

Ultrastructural analysis of berry skin from four grapes varieties at harvest and in relation to postharvest dehydration

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ABSTRACT

In several production areas, dessert (sweet) and reinforced wines are made after a more or less intense dehydration of harvested grapes. The dehydration process depends on several factors, including the size, morphology and anatomy of the berries, all genetically defined traits that can be affected by vineyard management and microclimate conditions. At harvest, berry outer surface and skin ultrastructural features of cvs Aleatico, Sagrantino, Sangiovese and Trebbiano berries were investigated in a frozen-hydrated state using cryoscanning electron microscopy (Cryo-SEM). The berries were subjected to postharvest dehydration at 23 °C for twelve days and the differences in weight loss were determined. In terms of weight loss rate, Aleatico and Trebbiano were the fastest and the slowest respectively. Therefore, the ultrastructural changes of berry skin of these two varieties were also investigated at the end of the dehydration process. At harvest, the structure of the epicuticular waxes on the skin outer surface differed between berries of different cultivars. The thickness of the cuticle, epidermis and hypodermis was also found to be significantly different, with Trebbiano berries having the thickest skin. At the end of the dehydration process all the measured parameters decreased, in particular Aleatico epicuticular wax, hypodermis and cuticle thickness, as well as the hypodermis cell wall and the mesocarp parenchyma cell area. The high weight loss rate recorded for Aleatico can be partly explained by the thickness of the berry skin at harvest, which was significantly thinner than that of Trebbiano, as well as by other skin-related morphological and histological factors possibly affecting permeability.

KEYWORDS

Vitis vinifera, epicuticular waxes, epidermis, hypodermis, water loss

Supplementary data can be downloaded through: <https://oenone.eu/article/view/4165>

INTRODUCTION

The production of specific wine types (sweet, reinforced) is based on the application of protocols that are purposefully aimed at allowing harvested grapes to lose water. Depending on the production area and oenological goals, different techniques are applied and variable levels and intensities of dehydration are attained (Mencarelli and Tonutti, 2013). In addition to an increase in solute concentration, a number of metabolic processes are affected resulting in marked changes in grape properties and, as a consequence, in the resulting wines (Rizzini *et al.*, 2009; D'Onofrio, 2013; Tonutti and Bonghi, 2013). Besides the intensity of the dehydration, weight loss (WL) rate plays an important role, as clearly demonstrated by Bonghi *et al.* (2012), who showed that flavonoid metabolism and related gene expression are variably affected by these two factors in cv 'Raboso Piave' berry skins. Bellincontro *et al.* (2004) reported that different dehydration rates were highly effective in modulating berry composition, including aromatic traits, of Malvasia, Trebbiano and Sangiovese grapes. Controlling WL intensity and rate is, therefore, of paramount importance to properly addressing the production of these wine types. The transpiration rate depends on different parameters, namely surface/fresh weight ratio, water vapor pressure at the evaporating surface of the produce and the diffusive resistance of all paths from inside the fruit to the outside (Burton, 1982; Díaz-Pérez, 2019). Although a portion of total water loss occurs through the pedicels and the rachis (Mencarelli and Bellincontro, 2013), in harvested grapes most water loss occurs via transpiration through the skin, which is the main barrier to water movement from the inner fruit tissues to the external environment (Becker and Knoche, 2011). Skin permeance to water vapor is reported to be primarily affected by the composition of the cuticle, which comprises two sections: the cuticular layer and the cuticular proper (Carrillo-López and Yahia, 2019). The former is composed by cutin with embedded polysaccharides, while the latter is formed by organic solvent-soluble lipids, most of them derived from long-chain fatty acids (C20-C34), including alkanes, ketones, aldehydes, alcohols, and esters (Carrillo-López and Yahia, 2019). Cuticle resistance to water loss seems to be mainly due to the presence and nature of waxes, which occur on the epidermis, but can be deposited in cells (Esau, 1977), giving the fruit a nice luster. This property appears to be primarily determined

by the particular mixture of intra-cuticular and epicuticular waxes and their arrangement, rather than their amount. The way in which the wax platelets are laid out and distributed on the berry surface depends on both genetic factors and (to a lesser extent) environmental/growing conditions in the vineyard. Cultivar-dependent composition of epicuticular waxes has been observed in apple (Belding *et al.*, 1998) and olives (Servili *et al.*, 2008; Lanza and Di Serio, 2015). In systematic botany, data on epicuticular waxes, as well as on other traits of fruit surface ultrasculpture, are used for taxonomic purposes as diagnostic features (Yembaturova and Konstantinova, 2013). Very limited information is available regarding the epicuticular waxes of wine grapes. Rustioni *et al.* (2012) reported that four Georgian varieties can be discriminated based on exocarp optical properties related to epicuticular waxes, while Muganu *et al.* (2011) demonstrated that cv Trebbiano berries grown under different sunlight exposures show differences in wax platelets shape and morphological features. Epicuticular waxes are considered important players in modulating transpiration flux; however, the roles of the cuticular layer thickness, the epidermis and the hypodermis structures in fruit transpiration is not clear. In a study on a tomato mutant characterised by a reduced cuticle thickness, Isaacson *et al.* (2009) reported that there is no correlation between the amount of cutin and the permeability of the cuticle to water. Atkinson *et al.* (2012) showed that by suppressing the polygalacturonase (PG1) gene, the hypodermis of ripe apple was characterised by more densely packed cells, resulting in reduced water loss during storage. The aim of the present paper was to identify the different morphological and histological features of the outer pericarp layers of four wine grape varieties at commercial harvest, as well as the changes occurring in these fruit tissues after partial postharvest dehydration.

MATERIALS AND METHODS

1. Plant material

Grape (*Vitis vinifera* L.) bunches from three red-skinned (cvs 'Aleatico', 'Sagrantino' and 'Sangiovese') and one white-skinned (cv 'Trebbiano') Italian cultivars were harvested from different vineyards located in Tuscany (Italy) with the following soluble solids concentration: Aleatico and Trebbiano, 22.1 ± 0.5 °Brix; Sangiovese and Sagrantino, 23.3 ± 0.7 °Brix. Only good quality and healthy bunches and berries were collected and used in the trials.

2. Grape berry dehydration in a controlled environment

Once harvested, the grape bunches were taken to the laboratory facilities. Single berries ($n = 100$ for each cultivar) of similar and representative size were removed from the base of the pedicel with scissors. Samples were placed in a single layer in open air boxes (60 x 40 x 15 cm) and kept at 23 °C (± 1 °C) and 45 % (± 5 %) RH for 12 days. The fresh weight of single berries was determined on a daily basis. Specific samples were collected at harvest (D0) and after 12 days of dehydration (D12) for Cryo-SEM analysis on frozen-hydrated berries.

3. Cryo-Scanning Electronic Microscopy analysis

The analysis of both berry surface and internal tissue ultrastructure exposed by freeze-fracture was carried out using cryo-scanning electronic microscopy (Cryo-SEM); the samples were cryo-fixed and frozen-hydrated throughout the analysis. The possibility of analysing biological samples in their natural hydrated state by means of the cryo-SEM allows researchers to study structural and physiological components that would otherwise be lost in the fixation and subsequent drying protocols usually applied for microscopy sample preparation (McCully *et al.*, 2009). Berry samples were only quickly cryo-fixed in liquid nitrogen, in order to maintain the fine surface components, such as epicuticular waxes, in their original state (Rogiers *et al.*, 2005), thus avoiding chemical treatments that would modify their nature (Casado and Heredia, 2001). Furthermore, we were able to freeze fracture the samples in order to analyse the internal tissues, cells and their ultrastructure in their natural state.

Sample preparation was carried out as reported by Celano *et al.* (2009). Briefly, portions of fresh D0 and D12 grape berry samples from Aleatico, Sagrantino, Sangiovese and Trebbiano cultivars (five replicates per sample) were excised in polyhedral pieces with a 5 x 5 mm outer base (placed on the external surface of the fruit) with a thickness of 8 mm (towards the inner tissues) and quickly frozen in liquid nitrogen. Samples were stored in liquid nitrogen to maintain their frozen-hydrated (FH) state until the Cryo-SEM analysis. At the moment of the analysis the FH sample was mounted, under liquid nitrogen vapors, on an aluminum stub with Tissue-Tek (Miles Inc., USA), and then moved to a cryo-preparation chamber

(SEM Cryo Unit, SCU 020, Bal-Tech, Balzers, Liechtenstein) and freeze-fractured by a motor-driven fracturing microtome at - 120 °C. During this step, the samples were surface-etched for 1 min at - 80 °C under high vacuum ($P < 2 \times 10^{-4}$ Pa) and sputter-coated with 8 nm of gold in an argon atmosphere ($P < 2.2 \times 10^{-2}$ Pa) to produce an electrically conductive surface. The FH specimen was then transferred to a cryo-stage (- 60 °C) inside a scanning electron microscope (Philips SEM 515, Eindhoven, the Netherlands). The grape tissue images (Figures 1, 2, 3, 6 and Figure Supplementary material S1) were selected for their representativeness of the different cultivars and sampling times. The external and internal characteristics of the berries were interactively measured on digital images by Analysis 2.02 software (Soft-Imaging Software GmbH, Germany). Each value reported in the boxplots (Figures 4 and 7) is the average of five measurements performed on four different biological samples for each thesis, so the values reported in Tables 1 and 2 are the average of twenty measurements. Epidermis and hypodermis thickness measurements were carried out on two and eight cell layers respectively. The hypodermis layers were measured from the first cell below the epidermis to the first mesocarp cells, recognisable by their larger area.

4. Statistical analysis

The data presented in this work were analysed using one-way analysis of variance (ANOVA) statistical tools ($p \leq 0.05$) and Tukey's honest significant difference (HSD) *post hoc* test, in order to identify significant differences of the thickness and area of the analysed layers between berries from different cultivars at harvest. Significant differences in the skin measurements among cultivars, and in layer thickness among D0 and D12 samples within the same cultivar were investigated by applying t-test or Wilcoxon's test (depending on the data distribution of the parameters) to run pair comparisons of the different cultivars and theses. Fold change analysis ($FC = \text{Log}_2 \text{D12/D0}$) and two-way ANOVA ($p \leq 0.05$) were also performed to find any differences between D0 and D12 samples and to study the interaction between the Cultivar and Treatment factors. Lastly, linear regression models were used to evaluate the different rate of weight loss among cultivars.

RESULTS AND DISCUSSION

1. Berry outer and inner characteristics at harvest

A Cryo-SEM analysis on frozen-hydrated grape berries from cultivars Aleatico, Sagrantino, Sangiovese and Trebbiano was carried out to investigate outer and inner tissue characteristics at harvest (Figures 1, 2 and 3). Both the external (surface) and the internal tissues were perfectly preserved at the end of the sample preparation procedure, maintaining their natural characteristics in terms of cellular architecture.

As regards the outer surface, the different cultivars showed high variability in epicuticular wax morphology at high magnification and resolution (Figure 1). In Aleatico, the epicuticular wax layer appeared to be structured in plate-like components smoothed at the edges (Figure 1a). Meanwhile, Sagrantino showed a completely different wax particle arrangement, with a higher number of thinner filaments (Figure 1b). Sangiovese and

Trebbiano waxes (Figures 1c and 1d) showed a similar composition of intricate vertical platelets, with fringed edges, but with different density and thickness. The epicuticular waxes of the analysed samples revealed genotype-related traits in their morphological features, as previously found for other cultivars (Rosenquist and Morrison, 1988; Commenil *et al.*, 1997; Rogiers *et al.*, 2004, 2005; Rustioni *et al.*, 2012).

Thickness measurements of the different grape berry skin tissue layers (Table 1) were performed on the images of the fractures (Figure 2), while ultrastructural data were obtained from the analysis of images at higher magnification (Figure 3). In these cryo-SEM images it is possible to recognise the different tissue layers of the skin, as well as to distinguish different cellular components (cell wall and protoplasts) in their natural hydrated state. Below the outer surface, which is made up of epicuticular waxes, the cuticle layer is clearly visible, below which the two cell layers of the epidermis and several cell layers of the hypodermis are also distinguishable (Figures 2 and 3).

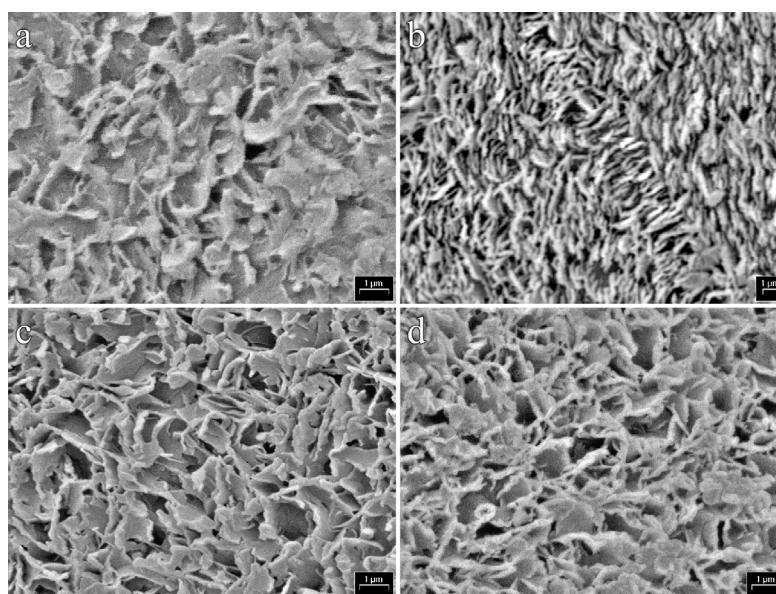


FIGURE 1. Cryo-SEM micrographs of epicuticular waxes of berry outer surface.

Aleatico (panel a), Sagrantino (panel b), Sangiovese (panel c) and Trebbiano (panel d) samples at harvest (D0).

TABLE 1. Thickness of grape berry skin tissue layers measured at harvest.

Measured parameters	Aleatico	Sagrantino	Sangiovese	Trebbiano
Epicuticular wax thickness (µm)	2.38 ± 0.40 a	1.66 ± 0.29 b	1.60 ± 0.09 b	1.38 ± 0.14 b
Cuticle thickness (µm)	1.48 ± 0.20 b	1.50 ± 0.26 b	1.58 ± 0.05 b	3.09 ± 0.16 a
Epidermis thickness (µm)	10.39 ± 1.87 b	11.12 ± 0.96 b	17.12 ± 2.12 a	16.84 ± 1.90 a
Hypodermis thickness (µm)	263.85 ± 20.16 b	235.96 ± 13.44 b	245.31 ± 10.13 b	346.68 ± 6.50 a

Data represent the mean (± SD) of twenty measurements performed on Aleatico, Sagrantino, Sangiovese and Trebbiano samples. Letters represent the result of a Tukey's HSD test after one-way ANOVA ($p \leq 0.05$) analysis to find differences between cultivars

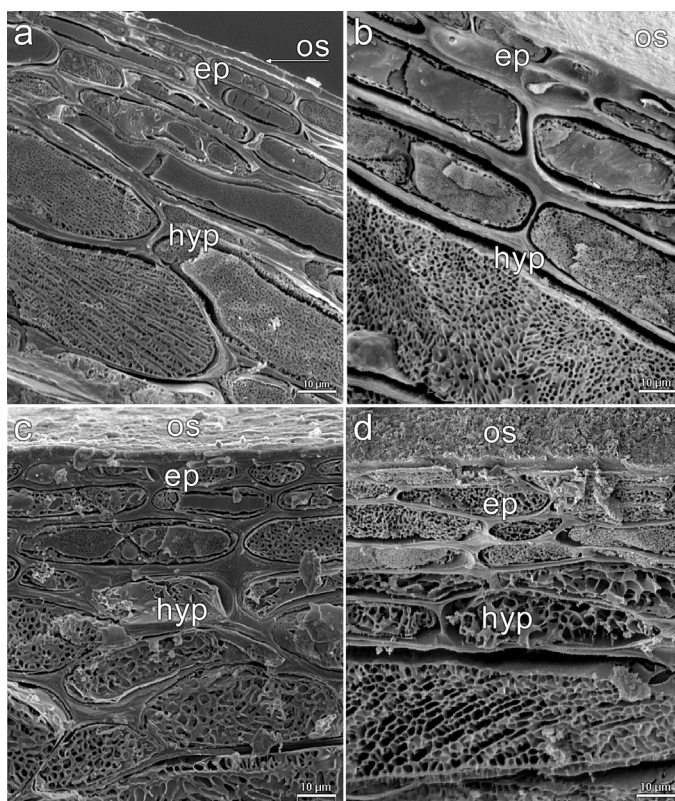


FIGURE 2. Cryo-SEM representative micrographs of skin fracture surfaces at harvest. Aleatico (panel a); Sagrantino (panel b); Sangiovese (panel c); Trebbiano (panel d). Epidermis (ep); hypodermis (hyp); outer surface (os).

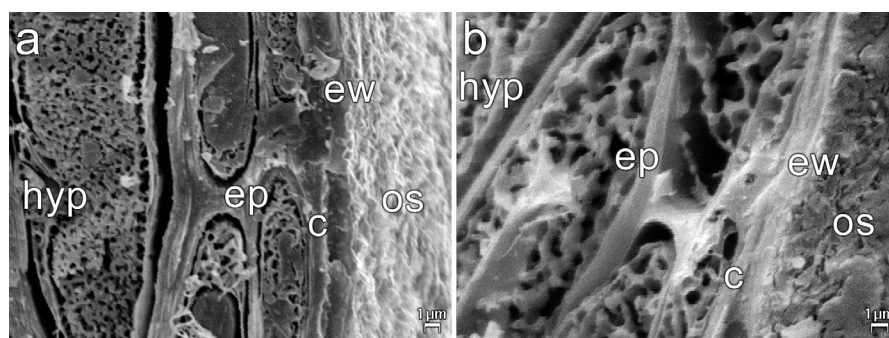


FIGURE 3. Cryo-SEM representative micrographs of skin fracture surfaces at harvest: details at higher magnification. Aleatico (panel a); Trebbiano (panel b).Epicuticular wax (ew); cuticle (c); epidermis (ep); hypodermis (hyp); outer surface (os).

Epicuticle wax thickness of Aleatico berries appeared to be significantly greater than the other cultivars, with Sagrantino, Sangiovese and Trebbiano showing similar thickness. For epidermis thickness, it was possible to cluster samples into two groups: Sangiovese and Trebbiano berries displayed thicker epidermal zones than Aleatico and Sagrantino. Meanwhile, Trebbiano berries showed the highest cuticle thickness, while the other three cultivars shared similar lower values. The Trebbiano cultivar was also characterised by having the thickest hypodermis, with the other cultivars showing non-significant differences between them.

The skin of a grape berry consists of a layer of epicuticular waxes, a layer of cuticle that covers the epidermis, and then an underlying outer hypodermis (Schlosser *et al.*, 2008; Battista *et al.*, 2015; Keller, 2020). Skin layer size (thickness) was obtained (Figure 4) as the sum of the thickness of all three layers (*i.e.*, cuticle + epidermis + hypodermis), as shown in Table 1. With the thickest skin, Trebbiano is clearly different from all the other samples, whereas no significant differences were observed among Aleatico, Sagrantino and Sangiovese berry skins. This result is mainly due to the fact that Trebbiano hypodermis is about 40 % thicker than that of the other three cultivars, and this specific

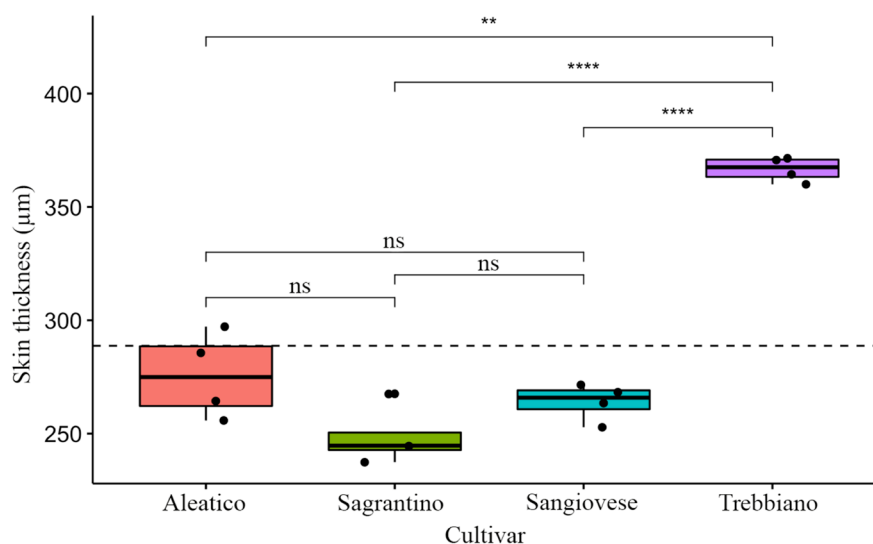


FIGURE 4. Skin (Cuticle + Epidermis + Hypodermis) thickness of grape berries at harvest.

Aleatico (red); Sagrantino (green); Sangiovese (light blue); Trebbiano (purple). Asterisks above the boxplot indicate significant differences between skin from different cvs (t-test; $p \leq 0.05$, *, $p \leq 0.01$; **, $p \leq 0.001$, ***, $p \leq 0.0001$, ****) while “ns” indicates non-significant difference.

cell layer constitutes most of the skin tissue, with epidermis and cuticle being much thinner.

These results clearly confirm that grape genotypes are characterised by differences in microstructural features of the skin, which most likely affect specific physical and mechanical properties of the outer layers of berries (Gabler *et al.*, 2003; Rolle and Gerbi, 2013).

2. Berry weight loss during dehydration

Berry WL increased linearly over time during dehydration, with significant differences among cultivars (Figure 5). Figure 5 also reports the formula for each specific linear model produced together with its R (correlation level between predicted and observed values) and p (statistical significance) values. In all models, a very high R value and a significant p value was observed, indicating in all cases that the models fitted very well with the measured weight losses of the different cultivars throughout the 12 days of dehydration. The Aleatico and Sagrantino berries showed an initial faster WL compared to the other two cultivars; this difference in cumulative WL was also found at the end of the 12 days trial. In fact, for Aleatico and Sagrantino, about 40 and 30 % of WL was recorded respectively. On the other hand, Sangiovese and, in particular, Trebbiano, showed more limited WL, reaching a value of about 18 % after 12 days of dehydration. As mentioned above, one of the factors affecting WL rate is fruit size and, consequently, the surface area/fresh weight ratio. Interestingly, the average weights of Aleatico

(showing the highest WL values) and Trebbiano (characterised by the lowest WL values) berries were quite similar (2.49 ± 0.34 and 2.66 ± 0.43 g respectively). Sagrantino and Sangiovese berries were the smallest, with an average fruit weight of 1.69 ± 0.19 and 1.96 ± 0.33 g respectively. This indicates that factors other than the surface area/fresh weight ratio play an important role in modulating the WL of berries.

It is well known that epicuticular waxes, together with the cuticle, act as a protective barrier against pathogenic fungi and reduce the loss of water due to transpiration (Commenil *et al.*, 1997), but our results do not show a link between wax thickness and the dehydration rate. In fact, despite Aleatico berries had the thickest wax layer, this cultivar also showed the fastest weight loss rate, with the other three cultivars possessing similar epicuticular wax thickness. With regards the wax layer in particular, our data suggest that other factors, such as wax composition and morphology, may affect the transpiration rate as recently demonstrated in apple fruit (Chai *et al.*, 2020). On the other hand, Trebbiano berries, which showed the lowest WL values, were characterised by the thickest skin, especially in terms of cuticle and hypodermis contribution.

In the following section of this paper, we focus on the histological features after dehydration of Trebbiano and Aleatico, the two cultivars that represent the two extremes in terms of WL performance, as well as skin thickness (Figures 4 and 5).

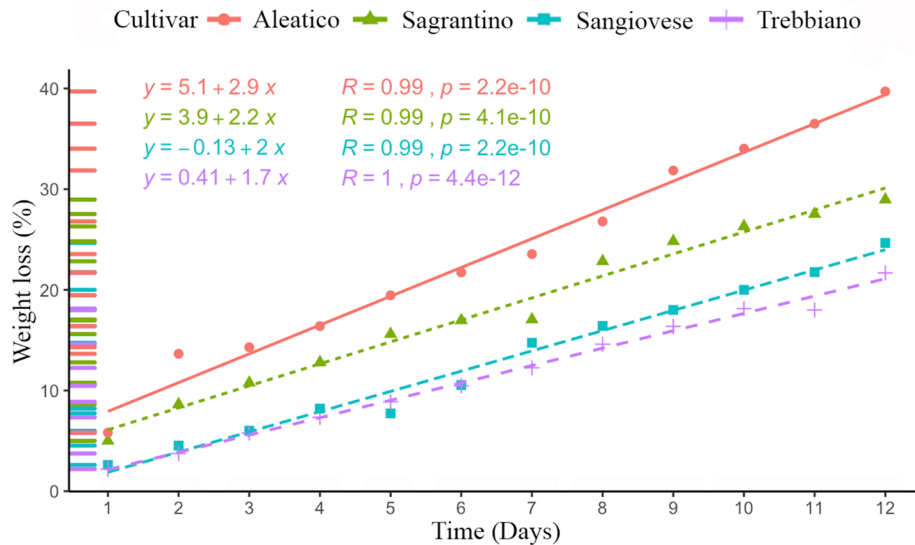


FIGURE 5. Time course of postharvest berry weight loss

Berries from Aleatico (red), Sagrantino (green), Sangiovese (light blue) and Trebbiano (purple) were maintained for 12 days in a controlled environment (23 ± 1 °C; 45 ± 5 % RH) to lose water. Regression line models and their R and p values are reported.

3. Berry characteristics after dehydration

As far as Aleatico and Trebbiano cultivars are concerned, images of fracture faces were used to study berry tissue histology after 12 days of dehydration (D12) (when they reached about 40 and 18 % of WL respectively), which was compared with their characteristics at harvest (D0). By comparing the D0 and D12 samples as shown in Figures 6 and S1 (Supplementary material) it is possible to observe the marked changes taking place in skin cell structure and layers after 12 days of dehydration. The alterations induced by WL are (as is to be expected considering the higher dehydration level) particularly evident in Aleatico berries, of which the vacuoles in both epidermal and hypodermal cells appeared to be compressed after the processing, thus becoming homogeneous.

Thickness measurements of the different grape skin tissue layers from Aleatico and Trebbiano were made on both D0 and D12 samples (Figure 7). All the measured parameters showed a general decreasing trend when comparing D0 and D12 samples. Skin, cuticle and hypodermis thickness revealed a significant decrease in both cultivars, regardless of dehydration intensity, whereas epicuticular wax thickness was only found to be significantly different for Aleatico out of all D0 and D12 samples; this might be the effect of the more pronounced dehydration level. The effect of the interaction between the investigated factors (Cultivar x Treatment) was analysed and a significant interaction (two-way ANOVA, $p \leq 0.05$) was detected for all

the parameters reported in Figure 7, except for epidermis thickness.

The effects of 12 days of dehydration on berry skin cell wall thickness and cell area, as well as on the mesocarp parenchyma cell area, were also studied (Table 2). It is evident that all the reported parameters decreased with water loss, except for epidermis cell wall thickness in both cultivars and hypodermis cell wall thickness in Trebbiano, which did not seem to be significantly affected by the postharvest dehydration level.

Epidermis and mesocarp parenchyma cell area appeared to be markedly affected by water loss in both cultivars, with higher FC values in Trebbiano epidermis, despite the lower level of dehydration. On the other hand, only Aleatico berries showed a significant decrease in the hypodermis cell wall thickness and the hypodermis cell area; once again, this might be due to the more pronounced level of dehydration compared to Trebbiano. A significant interaction between the Cultivar and Treatment factors was demonstrated by means of a two-way ANOVA test for hypodermis cell wall thickness and mesocarp parenchyma cell area. This suggests that the differing cell wall structure and composition of wine grape varieties (Ortega-Regules *et al.*, 2008; Apolinar-Valiente *et al.*, 2015) could also affect berry behaviour during postharvest dehydration.

Based on these observations, it can be hypothesised that a role in modulating postharvest water loss is played by the hypodermis, in addition to the outer structures (epicuticular waxes, cuticle).

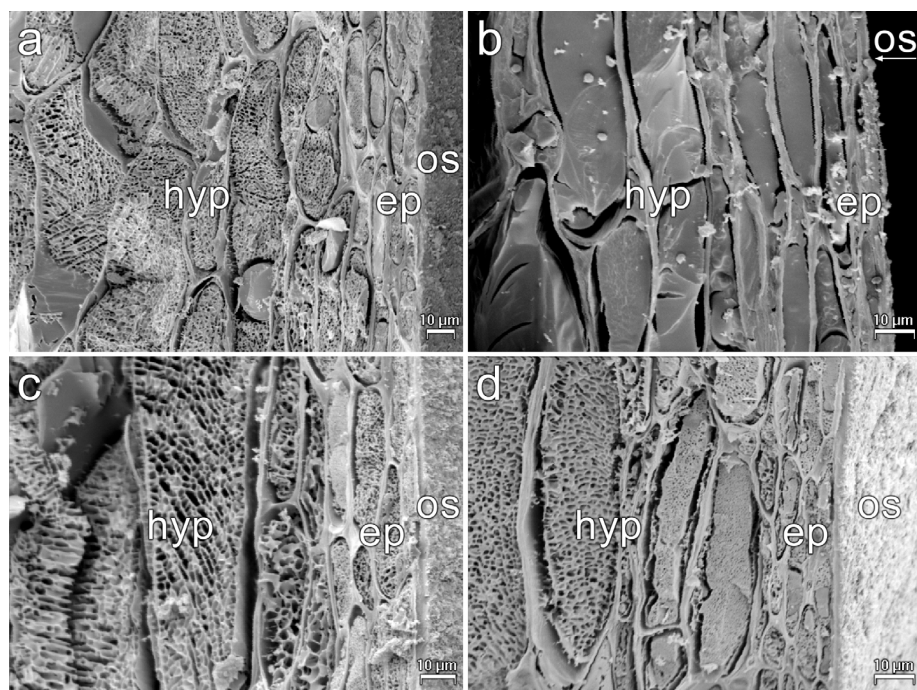


FIGURE 6. Cryo-SEM representative micrographs of skin fracture surfaces at D0 and D12 in Aleatico and Trebbiano. Aleatico (panels a and b); Trebbiano (panels c and d); D0 samples (panels a and c); D12 samples (panels b and d). Epidermis (ep); hypodermis (hyp); outer surface (os).

Atkinson *et al.* (2012) had already pointed out the importance of hypodermis cell wall modifications in relation to transpirational water loss in apple fruit. Only a few published studies have addressed this important cell layer in grape berry protection from different types of stress mainly related to infections, such as those caused by *Botrytis cinerea* (Gabler *et al.*, 2003). However, to the best of our knowledge, no data are available in the literature on possible hypodermis layer involvement in the response of grape berry to postharvest dehydration.

CONCLUSIONS

In a recently published review, Lufu *et al.* (2020) report that the factors which determine the difference in water loss among species and among cultivars of the same species are fruit surface-area-to-volume ratio, the surface structure of the fruit (including the number and size of stomata and lenticels), and cuticle thickness and composition. Our results indicate that these factors are indeed likely involved in the modulation of wine grape berry postharvest dehydration, along with the observed differences in epicuticular wax particle morphology, epidermis, and hypodermis thickness (which are determined by the genetic background and affected by agronomic and environmental conditions), as has already been observed for

table grapes and raisins (Riva and Peri, 1986; Crisosto *et al.*, 2001; Zhang *et al.*, 2001; Ramming, 2009).

Depending on the oenological goal (sweet, reinforced wines), the rate of WL in harvested grapes can be mainly controlled by setting appropriate environmental parameters (temperature, RH and ventilation), taking into consideration that different WL rates can result in wines with different styles and compositions. Therefore, the applied dehydration protocols must take into account the histology and morpho-anatomical differences in external pericarp features which exist among cultivars. The present paper clearly demonstrates that these differences, in particular those related to epicuticular waxes and hypodermis organisation, can explain the observed difference in WL rate between Aleatico and Trebbiano berries. The protocols applied in postharvest dehydration processes are often empirical, and the environmental parameters (if and when controlled) are modulated on the basis of the frequently measured weight loss parameter. It may be helpful for winemakers to have knowledge of how external berry tissue features determine (together with other factors) fruit permeability to water, to optimise and model the dehydration process by collecting physiological and technological data.

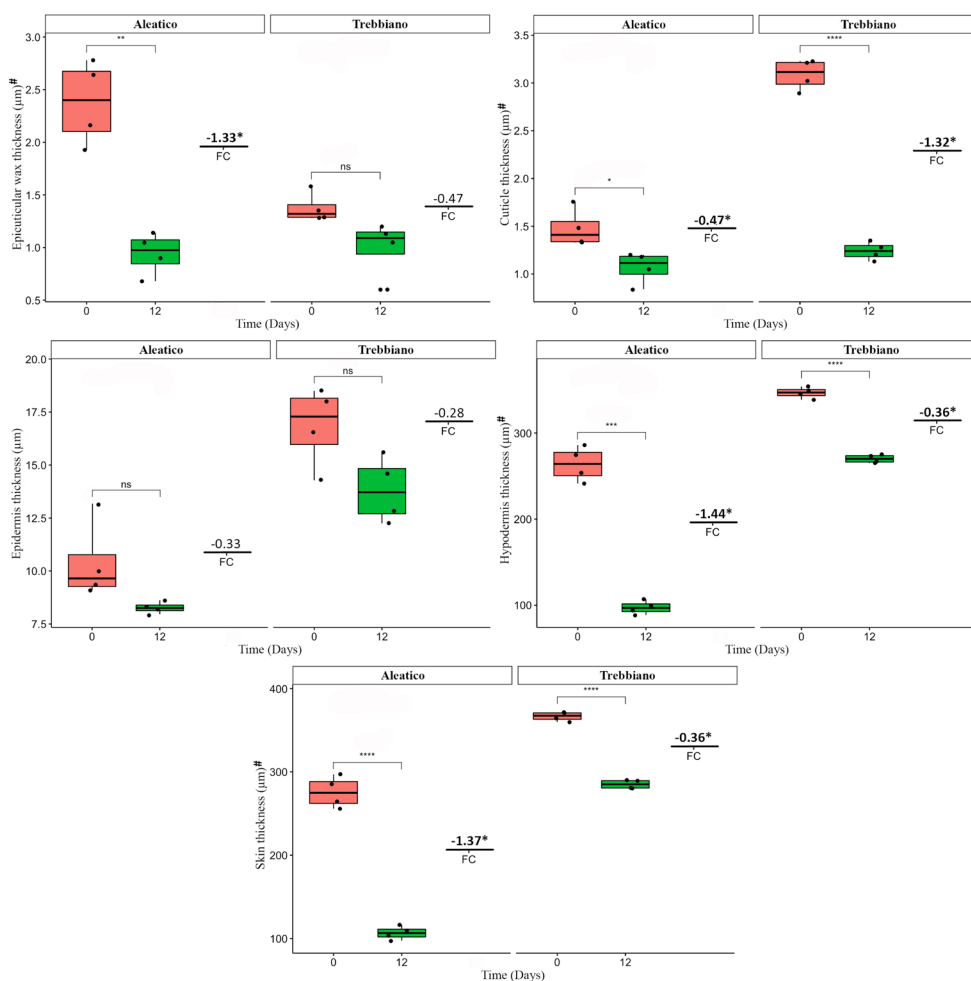


FIGURE 7. Thickness of grape berry epicuticular wax, cuticle, epidermis, hypodermis and skin (Cut + Epid + Hyp) at D0 and D12 in Aleatico and Trebbiano.

Asterisks above the boxplot indicate significant differences between D0 (red) and D12 (green) samples (t-test or Wilcoxon's test; $p \leq 0.05$, *, $p \leq 0.01$, **, $p \leq 0.001$, ***, $p \leq 0.0001$, ****; depending on parameters data distribution) while "ns" indicates a non-significant difference. FC represents the results of fold change analysis ($FC = \log_2 D12/D0$) and asterisks in bold indicate significant increase or decrease in thickness values (t-test $p \leq 0.05$). Hashtag symbols (#) on the different measured parameters indicate significant interaction between the Cultivar and Treatment factors (two-way ANOVA, $p \leq 0.05$). Colours depict D0 (red) and D12 (green) samples.

TABLE 2. Skin cell wall thickness and cell area, and mesocarp parenchyma cell area of Aleatico and Trebbiano at D0 and D12.

Measured parameters	Aleatico			Trebbiano		
	D0	D12	FC	D0	D12	FC
Epidermis cell wall thickness (µm)	0.71 ± 0.05	0.74 ± 0.04	0.05	0.60 ± 0.06	0.61 ± 0.05	0.04
Epidermis cell area (µm ²)	117.07 ± 23.29	63.30 ± 11.04	-0.89*	274.47 ± 108.26	96.53 ± 26.84	-1.51*
Hypodermis cell wall thickness (µm)#	1.34 ± 0.15	0.85 ± 0.19	-0.66*	0.82 ± 0.06	0.85 ± 0.08	0.06
Hypodermis cell area (mm ²)	0.0038 ± 0.0003	0.0012 ± 0.0005	-2.12*	0.0091 ± 0.0005	0.0059 ± 0.0005	-0.69
Mesocarp parenchyma cell area (mm ²)#	0.0732 ± 0.0336	0.0077 ± 0.0019	-3.15*	0.0532 ± 0.0096	0.0304 ± 0.0082	-0.81*

Average values ± SD of D0 and D12 samples are shown. Fold change ($FC = \log_2 D12/D0$) analysis is reported: values reported in bold and with the asterisk (*) indicate significant differences between D0 vs D12 (t-test or Wilcoxon's test, $p \leq 0.05$, depending on parameter data distribution). Hashtag symbols (#) associated with the different parameters indicate significant interaction between the factors Cultivar and Treatment (two-way ANOVA, $p \leq 0.05$).

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