C during all the maceration process (Figure 2).

The possible elicitor effect induced by the treatment on the synthesis of monomeric and oligomeric flavanols (assessed as FRV index) and polymeric flavanols (assessed by PRO index) was not clearly evident because it was only observed in 2015C, when also a higher extraction was found for these compounds, whereas the opposite effect was evidenced in 2016C. This lower influence of the treatment on the flavanols content could be due to the fact that the synthesis of skin tannins occurs mainly before veraison (Cadot, Miñana Castelló, & Chevalier, 2006). Portu et al. (2016) also found higher accumulation of anthocyanins in Tempranillo berries treated with YE but no elicitor effect on flavanols. Kogkou et al. (2017) reported YE effect on neither skin anthocyanins nor flavanols.

### 3.4 Wines

The Cortese and Nebbiolo grapes, harvested from control and treated parcels, were separately subjected to winemaking in order to assess the quality of wines produced from them, as a function of the foliar yeast treatment carried out on grapes. Technological parameters of Cortese and Nebbiolo wines are shown in Tables 4 and 5, respectively. In Cortese wines, although the sugars content was found to be non-significantly higher in grapes subjected to the foliar application of inactive dry yeasts, the ethanol production in the resulting treated wines was reduced due to a less effective fermentation of sugars. This reduction of about 0.5% v/v in the ethanol content is comparable to that corresponding to microbiological approaches based on mixed fermentations using non-*Saccharomyces* yeast strains (Rolle et al., 2018). Even though no effect of the treatment was observed on titratable acidity, significantly higher contents of malic and tartaric acids were found also in the 2016

vintage for Cortese wines, and of tartaric acid for Nebbiolo wines. Malic acid was not detected in Nebbiolo wines because of the completeness of malolactic fermentation. In other studies, slightly higher contents of tartaric acid were also evidenced in Tempranillo wines made from grapes treated with YE (Portu et al., 2016). In previous works on Agiorgitiko and Syrah varieties, the foliar application of yeast derivatives did not affect any of the wine technological parameters, namely alcohol and acidity (Kogkou et al., 2017; Villangó et al., 2015).

Regarding wine color characteristics, the treatment effect was vintage dependent. In Cortese wines (Table 4), the yellow/blue color coordinate (b*) decreased significantly in the 2015 vintage as a consequence of the foliar treatment whereas increased in the 2016 vintage. In the same way, Nebbiolo wines (Table 5) in the 2015 vintage had a more intense color when they were produced from treated berries in relation to control samples. Although the content of anthocyanins did not increase in these wines by the inactive dry yeast treatment, a different outcome to what could be expected from the effect on grapes shown in Table 3 since the representativeness of density class C in 2015 was about 70% of total berries weight, the wine content of flavonoids in "treated" samples did. Conversely, in the 2016 vintage the color of Nebbiolo wines from treated berries was significantly lighter and less intense. In this second year, higher differences were found in the phenolic composition of Nebbiolo wines made from control and treated berries with respect to the 2015 vintage. In 2016, the negative effect of the treatment on the wine color characteristics may be attributed to the lower contents of total anthocyanins present in these wines, although significant differences in these red pigments were not found between control and treated berries belonging to the most representative density class (accounting only for 51-56% of total berries weight). The same behavior was evidenced for monomeric anthocyanins and total flavonoids.

An important aspect to take into account is that Nebbiolo wines made from treated berries in 2016 showed higher percentages of malvidin-3-glucoside, which is considered a

highly stable form of anthocyanins, even though partially counter-balanced by a significantly lower relative abundance of cinnamoylated anthocyanins, which participate in intramolecular copigmentation reducing degradation processes. Portu et al. (2016) also reported that the foliar application of YE led to wines with an increased content of this anthocyanin compound, but it did not affect total contents of anthocyanins, hue, and color intensity. Villangó et al. (2015) observed higher anthocyanin contents and color intensity in the wines made from treated Syrah grapes only in some instances, and Kogkou et al. (2017) showed that there were no significant differences in anthocyanin contents or chromatic characteristics between Agiorgitiko wines made from control and treated berries. Therefore, the results obtained in the present work for the Nebbiolo cultivar in the 2015 vintage seem to be in agreement with those previously published for other red varieties. This confirms the strong vintage effect on the effectiveness of the treatment to improve the chromatic characteristics of wines.

Furthermore, in the 2016 vintage the contents of monomeric and oligomeric flavanols (FRV index) were significantly lower in Nebbiolo wines made from treated samples when compared to control wines, in agreement with the results found in grape berries (Tables 3 and 5). Portu et al. (2016) and Kogkou et al. (2017) pointed out that flavanol content and composition in the wines were unaffected by the yeast treatment as also occurred in the present work for the 2015 vintage.

From the aromatic point of view, Cortese and Nebbiolo are considered neutral varieties, and therefore the flavor of their wines is principally due to the presence of fermentative volatile compounds. YE treatment applied on white grapes was specifically designed to improve the aromatic complexity of the resulting white wines. Table 4 summarizes the volatile composition of Cortese wines made from control and treated grapes in 2015 and 2016 vintages, which showed no significant differences in total sum of free esters, alcohols, and acids. 2-Phenylethyl acetate contents still increased significantly in treated Cortese wines in 2015 and 2016 vintages (Table S1). Furthermore, isoamyl acetate

also showed greater contents in treated samples, even though the differences with respect to control wines were significant only in the 2015 vintage (Table S1). These two volatile compounds provide pleasant floral and fruity nuances. Instead, in 2016, the aforementioned increase was counterbalanced by decreasing ethyl-2-hydroxyisovalerate, ethyl-2-hydroxyhexanoate, isoamyl lactate, diethyl succinate, and ethyl malate contents (Table S1).

Although YE treatment applied on red varieties was specifically developed to enhance the phenolic maturity of red grapes, its effect on the volatile composition of Nebbiolo wines was also studied. A similar effect of inactive yeasts treatment, even significant in the 2015 vintage, was also observed in Nebbiolo wines, for which most of the esters detected increased their contents with the treatment, particularly isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, diethyl succinate, 2-phenylethyl acetate, and ethyl phenyllactate, whereas differences were not observed for free acetate and ethyl esters in 2016 (Tables 5 and S2). These free volatile compounds may contribute positively to the wine aroma. In 2015, hexanol was the second most abundant free alcohol in Nebbiolo treated wines, after 2-phenylethanol, because of the increase induced by the treatment. According to these results, the vintage effect was also evident on the volatile composition of wines.

Šuklje et al. (2016) pointed out that after two months of wine storage the dry yeast treatment on Sauvignon blanc grapes resulted in a significantly slower decrease of ethyl esters of straight chain fatty acids and to a lesser extent of higher alcohol acetates in the wines, as well as in a significantly slower synthesis of ethyl esters of branched acids.

An interesting aspect to take into account when volatile phenols were present (2016 vintage), which are characterized by unpleasant animal notes, is that the treatment significantly reduced the contents of some of these compounds in Cortese and Nebbiolo wines (Tables 4 and 5).

*3.5 Sensory analysis*

Cortese and Nebbiolo wines were subjected to a Duo-trio sensory test 6 months after the end of fermentation. For the 2015 vintage, the differences between Cortese wines made from control and treated grapes were correctly identified by 72% of the tasters ($\alpha = 0.05$) whereas only 38% identified correctly the different sample for Nebbiolo wines ($\alpha > 0.40$). In the 2016 vintage, the percentage decreased for Cortese wines up to 32% ($\alpha > 0.40$), but increased for Nebbiolo wines at 52% ($\alpha > 0.40$). Using a significance threshold of 0.05, the low percentages reported by the tasting panel were not sufficient to consider the control and treated wines significantly different, with the exception of Cortese wines in 2015 vintage. The results obtained are in agreement with those corresponding to the chemical and color analysis previously discussed (Tables 4 and 5), where it was found that 2015 Cortese wines were significantly different, among others, in terms of ethanol content, yellow/blue (b*) color coordinate, isoamyl acetate and 2-phenylethyl acetate contents.

## 4. CONCLUSIONS

The effect of foliar application of inactive dry yeasts on the technological chemical parameters defining grape ripeness seemed to be lower than the vintage effect, while it has entailed significant changes on skin mechanical properties. The increase induced by the treatment in berry skin thickness was accompanied by changes in the secondary metabolism, almost exclusively on anthocyanins. Particularly, the possible elicitor effect, or even the higher extractability, was observed in the less favorable climatic conditions for the accumulation of these compounds. The wines made from treated grapes showed higher contents of tartaric acid and lower ones of ethanol with respect to those produced from control grapes. Regarding color characteristics and phenolic composition of wines, the vintage effect prevailed on the treatment effect, while some fermentative volatile compounds significantly

increased in the wines made from treated grapes, particularly free acetate and ethyl esters. Nevertheless, the control and treated wines were significantly recognised different by the tasting panel in one case out of four. With the acquired knowledge in the present work, the foliar application of inactive dry yeasts may be effective when climate conditions are critical particularly for the synthesis and thermal degradation of anthocyanins without compromising their content and extractability, or when a dehydration process is planned without compromising the grapes sanity. Further research considering a wider range of cultivation, growing and environmental conditions is necessary to improve the knowledge about these treatments.

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**Figure 1.** Weight percentage distribution of control (dashed line) and treated (continuous line) berries by density sorting for Chardonnay, Cortese, and Nebbiolo cultivars.

**Figure 2.** Extraction kinetics of phenolic compounds during skin maceration of control (dashed line) and treated (continuous line) samples for Nebbiolo cultivar. Values are expressed as average $\pm$ standard deviation (n = 3). Sign: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively, for each maceration time. B = 1088-1094 kg/m$^3$; C = 1100-1107 kg/m$^3$; D = 1115-1119 kg/m$^3$.

**Table 1.** Standard chemical parameters of unsorted samples of Chardonnay, Cortese, and Nebbiolo control and treated grapes at harvest.

| Vintage | Cultivar | Harvest date | Reducing sugars (g/L) | | | pH | | | Titratable acidity (g/L)[a] | | | Tartaric acid (g/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control | Treated | *Sign.* | Control | Treated | *Sign.* | Control | Treated | *Sign.* | Control | Treated | *Sign.* |
| | Chardonnay | Sept. 2nd | 228 ± 4 | 227 ± 1 | ns | 3.35 ± 0.01 | 3.32± 0.02 | ns | 5.18 ± 0.05 | 5.48 ± 0.11 | ns | 6.91 ± 0.03 | 7.11 ± 0.15 | ns |
| 2015 | Cortese | Sept. 10th | 237 ± 1 | 237 ± 8 | ns | 3.21 ± 0.02 | 3.13 ± 0.01 | * | 5.08 ± 0.24 | 6.04 ± 0.16 | * | 7.19 ± 0.12 | 7.23 ± 0.11 | ns |
| | Nebbiolo | Sept. 29th | 250 ± 1 | 261 ± 1 | ** | 3.19 ± 0.02 | 3.28 ± 0.01 | * | 5.33 ± 0.05 | 4.61 ± 0.05 | ** | 6.70 ± 0.09 | 6.99 ± 0.24 | ns |
| | Chardonnay | Sept. 5th | 212 ± 1 | 208 ± 5 | ns | 3.07 ± 0.06 | 3.07 ± 0.03 | ns | 7.35 ± 0.48 | 7.73 ± 0.11 | ns | 6.72 ± 0.20 | 6.73 ± 0.16 | ns |
| 2016 | Cortese | Sept. 14th | 225 ± 1 | 232 ± 8 | ns | 3.08 ± 0.02 | 3.14 ± 0.02 | ns | 6.51 ± 0.03 | 6.30 ± 0.11 | ns | 7.51 ± 0.13 | 7.23 ± 0.32 | ns |
| | Nebbiolo | Oct. 5th | 257 ± 6 | 252 ± 5 | ns | 3.16 ± | 3.13 ± | ns | 5.79 ± | 6.00 ± | ns | 7.59 ± | 7.99 ± | ** |

|  | 0.01 | 0.02 |  | 0.08 | 0.11 |  | 0.02 | 0.04 |

Values are expressed as average ± standard deviation (n = 2). *Sign.*: *, **, and ns indicate significance at $p < 0.05$, 0.01, and not significant, respectively. [a]As tartaric acid.

**Table 2.** Berry skin texture parameters of Chardonnay, Cortese, and Nebbiolo control and treated grapes subdivided in density classes.

| Cultivar | Vintage | Density class | $Sp_{sk}$ (µm) Control | $Sp_{sk}$ (µm) Treated | Sign. | $F_{sk}$ (N) Control | $F_{sk}$ (N) Treated | Sign. | $W_{sk}$ (mJ) Control | $W_{sk}$ (mJ) Treated | Sign. | $E_{sk}$ (N/mm) Control | $E_{sk}$ (N/mm) Treated | Sign. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chardonnay | 2015 | A | 151 ± 31 | 190 ± 28 | *** | 0.489 ± 0.118 | 0.515 ± 0.126 | ns | 0.467 ± 0.188 | 0.557 ± 0.245 | ns | 0.242 ± 0.032 | 0.224 ± 0.035 | * |
| | | B | 178 ± 28 | 189 ± 24 | ns | 0.556 ± 0.112 | 0.586 ± 0.131 | ns | 0.592 ± 0.182 | 0.664 ± 0.226 | ns | 0.242 ± 0.040 | 0.237 ± 0.040 | ns |
| | | C | 186 ± 25 | 196 ± 29 | ns | 0.695 ± 0.136 | 0.663 ± 0.126 | ns | 0.803 ± 0.246 | 0.749 ± 0.228 | ns | 0.272 ± 0.037 | 0.267 ± 0.043 | ns |
| | 2016 | A | 196 ± 41 | 200 ± 36 | ns | 0.711 ± 0.186 | 0.866 ± 0.158 | * | 0.836 ± 0.304 | 1.033 ± 0.313 | * | 0.269 ± 0.047 | 0.316 ± 0.040 | * |
| | | B | 200 ± 30 | 230 ± 31 | * | 0.829 ± 0.127 | 0.884 ± 0.189 | ns | 1.023 ± 0.228 | 1.099 ± 0.355 | ns | 0.297 ± 0.036 | 0.318 ± 0.046 | ns |
| | | C | 229 ± 25 | 251 ± 32 | ** | 0.827 ± 0.125 | 0.910 ± 0.166 | * | 1.041 ± 0.243 | 1.145 ± 0.306 | ns | 0.293 ± 0.028 | 0.324 ± 0.046 | ** |
| Cort | 20 | B | 192 | 215 | * | 0.669 | 0.672 | ns | 0.837 | 0.877 | ns | 0.232 | 0.222 | ns |

| Sample | Dose | Group | | | sig | | | sig | | | sig | | | sig |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ese | 15 | | ± | ± | | ± | ± | | ± | ± | | ± | ± | |
| | | | 33 | 40 | | 0.138 | 0.133 | | 0.252 | 0.257 | | 0.047 | 0.032 | |
| | | | 207 | 234 | * | 0.666 | 0.651 | | 0.849 | 0.842 | | 0.223 | 0.221 | |
| | | C | ± | ± | * | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 41 | 34 | | 0.166 | 0.170 | | 0.279 | 0.312 | | 0.038 | 0.039 | |
| | | | 222 | 248 | | 0.694 | 0.632 | | 0.927 | 0.843 | | 0.218 | 0.202 | |
| | | D | ± | ± | * | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 43 | 41 | | 0.149 | 0.129 | | 0.303 | 0.219 | | 0.028 | 0.034 | |
| | | | 162 | 170 | | 0.656 | 0.674 | | 0.734 | 0.773 | | 0.266 | 0.264 | |
| | | B | ± | ± | ns | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 43 | 39 | | 0.161 | 0.180 | | 0.286 | 0.290 | | 0.053 | 0.054 | |
| | 20 | | 188 | 187 | | 0.711 | 0.688 | | 0.877 | 0.839 | | 0.251 | 0.251 | |
| | 16 | C | ± | ± | ns | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 31 | 37 | | 0.112 | 0.139 | | 0.209 | 0.242 | | 0.041 | 0.047 | |
| | | | 198 | 216 | | 0.679 | 0.732 | | 0.863 | 0.995 | | 0.232 | 0.237 | |
| | | D | ± | ± | * | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 39 | 36 | | 0.162 | 0.155 | | 0.329 | 0.351 | | 0.031 | 0.030 | |
| | | | 179 | 202 | * | 0.759 | 0.738 | | 0.794 | 0.796 | | 0.319 | 0.306 | |
| | | B | ± | ± | * | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 24 | 20 | * | 0.128 | 0.208 | | 0.197 | 0.371 | | 0.039 | 0.042 | |
| Neb | 20 | | 202 | 219 | | 0.790 | 0.807 | | 0.850 | 0.834 | | 0.327 | 0.346 | |
| biolo | 15 | C | ± | ± | ns | ± | ± | ns | ± | ± | ns | ± | ± | ns |
| | | | 25 | 39 | | 0.193 | 0.150 | | 0.307 | 0.226 | | 0.050 | 0.054 | |
| | | | 208 | 209 | | 0.843 | 0.871 | | 0.856 | 0.878 | | 0.365 | 0.378 | |
| | | D | | | ns | | | ns | | | ns | | | ns |
| | | | ± | ± | | ± | ± | | ± | ± | | ± | ± | |

| | | Sp$_{sk}$ | | | F$_{sk}$ | | | W$_{sk}$ | | | E$_{sk}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 26 | 35 | | 0.179 | 0.154 | | 0.299 | 0.235 | | 0.046 | 0.040 | |
| | B | 183 ± 28 | 196 ± 28 | ns | 0.666 ± 0.202 | 0.588 ± 0.141 | ns | 0.711 ± 0.312 | 0.578 ± 0.195 | ns | 0.276 ± 0.043 | 0.260 ± 0.036 | ns |
| 20 16 | C | 197 ± 24 | 192 ± 27 | ns | 0.819 ± 0.145 | 0.741 ± 0.206 | ns | 0.878 ± 0.221 | 0.756 ± 0.308 | ns | 0.327 ± 0.035 | 0.319 ± 0.049 | ns |
| | D | 176 ± 42 | 200 ± 29 | * | 0.942 ± 0.174 | 0.934 ± 0.173 | ns | 0.969 ± 0.267 | 0.999 ± 0.261 | ns | 0.376 ± 0.044 | 0.366 ± 0.047 | ns |

Values are expressed as average ± standard deviation (n = 30). *Sign.*: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. A = 1075-1081 kg/m$^3$; B = 1088-1094 kg/m$^3$; C = 1100-1107 kg/m$^3$; D = 1115-1119 kg/m$^3$. Sp$_{sk}$ = berry skin thickness; F$_{sk}$ = berry skin break force; W$_{sk}$ = berry skin break energy; E$_{sk}$ = berry skin resistance to the axial deformation.

**Table 3.** Berry skin phenolic composition at the end of maceration for Nebbiolo control and treated grapes subdivided in density classes.

| Cultivar | Vintage | Density class | TA (mg malvidin-3-O-glucoside chloride/kg berries) | | | FRV [mg (+)-catechin/kg berries] | | | PRO (mg cyanidin chloride/kg berries) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control | Treated | *Sign.* | Control | Treated | *Sign.* | Control | Treated | *Sign.* |
| | 2015 | C | 394 ± 25 | 476 ± 9 | ** | 985 ± 33 | 1237 ± 39 | ** | 2303 ± 107 | 2751 ± 48 | ** |
| Nebbiolo | | B | 423 ± 8 | 497 ± 40 | * | 1014 ± 77 | 900 ± 59 | ns | 2176 ± 136 | 2029 ± 167 | ns |
| | 2016 | C | 510 ± 13 | 475 ± 30 | ns | 1138 ± 55 | 995 ± 61 | * | 2554 ± 125 | 2214 ± 53 | * |
| | | D | 550 ± 14 | 562 ± 22 | ns | 1232 ± 88 | 1225 ± 7 | ns | 2823 ± 170 | 2712 ± 118 | ns |

Values are expressed as average ± standard deviation (n = 3). *Sign.*: *, **, and ns indicate significance at $p < 0.05$, 0.01, and not significant, respectively. B = 1088-1094 kg/m$^3$; C = 1100-1107 kg/m$^3$; D = 1115-1119 kg/m$^3$. TA = total anthocyanins; FRV = flavanols reactive to vanillin; PRO = proanthocyanidins.

**Table 4.** Technological parameters, color characteristics, and free volatile compounds of Cortese wines.

| Parameter | 2015 | | | 2016 | | |
| --- | --- | --- | --- | --- | --- | --- |
| | **Control** | **Treated** | *Sign.* | **Control** | **Treated** | *Sign.* |
| *Technological parameters* | | | | | | |
| Ethanol (% v/v) | 14.21 ± 0.02 | 13.71 ± 0.01 | ** | 12.89 ± 0.06 | 12.58 ± 0.04 | * |
| Residual sugars (g/L) | 1.67 ± 0.11 | 1.55 ± 0.01 | ns | 1.65 ± 0.01 | 1.76 ± 0.01 | ** |
| Glycerol (g/L) | 7.59 ± 0.04 | 7.45 ± 0.03 | ns | 7.13 ± 0.04 | 7.19 ± 0.02 | ns |
| pH | 3.24 ± 0.03 | 3.24 ± 0.02 | ns | 3.13 ± 0.02 | 3.16 ± 0.01 | ns |
| Titratable acidity (g tartaric acid/L) | 5.85 ± 0.04 | 5.74 ± 0.04 | ns | 6.30 ± 0.01 | 6.36 ± 0.03 | ns |
| Acetic acid (g/L) | 0.35 ± 0.01 | 0.35 ± 0.01 | ns | 0.28 ± 0.03 | 0.33 ± 0.01 | ns |
| Malic acid (g/L) | 1.30 ± 0.01 | 1.27 ± 0.02 | ns | 1.02 ± 0.01 | 1.34 ± 0.02 | ** |
| Lactic acid (g/L) | 0.13 ± 0.01 | 0.12 ± 0.02 | ns | 0.17 ± 0.01 | 0.15 ± 0.01 | *** |
| Tartaric acid (g/L) | 2.09 ± 0.03 | 2.09 ± 0.01 | ns | 2.40 ± 0.02 | 2.94 ± 0.01 | ** |
| *Color characteristics* | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| L* | 99.4 ± 0.0 | 99.5 ± 0.1 | ns | 98.8 ± 0.1 | 98.8 ± 0.0 | ns |
| a* | −0.95 ± 0.03 | −0.94 ± 0.01 | ns | −0.64 ± 0.05 | −0.75 ± 0.01 | ns |
| b* | 4.29 ± 0.05 | 4.05 ± 0.03 | * | 4.54 ± 0.04 | 4.96 ± 0.02 | * |
| $A_{420}$ (a.u., 10 mm optical path) | 0.062 ± 0.001 | 0.057 ± 0.001 | ns | 0.070 ± 0.002 | 0.074 ± 0.001 | ns |
| *Free volatile compounds* | | | | | | |
| Σ Free esters (mg/L) | 6.37 ± 0.16 | 6.24 ± 0.01 | ns | 21.38 ± 0.59 | 19.13 ± 1.30 | ns |
| Σ Free alcohols (mg/L) | 27.88 ± 5.29 | 31.54 ± 13.65 | ns | 73.10 ± 1.03 | 75.89 ± 6.02 | ns |
| Σ Free acids (mg/L) | 11.28 ± 0.58 | 11.25 ± 0.35 | ns | 7.73 ± 0.37 | 8.21 ± 0.03 | ns |

Values are expressed as average ± standard deviation (n = 2). *Sign.*:  *, **, ***, and ns indicate significance at $p$ <0.05, 0.01, 0.001 and not significant, respectively.  L* = lightness; a* = red/green color coordinate; b* = yellow/blue color coordinate; $A_{420}$ = absorbance measured at 420 nm and expressed in absorbance units (a.u.).

**Table 5.** Technological parameters, phenolic composition, color characteristics, and free volatile compounds of Nebbiolo wines.

| Parameter | 2015 | | | 2016 | | |
|---|---|---|---|---|---|---|
| | Control | Treated | *Sign.* | Control | Treated | *Sign.* |
| *Technological parameters* | | | | | | |
| Ethanol (% v/v) | 13.69 ± 0.02 | 13.61 ± 0.03 | ns | 13.63 ± 0.08 | 13.59 ± 0.05 | ns |
| Residual sugars (g/L) | nd | nd | - | 1.12 ± 0.01 | 1.39 ± 0.01 | *** |
| Glycerol (g/L) | 7.72 ± 0.10 | 7.67 ± 0.06 | ns | 9.11 ± 0.03 | 8.84 ± 0.01 | * |
| pH | 3.44 ± 0.02 | 3.42 ± 0.03 | ns | 3.27 ± 0.01 | 3.29 ± 0.01 | ns |
| Titratable acidity (g tartaric acid/L) | 5.59 ± 0.10 | 5.76 ± 0.10 | ns | 6.36 ± 0.03 | 6.38 ± 0.01 | ns |
| Acetic acid (g/L) | 0.43 ± 0.04 | 0.50 ± 0.08 | ns | 0.63 ± 0.01 | 0.60 ± 0.01 | * |
| Malic acid (g/L) | nd | nd | - | nd | nd | - |
| Lactic acid (g/L) | 0.90 ± 0.03 | 0.80 ± 0.04 | ns | 1.05 ± 0.01 | 1.04 ± 0.01 | ns |
| Tartaric acid (g/L) | 1.60 ± 0.07 | 1.69 ± 0.08 | ns | 2.09 ± 0.01 | 2.24 ± 0.01 | ** |
| *Phenolic compounds* | | | | | | |
| TA (mg malvidin-3-O-glucoside | 101 ± 1 | 101 ± 1 | ns | 160 ± 2 | 132 ± 3 | * |

chloride/L)

| | | | | | | |
|---|---|---|---|---|---|---|
| MA (mg malvidin-3-O-glucoside chloride/L) | 49 ± 1 | 48 ± 4 | ns | 86 ± 1 | 73 ± 1 | * |
| TF (mg (+)-catechin/L) | 1345 ± 3 | 1547 ± 9 | * | 1679 ± 15 | 1438 ± 17 | * |
| FRV (mg (+)-catechin/L) | 1128 ± 89 | 1211 ± 51 | ns | 1576 ± 7 | 1269 ± 13 | ** |
| PRO (mg cyanidin chloride/L) | 2467 ± 53 | 2617 ± 13 | ns | 2897 ± 79 | 2411 ± 1 | ns |
| FRV/PRO ratio | 0.46 ± 0.03 | 0.46 ± 0.02 | ns | 0.54 ± 0.02 | 0.53 ± 0.01 | ns |
| Delphinidin-3-glucoside (%) | 5.36 ± 0.05 | 5.24 ± 0.06 | ns | 6.87 ± 0.06 | 6.70 ± 0.01 | ns |
| Cyanidin-3-glucoside (%) | 2.43 ± 0.02 | 2.56 ± 0.03 | ns | 3.41 ± 0.02 | 3.34 ± 0.06 | ns |
| Petunidin-3-glucoside (%) | 7.57 ± 0.03 | 7.31 ± 0.14 | ns | 8.08 ± 0.01 | 8.04 ± 0.01 | ns |
| Peonidin-3-glucoside (%) | 21.64 ± 0.26 | 22.40 ± 0.33 | ns | 24.39 ± 0.03 | 23.23 ± 0.10 | * |
| Malvidin-3-glucoside (%) | 51.33 ± 0.01 | 50.20 ± 0.13 | ns | 45.92 ± 0.05 | 47.83 ± 0.03 | ** |
| Σ Acetylglucosides (%) | 5.87 ± 0.04 | 6.20 ± 0.12 | ns | 4.09 ± 0.02 | 4.11 ± 0.01 | ns |
| Σ Cinnamoylglucosides (%) | 5.80 ± 0.10 | 6.09 ± 0.11 | ns | 7.23 ± 0.01 | 6.74 ± 0.05 | * |

*Color characteristics*

| | | | | | | |
|---|---|---|---|---|---|---|
| L* | 44.2 ± 0.2 | 43.5 ± 0.2 | ns | 36.4 ± 0.0 | 40.7 ± 0.3 | * |
| a* | 56.95 ± 0.06 | 57.93 ± 0.24 | ns | 61.66 ± 0.26 | 61.26 ± 0.28 | ns |
| b* | 27.53 ± 0.06 | 29.07 ± 0.25 | ns | 35.76 ± 1.00 | 33.67 ± 0.17 | ns |
| Color hue | 0.76 ± 0.01 | 0.75 ± 0.01 | ns | 0.73 ± 0.01 | 0.74 ± 0.01 | * |
| Color intensity (a.u., 10 mm optical path) | 3.07 ± 0.02 | 3.20 ± 0.03 | * | 4.30 ± 0.05 | 3.72 ± 0.02 | * |

*Free volatile compounds*

| | | | | | | |
|---|---|---|---|---|---|---|
| Σ Free esters (mg/L) | 5.66 ± 0.21 | 11.50 ± 0.42 | * | 20.46 ± 0.12 | 21.55 ± 0.29 | ns |
| Σ Free alcohols (mg/L) | 20.90 ± 3.51 | 32.41 ± 1.80 | ns | 80.95 ± 4.99 | 77.00 ± 3.44 | ns |
| Σ Free acids (mg/L) | 3.16 ± 0.22 | 3.08 ± 0.22 | ns | 3.74 ± 0.03 | 3.88 ± 0.07 | ns |

Values are expressed as average ± standard deviation (n = 2). *Sign.*: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. nd = not detected. TA = total anthocyanins; MA = monomeric anthocyanins; TF = total flavonoids; FRV = flavanols reactive to vanillin; PRO = proanthocyanidins; L* = lightness; a* = red/green color coordinate; b* = yellow/blue color coordinate. a.u.= absorbance units.

Highlights

- Foliar treatments based on inactive dry yeasts were tested on grape varieties

- A tendency to increased berry skin thickness values in treated grapes was found

- Berry skin anthocyanin accumulation was affected by the treatment in some cases

- A vintage effect was observed on wine color characteristics and phenolic composition

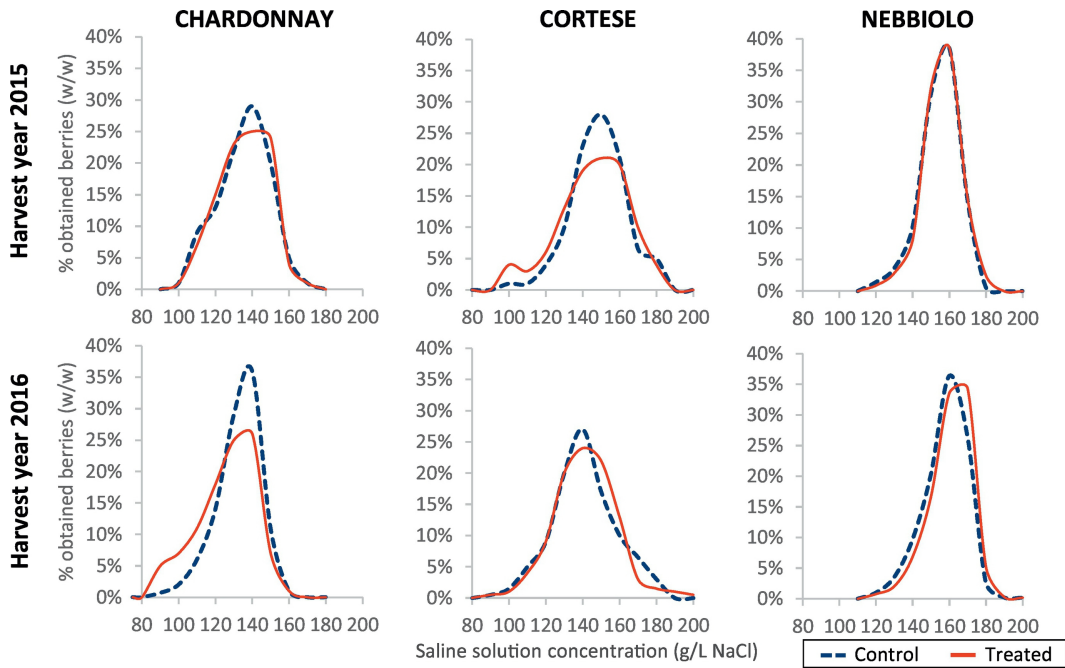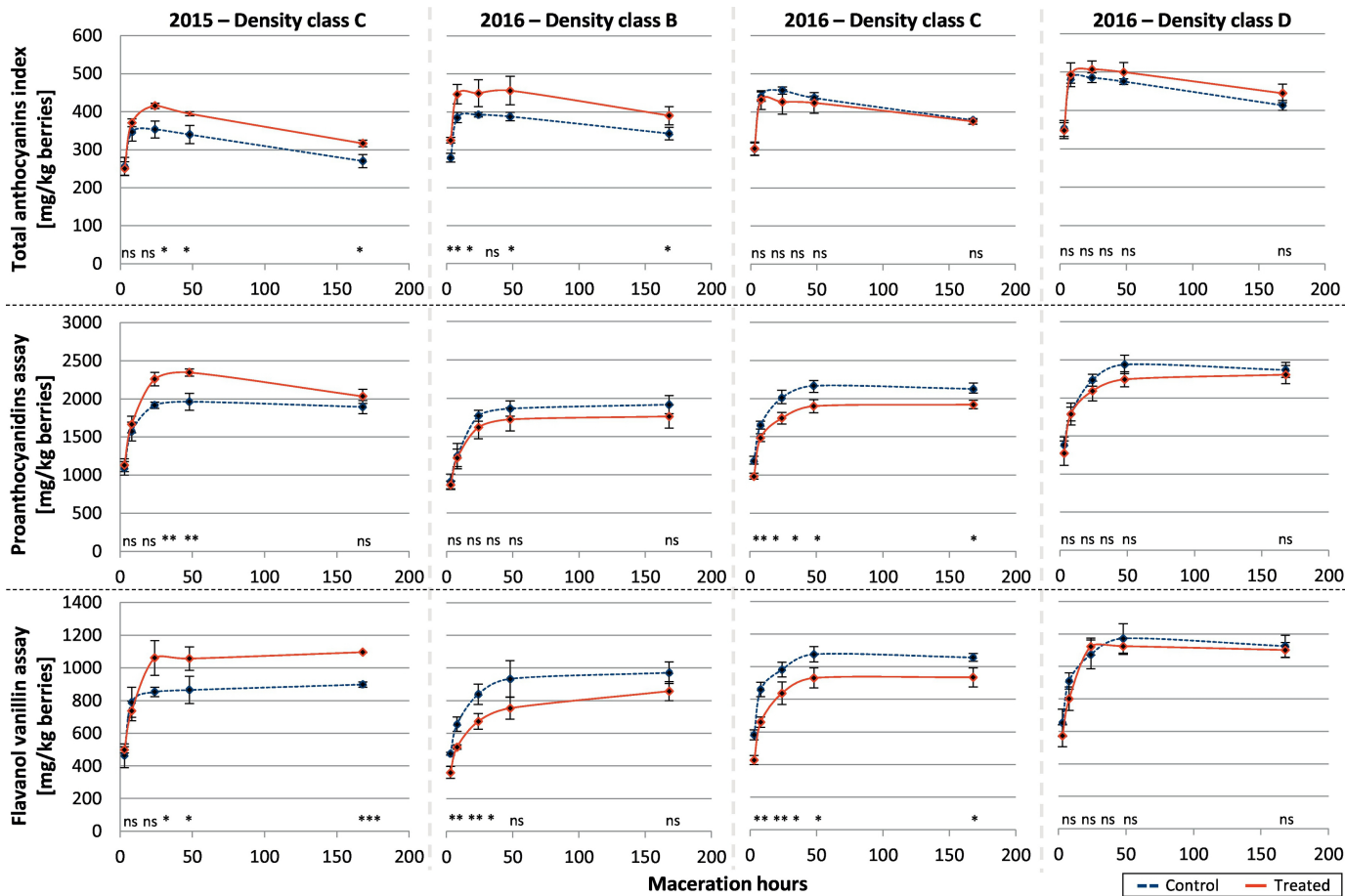- Nebbiolo wines from treated grapes were richer in free volatile esters

Figure 1

Figure 2