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Lipids in the seeds of wild grapes native to Japan: *Vitis coignetiae* and *Vitis ficifolia* var. *ganebu*

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ABSTRACT

The seed oils of *Vitis coignetiae* (Coignetiae) and *Vitis ficifolia* var. *ganebu* (Ganebu) at véraison and the ripe stage were evaluated for their fatty acid composition, and the content and profiles of phytosterols and tocopherols/tocotrienols. The oil content in Coignetiae seeds at ripe was almost same as that in 'Merlot' and 'Muscat Bailey A' seeds. The oil content in Ganebu seeds was 73% of that in Coignetiae seeds. Although linoleic acid was >64% in 'Merlot', 'Muscat Bailey A' and Coignetiae in each developmental stage, that in Ganebu at véraison and at ripe was 46.6 and 50.3%, respectively. The stigmasterol content in Ganebu seed oil at ripe was >2.6× higher than in the other grapes. The content and composition of tocopherols/tocotrienols in Coignetiae seed oil were similar to those in 'Merlot.' Although the total tocopherol/tocotrienol levels of Ganebu at ripe showed no significant differences compared with levels in the other grapes, the α-tocopherol level was >3.3× higher than in the other grapes. Our data suggest that Coignetiae is a common source of grape seed oil, but Ganebu may be a peculiar source of grape seed oil.

1. Introduction

The grape genus (*Vitis*) is classified into three species: Europe—West Asian species, North American species and East Asian species. Most of the East Asian species are wild, which have limited use compared with the other species. The wild grapes investigated in this study, *Vitis coignetiae* Pulliat (Coignetiae) and *Vitis ficifolia* Bunge var. *ganebu* Hatusima (Ganebu), are of the East Asian species and indigenous to Japan. Coignetiae exhibits low temperature tolerance and mainly inhabits subarctic wet climate areas of Japan. Ganebu is endemic to coastal areas of the subtropical southwestern islands of Japan. This subtropical grape has no endodormancy and can bear berries continuously in the habitat. The berry size of Coignetiae is as large as that of *Vitis vinifera*, but that of Ganebu is about a third of *V. vinifera* in terms of weight. Although there are no obvious differences in seed number per berry between the two species (average seed number per berry is ca. 2.8), the seed fresh weight of Ganebu is about 37% of that of Coignetiae (Mochioka, 1996).

Grapes, especially *V. vinifera* and the hibrids such as *V. labruscana*, are one of the important horticultural crops cultivated world widely and used for table, juice and wine. Wild grapes may be useful as processing materials, although most of them are not suitable for tabel grapes owing to the many seeds per berry or the small berry size. Coignetiae is actually cultivated in vineyards and the berries are mainly used for wine and juice as local products in the northern areas of Japan. However, effective utilization of the seed has not been considered. On the other hand, the cultivation of Ganebu in vineyards and its use are currently sparse. An exception to this is use of the extract of the leaf in a cosmetic in Japan. However, Ganebu may be a useful source for wine, juice and foods because of the high productivity of resveratrol, which is a characteristic polyphenol in grape berries and wine, with antioxidant activity, in the berry skin at the ripe (Shiozaki et al., 2013).

Grape seeds in the solid residues after the production of wine or juice are often used for the production of grape seed oil. The oil can be used for several purposes, including dietetic, because it is clean, light and neutral in smell and taste. The production of grape seed oil as a by-product in wineries and the juice industry constitutes an effective utilization of resources and provides economic advantages. In addition to obtaining grape seed oil from mature grapes, immature grapes, which are eliminated during optimization of a yield and harvest quality, may also be useful for oil production. Rubio et al. (2009) have demonstrated that the seed oil from immature grapes compares favorably with respect to the oil yield from and the content of mature grapes.

Grape seed oil is well known as one of the vegetable oils with a high amount of unsaturated fatty acids (Ahmadi and Siahsar, 2011;

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Baydar and Akkurt, 2001; Baydar et al., 2007; Demirtas et al., 2013; Hassanein and Abedel-Razek, 2009; Pardo et al., 2009). Linoleic acid, an essential fatty acid for human metabolism, is present in high levels in grape seed oil. Linolenic acid is present in low levels. A high level of linolenic acid can cause an unpleasant odor and taste in oil. Grape seed oil also contains phytosterols and tocopherols/tocotrienols, which are vitamin E active compounds and have antioxidant activity. β-Sitosterol and γ-tocotrienol have been reported, in several grape cultivars, to be the most abundant phytosterols and vitamin E active compounds (Baydar and Akkurt, 2001; Baydar et al., 2007; Beveridge et al., 2005; Crews et al., 2006; Hassanein and Abedel-Razek, 2009; Rubio et al., 2009).

Characterization of grape seed oil seems to be limited to *V. vinifera*; we have little information about the features of oil obtained from other species, including wild grapes. Characterization of the grape seed oil would provide useful information for the exploitation of low-use wild grape resources, leading the wild grapes to valuable horticultural crops.

This work describes the characteristics of the grape seed oil obtained from Coignetiae and Ganebu, both of which are promising wild grape resources in Japan. The fatty acid composition and the content and composition of phytosterols and tocopherols/tocotrienols were evaluated in the oil from the immature (at véraison) and mature (at ripe) seeds in each species, and then compared with *V. vinifera* (Merlot) and *V. labrusca* (Muscat Bailey A).

2. Materials and methods

2.1. Seeds

The grapes used in this study were grown in a research field at Osaka Prefecture University. Grape seeds were sampled from *V. coignetiae* (Coignetiae) and *V. ficifolia* var. *ganebu* (Ganebu) berries at véraison and at ripe in 2010. Seeds of *V. vinifera* cv. Merlot and *V. labrusca* cv. Muscat Bailey A, which were sampled at the same developmental stages, were used as comparison. The soluble solid content (Brix°) in the berry juice of Coignetiae, Ganebu, 'Merlot' and 'Muscat Bailey A' at ripe were 13.4°, 14.7°, 20.2° and 18.3°, respectively. Average seed fresh weight was 47.4, 76.9, 44.4 and 16.2 mg for 'Merlot', 'Muscat Bailey A,' Coignetiae and Ganebu, respectively, at véraison and 36.2, 70.1, 41.8 and 15.6 mg at ripe. Seeds were pulverized in liquid nitrogen with a mortar and pestle and stored at −30 °C until extraction.

2.2. Extraction of oil

Oil was extracted according to the method of Adhikari et al. (2008) and purified, with partitioning according to the method of Gómez-Brandón et al. (2008) with modification. Oil was extracted from 10 g pulverized seeds with 100 ml extraction solvent, comprising chloroform, methanol and 0.88% KCl (1:2:1 v/v). The homogenate was stirred overnight at 4 °C and filtered through a Whatman No. 2 filter paper. The samples were then centrifuged for 20 min at 1800 × *g* to separate the phases. The chloroform phase was collected. The aqueous phase was well mixed with 60 ml extraction solvent and centrifuged as described above. The chloroform phase was then collected. The aqueous residue was transferred to a separating funnel and 50 ml of 0.88% KCl was added. It was then partitioned against 50 ml chloroform and left until the two layers were clearly separated. The chloroform phase was collected and combined with the former two chloroform extracts. The combined chloroform phase was washed with 100 ml of 0.88% KCl to remove nonlipid compounds. The washed chloroform extract was dehydrated by passing it through a column containing 10 g Na_2SO_4 and then reduced *in vacuo* to dryness. After weighing, the dry sample was redissolved in 1 ml hexane. A 100 μl aliquot was separated for fatty acid analysis and the remainder was equally divided for phytosterol and tocopherol/tocotrienol analyses. Each sample was then reduced to dryness and stored at −30 °C until required for analysis.

2.3. Fatty acids: alkaline methanolysis and GC analysis

The oil sample was vortex mixed in 2 ml of 0.5 M methanolic KOH and heated for 5 min by placing a container holding the oil in boiling water. After cooling in air, the fatty acids were methylated with 1 ml of 14% methanolic boron trifluoride for 10 min at 40 °C. After cooling, the sample was mixed with 10 ml saturated NaCl and partitioned three times with 5 ml hexane. The combined hexane phase was also dehydrated by passing it through a column containing 3 g Na_2SO_4 and then filtered. The dehydrated hexane was reduced *in vacuo* to dryness.

GC analysis of methylated fatty acids was performed with a GC-14A (Shimazu, Kyoto, Japan) equipped with a FID detector in split mode. The oil samples were dissolved in 500 μl acetone and the fatty acids in the samples (2 μl) were separated using a FAMEWAX capillary column (0.25 mm i.d. × 30 m, 0.25 μm film thickness; Shimazu, Kyoto, Japan). Nitrogen was used as the carrier gas at a flow rate of 1.4 ml min^{-1}. The split ratio was 1:50. The column temperature was initially held at 130 °C for 1 min and then ramped to 225 °C at a rate of 6 °C min^{-1} with a final hold of 10 min. The temperature of the injector and detector was 220 °C and 230 °C, respectively. Palmitic, stearic, oleic, linoleic and linolenic acid were identified in the samples by comparing the retention times of the standards (typical retention times of palmitic, stearic, oleic, linoleic and linolenic were 12.3 min, 15.5 min, 15.8 min, 16.5 min and 17.4 min, respectively). The amount of fatty acids was determined by comparing the peak areas to the standard curves made using each fatty acids (palmitic acid: standard curve made over the range 0–25 mM with a regression constant $r^2 = 0.995$; stearic: 0–25 mM, $r^2 = 0.983$; oleic: 0–50 mM, $r^2 = 0.989$; linoleic: 0–100 mM, $r^2 = 0.975$; linolenic: 0–1 mM, $r^2 = 0.991$). The detection ranges of each fatty acid sample were as followes: palmitic acid: 1.8–15 mM; stearic: 0.7–5 mM; oleic: 3–28 mM; linoleic: 10–92 mM; linolenic: 0.2–0.8 mM. After conversion of the molar concentration into mg per 1 g oil, the composition of each fartty acid was caluculated by deviding the individual content by the total fatty acid content.

2.4. Sterols: hydrolysis, purification and GC analysis

An oil sample (0.5 g) was suspended in 5 ml of 6 N HCl and heated at 80 °C for 60 min, with vortex mixing for a few seconds every 10 min. After cooling in air, hydrolyzed oil was extracted with 20 ml of 50% diethyl ether in hexane and reduced *in vacuo* to dryness. To the dry sample was added 7 ml of 0.5 M ethanolic KOH, followed by refluxing for 20 min at 80 °C. After cooling, 12 ml distilled water was added and the sample partitioned against 20 ml cyclohexane. A 15 ml portion of the cyclohexane phase containing nonsaponifiables was evaporated to dryness.

The nonsaponifiables were purified using silica gel column chromatography in conjunction with a vacuum manifold (Bond Elut; Varian, Harbor City, CA, USA). The silica gel column was prepared as follows. Bondesil SI (0.5 g; Analytichem International, Harbor City, CA, USA) was packed in a Bond Elut empty reservoir (8 ml; Varian), preinstalled with a frit of 40 μm pore size. In addition, a frit of 70 μm pore size was installed on the resin to avoid disturbance of the resin. The sample flow rate was <1 ml min^{-1}. Dry samples were dissolved in 1 ml hexane with ultrasonication and then loaded onto the column, which had been previously activated with 5 ml hexane. After