TOWARD A MODEL OF GRAPE PROANTHOCYANIDIN EXTRACTION DURING VINIFICATION•

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1. INTRODUCTION

Within the grape berry, proanthocyanidin (PA or condensed tannin) differs in its composition and extractability depending upon the compartment in which it occurs, namely seed, flesh or skin. Following vinification the concentration and composition of wine PA will depend upon its extraction from grape compartments, but also modification or loss due to adsorption by suspended insoluble cell wall material (CWM) (Bindon *et al*., 2010 a,b). In research, PA is frequently analysed as a whole-berry or wine measure, allowing a limited understanding of how it is extracted from skin, seed or flesh and this may account for a lack of correlation often observed from grape to wine. In addition, little attention has been given to date to the role of suspended CWM in modifying or removing PA from the wine during fermentation or settling. This study aimed to improve knowledge of how PA is extracted and modified during vinification by incorporating these two aspects into a preliminary model, using *Vitis vinifera* 'Cabernet Sauvignon' as the study system.

2. MATERIALS AND METHODS

2.1. Materials. Grapes from *Vitis vinifera* 'Cabernet Sauvignon' were obtained at five different ripeness stages from a commercial vineyard at Langhorne Creek, SA, Australia. Grapes were sub-sampled and then vinified in 3 x 50 kg lots.

2.2. Model extraction and wine analysis. Fresh berries were separated into skin, seed and flesh components and then extracted for 68 h in either ethanol/water (v/v) 10 %, 20 % or 50 %. PA in extracts or from wines was analysed spectrophotometrically for concentration (Sarneckis *et al.*, 2006), using phloroglucinolysis for subunit composition, and gel permeation chromatography (GPC) for molecular mass (MM) distribution (Bindon *et al.*, 2010a).

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2.3. Cell wall binding assay. Purified skin PA and cell wall material (CWM) from flesh and skin was prepared according to Bindon *et al*. (2010a). CWM (10 mg) was with PA (2 mg) in a 1 mL reaction volume according to the conditions. PA composition analysis before and after reaction with CWM was as for 2.2.

3. RESULTS AND DISCUSSION

Model extractions of fresh grape material showed that skin PA was more highly extracted than seed PA irrespective of grape ripeness or solvent (fig. 1). Flesh PA was found to be inextractable in any ethanol-based solvent, but was extracted in 70 % acetone (data not shown). Skin PA extraction increased with ripeness, and was more highly extracted as ethanol concentration increased. On the other hand, seed PA was poorly extracted in 10 % or 20 % ethanol, and generally declined with ripeness. Using 50 % ethanol, seed extraction increased with ripeness.

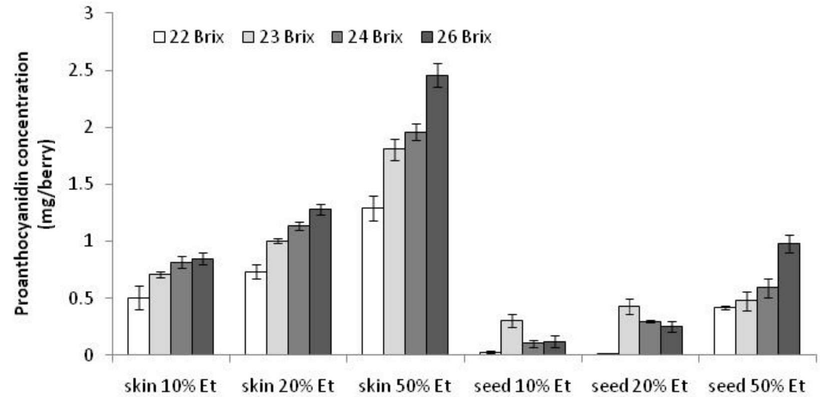

Fig. 1 - Recovery of PA in model ethanolic (Et) extracts of skin and seed at different grape ripeness stages.

When the extracts were compared for MM distribution, the MM of extracted PA increased from 20 % ethanol to 50 % ethanol (fig. 2). This indicated that a stronger solvent may extract a greater proportion of higher MM PA than would be extractable at wine ethanol concentrations, thus reflecting an over-estimation of PA extraction from the grape. In the model extracts, skin PA extraction increased with grape ripeness, as did its average MM (fig. 2).

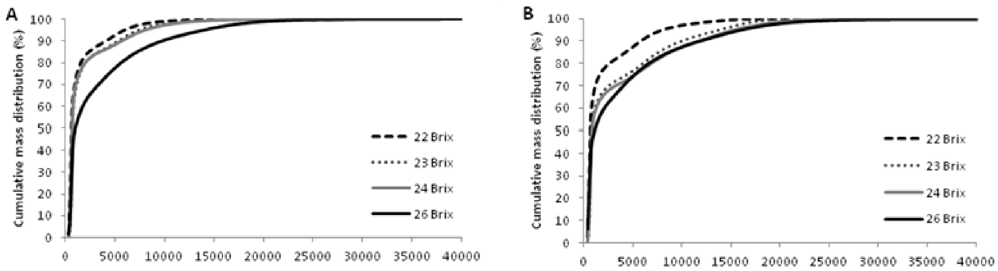

Fig. 2 - Cumulative molecular mass distribution of grape skin model extracts at different ripeness stages extracted in **A**. 20 % ethanol **B**. 50 % ethanol.

In the PA extracted from the corresponding wines, concentration followed the progression with ripeness observed for skin PA, with the lowest concentrations in wines of 12 % and 13 % ethanol, and highest in the 15 % ethanol wines (fig. 3). However, assay of the MM distribution of the wine PA showed only minor, insignificant differences in MM. The proportion of skin to seed PA extraction increased with grape ripeness, from 57 % skin in the 12 % wines to 72 % skin in the 15 % wines (fig. 3), which was consistent with the model extracts.

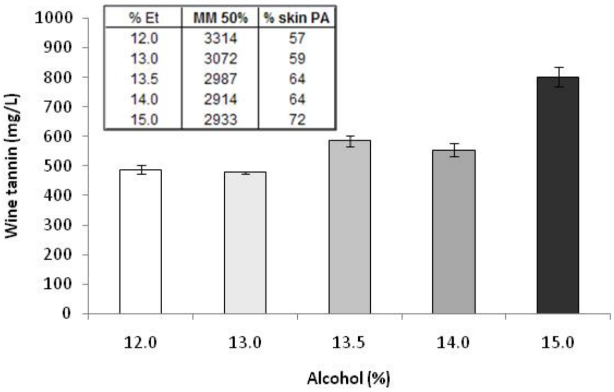

Fig. 3 - Wine PA concentration, average MM distribution (at 50 % elution) and % skin PA at different alcohol grape^{-1} ripeness levels.

The role of CWM in modifying PA MM distribution was assessed through binding experiments where purified skin PA was reacted with skin or flesh CWM in model wine solutions. This result was in agreement with previous work of Bindon *et al.* (2010a), where flesh CWM was shown to have a higher affinity for PA than skin CWM, preferentially removing higher MM PA (earlier elution by GPC) from solution (fig. 4A).

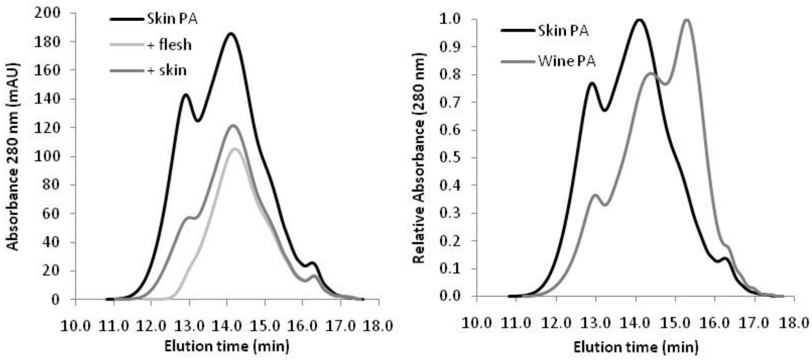

Fig. 4 - GPC elution profile of skin PA from grapes corresponding to 15 % alcohol.
A. Reaction of PA with either flesh or skin CWM
B. Comparison of skin PA and PA from the corresponding wine.

Comparison of skin PA and wine PA MM distribution showed that wine PA was more distributed toward the lower MM range than skin PA (fig. 4B). This may reflect fining of a proportion of higher MM PA from solution by suspended CWM during vinification, which is removed via the lees together with yeast debris during settling. However, this may also reflect limited extraction of higher MM PA from skin (or seed) during fermentation, due to its association with CWM within the cell, limiting PA desorption. Using the data generated in this study, it is possible to propose a preliminary model for PA movement from the grape cell during vinification (fig. 5). This reflects that extraction (k_E) may be limited by adsorption to CWM (k_A), which is higher for flesh than skin CWM. A further important factor is k_D which represents PA bound *in situ* to CWM within the berry, or PA to adsorbed suspended CWM during vinification which may be re-extracted. Future studies will seek to elucidate further factors contributing to this model, and it is discussed in greater detail in Bindon *et al.* (2010b).

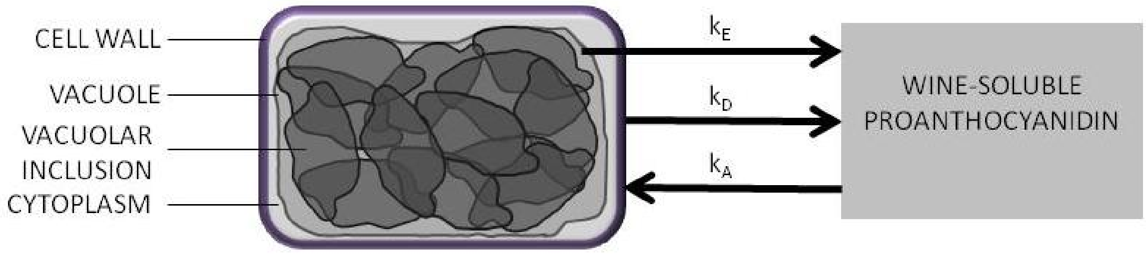

Fig. 5 - Conceptual model of PA movement during vinification showing rate constants for extraction from the vacuole (k_E), desorption from CWM (k_D) and adsorption to CWM (k_A) (adapted from Bindon *et al.*, 2010b).

Abstract

PAs are compartmentalised within the grape berry, and differ in their composition and degree of extractability. Within each compartment, the CWM limits PA extraction firstly by its degree of permeability and secondly its ability to complex with PA molecules. Grape CWM shows a high affinity for PA of higher MM. In addition, grape flesh cell walls bind PA more strongly than skin CWM, which renders flesh-derived PA inextractable in dilute alcohol solution. Analysis of wine PA has shown that skin-derived PAs are preferentially extracted over those derived from the seed in riper grapes, and PAs of higher MM are absent. The reason for the lack of high MM PA may be two-fold. Firstly, skin PAs of higher MM are poorly extracted in dilute alcohol, and require a strong solvent to break their association with the CWM. Secondly, suspended CWM modulates the MM distribution of extracted PA during fermentation, removing that of higher MM. Using 'Cabernet sauvignon' for a study of grape berry ripening, skin-derived PA was found to become more extractable in dilute alcohol solution as ripening progressed, and the MM of the extracted PA increased. This may reflect changes in both cell wall permeability and affinity for PA. A conceptual model for PA extraction is presented which proposes the inclusion of rate constants for total PA extraction (k_E), adsorption to suspended CWM (k_A) and desorption of CWM-bound PA (k_D).

Literature cited

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