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Article

Effects of Traditional and Modern Post-Harvest Withering Processes on the Composition of the Vitis v. Corvina Grape and the Sensory Profile of Amarone Wines

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Abstract: In the Valpolicella area (Verona - Italy) Vitis vinifera cv. Corvina is the main grape variety used to produce Amarone wine. Before starting the winemaking process, the Corvina grapes are stored in a withering (i.e., dehydrating) warehouse until about 30% of the berry weight is lost (WL). This practice is followed to have the chemical metabolites concentrate in the berry and enrich the Amarone wine in aroma and antioxidant compounds. In compliance with the guidelines and strict Amarone protocol set by the Consorzio of Amarone-Valpolicella, withering must be carried out by setting the grapes in a suitable environment, either under controlled relative air humidity (RH) conditions and wind speed (WS) – no temperature modification is to be applied – or, following the traditional methods, in open-air natural environmental conditions. In general, the two processes have different dehydration kinetics due to the different conditions in terms of temperature, RH, and WS, which affect the accumulation of sugars and organic acids and the biosynthesis of secondary metabolites such as stilbenes and glycoside aroma precursors. For this study, the two grape-withering processes were carried out under controlled (C) and not-controlled (NC) conditions and the final compositions of the Corvina dried grapes were compared also to evaluate the effects on the organoleptic characteristics of Amarone wine. The findings highlighted differences between the two processes mainly in terms of the secondary metabolites of the dried grapes, which affect the organoleptic characteristics of Amarone wine.

Keywords: post-harvest; grape; wine; withering; stilbenes; aroma; Amarone; Corvina

1. Introduction

Wine grapes are generally harvested at the proper technical ripening stage, which is when the major chemical ripening parameters (pH, acidity, soluble sugars, polyphenols and aromatic compounds) meet the oenological requirements of each type of wine, according to the oenological goal set by the winemakers [1]. Indeed, some characteristic wines (sweet or dry) are produced by drying the harvested grapes in the open air

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(Moscato di Pantelleria) or by storing them in withering (dehydration) chambers before vinification, to achieve a weight loss (WL) of around 30% (Pedro Ximenez of Montilla-Moriles, Riesling, Commandaria of Cipro, Vin Santo di Santorini, French Souternes, Valpolicella Amarone).

Worldwide, Amarone is considered one of the most important Italian wines (15 million 750ml bottles, with an average commercial price of 62 USD/bottle – Consortium for the protection of DOP Valpolicella Wine, 2019 data provided by Siquria). Amarone is produced from three native varieties: Corvina, Corvinone, and Rondinella (Consorzio Valpolicella, 2010), however, withered Corvina grapes are still the main variety used to craft this wine [2]. Corvina is a native variety of the Verona area; the wine it gives is characterised by hints of cherry, bitter almond, blossom aromas, and refreshing acidity; nevertheless, it lacks anthocyanins and has a moderate content of skin tannins. The withering process, in this sense, has been selected over the decades to concentrate and enrich the berry and wine composition.

The grape drying process involves two simultaneous transfer phenomena: i) heat (energy) transferred from the environment to the bunches and ii) water (mass) transfer from the inside to the outer surface of the berries, followed by evaporation. The drying conditions influence the properties of the resulting grapes and wines; slow drying at a low temperature (T) and aeration rate (W) and higher relative humidity levels (RH), provides more harmonious wines [3]. Whereas, fast drying (high T, low RH, high W), could lead to wines with unbalanced aromatic profiles [4]. Drying also causes cell damage and alters the overall structure of the berry due to the loss of moisture and consequent berry sanitary issues [5]. As a consequence of berry water loss, the skin/pulp ratio increases, moreover, withering causes compositional changes in the phenolic and aromatic composition to an extent that is variable depending on the specific drying conditions [6]. Biochemical changes have also been described in berries dehydrated in ventilated chambers, deriving from environmental parameters and endogenous factors (genotype) [7].

The most marked effect occurs on sugars. Their increase is mainly due to a concentration effect, however, the composition and nature of the sugars change throughout the drying process, particularly in terms of the glucose/fructose ratio variations [8]. The change in the glucose/fructose ratio in this phase is mainly due to respiration [9] which preferably uses glucose as a substrate. Organic acidity, as for sugars, during the drying process is influenced by the concentrative aspect and both malic and tartaric acid are affected by the metabolic respiration and precipitation processes. These changes may have a different intensity depending on the organic acid considered, and the stage and intensity within the dehydration process. It is reported that there are slight increases in the total acidity mainly due to the concentration of tartaric acid [10] as well as a slight decrease in malic acid [11]. Specifically, during the drying process, the sugar/acid ratio varies significantly, and this is linked to the marked concentration of sugars [12].

Alongside the primary metabolism compounds, the secondary metabolites, mainly present in the hypodermal layer of the berry skin and that affect colour, flavour and aroma of the wine, also need to be considered [13,14]. The withering process affects the development of dehydration-related aromas and polyphenolic compounds [6]. Following harvest, as the grape berry is metabolically active until cell death, it reacts to endogenous and exogenous stresses such as dehydration. In the early stages of the drying process, there is an enrichment of polyphenols [15–17] due to new synthesis, confirmed by the increase in the transcripts of their biosynthetic pathway [18]. At a later stage, an oxidative process of the polyphenols takes place, leading to a depletion of many of them [16,19]. And while withering does induce a general decrease of

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polyphenols, such as anthocyanins, flavanols, procyanidins and glycoside flavonols [19,20], an increment of other polyphenols, such as trans-resveratrol (3,5,4′-trihydroxystilbene), taxifolin, quercetin, some methoxylated flavanones and acylated anthocyanins, was observed [9,19,20,21].

Regarding polyphenols, the synthesis of stilbenes produced as a response to abiotic cell stress (i.e., *trans*-resveratrol, viniferins, *cis*- and *trans*-piceid) is crucial for the development of the nutraceutical properties of grapes and wine [15]. In particular, many studies have demonstrated the antioxidant, anti-inflammatory, and cardioprotective properties of *trans*-resveratrol, alongside its ability to inhibit platelet aggregation and antimutagenic and antiproliferative effects.

Likewise, piceatannol (3,4,3',5'-tetrahydroxy-*trans*-stilbene) has been shown to block LMP2A, a viral protein-tyrosine kinase associated with leukaemia, non-Hodgkin's lymphoma, and other diseases associated with the EBV virus, which also acts on human melanoma cells [22]. The withering process also affects the volatile organic compounds (VOCs) and their glycoside precursors in grapes [23,24]. Indeed, following harvest, the dehydrated berry VOCs increase not only as a concentration effect but also as a consequence of an active process [25]. It was observed that a dehydration temperature of above 30°C drastically reduces the share of primary and varietal aroma compounds (e.g., terpenes and norisoprenoids), which instead are maintained and concentrated slowly at temperatures of around 20°C and up to 40% of berry water loss [26]. Studies on aroma composition carried out during the dehydration of Pinot noir [27] and Pedro Ximenez [28] grapes showed that the aroma profiles are affected both qualitatively and quantitatively due to the development of new odorous compounds [23]. These new compounds confer notes of jam, raisins, plums, morello cherries, and almond. The main grape aroma compounds of Amarone wines belong to chemical classes of C_{13}-norisoprenoids, terpenes, benzenoids, furans, and aliphatic alcohols [29].

Current Amarone withering techniques require an average of 2.5 months (as stated by law, grape crushing cannot be done before 1st December, MIIPAF; Reg, n° 558533/2019) [30], yielding a final product that is richer in sugars, polyphenols, and aromatic compounds. In the past, the Amarone withering was carried out by setting the grapes on particular wooden trays known as arelle, inside dehydration chambers not provided with any climatic control; nowadays, air humidity and ventilation can easily be controlled (no extra temperature conditioning is allowed by the Amarone production regulations) [30]. Indeed, artificial environment conditioning provides faster withering and allows to achieve healthy dried grapes, though, it seems to be stressful for the berries [7]. Studies focusing on post-harvest events have confirmed that functional biochemical and molecular changes continue within the berries reflecting the environmental conditions of post-harvest ripening and/or senescence [18,31,32]. Moreover, Ferrarini [33] conducted a study to investigate the best timing for harvest and withering to achieve a more aromatic Amarone wine.

The primary purpose of this work is to compare natural and artificial Corvina withering processes to assess their effect on dried grape chemical composition and peculiar wine organoleptic evaluations. This aspect is particularly interesting for the few wineries that still adopt the traditional drying synthesis to pursue a true-to-varietal character and vintage-related wine.

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2. Materials and Methods

2.1 Experimental setup

The experimental data were collected during 2016 and 2017. The vineyard was selected the in Valpolicella classic area (Novare locality in the Negrar municipality, Verona) owned by a worldwide renowned Amarone wine producer, Bertani. The vine training system adopted was Guyot with a plant density of 4,444 vines per hectare; the variety raised was Corvina, clone ISV-CV48 grafted onto Kober 5 bb, grown predominantly in calcareous soil. Corvina was chosen as it is commonly the main variety for Amarone wine production. Harvest, following local tradition, was scheduled to take place ten days before the standard harvest period for Valpolicella fresh wine, occurring on 17 September in 2016 and on 6 September in 2017. In order to preserve their healthy status, the carefully selected grapes were placed in plastic trays – in the case of the modern warehouse drying process – and in the traditional arelle (wooden trays) to wither the grapes under natural conditions. In modern warehouses, air dehumidifiers maintained the air humidity between 60 and 70% (optimal range for grape dehydration) and fans were used to provide airflow through the drying trays. Instead, the natural and traditional dehydration process took place without any air humidity control system or mechanical airflow: the warehouse supervisor was in charge of opening or closing the windows to enable the high-moisture air to escape and the dry, fresh air to flow inside the drying chamber. In this study, the two withering chambers (C: controlled, NC: not-controlled environment) were close to each other on the winery premises (Bertani), though the NC chamber stood closer to the bottom of the hill where air movement is enhanced by the natural breeze which assists the discharge of the humid air from the chamber through the open windows. Berry chemical composition was analysed at four different stages: i) T0 fresh berry, ii) -10% of berry water loss (WL), iii) -20% WL, iv) -30% WL.

2.2 Withering environmental conditions

The climatic conditions in both environments, C and NC, were measured. Specifically, temperature and humidity were measured using air sensors (Lascar Electronics EL – USB - 2) located in each environment of study: four sensors per drying chamber, two in contact with the withering grapes and two 50 cm above the grapes. The daily values recorded were the average of the measurements collected every 15 minutes. The outdoor climatic conditions were measured by the weather station belonging to Avepa (Avepa.it) located in the Negrar area. In 2016, due to contingent technical problems during data download, the initial climatic records (first ten days) collected in the withering chamber were lost.

2.3 Kinetics of withering and grape samples

The kinetics of the withering process was evaluated by measuring the post-harvest weight loss; in the NC and C treatments, where values are expressed as the percentage (%) of weight loss during withering. Specifically, data were taken by weighing two arella trays (about 250 kg of grapes) in the case of the natural environment treatment and two pallets of trays (around 200 kg of grapes) as regards to the controlled environment. The arella trays and the pallets were chosen from two representative positions within the drying chambers. As last consideration, we report that these values does not come from replicates, since they represent the whole weight of a tray (with hundred bunches), only distinguished in C and NC.

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For both drying processes (C and NC), at harvest time (T0) and during withering (-10% WL, -20% WL, and -30%WL), three samples of grapes weighing 1.0 kg each were collected to determine the berry chemical composition. The samples were obtained from several portions of the grape bunches stored on the selected trays and pellets.

2.4 Accumulation of sugars and acids

The total soluble sugars in the berries were quantified using an ATAGO PR-32 digital refractometer (0-32%) and expressed in Total Soluble Sugars (TSS), Brix degree; in the same samples, the organic acid profile of the berries (specifically tartaric and malic acids, expressed in g/L), was determined by high-pressure liquid chromatography (HPLC Agilent 1220 infinity). The samples for HPLC were prepared taking 250 µL of grape must diluted 1:50 with distilled water. The sample was then filtered through a 0.2 µm filter and analysed. The grape must samples were prepared by pressing three subsamples of 250 g of berries.

2.5 Accumulation of colouring substances

Among the berry skin flavonoids, those belonging to the anthocyanins class are the most interesting in terms of direct feedback on the characteristics of a wine. Following the method described by Di Stefano [11], in 30-berry samples per withering process, the total flavonoids and total anthocyanins were quantified. The values were expressed in mg/kg of grapes.

2.6 Gas chromatography/mass spectrometry analysis of volatile compounds

The glycosidic bound aroma compounds were analysed by GC/MS after enzymatic hydrolysis according to the methods by Di Stefano (1991) e Mateo et al. and summarized by Flamini and Traldi [34-36]. The skins of 100 berries were separated from the pulp and extracted with 35 mL of methanol for 4 h in the dark. The extract was homogenized using an Ultra-Turrax and centrifuged. The volume of the supernatant was adjusted to 250 mL with water, and the solution was treated with 2 g of insoluble poly(vinylpyrrolidone) (PVP) to reduce the polyphenolic content. Also, the pulp was homogenized and centrifuged, and the volume was adjusted to 250 mL. The solutions were treated with 75 mg of pectolytic enzyme for 4 h at room temperature to release the free aroma compounds. Following the addition of 1-heptanol as internal standard, the aglycones were isolated using an SPE 10 g C_{18} cartridge (Waters Corporation, Milford, MA) previously activated by successive passages of 30 mL dichloromethane, 30 mL methanol, and 30 mL water. Salts, sugars, and other polar compounds were removed by washing the cartridge with 50 mL of water, and the fraction containing the free compounds was recovered using 50 mL of dichloromethane. The solution was concentrated to 2–3 mL by distillation using a 40 cm length Vigreux column then to 200 µL under a nitrogen flow prior to perform GC/MS analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using a 6850 gas chromatography system by Agilent Technologies (Santa Clara, CA, US), fitted with a fused silica HP-INNOWax polyethylene glycol capillary column (30 m × 0.25 mm, 0.25 µm inner diameter) (Agilent Technologies, Santa Clara, CA, U.S.A.), coupled with HP 5975C mass spectrometer and 7693A automatic liquid sampler injector (Agilent Technologies, Santa Clara, CA, U.S.A.). Compound identification was performed using the NIST Mass Spectral Libraries Database (rev08) and the in-house CREA-VE database. Compound content was calculated as µg IS/kg of dried grape (d.g.).

2.7 Analysis of stilbene compounds

Sample preparation for HPLC/DAD analysis was performed using the methods suggested by Bavaresco et al. and Repetto et al. modified to meet the study's goals [37,38]. The skins of 20 berries were homogenized with 45 mL of methanol using a T25 Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and kept under stirring for 20 min in the dark at room temperature. The extract was centrifuged, and to the supernatant of 200 µL of trans-4-hydroxystilbene, 9.6 mg/L solution in methanol was added as internal standard. The solution was evaporated to dryness under vacuum at 40 °C, and the residue was suspended in 10 mL of HCl 10^{-3} M in water. After the addition of 5 g of NaCl, the solution was extracted 3×5 mL with ethyl acetate. Ethyl acetate extract was evaporated to dryness under vacuum at 40°C and reconstituted with 4 mL of methanol/aqueous H_3PO_4 $5{\cdot}10^{-3}$ M 30:70 (v/v) solution. Finally, the sample was filtered through a 0.22 µm PTFE filter and analysed by High Pressure Liquid Chromatography (HPLC). The HPLC system used was a 1220 Infinity G4290B coupled with a Gilson 170 G1315A DAD-UV detector (Agilent Technologies, Santa Clara, CA, USA) equipped with an RP C18 column (ODS Hypersil® 200 mm×2.1 mm i.d., 5 µm, Thermo Hypersil-Keystone). Elution was performed using a binary solvent composed of A) methanol and B) H_3PO_4 $5{\cdot}10^{-3}$ M in water and the following gradient program: from 25 to 30% of A in 30 min, from 30 to 35% of A in 20 min, from 35% to 75% of A in 10 min, from 75% to 85% of A in 15, from 85% to 25% of A in 5 min and isocratic for 20 min (flow rate 0.25 mL/min, injection volume 10 ◎L). *trans*-Piceid, *trans*-resveratrol, *trans*-piceatannol, *trans*-ε-viniferin and δ-viniferin were quantified by recording the chromatograms at 307 nm, *cis*-piceid at 285 nm. The UV–Vis spectra in the 200–400 nm range were also recorded. Standard of *trans*-resveratrol, piceatannol, *trans*-piceid and *trans*-4-hydroxystilbene were purchased from Sigma–Aldrich (Milan, Italy); δ-viniferin was provided by CT Chrom (Marly, Switzerland). *cis*-Piceid was produced by photoisomerization of trans isomer; *trans*-ε-viniferin was extracted from a lignified vine cane of Gamaret following the method described by Pezet et al. [39].

Qualitative profiles of stilbenes were characterized by using an Ultra-High Performance Liquid Chromatography (UHPLC) Agilent 1290 Infinity system coupled with Agilent 1290 Infinity Autosampler (G4226A) and Agilent 6540 accurate-mass Quadrupole-Time of Flight (QTOF) Mass Spectrometer (nominal resolution 40.000) equipped with Dual Agilent Jet Stream Ionization source (Agilent Technologies, Santa Clara, CA) and the methods previously described [40-41].

2.8 Winemaking and wine tastings

The winemaking was made via micro-vinification carried out at the vinification centre of the cooperative nurseries of Rauscedo (VCR) where about 150/180 kg of dried grapes were used for each vinification obtaining about 60 L of vine, as previously described by Alessandrini et al. [42]. The vinification process started with the collection of withered grapes when the 30% weight loss was reached, after that the cluster were crushed and pressed to obtain the must. The must was put in a fermenter with the addition of 10 g/hl of potassium metabisulfite, 0.3 g/t of enzymes (Lysis first, Oenofrance®), 5 g/hl of ascorbic acid and 0.5 g/t of tannin. Afterwards, a sample of must has been picked up and inoculated with 20 g/hl of selected yeast (Zymaflore® FX10 and F83, Laffort®), with the addition of 2 g/t of Vivactiv® Premier (Oenofrance®), 2 g/t of Vivactiv® Arome (Oenofrance®) and 2 g/t of Philya cys (Oenofrance®), and then remixed incorporating it in the fermenter. After 48h from the inoculation, to favour malolactic fermentation, 1 g/hl of Oenococcus oeni bacteria (Lalvin VP41®, Lallemand®) has been added. Once a determinate alcoholic level has been reached in the must, a high-alcohol tolerant yeast at a concentration of 30 g/hl was added (Lalvin 2226®, Lallemand®), also adding 15 g/hl of

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mineral nutrients (Vivactiv® Performance, Oenofrance®). At the end of the fermentation, a soft pressing of grapes was performed and 5 g/hl of tannin (Tannino Perfect®), 5 g/hl of potassium metabisulfite, 15 g/hl of Vivactiv® Control (Oenofrance®) and 10 g/hl of Philya LF (Oenofrance®) was added. Successively, the malolactic fermentation was favoured bringing the must at 21 °C after a decanting of it 3 days later. At the end of the malolactic fermentation another decanting was performed and 2,5 g/hl of enzyme (Lallzyme MMX™) was added. The temperature was reduced at 10 °C and for four weeks a batonnage was performed twice per week. At the end of the batonnage, another decanting operation was performed, and the temperature was reduced at 10 °C for two weeks. At the end of this period, the wine has been filtered with cardboard filters, stabilised with Cryokappa (Oenofrance®), and brought at -5 °C for 3 weeks. After that, the wine was filtered again with a, firstly, 1 μm, and secondly, 0.45 μm, candle filter and bottled.

The tasting analysis was carried out in Conegliano (TV) at CREA-VE by a test panel made up of 10 expert judges plus a panel leader. For the quantitative evaluation of the intensity of attributes (olfactory, gustatory-tactile and retro-olfactory), Quantitative Descriptive Analysis was used [43] with the help of a question sheet providing discrete scale responses with intervals from 1 to 9. For each of the three attributes, the relative differences between wines were analysed and confirmed, submitting the judgements to statistical analysis using the ANOVA method (F-test p-values <0.0001;[44]). The 2016 and 2017 wines were tasted one year following vinification; the wines were stored in stainless steel tanks in a temperature-conditioned cellar at 12-14 °C.

2.9 Statistical analysis

One-way analysis of variance was performed using "R" freeware. Statistical analyse to determine significant differences between treatment means was carried out using the Student-Newman-Keuls test ($p \leq 0.05$).

3. Results

3.1 Temperature - humidity mean and kinetics of withering (WL) in two years of study, in C and NC environment

In order to describe the kinetics of withering, namely the velocity of WL and sugar concentration, using climate data collected inside and outside the withering chambers, mean temperature and mean humidity per day were measured throughout the two test years. The weather conditions of the test locality (Negrar) were also taken into account to understand the difference between treatments inside and outside the chambers. In 2016 (Fig. 1), the C chamber's average temperature values were higher than in the NC one. On average, the temperatures in the chamber conducted in NC were closest to the outside ones. The relative humidity was comparable in the two conditions C and NC though C presented a slightly lower level than NC, mainly at the beginning of the process when the WL from the berries was higher, while the external treatment followed the normal trend of the daily weather conditions.

As for 2017 (Fig. 2), the average temperature was again higher in C and lower in NC, and in this case, NC was also closest to the external one. As air humidity was controlled in the C withering chamber, this parameter was lower in C conditions and higher in NC conditions, while the external humidity level was lower or in the middle between that of the NC and C conditions and presented more abrupt variability.

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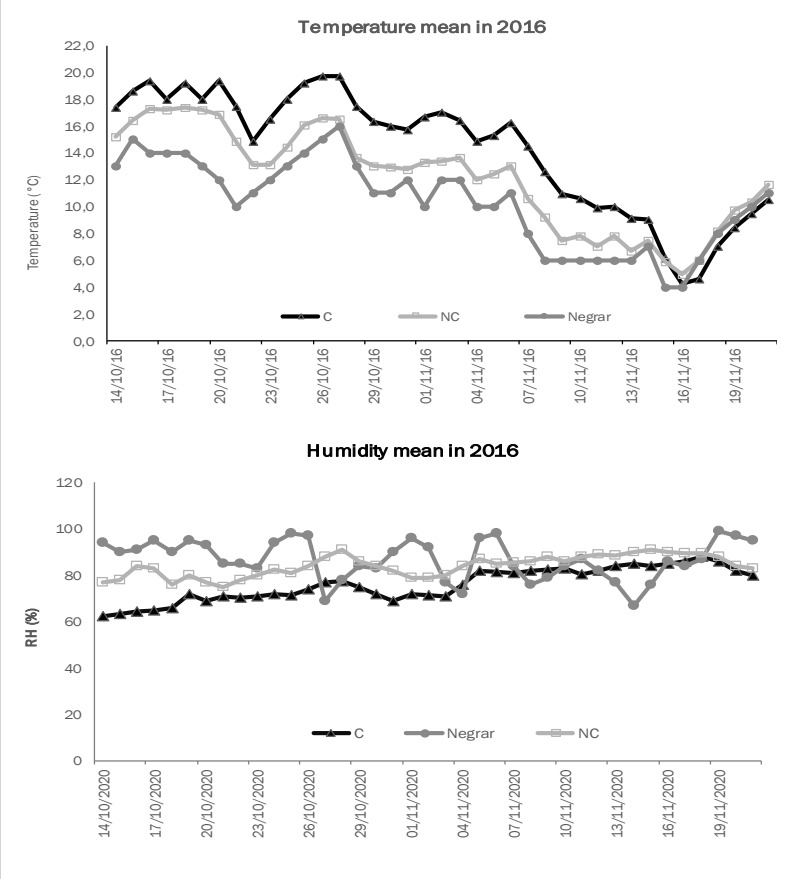

Figure 1 Daily mean air temperature and humidity in controlled (C) and not-controlled (NC) chambers and outside environment (Negrar) in 2016

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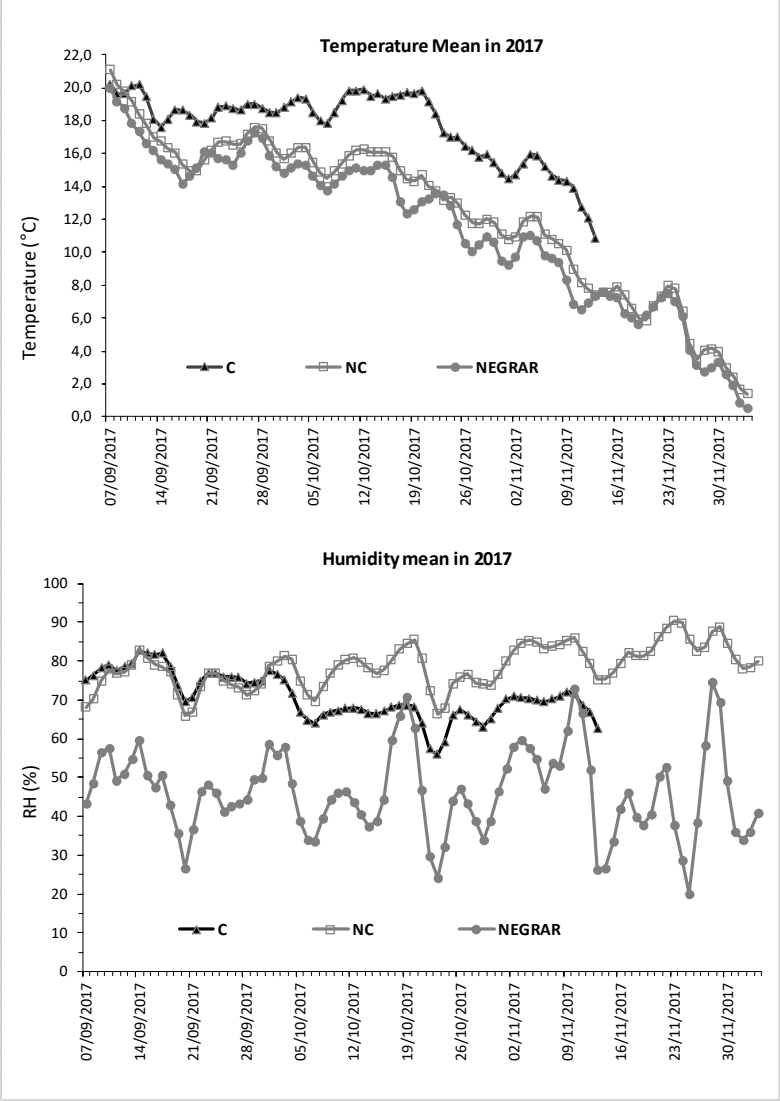

Figure 2 Daily mean air temperature and humidity in controlled (C) and not-controlled (NC) chambers and outside environment (Negrar) in 2017

As previously reported, T and H are fundamental for the kinetics of WL. Hence, measurements of the kinetics of withering were taken and are indicated as a percentage of weight loss during the period of withering, starting from time 0 (grape set – aside) to -30% WL (Fig. 3). 2016 evidenced a faster withering in C than NC throughout the entire period, and -30% WL was reached ± 17 days earlier in C than in NC. In 2017 there were no significant differences between treatments during the first period of withering (-20% WL), but at the end, -30% WL was once again reached earlier in C (± 14 days) than in NC.

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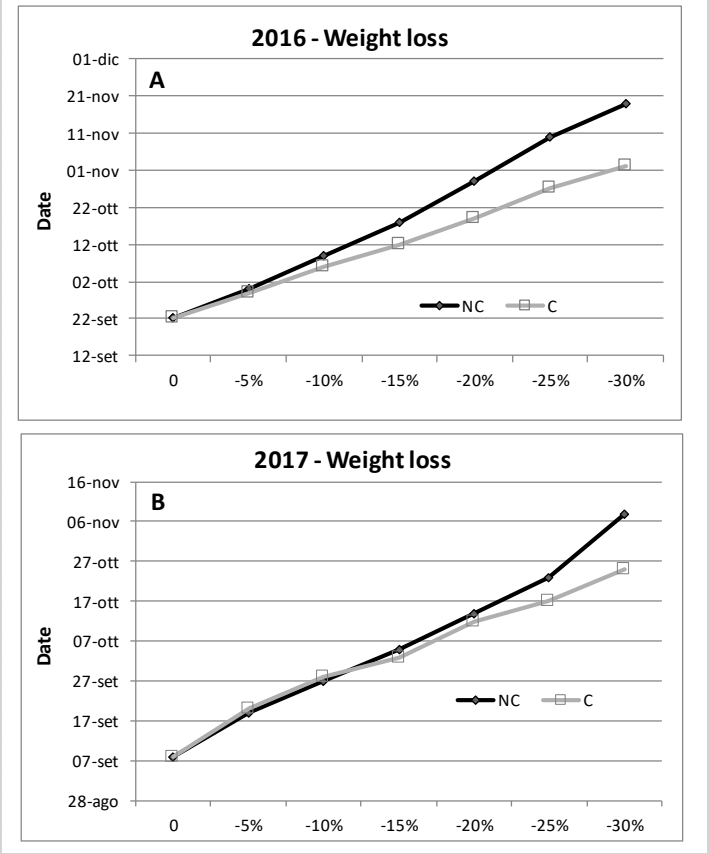

Figure 3 Kinetics of withering: WL in 2016 (A) and 2017 (B)

3.2 Sugar accumulation trend

During withering, generally, a concentration of sugars occurs due to the berry water loss. The two test years (Fig. 4) evidenced a gradual and constant accumulation of sugars with a similar trend between C and NC. In 2016 the grape set – aside started on 17 September; in this year, NC grapes showed a slower accumulation of sugars than C, but at the end (-30% WL), there were no differences in sugar concentration between treatments. In 2017 the grape set – aside started on 6 September, and the trend of sugar enrichment was similar. In this year, in NC there were more degrees than in C, during all the periods. The berry sugar content was 30.2 and 30.8 Brix degree in 2016, 27.0 and 28.6 in 2017, for the C and NC conditions, respectively. At -30% WL, the NC treatment showed a higher degree than C, explicitly highlighted in 2017. In 2016 the outside RH was higher than in 2017; as a consequence, C conditions showed markedly lower room RH compared to NC, which can explain the fact that C sugar accumulation was faster in C conditions. In 2017 the external weather conditions were different compared to those in 2016. Indeed, the air RH was much lower, and it was easier to control the room RH even in the NC treatment (see the similar level in RH between C and Nc reported in fig. 2), which can explain the similar trend in sugar accumulation. The last two considerations refer to the final sugar enrichment, which tends to be higher in NC conditions, and to the fact that the first stage of sugar enrichment tends to be more active in C conditions.

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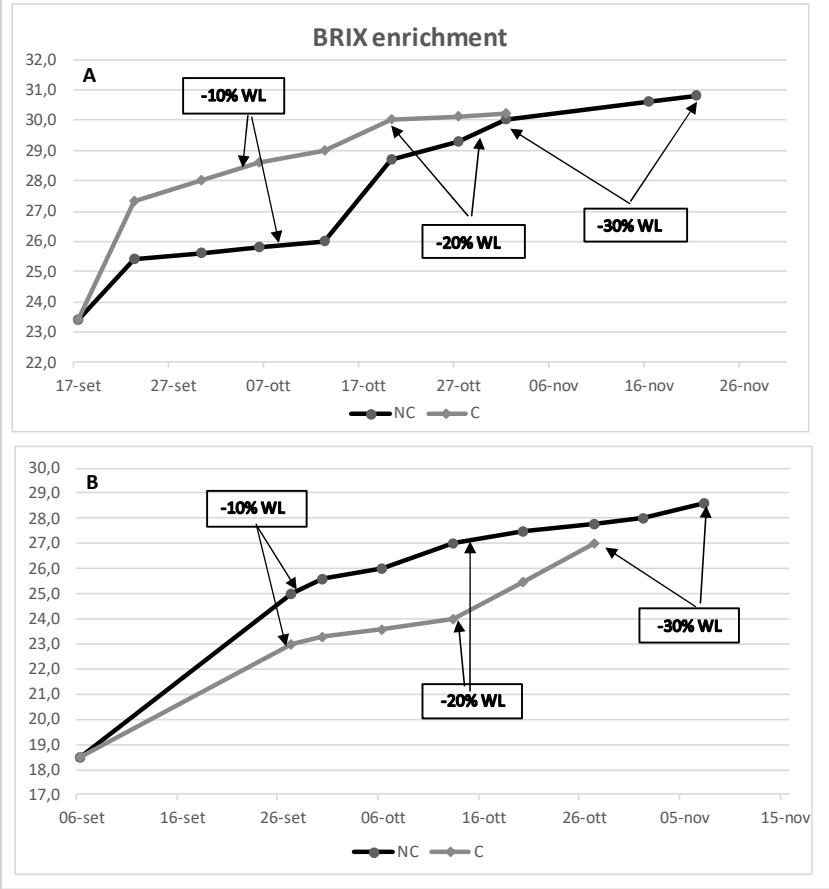

Figure 4 Brix degree enrichment in 2016 (A) and 2017 (B). Arrows means different WL values

3.3 Accumulation of acid trend and content in specific acid compounds

Because of water evaporation and WL, acids tend to be accumulated during withering. The analysis of the two tested years reported an almost specular behaviour in terms of total acidity (Fig. 5). In 2016 the trend was similar in both treatments, with only a minor decrease in NC at the beginning of the process, but the acidity in NC was slightly above C with no significant differences during the final stage of the process. The trend was confirmed in 2017, where acidity levels were very close during the whole period of the drying process, except for the last stage when the NC environment displayed higher acidy with a final significant difference. Moreover, regarding the components of acidity (Fig. 6), in 2016, the level of tartaric acid in NC remained the same, but during the final stage (-30% WL), it increased, while in C, there were no significant differences throughout the WL process. As for malic acid, there were no differences between C and NC at the different stages of WL. In 2017, at the different stages of WL, tartaric acid was consistently higher in NC than in C. The highest content was found at -30% WL for NC. Regarding malic acid, the concentration was higher in the second part of the drying process, with no difference between the two treatments.

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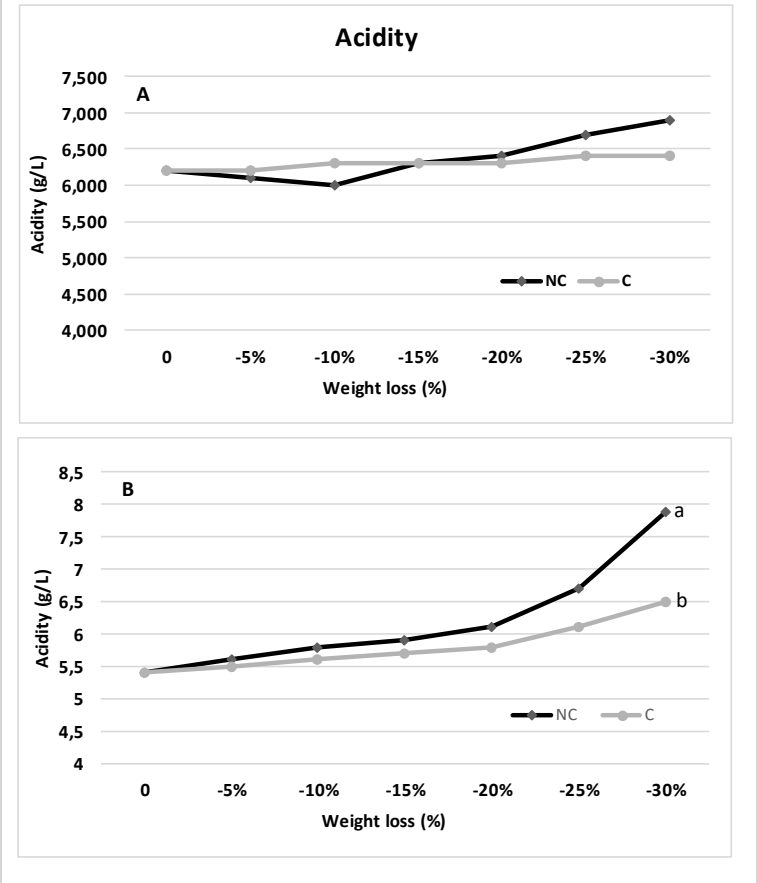

Figure 5 Total acidity in 2016 (A) and 2017 (B) at harvest date and different WL stages. Values indicated with different letters were significantly different ($p<0.05$)

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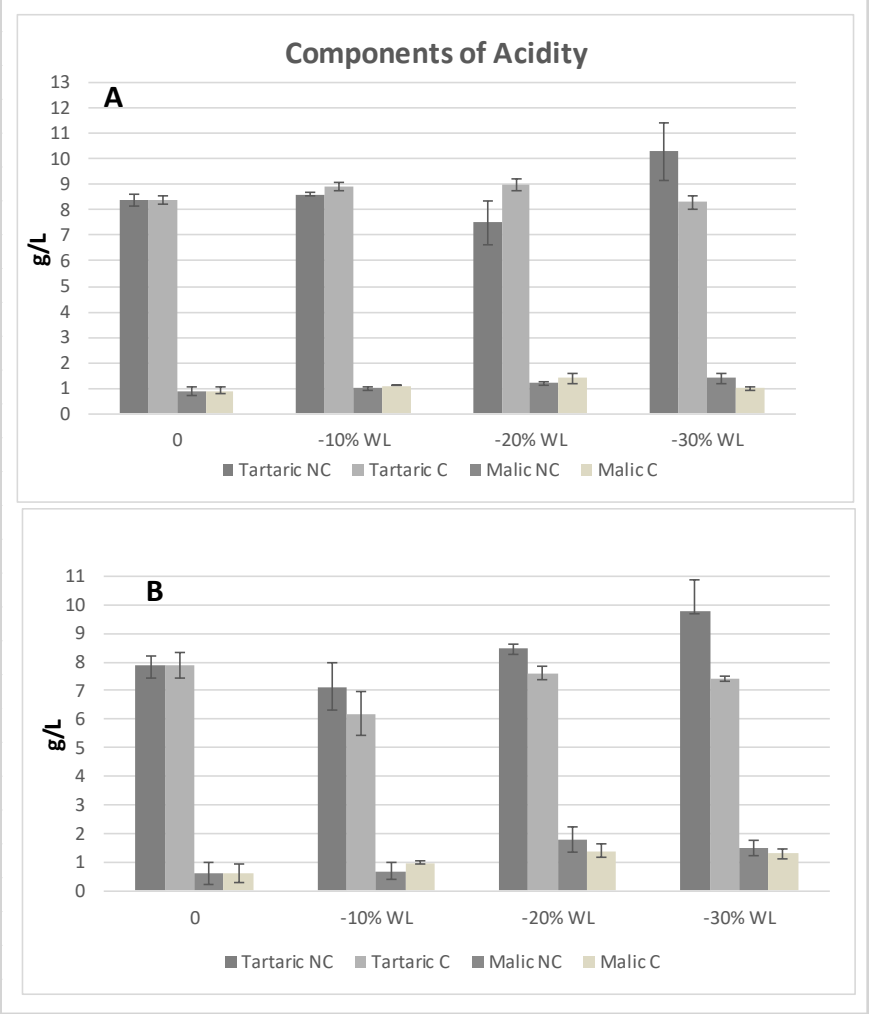

Figure 6 Components of total acidity in 2016 (A) and 2017 (B). Vertical bars indicate Standard Deviation. No statistical differences were found

3.4 Colour substances accumulation trend

Different climate conditions inside the withering chambers may affect the berry colour compounds. At the end of the process, the content in total polyphenols consistently predominated in NC in the two tested years of study (Fig 7 and 8). In both years, total polyphenols were higher in NC at -30% WL (2586 mg/kg berries t.q. in C vs 2933 in NC in 2016, 1550 in C vs 2.000 in NC in 2017). Specifically, in 2016, the accumulation trend was similar in C and NC, furthermore, the differences between the two treatments were significant only at -30% WL ($p \leq 0.05$). In 2017, the trend was again similar in C and NC but, the differences became highly significant starting from the 10% WL stage. As for anthocyanins concentration, in 2016, there were no differences during the initial stage (-10% WL), whilst at the end of the drying period, NC grapes had 11% more anthocyanins than C grapes (614 mg/kg berries t.q. and 549 for C and NC, respectively). In the second year (2017), there was a considerably higher and more meaningful content in NC than C at every stage of WL (at -30% WL: 242 mg/kg berries t.q. in C vs 380 mg/kg in NC, + 36%).

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The anthocyanin profile of Corvina is constituted by malvidin, peonidin, petunidin, delphinidin, and cyanidin monoglucosides and their acyl derivatives where malvidin-3-*O*-monoglucoside is the main compound. Findings did not show relevant differences between the two processes in terms of changes regarding single anthocyanins (data not showed).

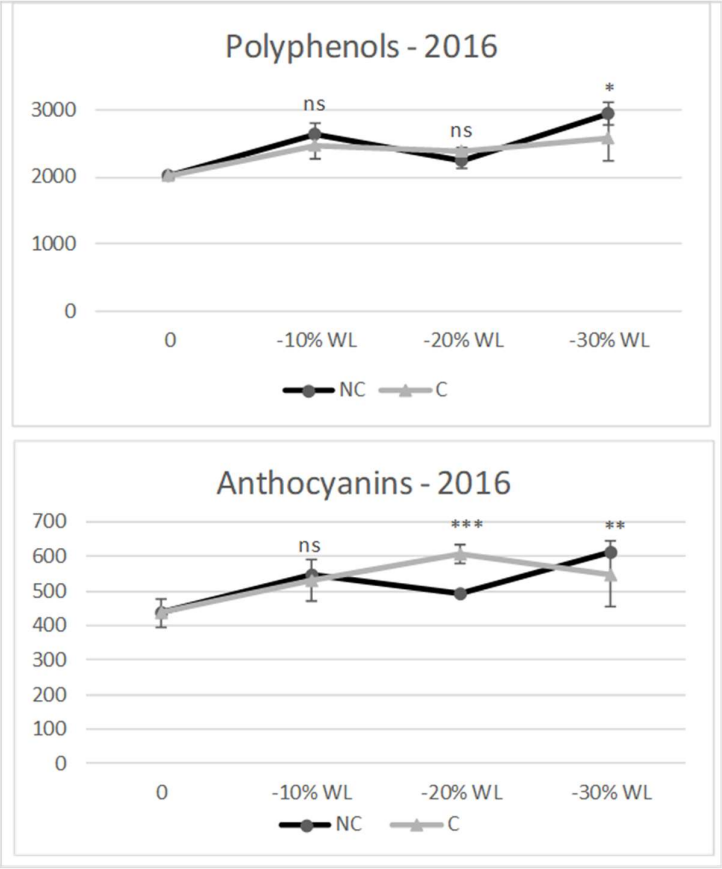

Figure 7 Content in polyphenols and anthocyanins in 2016. Data are the mean of three berry samples. *; **; *** indicate a significant difference ($p \leq 0.05, 0.01, 0.001$) between C and NC treatment. Vertical bars indicate standard deviation

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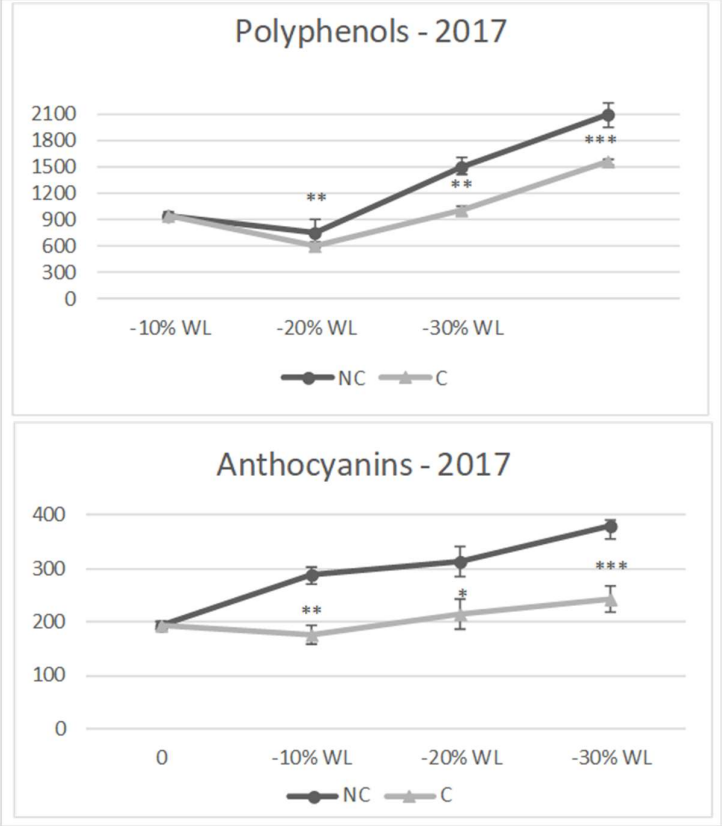

Figure 8 Content in polyphenols and anthocyanins in 2017. *; **; *** indicate a significant difference ($p \leq 0.05, 0.01, 0.001$) between C and NC treatment. Vertical bars indicate standard deviation

3.5 Synthesis of stilbenes

Withering induces specific chemical changes in the berry, and most of them involve secondary metabolites. In particular, the biosynthesis of stilbenes is promoted by abiotic stress – e.g., cell dehydration – and these compounds determine the nutraceutical properties of the grapes and wines. The profile of stilbenes in Corvina grape during withering was characterized by liquid chromatography/quadrupole-time of flight mass spectrometry (UHPLC/QTOF), and the 19 compounds reported in supplemental material Table S1 were identified. They included *trans*-resveratrol, *cis* and *trans*-piceid, piceatannol, *E* and *Z*-astringin, pallidol, four viniferins (*E*- and Z-ε-viniferin, *Z*-ω-viniferin, δ-viniferin) and another resveratrol dimer, caraphenol, pallidol-glucoside, ⌾-viniferin, two resveratrol trimers (*E*- and *Z*-miyabenol C) and two resveratrol tetramers. HPLC/DAD quantification of the compounds showing the higher signals, where the standards were available, was performed. Data in Figure 9 show an accumulation of *trans*-resveratrol and total stilbenes in the grapes during both processes carried out in two years.

In 2016, a constant increase in *trans*-resveratrol and total stilbenes were observed throughout both withering processes. In particular, until the -20% WL stage, stilbenes were higher in the NC samples. In the last stage (between -20-30% WL), the level of stilbenes in NC samples remained constant, while in the C samples, it continued to

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increase, reaching the other process. In the latter year, the trend was different: at -10% WL, both processes induced a low stilbene increase, and the levels remained similar until the -20% WL stage. A sudden increase in the synthesis of stilbenes was observed during the last stage in both processes, as well as higher accumulation in the C samples.

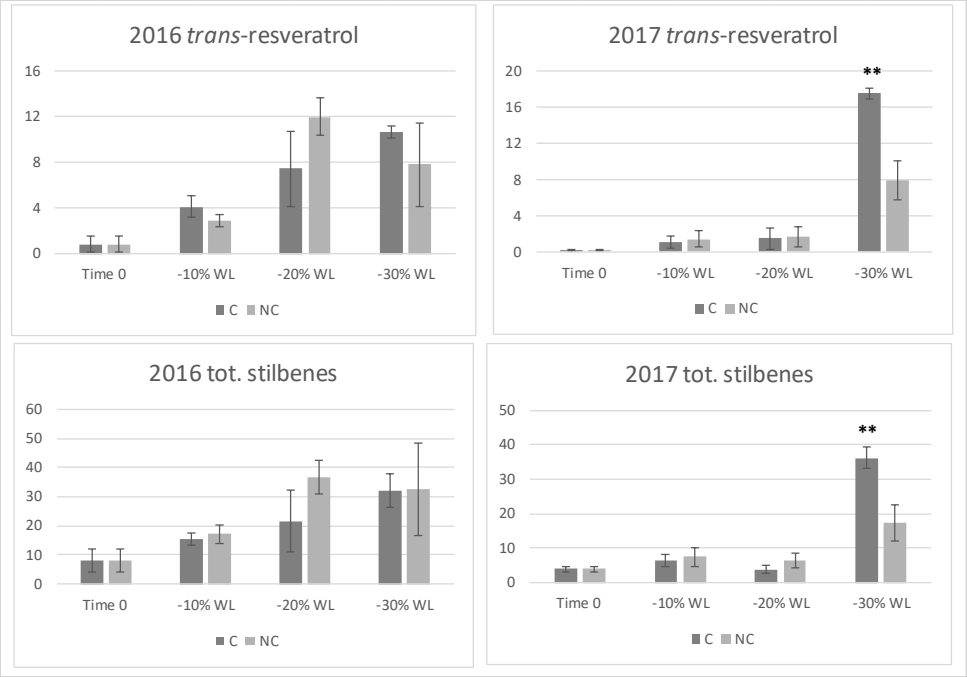

Figure 9. Levels of *trans*-resveratrol and total stilbenes calculated as mg *trans*-resveratrol/kg d.g. (dried grape) during two withering processes carried out in the two years. C, dehydration in RH and WS controlled warehouse; NC, dehydration carried out in the not-controlled environment. ** indicate a significant difference p≤0.01 between two processes. Vertical bars show the standard deviation of 3 data.

3.6 Accumulation of glycoside aroma precursors in berry

Glycoside aroma precursors identified in Corvina grape belong to different chemical classes of compounds, and the sums of their contents in the grape berry expressed as μg IS/kg d.g., are reported in Table 1. Tables S2 and S3 show the contents of the single compounds in the two years of study.

Glycoside aliphatic alcohols identified were 1-butanol, 3-methyl-1-butanol, 3-methyl-2-butene-1-ol, 3-methyl-3-butene-1-ol, 1-hexanol, *E*- and *Z*-3-hexen-1-ol, 2-butoxyethanol, and 2-hexenol. In 2016, C samples had higher levels of total aliphatic alcohols, but a significant difference (p≤0.05) between C and NC samples was found only at the highest content at -20% WL stage. In 2017, the total content was higher in NC with a significant difference at -20% and -30% WL, the highest aliphatic alcohols content was reached at -30% WL in both C and NC.

Glycosides of the C_6-aldehydes hexanal and 2-hexenal, were identified, these two compounds confer to the wines herbaceous/grassy notes. Their content was higher in

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NC in both years but, a significant difference among C and NC thesis, was found at -10% and -30% WL in 2016 and at -20/-30 WL in 2017. In both years, the highest concentration was achieved at -20% WL.

Fourteen glycoside monoterpenes were identified in Corvina grape which include *cis*- and *trans*-furanlinalool oxide, *trans*-pyranlinalool oxide, linalool, α-terpineol, nerol, geraniol, diendiol I, 8-hydroxylinalool *cis* and *trans*, hydroxygeraniol, 2-exo-2-hydroxycineol, 7-hydroxy-α-terpineol and geranic acid. The highest levels were found for geraniol and 7-hydroxy-α-terpineol. In general, the aglycones are characterized by low sensory thresholds and confer floral or citrus notes to the wines. In 2016, monoterpene total content was higher in C at all withering stages, a significant difference among C and NC was found at -10% and -20% WL. As expected, water loss increased the monoterpene concentration in the berry and the highest content was found thereabout at -30% WL in both C and NC, but no statistical difference among the two processes was found. Indeed, in 2017 the content was higher in NC at all withering stages and significant difference between two processes was found at -20% and -30% WL. In general, the highest concentration in the berry were reached in the last withering stages, showing that the process does not promote degradation processes of these glycoside precursors.

C_{13}-Norisoprenoids are correlated to floral/spicy notes developed in red wines specially during aging. Glycoside norisoprenoids identified in Corvina grape are 3-hydroxy-β-damascenone, 3-oxo-α-ionol, 3,9-dihydroxy-megastigma-5-ene, 3-hydroxy-7,8-dihydro-α-ionol and vomifoliol. Potentially, they are precursors of volatile compounds which contribute by conferring positive notes to the aroma of wines, such as β-damascone (fruity note), β-damascenone and 3-oxo-α-ionone (floral, tobacco). In 2016, total content of norisoprenoids at -10% WL was higher in C, instead at -20% and -30% WL the two processes showed similar levels. In 2017, the content was higher in NC samples at all withering stages with significant difference between the processes at -20% and -30% WL. In this year, the highest concentration of norisoprenoids was found at -30% WL in NC, instead in C at -20% WL.

Glycoside precursors of sixteen compounds belonging to the chemical class of benzenoid derivatives were identified: benzaldehyde (almond note), acetophenone, methyl salicylate, guaiacol, benzyl alcohol, ß-phenylethanol (rose), eugenol (clove), 4-vinylguaiacol, 4-vinylphenol, syringol, vanillin (vanilla), methyl vanillate, acetovanillone, 4-hydroxybenzeneethanol, vanillic and homovanillic alcohols. In 2016, their total content during the entire process was higher in NC, and a significant difference between the two processes was found at -30% WL. The highest concentration was reached at -30% WL in NC and at -20% WL in C samples. The second year of the study confirmed the higher contents in NC during withering, with a significant difference at -20% and -30% WL. In both processes, the highest accumulation of benzenoids was reached at -30% WL.

Moreover, regarding the total content of aromatic compounds at every stage, in 2016, at -10% and -20% WL the content was almost the same in both C and NC, while, at -30% WL, the content was higher in NC than in C, with a statistical difference between treatments. In 2017 there were more aromatic substances at every stage in NC than in C. Furthermore, at -20 and -30% WL, statistical differences were found between treatments, with the already reported higher content in NC.

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	T0	-10% WL		-20% WL		-30% WL	
		(μg/Kg d.g.)					
2016		C	NC	C	NC	C	NC
aliphatic alcohols	299	444	335	774**	570	575	535
C_6-aldehydes	27	43	55*	41	46	21	35*
monoterpenes	553	805*	692	862	877	857	813
C_{13}-norisoprenoids	785	1103*	914	1149	1177	1126	1144
benzenoids	2401	4637	4728	5286	5472	4611	5805**
Total	4065	7033	6724	8112	8142	7190	8332**
2017							
aliphatic alcohols	485	538	460	456	552*	641	820*
C_6-aldehydes	58	55	70	120	138*	65	104**
monoterpenes	738	625	829	640	912**	698	895*
C_{13}-norisoprenoids	1074	961	1462*	1485	1968**	1164	2702**
benzenoids	3257	3258	3696	4430	6156**	5937	8698***
Total	5612	5437	6517	7131	9726**	7865	12400***

Table 1. Glycoside aroma precursors in Corvina grape during withering. C, in-chamber dehydration under controlled conditions of relative humidity (RH) and wind speed (WS); NC, dehydration carried out in not-controlled environment. Contents are expressed as μg 1-heptanol (IS)/kg d.g. (dried grape). *, **, ***: significant difference between the C and NC process ($p \leq 0.05$, 0.01, and 0.001, respectively). Contents of singular compounds in the two years of study are reported in Tables S2 and S3 supplemental material.

3.7 Sensory analysis of the resulting wines

Results of the tastings carried out in 2016 and 2017 are reported in Figure 10. The 2016 vintage showed a great difference between the two wines. NC generally resulted richer and more interesting regarding the olfactory scents, with low herbaceous notes and higher scores for smoothness. In the mouth, the NC wine was smoother and better appreciated in terms of finesse, taste balance and full body. The C wine resulted achieving less score compared to NC, which displayed more character (see full-bodied, structure) and elegance (see finesse and olfactory quality less herbaceous)

As for the 2017 vintage, the NC wines confirmed the higher pleasantness with a peak in ripe fruit combined with spicy notes. Overall, the NC wine was more harmonious with good scores in terms of taste balance and finesse.

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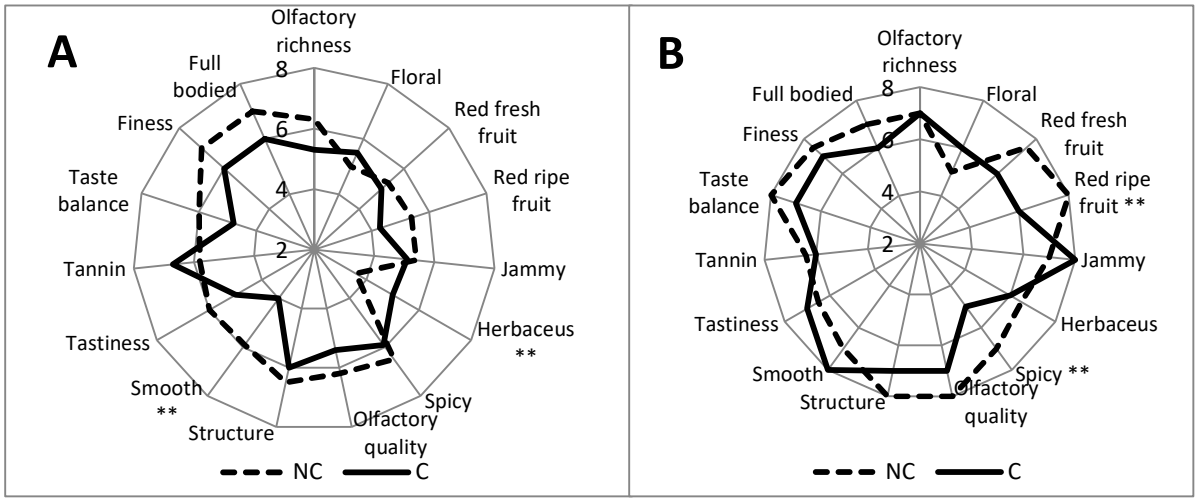

Figure 10 Results of tasting for wines in 2016 (A) and 2017 (B) years. *; **; *** indicate a significant difference ($p \leq 0.05, 0.01, 0.001$) between C and NC treatment.

4. Discussion

Post-harvest dehydration has a significant impact on berry physiology and metabolism, directly influencing physical, metabolic and metabolomics aspects. For the production of Amarone wine – obtained mainly from the Corvina variety – the grapes are harvested and stored in dehydration chambers and then vinified. This specific process, which is employed to obtain reinforced wines rich in alcohol, anthocyanins, flavour and structure, seems particularly suitable for the Corvina variety – characterized by thick skin with compact layers that slow down the dehydration process. Lower water stress plays an important role in terms of transcriptional modulation and metabolic change [32,44]. The process could be carried out either in dehydration chambers under natural environmental conditions, where the airflow from the outside towards the inside is promoted through careful window management, or in chambers with controlled environmental parameters (RH and wind speed). As known, dehydration of berries post-harvest impacts the compound content of the berries and wine [2,7]. In this context, a different level of dehydration-chamber control, in terms of environmental conditions, is important for WL kinetics (Fig. 3), also because this process can strongly influence the composition of the berries, as described previously (Fig. 4); these components, of course, also influence the wine profile. (Fig. 10). For this purpose, we investigated the composition of the berries before and after withering in two tested years and two different environmental conditions.

The microclimatic analysis of the chambers used for tests confirmed a different air thermal and humid regime in the two situations: lower temperatures and higher humidity in NC, higher temperatures, and lower humidity in C, for both tested years (Fig. 1 and Fig. 2). Furthermore, NC temperature was consistently closest to the external thermal conditions simulating the external regime. Thus, the NC must be conducted skilfully as the only action possible to counter the onset of Botrytis (favoured by high humidity) and regulate the dehydration kinetics is by careful management of the internal airflow, performed by opening and closing the windows of the dehydration chambers as needed. T and RH are directly related to the kinetics of withering; indeed,

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they entail the transference of energy and water from the berries into the environment. Concerning this parameter, in both years, the trend of weight loss was faster in C conditions than in NC, and consequently, -30% WL was reached earlier in C (2 weeks). This could have resulted because the highest T and lowest RH for C led to a faster transference of energy and water from the berries into the environment due to the higher value of vapour pressure deficit (VPD), resulting in a faster WL.

Moreover, higher ventilation in C can facilitate water loss from berries due to removing the boundary layer, [46] reducing the RH around the berry and accelerating the WL. It is also interesting to note that in 2017 the WL at the beginning of the drying process was very close between C and NC. Such evidence can be attributable to the similar thermal and humidity conditions in the two dehydration chambers and linked to the external low air humidity.

Regarding the berry chemical composition (Fig. 4), the sugar accumulation trend during the period of withering was different between tested years: in 2016, there were no significant differences between treatments, and there was a high enrichment in the first week following harvest, while in 2017, there was a slightly higher degree in NC than in C conditions, which can be related to the low RH of the and, consequently, a better condition in NC than in C (lower T and similar RH). Observing the rate of sugar increase between the two postharvest maintenance of the grapes, even if the trend is similar as well as the final values, throughout the drying period, the rate of increase in sugar cannot be explained only by the rate of WL, and indeed, for the same WL, there were different values in sugar enrichment between treatments. An explanation could be found in the consequences that different combinations of the climatic elements (T, RH, Wind) can produce during the dehydration process in terms of acceleration in sugar release from the cell wall, [47] mainly galactose [48].

Measurements taken included acidity (Fig. 5 and Fig. 6) and its component in tartaric and malic acid. The accumulation trend was similar between treatments in the period of withering, with a predominance of total acidity in NC, especially in 2017. More in detail, tartaric acid was more stable and tended to remain unaltered and even accumulate during the withering process at the different stages of WL, evidencing a greater concentration in NC conditions in almost every stage of weight loss. Malic acid performed differently, showing a smaller conservation capability due to its rapid consumption during the initial stage of grape dehydration [49]; its enrichment throughout the drying period was almost immeasurable or very minimum. These results suggested that the most significant accumulation of sugars and acidity could occur at -30 % WL. Considering acidity, the different content at -30% WL was entirely in favour of the NC conditions, mostly due to tartaric acid concentration. The faster withering in C likely led to less accumulation of these compounds due to the respiration of tartaric and malic acids as acidity strongly depends on water and temperature stress [33].

Many factors determine the accumulation of colouring substances: the level of ripeness of the grapes [50], thermal stress [51], light [52,53] and water [54]. However, the drying process affects the anthocyanins enrichment by concentration and neoformation (Fig. 7 and Fig. 8) [26,55]. Moreover, the drying process damages the cellular structure of the grape skin, which facilitates the extraction of anthocyanins [56–57]. We then performed a focused analysis of the secondary metabolism. The accumulation of polyphenols and anthocyanins evidenced an interesting trend showing an initial positive step linked to a new polyphenol and anthocyanin synthesis (such phenomenon regarding polyphenols did not occur in 2017) followed by a more stable phase (up to -20% WL). Finally, a third phase took place with the accumulation of new colour compounds due to the concentration process. Specifically, NC tended to have more of both components than C

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during the withering process and, at -30% WL, significant differences were found in favour of NC. Also, in this case, NC confirmed a higher accumulation capability, probably due to a slower and less stressful withering process linked to the higher temperature in C, capable of decreasing the synthesis or increasing the degradation of polyphenols and anthocyanins as confirmed previously by Shaked [59].

Stilbenes are characterized by several positive biological activities in human health [22,60]. Several studies have demonstrated that osmotic stress occurring during withering, which is induced by water loss and the higher temperatures of the chamber (30 °C), stimulates stilbenes biosynthesis [15,18-21,59,62]. Therefore, a higher stilbene level is considered positive as it increases the health benefits of wine, particularly red wine. For our study, only visually healthy grapes, including berries affected by little, or no stilbene-oxidase activity caused by (e.g.) botrytis infections, were collected. Consequently, differences observed in the two drying processes were mainly related to the different dehydration conditions. In general, accumulation of stilbenes in the berry (Table S1) up to -20% WL was higher in the NC samples compared to the C ones, but in the latter case, the biosynthesis of stilbenes accelerated suddenly in the final withering stage (between 20-30% WL) by increasing their level in the berry, in particular in 2017. Reasonably, the higher air humidity and lower temperature occurring in the NC process induce a longer time to reach a -30% WL in the berry (Figure 3) by reducing cell stress in the initial withering stages. On the other hand, marked differences were observed between the two harvests. Grapes collected in the first year had higher initial stilbene content (Figure 9), and the stilbene accumulation trend during withering was different: against a steady increase observed in 2016, low accumulation was found up to -20% WL in 2017.

In the glycoside aroma precursors profile aliphatic alcohols found in both years to be increased by withering were butanol, 3-methyl-1-butanol, hexanol and 2-hexenol (Table S3). In general, these compounds account for herbaceous and unripe fruit aromas, and the wine tasting can reveal their presence [22]. *cis*-8-Hydroxy-linalool, geranic acid and 7-hydroxy-α-terpineol were the main monoterpenes that increased at -30% WL in both withering processes in both years. This class of aroma compounds is characteristic of Amarone wines, with geraniol as the main compound [63]. Significant is the presence of α-terpineol in Corvina grapes (floral note); however, the slight increase observed at the end of withering can be linked to the chemical mechanisms of rearrangement of other monoterpenols occurring during the process [64,65].

C_{13}-Norisoprenoids contribute to the aromas of the Amarone with notes of ripe fruit, honey, jam, tea, and tobacco. In both years, the glycoside derivatives of 3-hydroxy-ß-damascenone, 3-oxo-α-ionol, and vomifoliol had the highest concentrations in NC (in 2017, at the end of the process, a 2-fold increase was found with a significant difference between the treatments).

In 2016, glycoside benzenoids (spicy, balsamic aromas) such as methyl salicylate, guaiacol, benzyl alcohol, ß-phenylethanol, syringol, vanillin, and vanillic alcohol had a 2-fold increase in both C and NC processes. In both the dehydration processes, the highest amount of benzenoids in the berry was found at -30% WL, particularly benzyl alcohol, guaiacol, syringol, vanillin, acetovanillone, and vanillic alcohol. In 2017 at -30% WL, the NC treatment evidenced the highest quantity in benzenoids due to the high level in benzyl alcohol, guaiacol, syringol, vanillin, acetovanillone, eugenol, and vanillic alcohol (Table S3), and all together can contribute to the typical aroma of Amarone wines.

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In both years, the content of 4-vinylguaiacol at -30% WL (spicy note; sensory threshold in wine 40 μg/L [66] was statistically different between the two processes (p<0.05 and p<0.001 in 2016 and 2017, respectively) with higher contents in NC samples (data not shown). The low benzaldehyde level found in the samples compared to benzyl alcohol (between 20-50 μg/kg d.g. and 800-2500 μg/kg d.g., respectively) indicates that low or no oxidative processes occurred due to Botrytis cinerea infection [67].

In general, the drying process carried out in NC conditions was slower than in C due to higher RH, lower T, and air forced movement in the C environment. This slower process led to a major accumulation of sugars (i.e., 2017 vintage), polyphenols and anthocyanins in NC. It is assumed that the slower process in NC was able to maintain vital cell structures for longer, with less water stress effects (lesser synthesis of stilbenes can confirm this observation). In terms of total aroma compounds (Table 1), we found a higher content in NC, also confirmed by the better wine profile of these wines. Finally, to reach a considerable content of compounds and obtain balanced wines, we can report that it is desirable to reach a -30% WL and adopt NC conditions. The present research confirmed that slowing down the drying process and creating less stressful conditions for the cells could be a strategic option to achieve a more traditional and well-appreciated Amarone wine and that choosing the traditional drying process carried out in natural conditions could also improve overall market appreciation.

5. Conclusions

The positive correlation between the slow rate of water loss and the total number of activated genes has already been reported for berries under different maintenance procedures affecting the total length of the post-harvest process [68,69]. These experiences emphasized that the longer the period of berry dehydration, the higher the transcriptional modulation, which in turn influences the metabolomic profile of the dried grapes.

Furthermore, we can assume that the grape ripening level at the beginning of the drying process can play a role in the subsequent berry chemical composition. In fact, in 2016, where we found more sugars and anthocyanins at T0, at -20% WL, we observed more colour, stilbenes and the final level of the aroma compounds. Specifically, this occurred mostly for C in terms of sugar, anthocyanins, and aroma composition. For NC, especially in the vintage less favourable in terms of grape quality (for instance, in 2017 sugar and anthocyanins T0 levels were lower than in 2016), the slow down drying resulted more suitable to produce grapes characterized by higher levels of VOC glycoside precursors, anthocyanins and polyphenols, and wines with higher complexity where olfactory notes harmonize well with wine alcohol content. Sensory evaluation of wines highlighted the positive sensory descriptors present in the wines produced adopting the NC process, since, as a general trend, the panel found this wine to be more harmonious, elegant, and balanced and displaying more ripe, red fruit and spicy notes and less herbaceous nuances (with significant scores for ripe fruit, smooth and spicy notes), than that wines from grapes dehydrated in C conditions.

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Supplementary Materials:

stilbenes identified	signal intensity
trans-resveratrol	169027
piceatannol	490372
cis-piceid	34732
trans-piceid	35455
E-astringin	19778
Z-astringin	5072
pallidol	11191
resveratrol dimer	5555
Z-ε-viniferin	122415
Z-ω-viniferin	19796
E-ε-viniferin	582239
δ-viniferin	8816
caraphenol	24940
pallidol-glucoside	24020
α-viniferin	1848
Z-miyabenol C	4538
E-miyabenol C	26439
resveratrol tetramer 1	5076
resveratrol tetramer 2	16480

Table S1. Stilbenes identified in Corvina grape before withering. Signal intensities are normalized to the internal standard.

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	(µg 1-heptanol/Kg d.g.)													
	T0		-10% WL				-20% WL				-30% WL			
2016			C		NC		C		NC		C		NC	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
aliphatic alcohols														
1-butanol	48	18	87	19	64	3	101	8	101	12	92	30	97	4
3-methyl-1-butanol	47	15	105	50	64	27	211	53	87	28	150	60	94	42
3-methy-3-buten-1-ol	25	4	33	3	30	6	36	4	38	3	33	11	36	1
1-pentanol	7	1	13	3	8	2	16	6	11	1	17	1	14	3
3-methyl-2-buten-1-ol	61	10	88	16	76	17	107	15	91	11	93	21	99	11
1-hexanol	69	18	77	19	56	4	110	18	74	17	113	71	119	55
3-hexen-1-ol (E)	1	0	1	0	1	0	3	1	1	1	2	1	2	1
3-hexen-1-ol (Z)	22	6	17	1	14	3	20	2	15	5	17	6	17	4
2-butoxyethanol	6	2	11	1	11	1	152	128	137	32	40	36	37	23
2-hexenol	11	2	12	2	11	2	16	9	15	5	18	8	18	2
C_6-aldehydes														
hexanal	13	3	23	2	29	11	27	11	25	11	13	5	20	3
2-hexenal	14	3	20	3	26	9	14	5	21	7	9	4	15	1
monoterpenes														
trans-furanlinalool oxide	2	0	3	1	2	1	3	1	3	1	3	1	3	2
cis-furanlinalool oxide	2	0	3	0	2	1	3	0	2	1	2	1	2	1
linalool	15	7	14	1	13	5	6	0	18	4	8	4	10	3
α-terpineol	3	0	5	1	3	1	5	1	4	1	4	1	4	3
trans-pyranlinalool oxide	8	2	11	2	8	5	12	2	11	4	11	4	11	7
nerol	16	1	24	4	18	4	21	6	22	2	19	6	20	1
geraniol	69	7	100	7	88	20	95	11	97	9	87	23	92	6
2-exo-2-hydroxycineol	9	3	11	2	11	5	13	3	13	4	14	7	13	4
diendiol I	10	3	10	2	11	5	10	3	12	4	11	6	9	3
trans-8-hydroxylinalool	39	17	73	17	50	13	68	21	61	4	77	7	59	20
hydroxygeraniol	19	2	37	6	26	8	44	3	36	5	37	10	41	5
cis-8-hydroxylinalool	104	31	132	24	90	48	128	33	143	20	134	32	125	45
geranic acid	56	6	116	11	91	12	107	11	120	9	106	25	116	9
7-hydroxy-α-terpineol	203	105	268	81	280	167	345	121	335	166	344	230	308	74
C_{13}-norisoprenoids														
3-hydroxy-β-damascenone	35	6	41	8	35	9	50	9	40	7	43	12	46	14
3-oxo-α-ionol	331	35	485	55	385	92	502	41	511	10	467	43	488	91
3,9-dihydroxy-megastigma-5-ene	28	5	35	4	26	6	38	3	35	1	37	6	36	8
3-hydroxy-7,8-dihydro-α-ionol	23	3	33	6	29	7	36	2	35	3	36	9	35	2
vomifoliol	368	81	510	115	439	92	524	85	556	39	543	90	538	107
benzenoids														
benzaldehyde	46	53	36	27	18	2	26	11	20	9	22	9	17	5
acetophenone	6	6	6	2	5	2	6	2	7	1	6	2	8	3
methyl salicilate	15	15	15	8	9	3	13	6	10	2	9	1	12	7
guaiacol	5	2	26	11	9	4	22	4	10	4	19	8	15	7
benzyl alcohol	590	173	799	207	1100	269	924	151	1345	590	964	213	1337	541
β-phenylethanol	276	26	391	96	404	85	478	64	447	63	418	135	436	39
eugenol	20	3	29	5	29	3	34	4	37	3	28	6	33	5
4-vinylguaiacol	375	76	808	186	800	107	993	68	900	42	841	201	999	161
syringol	58	12	275	86	83	41	228	70	87	33	176	75	127	56
4-vinylphenol	105	47	404	207	380	99	402	29	379	85	219	23	409	110
vanillin	30	6	54	15	48	5	66	8	51	6	56	16	55	2
methyl vanillate	42	15	54	13	51	21	67	16	59	13	59	23	53	8
acetovanillone	297	45	443	115	482	67	512	88	580	114	478	123	619	154
vanillic alcohol	267	48	620	335	625	42	760	213	724	155	601	111	815	243
homovanillic alcohol	196	46	416	134	394	51	437	85	509	59	467	131	539	11
4-hydroxy benzene ethanol	70	24	**260**	**107**	291	20	317	98	307	43	248	83	330	64
furanic compounds														
5-methylfurfural	27	8	43	7	42	3	48	6	57	1	43	15	54	1
2-furanmethanol	26	3	59	11	50	3	66	16	66	2	55	10	70	3
furaneol	**53**	9	102	18	92	5	114	22	123	1	99	22	124	4

Table S2. Contents of glycoside precursors of volatile organic compounds (VOCs) identified in Corvina grapes harvested in 2016 and withered in controlled (C) and not-controlled (NC) environments (data expressed as µg 1-heptanol/kg d.g.).

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	(µg 1-heptanol/Kg d.g.)													
	T0		-10% WL				-20% WL				-30% WL			
			C		NC		C		NC		C		NC	
2017	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
aliphatic alcohols														
1-butanol	74	24	76	17	68	9	76	9	94	49	112	41	174	58
3-methyl-1-butanol	79	19	117	111	76	16	99	30	118	54	161	109	225	39
3-methy-3-buten-1-ol	40	4	33	2	36	2	35	2	43	8	45	3	45	9
1-pentanol	11	5	5	0	7	2	8	1	10	4	8	1	16	7
3-methyl-2-buten-1-ol	108	14	101	11	104	12	108	19	136	30	112	17	87	77
1-hexanol	72	8	62	16	62	22	59	14	89	25	86	33	153	27
3-hexen-1-ol (E)	1	0	1	0	1	0	1	0	2	1	2	1	3	1
3-hexen-1-ol (Z)	33	4	16	3	23	10	13	5	21	4	14	9	23	2
2-butoxyethanol	62	45	123	69	78	96	51	32	30	15	95	79	76	71
2-hexenol	7	2	5	2	4	1	7	2	9	3	6	4	16	9
C_6-aldehydes														
hexanal	31	15	38	13	48	7	110	45	124	59	49	13	89	29
2-hexenal	28	7	18	6	22	2	11	4	14	1	16	4	16	2
monoterpenes														
trans- furanlinalool oxide	4	1	3	2	5	1	5	4	8	4	3	2	8	2
cis- furanlinalool oxide	3	0	3	1	5	2	6	4	8	4	3	2	8	3
linalool	49	12	34	11	40	4	36	19	39	6	19	7	39	37
α-terpineol	5	0	5	2	7	3	7	5	12	4	5	3	11	2
trans -pyranlinalool oxide	12	2	12	5	14	3	14	6	22	10	9	6	22	4
nerol	19	3	15	7	17	4	14	6	20	5	16	5	21	3
geraniol	79	14	66	28	76	19	61	26	82	19	80	23	87	21
2-exo-2-hydroxycineol	12	3	11	5	14	2	14	5	16	2	14	6	24	7
diendiol I	12	3	10	6	13	4	12	5	15	6	10	8	9	5
trans -8-hydroxylinalool	52	9	42	12	73	18	43	11	106	78	45	30	86	68
hydroxygeraniol	25	6	21	6	35	11	32	14	49	20	31	13	51	6
cis -8-hydroxylinalool	228	60	162	27	220	38	137	24	209	44	123	33	173	14
geranic acid	49	10	54	16	84	13	44	13	74	27	108	20	109	22
7-hydroxy-α-terpineol	188	46	185	120	225	30	214	92	251	45	232	117	249	31
C_{13}-norisoprenoids														
3-hydroxy-β-damascenone	51	7	55	8	64	5	66	13	84	22	47	10	109	46
3-oxo-α-ionol	541	63	466	178	737	49	733	150	878	214	562	100	1340	575
3,9-dihydroxy-megastigma-5-ene	31	3	35	5	36	4	31	5	41	17	25	9	40	6
3-hydroxy-7,8-dihydro-α-ionol	28	3	28	4	33	1	31	4	37	5	33	5	48	10
vomifoliol	422	101	377	52	592	79	623	202	928	308	497	99	1167	448
benzenoids														
benzaldehyde	39	17	30	7	20	2	50	19	32	15	34	16	43	25
acetophenone	6	1	5	2	5	4	8	3	7	6	6	3	15	14
methyl salicilate	4	2	6	1	3	1	8	3	6	1	6	0	24	18
guaiacol	125	24	111	104	139	32	243	259	576	324	222	192	799	266
benzyl alcohol	572	41	748	117	662	170	1263	259	1031	242	1484	360	1936	942
β-phenylethanol	370	41	412	99	456	49	368	54	453	113	457	90	593	60
eugenol	24	2	28	2	28	6	23	5	30	10	28	8	55	22
4-vinylguaiacol	578	99	493	120	583	85	467	94	746	196	736	159	1092	139
syringol	160	31	145	72	241	128	344	296	741	286	253	235	825	291
4-vinylphenol	265	126	135	103	228	54	281	129	579	219	688	312	513	192
vanillin	48	5	68	18	78	16	99	25	137	17	93	21	154	47
methyl vanillate	16	5	18	7	25	2	20	10	33	9	21	7	43	14
acetovanillone	268	24	240	192	377	68	321	14	467	132	340	61	744	262
vanillic alcohol	239	73	324	71	422	35	517	139	754	154	641	198	1079	430
homovanillic alcohol	349	65	381	67	358	114	390	42	518	136	518	61	624	93
4-hydroxy benzene ethanol	196	51	112	129	70	10	28	3	46	21	411	111	158	175
furanic compounds														
5-methylfurfural	9	5	7	1	6	1	7	1	7	2	7	1	9	3
2-furanmethanol	65	12	40	5	41	5	36	11	36	15	53	3	41	14
furaneol	29	6	29	5	20	10	25	4	26	4	45	8	32	9

Table S3. Contents of glycoside precursors of volatile organic compounds (VOCs) identified in Corvina grapes harvested in 2017 and withered in controlled (C) and not-controlled (NC) environments (data expressed as µg 1-heptanol/kg d.g.).

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