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Influence of different withering conditions on phenolic composition of Avanà, Chatus and Nebbiolo grapes for the production of 'Reinforced' wines

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Running title: **Grape withering conditions for the production of 'Reinforced' wines**

Abstract

The impact of postharvest withering rates on the phenolic composition of 'reinforced' wines produced with partially dehydrated grapes was evaluated. The study was performed on winegrape varieties with anthocyanin profiles differently constituted of di- and tri-substituted forms. Dehydration induced limited changes in the anthocyanin profile of berry skins. Nevertheless, the greatest abundance of total anthocyanins and their more stable forms (malvidin-3-glucoside and acylated glucosides) corresponded to the wines made from slow withered Chatus grapes, which were in turn the darkest. In contrast, the wines made from withered Avanà grapes did not meet good chromatic characteristics due to low contents of total anthocyanins and high ratios between di- and tri-substituted forms. Nebbiolo wines showed intermediate values of this ratio, and therefore of clarity and color intensity. The fast process is recommended because higher percentages of galloylated flavanols in the seeds of slow withered Nebbiolo grapes may have a negative influence on wine astringency.

Keywords: Phenolic composition; Anthocyanins; Flavanols; Withering process; Red winegrapes; Reinforced wines.

1. Introduction

In the enological sector, the withering process of winegrapes is of particular importance in the diversification of wine products. In almost all viticultural areas in the world, there are traditional wines locally produced using over-ripe and more or less withered grapes. Among these enological products, some famous sweet and fortified wines are included, such as ice wines, Passito wines, Sauternes, Tokaj, Porto, Pedro Ximénez, as well as many others (Mencarelli & Tonutti, 2013).

In Italy, in particular in the mountain area of Valtellina (Lombardy region), there are special dry wines called 'Sfursat', which are produced from partially dehydrated Nebbiolo grapes. These wines are classified as 'reinforced wines' and defined as dry wines (generally, but not exclusively, red) characterized by a higher alcohol and secondary metabolites content, and produced with partially dehydrated berries (weight loss less than 25% of initial fresh weight) (Mencarelli & Tonutti, 2013). After harvest, the bunches are dehydrated indoors in naturally ventilated rooms called '*fruttai*' until they reach the desired sugar content (Nicoletti et al., 2013). In the production of reinforced wines, when not regulated by specific rules imposed by a product specification (as in the case of Sfursat), the natural and uncontrolled withering process of grapes is often substituted by forced withering in chambers under controlled dehydration conditions: temperature, relative humidity, and air speed.

In any case, during postharvest grape dehydration, important metabolic changes occur due to water loss, leading to an active metabolism that affects the phenol composition and extractability, volatile profile, and mechanical properties (Rolle et al., 2012b; Rolle, Giacosa, Río Segade, Ferrarini, Torchio, & Gerbi, 2013b; Toffali et al., 2011; Zoccatelli et al., 2013). Different postharvest dehydration rates and conditions affect the quality characteristics of grapes and related wines (Bellincontro, De Santis, Botondi, Villa, & Mencarelli, 2004; Frangipane, Torresi, De Santis, & Massantini, 2012; Moreno, Cerpa-Calderón, Cohen, Fang,

Qian, & Kennedy, 2008; Rolle et al., 2013b), and also the grape cultivar could have a influence (Marquez, Serratosa, & Merida, 2012a).

On the basis of the above, the aim of this work was to study the influence of two different withering conditions on the phenolic composition of three red grape varieties with different phenolic profile (Nebbiolo, Chatus, and Avanà), and to evaluate the possible differences in the chemical composition and chromatic characteristics of the reinforced wines obtained from them.

Avanà, Chatus, and Nebbiolo are authochtonous varieties growing in northwest Italy Alps. They were used for the production of dry wines with fresh or partially dehydrated grapes. In particular, these varieties were selected because of their different ratios between di- and tri-substituted anthocyanins in order to investigate the impact of the anthocyanin profile on the chromatic properties of this specific wine (Rolle & Guidoni, 2007). The present study will determine whether the predominance of certain anthocyanin forms in fresh grapes is a determining factor in the protection of red-colored phenolic compounds, more so than oxidation, during the withering process and subsequent winemaking, or whether the postharvest dehydration conditions also play an important role in the stability of these phenolic compounds.

2. Materials and methods

2.1. Grape samples and dehydration process

In this study, whole bunches of red grape *Vitis vinifera* L. cv. Avanà, Chatus, and Nebbiolo were harvested from various vines in commercial vineyards located in the same mountainous growing zone (Piedmont, Turin province, north-west Italy) in 2012. The clusters were visually inspected, and those berries with damaged skins were discarded. For each variety, one set of 150 berries with attached short pedicels was randomly selected from

different positions in the cluster for fresh grape analysis. Afterwards, the clusters were randomly distributed in two batches, and each batch was subjected to different withering conditions of temperature and relative humidity (RH) in a thermohygrometrically controlled chamber. The first batch was subjected to 18 ºC and 40% RH (slow withering, SW, 32 days). The second batch was treated at 28 ºC and 40% RH (fast withering, FW, 24 days). The air speed used was 0.9 m/s. For all trials, the final weight loss was <25%. For each variety and withering process, one set of 150 berries with attached short pedicels was randomly selected at the end of the dehydration process for withered grape analysis.

For each set of fresh and withered grapes, three subsamples of 10 berries were accurately weighed and used for the determination of phenolic compounds (Rolle et al., 2013b). The remaining berries, subdivided in three replicates, were used to determine the standard physicochemical parameters in the grape must obtained by manual crushing and centrifugation.

2.2. Winemaking

The wines were made in the experimental cellar of the University of Turin. For each variety and withering process, partially dehydrated grape berries from about 200 kg of fresh grapes, subdivided in two replicates, were destemmed and crushed. Total sulphur dioxide (20 mg/L) was added to the grape must, which was then inoculated with *Saccharomyces cerevisiae* (Lalvin EC-1118, Lallemand, Montreal, Quebec, Canada) commercial yeast (20 g/hL). The fermentation was conducted in 100 L stainless steel tanks, and the temperature was kept at 28 °C. Punching-down was done twice a day during the first four days of fermentation, one punch-down and one pump-over were conducted during the fifth and sixth day, and two pump overs were performed in the last day of maceration. After seven days of maceration-fermentation, the grape pomace was pressed by a small pneumatic press (PMA 4, Velo SpA, Italy) with a maximum pressure of 1.20 bar, and free-run juice and press juice were mixed.

Subsequently, the malolactic fermentation was induced by inoculation with *Oenococcus oeni* lactic acid bacteria (Lactobacter SP1, Laffort, Bordeaux, France). After one month, the free sulphur dioxide content was then adjusted to 40 mg/L. Finally, the wines obtained were stored at 0 °C for 2 weeks (cold stabilization), filtered (Seitz K300 grade filter sheet, Pall Corporation, Port Washington, NY, USA), and bottled.

2.3. Chemical analysis

2.3.1. Reagents and standards

Solvents of HPLC–gradient grade and all other chemicals of analytical-reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Among phenolic standards, gallic acid (as GA), (+)-catechin (as C), (-)-epicatechin (as EC) and (-)-epicatechin gallate (as ECG) were obtained from Sigma, and caffeic acid, cyanidin chloride, procyanidins B_1 and B_2, delphinidin-3-glucoside chloride, malvidin-3-glucoside chloride, petunidin chloride, peonidin-3-glucoside chloride and cyanidin-3-glucoside chloride were purchased from Extrasynthèse (Genay, France). Phloroglucinol was supplied by Aldrich (Steinheim, Germany).

2.3.2. Standard parameters

In the must obtained and/or in the resulting wine, pH was determined by potentiometry using an InoLab 730 pHmeter (WTW, Weilheim, Germany). Titratable acidity (g/L tartaric acid, as TA), acetic acid (g/L), and ethanol (% v/v) were determined according to OIV (Organisation Internationale de la Vigne et du Vin) methods (OIV, 2008). Organic acids (citric acid, tartaric acid, and malic acid), glycerol, and reducing sugars (glucose and fructose) (g/L) were quantified using a HPLC (high performance liquid chromatography) system

equipped with a diode array detector (DAD) and a refractive index detector (Giordano, Rolle, Zeppa, & Gerbi, 2009).

2.3.3. Extraction and determination of phenolic compounds

The berry skins and seeds were manually removed from the pulp using a laboratory spatula. The berry skins were quickly immersed into 25 mL of a hydroalcoholic buffer solution of pH 3.2 containing 5 g/L tartaric acid, 2 g/L sodium metabisulphite, and 12% v/v ethanol (Río Segade et al., 2013). The skins were then homogenized at 8000 rpm for 1 min using an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany), and centrifuged in a PK 131 centrifuge (ALC International, MI, Italy) for 15 min at 3000×g at 20 °C. The supernatant was used for skin analysis. The berry pulp was introduced into a tube containing 100 mg sodium metabisulphite, and subsequently diluted (9:1, w/w) with 5 mol/L sulphuric acid (Río Segade et al., 2013). Afterwards, the pulp was homogenized at 9500 rpm for 30 s with an Ultraturrax T10 high-speed homogenizer (IKA Labortechnik), and centrifuged for 15 min at 3000×g at 20 °C. The resulting solution was used for pulp analysis. The berry seeds, after immersion into 10 mL of the same buffer solution used for skins, were maintained at 25 °C for one week (Río Segade et al., 2013). The solution was used for seed analysis.

Spectrophotometric methods were used to determine absorbance at 280 nm (as A_{280}/kg grape or L wine) and total flavonoids (mg (+)-catechin/kg grape or L wine, as TF) in the skin, pulp, seeds, and wine, proanthocyanidins (mg cyanidin chloride/kg grape or L wine, as PRO) and flavanols reactive to vanillin (mg (+)-catechin/kg grape or L wine, as FRV) in the skin, seeds, and wine, total anthocyanins (mg malvidin-3-glucoside chloride/kg grape or L wine, as TAI) in the skin and wine, and total hydroxycinnamic acids (mg caffeic acid/kg grape, as HCTA) in the pulp (Río Segade et al., 2013; Rolle, Torchio, Giacosa, Río Segade, Cagnasso, & Gerbi, 2012a). The wine color was assessed by the Glories chromatic parameters, such as

color intensity and tonality, and by the CIELab space, using a 2 mm path length cuvette, following OIV (2008) methods. The parameters that define the CIELab space are clarity (as L*), red/green color coordinate (as a*), and yellow/blue color coordinate (as b*), from which the parameters correlated with the color perception are obtained, such as chroma (as C*, referred also as C^*_{ab}) and hue angle (as H*). A UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) was used.

The determination of the anthocyanin profile was performed after the berry skin extracts or the wines have been diluted with 0.05 M H_2SO_4 to less than 4 % ethanol content, and 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 (Rolle et al., 2012a). The HPLC-DAD system and chromatographic conditions were previously reported in the literature (Rolle et al., 2012a). A LiChroCART analytical column (250 mm × 4 mm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA), was used. The mobile phases were: A = formic acid/water (10:90, v/v); B = formic acid/methanol/water (10:50:40, v/v/v), working at a flow-rate of 1 mL/min. After the identification, the amounts of individual anthocyanins were expressed as percentages, based on the concentration of the respective compounds (or as malvidin-3-glucoside for acylated compounds) evaluated at 520 nm wavelength. All analyses were performed in duplicate.

Individual flavanols were determined by liquid chromatography before and after acid-catalyzed degradation of polymeric proanthocyanidins in the presence of phloroglucinol. Phloroglucinolysis of the seed extracts was carried out according to the method proposed by Kennedy and Jones (2001) and slightly modified by Torchio, Río Segade, Giacosa, Gerbi, and Rolle (2013). The extract was dealcoholized by evaporation to dryness under reduced pressure at 35 °C. Thereafter, the residue was redissolved in phloroglucinol reagent consisting of 50 g/L phloroglucinol and 10 g/L ascorbic acid in methanol containing 0.1 mol/L

hydrochloric acid, and they were allowed to react for 20 min at 50 °C. The determination of gallic acid and individual flavanols was performed by the HPLC-DAD system, and chromatographic conditions were previously reported in the literature (Kennedy & Jones, 2001). The chromatographic separation was carried out at 25 °C on a LiChroCART analytical column (250 mm × 4 mm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA). The mobile phases consisted of A = 1% aqueous acetic acid; B = methanol, working at a flow-rate of 0.8 mL/min. After the identification, the contents of individual gallic acid, monomeric flavanols (catechin, epicatechin, epicatechin gallate), and dimeric flavanols (procyanidin B_1, procyanidin B_2) were quantified in mg/kg grape. The mean degree of polymerization (as mDP) was calculated as the molar ratio of the sum of all flavanol units produced by phloroglucinolysis (phloroglucinol adducts plus monomers) to the sum of monomeric flavanols. The percentage of galloylation (as G) was calculated as the ratio of the sum of galloylated flavanols to the sum of all flavanols. All analyses were performed in duplicate.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS Statistics software package version 19.0 (IBM Corporation, Armonk, NY, USA). The Tukey-b test at $p < 0.05$ was used to establish significant differences by one-way analysis of variance (ANOVA).

3. Results and discussion

3.1 Technological ripeness parameters

Table 1 shows the parameters that define the technological ripeness of fresh and withered Avanà, Chatus and Nebbiolo winegrapes. Regarding fresh grapes, significant

differences were observed in all the parameters studied between Chatus and the other two varieties, except for malic acid. The lower values of the parameters related to the accumulation of sugars in the grape must (reducing sugars content, G/F ratio), and the higher values of those associated with the acidity (titratable acidity, organic acids content) showed that Chatus berries were less ripe than Avanà and Nebbiolo.

Dehydrated grapes showed, with respect to each variety fresh ones, higher pH values and citric acid (although not significantly different for Chatus, and for the latter also for Nebbiolo), while lower titratable acidity and malic acid contents (except for Chatus faster condition) were achieved. After postharvest dehydration, the technological parameters did not differ significantly among berries of the same variety dehydrated under different thermohygrometric conditions (slow and fast withering processes) for Avanà and Nebbiolo varieties, with some exceptions. In the case of the Chatus variety, a significantly higher concentration of the must components occurred when the grape dehydration process was conducted at higher rate, excluding the tartaric acid content.

On average, at the end of the withering process, the percentages of weight loss were 19.2±0.4, 24.5±0.5 and 14±2 % for Avanà, Chatus and Nebbiolo, respectively. These differences in the dehydration rate among varieties by applying the same withering conditions can be imputable to different berry skin hardness (Giacosa, Torchio, Río Segade, Caudana, Gerbi, & Rolle, 2012). In particular, the varieties characterized by a high skin break force were associated with slower dehydration kinetics (Giacosa et al., 2012).

3.2 Phenolic composition of fresh berries

The phenolic composition of berry skin, pulp and seeds for fresh Avanà, Chatus and Nebbiolo winegrapes is shown in Table 2. Chatus berries were characterized by significantly higher values of the spectrophotometric indices A_{280}, TF, PRO, and TAI in the skin, whereas Avanà had the lowest values of TF and FRV. The Nebbiolo variety showed intermediate

values of the spectrophotometric indices of the skin, which were not significantly different to those of A_{280}, PRO, and TAI for Avanà, and those of FRV for Chatus. Significant differences were found in the anthocyanin profile of the three varieties studied. Malvidin-3-glucoside was the predominant anthocyanin compound in the Chatus variety with an anthocyanin profile mainly constituted of tri-substituted (delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside) anthocyanins (68.3%). In fact, significantly higher percentages of delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside were found in Chatus skins. Instead, for Avanà and Nebbiolo varieties, peonidin-3-glucoside was significantly more abundant with an important richness in di-substituted (cyanidin-3-glucoside and peonidin-3-glucoside) anthocyanins (71.9 and 51.7%, respectively, for Avanà and Nebbiolo). The second more abundant anthocyanin compound was delphinidin-3-glucoside in Chatus, cyanidin-3-glucoside in Avanà, and malvidin-3-glucoside in Nebbiolo. The ratios between di- and tri-substituted anthocyanins were 2.94±0.23, 0.14±0.01, and 1.57±0.28 for Avanà, Chatus, and Nebbiolo, respectively. A significant prevalence of acylated forms of anthocyanins was observed in Chatus skins, followed by Nebbiolo, when compared with Avanà. The results obtained agreed with those previously reported in the literature for TAI, TF, PRO, and FRV of Nebbiolo skins (Rolle et al., 2012a), and for the anthocyanin profile of Avanà, Chatus, and Nebbiolo skins (Ferrandino, Carra, Rolle, Schneider, & Schubert, 2012; Rolle & Guidoni, 2007; Zeppa, Rolle, Gerbi, & Guidoni, 2001). Regarding the pulp, the Nebbiolo variety had significantly higher values of A_{280} and TF when compared with Avanà and Chatus, whereas no significant difference was observed among varieties in the values of HCTA. Ferrandino et al. (2012), using a HPLC method, found lower contents of HCTA in the pulp of Avanà and Nebbiolo varieties (two-year average of 32.3 and 22.2 mg/kg, respectively).

In the seeds, the Avanà variety showed significantly lower values of the spectrophotometric indices A_{280}, TF, PRO, and FRV, as well as of the extractable content of

C, EC, and procyanidins B_1 and B_2, whereas the extractable content of ECG and the percentage of galloylation (total) were significantly higher when compared with Chatus and Nebbiolo seeds. The G value is related to the proportion of ECG subunits in polymeric flavanols, and Avanà seeds are characterized by a higher percentage of galloylated subunits in both terminal and extension units (Table 2), different with respect to Chatus. Nebbiolo seeds were significantly richer in TF, GA, and C, whereas procyanidin B_1 was significantly more abundant in Chatus seeds. The values found of A_{280}, TF, and FRV were in the range reported in the scientific literature for Nebbiolo seeds (Rolle et al., 2012a, 2013a), but they were lower for Avanà seeds than others published (Torchio, Giacosa, Río Segade, Gerbi, & Rolle, 2014). The values observed of PRO were higher than those previously published for Avanà and Nebbiolo seeds (Rolle et al., 2012a, 2013a; Torchio et al., 2014).

The contents obtained of the different flavanol compounds agreed with those previously reported for the same varieties (Rolle et al., 2013a; Torchio et al., 2014), with the exception of GA for Nebbiolo, and ECG for Avanà and Nebbiolo. A possible reason is the edaphoclimatic vineyard and vintage effect on the flavanolic composition of the seeds (Lorrain, Chira, & Teissedre, 2011). The compounds C and EC were by far the main constituents of seed monomeric flavanols. The most abundant monomeric flavanol in the seeds of the Nebbiolo variety was C, in agreement with a previous work (Rolle et al., 2013a), whereas similar percentages of C and EC on the total monomers were found in Avanà and Chatus seeds, ranging from 44.7% to 49.6%. Procyanidins B_1 and B_2 were equally present in Nebbiolo seeds (Rolle et al., 2013a). mDP and G_t values for the three varieties studied were consistent with the previously reported for the seeds of Nebbiolo and other winegrape varieties. In fact, other works showed mDP values ranging from 2.0 to 16.1, and G_t values ranging from 4.1% to 51.3% (Bordiga, Travaglia, Locatelli, Coïsson, & Arlorio, 2011; Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; Lorrain et al., 2011; Rolle et al., 2013a). No significant difference was found among the three varieties studied in the value of mDP,

although other researchers found that the variety is a factor influencing the seed flavanol composition including mDP values (Chira et al., 2009; Lorrain et al., 2011).

It was confirmed that the three varieties tested showed different characteristics regarding the phenolic profile. As discussed, anthocyanin content and profile were among the most variable parameters between these varieties.

3.3 Effect of postharvest dehydration on phenolic composition

The effect of postharvest dehydration of red winegrapes, conducted at two different controlled thermohygrometric conditions (slow and fast withering processes), on the phenolic composition of berry skin, pulp and seeds is shown in Table 2. The phenolic composition was differently affected by the dehydration process depending on the variety.

3.3.1 Skins

The dehydration process induced some modifications in the skin phenolic composition. The A_{280} index, recognizable as a fast generic index and presented as normalized by the weight of the berries used for the extraction, achieved significant differences before and after the dehydration process only for slow withered Avanà grapes, marking a decrease. Instead, in Chatus the A_{280} value increased with the dehydration, but the differences were not significant due to the high variability in the fast withering samples. Nebbiolo extracts showed non-significantly different slightly higher values after withering when the values were expressed per kg grapes. The aforementioned tendencies before and after the dehydration were found also for TF, FRV, and TAI indices, with non-significant differences except for slow withered Avanà grapes.

PRO values and the sum of acetyl-glucoside anthocyanins percentage seemed to be the skin parameters which changed more with the dehydration. In particular, the latter parameter achieved significant differences in Chatus and Nebbiolo, only before and after the fast

dehydration process. Regarding Avanà acetyl-glucoside anthocyanins, if we look closely to the results we can see that the significant differences were present only between the withered samples, and not in relation to the grape dehydration process.

In detail, regarding the differences between dehydration conditions, the values of the spectrophotometric indices determined in the skin were higher in fast withered grapes for the three varieties studied when compared with slow withered grapes, but the differences were significant only for A_{280}, PRO, and FRV in Avanà.

The important decrease caused by the dehydration process in the values of PRO and FRV for Avanà skins, agreed with the reported for these indices in Mondeuse winegrapes throughout the on-vine drying process (Rolle, Torchio, Giacosa, & Gerbi, 2009), and with the contents of monomeric and oligomeric flavanols in Raboso Piave berries during postharvest dehydration (Bonghi, Rizzini, Gambuti, Moio, Chkaiban, & Tonutti, 2012). The FRV index is sensitive to the presence of monomeric flavanols and it is partially related with the concentration of low molecular weight proanthocyanidins. However, fast withering appeared to be generally effective in delaying the reduction in the skin flavanol concentration (Bonghi et al., 2012), as occurred in the three varieties studied.

Anthocyanin content (as TAI) in the skin decreased only during slow withering of Avanà grapes in relation to fresh grapes, whereas this was not significantly affected during dehydration of Chatus and Nebbiolo. The fast withering process, although notably for having a TAI content (as mg/kg berries) not significantly higher than the slow process, may have reduced the degradation of anthocyanin compounds in Avanà berries in relation to the slow process, which is of great relevance in varieties characterized by an important presence of di-substituted anthocyanins. It is well known that di-substituted forms of anthocyanins are more unstable than those that are tri-substituted. In fact, the ratio between di- and tri-substituted anthocyanins was 1.6 times greater in fast withered Avanà grapes than in slow withered grapes. Nevertheless, the ratio observed was independent on the withering process for Chatus

and very little dependent for Nebbiolo. The ratio differences between the two withering trials, for each cultivar, were not significant ($p > 0.05$).

Nicoletti et al. (2013) observed a decrease in the anthocyanin content of withered Nebbiolo grapes in relation to fresh berries, although it was not significantly different among berries dehydrated at 20 and 30 °C. No significant variation in the value of TAI was found for Mondeuse winegrapes throughout the on-vine drying process (Rolle et al., 2009), or for Corvina during controlled postharvest dehydration at different rates (Rolle et al., 2013b). Bonghi et al. (2012) reported that key genes involved in anthocyanin biosynthesis were unaffected or down-regulated when Raboso Piave berries were dehydrated at different rates. In contrast, Mencarelli, Bellincontro, Nicoletti, Cirilli, Muleo and Corradini (2010) showed that the anthocyanin content increased significantly in Aleatico grapes after postharvest dehydration at 10 and 20 °C, whereas it diminished at 30 °C. The discrepancies are probably due to the different withering conditions, as well as to variety effects on the grape anthocyanin content and composition.

With regard to the anthocyanin profile, few significant differences were observed among berries dehydrated at different rates for the three varieties studied, and dehydration itself induced limited changes in the anthocyanin profile of grapes. Significant differences were found in Avanà skins: fast withering provided a higher relative abundance of peonidin-3-glucoside and acetylated glucosides. In withered Chatus grapes, the proportion of acetylated glucosides was significantly higher when the fast process was used, mainly at the expense of cinnamoylated forms of anthocyanins. Acylated anthocyanins participate in intramolecular copigmentation processes, protecting the flavylium cation (Gil-Muñoz, Moreno-Pérez, Vila-López, Fernández-Fernández, Martínez-Cutillas, & Gómez-Plaza, 2009). In agreement with the results reported from other researchers for Nebbiolo berries (Nicoletti et al., 2013), for each winegrape variety studied, the differences in the percentage of total acylated anthocyanins (acetyl plus cinnamoyl derivatives) among berries dehydrated by fast and slow

processes were not significant. Thermal stress has not induced the acylation of anthocyanins and, therefore, it has not increased the vacuolar content of stable pigments.

The limited variations in the anthocyanin profile among fresh and withered berries found here were consistent with those observed in the Mondeuse variety (Rolle et al., 2009).

3.3.2 Pulps

In the absence of reactions, the withering process should increase the content of the phenolic compounds present in the pulp, mainly due to the concentration effect associated with water evaporation. However, the changes induced by postharvest dehydration of grape berries are a balance between concentration, hydrolysis and oxidation processes (Serratosa, Lopez-Toledano, Merida, & Medina, 2008; Bonghi et al., 2012). In the present work, the concentration effect prevailed in the degradation reactions for these compounds, particularly for Chatus grapes, since all the reported values for dehydrated berries were higher than the fresh berries content (Table 2), and with the exception of Avanà slow withering sample which almost matched the HCTA content with the unwithered one, and slow-withered Nebbiolo TF value. However, only Chatus and Nebbiolo HCTA contents showed significant changes between fresh and dehydrated samples.

Few works are available in the scientific literature on the evolution of pulp phenolic compounds during off-vine dehydration of grapes. Frangipane et al. (2012) showed a slight decrease in the content of hydroxycinnamic acids in the juice during the dehydration process of Roscetto grapes, probably due to oxidative phenomena.

When the phenolic composition of the pulp in slow and fast withered berries was compared for each variety, the value of TF was found significantly higher in slow withered Avanà grapes, whereas the fast process gave withered Nebbiolo grapes a significantly higher value of A_{280}. No significant difference was found between slow and fast withered grapes in

the content of HCTA in the pulp, as well as in the values of A_{280} and TF for the Chatus variety.

A possible justification could be that thermally processed biomaterials support a tissue degradation closely related to enzymatic and nonenzymatic changes in the cell wall pectin (Chong, Law, Cloke, Abdullah, & Daud, 2008). This degradation depends on the dehydration rate of grape berries and is variety dependent (Rolle et al., 2013b). In addition, the skin cell wall degradation during the postharvest dehydration process facilitates the diffusion of phenolic compounds to the pulp (Márquez, Dueñas, Serratosa, & Mérida, 2012b). Furthermore, another hypothesis is related to the higher water evaporation occurring when the dehydration temperature is increased, leading to less oxygen entry (Márquez, Perez-Serratosa, Varo & Merida, 2014). In the cited study Tempranillo musts presented higher antioxidant activity after the grape dehydration conducted at 40 °C temperature with respect to 30 °C condition, but the phenolic classes here described (total flavonoids and hydroxycinnamic acids) were not determined.

3.3.3 Seeds

The effect of postharvest grape dehydration on the qualitative flavanolic composition of the seeds was small: Avanà and Chatus marked some differences with the dehydration in spectrophotometric parameters like A_{280}, TF and PRO, while the extraction from Nebbiolo seeds before and after dehydration did not appear to be different when analyzed by these parameters. Among them, the differences between fresh and withered grapes from both dehydration rates (slow and fast) were significant only for TF parameter in Chatus samples. Furthermore, the dehydration process influenced ECG content, which decreased significantly in Avanà. Procyanidin B_2 values were significantly different only in fast dehydrations for Chatus (as also B_1) and Nebbiolo (together with EC), marking an increase.

The thermohygrometric dehydration conditions affected the quantitative composition differently as a function of the variety. In Avanà grapes, higher values of the spectrophotometric indices A_{280} and TF, and non-significantly higher contents of monomeric (except ECG) and dimeric flavanols in the seeds were obtained with the slow withering process. By contrast, Chatus and Nebbiolo seeds showed higher indices and contents when the fast withering process was applied, but significant differences only for Nebbiolo EC and B_2 contents. Rolle et al. (2013b) also reported higher values of TF, PRO, and FRV in the seeds of Corvina winegrapes dehydrated at higher rates under controlled thermohygrometric conditions.

An important aspect to take into account is the increased contribution of proanthocyanidins (PRO index) and low molecular weight flavanols (FRV index) from the seeds to total content (seeds and skins) in withered Avanà grapes, particularly for the slow withering process, when compared with that in the fresh ones. This may have a notable influence on the future sensorial properties of red wines made with maceration, particularly astringency and bitterness (McRae, Schulkin, Kassara, Holt, & Smith, 2013).

mDP value was not significantly affected by the postharvest dehydration, nor by the variety or the dehydration rate. On the other hand, the values of G_t in the seeds from dehydrated grapes were higher in Avanà samples with respect to the other varieties, as a consequence of the higher value in the fresh grapes. However, the G_t value decreased with the dehydration for all the varieties and conditions, except for the only significant variation for Nebbiolo slow-withered samples, which showed a increase. The higher percentage of galloylated flavanolic compounds (G_t) found in the seeds from slow withered Nebbiolo grapes in relation to the fast withered and to the fresh grapes might directly be associated with higher astringency of wines made from them (Ferrer-Gallego, García-Marino, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2010).

Regarding phenolic acids of the seeds, with respect of fresh samples, slow withered Nebbiolo samples are the only ones that showed lower non-significantly different GA values, as all the other dehydrated samples were richer in GA, with Avanà seeds showing significant increases. Indeed, only the Nebbiolo variety showed significant differences in the content of GA among berries withered at different rates, with higher values in fast withered grapes.

3.4 Evaluation of wines produced with dehydrated berries

Because postharvest dehydration affected the secondary metabolism of grape berry, the impact of the changes induced by the different dehydration rate on phenolic compounds released into the wine was also studied for the three varieties (Table 3). For each variety, significant differences in the phenolic composition and chromatic characteristics were observed among the wines made from grapes dehydrated under different controlled thermohygrometric conditions.

The spectrophotometric indices A_{280}, TF, PRO, and FRV were significantly higher in the wines made from fast withered Chatus and Nebbiolo grapes when compared with those from the respective slow withered samples, amplifying the effect of the dehydration rate observed on the phenolic composition of the dehydrated berries (Table 2) and changing the astringency and bitterness properties of the wines from fast withered grape samples (Vidal, Francis, Noble, Kwiatkowski, Cheynier, & Waters, 2004). However, this effect was inverted and significant only for PRO in Avanà wines, but the values of these indices and TAI were very low in each case. A possible justification is the different ability of skins and seeds of the three winegrape varieties after dehydration to release phenolic compounds during maceration. The qualitative and quantitative composition of anthocyanins in the resulting wines was not directly related to that found in the respective withered grapes. This could be due to different berry skin hardness values that condition the diffusion of anthocyanins from the skin into the wine during maceration (Río Segade et al., 2014), although this kind of data were not

acquired. Only for the Chatus variety, significant differences were observed in TAI among the wines made from slow and fast withered grapes, showing higher values for the slow process.

Malvidin-3-glucoside was the predominant anthocyanin compound in the wines ranging from 50.0% to 67.1%. An important percentage of peonidin-3-glucoside was also found in Avanà and Nebbiolo wines (16.7 to 29.8%). In varieties characterized by an important presence of di-substituted anthocyanins, such as Avanà and Nebbiolo, a remarkable loss of these anthocyanin compounds has been also noticed during winemaking by oxidation, polymerization and insolubilization processes (Cagnasso, Rolle, Caudana, & Gerbi, 2008; Cheynier, Souquet, Kontek, & Moutounet, 1994). Therefore, the prevalence of malvidin-3-glucoside over peonidin-3-glucoside is possible in the resulting wines (Cagnasso et al., 2008; González-Neves, Gil, & Barreiro, 2008). When the anthocyanin profile was compared among the wines obtained from grapes dehydrated by the slow and fast processes, the greatest number of significant differences was found for the Chatus variety, corresponding the higher percentages of more stable anthocyanins (malvidin-3-glucoside and acylated forms) to the slow withering process, as also occurred for malvidin-3-glucoside in the Nebbiolo variety.

The chromatic characteristics showed that the significantly darker wines (lower L* values, higher color intensity) for each variety considered corresponded to those made from slow withered Avanà and Chatus grapes, and from fast withered Nebbiolo grapes. The fast withering process resulted in a significantly higher contribution of the components represented by coordinates a*, b*, and C* to the color of Chatus and Nebbiolo wines (thus more red and yellow color contributions), but lower contribution for Avanà wines, when compared with the slow withering process. For the Nebbiolo variety, the wines made from grapes dehydrated by the fast process had a significantly lower color tonality. It is important to indicate that Avanà wines made from dehydrated grapes did not meet good chromatic characteristics because of their high values of L*, H* (corresponding in degrees to 69.0°,

possible pyranoanthocyanin formation, orange hue), and color tonality, and low values of a* (low red color contribution), C*, and color intensity.

ΔE* parameter (OIV, 2008) was calculated from the average L*, a*, and b* coordinates values to show the overall colorimetric difference between slow and fast-withered wines, separately for each cultivar. The calculated ΔE* values were 5.54, 3.75, and 15.43, respectively for Avanà, Chatus and Nebbiolo. This confirms that Chatus wines showed the less color differences between slow and fast trials, while Nebbiolo wine color was the more influenced by the grape dehydration rate.

Achieving a good color intensity and tonality could be interesting in *Passito* and reinforced red wines, despite the presence of a postharvest process. In short cv. Tempranillo grapes dehydration trials, higher temperatures (40 °C with respect to 30 °C) increased the must color absorbance measurements at 420 and 520 nm wavelengths, thus lowering the color tonality (or hue, as Abs_{420nm}/Abs_{520nm}) due to the predominance of the latter contribution (Marquez et al., 2014). As previously mentioned, these authors indicated that faster dehydration rates could have prevented the oxygen entry in the grapes under dehydration. However, the cited study analyzed only the color of the must obtained by pressing of dehydrated grapes, and not the color of wines from a maceration and complete fermentation of the grapes.

Table 4 shows the standard parameters of the wines made from grapes dehydrated by slow and fast processes for each variety. Chatus wines were characterized by the highest values of TA, as well as the highest contents of malic acid (sign of the inability to complete the malolactic fermentation) and ethanol, but the lowest values of pH. Furthermore, Chatus wines made from slow withered grapes were more alcoholic, and less acid and glyceric than those made from fast withered grapes. No significant difference was found in these described parameters among wines made from differently dehydrated grapes for Avanà and Nebbiolo varieties, although about 9 g/L of residual sugars were present in Avanà wines produced with

slow withered grapes, resulting in significant differences when compared with the fast trials. Also Chatus fast trials showed more than 5 g/L of unfermented residual sugars.

4. Conclusions

Grape dehydration is an important technical method used to improve certain berry quality traits during the production of premium wines. This study improved our understanding of the changes occurring in phenolic compounds of the grapes during slow and fast withering processes under controlled thermohygrometric conditions, and allowed an evaluation of implications for the wines obtained. The three winegrape varieties used for the production of reinforced wines were selected on the basis of different anthocyanin profiles for di- and tri-substituted anthocyanins. The phenolic composition was differentially affected by the dehydration rate as a function of the genotype.

Avanà grapes were not suitable for the production of reinforced wines. The withering process caused an important decrease in the content of total anthocyanins in Avanà grapes, and the resulting wines showed similar percentages of di- and tri-substituted anthocyanins, which affected negatively the chromatic characteristics. In addition, high acetic acid contents and some differences in residual sugars depending by the withering trial could affect the sensory perception. Chatus grapes were very suitable for the production of reinforced wines, particularly slow withered grapes. In fact, the resulting wines showed higher amounts of stable red pigments, lower contents of phenolic compounds related to astringency and bitterness (proanthocyanidins and low molecular weight flavanols), lower pH, and the better chromatic characteristics. Nebbiolo grapes, fit for the production of reinforced wines, are better suited to the fast withering process when using longer maceration times, as this have

prevented a greater presence of seed galloylated flavanols, a factor that may affect negatively wine astringency.

References

Bellincontro, A., De Santis, D., Botondi, R., Villa, I., & Mencarelli, F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. *Journal of the Science of Food and Agriculture*, *84*, 1791–1800.

Bonghi, C., Rizzini, F. M., Gambuti, A., Moio, L., Chkaiban, L., & Tonutti, P. (2012). Phenol compound metabolism and gene expression in the skin of wine grape (*Vitis vinifera* L.) berries subjected to partial postharvest dehydration. *Postharvest Biology and Technology*, *67*, 102–109.

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

Cagnasso, E., Rolle, L., Caudana, A., & Gerbi, V. (2008). Relationship between grape phenolic maturity and red wine phenolic composition. *Italian Journal of Food Science*, *20*, 365–380.

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

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P.-L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry*, *57*, 545–553.

Chong, C. H., Law, C. L., Cloke, M., Abdullah, L. C., & Daud, W. R. W. (2008). Drying kinetics, texture, color, and determination of effective diffusivities during sun drying of Chempedak. *Drying Technology*, *26*, 1286–1293.

Ferrandino, A., Carra, A., Rolle, L., Schneider, A., & Schubert, A. (2012). Profiling of hydroxycinnamoyl tartrates and acylated anthocyanins in the skin of 34 *Vitis vinifera* genotypes. *Journal of Agricultural and Food Chemistry*, *60*, 4931−4945.

Ferrer-Gallego, R., García-Marino, M., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2010). Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Analytica Chimica Acta*, *660*, 22−28.

Frangipane, M. T., Torresi, S., De Santis, D., & Massantini, R. (2012). Effect of drying process in chamber at controlled temperature on the grape phenolic compounds. *Italian Journal of Food Science*, *24*, 26–31.

Giacosa, S., Torchio, F., Río Segade, S., Caudana, A., Gerbi, V., & Rolle, L. (2012). Varietal relationship between skin break force and off-vine withering process for winegrapes. *Drying Technology*, *30*, 726–732.

Gil-Muñoz, R., Moreno-Pérez, A., Vila-López, R., Fernández-Fernández, J. I., Martínez-Cutillas, A., & Gómez-Plaza, E. (2009). Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet Sauvignon wines. *European Food Research and Technology*, *228*, 777–788.

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

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

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

Lorrain, B., Chira, K., & Teissedre, P.-L. (2011). Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages. *Food Chemistry*, *126*, 1991−1999.

Marquez, A., Serratosa, M.P., & Merida, J. (2012a). Anthocyanin evolution and color changes in red grapes during their chamber drying. *Journal of Agricultural and Food Chemistry*, 61, 9908-9914.

Márquez, A., Dueñas, M., Serratosa, M. P., & Mérida, J. (2012b). Formation of vitisins and anthocyanin−flavanol adducts during red grape drying. *Journal of Agricultural and Food Chemistry*, *60*, 6866−6874.

Márquez, A., Perez-Serratosa, M., Varo, M. A., & Merida, J. (2014). Effect of temperature on the anthocyanin extraction and color evolution during controlled dehydration of Tempranillo grapes. *Journal of Agricultural and Food Chemistry*, 62, 7897-7902.

McRae, J. M., Schulkin, A., Kassara, S., Holt, H. E., & Smith, P. A. (2013). Sensory properties of wine tannin fractions: Implications for in-mouth sensory properties. *Journal of Agricultural and Food Chemistry*, *61*, 719−727.

Mencarelli, F., & Tonutti, P. (2013). *Sweet, reinforced and fortified wines: Grape biochemistry, technology and vinification*. Wiley-Blackwell, A John Wiley & Sons, Ltd., publication.

Mencarelli, F., Bellincontro, A., Nicoletti, I., Cirilli, M., Muleo, R., & Corradini, D. (2010). Chemical and biochemical change of healthy phenolic fractions in winegrape by means of postharvest dehydration. *Journal of Agricultural and Food Chemistry*, *58*, 7557–7564.

Moreno, J. J., Cerpa-Calderón, F., Cohen, S. D., Fang, Y., Qian, M., & Kennedy, J. A. (2008). Effect of postharvest dehydration on the composition of Pinot noir grapes (*Vitis vinifera* L.) and wine. *Food Chemistry*, *109,* 755–762.

Nicoletti, I., Bellincontro, A., De Rossi, A., De Sanctis, F., Tiberi, D., Pietromarchi, P., Botondi, R., Corradini, D., & Mencarelli, F. (2013). Postharvest dehydration of Nebbiolo grapes grown at altitude is affected by time of defoliation. *Australian Journal of Grape and Wine Research*, *19*, 358–368.

OIV (2008). *Recueil international des méthodes d'analyse des vins et des moûts*. Paris: Organisation Internationale de la Vigne et du Vin.

Río Segade, S., Giacosa, S., de Palma, L., Novello, V., Torchio, F., Gerbi, V., & Rolle, L. (2013). Effect of the cluster heterogeneity on mechanical properties, chromatic indices and chemical composition of Italia table grape berries (*Vitis vinifera* L.) sorted by flotation. *International Journal of Food Science & Technology*, *48*, 103–113.

Río Segade, S., Torchio, F., Giacosa, S., Ricauda Aimonino, D., Gay, P., Lambri, M., Dordoni, R., Gerbi, V., & Rolle, L. (2014). Impact of several pre-treatments on the extraction of phenolic compounds in winegrape varieties with different anthocyanin profiles and skin mechanical properties. *Journal of Agricultural and Food Chemistry*, *62*, 8437−8451.

Rolle, L., & Guidoni, S. (2007). Color and anthocyanin evaluation of red winegrapes by CIE L*, a*, b* parameters. *Journal International des Sciences de la Vigne et du Vin*, *41*, 193–201.

Rolle, L., Giacosa, S., Río Segade, S., Ferrarini, R., Torchio, F., & Gerbi, V. (2013b). Influence of different thermohygrometric conditions on changes in instrumental texture properties and phenolic composition during postharvest withering of ‘Corvina’ winegrapes (*Vitis vinifera* L.). *Drying Technology*, *31*, 549–564.

Rolle, L., Giacosa, S., Torchio, F., Perenzoni, D., Río Segade, S., Gerbi, V., & Mattivi, F. (2013a). Use of instrumental acoustic parameters of winegrape seeds as possible predictors of extractable phenolic compounds. *Journal of Agricultural and Food Chemistry*, *61*, 8752−8764.

Rolle, L., Giordano, M., Giacosa, S., Vincenzi, S., Río Segade, S., Torchio, F., Perrone, B., & Gerbi, V. (2012b). CIEL**a***b** parameters of white dehydrated grapes as quality markers according to chemical composition, volatile profile and mechanical properties. *Analytica Chimica Acta*, *732*, 105−113.

Rolle, L., Torchio, F., Giacosa, S., & Gerbi, V. (2009). Modifications of mechanical characteristics and phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying process. *Journal of the Science of Food and Agriculture*, *89*, 1973−1980.

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

Serratosa, M. P., Lopez-Toledano, A., Merida, J., & Medina, M. (2008). Changes in color and phenolic compounds during the raisining of grape cv. Pedro Ximenez. *Journal of Agricultural and Food Chemistry*, *56*, 2810–2816.

Toffali, K., Zamboni, A., Anesi, A., Stocchero, M., Pezzotti, M., Levi, M., & Guzzo, F. (2011). Novel aspects of grape berry ripening and post-harvest withering revealed by untargeted LC-ESI-MS metabolomics analysis. *Metabolomics*, *7*, 424–436.

Torchio, F., Giacosa, S., Río Segade, S., Gerbi, V., & Rolle, L. (2014). Berry heterogeneity as a possible factor affecting the potential of seed mechanical properties to classify wine grape varieties and estimate flavanol release in wine-like solution. *South African Journal of Enology and Viticulture*, *35*, 20–42.

Torchio, F., Río Segade, S., Giacosa, S., Gerbi, V., & Rolle, L. (2013). Effect of growing zone and vintage on the prediction of extractable flavanols in winegrape seeds by a FT-NIR method. *Journal of Agricultural and Food Chemistry*, *61*, 9076−9088.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004) Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta*, 513, 57-65.

Zeppa, G., Rolle, L., Gerbi, V., & Guidoni, S. (2001). Anthocyanin composition of four autochthonous *Vitis vinifera* grapevine varieties from the Piedmont. *Italian Journal of Food Science*, *13*, 405–412.

Zoccatelli, G., Zenoni, S., Savoi, S., Dal Santo, S., Tononi, P., Zandonà, V., Dal Cin, A., Guantieri, V., Pezzotti, M., & Tornielli, G. B. (2013). Skin pectin metabolism during the postharvest dehydration of berries from three distinct grapevine cultivars. *Australian Journal of Grape and Wine Research, 19*, 171–179.

Table 1
Technological ripeness parameters of fresh and withered Avanà, Chatus, and Nebbiolo grape berries.

Parameter	Avanà					Chatus					Nebbiolo					*Sign*[c]
	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	*fresh*
pH	3.37±0.03aB	3.62±0.05b	3.71±0.06b	ns	*	3.13±0.05A	3.26±0.03	3.18±0.01	ns	ns	3.29±0.03aB	3.51±0.00b	3.68±0.02c	**	***	*
TA (g/L tartaric acid)	6.61±0.27bA	5.32±0.42a	5.28±0.11a	ns	*	10.54±0.42bC	7.17±0.04a	11.51±0.11c	***	***	8.40±0.11bB	6.56±0.05a	6.34±0.27a	ns	**	**
Citric acid (g/L)	0.25±0.02aA	0.62±0.00b	0.78±0.16b	ns	*	0.29±0.00aB	0.34±0.01a	0.69±0.06b	*	**	0.42±0.01C	0.49±0.08	0.50±0.06	ns	ns	***
Tartaric acid (g/L)	6.21±0.26bA	6.39±0.05b	5.41±0.06a	**	*	8.35±0.22bB	6.91±0.20a	6.26±0.25a	ns	**	6.34±0.15aA	6.60±0.02ab	7.05±0.25b	ns	ns	**
Malic acid (g/L)	3.35±0.10bA	2.62±0.14a	2.76±0.09a	ns	*	4.76±0.27bB	2.73±0.12a	6.36±0.13c	**	***	4.49±0.15bB	3.48±0.14a	3.70±0.18a	ns	*	**
Reducing sugars (g/L)	231±2aB	285±14b	287±13b	ns	*	217±1aA	286±2b	290±5b	ns	***	232±2aB	266±5b	273±3b	ns	**	**
G/F ratio	0.96±0.00aB	0.92±0.01b	0.94±0.01b	ns	*	0.89±0.01bA	0.86±0.00a	0.84±0.00a	*	**	0.96±0.00cB	0.91±0.01a	0.92±0.00b	ns	**	***

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 differences, respectively, between withered samples in the same cultivar ([a]), fresh and withered samples in the same cultivar ([b]), and between fresh grapes cultivars ([c]). Different lowercase letters within the same row indicate significant differences ([b]) among fresh and withered grapes according to the Tukey-b test ($p < 0.05$). Different uppercase letters within the same row indicate significant differences ([c]) among cultivars fresh grapes values according to the Tukey-b test ($p < 0.05$). TA = titratable acidity, G/F = glucose/fructose, SW = slow withering, FW = fast withering.

Table 2
Phenolic composition of Avanà, Chatus, and Nebbiolo grape berries prior to dehydration and after slow and fast withering processes.

Phenolic compound	Avanà					Chatus					Nebbiolo					*Sign*[c]
	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	Fresh	SW	FW	*Sign*[a]	*Sign*[b]	*fresh*
Skin																
A_{280} (1/kg)	22.7±4.6bA	10.0±1.0a	18.3±3.9b	*	*	46.4±2.3B	62.7±2.7	63.4±15.5	ns	ns	25.7±2.2A	26.6±1.4	29.4±2.1	ns	ns	***
TF (mg (+)-catechin/kg)	1611±244bA	883±37a	1275±37ab	*	*	3023±200C	3699±141	4048±927	ns	ns	2051±123B	2133±25	2224±124	ns	ns	***
PRO (mg cyanidin chloride/kg)	1889±289cA	123±69a	805±268b	*	***	3013±45B	2116±210	3079±1001	ns	ns	1858±329bA	1224±86a	1582±167ab	*	*	***
FRV (mg (+)-catechin/kg)	457±35cA	117±27a	302±92b	*	**	826±123B	983±101	1299±579	ns	ns	682±78B	587±20	717±112	ns	ns	**
TAI (mg malvidin-3-glucoside chloride/kg)	431±123bA	108±11a	277±82ab	*	*	855±40B	1081±14	1097±290	ns	ns	457±52A	453±51	507±72	ns	ns	**
Delphinidin-3-glucoside (%)	4.8±0.5A	6.9±1.8	4.0±0.9	ns	ns	10.8±0.3B	8.8±1.7	11.8±2.3	ns	ns	4.4±0.5A	4.9±0.5	4.1±0.4	ns	ns	***
Cyanidin-3-glucoside (%)	28.2±3.7C	27.8±5.8	28.0±3.9	ns	ns	1.7±0.2A	1.2±0.3	1.7±0.2	ns	ns	10.7±1.7B	9.7±0.9	8.4±0.5	ns	ns	***
Petunidin-3-glucoside (%)	4.6±0.5B	5.1±1.7	4.0±0.7	ns	ns	7.8±0.2C	7.6±0.9	8.3±1.0	ns	ns	3.3±0.3A	3.7±0.5	3.3±0.2	ns	ns	***
Peonidin-3-glucoside (%)	43.7±2.3bB	36.4±3.1a	46.1±3.7b	*	*	7.3±0.6A	6.9±0.8	7.1±0.4	ns	ns	41.0±1.1B	35.7±3.5	36.3±0.5	ns	ns	***
Malvidin-3-glucoside (%)	15.3±1.1A	20.0±5.1	14.0±3.4	ns	ns	49.7±0.6C	50.9±1.9	48.5±2.3	ns	ns	25.2±2.3B	27.1±3.1	28.6±0.8	ns	ns	***
∑ Acetyl glucosides (%)	0.4±0.0abA	0.4±0.0a	0.5±0.1b	*	**	5.2±0.0aC	5.2±0.1a	6.4±0.2b	***	***	3.8±0.9aB	5.4±0.1a	5.8±0.4b	ns	**	***
∑ Cinnamoyl glucosides (%)	3.0±0.4A	3.4±0.2	3.4±0.6	ns	ns	17.5±1.1C	19.4±1.5	16.2±1.1	*	ns	11.6±0.7aB	13.5±0.3b	13.5±1.0b	ns	*	***
Pulp																
A_{280} (1/kg)	19.0±1.4A	24.3±3.1	20.1±1.5	ns	ns	17.2±0.6aA	29.7±1.2b	32.2±2.3b	ns	***	28.7±1.3aB	29.1±1.0a	34.4±2.6b	*	*	***
TF (mg (+)-catechin/kg)	1018±57aA	2149±355b	912±66a	**	***	1356±161aB	2288±172b	2647±245b	ns	***	2679±69C	2175±68	2680±441	ns	ns	***
HCTA (mg caffeic acid/kg)	100±1	99±12	112±4	ns	ns	97±2a	156±14b	161±11b	ns	***	100±3a	129±2b	131±9b	ns	***	ns
Seeds																
A_{280} (1/kg)	11.4±1.1aA	19.7±4.4b	17.4±2.4ab	ns	*	17.2±2.0B	26.4±5.6	28.2±5.1	ns	*	20.0±0.9B	20.6±3.5	25.9±3.5	ns	ns	***
TF (mg (+)-catechin/kg)	865±57aA	1486±242b	1257±171ab	ns	*	1325±147aB	1982±349b	2111±274b	ns	*	1558±45C	1581±152	1864±201	ns	ns	***
PRO (mg cyanidin chloride/kg)	2133±97bA	1430±287a	2050±222ab	*	*	3080±251B	2906±655	3933±614	ns	ns	3287±299B	2543±556	3543±573	ns	ns	**
FRV (mg (+)-catechin/kg)	515±60A	809±189	728±111	ns	ns	924±82B	1369±267	1484±310	ns	ns	945±71B	902±123	1176±170	ns	ns	***
GA (mg/kg)	1.53±0.19aA	6.59±2.33b	7.96±2.10b	ns	*	0.55±0.18A	1.96±1.00	1.64±1.19	ns	ns	9.87±1.14B	7.85±0.96	10.31±0.87	*	*	***
C (mg/kg)	34.5±4.3A	39.2±13.8	34.0±6.2	ns	ns	64.4±10.5B	65.7±13.5	85.7±10.7	ns	ns	105.5±5.2C	90.2±9.8	111.3±10.2	ns	ns	***
EC (mg/kg)	35.8±1.5A	47.8±13.9	43.2±7.7	ns	ns	62.5±9.5B	53.4±11.8	77.4±14.0	ns	ns	59.9±10.3aB	56.6±2.4a	81.4±12.6b	*	*	*
ECG (mg/kg)	6.90±0.79bB	2.78±0.34a	2.84±0.99a	ns	***	3.05±0.57A	3.67±1.51	3.77±1.13	ns	ns	2.91±0.70bA	0.93±0.32a	1.99±0.77ab	ns	*	***
B_1 (mg/kg)	15.7±1.7A	22.6±4.4	18.3±2.7	ns	ns	33.4±3.0aC	49.9±9.2ab	53.0±8.4b	ns	*	24.0±1.5B	22.0±1.8	27.2±2.9	ns	ns	***
B_2 (mg/kg)	12.6±1.2A	20.1±5.4	19.3±3.3	ns	ns	30.8±2.8aB	42.0±7.1ab	47.3±6.7b	ns	*	24.6±4.3aB	25.4±1.9a	36.6±2.0b	**	**	***
mDP	6.66±1.10	7.17±0.51	7.45±0.08	ns	ns	5.94±1.00	5.56±0.64	5.90±0.49	ns	ns	7.01±1.50	6.88±0.90	5.60±0.24	ns	ns	ns
G_{ext} (%)	6.3±1.4B	7.2±1.0	7.0±0.8	ns	ns	2.8±1.3A	3.3±0.9	3.5±0.3	ns	ns	3.9±0.5aAB	6.1±0.6b	3.4±0.8a	**	**	*
G_{ter} (%)	30.1±6.7B	23.4±3.5	26.4±1.7	ns	ns	15.4±2.3A	15.1±3.1	16.1±0.4	ns	ns	24.7±4.8bAB	21.3±1.8ab	16.4±1.3a	*	*	*
G_t (%)	9.9±1.7B	9.5±1.2	9.6±0.9	ns	ns	4.9±1.2A	5.4±1.0	5.6±0.0	ns	ns	6.8±0.5aA	8.3±0.5b	5.7±0.8a	**	**	**

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 differences, respectively, between withered samples in the same cultivar ([a]), fresh and withered samples in the same cultivar ([b]), and between fresh grapes cultivars ([c]). Different lowercase letters within the same row and cultivar indicate significant differences ([b]) among fresh and withered grapes according to the Tukey-b test ($p < 0.05$). Different uppercase letters within the same row indicate significant differences ([c]) among cultivars fresh grapes values according to the Tukey-b test ($p < 0.05$). A_{280} = absorbance at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, TAI = total anthocyanins, HCTA = total hydroxycinnamic acids, GA = gallic acid, C = catechin, EC = epicatechin, ECG = epicatechin gallate, B_1, B_2 = procyanidins B_1, B_2, mDP = mean degree of polimerization, G_{ext} = galloylation in extension units, G_{ter} = galloylation in terminal units, G_t = galloylation total, SW = slow withering, FW = fast withering.

Table 3
Wine phenolic composition of Avanà, Chatus, and Nebbiolo varieties after slow and fast withering process.

Parameter	Avanà			Chatus			Nebbiolo		
	SW	FW	*Sign*	SW	FW	*Sign*	SW	FW	*Sign*
A_{280} (1/L)	17.9±0.1	17.3±0.2	ns	43.4±0.6	47.4±0.3	*	35.8±1.1	43.4±0.4	*
TF (mg (+)-catechin/L)	510±7	494±10	ns	1351±40	1533±23	**	1351±50	1648±47	*
PRO (mg cyanidin chloride/L)	538±26	444±17	**	1566±63	1804±87	*	2201±97	2580±93	*
FRV (mg (+)-catechin/L)	179±5	188±4	ns	501±11	701±22	**	907±33	1112±28	*
TAI (mg malvidin-3-glucoside chloride/L)	29±1	24±1	ns	211±4	199±3	*	120±3	118±3	ns
Delphinidin-3-glucoside (%)	2.0±0.1	1.9±0.1	ns	3.6±0.3	6.9±0.3	***	3.4±0.3	3.2±0.3	ns
Cyanidin-3-glucoside (%)	6.0±0.3	5.5±0.2	*	0.6±0.0	1.1±0.1	***	2.1±0.2	2.4±0.1	ns
Petunidin-3-glucoside (%)	5.5±0.5	5.8±0.4	ns	5.4±0.3	6.6±0.3	***	5.1±0.3	5.1±0.4	ns
Peonidin-3-glucoside (%)	29.8±0.5	28.2±0.3	**	4.8±0.2	5.9±0.2	***	16.7±0.4	20.0±0.4	**
Malvidin-3-glucoside (%)	50.0±0.6	51.6±0.5	**	67.1±0.9	63.5±0.9	**	56.3±0.4	52.6±0.3	**
∑ Acetyl glucosides (%)	2.3±0.1	2.4±0.3	ns	10.9±0.3	8.7±0.3	***	7.0±0.3	8.2±0.7	ns
∑ Cinnamoyl glucosides (%)	4.4±0.4	4.6±0.1	ns	7.6±0.4	7.3±0.3	ns	9.4±0.4	8.5±0.3	*
L*	83.6±1.0	85.8±0.8	*	13.4±0.3	15.1±0.1	**	46.9±0.5	32.9±0.4	***
a*	10.60±0.10	8.80±0.07	**	44.75±0.37	46.85±0.41	**	48.05±0.41	53.45±0.47	***
b*	27.65±0.27	22.90±0.20	***	31.25±0.31	33.85±0.21	**	36.30±0.28	39.90±0.34	**
C*	29.65±0.27	24.50±0.30	***	54.55±0.51	57.85±0.61	**	60.20±0.58	66.75±0.71	**
H* (rad)	1.20±0.01	1.20±0.01	ns	0.61±0.02	0.63±0.02	ns	0.65±0.02	0.64±0.02	ns
Color tonality	2.01±0.03	2.00±0.03	ns	0.69±0.02	0.68±0.02	ns	1.04±0.03	0.94±0.02	***
Color intensity (AU, OP 10 mm)	0.89±0.05	0.74±0.03	***	11.38±0.18	9.27±0.22	***	3.02±0.12	4.53±0.11	***

All data are expressed as average value ± standard deviation (n = 2). Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant differences, respectively. A280 = absorbance at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, TAI = total anthocyanins, L* = clarity, a* = red/green color coordinate, b* = yellow/blue color coordinate, C* = chroma, H* = hue angle, SW = slow withering, FW = fast withering.

Table 4
Wine standard parameters of Avanà, Chatus, and Nebbiolo varieties after slow and fast withering process.

Parameter	*Avanà*			*Chatus*			*Nebbiolo*		
	SW	*FW*	*Sign*	*SW*	*FW*	*Sign*	*SW*	*FW*	*Sign*
pH	*4.08±0.02*	*4.12±0.01*	*ns*	*3.53±0.01*	*3.68±0.02*	*	*4.05±0.02*	*4.13±0.04*	*ns*
TA (g/L tartaric acid)	*4.76±0.11*	*4.65±0.08*	*ns*	*7.13±0.10*	*8.78±0.16*	**	*5.29±0.09*	*5.03±0.13*	*ns*
Citric acid (g/L)	*0.10±0.03*	*0.13±0.01*	*ns*	*0.14±0.01*	*nd*	***	*nd*	*0.15±0.02*	**
Tartaric acid (g/L)	*1.04±0.07*	*1.15±0.06*	*ns*	*1.10±0.03*	*0.87±0.06*	**	*0.87±0.09*	*0.88±0.07*	*ns*
Malic acid (g/L)	*nd*	*nd*	*ns*	*2.00±0.09*	*2.98±0.07*	**	*0.04±0.03*	*nd*	*ns*
Glycerol (g/L)	*13.91±0.08*	*14.15±0.10*	*ns*	*9.90±0.11*	*11.20±0.09*	***	*10.60±0.17*	*10.34±0.12*	*ns*
Acetic acid (g/L)	*1.03±0.03*	*0.97±0.04*	*ns*	*0.50±0.05*	*0.31±0.08*	*	*0.68±0.07*	*0.56±0.05*	*ns*
Ethanol (% v/v)	*15.7±0.1*	*15.8±0.1*	*ns*	*16.5±0.0*	*16.0±0.1*	*	*15.0±0.2*	*15.1±0.1*	*ns*
Sugars (g/L)	*8.86±0.07*	*1.55±0.04*	***	*1.97±0.08*	*5.25±0.08*	***	*1.12±0.06*	*1.20±0.01*	*ns*

All data are expressed as average value ± standard deviation (n = 2). Sign: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant differences, respectively. TA = titratable acidity, SW = slow withering, FW = fast withering. nd = not detected.