Scientia Horticulturae 243 (2019) 78–83

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Changes in pathogenesis-related proteins and phenolics in *Vitis vinifera* L. cv. 'Sauvignon Blanc' grape skin and pulp during ripening

Bin Tian*, Roland Harrison, James Morton, Marlene Jaspers

Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand

ARTICLE INFO

Keywords:
Phenolics
Pathogenesis-related proteins
Protein stability
Ripening
'Sauvignon Blanc'

ABSTRACT

Grape proteins, particularly pathogenesis-related (PR) proteins, are responsible for white wine haze formation which is of great concern to winemakers. Phenolic substances in grapes have also been reported with involvement in protein haze formation by interaction with proteins. The protein and phenolic substances in grapes are important in determining their concentrations in juice and resultant wine, and thus consequent wine protein stability. This study investigated the changes in these haze formation related components in 'Sauvignon Blanc' grape skin and pulp during ripening. The phenolic content on per berry basis generally increased in grape skin and pulp, but the phenolic concentration on per gram of berry basis showed a decreasing trend in grape skin, suggesting the accumulation effect of phenolics in skin is less than the dilution effect caused by berry growth. Tannin was only detected in grape skin but not in the pulp and the changes in tannin were similar to total phenolics. PR proteins were synthesized and accumulated along with the increase of total soluble solids (TSS) in both skin and pulp from véraison until harvest during ripening. The increase of PR proteins in grapes during ripening was also reflected in corresponding juice. The results suggested most of PR proteins in juice is likely extracted from grape pulp, and the riper the grapes the more PR proteins may be extracted into juice. The higher level of phenolics in riper grapes could increase the extraction of phenolics into juice and thus decrease the final concentration of PR proteins.

1. Introduction

Grape-derived pathogenesis-related (PR) proteins, particularly thaumatin-like proteins (TLPs) and chitinases, have been shown to be involved in the process of haze formation in white wine (Waters et al., 2005, 1996). Phenolic compounds have also been implicated in wine haze formation through their interaction with PR proteins (Siebert, 1999; Siebert et al., 1996a,b). The TLPs and chitinases are major soluble proteins remained in finished white wine and they have been identified present in both grape skin and pulp (Deytieux et al., 2007; Monteiro et al., 2007; Tian et al., 2015b). Increase of proteins in grapes during ripening has been observed previously (López-Miranda et al., 2011; Murphey et al., 1989; Serrano-Megías et al., 2006), and the concentration of PR proteins in grapes increases (Deytieux et al., 2007; Giribaldi et al., 2007; Pocock et al., 2000) but the diversity of PR proteins decreases during ripening (Monteiro et al., 2007). The concentration of PR proteins in grape are also influenced by factors such as UV radiation and fungal infection (Tian et al., 2015a), and their concentration in juice could be impacted by various harvesting and processing conditions (Tian et al., 2017).

In an early report by Singleton (1966), a general trend was found of reducing total phenolics per unit weight of berry over time as the berry grew, but the total phenolic content per berry actually increased quite rapidly over a considerable portion of the berry development and ripening period. The increase of total phenolics and anthocyanin in red grape skin during ripening was also observed in a later study (Pirie and Mullins, 1980). In white grapes, the major phenolic compounds were studied by Lee and Jaworski (1989) who observed that hydroxycinnamic tartrates decreased continuously to a low concentration at harvest, while flavan-3-ols, procyanidins and their gallates increased sharply at véraison and then decreased to their lowest concentrations at harvest. In a recent review with regard to phenolics and ripening in grape berries, Adams (2006) described changes in phenolics in grape berries during ripening for different tissues: in the skin, tannins changed very little from véraison to harvest on a per berry basis (Harbertson et al., 2002), indicating that synthesis of tannin in skin occurred very early in berry development, but the mean degree of polymerization (mDP) of skin tannin increased during ripening (Kennedy et al., 2001); in the pulp, the dominant phenolic compounds were hydroxycinnamates which increased during the early stage of

* Corresponding author.
E-mail address: bin.tian@lincoln.ac.nz (B. Tian).

https://doi.org/10.1016/j.scienta.2018.08.018
Received 25 June 2018; Received in revised form 9 August 2018; Accepted 10 August 2018

ripening towards véraison, then remained constant on a per berry basis as the fruit ripened (Romeyer et al., 1983); in the seed, studies have shown that most of soluble phenolics were tannins which declined in concentration during ripening possibly due to the reduced extractability (Downey et al., 2003; Kennedy et al., 2000; Ristic and Iland, 2005).

Most of previously published studies focused on either proteins or phenolic substances in intact grape berries or in one specific grape tissue (e.g. grape skin), but since TLPs and chitinases have been found in both grape skin and pulp, it's worth knowing how these PR proteins along with phenolic substances develop in grape skin and pulp respectively during grape ripening. Such information would be useful to understand the complex interaction between PR proteins and phenolics during juicing process. This may help winemakers better manage the extraction of haze formation related grape components into juice, and thus reduce their concentration in resultant wine in order to reduce the bentonite requirement for protein stabilization.

2. Materials and methods

2.1. Grape and juice samples

Grape berries were collected from *Vitis vinifera* L. cv. 'Sauvignon Blanc' grapevines (MS1 clone) planted in Pegasus Bay vineyard (Waipara, New Zealand) on 11 consecutive weeks from pre-véraison until harvest in 2012. Grape bunches were randomly collected from two rows of grapevines at each sampling occasion, and 60 berries were randomly chosen from all positions of clusters for determination of mean berry weight, and then hand-squeezed to get juice for pH and TSS (Total Soluble Solids; °Brix) measurement. There was 5 mL of juice being stored at −20 °C for further analysis of PR proteins. For the analysis of grape tissue components, twenty berries were randomly selected as above and frozen using liquid nitrogen. The grape skin was collected by hand-peeling 20 frozen grape berries and the pulp was accordingly obtained by removing the seeds. The total quantity of grape skin and pulp were ground into powder with mortar and pestle and used to provide three replicate samples for analysis.

2.2. Extraction of grape components

2.2.1. Phenolics

Approximately 0.5 g of skin powder and 1 g of pulp powder were weighted and added to 5 mL of 50% (v/v) ethanol/water in 15 mL centrifuge tubes (Corning Life Science, USA). After vortexing the contents for one hour, the supernatant was collected by centrifuge (Centrifuge 5810R, Eppendorf, Germany) at 3000*g* for 30 min at room temperature (Nawaz et al., 2006).

2.2.2. Proteins

The protein extraction from grape skin and pulp was adapted from previously described protocols (Vincent et al., 2006). Approximately 2 g of skin powder and 2 g of pulp powder were vortexed in 5 mL of sucrose buffer (0.7 M sucrose, 0.5 M Tris-HCl pH 7.5, 50 mM EDTA, 0.1 M potassium chloride, 2 mM PMSF, 2% 2-ME and 1% PVPP) and incubated for 30 min at 4 °C, respectively. An equal volume of 1 M Tris-saturated phenol (pH 7.9) was added. The mixture was stored at −20 °C for 30 min with vortexing every 10 min. The phases were separated by centrifugation (for 30 min at 0 °C and at 3210*g*). The upper phenol phase was collected and re-extracted twice with an equal volume of sucrose buffer. From 5 mL initially collected of the phenol phase, 2 mL was recovered after two re-extractions. Five volumes of 0.1 M w/v ammonium acetate in cold methanol were added to the phenol phase followed by incubation at −20 °C overnight to precipitate proteins. The pellet was washed three times with 5 mL of cold 0.1 M ammonium acetate/methanol (w/v) and once with 5 mL of cold acetone.

2.3. Quantification of phenolics and proteins

2.3.1. Total phenolics

The concentration of total phenolics was determined using a micro scale protocol for the Folin-Ciocalteau colorimetric reaction method (Waterhouse, 2002). Total phenolics were quantified against gallic acid standard curve (0–500 mg/L). Grape skin extracts were diluted with an equal volume of 50% ethanol before the measurement. The absorbance readings were taken at the wavelength of 765 nm on a Unicam Heλios UV-VIS Spectrophotometer (Cambridge, UK).

2.3.2. Tannin

Total tannin was determined using the 1 mL assay of the methylcellulose precipitation (MCP) method by Sarneckis et al. (2006) as modified by Mercurio et al. (2007). Methylcellulose solution (0.04% of the product; Sigma-Aldrich, M-0387, Sydney, Australia, 1500 cP viscosity at 2%) and saturated ammonium sulphate solution (Sigma-Aldrich A4915, Auckland, New Zealand) were prepared according to Sarneckis et al. (2006). The standard curve was provided by 0–125 mg/epicatechin (Sigma-Aldrich, Auckland, New Zealand). Grape skin extracts (25 µL) were used for analysis, but not grape pulp as it contained no tannin. Measurements were carried out at 280 nm in a Unicam Heλios UV–vis Spectrophotometer (Cambridge, UK).

2.3.3. Total proteins

Total protein content in grape skin and pulp was determined using the EZQ protein quantification kit (Invitrogen, New Zealand) following the manufacturer's instructions. The calibration curve was developed using dilution series of ovalbumin (0–500 mg/L). Fluorescence measurements were taken using excitation/emission settings of 485/590 nm with a 96-well micro plate reader (FLUOstar Omega, New Zealand).

2.3.4. PR proteins by HPLC

relative quantification of TLPs and chitinases in protein extracts of grape tissues were carried out according to Tian et al. (2015b), and quantification of TLPs and chitinases in juice was conducted based on method described by Marangon et al. (2009) using reverse phase (RP) HPLC. In brief, protein extracts (50 µL) and juice (50 µL) were loaded at 1 mL/min onto a C8 column (4.6 x 250 mm, Vydac 208TP54), fitted with a C8 guard column kit (4.6 x 5 mm, Vydac 208GK54) which was equilibrated in a mixture of 83% (v/v) solvent B [0.1% trifluoroacetic acid (TFA) in 8% acetonitrile] and 17% solvent A [80% acetonitrile, 0.1% (v/v) TFA] and held at 35 °C. Proteins were eluted by a gradient of solvent A, from 17% solvent A to 49% solvent A in the first 7 min, from 49 to 57% in 7 to 15 min, from 57 to 65% in 15 to 16 min, from 65 to 81% in 16 to 30 min, and then held at 81% for 5 min before re-equilibrating the column in the starting conditions for an additional 6 min. Elution was monitored by absorbance at 210, 220, 260, 280, and 320 nm. Identity of TLPs and chitinases in samples was assigned from the 210 nm chromatogram by comparison of peak retention times to those of purified TLPs and chitinases from Chardonnay juice. In this study, the peaks eluting between 9 and 12 min were assigned as TLPs and the peaks eluting between 18 and 25 min were assigned as chitinases (Marangon et al., 2009; Tattersall et al., 1997; Waters et al., 1992). Protein quantification was conducted through comparison with the peak area of thaumatin from *Thaumatococcus daniellii* (Sigma-Aldrich, New Zealand), and protein concentration was expressed as thaumatin equivalent.

3. Results and discussion

3.1. 'Sauvignon Blanc' berry development

'Sauvignon Blanc' grapes were harvested in May, which was much later than usual because the 2012 vintage had one of the coldest