Effect of Cap Management Technique on the Concentration of Proanthocyanidins in Muscat Bailey A Wine

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The concentrations of proanthocyanidins (PAs) were measured during red winemaking. In Cabernet Sauvignon (CS), PA concentration during maceration increased with increasing alcohol concentration and became constant after reaching a maximum. In contrast, in Muscat Bailey A (hybrid: Muscat Hamburg × Bailey, MBA), PA concentration increased, reached a maximum, and then decreased rapidly to below 20 mg catechin equivalent (CAE)/L. PA concentration was affected by the cap management method (submerged and punched down) and pressing. The decrease in PA concentration was suppressed in the submerged method compared to the punched down method. The final PA concentration in the submerged method was higher than that in the punched down method. Early pressing resulted in reduction on the rate of loss of PA.

Keywords: proanthocyanidin, Muscat Bailey A, cap management technique, red wine production

Introduction

Proanthocyanidins (PAs) are present in berry skin and seeds (Adams, 2006). Red wine contains more PAs than white wine because it is made from not only juice but also seed and skin.

PAs in grape berry are composed of flavan-3-ol subunits, such as catechin, epicatechin, epicatechin gallate, and epigallocatechin (Prieur et al., 1994; Souquet et al., 1996; Kennedy and Jones, 2001). Interflavan bonds are formed between the C4-C8 or C4-C6 positions of the monomer to produce polymerized compounds. The mean degree of polymerization (mDP) of PAs is reported to be approximately 2 to 17 in seed and 2 to 85 in skin (Prieur et al., 1994; Monagas et al., 2003; Mattivi et al., 2008). Grape variety, ripening stage, vineyard location, and vintage affect the amount and the chemical structure of PAs (Kennedy et al., 2001; Monagas et al., 2003; Mattivi et al., 2008; Hanlin and Downey, 2009).

PAs are the predominant polyphenols in red wines (Sacchi et al., 2005). PAs give an astringent mouth feel and markedly influence the quality of red wine (Fischer and Noble, 1994; Fontoin et al., 2008; Holt et al., 2008, Chira et al., 2009). They also play an important role in the stabilization of wine color (Remy et al., 2000; Romero and Bakker, 2000; Peng et al., 2002).

Some studies have reported the measurement of PA concentration in commercial red wine by the bovine serum albumin (BSA) precipitation method (Cliff et al., 2007; Harbertson et al., 2008). Harbertson et al. (2008) showed that PA concentrations in red wines were 672 mg catechin equivalent (CAE)/L (Cabernet Sauvignon, CS) and 559 mg CAE/L (Merlot, MER). The authors also showed that PA concentrations varied markedly (32-fold) even in wines made from the same variety.

In our former study, we measured PA concentrations in Japanese red wines by the BSA precipitation method (Ichikawa et al., 2011). The mean concentrations of the PAs were 65 mg CAE/L for Muscat Bailey A (hybrid: Muscat Hamburg × Bailey, MBA), 312 mg CAE/L for CS, and 356 mg CAE/L for MER. Many wines made from MBA showed very low concentrations of PAs. Indeed, most MBA wines have a light mouth feel, and that characteristic may be derived from their low PA concentrations.

PA concentrations varied by 13-fold in Vitis vinifera and more than 300-fold in MBA (Ichikawa et al., 2011). Most of the MBA wines used in this report were produced in the Ya-
manashi area, and therefore likely exposed to similar climate conditions and harvesting time. Thus, it is difficult to reason that the more than 300-fold difference in PA concentrations in MBA wines is due only to the grapes themselves. It has been reported that enological techniques (cold maceration, fermentation temperature, etc.) also influence PA concentrations in wines (Koyama et al., 2007; Busse-Valverde et al., 2011).

The general steps in red winemaking are as follows: after harvesting, clusters of grape are de-stemmed and crushed. The crushed grapes (must) are inoculated with yeast for alcohol fermentation. In some cases, the must is permitted to soak for a period of one to two days prior to inoculation of yeast (cold maceration). When the alcohol fermentation begins, carbon dioxide gas become trapped in the solids (mixture of skins and seeds), which are raised to the surface, forming a cap (1st fermentation). To prevent oxidation and acetic bacteria growth, and for extraction of phenolics (i.e. anthocyanins and PAs), the cap should be kept in the liquid (cap management). This step is called “maceration”. When desired components are extracted from skin or seed, solids are pressed and discarded. Pressed wine is further fermented (2nd fermentation).

The differences in these winemaking techniques may contribute to the variation in PA concentrations in MBA wines. PA concentrations in red wines obtained using different cap management techniques were measured with the aim to determine if this intrinsic variability could be explained by the effect of the cap management technique. Many cap management methods are developed and employed in red winemaking. The “Punch down method” is a traditional method in winemaking. The cap has to be punched down manually with a pole several times each day. The “Submerged method” is that the cap is kept submerged by placing a perforated screen just under the surface of the liquid. “Pumping over” is also commonly used for winemaking with relatively large scale fermentations. In this method, liquid is pumped up from bottom of the fermentation vat and sprayed over the cap. “Rotary mixing” of the cap is also used for large scale fermentation. The fermentation vessel itself is rotated horizontally to mix the cap with liquid. Punch down and submerged methods are compared in this report.

Materials and Methods

Grapes  Grapes (V. vinifera L. cv. Cabernet Sauvignon (CS) and hybrid: Muscat Hamburg × Bailey, Muscat Bailey A (MBA)) for 100 kg and 3.5 kg scale fermentation were harvested from the experimental vineyard of University of Yamanashi. In 2009, the grapes were harvested at commercial maturity (about 17 to 18 Brix for MBA and 18 to 20 Brix for CS) on September 28 (MBA) and October 5 (CS) and the corresponding total soluble solids were 17.8 and 21.0 Brix, titratable acidity were 6.1 and 6.9 g tartaric acid equivalent /L, and pH were 3.20 and 3.49, respectively. In 2010, the grapes were harvested on September 21 (MBA) and October 12 (CS) and the corresponding total soluble solids were 17.0 and 18.1 Brix, titratable acidity were 5.7 and 6.1 g tartaric acid equivalent /L, and pH were 3.45 and 3.61, respectively. Grapes used for 750 kg scale fermentation of MBA wine were products of a private vineyard in Yamanashi Prefecture, and were harvested on October 4, 2009.

Middle-scale fermentation  Wines were labeled according to variety, wet weight of grapes (in kg), and method of cap management (P, punched down).

CS (CS-100-P) and MBA (MBA-100-P) grapes were de-stemmed from bunches and crushed. Then, potassium pyrosulfite (100 mg/kg berry), which produces ≈ 50 mg/kg sulfur dioxide, was immediately added. The must was chaptalized with sucrose to give 21 Brix. Fermentation was accomplished in a stainless steel vessel by the addition of active dry yeast (Saccharomyces cerevisiae bayanus Lalvin strain EC1118, Lallemand Inc., Grenaa, Denmark) at 200 mg/kg. The cap was punched down twice a day (conventional method) and wine was sampled at this time. Meanwhile, MBA 750 kg scale experiments (MBA-750-P) were carried out in a private winery for a commercial product by a different enologist from that in the 100 kg scale experiments. Must was pressed on Day 7 (MBA-100-P), Day 9 (MBA-750-P), and Day 14 (CS-100-P). Middle-scale wine production tests were carried out in 2009.

Small-scale fermentation  Wines were labeled according to variety, wet weight of grapes (in kg), and method of cap management (P, punched down; S, submerged method; or S-pressed, submerged method and pressing).

Small-scale fermentation was carried out in a 5 L glass bottle using the cap submerged method as described by Sampio et al. (2007) or the punched down (twice a day) method. Grapes (3.5 kg) were hand-picked from bunches and crushed with a handmade mechanical rotor crusher. The must was sulfited with potassium pyrosulfite (150 mg/kg berry) and chaptalized with sucrose to give 21 Brix. Then, the must was divided into 3.5 kg lots in 5 L glass bottles and blanketed with CO2 by the addition of dry ice. Yeast (EC1118) was inoculated (200 mg/L) and fermentation was carried out in an air conditioned room at 25°C. Sampling was done once a day when a small piece of dry ice was added to prevent oxidation. Immediately, the samples were centrifuged at 15,000 × g for 5 min to obtain the supernatant for analysis. Must was pressed (HYDRO-PRESSE-40, SPEIDEI, Ofterdingen, Germany) on Day 4 (09MBA-3.5-S-Pressed), Day 6
(10MBA-3.5-S-Pressed), Day 8 (09CS-3.5-S-Pressed), and Day 9 (10CS-3.5-S-Pressed). In 2009 and 2010, 3.5 kg scale experiments were carried out in duplicate.

**General analysis** PA concentrations were measured by the BSA precipitation method as described by Harbertson et al. (2002). PA concentrations were expressed as CAE. Total phenols (TPs) were measured by the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as gallic acid equivalent (GAE). Alcohol concentration was measured using Alcomate (AL-3, Riken Keiki Co., Ltd., Tokyo, Japan). Brix was measured with a refractometer (MASTER-α, ATAGO Co., Ltd., Tokyo, Japan).

**Results**

**Middle-scale fermentation** PA concentrations increased with increasing alcohol concentration during maceration (Fig. 1 and 2). PAs are extracted during fermentation by increasing alcohol concentration, as reported by Singleton and Draper (Singleton and Draper, 1964) and Ozmianski et al. (1986). In CS-100-P, PA concentration increased during the first 6 days, after which it became constant. After pressing, the concentration did not change (Fig. 1). In MBA-750-P, PA concentration abruptly increased, reached a maximum at day 4, and then decreased rapidly to below 30 mg CAE/L at Day 7 (Fig. 2). MBA-100-P showed the same tendency as that observed for MBA-750-P (Fig. 2). The reproducibility of these PA and TP profiles was examined using CS and MBA with the microscale fermentation method (Sampaio et al., 2007) in 2009 and 2010. The submerged method and the conventional punched down method were also compared in terms of the extraction profiles of PAs.

**Small-scale fermentation** PA concentration increased abruptly after Day 2 for all experiments. At that time, the alcohol concentrations were approximately 6.0% (09CS), 7.0 – 9.0% (10CS), 7.0% (09MBA), and 4.5 – 7.0% (10MBA) (Fig. 3).

For CS wine made by the punched down method in both vintages, PA concentration increased during the first 8 days and leveled off thereafter (Fig. 3A and B). For CS wine made by the submerged method in both vintages, PA concentration increased with increasing alcohol concentration. Pressing increased PA concentration in CS wines at Day 8 (2009) and Day 9 (2010), after which it leveled off. PA concentration continued to increase in non-pressed lots. PA concentrations in MER and Syrah also showed a similar tendency to that in CS (data not shown).

In MBA wine made by the punched down method in both vintages, PA concentration abruptly increased and reached a maximum at Day 3. Then, it decreased rapidly after Day 4 (09MBA-3.5-P) or Day 6 (10MBA-3.5-P) and reached values that were below 20 mg CAE/L (Fig. 3C and D). These results were consistent with those of the middle-scale experiment described above (Fig. 2). In MBA wine made by the submerged method, PA concentration increased during the early stage of maceration and the tendency was similar to that observed in the punched down method. The final PA concentration, however, was higher than that of the punched down method. Notably, the decrease in PA concentration was better suppressed by the submerged method than the punched down method in 2010.

**Fig. 1.** Changes in PA concentration (mg CAE/L) (solid line) and alcohol concentration (%) (dotted line) during CS-100-P (○) middle-scale fermentation.

**Fig. 2.** Changes in PA concentration (mg CAE/L) (solid line) and alcohol concentration (%) (dotted line) during MBA-100-P (○) and MBA-750-P (●) middle-scale fermentation.
The change in PA concentration during maceration differed between CS and MBA. PA concentrations in *V. vinifera* (CS, MER, and Syrah) increased with increasing alcohol concentration and leveled off after reaching a maximum. In contrast, in MBA, PA concentration increased, reached a maximum, and then decreased rapidly during maceration. PAs in MBA grape might have unique characteristics compared with those in the other varieties, because the decrease in PA concentration in MBA wine during maceration was observed in two consecutive vintages despite the difference in enologists. The former report indicated that MBA wine had

The rate of decrease was suppressed by pressing (09MBA-3.5-S-Pressed and 10MBA-3.5-S-Pressed), as shown in Figures 3C and D. TP concentration showed the same tendency as PA concentration (Fig. 4). In CS 3.5 kg scale winemaking, TP concentration increased during maceration (Fig. 4A and B) and no reduction in TP concentration was observed for all CS wines.

In MBA 3.5 kg scale winemaking, TP concentration decreased after reaching a maximum at Day 5. The rate of decrease was obviously high in the punched down method and this decrease was equal in amount to the decrease in PA concentration.

**Discussion**

The change in PA concentration during maceration differed between CS and MBA. PA concentrations in *V. vinifera* (CS, MER, and Syrah) increased with increasing alcohol concentration and leveled off after reaching a maximum. In contrast, in MBA, PA concentration increased, reached a maximum, and then decreased rapidly during maceration. PAs in MBA grape might have unique characteristics compared with those in the other varieties, because the decrease in PA concentration in MBA wine during maceration was observed in two consecutive vintages despite the difference in enologists. The former report indicated that MBA wine had
and cell wall materials (Bindon et al., 2010) bind with PAs. The removal of skin and seed by pressing might have resulted in the elimination of compounds that have high binding affinity towards PAs, thereby removing them from wines, because the amount of decreased TP was almost the same as that of PAs, in this study. Although the skin of MBA grapes became pulpy during maceration, that of CS grapes did not. This difference in the physical properties of grape skin might affect the extraction of PA-binding compounds into wines. In addition, the final PA concentration in MBA wine made with the submerged method was higher than that in wine made with the punched down method. Compared to the submerged an extremely low concentration of PAs compared to the other varieties (Ichikawa et al., 2011). The decrease in PA concentration during maceration may be the cause of MBA wines having extremely low concentrations of PAs.

It is considered that the presence of skin and/or seed in must may have an effect on the decrease in PA concentration because the rate of decrease in PA concentration during maceration was suppressed by pressing. It was speculated that PA might be adsorbed onto the skin.

Some studies have reported that polysaccharide (Mercurio et al., 2007; Sarneckis et al., 2006), protein (Hagerman and Butler, 1978; Harbertson et al., 2002; Heredia et al., 2006), 

Fig. 4. Changes in TP concentration (mg GAE/L) during 3.5 kg scale fermentation experiments (3.5-S (○), 3.5-S-pressed (●), and 3.5-P (△)). (A) 2009 CS; (B) 2010 CS; (C) 2009 MBA; (D) 2010 MBA. Timing of pressing is shown by an arrow. Error bars indicate ± SD.
method, the punched down method might have physically damaged the grape skin tissue, resulting in the extraction of PA-binding compounds into wine. The chemical structure and extractability of PA is known to change during the ripening of grape (Kennedy et al., 2001; Hanlin and Downey, 2009). Further research should aim to make the relation clear between grape maturity and extractable PA, for both CS and MBA. Wine was blanketed with CO₂ from dry ice to prevent oxidation when the cap was punched down. However, it is possible that must was oxidized during the punching down procedure.

From the results described above, cap management techniques (strength, term, and method) might markedly affect PA concentration, particularly in MBA wines, and these differences in winemaking techniques might be responsible for the 300-fold difference in PA concentration in MBA wines (Ichikawa et al., 2011). For red wine, the viticultural area for MBA cultivation is the largest in Japan. Many Japanese wineries make MBA wine. From these results, it appears that to maintain a high PA concentration in the production of MBA wine, the submerged method is more effective than the punched down method, and wine pressing should be done earlier (at Day 5) than the conventional manner (at Day 8). It is necessary to identify the specific factors that affect the decrease in PA concentration during fermentation.

Conclusion

During maceration, PA concentration increased and reached a maximum, after which it became constant in CS. However, in MBA, PA concentration increased, reached a maximum, and then decreased rapidly.

In addition, the final PA concentration in MBA wine made with the submerged method was higher than that in wine made with the punched down method. Additionally, the pressing slowed down the rate of decrease in PA concentration. From the results described, cap management during maceration is important to control PA concentration, and thereby further improve wine quality.

References


