Skin pectin metabolism during the postharvest dehydration of berries from three distinct grapevine cultivars

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Abstract

Background and Aims: Postharvest dehydration is used to modify certain grape berry quality traits that affect the characteristics of wine. After harvesting, berries undergo several chemical and physical changes that often reflect ongoing or specifically activated metabolic processes, such as the modification of cell wall polymers in the skin. We compared berry skin pectin metabolism during postharvest dehydration in three Italian wine grape cultivars.

Methods and Results: Corvina, Oseleta and Sangiovese grapes were postharvest dehydrated under the same environmental conditions and displayed different dehydration rates. The quantitative characterisation of pectins by ion-exchange and size-exclusion chromatography revealed that more demethoxylation and depolymerisation occurred in the Corvina berries, which also showed the strongest induction of pectin methylesterase and polygalacturonase gene expression (by microarray analysis) and enzyme activity (by zymography).

Conclusions: Postharvest dehydration affected the skin pectin metabolism of grape berries to an extent different in each of the three cultivars, reflecting differences in water loss kinetics and metabolic processes.

Significance of the Study: This is the first description of dynamic cultivar-specific changes in pectin metabolism during the postharvest dehydration of grape berries.

Keywords: gene expression, pectin metabolism, pectin methylesterase, polygalacturonase, postharvest dehydration

Introduction

The structure and composition of cell walls in the pericarp of grape berries have a direct impact on textural characteristics that impact wine production by influencing the extractability of phenolics and other compounds (Ortega-Regules et al. 2006, Bindon et al. 2012). The cell wall comprises a mixture of hemicelluloses, pectins and structural proteins embedded in a network of cellulose microfibrils. The exocarp (skin) has a content of cell wall material higher than that of the mesocarp (pulp) and is richer in pectins (Vidal et al. 2001, Ortega-Regules et al. 2008). The structure and composition of cell walls in both tissues, however, vary significantly among different grape berry cultivars (Nunan et al. 1998, Ortega-Regules et al. 2008).

Previous reports describing the dynamic expression profiles of cell wall-modifying enzymes and corresponding changes in cell wall composition during berry ripening have shown that the modification of pectins is primarily responsible for the progressive loss of firmness in ripening fruits (Nunan et al. 1998, 2001, Vidal et al. 2001, Doco et al. 2003, Grimplet et al. 2007). The activity of pectin methylesterase (PME), α-galactosidase and β-galactosidase increases after veraison, whereas polygalacturonase (PG) activity is low or undetectable (Selvaraj et al. 1995, Nunan et al. 2001, Deytieux-Belleau et al. 2008, Ortega-Regules et al. 2008). Although cell wall modification during ripening has been investigated, it is unclear what happens during the postharvest dehydration of berries. This technique is used to modify specific berry quality traits, (e.g. the sugar and alcohol content and the abundance of particular aromatic compounds) reflecting physical and chemical changes associated with water loss (e.g. weight loss, shrinkage and solute concentration) and with ongoing or specifically activated metabolic processes (e.g. changes in polyphenol profiles, the accumulation of stilbenes, proline, acetaldehyde and C6 volatile compounds, and the loss of terpenoids and malic acid) (Versari et al. 2001, Bellincinto et al. 2004, Franco et al. 2004, Costantini et al. 2006). The activity of enzymes, such as lipoxigenase and alcohol dehydrogenase, has been shown to change during postharvest dehydration (Costantini et al. 2006). Furthermore, PME activity increases constantly in dehydrating Aleatico berries whereas PG activity reaches a peak (Botondi et al. 2011), and PME activity was also detected at the end of dehydration in Erbaluce berries (Vincenzi et al. 2012). Grape berries that are dehydrated on the vine also undergo cell wall modifications, suggesting that this is a general feature of post-ripening. For example, skin texture characteristics, such as hardness and thickness, are modified in dehydrating Mondèuse berries (Rolle et al. 2009), and the demethylation of skin pectins that occurs during the ripening of Shiraz berries continues for several weeks after the berries have reached full ripeness (Vicens et al. 2009).

The induction of stilbene synthase gene expression in postharvest berries, as reported by Versari et al. (2001), was the first indication of large-scale changes in the berry transcriptome, reflecting the induction of genes involved in stress responses.
carbohydrate metabolism and transport, secondary metabolism (particularly the phenylpropanoid and terpenoid pathways) and cell wall metabolism (Zamboni et al. 2008, 2010, Rizzini et al. 2009, Bonghi et al. 2012). The metabolic changes observed in dehydrating berries, including cell wall modifications, may therefore be regulated predominantly at the mRNA level.

We investigated the changing pectin content during the postharvest dehydration of the berries of three Italian grape cultivars, Corvina, Oseleta and Sangiovese, which are characterised by different dehydration rates. We analysed the activity of the PMEs and PGs and the transcriptional profiles of the corresponding genes. We discuss the impact of dehydration on berry traits highlighting the important role of pectin metabolism in quality traits with an impact on winemaking.

Materials and methods

Dehydration studies

Berries from the grapevine (Vitis vinifera) cultivars Corvina, Sangiovese and Oseleta grown in neighbouring vineyards in the Verona-Valpolicella region of north-east Italy were harvested on the same date in 2010, at a similar stage of ripening. Approximately 100 kg of grape bunches from each cultivar were placed on wooden trays and stored in the same dehydrating room of about 750 m³ equipped with a dehumidifying/refrigeration air-conditioning equipment (Zanoni Impianti, Verona, Italy). The imposed environmental conditions represented the average values registered in non-conditioned dehydrating rooms over the same period for the previous 10 years, i.e. a gradually decreasing temperature (from 16 to 7°C) and a gradually increasing relative humidity (from 55 to 80%) starting on day 0 and ending on day 100 after harvest.

Berry weight loss and quality

Samples from all three cultivars were taken on harvest day (T0), day 13 (T1), day 26 (T2), day 47 (T3), day 69 (T4) and day 100 (T5) to determine weight loss and provide material for molecular analysis. The drying process was stopped when the weight loss reached approximately 30%. About 400 berries were collected from each bunch at each time point and split into three subsamples for quality (total soluble solids, titratable acidity and pH), biochemical (pectin characterisation and enzymatic activity) and transcriptome analysis, respectively, the latter immediately frozen in liquid nitrogen without the pedicel. Each subsample was further divided into three biological replicates. Soluble solids (ºBrix) were measured with a bench-top digital refractometer (model DBR35; Tsingtao Unicom-Optics Instruments Co. Ltd, Shandong, China). Titratable acidity was determined by titrating 7.5 mL of berry juice to pH 8.1 with 0.1 N NaOH, using bromothymol blue as a colourimetric indicator.

Berry skin preparation

Berries were manually pressed through three layers of gauze. Any visible pulp tissue was separated from the skin using a scalpel. The skins were weighed, immersed in distilled water and homogenised for 2 min at 4°C with an Ultra-Turrax homogeniser (IKA, Staufen, Germany). The resulting suspension was centrifuged (3500 g for 15 min at 4°C), and the skin pellet was lyophilised and stored at −20°C.

Pectin extraction and fractionation

Chelator-soluble pectin was extracted from 750 mg of powdered skin tissue in Na2 ethylenediaminetetraacetic acid (EDTA) as described by Silacci and Morrison (1990). Briefly, the powder was mixed with 45 mL of 95% ethanol and boiled for 10 min, then centrifuged (3500 g for 10 min) and the supernatant discarded. The procedure was repeated three times. The washed pellet was resuspended in 30 mL 20 mM Na2EDTA (pH 8.0) and extracted by boiling for 10 min. The supernatant containing the pectin was collected, and the pellet was subjected to two additional extractions. The pooled supernatants were dialysed against 50 mM sodium acetate buffer (pH 6.0) containing 20 mM Na2EDTA and fractionated by anion-exchange chromatography on a diethylaminoethyl (DEAE) cellulose (Serva Electrophoresis, Heidelberg, Germany) packed column (1.5 x 30 cm) connected to a peristaltic pump with a flow rate of 1 mL/min. Unbound pectin (UP) was collected in the flow-through fraction, whereas bound pectin (BP) was eluted with 0.6 M NaCl in the same buffer after a washing step (five column volumes). The UP and BP fractions were concentrated with a rotary evaporator, dialysed against water and lyophilised.

Determination of methoxylation degree

Ten milligrams of each lyophilised pectin fraction were solubilised in 12.5 mL water and subjected to saponification adding 12.5 mL 1 M KOH. After 30 min at room temperature, the solution was titrated to pH 7.5 with phosphoric acid and diluted to a final volume of 50 mL. The determination of methanol produced in the previous reaction was carried out following the method described by Klavons and Bennett (1986). Aliquots of 0.3–0.6 mL of samples obtained after treatment with KOH were diluted to 1 mL with 0.1 M KH2PO4 buffer pH 7.5 and added to 1 mL of alcohol oxidase solution (1 U/mL KH2PO4 buffer pH 7.5, Sigma-Aldrich, St Louis, MO, USA) in a glass tube. After 15 min incubation at room temperature, 2.5 mL of 0.02 M 2,4-pentanediene in 2 M ammonium acetate and 0.05 M acetic acid was added. After 20 min incubation at 60°C, the absorbance was measured at 412 nm. Known amounts of methanol were used to prepare a calibration curve. The methanol content was expressed as µmoles per 50 mL of solution. On the same KOH-treated samples, the content of pectin was determined as described later. The methoxylation degree was expressed as the ratio of methanol/galacturonic acid x 100.

Gel filtration

UP and BP fractions prepared from samples collected at maturation (T0) and at the end of drying (T5 for Corvina and Sangiovese, T3 for Oseleta) were analysed by gel filtration using a Sepharose® CL-4B 1.5 x 63 cm column (Sigma, Milano, Italy) in 50 mM sodium acetate running buffer (pH 6.0) containing 20 mM Na2EDTA and 0.15 M NaCl. The samples were solubilised in distilled water at 6 mg/mL final concentration; 1 mL was loaded onto the column. The eluate was fractionated using a Gilson FC203B automatic collector (fraction volume = 1.3 mL). The column was calibrated using dextran with different molecular masses (150 and 50 kDa, Sigma-Aldrich). The void volume (V0) and total volume (Vt) of the column were determined using Dextran T2000 and galacturonic acid, respectively. Collected fractions were analysed using the carbazole and phenol-sulfuric acid methods (see later) to quantify the uronic acids and total sugars, respectively.

Pectin content

The pectin content was determined by the carbazole method (Dische 1947). We added 0.3 mL of the sample, 0.1 mL water, 40 µL 4 M sulfamic acid (pH 1.6), 2.4 mL sulfuric acid and 100 µL 0.1% w/v carbazole to a glass tube. After 22 min of boiling, absorbance (525 nm) was determined with an ATI
Unicam UV-2 spectrophotometer (Ati-Unicam, Cambridge, UK). The pectin content was expressed as mg/mL of galacturonic acid (used as standard).

**Total sugar content**

The total sugar content was determined by the phenol-sulfuric acid method (Southgate 1991). We added 0.5 mL of the sample, 0.5 mL 5% w/v phenol and 2.5 mL sulfuric acid to a glass tube, and incubated at 25°C for 10 min followed by a further 10 min in cold water. The absorbance was read at 490 nm. The total sugar content was expressed as mg/mL of glucose.

**Enzyme extraction**

Pectinolytic enzymes were extracted by mixing 500 mg of lyophilised skins with 25 mL 0.1 M potassium phosphate buffer and 0.1 M ascorbic acid (pH 5.5) containing 0.1 M NaCl, 10% glycerol and 0.2% Triton X-100. After 2 h of stirring at room temperature, the suspension was centrifuged at 3500 g for 15 min, and the supernatant was filtered through Whatman paper. An aliquot of extract was desalted on a PD10 column (GE Healthcare, Milano, Italy) equilibrated with 10% glycerol in water and stored at 4°C.

**Enzyme activity**

A lyophilised preparation representing 1 mL of desalted extract was dissolved in 200 μL of an appropriate sample buffer depending on the electrophoretic procedure. For PME detection the sample buffer contained 62.5 mM Tris-HCl (pH 6.8), 1.3% w/v SDS and 10% w/v glycerol. The running gel was polymerised at 10% acrylamide (acrylamide/bis acrylamide: 29/1) including 0.5 mL 5% w/v phenol and 2.5 mL sulfuric acid to a glass tube, and incubated overnight at room temperature. The gel was then stained for 30 min with 0.05% methylene blue (Sigma) and destained in water.

For PG detection a continuous pH electrophoretic separation in 40 mM Tris-20 mM Caps buffer was carried out (McElhaney 1982). The running gel was polymerised at 7.5%. After the separation the gel was immersed in 100 mL 0.1 M sodium acetate (pH 4.5) containing 0.2% polyacrylamide (Sigma) and incubated overnight at room temperature. The gel was stained for 30 min with 0.05% ruthenium red (Sigma) and destained in water.

**RNA extraction and cDNA synthesis**

Total RNA for microarray analysis was isolated from ~200 mg of the ground berry tissue without seeds using the Spectrum Plant Total RNA kit (Sigma-Aldrich). RNA quality and quantity were determined using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and a Bioanalyzer Chip RNA 6000 series II (Agilent, Santa Clara, CA, USA). The cDNA synthesis, labelling, hybridisation and washing reactions were performed according to the NimbleGen Arrays User Guide v3.2.

**Microarray hybridisations and statistical analysis**

We used the NimbleGen 090818 Vitis exp HX12 microarray (Roche, NimbleGen Inc., Madison, WI, USA), representing 29 549 predicted genes based on the 12X grapevine V1 gene prediction. The chip probe design is available at the following URL: http://diblab.sci.univr.it/FunctionalGenomics/.

The microarray was scanned using an Axon GenePix 4400A at 532 nm (Cy3 absorption peak) and GenePix Pro7 software (Molecular Devices, Sunnyvale, CA, USA) according to the manufacturers’ instructions. Images were analysed using NimbleScan v2.5 software (Roche), which produces Pair Files containing the raw signal intensity data for each probe and Calls Files with normalised expression data derived from the average of the intensity of the four probes for each gene. Values reported in the figures and supplementary information are the means of three biological replicates at each time point. A gene was considered to be expressed if the normalised expression value was higher than the value obtained by averaging the fluorescence of the negative control present on the chip, for at least two of the three biological replicates. Differentially expressed genes during postharvest drying were evaluated using the significance analysis of microarrays (SAMs) statistical approach in the TMev software suite v4.3 (http://www.tm4.org/mev) with a false discovery rate (FDR) of 0.1%.

**Results**

**Characterisation of postharvest drying in Corvina, Sangiovese and Oseleta berries**

We found that each berry type exhibited distinct properties by comparing the postharvest drying characteristics of the three cultivars. The average berry weight at harvest was 2.3 g for Corvina, 1.2 g for Oseleta and 2.8 g for Sangiovese. The berries were stored under identical controlled environmental conditions until ~30% weight loss, but the dehydration kinetics of the three berry types differed to the extent that the drying process was stopped after ~50 days for Oseleta, and after 100 days for Sangiovese and Corvina (Table 1). Berry samples were collected at four time points during the shorter postharvest drying of Oseleta, whereas two additional time points were required for Sangiovese and Corvina berries so that the entire process could be monitored. The soluble solids content, titratable acidity and pH were recorded during the drying process (Table 1). Water loss had a greater impact on the juice sugar concentration in Oseleta berries than in the other cultivars. Sangiovese berries had the highest total soluble solids content due to a slight overdrying effect compared with that of Corvina and Oseleta. A general increase in titratable acidity was observed in Corvina, whereas Oseleta and Sangiovese showed an initial drop followed by an increase. The pH value increased uniformly in the berries of all three cultivars.

**Change in berry skin pectin level and composition during dehydration**

Pectins extracted from the berry skin by Na2EDTA were fractionated by anion-exchange chromatography on DEAE cellulose. This produced BP and UP fractions, the UP in the flowthrough and the BP eluting when NaCl was added to the buffer. The different behaviour of the two fractions predominantly reflects the degree of methoxylisation (Figure S1), with the UP fraction containing more methoxyl-derivatised galacturonic acids, neutralising the negative charge of the carboxylic acids and reducing their affinity for the positively charged DEAE resin. Figure 1a–c shows the time-dependent quantitation of total pectins extracted from Corvina, Sangiovese and Oseleta berries, with the amount of pectin at each time point expressed as mg (UP + BP) per gram of dry skin material. At harvest, the skin of Corvina berries contained a quantity of extractable...
pectins larger than that of Sangiovese and Oseleta berries, but there was a continuous reduction in the pectin level during the dehydration of Corvina berries suggesting that pectinolytic enzymes become more active during this process (Figure 1a). In contrast, although the pectin level varied during the dehydration of Sangiovese and Oseleta berries, the overall level was similar at the beginning and end of the dehydration process albeit over a shorter timescale in the Oseleta cultivar (Figure 1b,c).

Figure 1d–f shows the individual time-dependent quantitation of the UP and BP fractions in the three cultivars. At harvest, the UP fraction was more prevalent than the BP fraction in

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days after harvest</th>
<th>Weight (%FW†)</th>
<th>Soluble solids content (°Brix)‡</th>
<th>Titratable acidity (g/L)‡</th>
<th>pH‡</th>
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<tbody>
<tr>
<td>Corvina T0</td>
<td>0</td>
<td>100</td>
<td>21.4 ± 1.2</td>
<td>8.52 ± 0.9</td>
<td>3.04 ± 0.1</td>
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<td>13</td>
<td>92.7</td>
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<td>Corvina T2</td>
<td>26</td>
<td>87.4</td>
<td>24.0 ± 1.3</td>
<td>9.11 ± 1.0</td>
<td>3.10 ± 0.1</td>
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<tr>
<td>Corvina T3</td>
<td>47</td>
<td>80.8</td>
<td>24.4 ± 0.8</td>
<td>9.03 ± 0.8</td>
<td>3.13 ± 0.9</td>
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<tr>
<td>Corvina T4</td>
<td>69</td>
<td>72.7</td>
<td>24.8 ± 1.4</td>
<td>9.97 ± 0.9</td>
<td>3.10 ± 0.0</td>
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<tr>
<td>Corvina T5</td>
<td>100</td>
<td>69.7</td>
<td>28.8 ± 1.4</td>
<td>9.86 ± 1.1</td>
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<td>Sangiovese T0</td>
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<td>20.6 ± 1.0</td>
<td>7.16 ± 0.7</td>
<td>3.21 ± 0.12</td>
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<td>6.21 ± 0.4</td>
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<td>7.61 ± 0.4</td>
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<td>8.11 ± 0.6</td>
<td>3.11 ± 0.11</td>
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<td>32.2 ± 1.3</td>
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</table>

†Fresh weight. ‡Values were obtained by the analysis of three biological replicates of 40 berries each.
Corvina and Sangiovese berry skins (Figure 1d,e), whereas the BP was more prevalent in the Oseleta cultivar (Figure 1f). The UP/BP ratio declined during the drying of Corvina and Oseleta berries, probably reflecting demethylation and depolymerisation catalysed by pectinolytic enzymes. In Sangiovese berries, this reduction was limited to the first 47 days (T3) of the dehydration process.

The impact of hydrolytic enzymes on the pectin level during dehydration was investigated by analysing the size distribution of isolated pectins by gel filtration, revealing cultivar-specific differences also depending on the charge (Figure 2). All three cultivars contained a high-molecular weight component in the BP fraction represented by V₀ (Figure 2a, e and i), which was all but absent in the UP fraction (Figure 2c, g and k). The

Figure 2. Size distribution of bound pectin (BP) and unbound pectin (UP) fractions analysed by gel filtration at harvest (T0) and at the end of dehydration (T5) in (a–d) Corvina, (e–h) Sangiovese and (i–l) Oseleta berry skins. Solid line: uronic acids (carbazole method); dashed line: total sugars (phenol-sulfuric method).
chromatograms of Corvina berries at T0 and T5 provide evidence of extensive depolymerisation by T5, shifting the peaks towards low-molecular weight pectins in the BP fraction (Figure 2a,b) and the UP fraction (Figure 2c,d).

The BP fraction of Sangiovese berries showed less evidence of depolymerisation, although low-molecular weight pectins were present in Sangiovese berries from T0 to T5 (Figure 2g,h). In this case the signal produced by the carbazole method can be detected even in fractions eluted after Vₜ, indicating that oligosaccharides containing uronic acids bind non-specifically to the Sepharose gel. The BP fraction of Oseleta berries showed an unexpected increase in V₀ and >150 kDa peaks in the latter, indicating that the high-molecular weight polymers become more abundant during dehydration (compare Figure 2i,j).

Activity of PME and PG during dehydration
The activity of the major pectinolytic enzymes was assayed by zymography, in which the renatured enzyme can degrade a substrate copolymerised in the gel or added after electrophoretic separation (Figure 3). Both enzymes showed a discontinuous but time-dependent trend in activity, with a peak corresponding to the samples collected at T3 for Corvina and Sangiovese (Figure 3a,b), and at T2 for Oseleta (Figure 3c). There was almost no PG activity at T0 in all cultivars but PME activity was already present at this stage (Figure 3a–c) and was consistently higher over the whole dehydration period in Corvina berries (Figure 3d). Multiple bands (indicated by the arrows) were observed particularly for PG activity suggesting the presence of several isoforms with different electrophoretic mobilities (Figure 3d–f).

Transcriptional profiling of PME and PG genes
We carried out a comparative microarray analysis of the Corvina, Sangiovese and Oseleta berries, focusing on genes involved in pectin degradation, to provide transcriptional data complementing the biochemical results. To find genes that were differentially expressed during dehydration, we performed a multiclass comparison analysis for each cultivar using the SAM procedure in TMev 4.3 with an FDR of 0.1%. Among the modulated transcripts annotated against the V1 version of the 12X draft annotation of the grapevine genome (http://genomes.criibi.unipd.it/DATA/), we extracted genes belonging to the PME and PG gene families (Table S1) and found that 13 PME genes and three PG genes were differentially expressed during the Corvina berry drying process (Figure 4a). Six PME genes were strongly upregulated (VIT_06s0009g02560, VIT_06s0009g02600, VIT_06s0009g02630, VIT_06s0009g02590, VIT_06s0009g02570 and VIT_07s0005g00720), with expression peaking at the end of the process, sometimes exceeding fold change (FC) = 50 with respect to T0 (Table S1). Another four PME genes increased moderately after harvest albeit with different expression profiles, whereas the remaining three PME
genes were initially downregulated but increased thereafter (Figure 4a). Two PG genes showed a shallow but constant increase in expression during dehydration, whereas the third was initially downregulated followed by an increase in expression until the end of the process.

In Sangiovese berries, 16 PME genes and seven PG genes were significantly modulated during postharvest drying. The same six PME genes that were strongly induced in Corvina berries were also upregulated in Sangiovese berries (Figure 4b), although the fold change by the end of drying was lower than in Corvina berries and the upregulation was initially weak, followed by a stronger increase late in the process (Table S1). Three of the PME genes showing a moderate, steady increase in drying Corvina berries (VIT_04s0044g01000, VIT_16s0098g01900 and VIT_09s0002g00320) were initially downregulated in drying Sangiovese grapes before recovering to achieve fivefold changes in expression at the end of drying (Table S1). Among the seven modulated PG genes in Sangiovese berries, the three identified in Corvina berries were also expressed, albeit with slightly different expression profiles. Four genes were initially downregulated but were weakly induced by the end of drying, two were marginally induced and the third showed a discontinuous expression pattern (Figure 4b).

In Oseleta berries, four PME genes were differentially expressed during drying, but no modulated PG genes were identified (Figure 4c). The four PME genes were among the group of six PME genes strongly induced in the other cultivars, and in Oseleta berries they were also induced starting from T2. The fold changes, however, achieved at the end of the drying process were much lower than we observed in the other two cultivars.

**Discussion**

Grape berry dehydration is an important technical method used to improve certain berry quality traits during the production of premium wines. We carried out a comparative analysis of berry skin pectin metabolism in three Italian grape cultivars, Corvina, Sangiovese and Oseleta, during postharvest dehydration. Corvina is the principal cultivar used for the production of passiti wines (e.g. Amarone and Recioto) from the Verona province (Paronetto and Della Glio 2011), whereas the suitability of the Sangiovese and Oseleta cultivars for postharvest dehydration is not fully established.

The berries from the three cultivars were stored after harvest under the same environmental conditions but showed different drying kinetics, with Corvina the slowest and Oseleta the fastest.
The soluble solids content also differed among the cultivars, with the Oseleta berries showing more effective sugar concentration during dehydration than the others, possibly reflecting the smaller berry size and lower juice content. The titratable acidity of Corvina berries increased consistently, whereas there was an initial loss of acidity in the other cultivars in agreement with previous reports (Vicens et al. 2009, Becatti et al. 2010). The reduction in titratable acidity is largely due to the respiration of malic acid but is counterbalanced by juice concentration due to water loss. Therefore, the different titratable acidity kinetics in the three cultivars is likely to reflect the cumulative effect of these two phenomena.

Corvina berry skins contain more pectin than that of the other cultivars at harvest, and the UP fraction (with its higher content of methylated residues) is more prevalent than the BP fraction (Figure 1d). The total pectin level and the relative amount of methylated pectins, however, decline significantly during dehydration, and reach about 50% of the initial quantity at the end of the process. This suggests that postharvest dehydration has a profound impact on pectin metabolism in Corvina berry skin and may represent one of the key changes that positively affect Corvina grape quality traits for wine production by increasing the extractability of phenolics and other important compounds (Goulao et al. 2012). Indeed, differences in berry skin cell wall morphology and composition may explain cultivar differences in anthocyanin extraction during winemaking, such as the relation between anthocyanin extractability and the low level of pectin methylation in berry skins (Romero-Cascales et al. 2005, Ortega-Regules et al. 2008).

Sangiovese and Oseleta berry skins contain a level of pectin lower than that of Corvina berries at harvest, and the amount does not change significantly during dehydration. In Sangiovese berries, there is little change in the UP/BP ratio, suggesting that the level of methylated residues remains constant. In Oseleta berries, the UP/BP ratio declines marginally during drying, but the abundance of methylated pectins is already <50% at harvest. This may reflect the greater activity of PME enzymes and thus a higher turnover of pectins during ripening compared with that in Corvina and Sangiovese.

Overall these data indicate that the postharvest dehydration process has a marginal impact on pectin skin metabolism in Sangiovese and Oseleta grapes but a more substantial effect in Corvina berries. This is supported by the size-exclusion chromatography results, which show a clear shift from high- to low-molecular weight pectins in Corvina berries indicating significant depolymerisation activity, whereas this is less appreciable in the other cultivars (Figure 3). The concurrent activity of PMEs and PGs in Corvina berries could explain this phenomenon, with both enzyme activities peaking at T3 (corresponding to about 20% weight loss) but persisting until the end of dehydration. In contrast, the activity of PMEs and PGs in the other cultivars is discontinuous, confirming that the dehydration of Sangiovese and Oseleta berries has only a marginal impact on skin pectin metabolism. In Corvina berries, the coordination of PME and PG activity leads to the degradation of skin pectins, whereas the two enzymes appear uncoupled throughout dehydration in the other cultivars and may serve a different role. For example, PMEs could modify the pH and cation exchange properties of the cell wall, affecting the activity of other wall-degrading enzymes including galactosidases (Botondi et al. 2011). The demethylation of pectin is one of the most relevant changes in the skin cell wall during ripening (Nunan et al. 1998), but our data combined with the demethylation of pectins during the on-vine drying of Shiraz grapes (Vicens et al. 2009) and the increasing PME activity in Aleatico grapes undergoing postharvest dehydration (Botondi et al. 2011) strongly support an additional role for PMEs in the modification of cell walls after ripening.

PG activity may contribute to the pectin degradation during ripening, although previous reports suggest that the enzyme is not active at this stage (Nunan et al. 2001, Ortega-Regules et al. 2008). Our data confirm the absence of PG activity in harvested grapes but indicate that the enzyme is induced after harvest, particularly in Corvina berries, in agreement with previous experiments using Aleatico grapes (Botondi et al. 2011).

PME and PG gene expression profiles have been reported in ripening grapes (Barnavon et al. 2001, Nunan et al. 2001, Deytieux-Belleau et al. 2008), but few studies have considered post-ripening berries. The V. vinifera genome sequence and corresponding microarrays allowed us to identify PME and PG genes whose expression is significantly modulated during postharvest dehydration. We identified six PME and three PG genes that represent putative candidates involved in pectin demethylation and depolymerisation in Corvina berry skins. The expression of the PME genes increased constantly, achieving an up to 70-fold induction at the end of dehydration, whereas PG gene expression increased only marginally over the same period. The same PME genes were also modulated in Sangiovese grapes but not to the same extent, whereas the PG genes showed weak and variable expression. Four of the PME genes were also induced in Oseleta berries, albeit only weakly, and no modulated PG genes were identified.

Several studies have found that the final characteristics of dried berries are strongly dependent on the drying technique and the grapevine genotype (Bellincontro et al. 2004, Chkaiban et al. 2007, Barbanti et al. 2008). One key parameter that influences the quality traits of dried berries is the dehydration rate (Versari et al. 2001, Rizzini et al. 2009, Mencarelli et al. 2010). Grapes that dehydrate slowly rather than quickly need more time to lose weight and may thus be more subject to a senescence syndrome that may account for different metabolic and transcriptional responses. We have shown that postharvest dehydration affects berry skin pectin metabolism in a cultivar-specific manner based on different water loss kinetics and metabolic activity. Although these differences may rely in part on the genotype-dependent responsiveness to dehydration, our data once more suggest the importance of the dehydration rate in determining the quality traits of dried grapes.

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Pectin metabolism in grape postharvest dehydration


Supporting information

Additional Supporting Information may be found in the online version of this article: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12014/abstract

Figure S1. Methoxylation degree of purified unbound pectin (UP) and bound pectin (BP) fractions during dehydration of the three varieties Corvina, Sangiovese and Oseleta. The data obtained clearly show that UP fraction is characterised by a higher methoxylation degree that helps in explaining why this fraction is not retained in the column. On the contrary, BP fraction, due to a lower amount of methoxyl groups, and hence a higher degree of negatively charged residues, is bound to the diethylaminoethyl (DEAE) cellulose.

Table S1. Differentially expressed pectinesterase and polygalacturonase genes during dehydration of Corvina, Sangiovese and Oseleta berries. For each gene the gene ID, the gene description, the fluorescence values at each time point and the fold changes respect to T0 are reported.