［Original Paper］

Extraction of Proanthocyanidins during Fermentation of Muscat Bailey A and Cabernet Sauvignon Wines

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Abstract: As we reported earlier, Muscat Bailey A (MBA) wine has very low proanthocyanidin (PA) content compared with Cabernet Sauvignon (CS) and Merlot wines. Whereas PA concentration in CS was maintained at the maximum level during maceration, that in MBA reached a maximum approximately four days after maceration and drastically decreased thereafter. In this study, the model wine extraction profiles of seed total phenols (TPs) and PAs in MBA and CS were compared. The final TP concentration extracted from seeds obtained from 1 kg of grape berry with 1 L of model wine was 256 mg/L and 1,428 mg/L for MBA and CS, respectively. The final PA concentration was 29 mg/L for MBA and 738 mg/L for CS. Changes in TP and PA concentration in fermenting must were also compared. Wines were made with/without skins and seeds. For wines fermented without seeds (skin samples), TP concentration peaked at days 4 to 6 and declined thereafter for both cultivars. For wines fermented without skin (seed samples), TP concentration started to increase from day 7 to reach the maximum concentration of 1,191 mg/L for CS, but not for MBA. The same tendency was shown for PA concentration. These findings suggest that the extractable PA content in grape berry differs between the two cultivars. The final TP concentration was 2,107 mg/L for CS wine and 1,013 mg/L for MBA wine, and the difference was only twofold. In contrast, the final PA concentration was 485 mg/L for CS wine and 19 mg/L for MBA wine, and the difference was 25-fold.

Abbreviations: CS, Cabernet Sauvignon; MBA, Muscat Bailey A; PA, proanthocyanidin; TP, total phenol.

Key words: proanthocyanidin, tannin, wine, grape, fermentation

Introduction

Astringency is an important aspect of red wine quality, and PA (condensed tannin) is responsible for this attribute (Gawel 1998). PAs are flavonoid compounds consisting of polymeric flavan-3-ol subunits, and are extracted from skins, seeds, and stems during maceration. PA quantity and composition in wine vary due to differences in winemaking practices, and have been associated with differences in astringency. It has been shown that wine grade is related to skin-derived PAs, suggesting that the maximization of skin PA concentration and/or proportion is related to the increase in projected wine bottle quality (Kassara and Kennedy 2011). Harbertson et al. (2008) measured PA concentration in red wine and showed that statistical differences existed among cultivars. They also showed that within a single variety, the variation in PA concentration was larger than one order of magnitude, and in two varieties (CS and Pinot Noir), the variation was 32-fold. Those findings suggested that controlling PA concentration is important for red

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winemaking.

Muscat Bailey A (hybrid grape: Muscat Hamburg × Bailey, MBA) is native to Japan and its wine is very popular in Japan. MBA wine has a very low concentration of bovine serum albumin (BSA)-precipitated PAs (65 mg/L) compared with wines made from other varieties, such as CS (312 mg/L) and Merlot (356 mg/L), in Japan (Ichikawa et al. 2011). To clarify why MBA wine has such a low PA concentration, PA concentration was measured during red winemaking in MBA and other varieties. Although PA concentration in MBA must was relatively low, it was increased during the first four days of maceration (punchdown method) and was decreased steeply thereafter. This phenomenon was reproducibly observed in laboratory-scale (3.5 – 80 kg) and commercial-scale (750 kg) MBA wines. TP concentration was also decreased after maceration for five days. Such decreases in PA and TP concentrations were not detected in CS wines. Although alcohol content is known to affect the extraction rate of PAs from skins or seeds into must (Hernández-Jiménez et al. 2012), the extraction mechanisms of PAs are not precisely known.

To clarify the phenomenon observed in MBA, model wine extraction and fermentation experiments were conducted for both MBA and CS.

Materials and Methods

Grape materials. Grapes were grown in the experimental vineyard of the University of Yamanashi in 2012. MBA was harvested on 28th September (17.2 °Brix) and CS was harvested on 16th October (21.4 °Brix).

Measurements of PA and TP concentrations and color intensity. PA and TP concentrations were measured using the BSA precipitation method and the Folin-Ciocalteu method, as described previously (Ichikawa et al. 2011). Color intensity (sum of absorbance at 420, 520, and 620 nm) was monitored as described by Yokotsuka (Yokotsuka 2000).

Extraction of seed TP and PA in model wine. Seeds were air-dried and used for experiments. The model wine consisted of 5 g/L potassium hydrogen tartrate and 12% (v/v) ethanol, and pH was adjusted to 3.3 with hydrochloric acid. Seeds obtained from 1 kg of grape berry (10.0 g for MBA and 18.9 g for CS) were soaked in 1 L of model wine in a 1 L glass bottle and were shaken at 25 °C in the dark. The headspace was substituted with N_2 gas to prevent oxidation. Two independent experiments were conducted.

Separate fermentation experiments. Grapes were harvested and destemmed by hand. Berries (47 kg for MBA and 51 kg for CS) were divided into three portions and crushed by hand, and 75 mg/L SO_2 was added as potassium pyrosulfite immediately. Fermentation was conducted at 3.5 kg scale in a 5 L glass bottle as described by Sampaio et al. (2007). Control samples were fermented with skins, seeds, and pulp as in conventional red winemaking. Skin samples were fermented with skins and pulp (without seeds). Seed samples were fermented with seeds and pulp (without skins). Because MBA had a low Brix, the obtained must was ameliorated to give a final °Brix of 21 with sugar. Fermentation was started by adding hydrated dry yeast (EC1118) on day 1. The must was mixed twice a day by rotating the fermentation bottles. Three independent fermentation experiments were carried out.

Results and Discussion

Extraction of seed TP and PA in model wine. Seed phenols were extracted with model wine, and TP and PA concentrations were measured during extraction. Because of the difference in berry diameter between the two cultivars (20 mm for MBA and 13 mm for CS), the weight of seeds obtained from 1 kg of berries was higher for CS (18.9 g) than for MBA (10.0 g). The final concentrations of TP and PA were 256 mg/L and 29 mg/L for MBA, respectively (Fig. 1A). In contrast, those were 1,428 and 738 mg/L for CS, respectively (Fig. 1B). This is one of the reasons why MBA

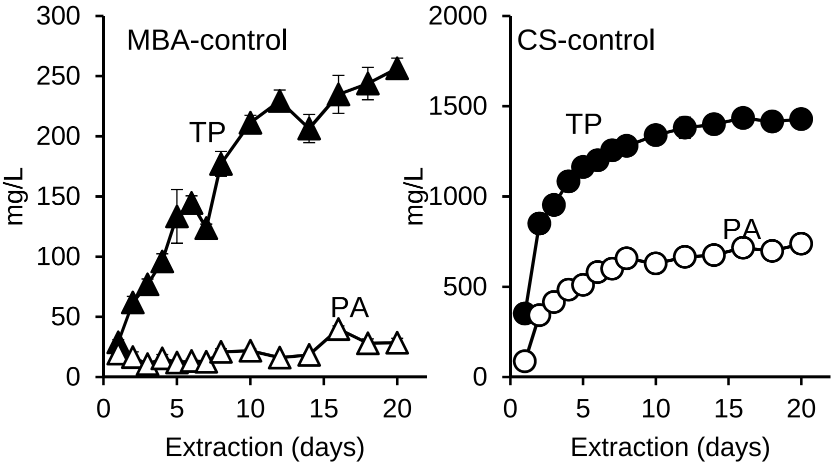

Fig. 1 TP and PA extraction from MBA (triangles) and CS (circles) grape seeds in model wine. Bars show standard deviations.

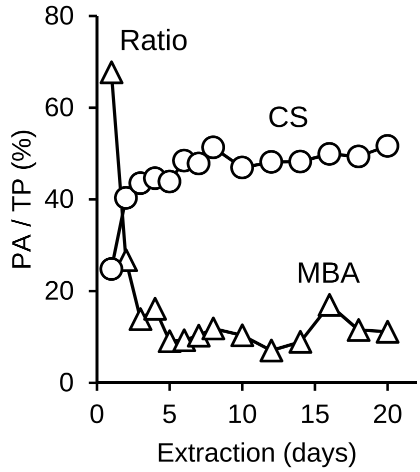

Fig. 2 Percentage of PA in TP. MBA, triangles; CS, circles.

wine has a very low concentration of PA compared with CS wine. The extraction rate of TP was also lower for MBA than for CS. The percentage of PA in TP was also significantly different between the two cultivars (Fig. 2). The results suggested that seed phenol composition differed between MBA and CS.

Separate fermentation experiments. To elucidate why PA concentration in MBA wine was extremely low, separate fermentation experiments on seeds and skins were conducted. Although alcohol formation was faster in MBA than in CS, no distinct differences were observed among the three samples (control, skin, and seed samples), and alcohol concentration reached approximately 12% (Fig. 3). During fermentation, color intensity showed an increase during the first 4 to 6 days and a decrease thereafter for both cultivars (Fig. 4). This decay of the color intensity is partly considered to be the effect of copigmentation (Boulton 2001). Compared with the skin sample, the color intensity was slightly high in the control sample. The color intensity was quite low in the seed samples of both cultivars because of the absence of skins from which anthocyanins could be extracted.

In control samples, TP and PA concentrations were increased up to fermentation days 4 to 5 for both cultivars, although the concentrations were low for MBA (Fig. 5

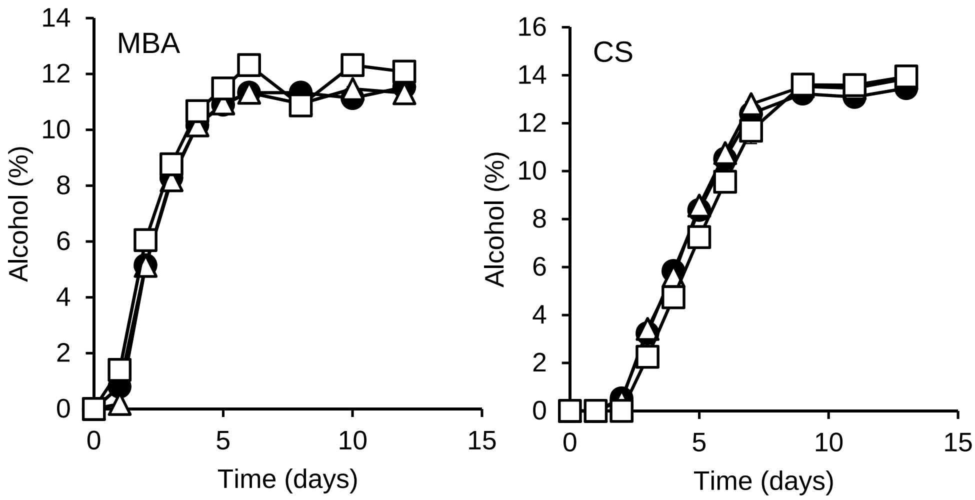

Fig. 3 Changes in alcohol concentrations during fermentation of MBA and CS wines. Control sample, circles; skin sample, triangles; seed sample, squares. Bars show standard

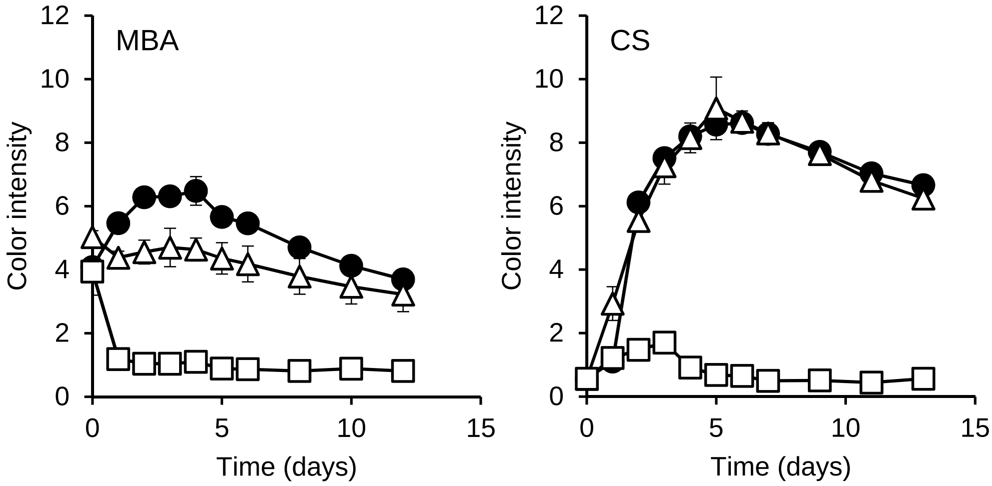

Fig. 4 Changes in color intensity during fermentation of MBA and CS wines.
Control sample, circles; skin sample, triangles; seed sample, squares. Bars show standard

upper). In MBA, PA concentration peaked (97 mg/L) on day 4 and decreased thereafter to reach almost zero on day 8; this result was included in a previous report (Ichikawa et al. 2012). TP concentration was approximately 1,490 mg/L on day 4 but decreased gradually thereafter. In CS, TP concentration reached a maximum (2,315 mg/L) on day 5, and the concentration was maintained thereafter. PA concentration peaked (536 mg/L) on day 11, although some bumps were noted.

In skin samples, TPs were expected to be derived from pulp and skins (because seeds were removed), and most of the PAs were derived from skins (Fig. 5 middle). In MBA, TP concentration peaked on day 4 at 1,241 mg/L, and then decreased gradually. PA concentration peaked on day 3 (59 mg/L) and decreased thereafter to reach 0 mg/L on day 8. TP and PA concentrations in MBA were lower than those in CS, indicating that the concentration of phenols extracted from the skin including PA was low in MBA grape. In CS, the concentration of TP increased until day 6 but decreased thereafter. The concentration of PA increased from day 4, peaked on day 9 (379 mg/L), and abruptly decreased on day 11.

In seed samples, TPs were derived from pulp and seeds. In MBA, both TP and PA concentrations were low (Fig. 5 lower), consistent with the results of the model wine extraction experiment (Fig. 1). PA concentration was also quite low. In CS, TP concentration increased from days 7 to 9, and was 1,191 mg/L on day 13. PA concentration also increased from day 9 to reach 183 mg/L on day 13. This slow extraction of seed PA was consistent with the observation of Koyama et al. (2007).

Taken together, the combination of skin and seed sample data roughly explained the behavior of control wine. Theoretically, the concentrations of TP and PA derived from pulp can be calculated from the difference between skin sample plus seed sample and the control sample. In reality, some skin phenols might contaminate the must particularly for CS because TP concentrations on day 0 for all CS samples were higher than expected. This was because of the difficulty of removing skins from the crushed berries. In MBA control must on day 12, most TPs were derived from skin, and TPs from seed were present in a very small amount compared with those in CS control wine. In contrast, in CS control wine on day 13, a considerable amount (approximately half) of TPs might be derived from seeds.
Similar results were obtained from the model wine extraction experiments described above.

Seed PA concentration in CS wine was increased continuously during the 13-day maceration period. In contrast, skin PA concentration peaked on maceration day 9 and decreased thereafter. The skin/seed PA ratio in must was considered to decrease with fermentation time as reported by Peyrot Des Gachons and Kennedy (2003). Preventing the decrease of skin PA concentration is a very important issue

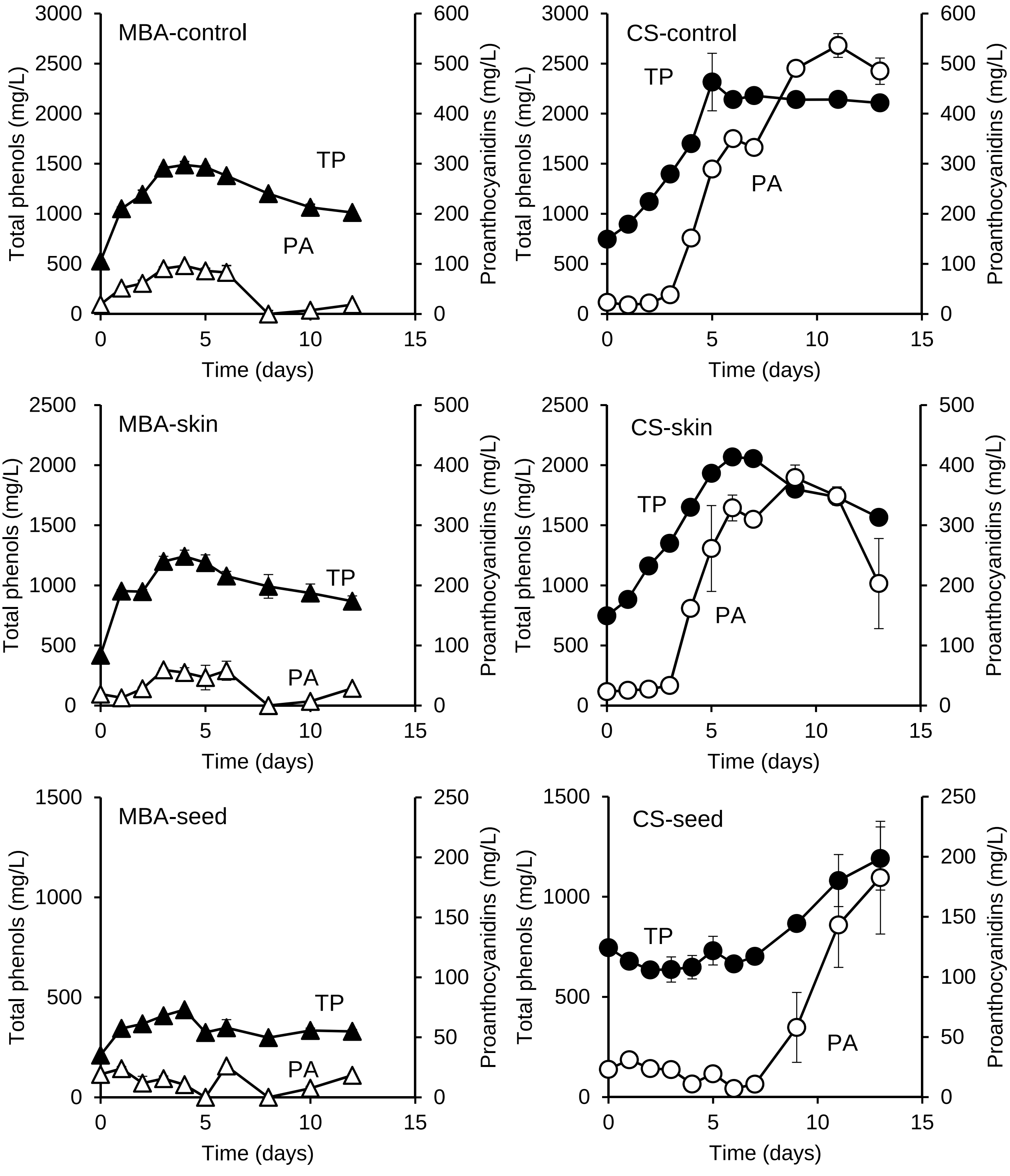

Fig. 5 Changes in TP (closed symbols) and PA (open symbols) during winemaking. Control sample, top; skin sample, middle; seed sample, bottom. MBA, left column; CS, right column. Bars show standard deviations.

in winemaking because skin PA is considered to be positively related to wine quality (Kassara and Kennedy 2011). Because seed PA extraction compensates the decrease of skin PA concentration, it is difficult to detect this phenomenon in commercial red winemaking. Cap management and press timing are very important to improve wine quality.

The final TP concentration was 2,107 mg/L for CS wine and 1,013 mg/L for MBA wine, and the difference was only two-fold. In contrast, the final PA concentration was 485 mg/L for CS wine and 19 mg/L for MBA wine, and the difference was 25-fold. This difference may affect the sensory perception of wines, particularly astringency.

In MBA, the concentrations of PA extracted from skins and seeds were low, and skin PA concentration decreased on day 7. These results explain why MBA wine has low PA concentration. In CS control wine, TP and PA concentrations increased continuously, whereas skin phenols decreased and the decrease was compensated by seed phenols. The color intensity also showed the same profile as the skin phenols. This phenomenon can be partly explained by skin cell wall material absorption (Bindon et al. 2010). To control the color and taste of red wines, clarification of the skin phenol extraction mechanism is important.

Conclusion

1. The amounts model wine extractable TP and PA in seeds of MBA were significantly lower than those of CS.
2. The amounts of extractable PA during vinification were very low from both skins and seeds of MBA berries. This is the reason why MBA wine has extremely low PA concentration.
3. Skin polyphenols, including PAs and anthocyanins, were extracted during the early stage of fermentation, but their concentrations decreased steeply. This phenomenon was observed in both MBA and CS wines.

要　約

MBA ワインの PA 濃度が低い理由を調べるため、二つの実験を行った。MBA と CS ブドウの種子をモデルワインで抽出した場合、TP の濃度はそれぞれ 256 および 1428 mg/L となり、最終的な PA 濃度は、29 および 738 mg/L となった。また、ワイン製造中の TP および PA 濃度の変化を調べた。種子を除去したワインでは、発酵 4〜6 日で TP 濃度が増加したが、その後減少した。一方、果皮を除去したワインでは、CS の場合は TP 濃度が７日目より増加し、その後も増加が見られたが、MBA ではこの増加が見られなかった。同様の傾向は PA 濃度においても見られた。以上の事から果実中の PA 濃度は品種によって大きく異なることが明らかとなった。最終的な TP 濃度の差は、２品種間で２倍程度であったが、PA 濃度の差は 25 倍も異なった。

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