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Impact of harvest timing on 4-hydroxy-2,5-dimethyl-3(2H)-furanone concentration in 'Muscat Bailey A' grape berries

H. Kobayashi[1)], K. Sasaki[2)], F. Tanzawa[2)], S. Matsuyama[1)], S. Suzuki[3)], R. Takata[2)] and H. Saito[1)]

[1)] Château Mercian, Mercian Corporation, Yamanashi, Japan
[2)] Product Development Research Laboratory, Mercian Corporation, Kanagawa, Japan
[3)] Laboratory of Fruit Genetic Engineering, Institute of Enology and Viticulture, University of Yamanashi, Yamanashi, Japan

Summary

We analyzed the effect of harvest timing on the concentration of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) concentration in 'Muscat Bailey A' grape berries. 'Muscat Bailey A' is one of the wine produced most in Japan, and the furaneol concentration in these wines were much higher than those of wine made from other black *Vitis vinifera* grape varieties regardless of the growing region in Japan. The furaneol concentration in 'Muscat Bailey A' grape skin increased during the grape developmental period. Indeed, 'Muscat Bailey A' wines made from grape berries harvested at 9 weeks post-véraison contained a larger amount of furaneol than those made from grape berries harvested at 7 weeks post-véraison. These findings could contribute to improving viticultural and enological practices for the unique strawberry-like fruity aroma of 'Muscat Bailey A' wine.

Key words: 4-hydroxy-2,5-dimethyl-3(2H)-furanone, furaneol, 'Muscat Bailey A', flavor, harvest timing, winemaking.

Introduction

4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) is an important component that gives the characteristic aroma of strawberries, pineapples, and mangoes (Pickenhagen *et al*. 1981). Zabetakis and Holden (1997) reported that furaneol is responsible for the key aroma of strawberries and confers the characteristic strawberry-like flavor. Furaneol, as well as *o*-aminoacetophenone and methyl anthranilate, also provides the characteristic aroma (*i.e*. the American character) of *Vitis labruscana* (Shure and Acree 1994).

'Muscat Bailey A' is a hybrid grape variety [*Vitis labrusca* (Bailey) × *Vitis vinifera* ('Muscat Hamburg')]. The cross-hybridization was developed in Japan in 1927, and since then, 'Muscat Bailey A' has been cultivated for table use and the wine from it is one of the Japanese wines produced most. In 2009, the Ministry of Agriculture, Forestry and Fisheries showed that the cultivation area, crop yield, and wine production of 'Muscat Bailey A' in Japan were 104 ha, 1382.8 t, and 1173.7 t, respectively (http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001084649). To date, 'Muscat Bailey A' is one of the most popular wine grapes in Japan. In addition, 'Muscat Bailey A' wine has unique characteristics; specifically, the amount of proanthocyanidins in this wine is significantly lower than those in other red wines made from *V. vinifera* grape cultivars such as 'Merlot', 'Cabernet Sauvignon', and 'Zweigeltrebe' (Ichikawa *et al*. 2011). However, there are few reports about the typical aroma of 'Muscat Bailey A' wine. In this study, we examined 1) the furaneol concentrations in 'Muscat Bailey A' grape berries and wines, and 2) the effect of harvest timing on furaneol concentration in 'Muscat Bailey A' grape berries.

Material and Methods

Chemicals: 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (98 %) and 2-methyl-3-propyl pyrazine (97 %) were purchased from Sigma-Aldrich (Tokyo, Japan).

Plant materials: Vineyards cultivating 'Muscat Bailey A' grapevines were located in the Katsunuma region (altitude, 35°39’8”N; longitude, 138°43’52”E; elevation, 396 m) of Yamanashi Prefecture in Japan. One hundred 'Muscat Bailey A' grape berries were randomly handpicked from August 8 (start of véraison) to November 11 in the 2010 growing season.

Wines: To determine furaneol concentration, 2009 vintage wines from 'Merlot' (n = 5), 'Cabernet Sauvignon' n = 4), 'Pinot Noir' (n = 3), and 'Muscat Bailey A' (n = 14) grape berries cultivated in Japan were collected. 'Muscat Bailey A' wine was prepared from grape berries cultivated in various growing regions in Japan such as Yamagata (n = 3), Niigata (n = 3), Nagano (n = 3), and Yamanashi (n = 5) Prefecture.

Quantitative analysis of furaneol in grape skin and wine: To evaluate the furaneol concentration in 'Muscat Bailey A' grape skin and wine, the skin was pulverized in liquid nitrogen. One gram of the powder was added in 49 mL of distilled water. To this resulting dilute solution or 50 mL of wine, 4 mL of dichloromethane were added to extract volatiles, and the mixture was mixed for 5 min in a separating funnel. 2-Methyl-3-propyl pyrazine was used as the internal standard (final concentration, 100 $\mu g \cdot L^{-1}$). The extract was quantitatively

Correspondence to: Dr. H. Kobayashi, Château Mercian, Mercian Corporation, 1425-1 Shimoiwasaki, Katsunuma, Koshu, Yamanashi 409-1313, Japan. FAX: +81-553-44-3061. E-mail: kobayashi-hr@mercian.co.jp

analyzed for furaneol by gas chromatography-tandem mass spectrometry (GC-MS) as described below.

GC-MS conditions: GC-MS was performed using a GC system (6890N, Agilent Technologies Japan Ltd., Tokyo, Japan) and an MS detector system (5975 *inert* mass selective detector, Agilent Technologies) as described previously (SHURE and ACREE 1994) with slight modification. Briefly, a fused silica capillary column (BP-20, 50 m × 0.22 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used and the temperature program for BP-20 was a cycle of 60 °C (3 min), 5 ºC·min^{-1} to 240 °C, and 230 °C (10 min). The ions monitored in single-ion-mode runs corresponded to *m/z* 57, 85, and 128 for furaneol. Means and standard deviations were calculated from triplicate experiments.

Vinification: Approximately 1,000 kg of 'Muscat Bailey A' grape berries were harvested on September 24 (7 weeks post-véraison) and October 8 (9 weeks post-véraison) in 2011. The commercial *Saccharomyces cerevisiae* strain BM 4 × 4 (Lallemand, Montreal, Canada) was used in vinification. Dry yeast was reactivated by soaking it in distilled water (40 °C) before use in alcoholic fermentation. The cap punching operations were carried out twice a day during maceration.

Seven days after alcoholic fermentation, wine was obtained by pressing at 75 % of the total weight using a press machine (SUTTER, Gallen, Switzerland). Sulfur dioxide was added to the wine at 60 mg·L^{-1} and the wine was stored at 4 °C until further analyses.

Grape and wine analysis: Titratable acidity (TA) was determined by adding 10 mL of distilled water to 10 mL of wine and the solution was subjected to neutralization titration to pH 7.0 using 0.1 N NaOH. TA is expressed as g of tartaric acid·L^{-1}. Total phenol concentration in wine was determined by the Folin-Ciocalteau method (SINGLETON and ROSSI 1965). pH was measured using a pH meter (MH-60S, Toakogyo, Japan). Total anthocyanin concentration in the grape skin and wine were determined with the method based on RIBÉREAU-GAYON and STONESTREET (1965) and expressed as mg of malvidin-3-glucoside·L^{-1}. Briefly, 1 g of grape skin and 1 mL of wine samples were added to 20 mL of 0.1 N HCl, and the mixture was incubated for 4 h at room temperature in the dark. The mixture was filtered through a 0.45 μm cellulose acetate filter (Advantec Toyo, Japan) and its OD at 520 nm was measured using a spectrometer (UV-1800 Shimadzu, Japan). Means and standard deviations of total anthocyanin concentration in grape skins were calculated from triplicate experiments.

Statistical analysis: Student's t-test was used to evaluate the significantly difference between furaneol concentrations for each grape cultivar.

Results and Discussion

The furaneol concentration in 'Muscat Bailey A' wine was much higher than those of wine made from other black *Vitis vinifera* grape varieties (Figure A). Generally, many

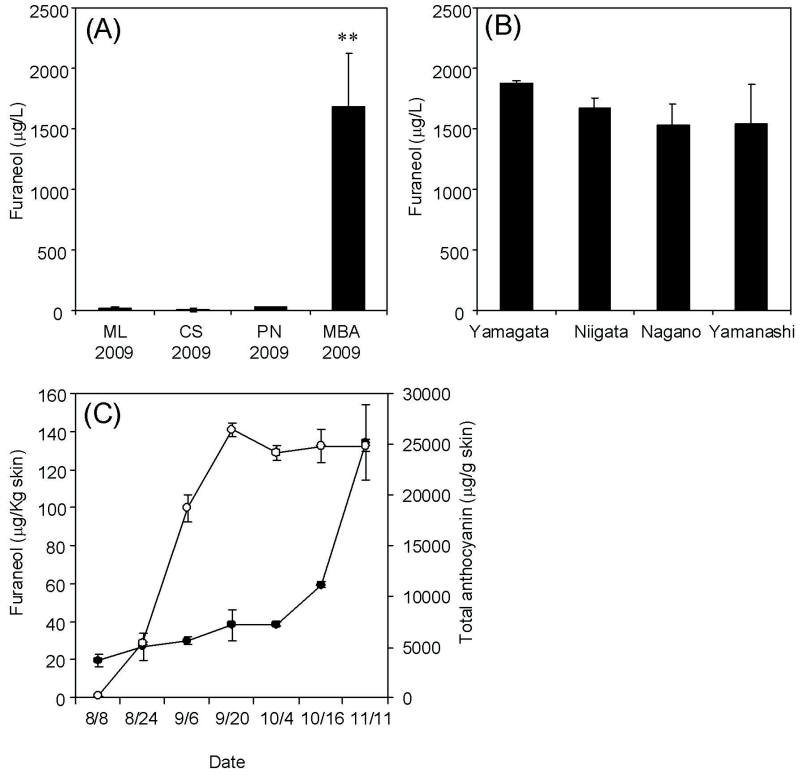

Figure: (**A**) Furaneol concentration in wines made from various grape cultivars in 2009. The numbers below the cultivar abbreviations indicate the vintages of the wines. ML: 'Merlot', CS: 'Cabernet Sauvignon', PN: 'Pinot Noir', and MBA: 'Muscat Bailey A'. **: indicates significant difference between grape cultivars determined by the t-test ($p < 0.01$). (**B**) Furaneol concentration in the wines made from MBA grape berries grown in the indicated regions in 2009. (**C**) Furaneol and total anthocyanin concentrations during developmental period of 'Muscat Bailey A' grape berries grown in 2010. Closed circles indicate furaneol concentration. Open circles indicate total anthocyanin concentration. (n = 3).

Table

Impact of harvest timing on 'Muscat Bailey A' wine composition in 2011

Harvest timing (weeks post-véraison)	Alcohol (%) v/v	Titratable acidity ($g \cdot L^{-1}$)	pH	Total phenolics concentration ($mg \cdot L^{-1}$)	Total anthocyanin concentration ($mg \cdot L^{-1}$)	Furaneol concentration ($\mu g \cdot L^{-1}$)
7	11.8	5.2	4.00	2269	628	1130
9	12.3	5.1	3.97	2157	765	3130

'Muscat Bailey A' wines also contained large amounts of furaneol regardless of the region the grapevines were grown in Japan (Figure B). These findings suggest that this compound might be essential for the characteristic flavor of 'Muscat Bailey A' wines. Thus, accumulation of furaneol might be a genetic component of 'Muscat Bailey A', which is only marginally influenced by environmental conditions. The furaneol concentration in 'Muscat Bailey A' grape berries increased during the grape developmental period (Figure C), suggesting that late harvest might be more effective for obtaining higher concentrations of furaneol in 'Muscat Bailey A' wines than the conventional time of harvest. Indeed, 'Muscat Bailey A' wines made from grape berries harvested at 9 weeks post-véraison had 2.8-fold furaneol concentrations than those made from grape berries harvested at 7 weeks post-véraison (Table). These findings are expected to lead to the improvement of viticultural practices related to harvesting of 'Muscat Bailey A' grape berries for better strawberry-like aroma for 'Muscat Bailey A' wine production. However, furaneol concentration of the skin was low compared with wine. This result supported that furaneol was provided without skin according to a previous report (Gueded de Pinho and Bertrand 1995). Further studies investigating the relationship between the analogs such as 4hyroxy-5-methyl-2-methylene-3(2H)-furanone and furaneol might be more clarified the biosynthesis of furaneol in grape berries as well as a previous report (Raab *et al*. 2006). On the other hand, it was reported that carbon dioxide treatment increases furaneol concentration in strawberries (Wang and Bunce 2004). Therefore, in addition to the optimal harvest timing, the use of techniques such as carbonic maceration might further improve the unique fruity aroma of 'Muscat Bailey A' wine.

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