

Research Note

Juice constituents and skin pigments in *Vitis coignetiae* Pulliat grapevines

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Introduction: *Vitis coignetiae* Pulliat is a wild grape species widely distributed in mountainous regions of Japanese Islands. In the Hiruzen Highlands, located in the northern Okayama Prefecture, *V. coignetiae* grapevines have been domesticated during the last two decades mainly for wine making.

Vines had been raised from cuttings of dormant canes collected from several wild vines during 1980s. Nowadays, the cultivated area is about 10 ha. The berries contain a peculiar flavor and abundant red pigments, but a very high acidity and low sugar level which are thought to be deteriorating the wine's taste. In the Hiruzen vineyards, grapes are usually harvested in late September, *i.e.* 6 weeks after veraison, primarily to avoid fungal damages of berries such as ripe rot and downy mildew (UEKI *et al.* 2001). However, it has not yet been established when *V. coignetiae* berries reach full ripeness and what are the constituents of juice and skins at this stage.

The aim of this study was to elucidate the optimum harvest time for wine making as well as the characteristics of juice and skin constituents in *V. coignetiae* grape berries as compared to Cabernet Sauvignon (*V. vinifera* L.).

Material and Methods: Six and five 15-20-year-old *V. coignetiae* vines, exhibiting the typical color in young leaves and cluster stems, were chosen from 6 commercial vineyards in the Hiruzen Highlands in 2000 and 2001. They were planted 4x2.5 m and trained to a multiple scaffold branch system on a horizontal trellis (year 2000) and to a bilateral cordon system on a vertical trellis (year 2001). 20-30 berries were sampled from each vine at 2 to 3-week intervals from early August (before veraison) until late October. Berries of 8-year-old vines of Cabernet Sauvignon (*V. vinifera*), cultivated in the same district, were also sampled in early October 2001, *i.e.* at the usual harvest time of Hiruzen.

Juice constituents: Berry samples were pressed by hand through doubled gauze to obtain the juice. Total

soluble solids (TSS) were measured by a refractometer (Atago-30) and titratable acidity (TA) by titrating the juice with 0.1 N NaOH until neutralization. Sugar and acid contents were determined by GC (Shimadzu GC-14A) after purifying the sample juice by ion exchange resin and silylating with hexamethyldisilazane and trimethylchlorosilane. For amino acid analysis, 0.5 ml of juice was filtered through a ϕ 0.45 μ m filter and injected into a HPLC (JLC-300).

Skin anthocyanin: 10 mg of lyophilized skin sample was homogenized with 25 ml of 80 % methanol containing 1 % HCl. Anthocyanins were extracted for 1 h at 25 °C twice. OD₅₃₅ of the extracts was determined with a spectrophotometer (Beckmann DU530). For the identification of skin anthocyanins, 1 kg of fresh *V. coignetiae* berries were extracted with 4000 ml of 50 % ethanol containing 0.1 % trifluoroacetic acid (TFA) for 1 d. The extract was concentrated under reduced pressure at 40 °C and applied to a 1-liter HP-20 column. The adsorbed pigments were eluted with 60 % EtOH containing 0.1 % TFA. The eluent was concentrated under reduced pressure at 40 °C and 204.3 g of berry colorant were obtained. The colorant was purified with YMC-ODS-L80 (30 i.d.x400 mm, YMC) column and Sephadex LH-20 (40 i.d.x600 mm, Pharmacia) column. HPLC chromatograms of the colorant revealed that it consists of about 7 pigments (Figure). Three main pigments, Pig-1, Pig-2 and Pig-3, were isolated, and the structures of these pigments were identified using ESI-LC-MS and NMR (1D and 2D NMR, HMBC, HMQC).

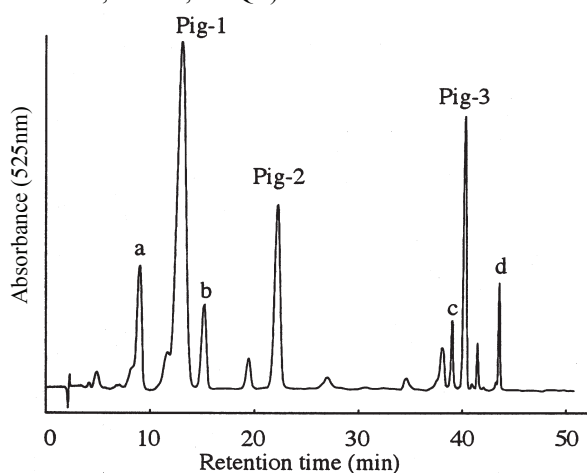


Figure: HPLC chromatogram of the colorant extracted from *V. coignetiae* berry skins.

Results and Discussion: In 2000, TSS of berry juice, sampled from 6 *V. coignetiae* vines, were about 15 °Brix in late September and 18-20 °Brix in late October (Table). These data suggest that in Hiruzen *V. coignetiae* grapes are generally harvested before the stage of full ripeness. GC analyses of sugars revealed that the levels of fructose were significantly higher than of glucose. TA, expressed as tartaric acid equivalent, decreased rapidly after veraison. However, the final TA level was higher than 1.6 g 100 ml⁻¹ for each vine. Malate decreased continuously until late October while tartarate did not decrease after late September. Total amino acid content increased significantly until late October, but the final levels were about 5 mmol l⁻¹ or lower, much lower than in berries of *V. vinifera* wine grapes (HUANG and OUGH

Table

Seasonal changes in the juice constituents and skin anthocyanins of *Vitis coignetiae* berries in comparison to *V. vinifera*

Species cultivar	Date of harvest	Berry weight (g)	TSS (°Brix)	TA (g 100 ml ⁻¹)	Juice pH	Total amino acid (mmol l ⁻¹)	Skin Anthocyanin ^z (O.D. 535)
<i>V. coignetiae</i> ^y							
	2000 Sep. 12	1.10 b	13.8 c	2.38 a	2.63 b	1.8 c	0.36 c
	Sep. 26	1.13 ab	15.1 b	2.19 ab	2.68 ab	2.4 b	0.65 bc
	Oct. 10	1.16 a	17.8 ab	1.86 b	2.72 ab	3.4 ab	0.90 b
	Oct. 10	1.18 a	19.2 a	1.76 b	2.84 a	4.5 a	1.16 a
<i>V. coignetiae</i> ^x							
	2001 Oct. 2	1.10 b	18.8	1.89 a	2.64 b	5.1 b	0.86 a
Cabernet Sauvignon (<i>V. vinifera</i>) ^x							
	2001 Oct. 10	1.40 a	18.5	0.82 b	3.08 a	12.0 a	0.44 b

^z Anthocyanins extracted from 1 g of lyophilized samples were suspended into 100 ml of 80 % ETOH.^y Berries were harvested from 6 *V. coignetiae* vines. Means were separated by DMRT at $p < 0.05$.^x Berries were harvested from 5 *V. coignetiae* and 3 Cabernet Sauvignon vines. Means were separated by DMRT at $p < 0.05$.

1991). Furthermore, the major constituents were proline and alanine indicating that the amino acid profiles of *V. coignetiae* berries resemble those of *V. labrusca* varieties rather than *V. vinifera* grapes (SPAYD and ANDERSEN-BAGGE 1996).

V. coignetiae berries contain far higher TA and lower pH levels than Cabernet Sauvignon berries. The extraordinary high level of tartaric acid, compared to that of most wine grape cultivars (LAVEE and NIR 1986), might be specific for this species. It is generally accepted that grape berries for wine making should contain 20–25 °Brix of TSS and 0.6–0.8 mg 100 ml⁻¹ of TA (WINKLER 1974). The lower levels of TSS in both *V. coignetiae* and Cabernet Sauvignon berries harvested in the Hiruzen vineyards may have resulted from insufficient sunshine and frequent rainfall during autumn.

The significantly higher level of skin anthocyanins is a characteristic of *V. coignetiae* berries. The molecular formula of three main constituents, Pig-1, Pig-2 and Pig-3, shown in the Figure, were determined as C₂₉H₃₅O₁₇, C₂₃H₂₅O₁₂, C₃₈H₄₁O₁₉, respectively, by positive-ion ESI-LC-MS (molecular ion m/z 655, 493, 801). ESI-LC-MS spectra of the three pigments indicated that malvidin (m/z 331) is a common aglycone unit in those pigments and that there are two glucose units in Pig-1 (m/z 655), one glucose unit in Pig-2 (m/z 493), and two glucoses and one *p*-coumarate in Pig-3 (m/z 801). On the basis of these results and spectral data of NMR, the structures of Pig-1, Pig-2 and Pig-3 were finally confirmed as malvidin-3,5-diglucoside, malvidin-3-glucoside, and malvidin-3,5-diglucoside-coumarate, respectively. The other pigments, denoted as a, -b, -c, and -d in the Figure can be estimated to be delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside-acetate, and malvidin-3-glucoside-coumarate, respectively, by referring to WULF and NAGEL (1978). The major anthocyanins in *V. coignetiae* berry skin, identified as malvidin-3,5-diglucoside and malvidin-3,5-diglucoside-*p*-coumarate are usually not found in berries of *V. vinifera* cultivars but are common in *V. labrusca* and hybrids between *V. vinifera* and *V. labrusca* (GALVI and FRANCIS

1978; YOKOTSUKA and SINGLETON 1997). IGARASHI *et al.* (1986) have already shown the presence of malvidin-3,5-glucoside in *V. coignetiae* berries and noted a high antioxidative activity of this pigment. As shown in our previous report (UEKI *et al.* 2002), the total anthocyanin content in *V. coignetiae* berry skins is 2–4 times higher than that in *V. vinifera* cultivars such as Cabernet Sauvignon, Cabernet Franc, and Pinot noir. The anti-oxidative activity of *V. coignetiae* juice should be further investigated.

There may be some genetic diversity in the *V. coignetiae* vines cultivated in Hiruzen, because they have been propagated by cuttings collected from several wild vines. Juice and skin constituents among the vines should be examined and they should be genetically identified.

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