JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Characterization of Polyphenolic Metabolites in the Seeds of *Vitis* Germplasm

Zhenchang Liang,[†] Yingzhen Yang,[‡] Lailiang Cheng,[†] and Gan-Yuan Zhong*[,‡]

[†]Department of Horticulture, Cornell University, Ithaca, New York 14853, United States

[‡]USDA-ARS Grape Genetics Research Unit, Geneva, New York 14456, United States

Supporting Information

ABSTRACT: The composition and content of polyphenols in the seeds of 91 grape accessions from 17 *Vitis* species were characterized. Eleven compounds, including 2 gallic derivatives, 3 monomeric flavan-3-ols, 3 flavonols, resveratrol, and procyanidin B1 and B2, were identified via HPLC−MS and quantified by HPLC−DAD. In addition, seventeen dimeric and trimeric flavan-3-ols were also quantified. Tremendous variation was observed both among and within species for these compounds. Monomeric flavan-3-ols were the most abundant polyphenols in seeds, followed by dimeric and trimeric flavan-3-ols, which collectively accounted for more than 96% of the total polyphenols. *V. palmata*, *V. vinifera*, and *V. vulpina* had significantly higher content of total polyphenols than other species. A number of *Vitis* accessions with high content of various types of seed polyphenols were identified, and they can serve as potential germplasm for improving the composition and content of seed polyphenols in cultivated grapes.

KEYWORDS: *Vitis, grape, seed polyphenols, flavan-3-ols, procyanidin, flavonols*

INTRODUCTION

Grapes (*Vitis* sp.) are rich in polyphenols, which represent the third most abundant constituent in grape berries, after carbohydrates and fruit acids,[1] and offer many potential health benefits.[2] Among the three berry tissue components of skin, flesh, and seed, seed has the most abundant polyphenols, accounting for about 60−70% of the total polyphenols in grape berries.[3] Polyphenols in grape seeds are a significant organoleptic component of wines and an important contributor to wine quality.[4,5] They affect the taste and mouthfeel of wine bitterness and astringency and are responsible for aging potentials of wine.[6] In addition, polyphenols are the key wine preservative and the basis of long aging.[7] They are the components that turn brown in wine (and other foods) when exposed to air due to oxidization.[8] In addition to affecting wine quality, grape seed polyphenols are also a class of natural antioxidants offering many health benefits. Grape seed polyphenols have strong free radical scavenging capabilities, and their antioxidant activities are superior to those of many other well-known antioxidants, such as vitamin C, vitamin E, and beta-carotene.[9] Various biological activities of grape polyphenolic compounds and the potential health benefits of those compounds, including protection from cardiovascular diseases and anticarcinogenic and antimicrobial effects, have been well recognized and documented.[9,10] Flavan-3-ol monomers and oligomers are the most abundant polyphenolic compounds in seeds, and these polyphenols were demonstrated to have neuroprotective, cardioprotective, and cancer-inhibiting effects and the effect of mitigating the damage caused by radiation and cisplatin in animal studies.[11−13]

There has been considerable interest in determining the composition and content of polyphenolic compounds in grape seeds.[14] It has been determined that polyphenols in grape seeds are mainly flavonoids, including flavan-3-ol monomers ((+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin 3-*O*-gallate), and procyanidin dimers, trimers, and more highly polymerized procyanidins. Oligomeric flavan-3-ols, including procyanidin B (dimers) and C (trimers), consist of monomers, linked though C4−C6 and/or C4−C8 interflavan bonds.[15] In addition, oligomeric flavan-3-ols can couple with gallic acid. It was found that monomeric flavan-3-ols are more bitter than astringent, whereas the reverse is true for larger molecular weight derivatives.[16] Galloylation has been shown to increase tannin interactions with various proteins, suggesting that galloylation may also enhance astringency.[17,18] It was observed that procyanidins were the main polyphenolic component in seeds and mainly localized in the outer seed coats that contained the majority of both monomers and polymers of flavan-3-ols (2 to 5 times more than the endosperm) in *Vitis vinifera* cv. Cabernet Sauvignon.[19]

Characterization of the composition and content of polyphenolic compounds in grape seeds has been largely limited to *V. vinifera*,[20,21] the most widely cultivated grape species. There are many wild grape species in the genus *Vitis*. Many of these *Vitis* wild species can be hybridized with *V. vinifera* and thus offer potential germplasm for the improvement of *V. vinifera* grapes. To explore these wild genetic resources for improving fruit and processing quality of the cultivated grapes, we recently evaluated the composition and content of polyphenolic compounds in the ripe berries of 16 wild *Vitis* species.[22] In this study, we analyzed the polyphenolic profiles in the seeds of these species. We also included in this study 10 common *V. vinifera* cultivars for comparison. This

Received: August 28, 2011
Revised: January 6, 2012
Accepted: January 9, 2012
Published: January 9, 2012

dx.doi.org/10.1021/jf2046637 | *J. Agric.Food Chem.* 2012, 60, 1291−1299

work provided a significant addition to the knowledge of polyphenolic composition and content in the *Vitis* germplasm and will contribute to our ongoing effort for establishing a database of health-related phytochemicals preserved in the US Department of Agriculture-Agricultural Research Service (USDA-ARS) *Vitis* germplasm.[22,23]

MATERIAL AND METHODS

Plant Material. Ninety-one accessions representing 17 *Vitis* species were assayed in this study (S Table 1 in the Supporting Information). All the berry samples were collected from the USDA-ARS *Vitis* Clonal Repository in Geneva, NY, except for the *V. vinifera* accessions which were not available in Geneva and collected from the USDA-ARS *Vitis* Clonal Repository in Davis, CA. Among the 17 *Vitis* species, *V. vinifera* originated in Europe/Middle East, *V. amurensis*, *V. coignetii*, and *V. yenshanensis* in East Asia, and the rest in North America. Some of these 17 *Vitis* species were represented by only one or a few accessions in the repository, but most others by 5 or more (S Table 1 in the Supporting Information). For each accession, two grapevines were available for sampling. Berry samples of individual vines were harvested upon their ripening in the two consecutive years 2008 and 2009. Berry ripening was mainly determined on the basis of berry firmness and changes of seed color. We observed that berry ripening varied significantly among species and also among accessions within the same species. To reduce sampling variation due to ripening, we harvested ripening berries from vines in which 50% or more berries had reached ripening. Two vines of the same accessions were sampled at the same time. Total soluble solids (Brix) data were collected at the time of harvest using a hand-held Atago PR-32α Palette digital refractometer. Because Brix values varied significantly among wild species, they were used only as a general reference of ripening for the same accessions across two different seasons. All the vines received standard fertilization, irrigation, pruning, and insect and disease control.

For each accession, about 100 representative berries were collected from each of two individual grapevines. There were four biological replicates for each accession (2 vines × 2 years). The fresh berries were frozen and stored at −80 °C for further processing. The frozen berries were crushed using mortar and pestle in liquid nitrogen, and berry seeds were separated from skins and pulps. Frozen seeds were ground into powder in an IKA A11 mill (IKA Works, Inc., NC, USA). For each sample, 0.2 g of powder was used for extraction.

Extraction of Polyphenols. The powdery samples were ground in 2 mL of extract solution (2:28:70, formic acid:water:methanol) using mortar and pestle. The extracts were shaken for 10 min in a thermo mixer (Eppendorf, USA). Then the extracts were centrifuged at 13000*g* at 4 °C for 10 min. About 1 mL of extract was filtered through a 0.2 μm membrane filter (Agilent Technologies, USA) for analysis.

Qualitative and Quantitative Analysis of Polyphenols. High performance liquid chromatography/quadrupole-time-of-flight mass spectrometer (HPLC/Q-TOF MS/MS) (Micromass Q-TOF micro, Waters, USA) was employed for identifying polyphenols.[22,23] The system was equipped with a Waters Alliance 2695 HPLC pump, Waters Alliance 2695 autosampler and Waters 996 photodiode array detector, which were coupled directly to the sprayer needle where ions were generated by electrospay ionization (ESI) in both positive and negative ionization modes. A reverse-phase C18 column Inertsil ODS-3 (5 μm particle sizes, 250 mm × 4.6 mm i.d.) from GL Sciences (Japan) and a C18 Nova Pack guard column (Waters, USA) were used for the analysis. The mobile phase consisted of water−formic acid (90:10) as solvent A and acetonitrile−formic acid (90:10) as solvent B. The gradient profile began at 95% A, to 85% A at 25 min, 73% A at 53 min, then A went back to 95% at 57 min, and was kept for 5 min. The flow rate was 1.0 mL min^{-1}, and the column temperature was set at 30 °C. The injection volume was 10 μL. Polyphenolic compounds were detected at 280, 320, and 360 nm on the diode array detector, and at the same time, spectrum scans were made from 210 to 600 nm. For MS analyses, nitrogen was used as drying and nebulizing gas, and nebulizer pressure was 380 Pa. Gas flow was set at 10 L min^{-1}, and temperature was 350 °C. The capillary voltage was 3000 V. Mass spectra of polyphenolic compounds in the range of m/z 100 and 1000 were recorded in both positive and negative ionization modes.

The same HPLC protocol was used by the Agilent 1100 HPLC system (Agilent Corporation, CA, USA) fitted with an Agilent 1100 diode array detector and autosampler for quantifying of polyphenolic compounds for all samples. The concentration of individual polyphenolic compounds was quantified on the basis of peak area and calibration curves derived from corresponding authentic polyphenolic compounds or standards at the same wavelength. Standards for eleven polyphenolic compounds, obtained from Sigma-Aldrich (St. Louis, MO, USA) and AApin Chemicals (Abingdon, Oxon, U.K.), were available for this study. They were gallic acids, galloyl glucoside, procyanidin B1, procyanidin B2, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-*O*-gallate, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, syringetin 3-*O*-glucoside, and resveratrol. Standards for dimeric and trimeric flavan-3-ols, except for procyanidin B1 and procyanidin B2, were not available in this study. Therefore the true identities for these dimers and trimers could not be positively determined. The contents of these compounds were quantified using (+)-catechin as external standard.

Data Analysis. Data analysis was carried out using the SAS 9.2 package (SAS Institute Inc. USA). Accession means over years and plants were used in correlation analysis. Boxplots were developed by using Sigmaplot 10.0 for Windows (SPSS, USA). Because the *V. vinifera* berry samples were collected from a different planting location, location effect was confounded with the true genetic difference when the results of *V. vinifera* were compared with those from other species. Therefore, comparisons of *V. vinifera* with other species were statistically compromised. Nevertheless, we included *V. vinifera* in this study as a general reference for assessing the range of variation of seed polyphenols in both cultivated and wild grape species.

RESULTS AND DISCUSSION

Identification of Polyphenolic Compounds by HPLC−MS. The identities of polyphenolic compounds were determined from the MS and HPLC profiles of retention time, molecular ions, fragment ions, and UV−Vis spectra absorbance maxima (Table 1). Under the experimental conditions established, the peaks corresponding to 11 compounds were satisfactorily separated, identified, and quantified in response to their analytical standards. They included gallic acid (peak no. 1), galloyl glucoside (peak no. 2), procyanidin B1 (peak no. 3), procyanidin B2 (peak no. 5), (+)-catechin (peak no. 6), (−)-epicatechin (peak no. 14), (−)-epicatechin 3-*O*-gallate (peak no. 20), quercetin 3-*O*-glucoside (peak no. 23), quercetin 3-*O*-galactoside (peak no. 24), syringetin 3-*O*-glucoside (peak no. 25) and resveratrol (peak no. 28). Seventeen di- and trimers were also quantified, although their exact identities were not accurately determined due to the lack of appropriate standards. These 28 polyphenolic compounds were classified into six groups on the basis of their chemical structures: gallic derivatives (gallic acid and galloyl glucose), flavan-3-ol monomers ((+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-*O*-gallate), flavan-3-ol dimers (procyanidin B1 and B2 and other dimer derivatives), flavan-3-ol trimers, resveratrol, and flavonols (quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and syringetin 3-*O*-glucoside). These similar groups of polyphenolic compounds were also described in previous studies of grape seed extracts.[24−26]

Total Detected Polyphenolic Content. The total detected polyphenolic content was calculated as sum of the 28 individual compounds observed in the study. It ranged from 0.950 to 41.315 mg g^{-1} FW among accessions, with an overall mean 8.770 mg g^{-1} FW (Figure 1A; S Table 1 in the

Table 1. Polyphenolic Compounds Identified in This Study through Chromatography and Mass Spectrometry

peak no.	t_R (min)	abs max[a] (nm)	fragment ions M^- (m/z)	identity[b]
1	4.31	280	125, 169	gallic acid
2	5.33	280	169, 331	galloyl glucose
3	7.24	280	289, 425, 577	procyanidin B1
4	7.54	280	289, 425, 577	dimer1
5	9.32	280	305, 577	procyanidin B2
6	10.23	280	289	(+)-catechin
7	11.90	280	289, 577, 785	dimer2
8	12.17	280	289, 425, 577	dimer3
9	12.42	280	289, 425, 577	dimer4
10	13.64	280	577, 729	dimer5
11	14.02	280	577, 729, 865	trimer1
12	14.80	280	304, 577, 865	trimer2
13	15.63	280	577, 729	dimer6
14	16.63	280	289	(−)-epicatechin
15	17.66	280	289, 425, 577, 631	dimer7
16	18.48	280	289, 577, 631, 865	trimer3
17	19.29	280	635	dimer8
18	22.73	280	765, 783	dimer9
19	23.35	280	783, 881	dimer10
20	24.47	280	289, 442	(−)-epicatechin 3-*O*-gallate
21	26.78	280	169, 289, 441, 577	dimer11
22	29.94	280	577	dimer12
23	31.96	350	301, 463	quercetin 3-*O*-glucoside
24	34.47	350	463	quercetin 3-*O*-galactoside
25	37.32	350	369, 507	syringetin 3-*O*-glucoside
26	38.12	280	369, 533	dimer13
27	40.50	280	369, 881	trimer4
28	43.11	320	169, 219	resveratrol

[a]Absorbance maxima. [b]Dimer1 to dimer13 refer to thirteen different chemical forms of dimers each consisting of one (+)-catechin (or its derivative) and one (−)-epicatechin (or its derivative); trimer1 to trimer4 refer to four different forms of trimers each consisting of two (+)-catechins (or derivatives) and one (−)-epicatechin (or its derivative) or one (+)-catechin (or its derivative) and two (−)-epicatechnins (or their derivatives).

Supporting Information). Among the 17 species, *V. palmata* had the highest mean polyphenolic content (21.017 mg g^{-1} FW), which was significantly higher than that of other species ($P < 0.05$). *V. vinifera* (17.629 mg g^{-1} FW) and *V. vulpina* (19.490 mg g^{-1} FW) had the next highest content of the total detected polyphenols, significantly higher than that of the rest of the species. *V. champinii* had the lowest polyphenolic content (0.950 mg g^{-1} FW) (Figure 1A). The top 5 accessions with high content of polyphenols were DVIT 0915 (Pinot Noir, *V. vinifera*), 588679 (*V. vulpina*), DVIT0944 (Salvador, *V. vinifera*), 588677 (*V. aestivalis*), and 588133 (*V. vulpina*) (Table 2).

Among the 6 groups of individual compounds, flavan-3-ol monomers were the most abundant type of polyphenols detected, on average accounting for about 46.9% of the total, followed by the dimers and trimers of flavan-3-ol, accounting for 35.1 and 14.4% of the total, respectively (Figure 2A). The remaining polyphenols all together were no more than 4% of the total detected polyphenols. These results were in general agreement with some of the previous studies. For example, it was reported that monomers, dimers, and trimers respectively accounted for 47.9, 40.2, and 6.9% of the total flavan-3-ols in *V. vinifera* cv. Tempranillo, 57.1, 28.4, and 9.1% in *V. vinifera* cv. Graciano, and 71.4, 19.4, and 5.9% in *V. vinifera* cv. Cabernet Sauvignon.[27]

Among the 17 species, *V. vulpina*, *V. aestivalis*, and *V. yenshanensis* had the highest content of flavan-3-ol monomers, accounting for 70.4, 68.9, and 62.6% of the total detected polyphenols, respectively. *V. vinifera* and *V. coignetiae*, on the other hand, had the highest content of flavan-3-ol dimers, accounting for 52.9, 44.0, and 62.4% of the total detected polyphenols, respectively (Figure 2D). *V. champinii* had the highest content of flavan-3-ol trimers (45.2% of the total detected polyphenols). Clearly, the content of the three major seed polyphenolic compounds, monomers, dimers, and trimers of flavan-3-ol, varied significantly among different species (Figure 2D).

Gallic Derivatives. Gallic derivatives ranged from 0.010 to 1.182 mg g^{-1} FW among accessions with a mean content of 0.230 mg g^{-1} FW (Figure 1B). *V. palmata* had the highest content of gallic derivatives (0.628 mg g^{-1} FW), significantly higher than that of other species. *V. vinifera* (0.466 mg g^{-1} FW) and *V. vulpina* (0.434 mg g^{-1} FW) had significantly higher gallic derivative content than the rest of the species. *V. doaniana* and *V. champinii* had the least gallic derivative content, 0.035 and 0.016 mg g^{-1} FW, respectively. The top 5 accessions with the highest content of gallic derivatives were DVIT0944 (Salvador, *V. vinifera*), 588233 (*V. palmata*), DVIT0468 (Muscat Hamburg, *V. vinifera*), 588679 (*V. vulpina*), and DVIT0947 (Sauvignon Blanc, *V. vinifera*) (Table 2).

Gallic derivatives mainly comprised gallic acid and galloyl glucose. The content of gallic acid ranged from 0.003 to 0.760 mg g^{-1} FW among accessions (data not shown) and from 0.004 to 0.584 mg g^{-1} FW among species (S Table 2 in the Supporting Information). On average, gallic acid accounted for about 45.9% of the total gallic derivatives (Figure 2B). In most species, gallic acid accounted for no more than 50% of the total gallic derivatives except for *V. palmata* (93.0%), *V. vulpina* 82.2%), *V. aestivalis* (77.8%) and *V. cinerea* (66.8%) (Figure 2B). The content of galloyl glucose ranged from 0.008 to 1.017 mg g^{-1} FW among accessions (data not shown) and from 0.012 to 0.377 mg g^{-1} FW among species (S Table 2 in the Supporting Information). The highest content of galloyl glucose was found in *V. vinifera*, accounting for about 80% of the total gallic derivatives (Figure 2B). Our results were in the general range of what were previously observed in various grape seed extracts studied. Guendez et al.[21] reported that the average content of gallic acid in the seeds of 12 white and 15 red grapes were respectively 0.036 and 0.052 mg/g FW; Yilmaz and Toledo[20] observed that the seeds of *V. muscadine* grapes contained 0.99 mg/g gallic acid; and Applequist et al.[14] reported that gallic acid ranged from 0.10 to 0.17 mg/g in hybrid grape seeds.

Monomers of Flavan-3-ol. The content of monomeric flavan-3-ols ranged from 0.017 to 20.954 mg g^{-1} FW among accessions with a mean of 4.746 mg g^{-1} FW (Figure 1C). *V. vulpina* had the highest content of monomeric flavan-3-ols (13.720 mg g^{-1} FW), significantly higher than other species. *V. palmata* (11.642 mg g^{-1} FW) and *V. aestivalis* (10.851 mg g^{-1} FW, Figure 1C) had the second and third highest, respectively. *V. vinifera* and *V. monticola* also had relatively high content of monomeric flavan-3-ols with the mean content of 6.885 mg g^{-1} FW and 6.882 mg g^{-1} FW, respectively. The lowest content of

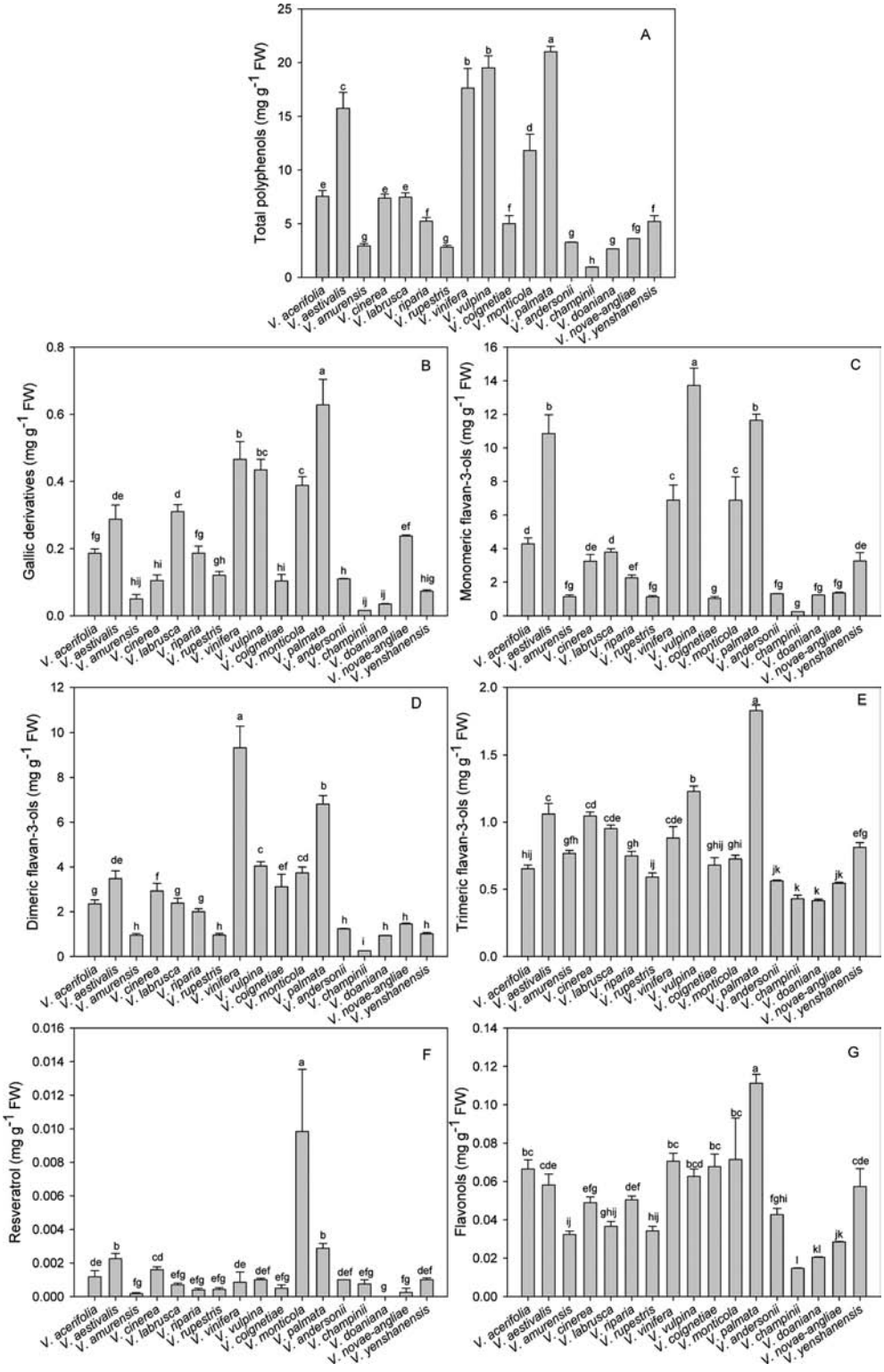

Figure 1. Polyphenolic content of seeds in 17 *Vitis* species: total polyphenols (A), gallic derivative (B), monomeric flavan-3-ols (C), dimeric flavan-3-ols (D), trimeric flavan-3-ols (E), resveratrol (F), and flavonols (G). Bars with no letters in common are significantly different at $P < 0.05$. Caution should be taken when *V. vinifera* is compared with other species, as explained in the Material and Methods.

monomeric flavan-3-ols was found in *V. coignetiae* (1.023 mg g^{-1} FW) and *V. champinii*, (0.237 mg g^{-1} FW). The top 5 accessions with the highest content of monomeric flavan-3-ols were 588679 (*V. vulpina*), DVIT0915 (Pinot Noir, *V. vinifera*), 588677 (*V. aestivalis*), 588133 (*V. vulpina*), and 588680 (*V. vulpina*) (Table 2).

(+)-Catechin was the most abundant monomers of flavan-3-ols, accounting for 55.9% of the total (Figure 2C). The content of (+)-catechin ranged from 0.070 to 12.400 mg g^{-1} FW among accessions (data not shown) and from 0.134 to 5.242 mg g^{-1} FW among species (S Table 2 in the Supporting Information). *V. yenshanensis*, *V. doaniana*, and *V. acerifolia* had the highest

dx.doi.org/10.1021/jf2046637 | *J. Agric.Food Chem.* 2012, 60, 1291−1299

Table 2. Representative Accessions Containing One or More of the Top 5 Most Abundant Polyphenolic Compounds (mg g^{-1} FW) (in Bold) in the 17 *Vitis* Species Studied

species	IVNO[a]	cultivar	gallic derivatives	flavan-3-ols			resveratrol	flavonols	total polyphenols
				monomeric	dimeric	trimeric			
V. acerifolia	588646		0.208	2.819	1.112	0.525	**0.006**	0.073	4.743
V. aestivalis	588677		0.240	**17.772**	5.342	1.221	**0.003**	0.048	**24.628**
V. monticola	588454		0.388	6.882	3.733	0.726	**0.010**	0.072	11.810
V. palmata	588467		0.440	11.487	**7.760**	**1.820**	0.002	**0.118**	21.627
V. palmata	588233		**0.816**	11.794	5.848	**1.841**	**0.004**	**0.105**	20.408
V. riparia	313922	Tarnau	0.382	6.379	6.669	1.252	**0.010**	0.054	14.744
V. vinifera	DVIT0468	Muscat Hamburg	**0.628**	3.888	4.550	0.568	0.000	0.059	9.693
V. vinifera	DVIT0677	Cabernet Sauvignon	0.459	7.773	**8.087**	0.938	0.001	**0.120**	17.377
V. vinifera	DVIT0915	Pinot Noir	0.406	**19.621**	**19.822**	**1.372**	0.001	**0.095**	**41.315**
V. vinifera	DVIT0944	Salvador	**1.182**	8.738	**15.089**	0.701	0.000	0.080	**25.789**
V. vinifera	DVIT0947	Sauvignon Blanc	**0.607**	8.316	**10.635**	1.016	0.001	0.062	20.634
V. vulpina	483180	Rem 15-77	0.431	10.063	3.380	**1.420**	0.002	0.076	15.370
V. vulpina	483187	Rem 36-77	0.316	5.025	4.271	1.225	0.001	**0.101**	10.938
V. vulpina	483188	Rem 38-77	0.222	14.241	4.537	**1.391**	0.001	0.069	20.459
V. vulpina	588133	GBC 5	0.324	**17.162**	4.721	1.130	0.001	0.053	**23.390**
V. vulpina	588679		**0.607**	**20.954**	3.461	1.101	0.001	0.056	**26.179**
V. vulpina	588680		0.549	**16.995**	4.299	1.357	0.001	0.040	23.241

[a]Inventory number in the USDA-ARS Germplasm Resources Information Network (GRIN).

relative content of (+)-catechin, accounting for more than 80% of the total monomeric flavan-3-ols in these species. (−)-Epicatechin was the second most abundant monomer of flavan-3-ols. It ranged from 0.060 to 13.437 mg g^{-1} FW among accessions (data not shown) and from 0.093 to 8.394 mg g^{-1} FW among species (S Table 2 in the Supporting Information). (−)-Epicatechin on average accounted for 40.2% of the total monomeric flavan-3-ols. *V. vulpina* and *V. palmata* had the highest relative (−)-epicatechin content, which accounted for 61.2 and 57.7% of total monomeric flavan-3-ols in the two species, respectively. In comparison, (−)-epicatechin 3-*O*-gallate was relatively less abundant and it ranged from 0.007 to 1.240 mg g^{-1} FW among accessions (data not shown) and from 0.011 to 0.989 mg g^{-1} FW among species (S Table 2 in the Supporting Information). (−)-Epicatechin 3-*O*-gallate accounted for no more than 4% of the total monomeric flavan-3-ols. The highest content of (−)-epicatechin 3-*O*-gallate was found in *V. palmata* (0.989 mg g^{-1} FW). Obreque-Slier et al.[28] reported that the mean content of (+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-*O*-gallate were respectively 0.467, 0.379, and 0.182 mg/g in *V. vinifera* cv. Carmenere, and 0.96, 0.36, and 0.052 mg/g in *V. vinifera* cv. Cabernet Sauvignon at the maturity stage. Guendez,[21] on the other hand, reported that the contents of these three monomeric flavan-3-ols were respectively 0.387, 0.28, and 0.122 mg g^{-1} FW in *V. vinifera* cv. Chardonnary, 2.15, 0.893, and 0.279 mg g^{-1} in *V. vinifera* cv. Cabernet Sauvignon, and 2.285, 1.205, and 0.206 mg/g in *V. vinifera* cv. Muscat Hamburg. Our results suggested that the mean concentrations of (+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-*O*-gallate in the 17 *Vitis* species obtained in this study were much higher than what were observed in *V. vinifera* by Guendez[21] or Obreque-Slier et al.[28]

Dimers of Flavan-3-ol. We quantified 15 dimeric flavan-3-ols which included procyanidin B1 and B2 and other dimers of (+)-catechin and (−)-epicatechin and their esterified forms with one or two gallic acids (S Tables 2 and 3 in the Supporting Information). The total dimeric flavan-3-ol content ranged from 0.253 to 19.822 mg g^{-1} FW among accessions with a mean of 3.090 mg g^{-1} FW. Among the 17 species, *V. vinifera* had the highest content of the total dimers of flavan-3-ol (9.325 mg g^{-1} FW, Figure 1D). *V. palmata* had the second highest content (6.804 mg g^{-1} FW). The lowest content of dimeric flavan-3-ols was found in *V. champinii* (0.253 mg g^{-1} FW). The top 4 accessions with the highest content of dimeric flavan-3-ols (more than 10 mg g^{-1} FW) all came from *V. vinifera* (Table 2). Procyanidin B1 was the predominant dimer type and accounted for 25% of the total dimeric flavan-3-ols. The content of procyanidin B1 ranged from 0.021 to 9.556 mg g^{-1} FW among accessions (data not shown) and from 0.021 to 3.410 mg g^{-1} FW among species (S Table 2 in the Supporting Information). *V. vinifera* had the highest content of procyanidin B1 (3.41 mg g^{-1} FW). Procyanidin B2 accounted for no more than 3.5% of the total dimeric flavan-3-ols. *V. cinerea* had the highest content of procyanidin B2 (0.421 mg g^{-1} FW, S Table 2 in the Supporting Information). Other dimers accounted for the remaining 71.5% of the total dimeric flavan-3-ols. Fuleki et al.[29] reported that the contents of procyanidin B1 and B2 were respectively 0.17 and 0.5 mg/g in the seeds of *V. vinifera* cv. Cabernet Sauvignon, 0.56 and 0.9 mg/g in *V. vinifera* cv. Pinot Noir, and 0.25 and 0.62 mg/g in *V. vinifera* cv. Chardonnay. Similarly, Monagas et al.[27] reported that the contents of procyanidin B1 and B2 were respectively 0.42 and 0.8 mg/g in the seed extracts of the *V. vinifera* cv. Carbernet Sauvignon. However, very low contents of procyanidin B1 (0.029 mg/g) and B2 (0.072 mg/g) were reported in Cabernet Sauvignon by Obreque-Slier et al.[28] All these results were much lower than what we observed in this study. The discrepancies of these results could be due to different analytical methods used, grapevine growing locations, or physiological conditions of berries and sampling errors, but certainly also due to the genetic differences among different species and varieties.

Trimers of Flavan-3-ol. We quantified four trimeric flavan-3-ols which are composed of three monomers and their esterified forms with gallic acids (S Tables 2 and 3 in the Supporting Information). The content of the total trimeric flavan-3-ols ranged from 0.343 to 1.841 mg g^{-1} FW among accessions, and the average content was 0.861 mg g^{-1} FW (Figure 1E). *V. palmata* had the highest content of the total

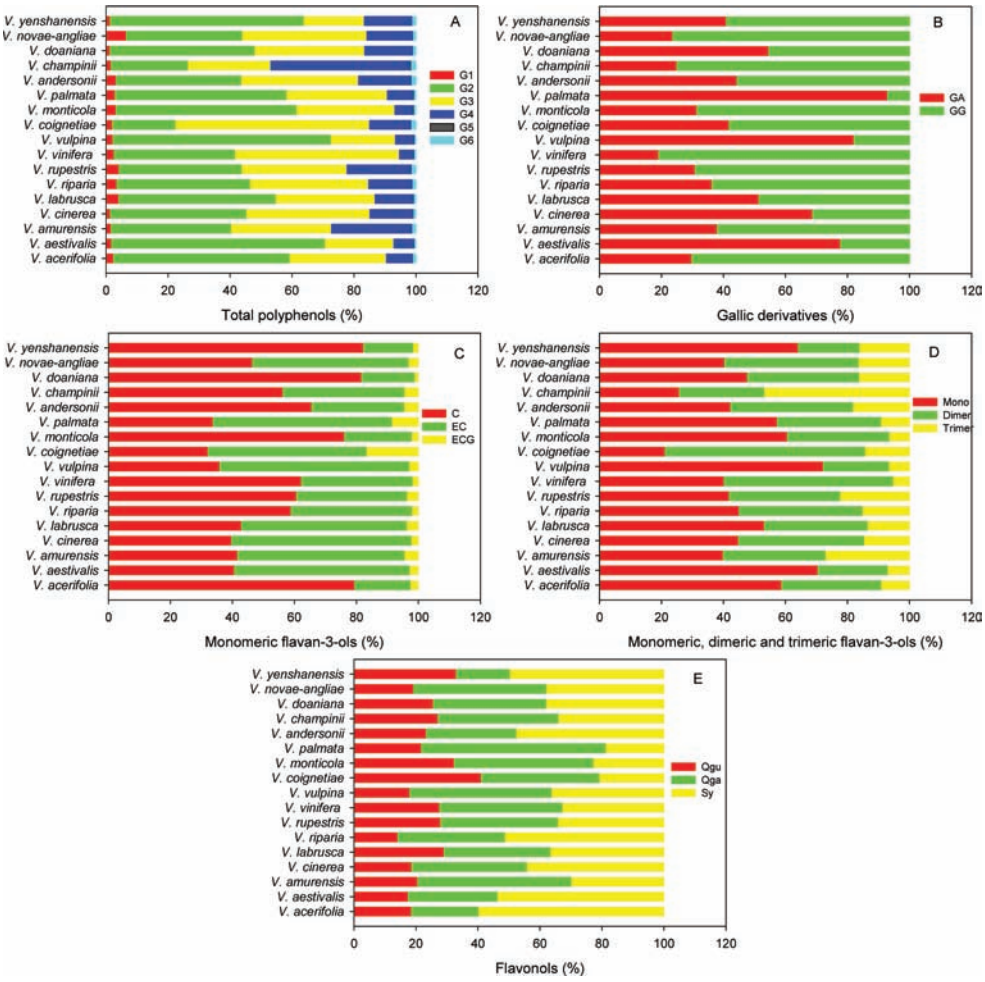

Figure 2. Percentages of individual components contributing to the total variation of polyphenolic compounds: total polyphenols (A), gallic derivatives (B), monomeric flavan-3-ols (C), and monomers, dimers, and trimers of flavan-3-ols (D) and flavonols (E) in 17 *Vitis* species. G1 = gallic derivatives; G2 = monomeric flavan-3-ols; G3 = dimeric flavan-3-ols; G4 = trimeric flavan-3-ols; G5 = resveratrol; G6 = flavonols; GA = gallic acid; GG = galloyl glucoside; C = (+)-catechin; EC = (−)-epicatechin; ECG = (−)-epicatechin 3-*O*-gallate; Qgu = quercetin 3-*O*-glucoside; Qga = quercetin 3-*O*-galactoside; Sy = syringetin 3-*O*-glucoside.

trimeric flavan-3-ols (1.830 mg g^{-1} FW), followed by *V. vulpina* (1.229 mg g^{-1} FW). The lowest content was found in *V. champinii* and *V. doaniana* (0.430 and 0.415 mg g^{-1} FW, respectively). The top 5 accessions with the highest content of trimeric flavan-3-ols were 588233 (*V. palmata*), 588467 (*V. palmata*), 483180 (*V. vulpina*), 483188 (*V. vulpina*), and DVIT0915 (*V. vinifera*) (Table 2). De Freitas et al.[30] reported that the content of trimeric flavan-3-ols was 12.5 mg/g in *V. vinifera* cv. Cabernet Sauvignon dry seeds and 6.54 mg/g in *V. vinifera* cv. Merlot dry seeds. Mateus et al.[31] found that the procyanidin C content was 1.43 mg/g in *V. vinifera* cv. Touriga Nacional. These results were higher than what we found in this study. One major factor contributing to these differences was that we used fresh seed material for the analysis, while others used dried seed material.

Resveratrol. The content of resveratrol ranged from 0 (undetectable) to 0.010 mg g^{-1} FW among accessions (data not shown) and from 0 (undetectable) to 0.010 mg g^{-1} FW among species (Figure 1F, S Table 2 in the Supporting Information). *V. monticola* had the highest content of resveratrol (0.010 mg g^{-1} FW), followed by *V. palmata* (0.003 mg g^{-1} FW) and *V. aestivalis* (0.002 mg g^{-1} FW). We did not detect resveratrol in *V. doaniana*. The top 5 accessions with the highest content of resveratrol were 588454 (*V. monticola*), 313922 (*V. riparia*), 588646 (*V. acerifolia*), 588233 (*V. palmata*), and 588677 (*V. aestivalis*) (Table 2).

Flavonols. Flavonols included quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and syringetin 3-*O*-glucoside. The content of flavonols ranged from 0.015 to 0.120 mg g^{-1} FW among accessions, and the mean content was 0.051 mg g^{-1} FW (Figure 1G). *V. palmata* had the highest content (0.111 mg g^{-1} FW), followed by *V. monticola* (0.072 mg g^{-1} FW), *V. vinifera* (0.071 mg g^{-1} FW), *V. coignetiae* (0.068 mg g^{-1} FW), and *V. rupestris* (0.034 mg g^{-1} FW). The lowest content of flavonols was found in *V. champinii* (0.015 mg g^{-1} FW). The top 5 accessions with the highest content of flavonols were DVIT0677 (Cabernet Sauvignon, *V. vinifera*), 588467 (*V. palmata*), 588233 (*V. palmata*), 483187 (*V. vulpina*), and DVIT0915 (*V. vinifera*) (Table 2).

On average, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and syringetin 3-*O*-glucoside accounted for 24.8, 37.1, and 38.2% of the total flavonols, respectively (Figure 2E). *V. coignetiae* and *V. palmata* had the highest content of quercetin 3-*O*-glucoside with 0.028 and 0.024 mg g^{-1} FW, respectively (S Table 2 in the Supporting Information). Quercetin 3-*O*-glucoside accounted for 41.1% of the total flavonols in *V.*

Table 3. Mean Square for the Six Groups of Polyphenols Attributable to the Variation Sources of Year, Species, Accessions within Species, and Plants within Species and Accessions

	variation source			
polyphenolic compds	year	species	accession (species)	plant (species, accessions)
gallic derivatives	0.032**[a]	0.454**	0.073**	0.001
monomeric flavan-3-ols	24.054**	315.243**	26.522**	1.759
dimeric flavan-3-ols	5.131**	94.544**	14.526**	0.089
trimeric flavan-3-ols	0.325**	1.298**	0.163**	0.009
resveratrol	5.00×10^{-8}	3.78×10^{-5}**	6.67×10^{-6}**	4.58×10^{-6}**
flavonols	1.42×10^{-4}	0.006**	0.001**	0.001**
total polyphenols	62.535**	697.347**	63.409**	2.205

[a]**Significance at $P < 0.01$.

coignetiae and 21.8% in *V. palmata*. Similarly, *V. palmata* and *V. monticola* had highest content of quercetin 3-*O*-galactoside, 0.066 and 0.032 mg g^{-1} FW, respectively (S Table 2 in the Supporting Information). Quercetin 3-*O*-galactoside accounted for 59.6% of the total flavonols in *V. palmata* and 22.4% in *V. monticola*. In the case of syringetin 3-*O*-glucoside, *V. acerifolia* and *V. aestivalis* had the highest content with 0.040 and 0.031 mg g^{-1} FW, respectively (S Table 2 in the Supporting Information). Syringetin 3-*O*-glucoside accounted for 59.6% of the total flavonols in *V. acerifolia* and 53.6% in *V. aestivalis*. Karmmerer et al.[32] reported that quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside were 0.033 and 0.015 mg/g, respectively, in the *V. vinifera* cv. Weisser Riesling seed.

Variation Patterns among and within *Vitis* Species. The results of analysis of variance are summarized in Table 3. Significant variation was observed at $P < 0.01$ among species and also among accessions within species for the total detected polyphenolic content. On average, 84.5% of the total variation was distributed among species, 7.7% among accessions within species, 7.6% between years, and only 0.3% between plants within accessions. The low plant-to-plant variation indicated that the clonal variation between samples was very limited. Significant variation was also observed at $P < 0.01$ among species and among accessions within species for the content of the six groups of individual compounds: gallic deivatives, monomeric flavan-3-ols, dimeric flavan-3-ols, trimeric flavan-3-ols, resveratrol, and flavonols. Among the six groups of polyphenolic compounds, the total variation explained by the difference among species was more than 72.3% (from 72.3 to 85.8%) of the total variation. The among-accession variation within species ranged from 7.6 to 13.2%. The variation between clonal plants within accessions, as expected, was rather small (no more than 1%), except for resveratrol and flavonol (9.3 and 12.3%, respectively). The relative amount of variation between years, however, was quite significant, particularly for trimeric flavan-3-ols (18.1%), suggesting that growing conditions between years had significant impact on the content of these compounds.

Although among-species variation was predominant, variation among accessions within species was also significant. For example, the total polyphenolic content among different accessions ranged from 1.358 to 10.728 mg g^{-1} FW in *V. acerifolia* and from 1.793 to 14.744 mg g^{-1} FW in *V. riparia* (S Table 1 in the Supporting Information). Similar magnitude of among-accession variation was also observed for polyphenolic compounds in other species. The observation of such large variation within species reinforced the importance of preserving diverse accessions to capture the majority of within-species genetic diversity for the purpose of germplasm conservation and utilization.

It was interesting to note that the total detected polyphenolic content in the seeds of the cultivated species *V. vinifera* was several times higher than that of most wild species. This could be partly explained by the fact that *V. vinifera* in general had smaller seeds than most wild *Vitis* species and there was a weak, but significantly negative correlation between seed weight and the total content of polyphenolic compounds in seeds ($r = -0.307$). Because most polyphenolic compounds reside in the seed coat,[19] a large seed is expected to have less content of phenolic compounds per unit weight. Another possible factor contributing to this observation is that *V. vinifera* has been under extensive human selection for winemaking. Polyphenolic compounds, tannins in particular, in seeds might be enriched in *V. vinifera* by such selection due to their importance in wine quality. Knowing that *V. vinifera* has higher content of seed polyphenols than most wild species is important, as it will provide us some guidance on how wild germplasm may affect the content of seed polyphenolic compounds in the crossing progeny when wild germplasm is introduced into *V. vinifera*.

The correlation coefficients among various polyphenolic compounds in the 17 *Vitis* species are shown in S Table 4 in the Supporting Information. As expected, the individual groups of polyphenolic compounds were all positively correlated with the total polyphenols (r = 0.385 to 0.917). Since monomeric flavan-3-ol was the most predominant type of polyphenol, it had the highest correlation coefficient with the total polyphenols (r = 0.917). Among the 6 groups of compounds, resveratrol had the lowest coefficients with other polyphenolic groups (r = 0.274 to 0.391). The correlation coefficients among the other five groups of compounds were medium to high (r = 0.439 to 0.652).

This study was the first to document the composition and content of seed polyphenols in wild *Vitis* germplasm. The information obtained in this study will be an important addition to our ongoing effort of developing a comprehensive database of health-related secondary metabolites in the *Vitis* germplasm for grape improvement.[22,23] Many *Vitis* accessions with high content of various individual groups of seed polyphenols were identified in this study. These accessions can serve as a potential gemplasm resource for improving the composition and content of seed polyphenols in cultivated wine grapes in the future.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Four tables providing information about the source of germplasm accessions, mean contents of various phenolic

compounds for individual species, and correlation coefficients among six groups of polyphenolic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*USDA-ARS Grape Genetics Research Unit, Geneva, NY 14456. Tel: 1-315-787-2482. Fax: 1-315-787-2339. E-mail: ganyuan.zhong@ars.usda.gov.

Funding

This work was supported through a Special Cooperative Agreement between USDA-ARS and Cornell University.

ACKNOWLEDGMENTS

We are grateful to Dawn Dellefave, Heidi Schwaninger, and Bill Srmack of the USDA-ARS Plant Genetic Resources Unit for their assistance in collecting berry samples of the *Vitis* species preserved in the *Vitis* Clonal Repository in Geneva, NY, and to Bernard Prins of the USDA-ARS National Clonal Germplasm Repository for Fruit and Nut Crops in Davis, CA, and Dr. Christopher Owens of the USDA-ARS Grape Genetics Research Unit in Geneva, NY, for providing the *V. vinifera* berry samples. USDA is an equal opportunity provider and employer.

REFERENCES

(1) Singleton, V. L. Grape and wine phenolics: background and prospects. In *Proceedings of Grape and Wine Centennial Symposium University of California*; Davis, CA, USA, 1980; pp 215−227.

(2) Xia, E. Q.; Deng, G. F.; Guo, Y. J.; Li, H. B. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.* **2010**, *11*, 622−646.

(3) Hernandez-Jimenez, A.; Gomez-Plaza, E.; Martinez-Cutillas, A.; Kennedy, J. A. Grape skin and seed proanthocyanidins from Monastrell x Syrah grapes. *J. Agric. Food. Chem.* **2009**, *57*, 10798−10803.

(4) Vernhet, A.; Pellerin, P.; Prieur, C.; Osmianski, J.; Moutounet, M. Charge properties of some grape and wine polysaccharide and polyphenolic fractions. *Am. J. Enol. Vitic.* **1996**, *47*, 25−30.

(5) Riou, V.; Vernhet, A.; Doco, T.; Moutounet, M. Aggregation of grape seed tannins in model wine - effect of wine polysaccharides. *Food Hydrocolloids* **2002**, *16*, 17−23.

(6) Brossaud, F.; Cheynier, V.; Noble, A. C. Bitterness and astringency of grape and wine polyphenols. *Aust. J. Grape Wine Res.* **2001**, *7*, 33−39.

(7) Sidari, R.; Postorino, S.; Caparello, A.; Caridi, A. Evolution during wine aging of colour and tannin differences induced by wine starters. *Ann. Microbiol.* **2007**, *57*, 197−201.

(8) Waterhouse, A. L. Wine Phenolics. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 21−36.

(9) Shi, J.; Yu, J.; Pohorly, J. E.; Kakuda, Y. Polyphenolics in grape seeds: Biochemistry and functionality. *J. Med. Food* **2003**, *6*, 291−299.

(10) Amarowicz, R.; Weidner, S. Biological activity of grapevine phenolic compounds. In *Grapevine Molecular Physiology & Biotechnology*, 2nd ed.; Roubelakis-Angelakis, K.A., Ed.; Springer Science and Business Media B.V.: 2009.

(11) Quesada, I. M.; Del Bas, J. M.; Blade, C.; Ardevol, A.; Blay, M.; Salvado, M. J.; Pujadas, G.; Fernandez-Larrea, J.; Arola, L. Grape seed procyanidins inhibit the expression of metallothione in genes in human HepG2 cells. *Genes Nutr.* **2007**, *2*, 105−109.

(12) Urpi-Sarda, M.; Monagas, M.; Khan, N.; Lamuela-Raventos, R. M.; Santos-Buelga, C.; Sacanella, E.; Castell, M.; Permanyer, J.; Andres-Lacueva, C. Epicatechin, procyanidins, and phenolic microbial metabolites after cocoa intake in humans and rats. *Anal. Bioanal. Chem.* **2009**, *394*, 1545−1556.

(13) Terra, X.; Palozza, P.; Fernandez-Larrea, J.; Ardevol, A.; Blade, C.; Pujadas, G.; Salvado, J.; Arola, L.; Blay, M. T. Procyanidin dimer B1 and trimer C1 impair inflammatory response signalling in human monocytes. *Free Radical Res.* **2011**, *45*, 611−619.

(14) Applequist, W. L.; Johnson, H.; Rottinghaus, G. (+)-Catechin, (-)-epicatechin, and gallic acid content of seeds of hybrid grapes hardy in Missouri. *Am. J. Enol. Vitic.* **2008**, *59*, 98−102.

(15) Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* **2000**, *55*, 77−85.

(16) Ricardo-Da-Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* **1991**, *30*, 1259−1264.

(17) Vidal, S.; Francis, L.; Guyot, S.; Marnet, N.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. J. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food. Agric.* **2003**, *83*, 564−573.

(18) Cheynier, V.; Prieur, C.; Guyot, S.; Rigaud, J.; Moutounet, M. The structures of tannins in grapes and wines and their interactions with proteins. *Wine* **1997**, *661*, 81−93.

(19) Thorngate, J. H.; Singleton, V. L. Localization of procyanidins in grape seeds. *Am. J. Enol. Vitic.* **1994**, *45*, 259−262.

(20) Yilmaz, Y.; Toledo, R. T. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem.* **2004**, *52*, 255−260.

(21) Guendez, R.; Kallithraka, S.; Makris, D. P.; Kefalas, P. An analytical survey of the polyphenols of seeds of varieties of grape (*Vitis vinifera*) cultivated in Greece: Implications for exploitation as a source of value-added phytochemicals. *Phytochem. Anal.* **2005**, *16*, 17−23.

(22) Liang, Z. C.; Yang, Y. Z.; Cheng, L. L.; Zhong, G.-Y. Polyphenolic composition and content in the ripe berries of wild *Vitis* species. *Food Chem.* **2012**, *132* (2), 730−738.

(23) Liang, Z. C.; Christopher, L. O.; Zhong, G. Y.; Cheng, L. L. Polyphenolic profiles detected in the ripe berries of *Vitis vinifera* germplasm. *Food Chem.* **2011**, *129*, 940−950.

(24) Monagas, M.; Gomez-Cordoves, C.; Bartolome, B.; Laureano, O.; Ricardo-Da-Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475−6481.

(25) Montagut, G.; Baiges, I.; Valls, J.; Terra, X.; del Bas, J. M.; Vitrac, X.; Richard, T.; Merillon, J. M.; Arola, L.; Blay, M.; Blade, C.; Fernandez-Larrea, J.; Pujadas, G.; Salvado, J.; Ardevol, A. A trimer plus a dimer-gallate reproduce the bioactivity described for an extract of grape seed procyanidins. *Food Chem.* **2009**, *116*, 265−270.

(26) Ricardo-Da-Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Mourgues, J.; Moutounet, M. Dimer and trimer procyanidins in Carignan and Mourvedre grapes and red wines. *Vitis* **1992**, *31*, 55−63.

(27) Monagas, M.; Gomez-Cordoves, C.; Bartolome, B.; Laureano, O.; Ricardo-Da-Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475−6481.

(28) Obreque-Slier, E.; Pena-Neira, A.; Lopez-Solis, R.; Zamora-Marin, F.; Ricardo-da Silva, J. M.; Laureano, O. Comparative study of the phenolic composition of seeds and skins from Carmenere and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *J. Agric. Food Chem.* **2010**, *58*, 3591−3599.

(29) Fuleki, T.; Ricardo-da-Silva, J. M. Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *J. Agric. Food Chem.* **1997**, *45*, 1156−1160.

(30) De Freitas, V. A. P.; Glories, Y.; Monique, A. Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *Am. J. Enol. Vitic.* **2000**, *51*, 397−403.

(31) Mateus, N.; Marques, S.; Goncalves, A. C.; Machado, J. M.; De Freitas, V. Proanthocyanidin composition of red *Vitis vinifera* varieties from the Douro valley during ripening: Influence of cultivation altitude. *Am. J. Enol. Vitic.* **2001**, *52*, 115−121.

(32) Kammerer, D.; Claus, A.; Carle, R.; Schieber, A. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera*

L.) by HPLC-DAD-MS/MS. *J. Agric. Food Chem.* **2004**, *52*, 4360−4367.