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Survey of Polyphenol Constituents in Grapes and Grape-Derived Products

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ABSTRACT: A rapid and comprehensive qualitative method has been developed to characterize the different classes of polyphenols, such as anthocyanins, flavonols, phenolic acids, and flavanols/proanthocyanidins, in grape products. The detection was achieved by two runs with the same LC gradient in different MS ionization modes and mobile phase modifiers (positive ionization mode and 0.4% trifluoroacetic acid for anthocyanins and flavonols; negative ionization mode and 0.1% formic acid for phenolic acids and flavanols). From an analysis of the MS and UV data and in comparison with the authenticated standards, a total of 53 compounds were identified, including 33 anthocyanins, 12 flavonols, 4 phenolic acids, and 4 flavanols/proanthocyanidins. With the method developed, a survey was then conducted to qualitatively assess the composition of polyphenols among 29 different grape products including original grape, grape juice, grape wine, and grape-derived dietary supplements, and their chemical profiles were systematically compared. This method provided a comprehensive qualitative insight into the composition of polyphenols in grape-derived products.

KEYWORDS: grape, LC-MS, polyphenols, anthocyanins, flavonoids, proanthocyanidin, phenolic acid

INTRODUCTION

Grapes and grape-derived products are rich in numerous bioactive dietary polyphenols.[1,2] Polyphenols can be broadly divided into two classes: flavonoids and nonflavonoids.[3] The nonflavonoids include several major subgroups, such as hydroxycinnamic acids and derived lignans and coumarins, benzoic acid, hydrolyzable tannins, and stilbenes. Flavonoids consist of flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanidins (listed in ascending order of oxidation), as well as quinones, a class of oxidized derivatives of polyphenols.[1] The major classes of flavonoids in grapes and grape-derived products are the flavanol/proanthocyanidins, flavonols, and anthocyanins as shown in Figure 1.[3]

Polyphenols from grapes and grape-derived products have been associated with the prevention of numerous diseases including cardiovascular and neurodegenerative diseases as well as several forms of cancers.[4−6] Epidemiological and experimental evidence supports hypotheses that specific grape polyphenol forms may serve as disease preventative agents.[5−9] These include resveratrol,[10] proanthocyanidins,[11] and anthocyanins,[12] which are common in grapes, juice, wines, and dietary supplements including grape seed extracts.[4,13−16]

Because of the potential beneficial roles of grape-derived polyphenols, as well as their popular use in foods, drinks, and beverages, interest in the polyphenol composition of grapes, grape

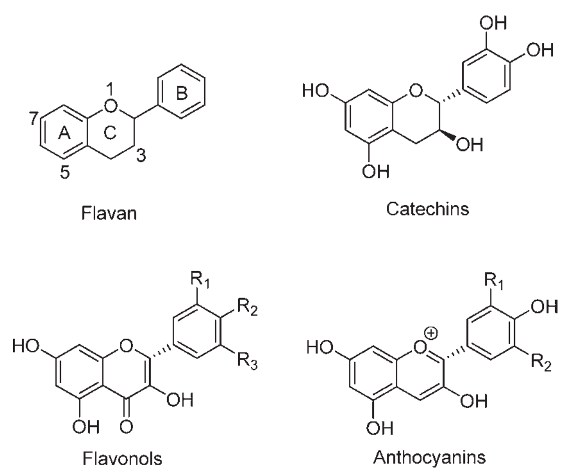

Figure 1. General structures of flavan and examples of flavonoids.

seed extracts, juices, and wines has grown in recent decades.[17−21] Many efforts have been limited by a focus on one type of grape

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Table 1. Information on Experimental Samples of Grapes and Grape-Derived Products

sample code	sample name	original source
1	Welch's Concord grape juice 100% concentrated	Welch's Inc.[a]
2	Welch's Concord grape juice cocktail	ACME
3	Welch's light concord grape juice beverage	ACME
4	Welch's 100% juice black cherry Concord grape juice	ACME
5	Langers pomegranate grape juice	ACME
6	Healthy Balance grape juice	ACME
7	Wild Harvest organic grape juice	ACME
8	Shoppers Value grape drink	ACME
9	ACME grape juice cocktail	ACME
10	ShopRite pasteurized grape juice	ShopRite
11	Santa Cruz organic Concord grape juice	ShopRite
12	Snapple naturally flavored grapeade juice	ShopRite
13	Walgreens grape juice	Walgreens
14	Manischewitz premium grape juice	A&P
15	Kedem Concord grape juice	A&P
16	Cabernet franc grape berries	Purdue University
17	Cabernet franc grape skins	Purdue University
18	Cabernet franc wine	Purdue University
19	Noiret grape berries	Purdue University
20	Noiret grape skins	Purdue University
21	Noiret wine	Purdue University
22	Cabernet Sauvignon wine	Purdue University
23	Pandol Red Seedless table grape berries	Costco
24	Pandol Red Seedless table grape skins	Costco
25	Pandol Black Seedless table grape berries	Costco
26	Pandol Black Seedless table grape skins	Costco
27	grape complete with pine bark	Country Life
28	best French red wine	Doctor's Best
29	herbal actives red wine	Nature's Plus

[a] Prepared for this research program.

product[18,19] or the characterization of one specific category of polyphenols[18,19,21] or other natural products. Furthermore, one of the major obstacles in studying the health benefits of grape and grape-derived products is that grapes and grape-derived products typically contain highly complicated and variable compositions of polyphenols. This is due, in part, to the fact that polyphenol contents and composition vary among different grape species as well as different cultivars within each species.[22,23] Moreover, the content and composition of polyphenols in grape and grape-derived products are highly affected by the climate, soil, and other environmental conditions in which grapes are grown, as well as postharvest handling, processing, and storage conditions.[24] This variability in polyphenol profile may also affect biological assessment

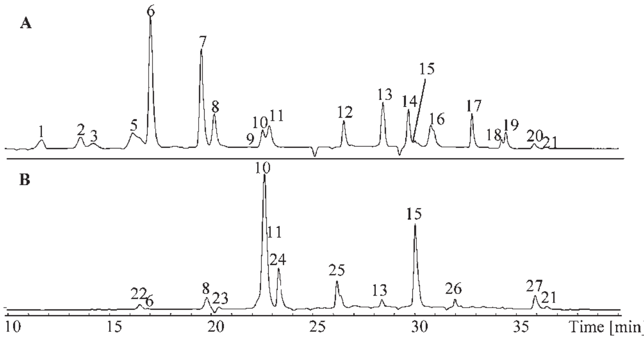

Figure 2. Representative UV chromatograms (520 nm) of (A) Welch's Concord grape juice 100% concentrated and (B) Cabernet franc wine. Peak assignments, t_R, and identities are listed in Table 2. The presence of the anthocyanin profile for each sample is listed in Table 3.

and outcomes. Thus, investigations on the health benefits of grapes and grape-derived products and on the mechanisms underlying these benefits must be accompanied by comprehensive characterizations of grape polyphenol profiles for individual grape and grape-derived products under investigation. Therefore, a robust and precise assessment method that can rapidly and accurately determine the polyphenolic composition in various grapes and grape-derived products is critical for supporting continued biological assessment of grape-derived products, identifying specific bioactive grape-derived polyphenolic components responsible for the health benefits, and understanding their mechanisms of action. The aim of this work was to develop a simple, effective, and comprehensive LC-MS method to characterize the profile of polyphenolic compounds, such as phenolic acids, flavanols/proanthocyanidins, flavonols, and anthocyanins. We then demonstrated the feasibility and efficacy of using this new LC-MS method to characterize the comprehensive polyphenolic profiles from a wide range of grapes, commercial grape juices, grape wines, and grape-derived products as well as to assess the changes of polyphenol compositions after the grapes are processed to wines.

MATERIALS AND METHODS

Materials. Standard compounds including caffeic acid, 4-hydroxybenzoic acid, (−)-epicatechin, *p*-coumaric acid, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Procyanidin B2, caftaric acid, and cyanidin-3-glucoside were purchased from ChromaDex (Irvine, CA). The HPLC grade acetonitrile (ACN), methanol (MeOH), and trifluoroacetic acid (TFA) were obtained from Fisher Scientific Co. (Fair Lawn, NJ). HPLC grade formic acid was obtained from Acros Organics (Morris Plains, NJ). HPLC grade water was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA). Fifteen retail grape juices were purchased from several food supermarkets, and they covered brands such as Welch's, Langers, Healthy Balance, Wild Harvest, Shoppers Value, ACME, ShopRite, Santa Cruz, Snapple, Walgreens, Manischewitz, and Kedem as shown in Table 1. Welch's Concord grape juice 100% concentrated (originally from Welch's Inc.), Cabernet franc grape berries, and Noiret grape berries as a whole fruit were provided by Jill Blume, Department of Food Science, Purdue University. Pandal Red Seedless table grape, Pandal Black Seedless table grape, and three grape-produced dietary supplements (samples 27−29 in Table 1) were commercially purchased. The grape skins were manually peeled from fresh grape berries. All grape

Table 2. Peak Assignments for the Analysis of Grapes and Grape-Derived Products[a]

peak	t_R(min)	identity	molecular and fragment ions (m/z)	compd code
1	11.7	Dp-G-G	627, 465, 303	**1**
2	13.6	Cy-G-G	611, 449, 287	**2**
3	14.3	Pt-G-G	641, 479, 317	**3**
4	15.4	Pg-G-G	595, 433, 271	**4**
5a	16.1	Pn-G-G	625, 463, 301	**5**
5b	16.5	Mv-G-G	655, 493, 331	**6**
6	17.1	Dp-G	465, 303	**7**
7	19.6	Cy-G	449, 287	**8**
8	20.1	Pt-G	479, 317	**9**
9	21.8	Pg-G	433, 271	**10**
10	22.6	Pn-G	463, 301	**11**
11	22.9	Mv-G	493, 331	**12**
12	26.4	Dp-G-Ac	507, 303	**13**
13a	28.2	Dp-G-G-Co	773, 611, 465, 303	**14**
13b	28.3	Cy-G-Ac	491, 287	**15**
13c	28.5	Pt-G-Ac	521, 317	**16**
14a	29.6	Cy-G-G-Co	757, 595, 449, 287	**17**
14b	29.7	Pt-G-G-Co	787, 625, 479, 317	**18**
15a	30.1	Pn-G-Ac	505, 301	**19**
15b	30.1	Mv-G-Ac	535, 331	**20**
16a	30.7	Mv-G-G-Co	801, 639, 493, 331	**21**
16b	31.0	Pn-G-G-Co	771, 609, 463, 301	**22**
17	32.9	Dp-G-Co	611, 303	**23**
18	34.4	Cy-G-Co	595, 287	**24**
19	34.5	Pt-G-Co	625, 317	**25**
20	35.9	Mv-G-Co	639, 331	**26**
21	36.5	Pn-G-Co	609, 301	**27**
22	16.8	Dp-G-Py	533, 371	**28**
23	20.1	Pt-G-Py	547, 385	**29**
24	23.2	Mv-G-Py	561, 399	**30**
25	26.2	Mv-G-Ac-Py	603, 399	**31**
26	32.0	Mv-G-Co-Py	707, 399	**32**
27	36.0	Mv-G-VP	609, 447	**33**
28	25.2	myricetin-G	481, 319	**34**
29	26.0	myricetin-GR	495, 319	**35**
30	28.8	quercetin-G	465, 303	**36**
31	29.4	quercetin-GR	479, 303	**37**
32	29.8	syringetin-G	509, 347	**38**
33	31.8	isorhamnetin-GR	493, 317	**39**
34	34.0	syringetin-G-Ac	551, 347	**40**
35	41.0	myricetin	319	**41**
36	48.5	laricitrin	333	**42**
37	51.6	quercetin	303	**43**
38	52.1	syringetin	347	**44**
39	54.7	isorhamnetin	317	**45**
40	5.7	gallic acid	169	**46**
41	11.7	PAC dimer	577	**47**
42	13.1	catechin	289	**48**
43	15.2	vanillic acid	167	**49**
44	16.3	epicatechin	289	**50**
45	16.7	PAC dimer	577	**51**
46	18.5	caffeic acid	179	**52**
47	26.8	*p*-coumaric acid	163	**53**

[a] The phenolic acids (gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid), proanthocyanidins, and cyanidin-3-glucoside were compared with the retention time of the authenticated standard. Dp, delphinidin; Pt, petunidin; Cy, cyanidin; Mv, malvidin; Pn, peonidin; Pg, pelargonidin; G, glucosyl or galactosyl moiety; GR, glucuronosyl; Ac, acetyl; Co, coumaroyl; Py, pyruvate; VP, vinylphenol; PAC, proanthocyanidin. For the flavonoid glycosides, in general, the glucosyl group, occasionally the galactosyl group, was substituted on the 3/5-position of the aglycone and the acetyl/coumaroyl group was linked to the 6′-position of the sugar moiety.

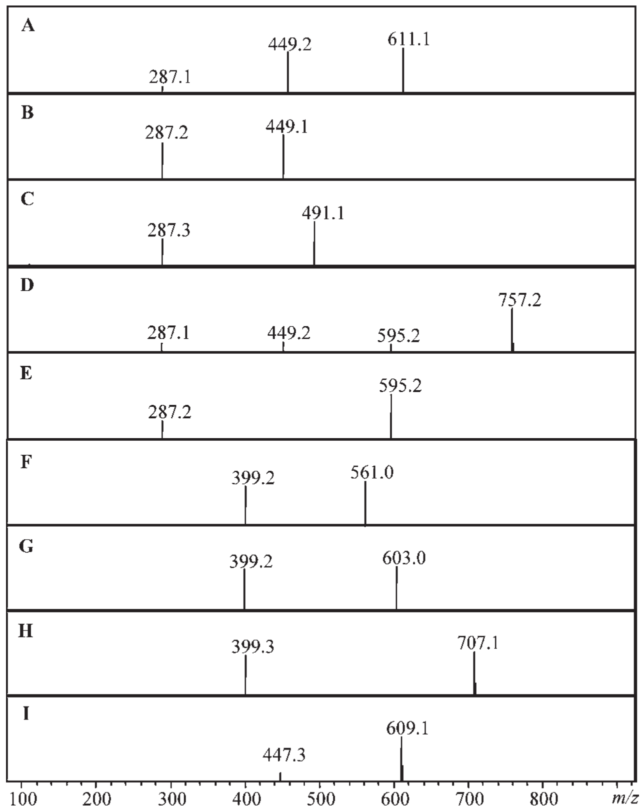

Figure 3. Representative MS spectra of cyanidin derivatives of compounds **2** (A), **8** (B), **15** (C), **17** (D), and **24** (E) in Welch's Concord grape juice 100% concentrated and malvidin pyruvate derivatives of compounds **30** (F), **31** (G), **32** (H), and **33** (I) in Cabernet franc wine.

juices and wines were stored at 4 °C, and grapes berries and skins were stored at −20 °C.

Sample Preparations. For qualitative identification, the grape wines and grape juices (samples 1−15, 18, 21, and 22 in Table 1) were filtered through a 0.45 μm filter into HPLC vials and directly injected for LC-MS analysis. The grape berries (samples 16, 19, 23, and 25 in Table 1) as a whole fruit were frozen at −20 °C and then ground. Around 1 g of grape berries was extracted with 10 mL of 70% MeOH containing 1% acetic acid solution and sonicated for 20 min. The extraction was conditioned to room temperature, and then approximately 1 mL samples were filtered through a 0.45 μm filter and transferred into HPLC vials. Grape skins (samples 17, 20, 24, and 26 in Table 1) were manually peeled from fresh grape berries and ground and then prepared using the

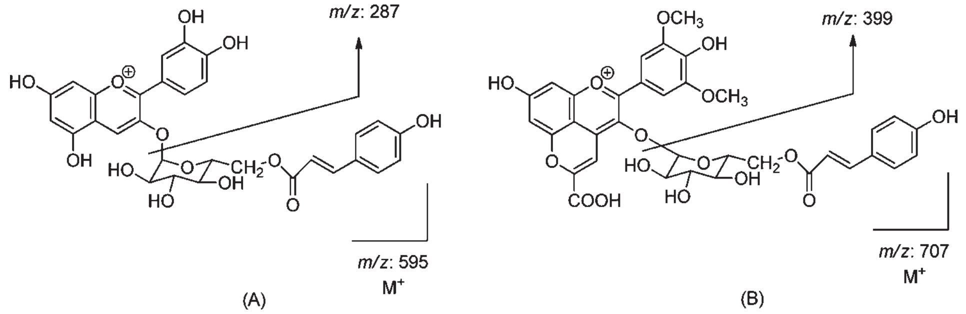

Figure 4. Structures of selected anthocyanins of (A) compound **24** (MS spectrum is shown in Figure 3E) and (B) compound **32** (MS spectrum is shown in Figure 3H) and their fragment pathway.

same procedure as for grape berries. Around 100 mg of grape-derived dietary supplements (samples 27−29 in Table 1) were dissolved in 10 mL of 70% MeOH containing 1% acetic acid solution and sonicated for 20 min. The extraction was conditioned to room temperature, and then around 1 mL was filtered into vials through a 0.45 μm filter into HPLC vials prior to the injection.

Equipment and HPLC-MS Conditions. HPLC separation was performed on a Polaris amide-C18 column, 250 × 4.6 mm, 5 μM (Varian Inc.). For LC-MS analysis, a Hewlett-Packard Agilent 1100 series LC-MS (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, a quaternary pump system, a DAD detector, a degasser, an MSD trap with an electrospray ion source (ESI), and HP ChemStation software, Bruker Daltonics 4.2 and Data Analysis 4.2, was used. The identification of anthocyanins, flavonols, phenolic acids, and flavanols was achieved by using the same LC gradient, but with different MS ionization modes and mobile modifiers. Anthocyanins and flavonols were detected under positive ion mode with 0.4% TFA (v/v) in water and ACN. Phenolic acids and flavanols/proanthocyanidins were detected under negative ion mode with 0.1% FA (v/v) in water and ACN. HPLC separation was performed with the mobile phase containing solvents A (0.4% TFA or 0.1% FA in water) and B (0.4% TFA or 0.1% FA in ACN) in the following gradient: 0−20 min, linear gradient from 10 to 20% B; 20−30 min, linear gradient from 20 to 30% B; 30−40 min, isocratic elution at 30% B; 40−50 min, linear gradient from 30 to 50% B; 50−60 min, linear gradient from 50 to 60% B. The flow rate was set at 1.0 mL/min. The injection volume was 20 μL, and the UV detector was set at 254, 280, 370, and 520 nm. The eluent was monitored by electrospray ion mass spectrometer (ESI-MS) under positive ion mode for anthocyanins and flavonols and under negative ion mode for phenolic acids and flavanols/proanthocyanins. The samples were scanned from m/z 100 to 1200. ESI was conducted by using needle voltages of 3.5 kV (positive) and −3.5 kV (negative). High-purity nitrogen (99.999%) was used as dry gas at a flow rate of 12 L/min, and the capillary temperature was 350 °C. Nitrogen was used as nebulizer at 60 psi and helium as collision gas.

RESULTS AND DISCUSSION

An LC-MS method was developed and applied to the survey of the polyphenol compositions of 29 samples, including grape berries, grape skins, grape juices, grape wines, and grape-produced dietary supplements. The same LC gradient using different MS ion modes and mobile phase modifiers was applied to detect different subgroups of polyphenols (positive ion mode and 0.4% TFA for anthocyanins and flavonols, negative ion mode and 0.1% FA for phenolic acids and flavanols). Under the optimized LC-MS conditions and on the basis of analysis of the MS and UV data and in comparison with the authenticated standards, a total of 53 compounds, including 33 anthocyanins, 12 flavonols, 4 phenolic acids, and 4 flavanols/proanthocyanidins were successfully separated and indentified.

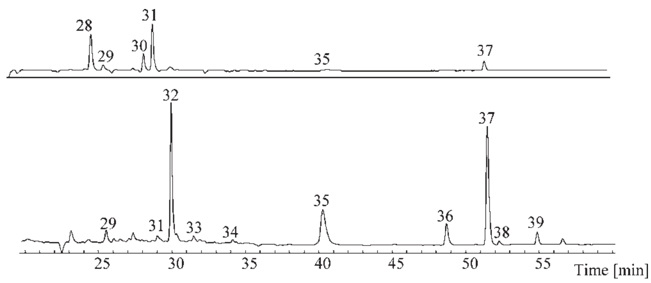

Figure 5. Representative UV chromatograms (370 nm) of (A) Welch's Concord grape juice 100% concentrated and (B) Cabernet Sauvignon wine. Peak assignment, t_R, and identities are listed in Table 2. The presence of flavonol profile for each individual sample is listed in Table 4.

Anthocyanin Identification and Profile Comparison. The representative UV chromatograms at 520 nm of Welch's Concord grape juice 100% concentrated (sample 1 in Table 1) and Cabernet franc wine (sample 18 in Table 1) are illustrated in Figure 2. The identities, retention times, peak assignments, molecular ions, and characteristic fragment ions for individual compounds are listed in Table 2. On the basis of the analysis of MS and UV data and in comparison with authenticated standards and reported data,[17−19] a total of 33 anthocyanins were simultaneously identified as anthocyanidin diglycosides, glucoside, acetylglucoside, coumaroylglucoside, coumaroyldiglucoside, and anthocyanin pyruvate derivatives (Table 2). The structures and fragment pathways of selected anthocyanins (compounds **24** and **32**) are illustrated in Figure 4. The representative MS spectra of cyanidin derivatives (compounds **2**, **8**, **15**, **17**, and **24**) detected in Welch's Concord grape juice 100% concentrated and malvidin derivatives (compounds **30**−**33**) detected in Cabernet franc wine are shown in Figure 3.

Previous research has identified many anthocyanins in grapes and grape-derived products.[17−19] In general, the glucosyl group, and occasionally the galactosyl group, is substituted on the 3- and 5-positions of the aglycone. The acetyl and coumaroyl groups are attached on the 6′-position of the sugar moiety. On the basis of the UV spectrum and molecular ions and their corresponding fragment ions, most of the structures could be tentatively determined. For example, the MS spectrum (Figure 3A) of compound **2** (t_R, 13.6 min) indicates that it has a molecular ion at m/z 611 and is fragmented to m/z 499 ([M − glucosyl]$^+$)

Table 3. Presence of Anthocyanins in Individual Grape Samples[a]

	sample code																												
compd code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	+	+	+	+	+	+	+	+	T	+	+	+	+	-	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
2	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
4	−	−	−	−	T	−	−	−	−	−	−	−	−	−	−	−	−	−	T	T	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	+	+	T	+	T	+	+	+	+	+	+	+	T	T	T	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	T	+	T	+	+	+	−	+	+	T	−	T	T	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	T	+	−	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+
10	T	T	T	T	T	−	T	T	−	T	T	T	−	−	T	T	T	−	T	T	−	−	T	T	T	T	T	T	T
11	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	+	+	T	+	+	T	−	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	−	+	+	+	+	+	−	−	+	+	−	+	−	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−
14	+	+	+	+	+	+	+	−	−	+	+	T	T	−	+	−	−	−	T	T	T	−	−	−	−	−	−	−	−
15	+	T	+	+	+	+	+	−	−	+	+	−	−	−	+	+	+	T	+	+	+	−	−	−	−	−	−	−	−
16	+	T	+	+	+	+	+	−	−	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
17	+	+	+	+	+	+	+	+	T	+	+	T	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
18	+	+	+	+	+	+	+	+	T	+	+	T	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
19	+	−	+	T	T	+	+	−	−	T	+	−	−	T	+	+	+	+	+	+	+	−	T	T	+	+	−	−	−
20	+	−	+	T	T	+	+	−	−	T	+	−	T	−	+	+	+	+	+	+	+	−	−	−	+	+	−	−	−
21	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
22	+	+	+	+	+	+	+	+	T	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
23	+	+	+	+	+	+	+	T	T	+	+	−	T	T	+	+	+	−	+	+	+	−	−	−	+	+	T	T	T
24	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	+	+	−	+	+	+	−	+	+	+	+	−	−	−
25	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	+	+	−	+	+	+	−	T	T	+	+	T	T	T
26	+	+	+	+	+	+	+	−	−	+	+	−	+	−	+	+	+	+	+	+	+	+	T	T	+	+	+	+	+
27	+	−	+	+	+	+	+	−	−	+	+	−	T	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	T	T	−	−	−	−	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	+	+	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	T	+	−	−	−	−	T	T	+
31	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−
32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	T
33	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	T	+	−	−	−	−	−	−	−
total	26	22	26	26	27	25	26	16	15	26	26	16	22	4	26	16	16	16	27	27	29	10	11	11	13	13	11	11	12

[a] Sample codes are the same as in Table 1. Compound codes refer to Table 2. +, present; −, not detectable; T, trace; total, total number of anthocyanins present in individual samples.

and m/z 287 ([M − glucosyl − glucosyl]$^+$), which corresponds to cyanidin diglucoside. Compound **8** (Figure 3B, t_R, 19.6 min) has a molecular ion at m/z 449 and is fragmented to m/z 287 ([M − glucosyl]$^+$), which corresponds to cyanidin glucoside. The same identifications are applied to the derivatives of acetylglucoside and coumaroylglucoside. For example, compound **15** (Figure 3C, t_R, 28.3 min) has a molecular ion at m/z 491 and a fragment ion at m/z 287 ([M − acetylglucosyl]$^+$). Compound **24** (Figure 3E, t_R, 34.4 min) has a molecular ion at m/z 595 and a fragment ion at m/z 287 ([M − coumaroylglucosyl]$^+$). As such, they were identified as cyanidin acetylglucoside and cyanidin coumaroylglucoside, respectively. Compound **30** (Figure 3F, t_R, 23.2 min) with the molecular ion at m/z 561 and a fragment ion at m/z 399 was identified as malvidin glucoside-pyruvate, formed through the interaction between malvidin glucoside and pyruvic acid. Compounds **31** (Figure 3G, t_R, 26.2 min) and **32** (Figure 3H, t_R, 32.0 min) have the same fragment ion at m/z 399, but different molecular ions at m/z 603 and at m/z 707 are assigned as malvidin acetylglucoside-pyruvate and malvidin coumaroulglucoside-pyruvate, respectively. Compound **33** (Figure 3I) has a molecular ion at m/z 609 and a fragment ion at m/z 447 and is elucidated as malvidin glucoside-vinylphenol. The assignment for individual compounds agrees with previous studies.[17−19]

Comparison of the anthocyanin profiles of 15 grape juices (Table 3) revealed that most were found to exhibit similar anthocyanin compositions as Welch's Concord grape juice 100% concentrated (Figure 2A), with the exception of Manischewitz premium grape juice. In Manischewitz premium grape juice, only trace amounts of delphinidin glucoside, cyanidin glucoside, petunidin acetylglucoside, and delphinidin coumaroylglucoside

Table 4. Presence of Flavonols, Phenolic Acids, and Flavanols/Proanthocyanidins in Individual Grape Samples[a]

	sample code																												
compd code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
34	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+	T	T	+	+	+	+	−	−	−	+	+	T	T	+
35	+	+	+	+	−	+	+	−	T	+	+	T	+	+	+	+	+	+	+	+	T	T	−	−	+	+	−	−	−
36	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+	+	T	T	+	+	−	−	+	+	+	+	T	T	+
37	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+
38	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
39	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	−	−	−	−	−
41	+	+	+	+	T	+	+	T	+	+	+	+	+	+	+	T	T	+	+	+	+	+	−	−	T	T	T	T	T
42	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
43	+	+	+	+	+	+	+	T	+	+	+	+	+	+	+	T	T	+	+	+	T	+	+	+	+	+	+	+	+
44	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	T	−	−	−	−	−	−	−
45	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
46	+	T	+	+	T	+	+	−	T	T	T	T	T	T	T	T	T	T	T	T	T	+	−	−	−	−	+	+	+
47	+	+	+	+	+	+	+	T	+	+	+	−	+	+	+	T	T	T	−	−	−	+	+	+	+	+	+	+	+
48	+	+	+	+	T	+	+	−	−	+	+	+	+	+	+	T	T	T	−	−	+	+	−	−	−	−	+	+	+
49	T	−	−	−	−	−	−	−	−	−	T	T	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
50	+	+	+	+	T	+	+	−	−	+	+	T	T	−	+	−	−	T	−	−	T	+	−	−	−	−	−	−	−
51	+	+	+	+	T	+	+	−	−	+	+	−	−	+	+	−	−	+	−	−	+	+	−	−	−	−	+	+	+
52	T	T	T	+	T	+	+	−	−	+	+	T	T	T	+	−	−	+	−	−	+	+	−	−	−	−	−	−	−
53	+	+	+	+	T	+	+	−	−	+	+	+	−	−	+	−	−	+	−	−	+	+	−	−	−	−	−	−	−
total	14	13	13	13	12	13	13	6	8	13	14	12	11	11	13	9	9	13	7	7	11	17	4	4	7	7	9	9	9

[a] Sample codes are the same as in Table 1. Compound codes refer to Table 2. +, present; −, not detectable; T, trace; total, total number of flavonols, phenolic acids, and flavanols/proanthocyanidins present in individual samples.

were detected. The major anthocyanins in the majority of the grape juices were cyanidin glucoside and delphinidin glucoside (peaks 6 and 7 in Figure 2A). However, peonidin diglucose and malvidin diglucose dominate the anthocyanins in 5 of 15 grape juice samples (samples 2, 4, 8, 9, and 12 in Table 1). The presence of the identified anthocyanins, as well as the other polyphenols in grape juices and other grape samples, is shown in Table 3.

Four different fresh grape berries were included to assess the heterogeneity of anthocyanin profiles, including one of the major red grape varieties, Cabernet franc, a newly developed hybrid variety, Noiret, and Red Seedless table grape and Black Seedless table grape from Pandol. Noiret was developed by Cornell University.[25,26] It is a hybrid variety with predominant ancestors *Vitis vinifera* and *Vitis labrusca* and has a black color and moderately large-sized berries.[25,26] Qualitative distributions of anthocyanins in four grape berries are quite different (Table 3). Noiret grape berries contained the highest variety of anthocyanin compounds, followed by Cabernet franc grape berries, Pandol Black Seedless grape berries, and Pandol Red Seedless grape berries. The anthocyanin qualitative profile of Noiret grape berries is similar to that observed for Welch's Concord grape juice 100% concentrated, with delphinidin glucoside (peak 6 in Figure 2A) being observed to be the most abundant form. In contrast to Concord juice, malvidin glucoside (peak 11 in Figure 2A) was most abundant in Cabernet franc grape berries and Pandol Red Seedless grape berries, and malvidin glucoside and malvidin coumaroylglucoside (peaks 11 and 20 in Figure 2A) were most abundant in Pandol Black Seedless grape berries. The anthocyanin compositional difference between Noiret grape berries and the other three berries is also characteristically distinguished by verifying the identities of the diglucoside derivatives (Table 3). In the Cabernet franc grape berries, the diglucoside of anthocyanidins (peaks 1−5 in Figure 2A) and coumaroylated derivatives of diglucosides (peaks 13a, 14a, 14b, 16a, and 16b in Figure 2A) were not detected. There were also no detectable diglucosides of anthocyanidins and coumaroylated derivatives of diglucosides in the Pandol Red Seedless grape berries and Pandol Black Seedless grape berries (Table 3).

The qualitative distributions of anythocyanins between grape berries (whole fruit) and grape skins (peeled from the fresh fruit) were quite similar among the four analyzed grapes. However, anthocyanins are known to be concentrated in the fruit skins. In this study, anthocyanins are asymmetrically distributed in the Cabernet franc grape berries and Noiret grape berries. Subsequently, the grape skins from these two grape berries have an anthocyanin profile closer to that shown in the berries, but easily distinguished from each other by verifying the identities of the diglucoside derivatives (Table 3).

The color evolution of red wines is a complex process that is partially attributed to the progressive displacement of original anthocyanins by newly formed pigments. These pigments usually arise from the interaction between anthocyanins and other phenolic compounds, such as phenolic acid (pyruvic acid) and flavan-3-ols (catechins and procyanindins). Here, Cabernet franc wine, Noriet wine, and Cabernet Sauvignon wine were investigated to characterize their anthocyanin profiles. In all three wines, we observed newly formed pigments, among which malvidin glucose-pyruvate (peak 24 in Figure 2B) had the highest proportion. Cabernet Sauvignon wine has a distinguished anthocyanin profile, and most of them are malvidin-related compounds,

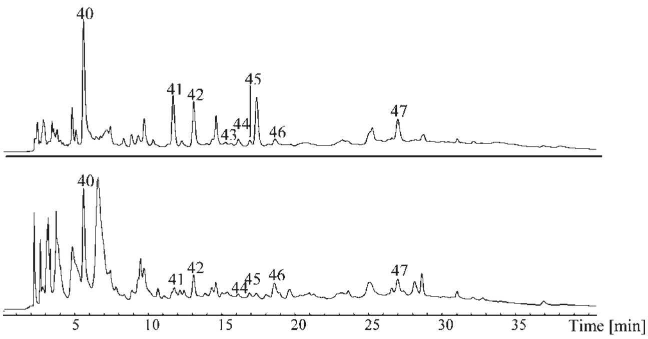

Figure 6. Representative UV chromatograms (280 nm) of (A) Welch's Concord grape juice 100% concentrated and (B) Cabernet Sauvignon wine. Peak assignment, t_R, and identities are listed in Table 2. The presence of phenolic acid and flavanol/proanthocyandin profile for each individual samples is listed in Table 4.

including malvidin glucose and other newly formed pigments, namely, malvidin glucoside-pyruvate, malvidin acetylglucoside-pyruvate, malvidin coumaroylglucoside-pyruvate, and malvidin glucoside-4-vinylphenol (Table 3). Only relatively low intensities of delphinidin glucoside-pyruvate and petunidin glucoside-pyruvate were identified in the Cabernet Sauvignon wine. The anthocyanin profile for the Noiret wine and Cabernet franc wine is close to those of their grape berries and corresponding grape skins (Table 3); only small amounts were transferred to the newly formed pigment by interacting with other phenolic compounds in the samples. This may be due to the young nature of this wine. In three grape-derived dietary supplements (samples 27−29 in Table 1), we observed glucoside derivatives and coumaroylglucoside derivatives of anthocyanidins, as well as trace amounts of newly formed anthocyanin pigments.

Flavonol Identification and Profile Comparison. By comparing the flavonol profiles of 15 grape juice samples at a UV wavelength of 370 nm, we observed that they have flavonol compositions very similar to that of Welch's Concord grape juice 100% concentrated (Figure 5A and Table 4). The major free flavonol aglycones in the various commercial grape juices are myricetin and quercetin, which are at 41.0 and 51.6 min in Figure 5A. Their glucoside or glucuronide was eluted much earlier due to the addition of very hydrophilic molecular glucose and glucoronic acid. Compound **34** (t_R, 25.2 min) has the protonated molecular ion at m/z 481 and fragment ion at m/z 319 ($[M + H - \text{glucosyl}]^+$) and, therefore, is assigned as myricetin glucoside. Compound **35** also has the fragment ion at m/z 319, but it is produced from a protonated molecular ion at m/z 495 by losing 176, which is usually from glucuronide, and is identified as myricetin glucuronide. The same identification could be achieved for quercetin glucoside and quercetin glucuronide.

The qualitative distributions of flavonols in Cabernet franc grape berries and skins, Noiret grape berries and skins, and Pandol Black Seedless grape berries and skins were compared (Table 4). The majority of the flavonols in the original grape samples are myricetin glucoside, myricetin glucuronide, quercetin glucoside, and quercetin glucuronide. In Pandol Red Seedless grape berries and skins only quercetin glucoside and quercetin glucuronide were detected. In this study, Cabernet franc wine, Noiret wine, and Cabernet Sauvignon wine were investigated to establish their flavonol profiles. Cabernet franc wine and Noiret wine show profiles similar to that of the corresponding grape berries and skins and grape juice products, with the major flavonals of either aglycons (myricetin and quercetin) or the corresponding glucoside and glucuronide. However, in Cabernet Sauvignon wine, the flavonol profile is significantly different from the grape juice products, Cabernet franc wine, and Noiret wine (Table 4). Syringetin glucoside (peak 32) is the dominant flavonol in the Cabernet Sauvignon wine (Figure 5B). Other flavonols, such as syringetin acetylglucoside, isorhamnetin glucuronide, syringetin, laricitrin, and isorhamnetin (peaks 33, 34, 36, 38, and 39) were detected only in the Cabernet Sauvignon wine (Figure 5B). Additionally, myricetin glucoside and quercetin glucoside (peaks 28 and 30) were not detected in the Cabernet Sauvignon wine (Figure 5B).

Phenolic Acid and Flavanol Identification and Profile Comparison. The detection of phenolic acids and flavanols was achieved under negative mode with the same LC gradient as the anthocyanins and flavonols. The representative UV chromatograms (280 nm) of Welch's Concord grape juice 100% concentrated and Cabernet Sauvignon wine are illustrated in Figure 6. On the basis of UV and MS spectral data and by comparison to the retention time of the authentic standards, we were able to detect and tentatively identify four phenolic acids, catechin, epicatechin, and proanthocyanidin dimers. Thirteen of 15 grape juices exhibited similar phenolic acid and proanthocyanin profiles. In the Shoppers Value grape drink, only trace amounts of proanthocyanidin dimer were identified. In ACME grape juice cocktail, only gallic acid and proanthocyanidin dimer were detected (Table 4). Grape berries and their corresponding grape skins contain the same composition of phenolic acids, whereas in their corresponding wine samples, more phenolic acids were found (Table 4).

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