Research Article

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Postharvest dehydration of red grapes: impact of temperature and water-loss conditions on free and glycosylated volatile metabolites of exocarp and epicarp of Nebbiolo and Aleatico varieties

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Abstract

BACKGROUND: Postharvest dehydration affects the metabolism of grapes, impacting odorous secondary metabolites and therefore the features of the corresponding *passito* wines – high-quality products with winemaking practices linked to specific territories and related autochthonous grape varieties. Water loss and temperature conditions are the main variables of the dehydration process. This study assessed how they impacted the patterns of free and glycosylated volatile organic compounds (VOCs) of the exocarp (pulp) and epicarp (skin) in Nebbiolo and Aleatico, a neutral and semi-aromatic red grape variety, respectively. Dehydration parameters were set in tunnel conditions, and VOCs were quantitatively analyzed by solid phase extraction–gas chromatography–mass spectrometry.

RESULTS: For Nebbiolo grapes, weight loss had a greater impact on free volatiles than dehydration temperature, with a 20% weight loss increasing total VOCs in both exocarp and epicarp. Low temperature (10 °C) significantly increased ($P < 0.05$) the glycosylated VOCs' terpene content. In Aleatico grapes, weight loss was key in modulating free volatiles, with 30% weight loss and 15 °C leading to significant increases in VOCs, especially exocarp terpenes, acids and benzenoids. More stressful dehydration (30% weight loss at 25 °C) resulted in higher aroma precursor concentrations.

CONCLUSION: These findings can assist *passito* wine production in preserving varietal aromas of original grapes trough optimized dehydration conditions, preventing sensory homologation occurring because of strong uncontrolled dehydration. They can also promote optimization of energy consumption, thus fostering financial and environmental sustainability.

Supporting information may be found in the online version of this article.

Keywords: postharvest tunnel withering; Italian grapes; pulp; skin; aromas; SPE-GC-MS

INTRODUCTION

Questioning the idea that dehydration is just a concentration process linked to water loss, some studies have tried to understand how different dehydration conditions can impact the volatile composition of grapes[1-12] and wines.[4,6,8,9,13-18] Different results were found, depending on the grape variety investigated and the conditions under which dehydration was applied. It is now understood that precise temperature regulation during berry dehydration is crucial because it significantly influences the speed of water loss and subsequent reduction in berry weight. The extent of water/weight loss, closely tied to the dehydration method applied, induces changes in berry rheology and chemical

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composition, triggering mass transfer within different berry tissues as well as enzymatic and non-enzymatic reactions, influencing cell metabolism.[5,19-21] These reactions affect primary and secondary metabolites of grapes, resulting in the synthesis or modification (increase or decrease) of important free volatiles, but also of glycosylated aroma precursors. Biochemical studies have been conducted to investigate possible effects of dehydration on the metabolism of grape berry and its effect on volatile organic compounds (VOCs).[1,7,22-24] It has been observed that the activity of enzymes such as lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH) are deeply influenced by the dehydration process.[1,22] Dehydration leads to an increase in membrane permeability and LOX activity, the primary enzyme involved in the production of fatty acid-derived volatiles, through the deoxygenation of unsaturated fatty acids (e.g., linoleic and α-linolenic acids) and the production of oxygen free radical molecules. HPL cleaves LOX products, initially producing hexanal and (*Z*)-3-hexenal, from linoleic acid and α-linolenic acid, respectively. Finally, as metabolism switches from aerobic to anaerobic, ADH converts the aldehydes into their corresponding alcohols: 1-hexanol and (*Z*)-3-hexenol.[1,7,22] Therefore, the increase in the activity of these enzymes, as well as the accumulation of amino acids, and other physiological and biochemical changes described in postharvest berry dehydration, lead to changes in the production pattern of several volatile grape compounds, such as C6 compounds, terpenes, benzenoids, norisoprenoids, alcohols, esters and acids.[1-11]

In recent reviews on the impact of postharvest dehydration methods on the chemical properties of grapes and wines, Mencarelli and Bellincontro[25] have hypothesized the potential changes of the main classes of volatile compounds of grapes during postharvest partial dehydration at different temperatures and under different water loss conditions. Higher temperatures generally provoke varietal VOC loss and increase VOC oxidation. Temperatures of 30 °C and above lead to strong oxidation and tend to homologate the volatile features of all the varieties and of the future wines, being then characterized by oxidative notes (i.e., dried figs, apricots, caramelized and honey odors). Moreover, Sanmartin et al.[26] reported that the volatile pattern linked to grape dehydration under sun-drying is characterized by specific aromas (i.e., octanoic acid, ethanol, isobutanol, γ-valerolactone and γ-butyrolactone, vinylguaiacol, vanillin, furfural and 5-methylfurfural), as well as those under controlled conditions (i.e., ethanol, isobutanol, linalool oxides, ethyl acetate, isoamyl acetate, vinylguaiacol and vanillin).

Based on the aforementioned studies, the aim of the current research was to assess, for the first time, the impact of different controlled dehydration conditions on the free and glycosylated volatiles in specific tissues of the grape berry, namely exocarp (pulp) and epicarp (skin). The study has been conducted by considering two different Italian grape varieties (*Vitis vinifera* L.): the neutral cv. Nebbiolo and the semi-aromatic Aleatico. These grapes are both used to produce two of the rare cases of red dessert wines. From Nebbiolo grapes are produced Barolo and Barbaresco (Langhe, Piemonte, Italy) – world-renowned wines with specific in-mouth features[27] – as well as 'Sforzato di Valtellina' (Valtellina, Lombardia, Italy) made with off-vine dehydrated Nebbiolo grapes. Aleatico is a semi-aromatic red grape variety particularly cultivated in the northern Lazio (Italy), but mainly on Elba Island and along the coastline of Tuscany (Italy) to produce the dessert wine 'Aleatico Passito dell'Elba', by a partial postharvest berry dehydration.

The experiments were performed in tunnels with fixed relative humidity, managed ventilation and temperatures, with grapes sampled at different weight losses. The interest is to contribute to the optimization of grape dehydration in terms of aromatic quality.

MATERIALS AND METHODS

Grape samples and dehydration

Red grapes *Vitis vinifera* L. cv. Nebbiolo and Aleatico were grown in Valtellina area (Cooperativa Villa di Tirano e Bianzone, Sondrio) and Lazio region (Tenuta Pomele, Gradoli), respectively. The postharvest dehydration experiment was conducted as previously reported on 'Nebbiolo' grapes[11] and applied to Aleatico grapes with some modifications. Briefly, grape bunches, harvested in different times of the year when berries reached a total soluble solids content (TSS) of 23 ± 1 °Brix, were shipped to the laboratory of the Department for Innovation in Biological, Agro-Food and Forest Systems (University of Tuscia, Viterbo, Italy) for dehydration tests. After 6 h overnight transport in a closed van from Valtellina for Nebbiolo and 1 h of shipping for Aleatico coming from Tuscany (temperature at arrival between 20 and 23 °C), the bunches were placed in perforated boxes (60 × 40 × 15 cm) in a single layer. For each test, two perforated boxes, each containing 6 kg of fruit, one on top of the other, were placed in a small metallic tunnel (45 × 45 × 100 cm) fitted with an exhaust fan with regulated airflow. The small tunnels were placed in two thermohygrometric controlled rooms (12 m^3) at either 10 ± 1 or 20 ± 1 °C for 'Nebbiolo' grapes and 15 ± 1 or 25 ± 1 °C for Aleatico grapes: the relative humidity (RH) was set at 60 ± 5% and airflow at 1.5 ± 0.3 $m\ s^{-1}$. The different temperatures used for the two grapes in the dehydration process were due to the final aim of vinification: slow dehydration for Nebbiolo to make dry wine and faster dehydration for Aleatico to make sweet wine. The duration of the experiment was the time taken to achieve 20% weight loss (WL) for Nebbiolo and 30% WL for Aleatico in the bunches. Bunches were sampled prior to dehydration (control samples corresponding to 0% WL) and then after WL of 10 ± 2% and 20 ± 4% for Nebbiolo grapes and 20 ± 4% and 30 ± 6% for Aleatico grapes. Bunch mass (using the same ten bunches) was determined using a technical balance (Adam Equipment, Milton Keynes, UK). During dehydration, thermohygrometric conditions were monitored with a HYGROclip model probe (Rotronic, Bassersdorf, Switzerland) connected to HYGROwin software to record the data. Air speed was measured by means of a Terman hotwire anemometer (LSI, Milan, Italy).

VOCs analysis

VOCs were analyzed as recently reported.[28] Briefly, 400 g berries for each treatment were processed and analyzed with a method previously optimized by Genovese et al.[29] and used for both grape[30] and wine analyses.[15] The berries were prepared by removing their peduncles, and then the skins and pulp were meticulously separated using tweezers. To prevent oxidation, the skins were immediately placed in bottles containing a must-like buffer solution (5 g L^{-1} of tartaric acid, 10 g L^{-1} polyvinylpolypyrrolidone and 2 g L^{-1} sodium azide, and pH was adjusted to 3.2 with 1 mol L^{-1} NaOH) for extraction. The skins were stirred for 24 h at 20 ± 1 °C in the absence of light and then centrifuged at 10 000 × *g* for 20 min at 20 ± 1 °C. After separating the skins, the deseeded pulp was homogenized using a CJ60 homogenizer (Black & Decker, Towson, MD, USA) for 2 min, with the addition of

2 g L^{-1} sodium azide. Subsequently, it was centrifuged at 10 000 × *g* for 10 min at 10 °C using an ALC 4239R centrifuge (Daihan Scientific, Wonju, South Korea). The resulting liquids were filtered through cellulose paper to obtain sample solutions, which were then stored at −20 °C until analysis (two replicates). For each grape sample, 50 mL of the sample solution was spiked with 250 μL 2-octanol (200 mg L^{-1} in methanol) and passed through a C18 reversed-phase solid-phase extraction (SPE) column (1-g C18 cartridge, Phenomenex, Torrance, CA, USA). The column was rinsed with Milli-Q water, and the adsorbed volatiles were eluted with dichloromethane, while bound volatiles were eluted with methanol. The methanol was evaporated under reduced pressure at 37 °C, and the residue was dissolved in 5 mL citrate–phosphate buffer (pH 5.0) containing 80 mg Rapidase AR 2000 pectolytic enzyme with secondary glycosidase activities (DSM, Delft, Holland) before incubating for 16 h at 40 °C. The volatiles released by enzymatic hydrolysis were eluted with dichloromethane on preconditioned C18 cartridges after the addition of 250 μL 2-octanol as an internal standard. The extracts were dried over Na_2SO_4 and then concentrated to 50 μL under an N_2 stream. Both free and bound VOCs were analyzed by gas chromatography–mass spectrometry (GC-MS) for identification and GC–flame ionization detection (GC-FID) for quantification, following a method previously described.[31] For each sample, the SPE procedure was duplicated. GC-MS analysis was performed using a GCMS-QP2010 mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a split/splitless injector and a DBWAX column (60 m × 0.250 i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). The temperature program involved an initial 40 °C for 5 min, followed by an increase to 220 °C at 2 °C min^{-1}, and then held at 220 °C for 20 min. Helium was used as the carrier gas at a flow rate of 1.02 mL min^{-1}. The samples (approximately 1.2 μL) were injected in splitless mode, with the injector port and ion source maintained at 250 and 230 °C, respectively. Positive electron impact spectra were recorded in the range of *m/z* 33–350. Compound identification was confirmed by injecting pure standards and comparing retention times and mass spectra with those in the NIST 2.0 library. For GC-FID analysis, an Agilent 7890 A chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a split/splitless injector and a J&W DB Wax column was employed. The same temperature program used for GC-MS analysis was followed. Helium served as the carrier gas, with a flow rate of 2.20 mL min^{-1}. Two replicates of each volatile extract (1.2 μL) were injected in splitless mode, and the detector and injector were maintained at 250 °C. Volatile compounds were quantified by GC-FID using calibration curves, with peak areas normalized relative to the internal standard peak area and interpolated using the calibration curve. Calibration graphs were generated by analyzing a blank solution as previously described[29] and spiking it with known amounts of each analyte and internal standard. The solution was then diluted to obtain calibration points for each analyte, with the concentration range aligning with values typically found in Italian grape cultivars.[30] The linear regression coefficient (r^2) for each volatile compound was ≥0.9918, consistent with previous literature. Dry mass (DM) was used instead of fresh mass to avoid concentration effects during computation, considering water loss through a moisture content measurement conducted via an oven-dry method.

Statistical analysis

VOC chemical data (mean values obtained by 2 extractions × 2 injections; $n = 4$) were treated by analysis of variance (ANOVA) and post hoc Tukey test for multiple comparison ($P < 0.05$) to test significant differences among the treatments. Principal component analysis (PCA) was used to study the relationships between the different dehydration treatments (observations) and volatile compounds (variables).

Computations were made using XLStat 2012.6.02 (Addinsoft Corp., Paris, France).

RESULTS AND DISCUSSION

Six classes of free volatiles (Supporting Information, Table S1) and four of glycoconjugate (Supporting Information, Table S2) – 47 volatiles in total – were identified in Nebbiolo grapes. Specifically, GC-MS analyses allowed the detection of C6 compounds, alcohols, terpenes and benzenoids in both free and bound forms, with butyl acetate and hexanoic acid detected only in the free form.

In Figs 1 and 2, the PCAs of free (Fig. 1) and glycosylated (Fig. 2) volatile fractions of Nebbiolo exocarp and epicarp are represented. Accounting for 56.92% of the total variance on the first two components (F1: 33.92%; F2: 23.00%) for the free fraction and 81.26% (F1: 53.75%; F2: 27.51%) for the glycosylated one, both PCAs show that exocarp and epicarp samples are well separated (Figs 1(a) and 2(a)) and thus are characterized by a different composition of the volatile fraction (Figs 1(b) and 2(b)).

In the case of free volatiles (Fig. 1), the PCA shows that the different dehydration conditions changed the volatile composition both in the exocarp and the epicarp. While control samples corresponding to fresh grapes not subjected to dehydration (0% WL) (Control_EP and Control_EX) are poorly correlated with volatile compounds, the dehydration process, and in particular samples corresponding to the dehydration conditions of 20% WL (10 °C and 20 °C), modified the free volatile composition of the grapes by enriching their volatile fraction. Indeed, these samples (20% _10 °C_EX, 20%_20 °C_EX, 20%_10 °C_EP, and 20%_20 °C_EP) correlate with several VOCs, such as some terpenes (*cis*- and *trans*-linalool oxide, and 4-terpineol for the exocarp, nerol for the epicarp), C6 compounds (*cis*-2-hexen-1-ol and 1-hexanol for the exocarp, *cis*-3-hexen-1-ol, hexanal and *trans*-2-hexenal for the epicarp), some alcohols (i.e., 1-butanol and 3-methyl-1-butanol for the exocarp, 1-octanol, 2-ethyl-1-hexanol and 1-heptanol for the epicarp) and benzenoids (i.e., benzaldehyde for the exocarp, syringol, phenylethyl and benzyl alcohols and vanillin for the epicarp). Furthermore, in both epicarp and exocarp, samples dehydrated up to 20% WL cluster together regardless of the dehydration temperature, suggesting that the impact of the weight loss on the free volatile fraction appears to be greater than that of the dehydration temperature. The epicarp sample corresponding to the mildest dehydration conditions (10%_10 °C_EP) appears to be the least correlated with free volatile compounds. Conversely, the corresponding exocarp sample (10%_10 °C_EX) did not show the same behavior. It could be hypothesized that skins are the outermost part of the berry and therefore more exposed to volatile loss through evaporation. The same trend would have been expected for the 10%_20 °C_EX sample, but, contrary to expectations, it behaved differently. Two possibilities can be considered. First, the initial temperature gap between the arrival temperature of the grapes (20–23 °C) and the dehydration temperature of 10 °C could have led to a very high initial vapor pressure deficit in the 10%_10 °C_EP sample compared to the 10%_20 °C one. This difference could have affected VOC diffusion rate from the berry, in which transpiration

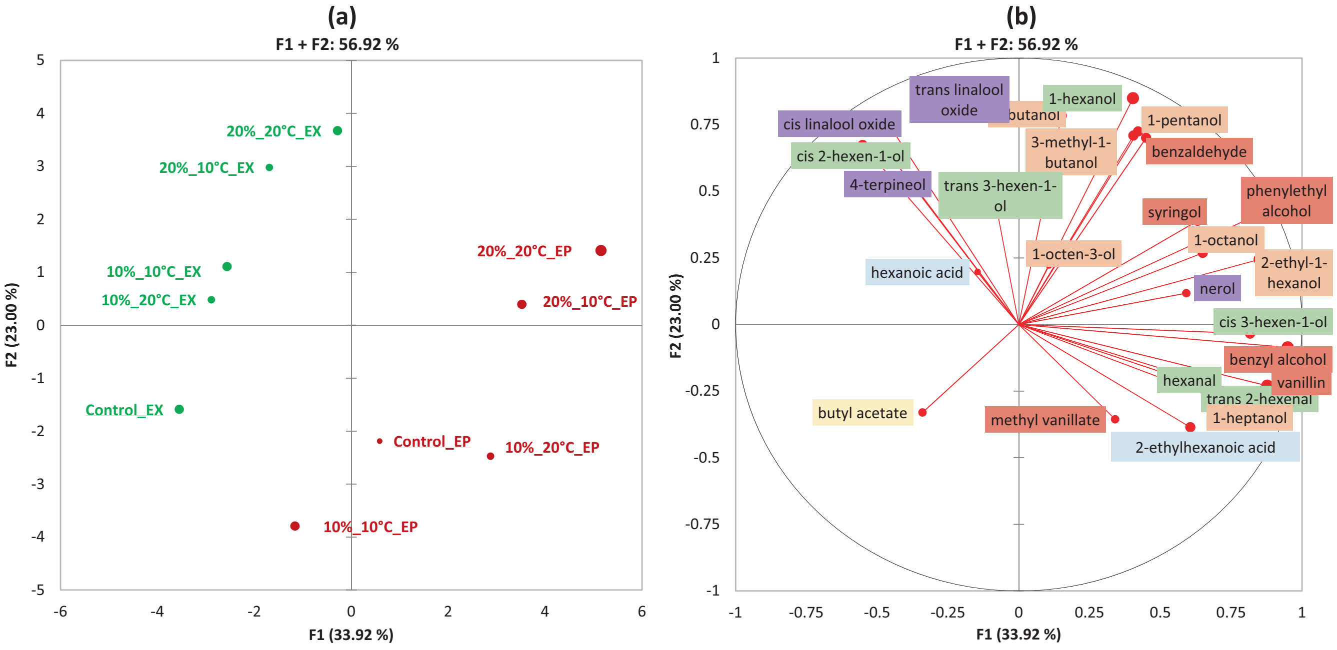

Figure 1. Principal component analysis plots of (a) sample observations and (b) volatile compound variables. Variables are free VOCs detected in exocarp and epicarp of Nebbiolo grapes dehydrated at: 0% WL (Control_EX and _EP), 10% WL and 10 °C temperature (10%_10 °C_EX and EP), 10% WL and 20 °C temperature (10%_20 °C_EX and EP), 20% WL and 10 °C temperature (20%_10 °C_EX and EP), 20% WL and 20 °C temperature (20%_20 °C_EX and EP). Colors of variables refer to VOC classes shown in Figs 3, 5 and 6.

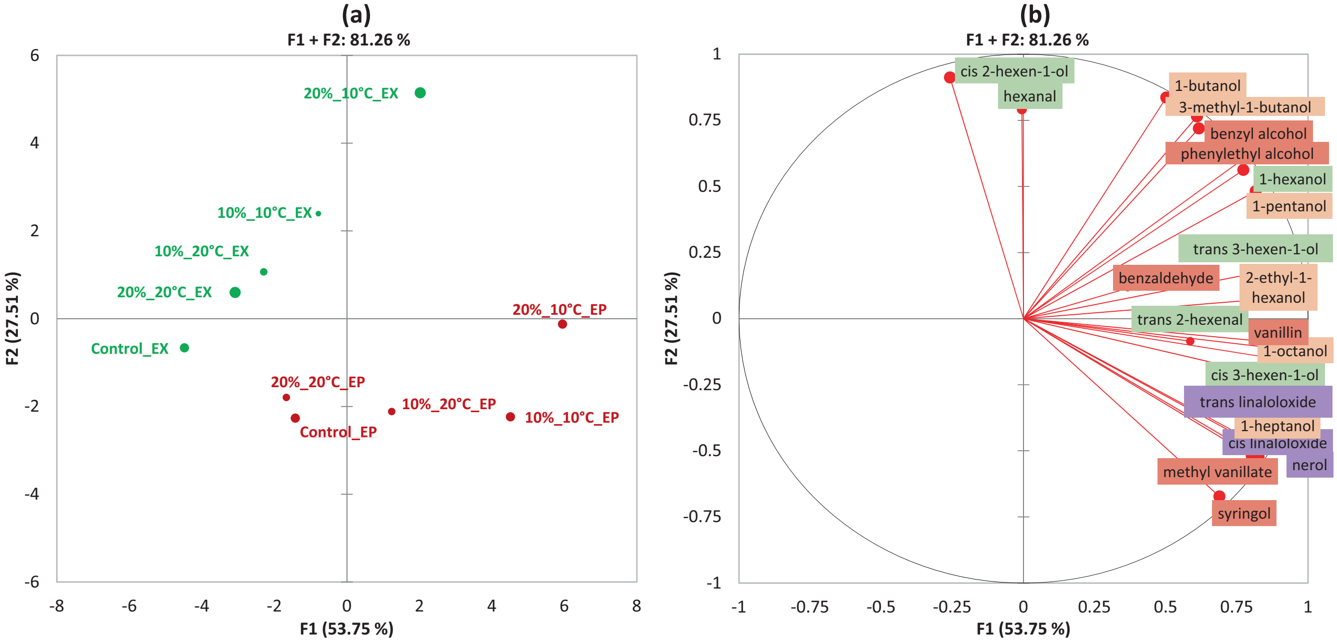

Figure 2. Principal component analysis plots of (a) sample observations and (b) volatile compound variables. Variables are glycosylated VOCs detected in exocarp and epicarp of Nebbiolo grapes dehydrated at: 0% WL (Control_EX and _EP), 10% WL and 10 °C temperature (10%_10 °C_EX and EP), 10% WL and 20 °C temperature (10%_20 °C_EX and EP), 20% WL and 10 °C temperature (20%_10 °C_EX and EP), 20% WL and 20 °C temperature (20%_20 °C_EX and EP). Colors of variables refer to VOC classes shown in Figs 3, 5 and 6.

(water vapor diffusion) mainly occurs through the surface.[32] The berry's limited stomata and lenticels mean that water vapor and volatile molecules primarily escape through the cuticle by diffusion.[33] Second, a weight loss of 10% is achievable more rapidly at 20 °C than at 10 °C, likely changing the dehydration kinetics and the rheology of the berry, which in turn could affect the volatilization of VOCs. Rolle et al.[21] observed that during postharvest withering (15 °C, 45% relative humidity with airflow) of Corvina wine grapes, berry skin break force decreased ~50% without change in the skin thickness while berry hardness decreased ~80%. Specific investigations could be useful to support our considerations.

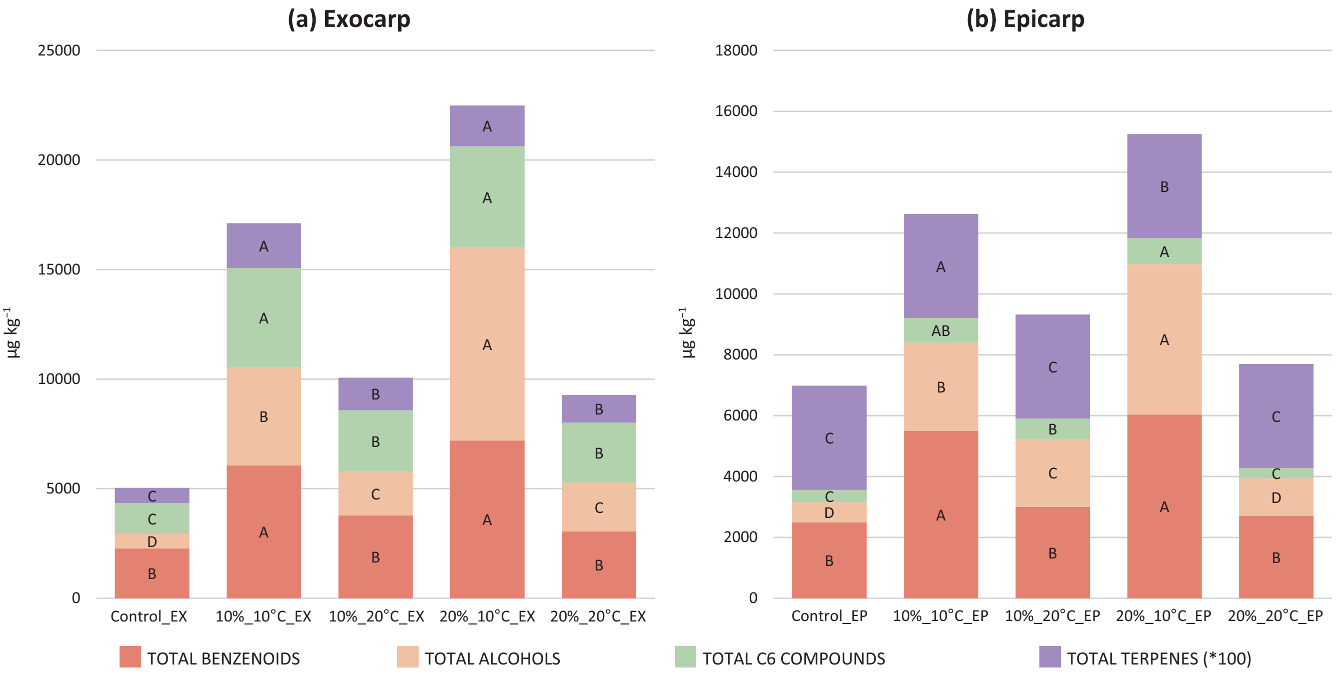

Figure 3. Representation of the concentrations (µg kg^{-1}) of berry weight of the total glycosylated volatiles detected in the exocarp (a) and epicarp (b) tissues of Nebbiolo grapes dehydrated at 0% WL (Control_EX and _EP), 10% WL and 10 °C temperature (10%_10 °C_EX and EP), 10% WL and 20 °C temperature (10%_20 °C_EX and EP), 20% WL and 10 °C temperature (20%_10 °C_EX and EP), 20% WL and 20 °C temperature (20%_20 °C_EX and EP). Values with different letters are significantly different ($P < 0.05$).

As for the free fraction, the dehydration process seems to have enriched grape berries with glycosylated volatile compounds (Fig. 2). The non-dehydrated samples of both exocarp and epicarp (Control_EP and Control_EX) are poorly correlated with bound volatiles. This is detailed in the histograms shown in Fig. 3. In those graphs, representing the global trend and the pattern of the aglycons in exocarp (Fig. 3(a)) and epicarp (Fig. 3(b)) in the different samples subjected to different dehydration conditions, control samples show a significantly lower quantity ($P < 0.05$) of all the classes of detected bound VOCs (total benzenoids, total alcohols, total C6 compounds and total terpenes). With the dehydration process, the bound fraction of volatiles seems enriched both in the exocarp and in the epicarp, with the same trend in the different parts of the grape berry.[11] This increase appears to be modulated by temperature conditions, playing therefore a key role for the glycosylated fraction of both exocarp and epicarp, in line with data recently reviewed.[24,25,34] Those authors concluded that temperature changes from 10 to 30 °C result in a loss of glycosylated varietal volatiles and generation of compounds from amino acids and fatty acid catabolism. However, in our case, where temperatures did not exceed 20 °C, samples dehydrated at lower temperatures (10 °C) were richer than those obtained at 20 °C. This suggests that, regardless the level of weight loss, the rise of glycosylated volatiles in Nebbiolo grapes is less evident at the more stressful dehydration conditions of 20 °C. Our result on glycosylated volatiles is in line with previous findings on other glycosylated compounds, such as anthocyanins. Indeed, Muche et al.[35] observed a lower rate of decay of these compounds at 5° C than at 25 and 35 °C, suggesting that storage of grape juice at lower temperature could reduce the loss of biologically active anthocyanins in red grape cultivars.

In general, the rise in both free and bound volatiles in Nebbiolo exocarp and epicarp in response to dehydration conditions is in line with previous observations on C6 compounds,[1,2,5,9,11] terpenes[8-10,36] and benzenoids.[4,36] Various researchers noted a higher concentration of free and glycosylated C6 compounds in dehydrated berries compared to fresh fruit. These compounds include free 1-hexanol, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, glycosylated 1-hexanol and (*E*)-2-hexen-1-ol,[1,2,5,9] which in the free form can be attributed to the changes in membrane permeability, which trigger LOX activity, leading to the formation of C6 volatiles.[1,2]

The increased content of total free and bound terpenes observed in grape berries exposed to dehydration treatments (mainly at 10 °C) has an oenological interest.[8,36] It is important for the winemaker to manage terpene accumulation and formation in grapes since they are important varietal volatile compounds of wine that could counterbalance the homologizing impact of dehydration on the aromatic varietal character of sweet wines from dehydrated grapes.

Moving to Aleatico grapes, as expected for a semi-aromatic grape variety, and as recently observed by Piombino et al.,[28] a greater number of free and bound volatile molecules have been identified compared to Nebbiolo, which is a neutral grape. Nine free and 16 bound terpenes were identified and quantified in Aleatico grapes, while only two free and three bound were detected in Nebbiolo. In total, GC-MS analyses allowed the detection of 38 free VOCs (Supporting Information, Table S3) and 46 glycoconjugate VOCs (Supporting Information, Table S4) belonging to seven chemical classes: C6 compounds, alcohols, terpenes, benzenoids, esters, acids and other compounds (the free furfural and the glycoconjugate 3-hydroxy-*β*-damascone).

In Fig. 4, the PCA of Aleatico free volatile fraction detected in exocarp and epicarp is represented, accounting for 76.22% of the total variance on the first two components (F1: 51.84%; F2: 24.37%).

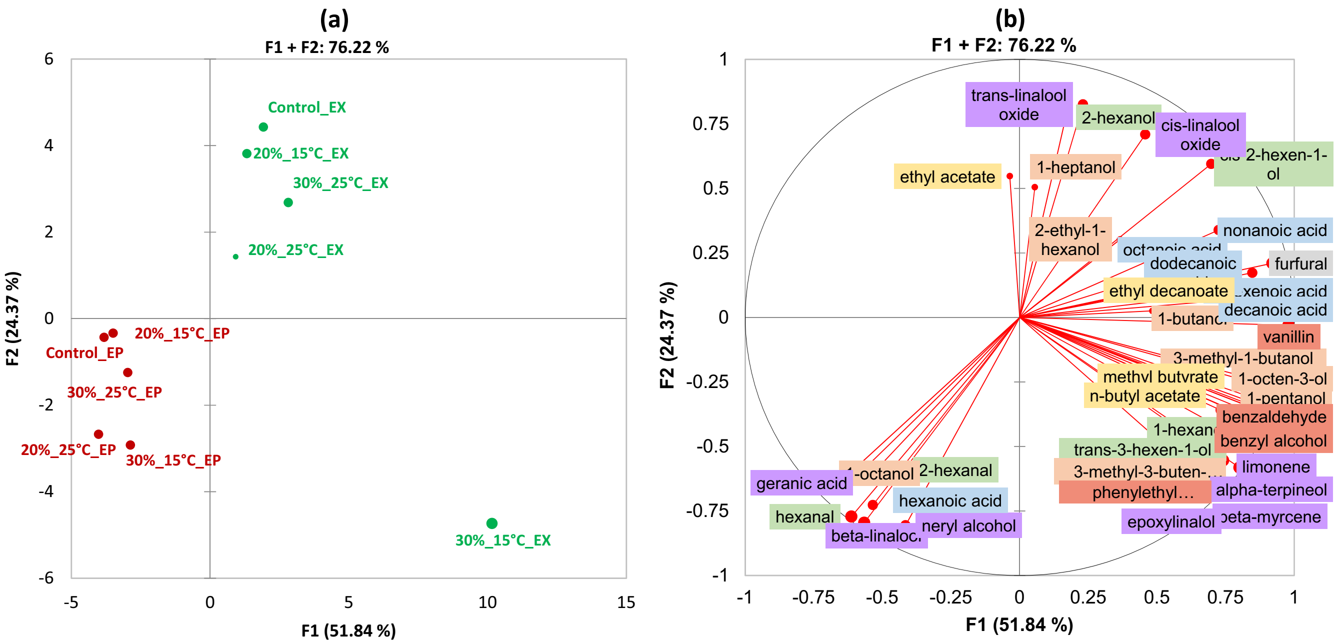

Figure 4. Principal component analysis plots of (a) sample observations and (b) volatile compound variables. Variables are free VOCs detected in exocarp and epicarp of Aleatico grapes dehydrated at: 0% WL (Control_EX and _EP), 20% WL and 15 °C temperature (20%_15 °C_EX and EP), 20% WL and 25 °C temperature (20%_25 °C_EX and EP), 30% WL and 15 °C temperature (30%_15 °C_EX and EP), 30% WL and 25 °C temperature (30%_25 °C_EX and EP). Colors of variables refer to VOC classes shown in Figs 3, 5 and 6.

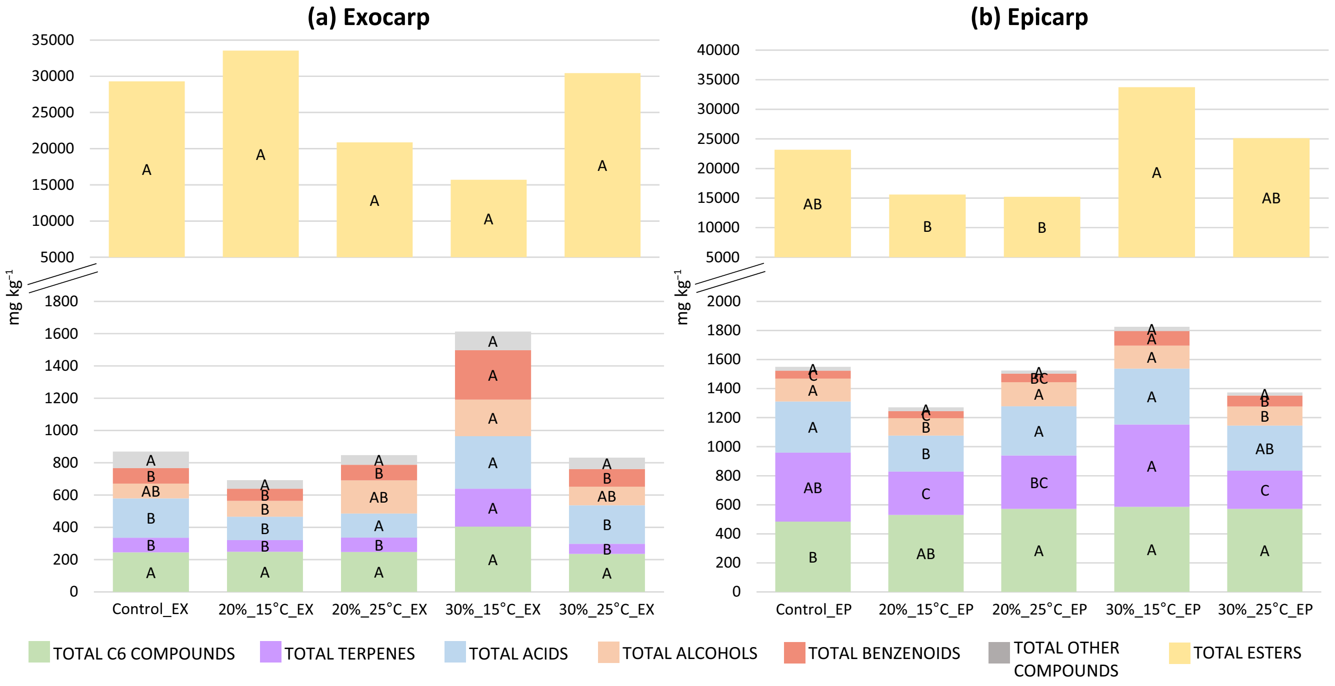

Figure 5. Representation of the concentrations (mg kg^{-1}) of berry weight of the total free volatiles detected in the exocarp (a) and epicarp (b) tissues of Aleatico grapes dehydrated at 0% WL (Control_EX and _EP), 20% WL and 15 °C temperature (20%_15 °C_EX and EP), 20% WL and 25 °C temperature (20%_25 °C_EX and EP), 30% WL and 15 °C temperature (30%_15 °C_EX and EP), 30% WL and 25 °C temperature (30%_25 °C_EX and EP). Values with different letters are significantly different ($P < 0.05$).

PCA shows that the exocarp and epicarp samples are well separated along F1, and that F2 separates the 30%_15 °C_EX sample from the rest of the pulp samples. The histograms in Fig. 5(a) show the specific pattern of the different classes of free volatiles in exocarp; the only sample showing a significant variation ($P < 0.05$) is 30%_15 °C_EX, confirming the key role played by the amount of weight loss in modulating the free volatile fraction, as already observed in Nebbiolo grape variety. At 30% of weight loss, a significant increase ($P < 0.05$) mainly in terms of exocarp terpenes, acids and benzenoids is observed. Figure 4 shows good

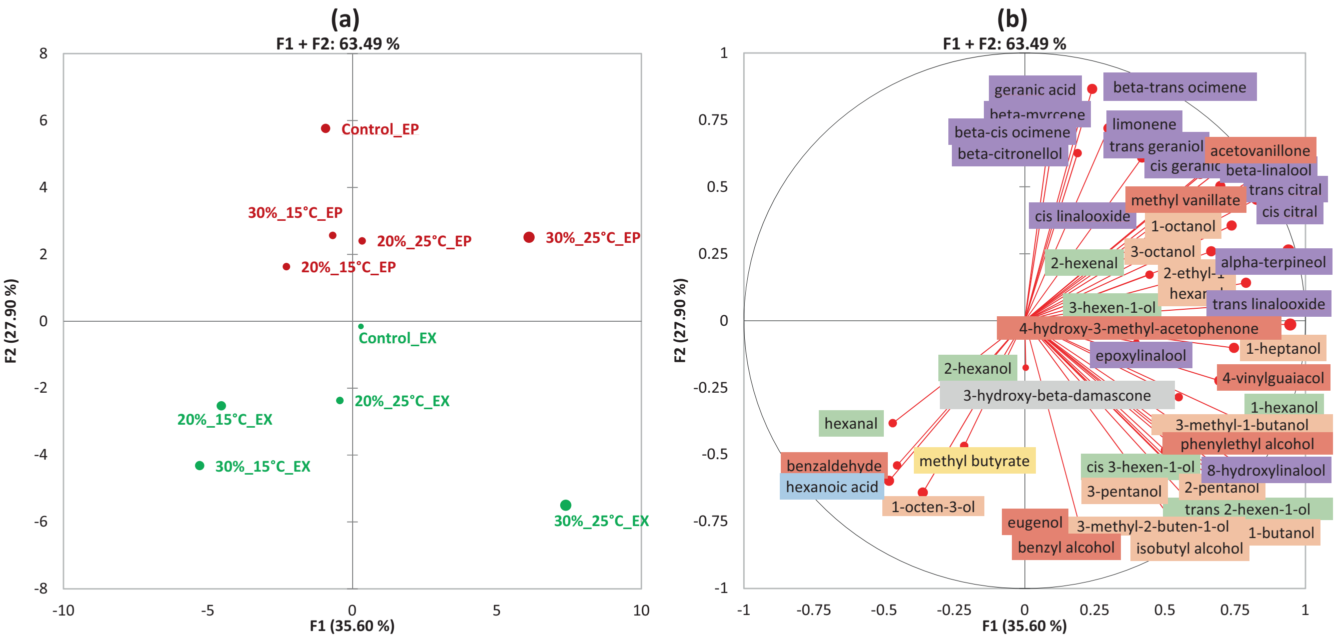

Figure 6. Principal component analysis plots of (a) sample observations and (b) volatile compound variables. Variables are glycosylated VOCs detected in exocarp and epicarp of Aleatico grapes dehydrated at: 0% WL (Control_EX and _EP), 20% WL and 15 °C temperature (20%_15 °C_EX and EP), 20% WL and 25 °C temperature (20%_25 °C_EX and EP), 30% WL and 15 °C temperature (30%_15 °C_EX and EP), 30% WL and 25 °C temperature (30%_25 °C_EX and EP). Colors of variables refer to VOC classes shown in Figs 3, 5 and 6.

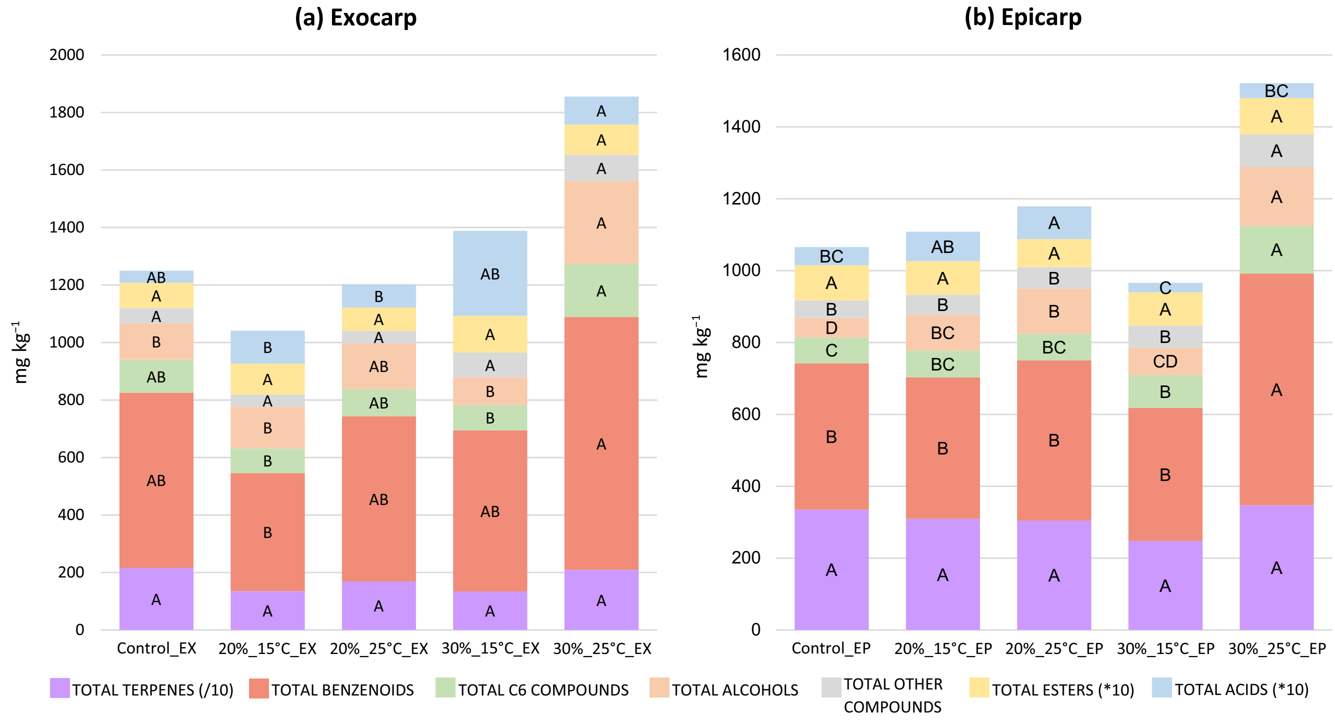

Figure 7. Representation of the concentrations (mg kg^{-1}) of berry weight of the total glycosylated volatiles detected in the exocarp (a) and epicarp (b) tissues of Aleatico grapes dehydrated at 0% WL (Control_EX and _EP), 20% WL and 15 °C temperature (20%_15 °C_EX and EP), 20% WL and 25 °C temperature (20%_25 °C_EX and EP), 30% WL and 15 °C temperature (30%_15 °C_EX and EP), 30% WL and 25 °C temperature (30%_25 °C_EX and EP). Values with different letters are significantly different ($P < 0.05$).

correlations between the 30%_15 °C_EX sample and three esters (i.e., methyl butyrate, *n*-butyl acetate and ethyl decanoate), four terpenes (i.e., α-terpineol, β-myrcene, limonene and epoxylinalool), five acids (decanoic, 2-hexenoic, dodecanoic, octanoic and nonanoic acids) and four benzenoids (i.e., phenylethyl alcohol, benzyl alcohol, benzaldehyde and vanillin). These observations suggest that high water loss and low dehydration temperatures could have a positive effect on the formation/accumulation of many VOCs in this grape variety. Previous findings on Aleatico grapes dehydrated in-tunnel at 12 °C and 60% RH with 31% WL, observed an increase in VOCs of 35% per berry weight (45% of free fraction and 33% of glycosylated fraction).[34] In terms of epicarp composition, Fig. 4 shows that the effects of dehydration on free VOCs are similar in the exocarp and epicarp: the 30%_15 °C_EP sample shows a better correlation with VOCs projected on F2 (β-linalool, neryl alcohol and geranic acid as terpenes, hexanal and 2-hexanal as C6 compounds, and 1-octanol and hexanoic acid). 30%_15 °C samples are the richest in free VOCs (Fig. 5), with esters showing the greatest impact. In the sample 30%_15 °C_EP, corresponding to the higher 'stress' conditions, the level of free ethyl acetate increased significantly ($P < 0.05$). Previous works have shown an increase of this compound in grapes subjected to dehydration, suggesting a correlation with ADH enzyme activity.[1,2,37]

Globally, except for 30%_15 °C, the dehydration conditions seem less impacting for the free VOCs of the semi-aromatic Aleatico grapes compared to the neutral Nebbiolo.

Finally, in Fig. 6 is represented the PCA (63.49% of the variance; F1: 35.60%; F2: 27.90%) of the glycosylated volatiles detected in Aleatico grapes. The second component separates exocarp and epicarp samples, suggesting different compositions of the berry portions. The first component mainly separates the 30%_25 °C samples from the rest (Fig. 6) with positive correlations with a higher number of VOCs than the other samples. These samples (30%_25 °C_EX and 30%_25°C_EP), are well correlated with seven terpene compounds (i.e., *cis*-geraniol, β-linalool, *trans*- and *cis*-citral, α-terpineol, *trans*-linalool oxide and 8-hydroxylinalool), six alcohols (i.e., 1-octanol, 3-octanol, 2-ethyl-1-hexanol, 1-heptanol, 2-pentanol and 3-methyl-1-butanol), five benzenoids (i.e., acetovanillone, methyl vanillate, 4-hydroxy-3-methylacetophenone, 4-vinylguaiacol and phenylethyl alcohol) and three C6 compounds (i.e., 1-hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol). This result highlights a significant ($P < 0.05$) increase in volatile precursors under more stressful dehydrating conditions. According to the histogram patterns in Fig. 7, this increase affects all the chemical classes expect for terpenes. This observation was more evident in the epicarp than in the exocarp, likely due to a mass transfer of VOCs (i.e., 1-hexanol, *cis*-3-hexen-1-ol, *trans*-linalool oxide, benzyl alcohol and phenylethyl alcohol) from the exocarp to the epicarp tissue during postharvest dehydration, as already observed by Centioni *et al.*[5] in Cesanese red grapes. These results suggest that, unlike Nebbiolo grapes, Aleatico glycosylated compounds showed a greater accumulation in the samples dehydrated under the most stressful conditions of temperature and weight loss.

CONCLUSIONS

For the first time, the impact of two important variables of dehydration (percent water loss and dehydration temperature) on the free and glycosylated volatiles of grapes was separately assessed on the two tissues of the grape berry, namely exocarp and epicarp. The study was conducted on Nebbiolo and Aleatico grapes, for which both common or varietal-dependent trends were observed, likely also by reason of their natural difference in volatile richness as neutral and semi-aromatic varieties.

In both grapes the VOCs composition of exocarp and epicarp were discriminable before and after dehydration, with a general greater compositional richness of epicarp. The different dehydration conditions significantly modulated the patterns of free volatiles as well as their glycosylated precursors in both grapes.

For Nebbiolo free volatiles, the impact of the weight loss was greater than that of the dehydration temperature in both exocarp and epicarp. Samples subjected to 20% WL at both 10 and 20 °C showed enriched volatile fractions (+47–48% and +6–13% per berry weight in the exocarp and epicarp, respectively). The impact of dehydration on free volatiles was less significant for Aleatico, except under conditions of high WL (30%) at 15 °C, which resulted in a significant ($P < 0.05$) increase in various volatile compounds, especially in terms of total exocarp terpenes (+158%), acids (+33%) and benzenoids (+200%), suggesting that high water loss and low dehydration temperatures could have a positive effect on the accumulation of many free VOCs in this grape variety.

For the glycosylated fraction, low temperatures (10 °C) led to higher contents of all the identified VOCs in Nebbiolo grapes, suggesting therefore a key role of this parameter. In the samples dehydrated at 10 °C, an increase in total bound terpenes (*cis*-linalool oxide + *trans*-linalool oxide + nerol) of 168–193% and 81–138% per berry weight in the exocarp and epicarp, respectively, was observed. This latter finding is of a particular oenological interest since terpenes are important varietal volatile compounds that could counterbalance the homologizing impact of dehydration on the aromatic varietal character of wines produced from dehydrated grapes. In a different way, in the semi-aromatic Aleatico, the more stressful dehydrating conditions (30% WL, 25 °C) led to significantly ($P < 0.05$) higher concentrations of glycosylated volatile precursors of all detected chemical classes except for terpenes and acids, suggesting that dehydration does not impact the semi-aromatic character of this grape variety.

Overall, the study highlights the influence of dehydration on the free and bound volatile composition of grape exocarp and epicarp and likely on the actual and potential aroma of corresponding wines. Further research would be useful to explore the mechanisms underlying the observed trends. These findings are of interest in the production of high-quality wines from dehydrated grapes such as sweet *passito* wines, in preserving free and bound varietal volatiles of original grapes trough optimized dehydration conditions, enhancing aging potential and preventing sensory homologation occurring because of strong uncontrolled dehydration. They could also promote a more efficient use of energy, thereby supporting financial and environmental sustainability during wine production.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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