JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Polyphenol Screening of Pomace from Red and White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS

DIETMAR KAMMERER, ACHIM CLAUS, REINHOLD CARLE, AND ANDREAS SCHIEBER*

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

Phenolic compounds of 14 pomace samples originating from red and white winemaking were characterized by HPLC-MS. Up to 13 anthocyanins, 11 hydroxybenzoic and hydroxycinnamic acids, and 13 catechins and flavonols as well as 2 stilbenes were identified and quantified in the skins and seeds by HPLC-DAD. Large variabilities comprising all individual phenolic compounds were observed, depending on cultivar and vintage. Grape skins proved to be rich sources of anthocyanins, hydroxycinnamic acids, flavanols, and flavonol glycosides, whereas flavanols were mainly present in the seeds. However, besides the lack of anthocyanins in white grape pomace, no principal differences between red and white grape varieties were observed. This is the first study presenting comprehensive data on the contents of individual phenolic compounds comprising all polyphenolic subclasses of grapes including a comparison of several red and white pomaces from nine cultivars. The results obtained in the present study confirm that both skins and seeds of most grape cultivars constitute a promising source of polyphenolics.

KEYWORDS: Grape pomace; grape skin; grape seed; screening; HPLC-DAD-MS/MS; anthocyanins; hydroxybenzoic acids; hydroxycinnamic acids; flavanols; flavonols; stilbenes

INTRODUCTION

Grapes and products obtained therefrom, such as wine, grape juice, jams, and raisins, constitute an economically important factor. As can be seen from the annual world production of 61 million tons in 2002, grapes are the world's largest fruit crop apart from oranges, with Italy, France, Spain, and the United States being among the most important producers (*1*). About 80% is used in winemaking (*2*). Because ∼20% of the weight of grapes processed remains as pomace, some 10 million tons of byproducts annually result from wineries. However, no statistical data are available concerning grape pomace resulting from winemaking, the data reported in the literature being inconsistent, ranging from 5−7 million tons (*3*) to 14.5 million tons solely in Europe (*4*). Grape pomace composition and water contents may considerably vary, depending on grape variety and technology of vinification (*5*).

Owing to disposal problems evolving from large amounts of winery byproducts arising within a few weeks, alternatives to their utilization as soil conditioner or to make fertilizers are required because problems concerning germination properties due to high levels of phenolic compounds have been reported (*6*). Additionally, grape pomace is poorly digested when used as a feed (*7*). On the other hand, grape pomace represents a rich source of various high-value products such as ethanol (*8*), tartrates and malates (*9*−*11*), citric acid (*12*), grape seed oil (*13*, *14*), hydrocolloids (*15*), and dietary fiber (*16*−*18*).

Furthermore, grape pomace is characterized by high phenolic contents because of poor extraction during winemaking. Their extractability mainly depends on the technological parameters applied during vinification. Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols, and stilbenes are the principal phenolic constituents of grape pomace (*5*). Anthocyanins from these byproducts have long been used as natural food colorants. Thus, several methods for their extraction have been described using sulfite-containing water or acidified alcohols (*19*−*21*).

Beginning with the "French paradox" observations (*22*), numerous studies have been initiated dealing with the antioxidative and health-promoting effects of plant secondary metabolites in grapes and wine, revealing the inhibition of human low-density lipoprotein oxidation by grape and wine phenolics (*23*, *24*). As a consequence, grape pomace is considered to be a valuable source of phenolic compounds, which could be recovered as functional food ingredients.

Despite detailed studies on the phenolic profile of grape pomace (*25*, *26*), quantitative data were mostly expressed as total phenolic contents and often correlated with the antioxidant activity of grape pomace extracts (*3*, *27*−*30*). Such extracts have also been shown to exert free radical scavenging activity; however, a correlation with total phenolic amounts or with individual compounds thereof was not established (*31*). When

* Corresponding author [telephone ++49-(0) 711-459-3125; fax ++49-(0) 711-459-4110; e-mail schieber@uni-hohenheim.de].

10.1021/jf049613b CCC: $27.50
Published on Web 06/11/2004

individual substances were quantified, only limited data concerning some compounds or substance groups were reported (*32*−*37*).

However, a systematic comparison of the phenolic contents of the pomace derived from different grape varieties has not yet been presented. Therefore, the main objective of the present study was to determine the amounts of individual phenolic compounds in the skins and seeds of 14 different press residues originating from winemaking. Such studies are of particular importance because polyphenols have been shown to differ considerably in their bioavailability and to exert different biological activities in vivo. Thus, these data may contribute to the selection of suitable plant materials for the extraction of phytochemicals as ingredients of functional foods (*38*).

EXPERIMENTAL PROCEDURES

Materials. All reagents and solvents of analytical or HPLC grade were purchased from VWR (Darmstadt, Germany). C_{18} reversed-phase cartridges (Chromabond, 1000 mg) were obtained from Macherey-Nagel (Düren, Germany).

Standards used for identification and quantification purposes with HPLC-MS and HPLC-DAD, respectively, were as follows: cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, petunidin 3-*O*-glucoside (Polyphenols, Sandnes, Norway); (+)-catechin, *p*-coumaric acid, (−)-epicatechin, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, caffeic acid, kaempferol (K), protocatechuic acid, quercetin (Q), sinapic acid (Roth, Karlsruhe, Germany); K 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, Q 3-*O*-galactoside, Q 3-*O*-glucoside, Q 3-*O*-rhamnoside, procyanidin B1, procyanidin B2 (Extrasynthèse, Lyon, France); epicatechin gallate, *trans*-resveratrol (Sigma, St. Louis, MO); 5-(hydroxymethyl)furfural, syringic acid (Fluka, Buchs, Switzerland); *trans*-resveratrol 3-*O*-glucoside (*trans*-polydatin) (Sequoia Research Products, Oxford, U.K.).

Grape pomace was obtained from Felsengartenkellerei Besigheim e.G. (Hessigheim, Germany). Nine different samples from red wine production (vintages 2001 and 2002) were used for polyphenol analyses (cultivars Cabernet Mitos, Lemberger, Spätburgunder, and Trollinger). The red wines were produced using high-temperature−short-time treatment of the mash followed by enzymatic degradation of grape pectins. Musts were obtained by use of a hose press. Additionally, Schwarzriesling pomace (rosé wine production) and four pomace samples from white wine production (cultivars Kerner, Müller-Thurgau, and Weisser Riesling; vintages 2001 and 2002), originating from the same process but without mash heating, were included in the study. One white wine pomace (cultivar Merzling; vintage 2002) was supplied by the Institute for Special Crop Cultivation and Crop Physiology, Hohenheim University. Pomace samples were sealed in polyethylene bags after pressing and kept at −20 °C until analyzed.

Sample Preparation. Frozen grape pomace samples were manually separated into skins and seeds using a sieve, lyophilized, and finely ground using an S 1/2 ball mill (Retsch, Haan, Germany). Aliquots of 5 g of the pulverized skins and seeds, respectively, were weighed into Erlenmeyer flasks and extracted with 100 mL of methanol/0.1% HCl (v/v) for 2 h under stirring after flushing with nitrogen in order to prevent oxidation during extraction. The extracts were centrifuged (10 min, 4000 rpm), and the material was re-extracted with 100 mL of the organic solvent (15 min). The combined supernatants were evaporated to dryness in vacuo at 30 °C, and the residue was dissolved in 20 mL of acidified water (pH 3.0). Anthocyanins were analyzed by direct injection of the solutions. Non-anthocyanin phenolics in red grape skin extracts were extracted with ethyl acetate before fractionation via solid phase extraction (SPE). For this purpose, aliquots of 5 mL of the skin extracts were made up to 20 mL. After the pH had been adjusted to 1.5, the solution was extracted four times with 50 mL of ethyl acetate each. The combined extracts were evaporated to dryness, dissolved in water, and applied to the SPE cartridges after the pH had been adjusted to 7.0. All other extracts were directly used for SPE. Aliquots of 5 mL were adjusted to pH 7.0 and applied to the cartridges, which were activated with 3 mL of methanol and rinsed with 10 mL of deionized water. Phenolic acids were subsequently eluted with 10 mL of deionized water and 10 mL of 0.01% HCl (fraction I); anthoxanthins and stilbenes were eluted with 20 mL of ethyl acetate. Fraction III containing the anthocyanins, which was eluted with methanol/0.01% HCl (v/v), was discarded. The eluates were concentrated in vacuo, and the residues obtained were dissolved in 2% acetic acid (fraction I) and in methanol (fraction II), respectively, membrane-filtered (0.45 μm), and used for LC analyses.

HPLC Analysis. Polyphenol analyses were carried out using an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/G1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector. The separation was performed with a Phenomenex (Torrance, CA) Aqua C18 column (250 × 4.6 mm i.d.; 5 μm particle size), with a C18 ODS guard column (4.0 × 3.0 mm i.d.), operated at a temperature of 25 °C. The diode array detector was set to an acquisition range of 200−600 nm at a spectral acquisition rate of 1.25 scans/s (peak width = 0.2 min).

System I (Anthocyanins). The mobile phase consisted of water/formic acid/acetonitrile (87:10:3, v/v/v; eluent A) and water/formic acid/acetonitrile (40:10:50, v/v/v; eluent B) using a gradient program as follows: from 10 to 25% B (10 min), from 25 to 31% B (5 min), from 31 to 40% B (5 min), from 40 to 50% B (10 min), from 50 to 100% B (10 min), from 100 to 10% B (5 min). Total run time was 50 min. The injection volume for all samples ranged from 1 to 25 μL. Monitoring was performed at 520 nm at a flow rate of 0.8 mL/min.

System II (Phenolic Acids). The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B) using a gradient program as follows: from 10 to 15% B (10 min), 15% B isocratic (3 min), from 15 to 25% B (7 min), from 25 to 55% B (30 min), from 55 to 100% B (1 min), 100% B isocratic (5 min), from 100 to 10% B (0.1 min). Total run time was 60 min. The injection volume for all samples ranged from 5 to 10 μL. Simultaneous monitoring was performed at 280 nm (hydroxybenzoic acids) and at 320 nm (hydroxycinnamic acids) at a flow rate of 1.0 mL/min.

System III (Anthoxanthins and Stilbenes). The mobile phase consisted of the same eluents as described for system II using a gradient program as follows: from 10 to 24% B (20 min), from 24 to 30% B (20 min), from 30 to 55% B (20 min), from 55 to 100% B (15 min), 100% B isocratic (8 min), from 100 to 10% B (2 min). Total run time was 90 min. The injection volume for all samples was 10 μL. Simultaneous monitoring was performed at 280 nm (flavanols), at 320 nm (stilbenes), and at 370 nm (flavonols) at a flow rate of 1.0 mL/min.

LC-MS Analysis. LC-MS analyses were performed with the HPLC system described above coupled on-line to a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. Data acquisition and processing were performed using Esquire Control software. Negative ion (phenolic acids, stilbenes, anthoxanthins) and positive ion (anthocyanins) mass spectra of the column eluate were recorded in the range *m*/*z* 50−1000 at a scan speed of 13000 *m*/*z*/s. Nitrogen was used both as drying gas at flow rates of 11.0 (system I) and 12.0 L/min (systems II and III) and as nebulizing gas at pressures of 65.0 (system I) and 70.0 psi (systems II and III). The nebulizer temperature was set at 365 °C. Helium was used as collision gas for collision-induced dissociation (CID) at a pressure of 4.0×10^{-6} mbar.

Quantification of Individual Compounds. Individual compounds were quantified using a calibration curve of the corresponding standard compound. When reference compounds were not available, the calibration of structurally related substances was used, including a molecular weight correction factor (*39*). All determinations were performed in duplicate.

RESULTS AND DISCUSSION

Fractionation of Grape Pomace Polyphenolics. Fractionation of the phenolic compounds proved to be a prerequisite for their unambiguous identification and quantification. Due to the complex profile of grape polyphenolics, which include phenolic acids, anthoxanthins, stilbenes, and anthocyanins, SPE

Table 1. LC-MS Data of Phenolic Compounds[a] Extracted from Grape Pomace (*V. vinifera* L.)

no.	compound	retention time (min)	$[M]^+$ $[M-H]^-$ *m/z*[b]	MS/MS fragments *m/z*[b]
	Anthocyanins			
1	delphinidin 3-*O*-glucoside	9.7	465	303
2	cyanidin 3-*O*-glucoside	11.8	449	287
3	petunidin 3-*O*-glucoside	13.2	479	317
4	peonidin 3-*O*-glucoside	15.6	463	301
5	malvidin 3-*O*-glucoside	16.8	493	331
6	delphinidin 3-*O*-acetylglucoside	18.2	507	303
7	petunidin 3-*O*-acetylglucoside	22.3	521	317
8	peonidin 3-*O*-acetylglucoside	24.6	505	301
9	malvidin 3-*O*-acetylglucoside	25.8	535	331
10	cyanidin 3-*O*-*p*-coumaroylglucoside	27.0	595	287
11	petunidin 3-*O*-*p*-coumaroylglucoside	28.1	625	317
12	peonidin 3-*O*-*p*-coumaroylglucoside	31.4	609	301
13	malvidin 3-*O*-*p*-coumaroylglucoside	32.3	639	331
	Phenolic Acids[a]			
14	gallic acid	5.9	169	125
15	5-(hydroxymethyl)furfural	7.7		
16	protocatechuic acid	12.7	153	109
17	caftaric acid	13.8	311	179
18	*p*-hydroxybenzoic acid	18.0	137	93
19	coutaric acid	20.8	295	163
20	caffeic acid	23.4	179	135
21	fertaric acid	24.3	325	193
22	syringic acid	25.7	197	153/182
23	*p*-coumaric acid	31.0	163	119
24	sinapic acid	32.1	223	164/208
25	ferulic acid	32.5	193	134
	Anthoxanthins and Stilbenes			
26	procyanidin B1	13.7	577	407/425
27	catechin	17.5	289	245
28	procyanidin B2	21.4	577	407/425
29	epicatechin	25.4	289	245
30	epicatechin gallate	41.9	441	289
31	*trans*-polydatin	42.5	389	227
32	quercetin 3-*O*-galactoside	49.5	463	301
33	quercetin 3-*O*-glucoside	50.4	463	301
34	quercetin 3-*O*-glucuronide	50.9	477	301
35	quercetin 3-*O*-rhamnoside	53.4	447	301
36	kaempferol 3-*O*-glucoside	56.4	447	284/285
37	isorhamnetin 3-*O*-glucoside	57.4	477	314/315
38	*trans*-resveratrol	59.2	227	185
39	quercetin	66.2	301	151/179
40	kaempferol	71.1	285	257

[a] And 5-(hydroxymethyl)furfural. [b] Positive ion mode, anthocyanins; negative ion mode, phenolic acids, anthoxanthins, and stilbenes.

and subsequent elution with acidified water, ethyl acetate, and acidified methanol according to a modified method for the fractionation of phenolic compounds in red wine (*40*) was required to avoid coelution and thus inaccurate peak integration.

HPLC-DAD and HPLC-DAD-MS/MS. A C_{18} stationary phase with hydrophilic endcapping was used, which has been shown to be highly suitable for the separation of polyphenolics in various matrices, such as apple and pear (*41*), mango (*42*), strawberry (*43*), and black carrots (*44*) using an MS compatible mobile phase system (*41*, *45*). However, due to similar spectral characteristics of individual compounds belonging to the same subclass and because of the limited availability of reference compounds, HPLC coupled to mass spectrometry proved to be an indispensable tool for unambiguous peak assignment. The LC-MS data of all quantified compounds are presented in **Table 1**. The mass-to-charge ratios of the fragments obtained by collision-induced dissociation in the MS^2 experiment are also included. Individual components were analyzed after methanolic extraction of pomace samples, which is often applied for polyphenol analysis; however, it must be noted that some minor amounts of phenolics may escape from extraction due to interaction with dietary fibers, proteins, and other polymerized structures.

Anthocyanins. The separation of anthocyanins from a Cabernet Mitos skin extract is presented in **Figure 1**. As can be seen, baseline separation of 13 anthocyanins was achieved within 33 min, 5 of them being identified as the 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, on the basis of their UV−Vis and mass spectra and by comparison with reference compounds (peaks 1−5). Additionally, 8 further compounds were detected, all of which represented acylated anthocyanins (peaks 6−13). Four of them (peaks 6−9) were identified as 3-*O*-acetylglucosides of delphinidin, petunidin, peonidin, and malvidin, whereas peaks 10−13 were assigned to the 3-*O*-*p*-coumaroylglucosides of cyanidin, petunidin, peonidin, and malvidin, on the basis of their molecular ions and the corresponding anthocyanidin fragments produced in the MS^2 experiment (**Table 1**) (*46*). In contrast to the complex anthocyanin pattern of Cabernet Mitos peel extracts, the skins of all other cultivars were devoid of the 3-*O*-acetylglucosides of delphinidin and petunidin and the 3-*O*-coumaroylglucosides of cyanidin and petunidin. Moreover, the peels of Spätburgunder and Schwarzriesling were characterized by the complete absence of acylated anthocyanins (**Table 2**). Accordingly, this lack of specific pigments in some grape cultivars has been used for their classification and for authenticity control (*45*, *47*−*48*).

The presence of minute amounts of anthocyanins detected in the peels of some white cultivars (data not shown) cannot be ascribed to an admixture of red grapes during mashing because the pomace samples were visually controlled during sieving to separate the skins from the seeds. This observation is not surprising because white grape cultivars have also been shown to synthesize anthocyanins during the final period of ripening (*49*). Furthermore, the seeds of the red cultivars, especially of the two samples from Cabernet Mitos, contained minor amounts of anthocyanins. Because seed anthocyanins generally comprised all of those compounds detected in the skins, artifact formation during extraction and concentration due to the high contents of proanthocyanidins in the seeds can be excluded. This conclusion is supported by the lack of anthocyanins in the seeds of white cultivars and the red shade of seeds from red grapes. Thus, the presence of pigments in the seeds must be ascribed to diffusion of the anthocyanins from the skins into the seeds during mashing and vinification. Accordingly, the Cabernet Mitos seeds showed the highest anthocyanin amounts because this cultivar is characterized by the presence of anthocyanins not only in the skins but also in the pulp, thus enhancing pigment diffusion.

The contents of individual anthocyanins in the skins of the nine red grape pomace samples are presented in **Table 2**. As expected, malvidin 3-*O*-glucoside was the predominant compound, mostly followed by peonidin 3-*O*-glucoside. Most strikingly, large amounts of anthocyanins were detected in the pomace samples of the cultivar Cabernet Mitos. However, marked differences were observed between the anthocyanin contents of Cabernet skins from different vintages; only 38% of the total amounts of 2002 were found in the skins of 2001. Similar results were observed for Spätburgunder and Trollinger, whereas the pomace of the poorly colored Lemberger grapes from the vintages 2001 and 2002 showed nearly the same total anthocyanin contents.

Because all press residues were obtained from the same winery and processed identically with the exception of Schwarzriesling, climatic and microclimatic factors must be responsible for the variability of pigment concentrations. The

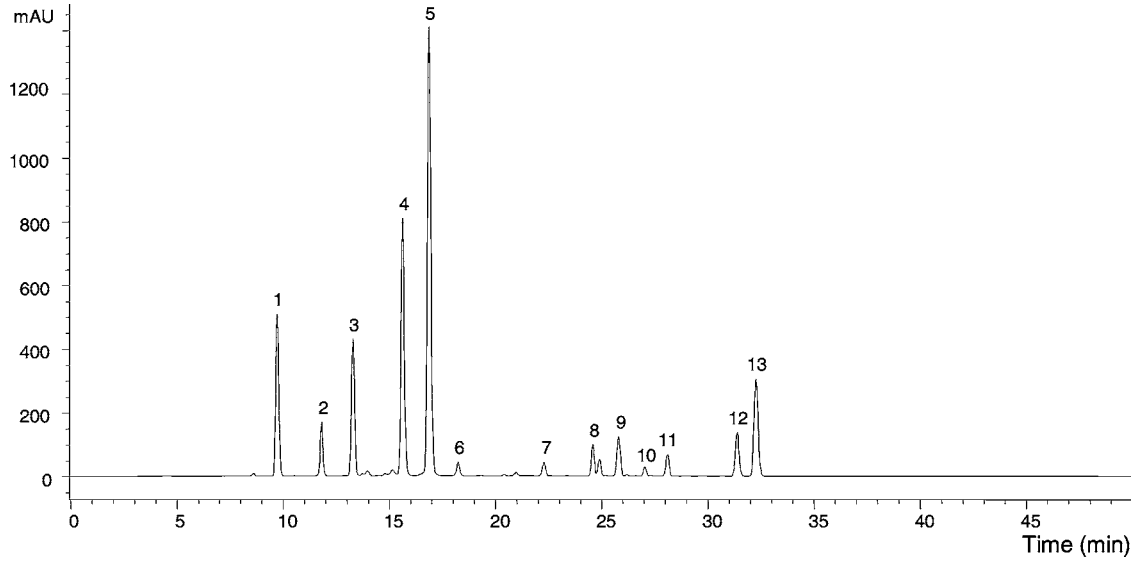

Figure 1. HPLC separation of anthocyanins from a Cabernet Mitos extract (520 nm). For peak assignment see **Table 1**.

Table 2. Anthocyanin Contents (mg/kg DM) of Peels Separated from Red Grape Pomaces from Vintages 2001 and 2002

	Cabernet Mitos		Lemberger		Spätburgunder		Schwarzriesling	Trollinger	
	2001	2002	2001	2002	2001	2002	2002	2001	2002
del 3-*O*-glc	2213 ± 38	5552 ± 48	470 ± 25	431 ± 19	183 ± 5	219 ± 21	81 ± 4	68 ± 3	105 ± 5
cya 3-*O*-glc	759 ± 35	1903 ± 11	79 ± 8	85 ± 2	80 ± 1	98 ± 2	37 ± 3	207 ± 11	382 ± 9
pet 3-*O*-glc	2643 ± 18	6680 ± 63	606 ± 15	65 ± 5	283 ± 9	463 ± 26	186 ± 4	116 ± 5	181 ± 10
peo 3-*O*-glc	4960 ± 16	12450 ± 158	515 ± 14	729 ± 35	678 ± 10	1786 ± 26	552 ± 14	715 ± 37	1371 ± 20
mal 3-*O*-glc	20533 ± 92	50981 ± 1000	7106 ± 298	7536 ± 343	4743 ± 104	10995 ± 142	4364 ± 118	1117 ± 44	1736 ± 19
del 3-*O*-acglc	392 ± 26	956 ± 8	nd	nd	nd	nd	nd	nd	nd
pet 3-*O*-acglc	545 ± 32	1375 ± 11	nd	nd	nd	nd	nd	nd	nd
peo 3-*O*-acglc	1371 ± 82	1484 ± 67	32 ± 11	nd	nd	nd	nd	27 ± 1	43 ± 6
mal 3-*O*-acglc	3110 ± 106	8688 ± 313	109 ± 0	nd	nd	nd	nd	45 ± 2	71 ± 3
cya 3-*O*-*p*cmglc	374 ± 8	1071 ± 50	nd	nd	nd	nd	nd	nd	nd
pet 3-*O*-*p*cmglc	974 ± 26	2458 ± 0	nd	nd	nd	nd	nd	nd	nd
peo 3-*O*-*p*cmglc	2151 ± 94	6828 ± 57	68 ± 8	77 ± 12	nd	nd	nd	178 ± 19	398 ± 32
mal 3-*O*-*p*cmglc	10591 ± 201	31442 ± 192	877 ± 175	774 ± 77	nd	nd	nd	271 ± 22	458 ± 55
total AC content	50616 ± 774	131868 ± 1978	9862 ± 554	9697 ± 493	5967 ± 129	13561 ± 217	5220 ± 143	2744 ± 144	4745 ± 159

[a] Abbreviations: del, delphinidin; cya, cyanidin; pet, petunidin; peo, peonidin; mal, malvidin; glc, glucose; ac, acetyl; *p*cm, *p*-coumaroyl; AC, anthocyanin; nd, not detected.

data presented clearly demonstrate that quality control of winery byproducts is a prerequisite for a profitable extraction of food colorants and bioactive compounds. The pomace of Cabernet Mitos proved to be a promising source of anthocyanins because the pigment concentration is higher than reported for other grape varieties (*2*). Furthermore, its pigment stability is enhanced due to the high percentage of acylated anthocyanins, because the latter are known to exhibit increased stability toward nucleophilic attack of water at raised pH values (*50*). Therefore, this source may particularly be suitable for low-acid food applications (*51*).

Phenolic Acids. The separation of the phenolic acids from a Cabernet Mitos skin extract is presented in **Figure 2**. Among the hydroxybenzoates, gallic, protocatechuic, *p*-hydroxybenzoic, and syringic acids were identified by their UV spectra and by comparison of their retention times with reference compounds. Their pseudomolecular ions and the fragments released after collision-induced dissociation (CID) in the MS^2 experiment confirmed the peak assignment (**Table 1**). The hydroxycinnamates caffeic, *p*-coumaric, ferulic, and sinapic acids were identified accordingly. Additionally, caftaric, coutaric, and fertaric acids were identified by their pseudomolecular ions at *m*/*z* 311, 295, and 325, respectively. CID in the MS^2 experiment revealed a loss of 132 Da, corresponding to a tartaric acid moiety and releasing caffeic, *p*-coumaric, and ferulic acids, respectively. In most pomace samples trace amounts of 5-(hydroxymethyl)-furfural were detected, probably as a reaction product resulting from the high-temperature−short-time treatment of the red grape mash, which is commonly applied for an accelerated anthocyanin extraction during winemaking.

Compared to the anthocyanins, the phenolic acids were present in considerably lower amounts, with caftaric acid being the predominant compound in all samples. The contents of caftaric, coutaric, and fertaric acids in the skins are listed in **Table 3**. As can be seen, great variabilities in the phenolic acid contents of both different cultivars and samples of different vintages were observed. However, differences between vintages 2001 and 2002 were not consistent because only in some cases were the contents higher in 2002. Thus, effects of various climatic conditions on the phenolic acid contents are doubtful. More likely, different ripening stages of the grapes processed may be responsible for varying phenolic acid contents, thus confirming earlier studies (*52*). However, differences in the contents were not as pronounced as described for the anthocyanins. Compared to peels of red grape varieties, contents of white grapes were generally lower. Total phenolic acid contents (compounds **14** and **16**−**25**) ranged from 60.5 (Schwarzriesling, 2002) to 973.5 mg/kg of dry matter (DM) (Lemberger, 2002) for red grape peels and from 104.7 (Weisser Riesling, 2001) to 227.0 mg/kg of DM (Merzling, 2002) for white grape peels.

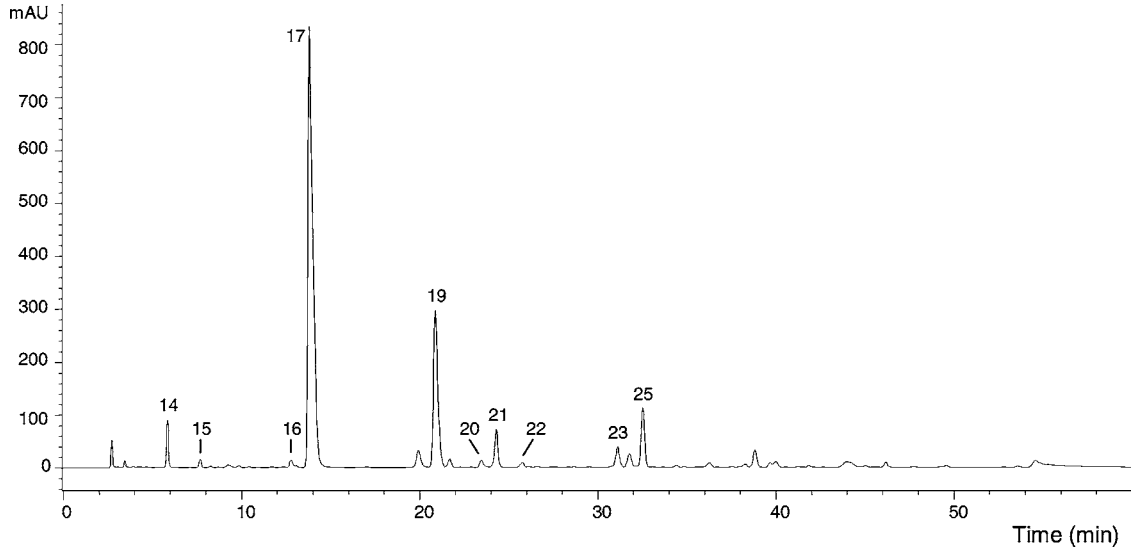

Figure 2. HPLC separation of phenolic acids from a Cabernet Mitos extract (280 nm). For peak assignment see **Table 1**.

Table 3. Contents of Caftaric, Coutaric, and Fertaric Acids (mg/kg DM) of Skins from Red and White Grape Cultivars from Different Vintages

cultivar (vintage)	caftaric acid	coutaric acid	fertaric acid
Cabernet Mitos (2001)	624.3 ± 10.1	178.6 ± 1.5	13.2 ± 1.0
Cabernet Mitos (2002)	217.7 ± 77.3	60.2 ± 17.9	7.1 ± 1.2
Lemberger (2001)	337.6 ± 26.5	91.8 ± 3.9	12.1 ± 0.3
Lemberger (2002)	447.6 ± 19.6	133.9 ± 3.5	15.9 ± 0.1
Spätburgunder (2001)	374.1 ± 34.2	183.8 ± 15.4	9.9 ± 1.1
Spätburgunder (2002)	33.2 ± 0.1	10.0 ± 0.4	5.3 ± 0.0
Schwarzriesling (2002)	16.3 ± 0.6	6.9 ± 0.3	4.4 ± 0.2
Trollinger (2001)	216.3 ± 2.0	119.7 ± 2.6	5.3 ± 0.0
Trollinger (2002)	176.9 ± 24.6	97.2 ± 13.3	4.9 ± 0.0
Kerner (2002)	36.9 ± 1.0	15.3 ± 0.2	15.4 ± 0.1
Merzling (2002)	61.0 ± 2.8	54.5 ± 1.4	17.3 ± 1.1
Müller-Thurgau (2002)	48.8 ± 4.1	48.8 ± 1.9	4.4 ± 0.4
Weisser Riesling (2001)	30.6 ± 1.8	10.0 ± 0.9	15.5 ± 0.9
Weisser Riesling (2002)	29.8 ± 2.4	9.8 ± 0.8	15.1 ± 1.0

Table 4. Phenolic Acid Contents (mg/kg DM) of Skins and Seeds from a White Grape Pomace (*V. vinifera* L. Cv. Merzling)

	Merzling (2002)	
	skins	seeds
gallic acid	15.0 ± 0.2	106.5 ± 8.8
protocatechuic acid	42.8 ± 0.5	102.8 ± 25.5
caftaric acid	61.0 ± 2.8	9.3 ± 3.4
p-hydroxybenzoic acid	31.1 ± 0.1	13.8 ± 0.9
coutaric acid	54.5 ± 1.4	30.2 ± 16.3
caffeic acid	1.7 ± 0.2	1.9 ± 0.6
fertaric acid	17.3 ± 1.1	3.0 ± 0.1
syringic acid	1.0 ± 1.2	1.1 ± 0.1
p-coumaric acid	nd[a]	7.2 ± 0.7
ferulic acid	2.6 ± 0.0	3.9 ± 0.4
sinapic acid	nd[a]	1.0 ± 0.1

[a] Not detected.

Therefore, the view that white grape pomace is characterized by higher total phenolic contents due to the different processes used for red and white winemaking (*53*) should be revised.

The phenolic acid contents of the seeds ranged from 115.9 (Trollinger, 2002) to 528.6 mg/kg of DM (Spätburgunder, 2001) for red grape seeds and from 167.8 (Weisser Riesling, 2001) to 326.6 mg/kg of DM (Müller-Thurgau, 2002) for white grape seeds. Most strikingly, levels were generally higher in the seeds compared to the skins for all white grape cultivars, whereas this was the case with only Spätburgunder (2002) and Schwarzriesling pomaces. Furthermore, the phenolic patterns of the skins and seeds differed significantly. Whereas the red grape cultivars Cabernet Mitos, Lemberger, and Trollinger exhibited comparatively high proportions of the hydroxycinnamates caftaric, coutaric, and fertaric acids in the seeds, which probably result from adhering residual skin and pulp, all other seed extracts showed a predominance of gallic and protocatechuic acids. The phenolic acid contents of the peels and seeds from a white grape pomace (cv. Merzling) are exemplified in **Table 4**.

Anthoxanthins and Stilbenes. The separation of the anthoxanthins and stilbenes from a Cabernet Mitos skin extract is presented in **Figure 3**. The flavan-3-ols catechin and epicatechin and the dimeric procyanidins B1 and B2 as well as epicatechin gallate were readily identified by comparison of their UV spectra and retention times with those of reference compounds. Their characteristic mass spectra in the MS^1 and MS^2 experiments further confirmed the peak assignments. Quercetin (Q), Q 3-*O*-galactoside, Q 3-*O*-glucoside, Q 3-*O*-rhamnoside, kaempferol (K), K 3-*O*-glucoside, and isorhamnetin 3-*O*-glucoside were identified in the same way. Additionally, Q 3-*O*-glucuronide was tentatively identified on the basis of its UV spectrum and its mass spectral behavior, revealing a loss of 176 Da in the MS^2 experiment, corresponding to a cleavage into the quercetin aglycon and a hexuronide moiety. Furthermore, two stilbenes, *trans*-resveratrol and its glucoside *trans*-polydatin, were identified via commercially available reference compounds (**Table 1**).

Compared to the anthocyanins, anthoxanthins and stilbenes were minor compounds, with the exception of the flavan-3-ols in the seeds, which ranged up to several grams per kilogram of dry matter, which are frequently available in grape seed extracts as food supplement preparations. Beginning with the "French paradox" observations, much attention has been paid to bioactive ingredients of grapes and wine. In this context, a lot of studies on the bioactive properties of resveratrol and its derivatives have been initiated. The amounts of resveratrol and polydatin in the skins of all pomace samples investigated are shown in **Table 5**. The contents ranged from 11.1 to 123.0 mg/kg of DM for resveratrol and from 5.1 to 148.0 mg/kg of DM for polydatin, showing only minor vintage-related differences with the exception of the Cabernet Mitos and the Spätburgunder pomaces.

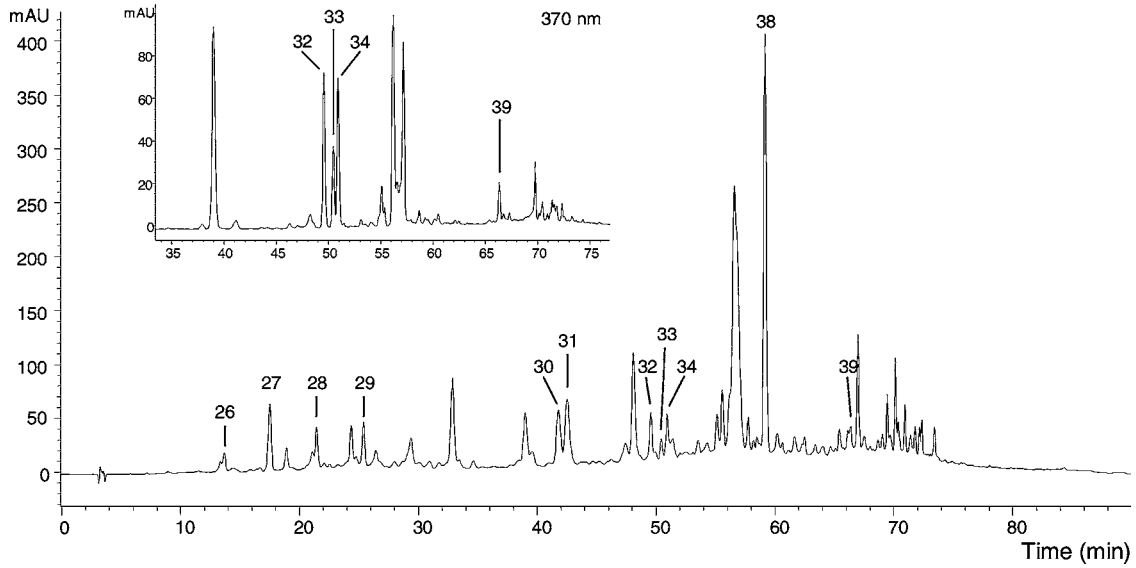

Figure 3. HPLC separation of anthoxanthins and stilbenes from a Cabernet Mitos extract (280 and 370 nm). For peak assignment see **Table 1**.

Table 5. *trans*-Resveratrol and *trans*-Polydatin Contents (mg/kg DM) of Skins from Red and White Grape Cultivars

cultivar (vintage)	*trans*-resveratrol	*trans*-polydatin
Cabernet Mitos (2001)	123.0 ± 5.1	75.8 ± 0.6
Cabernet Mitos (2002)	11.1 ± 1.6	35.6 ± 33.9
Lemberger (2001)	22.7 ± 1.0	101.9 ± 0.8
Lemberger (2002)	22.4 ± 1.5	148.0 ± 4.7
Spätburgunder (2001)	30.0 ± 2.9	124.5 ± 1.9
Spätburgunder (2002)	28.7 ± 2.3	28.5 ± 0.8
Schwarzriesling (2002)	18.8 ± 3.2	5.1 ± 0.1
Trollinger (2001)	50.0 ± 3.5	24.9 ± 2.4
Trollinger (2002)	37.9 ± 4.7	32.1 ± 0.3
Kerner (2002)	41.4 ± 2.7	12.4 ± 0.6
Merzling (2002)	16.9 ± 1.7	7.5 ± 0.7
Müller-Thurgau (2002)	54.7 ± 7.0	24.4 ± 1.4
Weisser Riesling (2001)	54.5 ± 1.1	10.0 ± 0.1
Weisser Riesling (2002)	86.4 ± 4.5	15.5 ± 0.1

Table 6. Anthoxanthin and Stilbene Contents (mg/kg DM) of Skins and Seeds from a White Grape Cultivar (*V. vinifera* L. Cv. Weisser Riesling)

	Weisser Riesling (2002)	
	skins	seeds
catechin	226.7 ± 24.6	790.2 ± 11.2
epicatechin	134.6 ± 12.1	674.5 ± 24.9
epicatechin gallate	35.5 ± 3.6	457.9 ± 35.8
procyanidin B1	191.5 ± 6.6	1053.7 ± 29.3
procyanidin B2	91.0 ± 2.5	506.2 ± 41.1
quercetin	nd[a]	nd[a]
quercetin 3-*O*-galactoside	156.7 ± 9.3	14.7 ± 0.9
quercetin 3-*O*-glucoside	351.7 ± 23.3	32.6 ± 3.0
quercetin 3-*O*-glucuronide	509.9 ± 30.4	38.0 ± 0.8
quercetin 3-*O*-rhamnoside	57.7 ± 3.5	14.4 ± 1.0
kaempferol	nd[a]	nd[a]
kaempferol 3-*O*-glucoside	247.6 ± 15.6	20.0 ± 1.4
trans-resveratrol	86.4 ± 4.5	14.2 ± 1.8
trans-polydatin	15.5 ± 0.1	4.7 ± 0.2
isorhamnetin 3-*O*-glucoside	35.5 ± 0.1	nd[a]

[a] Not detected.

However, large variabilities were observed between the cultivars, which is in accordance with previous data reported for the skin resveratrol contents of different grape varieties (*54*). Stilbenes are known to be phytoalexins, and their biosynthesis has been shown to be induced by abiotic stress such as ultraviolet light and fungal infection, for example, by *Botrytis cinerea* (*55*). Thus, differences in microclima and phytosanitary conditions of the grapes may account for the observed stilbene variability.

The total amounts of all identified anthoxanthins and stilbenes (compounds **26**−**40**) ranged from 297.3 (Cabernet Mitos, 2002) to 1857.8 mg/kg of DM (Lemberger, 2002) for red grape peels and from 1560.2 (Kerner) to 6571.2 mg/kg of DM (Merzling) for the white cultivars. White grape skins had generally higher yields than red ones, which again may be attributed to both cultivar-related variabilities and differences in red and white winemaking techniques. Whereas in most red grapes the phenolic composition of this fraction was mainly dominated by the flavanols, some of the flavonols such as isorhamnetin 3-*O*-glucoside could not even be detected. In contrast, the white peels exhibited a predominance of the flavonols, but also contained flavanols in considerable amounts. The yields of a typical white grape pomace sample (cv. Weisser Riesling, 2002) are presented in **Table 6**.

The seeds generally exhibited higher polyphenol contents than the skins. Their contents ranged from 2.28 (Trollinger, 2002) to 18.76 g/kg of DM (Spätburgunder, 2002) for red grape cultivars and from 3.52 (Weisser Riesling, 2001) to 13.63 g/kg of DM (Merzling, 2002) for white grapes. Differences between red and white grape seeds were not significant. This may be due to the fact that phenolic compounds are only extracted from the grape seeds after ethanol formation during red wine fermentation (*56*). Because in the present study mash heating and subsequent pressing were applied for red wine production, the seeds phenolics were poorly extracted into the must. Thus, the different seed phenolic contents should rather reflect cultivar-dependent variations. Furthermore, contents of grape seed polyphenols have been shown to depend on maturity stage (*57*). The phenolic profile of the seeds was dominated by flavanols, whereas the flavonols and stilbenes were detected in minor amounts and must be attributed to mash constituents adhering to the seeds.

In accordance with previous papers (*26*), the results presented in this study demonstrate that grape pomaces have generally very high polyphenolic contents, making their utilization worthwhile and thus supporting sustainable agricultural production. However, the data reveal great differences in the anthocyanin, phenolic acid, anthoxanthin, and stilbene contents of

the press residues, primarily depending on cultivar and vintage. Furthermore, the ripening status of the grapes may also be responsible for these findings. This investigation provides useful information for selecting suitable byproducts for a profitable extraction of potential health-promoting compounds and underscores the necessity of a polyphenol screening because differences between the polyphenol subclasses were not uniform. Technological factors such as conditions of grape extraction and pressing may be of importance, so further studies need to be conducted taking into consideration the effects of different vinification techniques on the phenolic contents of the pomaces. Taking together these data and studies on the bioavailability and in vitro and in vivo bioactivity of individual phenolic compounds, tailor-made extracts may be obtained by selecting byproducts from vinification that are suitable as dietary supplements or as ingredients in functional foods (*38*), even though it will not be easy to do this economically on an industrial scale.

ACKNOWLEDGMENT

We are grateful to the Felsengartenkellerei Besigheim e.G. (Hessigheim, Germany) and to the Institute for Special Crop Cultivation and Crop Physiology, Hohenheim University, for providing grape pomace.

LITERATURE CITED

(1) FAOSTAT−FAO Statistical Database, http://www.fao.org.
(2) Mazza, G.; Miniati, E. Grapes. In *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, 1993; pp 149−199.
(3) Meyer, A. S.; Jepsen, S. M.; Sørensen, N. S. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. *J. Agric. Food Chem.* **1998**, *46*, 2439−2446.
(4) Torres, J. L.; Bobet, R. New flavanol derivatives from grape (*Vitis vinifera*) byproducts. Antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *J. Agric. Food Chem.* **2001**, *49*, 4627−4634.
(5) Schieber, A.; Stintzing, F. C.; Carle, R. By-products of plant food processing as a source of functional compounds−recent developments. *Trends Food Sci. Technol.* **2001**, *12*, 401−413.
(6) Negro, C.; Tommasi, L.; Miceli, A. Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresour. Technol.* **2003**, *87*, 41−44.
(7) Famuyiwa, O. O.; Ough, C. S. Effect of structural constituents of cell wall on the digestibility of grape pomace. *J. Agric. Food Chem.* **1990**, *38*, 966−968.
(8) Silva, M. L.; Macedo, A. C.; Malcata, F. X. Steam distilled spirits from fermented grape pomace. *Food Sci. Technol. Int.* **2000**, *6*, 285−300.
(9) Nurgel, C.; Canbas, A. Production of tartaric acid from pomace of some Anatolian grape cultivars. *Am. J. Enol. Vitic.* **1998**, *49*, 95−99.
(10) Braga, F. G.; Lencart e Silva, F. A.; Alves, A. Recovery of winery by-products in the Douro Demarcated region: production of calcium tartrate and grape pigments. *Am. J. Enol. Vitic.* **2002**, *53*, 41−45.
(11) Palma, M.; Barroso, C. G. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. *Anal. Chim. Acta* **2002**, *458*, 119−130.
(12) Hang, Y. D.; Woodams, E. E. Grape pomace: a novel substrate for microbial production of citric acid. *Biotechnol. Lett.* **1985**, *7*, 253−254.
(13) Schieber, A.; Müller, D.; Röhrig, G.; Carle, R. Effects of grape cultivar and processing on the quality of cold-pressed grape seed oils. *Mitt. Klosterneuburg* **2002**, *52*, 29−33.
(14) El-Shami, S. M.; El-Mallah, M. H.; Mohamed, S. S. Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing. *Grasas Aceites* **1992**, *43*, 157−160.
(15) Stredansky, M.; Conti, E. Xanthan production by solid-state fermentation. *Process Biochem.* **1999**, *34*, 581−587.
(16) Bravo, L.; Saura-Calixto, F. Characterization of dietary fiber and the *in vitro* indigestible fraction of grape pomace. *Am. J. Enol. Vitic.* **1998**, *49*, 135−141.
(17) Martin-Carron, N.; Garcia-Alonso, A.; Goni, I.; Saura-Calixto, F. Nutritional and physiological properties of grape pomace as a potential food ingredient. *Am. J. Enol. Vitic.* **1997**, *48*, 328−332.
(18) Valiente, C.; Arrigoni, E.; Esteban, R. M.; Amado, R. Grape pomace as a potential food fiber. *J. Food Sci.* **1995**, *60*, 818−820.
(19) Metivier, R. P.; Francis, F. J.; Clydesdale, F. M. Solvent extraction of anthocyanins from wine pomace. *J. Food Sci.* **1980**, *45*, 1099−1100.
(20) Bocevska, M.; Stevcevska, V. Quality evaluation of anthocyanin extract obtained from wine grape pomace. *Food Technol. Biotechnol.* **1997**, *35*, 139−143.
(21) Ayed, N.; Yu, H.-L.; Lacroix, M. Improvement of anthocyanin yield and shelf-life extension of grape pomace by gamma irradiation. *Food Res. Int.* **1999**, *32*, 539−543.
(22) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.
(23) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.
(24) Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.
(25) Foo, L. Y.; Lu, Y.; Wong, H. Biphenyl-linked biflavonoids from grape pomace. *Phytochemistry* **1998**, *47*, 1137−1140.
(26) Lu, Y.; Foo, L. Y. The polyphenol constituents of grape pomace. *Food Chem.* **1999**, *65*, 1−8.
(27) Chidambara Murthy, K. N.; Singh, R. P.; Jayaprakasha, G. K. Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. *J. Agric. Food Chem.* **2002**, *50*, 5909−5914.
(28) Wood, J. E.; Senthilmohan, S. T.; Peskin, A. V. Antioxidant activity of procyanidin-containing plant extracts at different pHs. *Food Chem.* **2002**, *77*, 155−161.
(29) Arce, L.; Lista, A. G.; Ríos, A.; Valcárel, M. Screening of polyphenols in grape marc by on-line supercritical fluid extraction-flow through sensor. *Anal. Lett.* **2001**, *34*, 1461−1476.
(30) Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J. Agric. Food Chem.* **1997**, *45*, 1390−1393.
(31) Larrauri, J. A.; Sánchez-Moreno, C.; Saura-Calixto, F. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels. *J. Agric. Food Chem.* **1998**, *46*, 2694−2697.
(32) Bonilla, F.; Mayen, M.; Merida, J.; Medina, M. Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chem.* **1999**, *66*, 209−215.
(33) Alonso, Á. M.; Guillén, D. A.; Barroso, C. G.; Puertas, B.; García, A. Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *J. Agric. Food Chem.* **2002**, *50*, 5832−5836.
(34) Torres, J. L.; Varela, B.; García, M. T.; Carilla, J.; Matito, C.; Centelles, J. J.; Cascante, M.; Sort, X.; Bobet, R. Valorization of grape (*Vitis vinifera*) byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *J. Agric. Food Chem.* **2002**, *50*, 7548−7555.

(35) Alonso, E.; Bourzeix, M.; Revilla, E. Suitability of water/ethanol mixtures for the extraction of catechins and proanthocyanidins from *Vitis vinifera* seeds contained in a winery by-product. *Seed Sci. Technol.* **1991**, *19*, 545−552.

(36) Li, W.; Fong, H. H. S.; Singletary, K. W.; Fitzloff, J. F. Determination of catechins in commercial grape seed extract. *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25*, 397−407.

(37) Careri, M.; Corradini, C.; Elviri, L.; Nicoletti, I.; Zagnoni, I. Direct HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts. *J. Agric. Food Chem.* **2003**, *51*, 5226−5231.

(38) Lu, Y.; Foo, L. Y. Review of polyphenols from some agricultural by-products. In *Recent Research Developments in Phytochemistry*; Pandalai, S. G., Ed.; Research Signpost: Kerala, India, 2001; Vol. 5, pp 139−151.

(39) Chandra, A.; Rana, J.; Li, Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515−3521.

(40) Oszmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of phenolic compounds in red wine. *Am. J. Enol. Vitic.* **1988**, *39*, 259−262.

(41) Schieber, A.; Keller, P.; Carle, R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *910*, 265−273.

(42) Schieber, A.; Berardini, N.; Carle, R. Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 5006−5011.

(43) Hilt, P.; Schieber, A.; Yildirim, C.; Arnold, G.; Conrad, J.; Klaiber, I.; Beifuss, U.; Carle, R. Detection of phloridzin in strawberries (*Fragaria* × *ananassa* Duch.) by HPLC-PDA-MS/MS and NMR spectroscopy. *J. Agric. Food Chem.* **2003**, *51*, 2896−2899.

(44) Kammerer, D.; Carle, R.; Schieber, A. Detection of peonidin and pelargonidin glycosides in black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2407−2412.

(45) Holbach, B.; Marx, R.; Ackermann, M. Bestimmung der Anthocyanzusammensetzung von Rotweinen mittels Hochdruckflüssigkeitschromatographie (HPLC). *Lebensmittelchemie* **1997**, *51*, 78−80.

(46) Bejaoui-Kefi, B.; Sabbah, S.; Murkovic, M.; Dachraoui, M.; Pfannhauser, W. Red wines anthocyanins analyses by HPLC using different detection modes. *Ernaehrung* **2003**, *27*, 197−207.

(47) Wenzel, K.; Dittrich, H. H.; Heimfarth, M. Die Zusammensetzung der Anthocyane in den Beeren verschiedener Rebsorten. *Vitis* **1987**, *26*, 65−78.

(48) Hong, V.; Wrolstad, R. E. Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J. Agric. Food Chem.* **1990**, *38*, 698−708.

(49) Gholami, M.; Coombe, B. G. Occurrence of anthocyanin pigments in berries of the white cultivar Muscat Gordo Blanco (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* **1995**, *1*, 67−70.

(50) Malien-Aubert, C.; Dangles, O.; Amiot, M. J. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. *J. Agric. Food Chem.* **2001**, *49*, 170−176.

(51) Giusti, M. M.; Wrolstad, R. E. Acylated anthocyanins from edible sources and their applications in food systems. *Biochem. Eng. J.* **2003**, *14*, 217−225.

(52) Dokoozlian, N. K.; Kliewer, W. M. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 869−874.

(53) Larrauri, J. A.; Ruperez, P.; Saura Calixto, F. Antioxidant activity of wine pomace. *Am. J. Enol. Vitic.* **1996**, *47*, 369−372.

(54) Okuda, T.; Yokotsuka, K. *Trans*-resveratrol concentrations in berry skins and wines from grapes grown in Japan. *Am. J. Enol. Vitic.* **1996**, *47*, 93−99.

(55) Langcake, P.; Pryce, R. J. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, *9*, 77−86.

(56) Peyrot des Gachons, C.; Kennedy, J. A. Direct method for determining seed and skin proanthocyanidin extraction into red wine. *J. Agric. Food Chem.* **2003**, *51*, 5877−5881.

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

Received for review March 9, 2004. Revised manuscript received May 5, 2004. Accepted May 8, 2004. We are grateful to Jahncke Fruchtsäfte-Konzentrate GmbH & Co. KG, Drochtersen, Germany, for financial support.

JF049613B