JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Characterization of Anthocyanins by High-Performance Liquid Chromatography−Electrospray Ionization−Tandem Mass Spectrometry in Common Foods in the United States: Vegetables, Nuts, and Grains

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Anthocyanins in common foods in the United States, other than fruits and berries, were identified and characterized by high-performance liquid chromatography (HPLC)−electrospray ionization−tandem mass spectrometry coupled with diode array detection. Of all of the 40+ vegetables, nuts, and grains screened, seven vegetables, one nut, and one grain were found to contain anthocyanins; the number of anthocyanins detected varied from two in pistachio nuts to 34 in red radishes. The individual anthocyanins were identified by comparing their mass spectrometric data and retention times with those of standards, published data, and reference food samples. In all of the samples analyzed, except for sorghum, only six common anthocyanidins (delphinidin, cyanidin, pelargonidin, petunidin, peonidin, and malvidin) were found as their glycosides. Anthocyanins in certain vegetables such as red cabbage and red radish were highly conjugated with sugars and acylated groups, and thus, their structures were very complicated. Eight different either aliphatic or aromatic acylated groups (acetoyl, coumaroyl, malonoyl, *p*-hydroxybenzoyl, feruoyl, caffeoyl, sinapoyl, and oxaloyl) were identified in the anthocyanins. In addition to glucose, six other sugar moieties (galactose, xylose, rhamnose, rutinose, sambubiose, and laminaribiose) were observed. Three varieties of sorghum were found to contain 3-deoxyanthocyanidins and their derivatives as major anthocyanins. A number of new anthocyanins were identified in the foods studied. This paper presents complete HPLC profiles and MS spectrometric data, obtained under the same experimental conditions, for common vegetables, pistachio nuts, and sorghum that contain anthocyanins.

KEYWORDS: HPLC-ESI-MS/MS; anthocyanin; 3-deoxyanthocyanidin; black bean; eggplant; pistachio; red cabbage; red leaf lettuce; red onion; red radish; small red bean; sorghum

INTRODUCTION

Anthocyanins are a group of plant pigments that are widely distributed in nature. They generally occur in the plant as glycosides and acylglycosides of anthocyanidins, the aglycones. Anthocyanidins vary in the different hydroxyl or methoxyl substitutions in their basic flavylium (2-phenylbenzopyrilium) structure (*1*). Anthocyanins have been demonstrated to play a very important role in plant physiology and are important to the food industry and in human health as well (*2*−*4*). Thus, understanding the chemical structures of anthocyanins and their distribution in foods are critical to anthocyanin-related studies. In addition to fruits and berries (*1*, *5*), some vegetables can be major sources of anthocyanins in the diet. Red cabbage, red radish, and red potato were found to contain highly conjugated anthocyanins (*6*). These highly conjugated anthocyanins are believed to play a more important role in the food industry due to their increased stability.

In our previous study, we identified and characterized anthocyanins in common fruits and berries (*5*). The foods surveyed in the previous and the current studies were listed previously (*7*). The foods sampled are those commonly available in the United States and, in most cases, represented multiple samplings from different areas of the United States and at different times, and in most cases, the variety was not specified. In this investigation, the objective was to identify and characterize the anthocyanins in foods other than fruits and berries including vegetables, nuts, and grains. Consistent patterns between anthocyanin profiles that may help in identifying anthocyanins are also discussed.

MATERIALS AND METHODS

Standards and Solvents. Standards of 3-*O*-*β*-glucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin [six mixed anthocyanin standards, high-performance liquid chromatography

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10.1021/jf0478861 CCC: $30.25
Published on Web 03/26/2005

(HPLC) grade], cyanidin 3-*O*-*β*-glucoside, cyanidin 3-*O*-*β*-(coumaroyl)-sambubioside-5-*O*-*β*-glucoside, and peonidin 3,5-di-*O*-*β*-glucoside (HPLC grade) were obtained from Polyphenols Laboratories (Sandnes, Norway). The standards of apigeninidin and luteolinin (HPLC grade) were kindly provided by Dr. Joseph M. Awika (Texas A&M Univeristy, College Station, TX). Formic acid was purchased from Aldrich (St. Louis, MO). All other solvents were purchased from Fisher (Fair Lawn, NJ).

Sample Preparation. The sources of red leaf lettuce, red cabbage, red radish, and pistachio were from the U.S. Department of Agriculture database samples and have been described previously (*7*). Eggplant, black bean, small red bean, and red onion were purchased from a local supermarket and freeze-dried. Both grain and bran powders of black sorghum (Tx430), hi-tannin sorghum (Sumac), and red sorghum (Tx2911) were generously provided by Dr. Lloyd W. Rooney (Texas A&M University). All powders were kept at −70 °C until analyzed.

Sample powders were extracted using methanol:water:acetic acid (85:15:0.5; v/v, MeOH:H_2O:AcAc) as reported previously (*8*). All samples were diluted with acidic methanol and filtered using 0.22 *μ*m Teflon syringe filter (Cameo, MN) for anthocyanin analysis.

HPLC−Diode Array Detection−Electrospray Ionization (ESI)−Tandem Mass Spectrometry (MS/MS) Analysis of Anthocyanins. Chromatographic analyses were performed on an HP 1100 series HPLC (Hewlett-Packard, Palo Alto, CA) equipped with an autosampler/injector and diode array detector. A 4.6 mm × 250 mm, 5 *μ*m, Zorbax Stablebond Analytical SB-C_{18} column (Agilent Technologies, Rising Sun, MD) was used for separation. Elution was performed using mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol). The flow rate was 1 mL/min, and detection was set at 520 nm for all samples except for sorghum and at 480 nm for sorghum. Three different gradients were used. Gradient I was used to separate all samples except for red cabbage and red radish, which is same as "gradient I" in a previous paper (*5*). Gradient II, identical to "gradient III" in a previous paper (*5*), was used to separate red cabbage. Gradient III was used exclusively to separate red radish anthocyanins. It is described as follows: 0−2 min, 25% B; 2−10 min, 25−28% B; 10−15 min, 28% B; 15−30 min, 28−32% B; 30−50 min, 32% B; 50−55 min, 32−37% B; 55−65 min, 37% B; 65−70 min, 37−42% B; 70−75 min, 42% B; 75−80 min, 42−50% B; 80−85 min, 50% B; 85−90 min, 50−55% B; 90−95 min, 55% B; 95−100 min, 55−25%; 100−102 min, 25% B. Low-resolution electrospray mass spectrometry was performed with an Esquire 3000 Ion Trap Mass Spectrometer (MS) (Bruker Daltoniks, Billerica, MA). The experimental conditions were the same as previously described (*5*)

RESULTS AND DISCUSSION

Peak Identification and Assignment. Identification and peak assignment of anthocyanins were primarily based on comparison of their retention time (t_R) and mass spectrometric data with that of standards, references, and with guidelines presented previously by Mazza and Miniati (*1*), Giusti et al. (*9*), and Wu and Prior (*5*). One representative chromatogram from each different type of food is presented. As references, the chemical structures and molecular weights of six common anthocyanidins and the most common sugars are summarized in **Figure 1** and **Table 1**. All food samples presented in this paper were divided into three groups based on their anthocyanin composition: Group 1 consisted of foods containing simple anthocyanins; group 2 consisted of foods containing highly conjugated anthocyanins; and group 3 consisted of sorghums containing 3-deoxyanthocyanidins.

Group 1: Foods Containing Simple Anthocyanins. Six foods included in this group were eggplant, red leaf lettuce, red onion, black bean, small red bean, and pistachio.

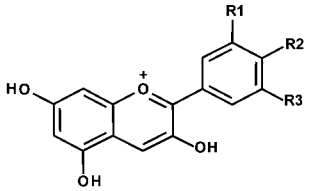
Eggplant. Eggplant was found to contain four delphinidin anthocyanins (**Figure 2A** and **Table 2**). By comparing their MS data and retention times with standards and published data (*10*, *11*), peaks 3 and 4 were identified as delphinidin 3-glucoside and delphinidin 3-rutinoside. Peaks 1 and 2 had the same MS profile but different retention times (13.5 vs 17.6 min). MS data indicated that both of these two anthocyanins contained delphinidin plus one hexose and one rutinose ($[M]^+$, *m*/*z* 773; MS/MS, *m*/*z* 611/463/303). They were tentatively identified as delphinidin 3-rutinoside-5-galactoside (peak 1) and delphinidin 3-rutinoside-5-glucoside (peak 2) from their retention times (*5*). An acylated anthocyanin, delphinidin 3-(coumaroyl)rutinoside-5-glucoside (nasunin), was previously found to be the major anthocyanin in eggplant (*12*). However, we were not able to detect this anthocyanin, possibly due to varietal differences (*13*).

Red Leaf Lettuce. Two major anthocyanins, cyanidin 3-glucoside and cyanidin 3-(6″-malonoyl)glucoside, were previously found in red leaf lettuce (*Lactuca sativa*) (*14*). In addition to being an antioxidant, cyanidin 3-(6″-malonoyl)glucoside in red leaf lettuce has been shown to attenuate light and offer effective and versatile protection to leaves without compromising photosynthesis (*15*). Four anthocyanins were found in red leaf lettuce in this study (**Figure 2B** and **Table 2**). Peaks 1, 3, and 4 were identified as cyanidin 3-glucoside, cyanidin 3-(6″-malonoyl)-glucoside, and cyanidin 3-(6″-acetoyl)glucoside, respectively, by comparing their retention times and MS data to standards and published data (*5*, *14*). Peak 2 had the same molecular weight and aglycone (cyanidin) as peak 3 but a much shorter retention time (32.3 vs 42.5 min). Similar to that found in blackberry (*5*), this anthocyanin was tentatively identified as cyanidin 3-(3″-malonoyl)glucoside.

Table 1. MW of Common Anthocyanidins, Sugars, and Acylated Groups Found in Anthocyanins

compound	MW	MW-H_2O
anthocyanidin		
pelargonidin	271	
cyanidin	287	
peonidin	301	
petunidin	303	
delphinidin	317	
malvidin	331	
monosaccharide		
pentose		
arabinose	150	132
xylose	150	132
rhamnose	164	146
hexose		
glucose	180	162
galactose	180	162
disaccharide		
sambubiose (2-*O*-*β*-D-xylosyl-D-glucose)	312	294
lathyrose (2-*O*-*β*-D-xylosyl-D-galactose)	312	294
rutinose (6-*O*-α-L-rhamnosyl-D-glucose)	326	308
sophorose (2-*O*-*β*-D-glucosyl-D-glucose)	342	324
laminaribiose (3-*O*-*β*-D-glucosyl-D-glucose)	342	324
gentiobiose (6-*O*-*β*-D-glucosyl-D-glucose)	342	324
acylated group		
aliphatic acid		
acetic acid	60	42
propionic acid	74	56
oxalic acid	90	72
malonic acid	104	86
succinic acid	118	100
malic acid	134	116
aromatic acid		
p-hydroxybenzoic acid	138	120
p-coumaric acid	164	146
caffeic acid	180	162
ferulic acid	194	176
sinapic acid	224	206

Six Common Anthocyanidins



Anthocyanidin	R1	R2	R3	MW
Pelargonidin	H	OH	H	271
Cyanidin	OH	OH	H	287
Delphinidin	OH	OH	OH	303
Peonidin	OMe	OH	H	301
Petunidin	OMe	OH	OH	317
Malvidin	OMe	OH	OMe	331

Common Mono- and Disaccharides

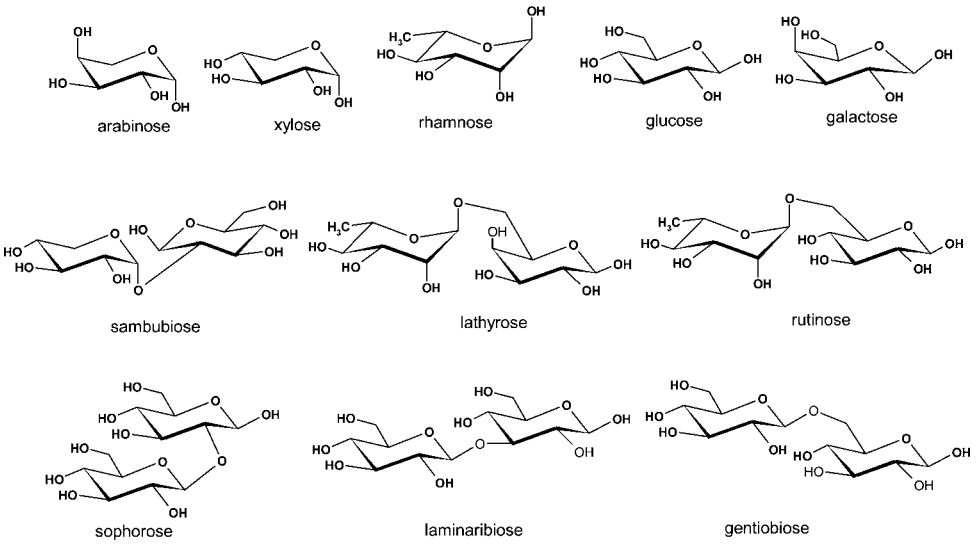


Figure 1. Chemical structures and molecular weights (MW) of six common anthocyanidins and mono- and disaccharides.

Red Onion. Red onion was shown to contain 10 anthocyanins (**Figure 2C** and **Table 2**); seven of them were identified from MS data, and retention times were compared with standards and published data. Their identities were as follows: peak 2, cyanidin 3-glucoside; peak 3, cyanidin 3-laminaribioside; peak 4, cyanidin 3-(3″-malonoyl)glucoside; peak 5, peonidin 3-glucoside; peak 7, cyanidin 3-(6″-malonoyl)glucoside; peak 8, cyanidin 3-(6″-malonoyl)laminaribioside; and peak 9, peonidin 3-(malonoyl)glucoside (*16*). Three other anthocyanins, corresponding to peaks 1, 6, and 10, were found in red onion for the first time. The MS data of peak 1 ($[M]^+$ *m*/*z* 697, MS/MS *m*/*z* 535/449/287) indicated that this anthocyanin contained one cyanidin, two hexose, and one malonylated group. Combining the fact that glucose is the only hexose found in red onion and the MS/MS pattern previously discussed (*5*, *11*), this anthocyanin was tentatively identified as cyanidin 3-(malonoyl)-glucoside-5-glucoside. Peak 6 had the same MS data as cyanidin 3-(6″-acetoyl)glucoside ($[M]^+$ *m*/*z* 491, MS/MS *m*/*z* 287) but a much shorter retention time (*5*). Considering that the acylated substitution at the 3-position of sugar that was found in red onion had a shorter retention time than that with same acylated group at the 6-position [cyanidin 3-(3″-malonoyl)glucoside (peak 4) vs cyanidin 3-(6″-malonoyl)laminaribioside (peak 7)], this anthocyanin was tentatively identified as cyanidin 3-(3″-acetoyl)glucoside. MS data of peak 10 indicated that this anthocyanin had a molecular weight of 577 ($[M]^+$) and a glucose plus at least two acylated group(s) (MS/MS *m*/*z* 491/287). Fragment ion *m*/*z* 491 had a $[M]^+ - 86$ peak. On the basis of mass spectrometric regularities discussed previously (*5*), this anthocyanin most likely contained one malonoyl group; thus, the other acylated group must be an acetoyl group. The retention time (48.8 min) also indicated that this anthocyanin was a highly acylated anthocyanin, which we identified as cyanidin 3-(malonoyl)(acetoyl)glucoside. Anthocyanins with unusual aglycone or sugar connections such as 4′-glucosidation (*18*) were not found in red onion under the conditions used.

Black Bean. Nine anthocyanins were detected (**Figure 2D** and **Table 2**). By comparing the MS data and retention times with standards (*19*), three major anthocyanins were found to be identical with what was found previously: peak 4, delphinidin 3-glucoside; peak 7, petunidin 3-glucoside; and peak 9, malvidin 3-glucoside. Six other anthocyanins were found in the black bean for the first time. Peaks 1, 2, and 5 shared the same MS pattern, and all contained an anthocyanidin plus two hexoses and were identified as the 3,5-diglucosides of delphinidin (peak 1), petunidin (peak 2), and malvidin (peak 5) (*5*, *11*). Peak 3

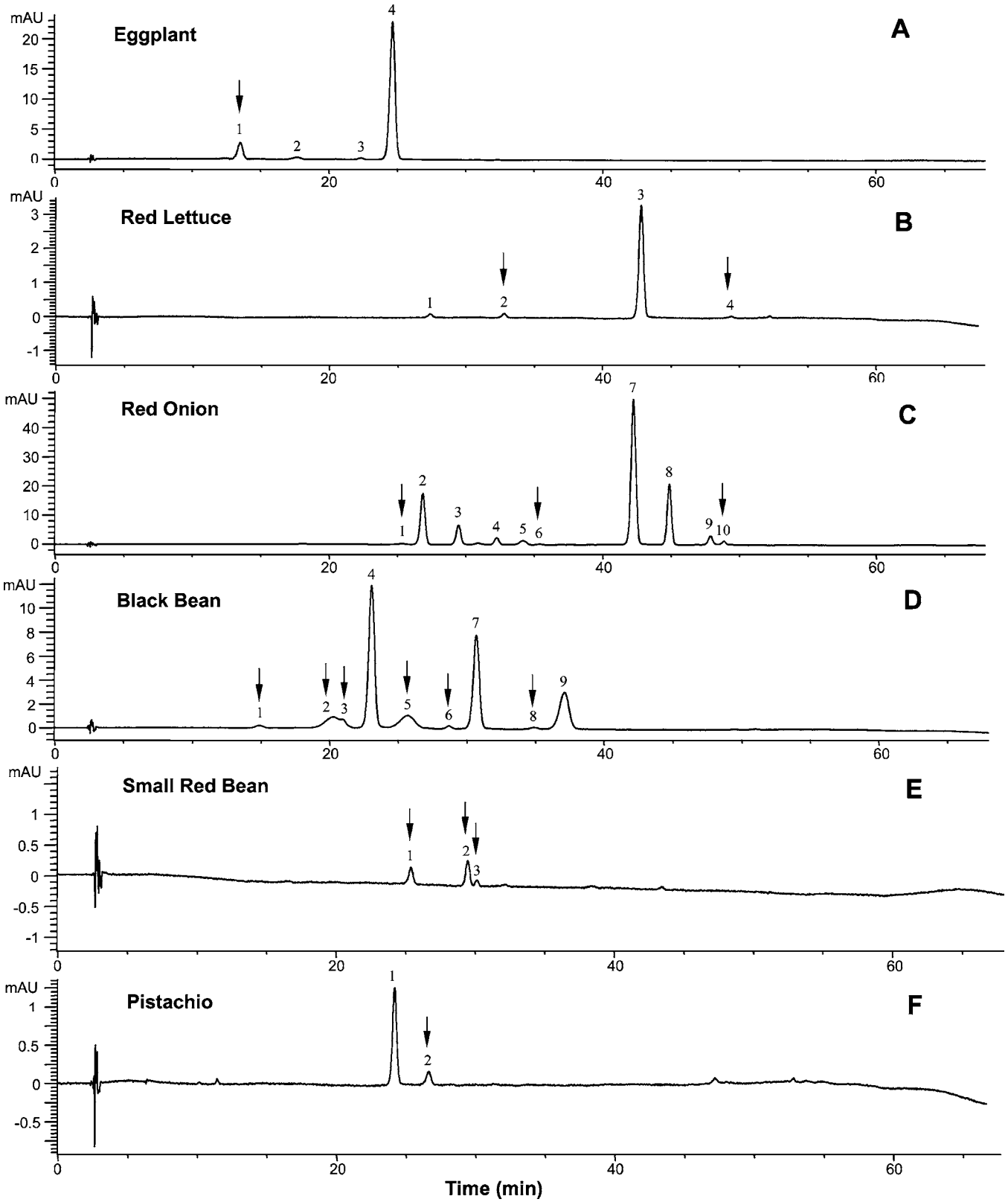


Figure 2. Reverse-phase HPLC chromatograms of anthocyanin profiles of eggplant (**A**), red leaf lettuce (**B**), red onion (**C**), black bean (**D**), small red bean (**E**), and pistachio (**F**). Elution gradient I was used to separate anthocyanins. Refer to **Table 2** for the identification of each numbered peak. Anthocyanin peaks detected for the first time in the given food were highlighted with arrows.

had the same MS data as peak 4 but a shorter retention time and, thus, was identified as delphinidin 3-galactoside (*5*). Similarly, peaks 6 and 8 were identified as petunidin 3-galactoside and malvidin 3-galactoside, respectively. Notably, black bean is one of the very few foods, which contained diverse anthocyanidins but no cyanidin.

Small Red Bean. To our knowledge, no published data of anthocyanins in the small red bean are available. Three anthocyanins were detected at low concentrations (**Figure 2E** and **Table 2**). Peaks 1 and 3 were identified as cyanidin 3-glucoside and pelargonidin 3-glucoside. Peak 2 was shown to have a pelargonidin and a sambubiose from its MS data ($[M]^+$

Table 2. Identification of Anthocyanins in Selected Vegetables and Pistachios (Gradient I)

peak no.	t_R (min)	$[M]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	anthocyanin
			eggplant	
1[a]	**13.5**	**773**	**611/465/303**	**delphinidin 3-rutinoside-5-galactoside**
2	17.6	773	611/465/303	delphinidin 3-rutinoside-5-glucoside
3	22.3	465	303	delphinidin 3-glucoside
4[b]	24.7	611	465/303	delphinidin 3-rutinoside
			red leaf lettuce	
1	26.8	449	287	cyanidin 3-glucoside
2[a]	**32.1**	**535**	**287**	**cyanidin 3-(3′′-malonoyl)glucoside**
3[b]	42	535	449/287	cyanidin 3-(6′′-malonoyl)glucoside
4[a]	**49.1**	**491**	**287**	**cyanidin 3-(6′′-acetoyl)glucoside**
			red onion	
1[a]	**26.2**	**697**	**535/449/287**	**cyanidin 3-(malonoyl)-glucoside-5-glucoside**
2	26.7	449	287	cyanidin 3-glucoside
3	29.3	611	287	cyanidin 3-laminaribioside
4	31.9	535	287	cyanidin 3-(3′′-malonoylglucoside)
5	34.1	463	301	peonidin 3-glucoside
6[a]	**35.1**	**491**	**287**	**cyanidin 3-(3′′-acetoyl)glucoside**
7[b]	41.7	535	287	cyanidin 3-(6′′-malonoylglucoside)
8	44.6	697	287	cyanidin 3-(6′′-malonoyl-laminaribioside)
9	47.8	549	505/301	peonidin 3-(malonoyl)glucoside
10[a]	**48.8**	**577**	**491/287**	**cyanidin 3-(malonoyl)(acetoyl)glucoside**
			black bean	
1[a]	**14.9**	**627**	**465/303**	**delphinidin 3,5-diglucoside**
2[a]	**20.2**	**641**	**479/317**	**petunidin 3,5-diglucoside**
3[a]	**21.0**	**465**	**303**	**delphinidin 3-galactoside**
4[b]	23.1	465	303	delphinidin 3-glucoside
5[a]	**25.7**	**655**	**493/331**	**malvidin 3,5-diglucoside**
6[a]	**28.7**	**479**	**317**	**petunidin 3-galactoside**
7[b]	30.7	479	317	petunidin 3-glucoside
8[a]	**34.7**	**493**	**331**	**malvidin 3-galactoside**
9[b]	37.1	493	331	malvidin 3-glucoside
			small red bean	
1[a]	**26.5**	**449**	**287**	**cyanidin 3-glucoside**
2[a,b]	**31.5**	**565**	**271**	**pelargonidin 3-sambubioside**
3[a]	**31.8**	**433**	**271**	**pelargonidin 3-glucoside**
			pistachio	
1[b]	24.6	449	287	cyanidin 3-galactoside
2[a]	**27.1**	**449**	**287**	**cyanidin 3-glucoside**

[a] Anthocyanins identified for the first time (bold). [b] Anthocyanins underlined are the predominant anthocyanins in the food.

m/*z* 565, MS/MS *m*/*z* 271, $[M - 294]^+$). The HPLC profile of peaks 2 and 3 was very similar to that of cyanidin 3-sambubioside and cyanidin 3-glucoside in elderberry under similar experimental conditions (*8*), suggesting that peak 2 was pelargonidin 3-sambubioside.

Pistachio. One anthocyanin, cyanidin 3-galactoside, was found in pistachio nuts in an earlier paper (*20*). However, two anthocyanins were detected from pistachio in this study (cyanidin 3-galactoside and cyanidin 3-glucoside) (**Figure 2F** and **Table 2**).

Group 2: Foods Containing Highly Conjugated Anthocyanins. Two vegetables, red cabbage and red radish, were assigned to this group. Anthocyanins in each vegetable shared very similar structural patterns, namely, various acylated group(s) conjugated with a specific anthocyanidin glycoside. The molecular weights of these anthocyanins varied from several hundred to more than a thousand. Acylated anthocyanins are considered to be better candidates than nonacylated anthocyanin for food colorants because of their increased stability (*6*, *21*).

Red Cabbage. A total of 23 anthocyanins were detected in red cabbage (**Figure 3** and **Table 3**). According to published data (*11*, *22*−*27*), only cyanidin was found in red cabbage. The major acylated anthocyanins in red cabbage were cyanidin 3-diglucoside-5-glucoside derivatives with various acylated groups connected to the diglucoside. Our data supported this observation. MS/MS of most of the high molecular weight acylated anthocyanins gave two major fragment peaks, *m*/*z* 449, a cyanidin 5-glucoside residue, and *m*/*z* 611 + acylated group, a cyanidin 3-(acyl)diglucoside residue. Consequently, the peak identification of the unknown acylated anthocyanins was made largely based on this structural pattern.

Peaks 1 and 2 are two known anthocyanins in red cabbage: cyanidin 3-diglucoside-5-glucoside and cyanidin 3,5-diglucoside (*22*). Peak 3 was found in red cabbage for the first time, and its MS data indicated that this anthocyanin had the cyanidin 3-diglucoside-5-glucoside backbone and acylated group(s) ($[M]^+$ *m*/*z* 965, MS/MS *m*/*z* 803, $[M - \text{glucose}]^+$, 449 $[M - \text{diglucose} - 192]^+$, and 287 $[\text{cyanidin}]^+$). The molecular weight of the acylated group(s) residue was 192. By calculating the possible combinations of acylated groups from **Table 1**, this anthocyanin was tentatively identified as cyanidin 3-(*p*-hydroxybenzoyl)-(oxaloyl)diglucoside-5-glucoside. Peaks 4 and 14 had the same MS data ($[M]^+$ *m*/*z* 935, MS/MS *m*/*z* 773, $[M - \text{glucose}]^+$, 449 $[M - \text{diglucose} - 162]^+$, and 287 $[\text{cyanidin}]^+$). Considering the notable difference in retention times between them (18.8 vs 54.1 min), peak 4 must be an anthocyanin with a much higher polarity. Considering the molecular weight, peak 4 was tentatively identified as cyanidin 3-triglucoside-5-glucoside and peak 14 as cyanidin 3-(caffeoyl)diglucoside-5-glucoside. Both were found in red cabbage for the first time. Peaks 5, 12, 15, and 17 had the same molecular weight of 979. Among them, peaks 5 and 12 shared the same MS data ($[M]^+$ *m*/*z* 979, MS/MS *m*/*z*

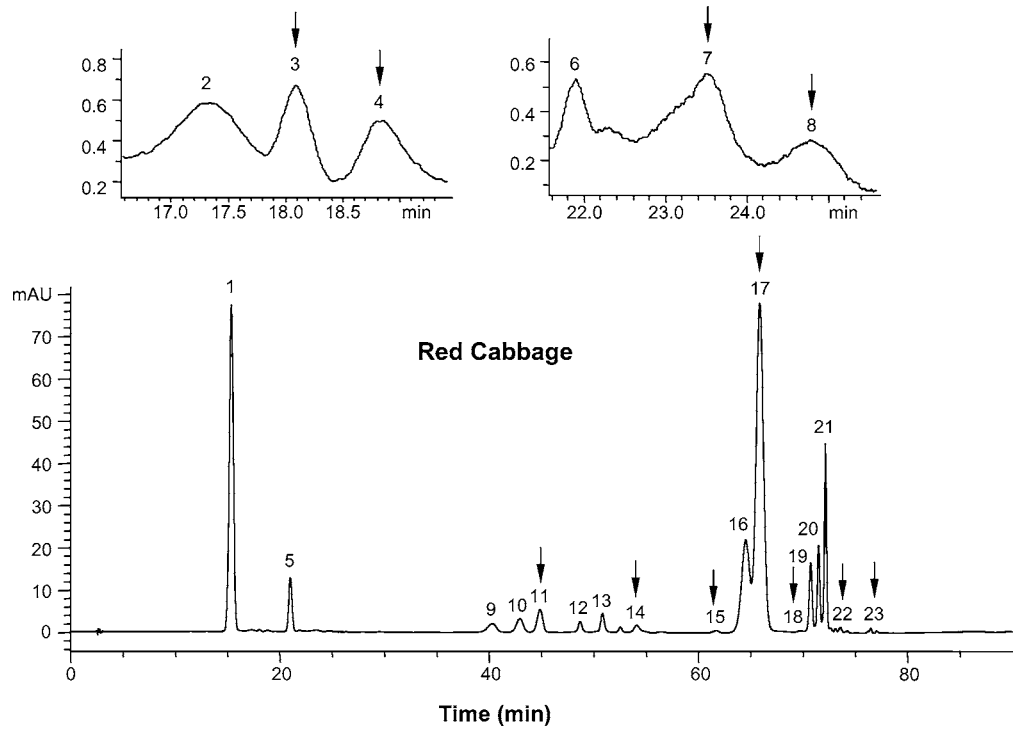


Figure 3. Reverse-phase HPLC chromatograms of anthocyanin profiles of red cabbage detected at 520 nm. Elution gradient II was used to separate the anthocyanins. Refer to **Table 3** for the identification of each numbered peak. Anthocyanin peaks detected for the first time in red cabbage were highlighted with arrows.

Table 3. Identification of Anthocyanins in Red Cabbage (Gradient II)

peak no.	t_R (min)	$[M]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	anthocyanin
1[b]	15.4	773	611/449/287	cyanidin 3-diglucoside-5-glucoside
2	17.3	611	449/287	cyanidin 3,5-diglucoside
3[a]	**18.1**	**965**	**803/449/287**	**cyanidin 3-(*p*-hydroxybenzoyl)(oxaloyl)diglucoside-5-glucoside**
4[a]	**18.8**	**935**	**449/287**	**cyanidin 3-(caffeoyl)diglucoside-5-glucoside**
5	21.0	979	817/449/287	cyanidin 3-(sinapoyl)diglucoside-5-glucoside
6	21.9	949	787/449/287	cyanidin 3-(feruloyl)diglucoside-5-glucoside
7[a]	**23.6**	**743**	**611/419/287**	**cyanidin 3-diglucoside-5-xyloside**
8[a]	**24.8**	**611**	**287**	**cyanidin 3-diglucoside**
9	40.3	1081	919/449	cyanidin 3-(caffeoyl)(*p*-coumaroyl)diglucoside-5-glucoside
10	42.9	1111	949/703/449	cyanidin 3-(glycopyranosyl-feruloyl)diglucoside-5-glucoside
11[a]	**44.9**	**1141**	**979/817/449**	**cyanidin 3-(glycopyranosyl-sinapoyl)diglucoside-5-glucoside**
12	48.7	979	817/449/287	cyanidin 3-(sinapoyl)diglucoside-5-glucoside
13	50.8	1125	963/449	cyanidin 3-(sinapoyl)(*p*-coumaroyl)diglucoside-5-glucoside
14[a]	**54.1**	**935**	**773/449/287**	**cyanidin 3-(caffeoyl)diglucoside-5-glucoside**
15[a]	**61.7**	**979**	**817/655/449/287**	**cyanidin 3-(sinapoyl)diglucoside-5-glucoside**
16[b]	64.5	919	757/449/287	cyanidin 3-(*p*-coumaroyl)diglucoside-5-glucoside
17[a,b]	**65.9**	**979**	**817/655/449/287**	**cyanidin 3-(sinapoyl)diglucoside-5-glucoside**
18[a]	**69.8**	**817**	**655/449/287**	**cyanidin 3-(sinapoyl)glucoside-5-glucoside**
19	70.7	1125	963/449	cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside
20	71.5	1155	993/899/449	cyanidin 3-(sinapoyl)(feruloyl)diglucoside-5-glucoside
21	72.1	1185	1023/449	cyanidin 3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside
22[a]	**73.6**	**949**	**817/419/287**	**cyanidin 3-(sinapoyl)diglucoside-5-xyloside**
23[a]	**76.5**	**1185**	**817/655**	**cyanidin 3-(sinapoyl)diglucoside-5-(sinapoyl)glucoside**

[a] Anthocyanins identified in red cabbage for the first time (bold). [b] The three anthocyanins underlined are the predominant anthocyanins in red cabbage.

817, [M − glucose]$^+$, 449 [M − diglucose − 206]$^+$, and 287 [cyanidin]$^+$). By comparing with published data (*23*, *24*, *27*), they were identified as isomers of cyanidin 3-(sinapoyl)-diglucoside-5-glucoside. In the MS data from peaks 15 and 17, one more fragment ion *m*/*z* 655 was observed besides the ions mentioned earlier. Although these two peaks were still identified as isomers of cyanidin 3-(sinapoyl)diglucoside-5-glucoside, their structures ought to be much different from those of peaks 5 and 12 in terms of the position where the sinapoyl group is connected to the sugar. However, the exact structures of these anthocyanins cannot be determined due to the limited information available. Peak 6 also followed the structural pattern discussed above ($[M]^+$ *m*/*z* 949, MS/MS *m*/*z* 787, [M − glucose]$^+$, 449 [M − diglucose − 176]$^+$, and 287 [cyanidin]$^+$); thus, it was identified as cyanidin 3-(feruloyl)diglucoside-5-glucoside based upon the molecular weight difference of 30 between ferulic and sinapic acids and information available in the literature (*24*, *27*). Peak 7 had a molecular weight of 743 ($[M]^+$ *m*/*z*) and two fragment ions *m*/*z* 611 [M − 132]$^+$ and *m*/*z* 419 [M − 324]$^+$, which clearly indicated that this anthocyanin had one pentose and one dihexose in different positions. This is the first time that a pentose-substituted

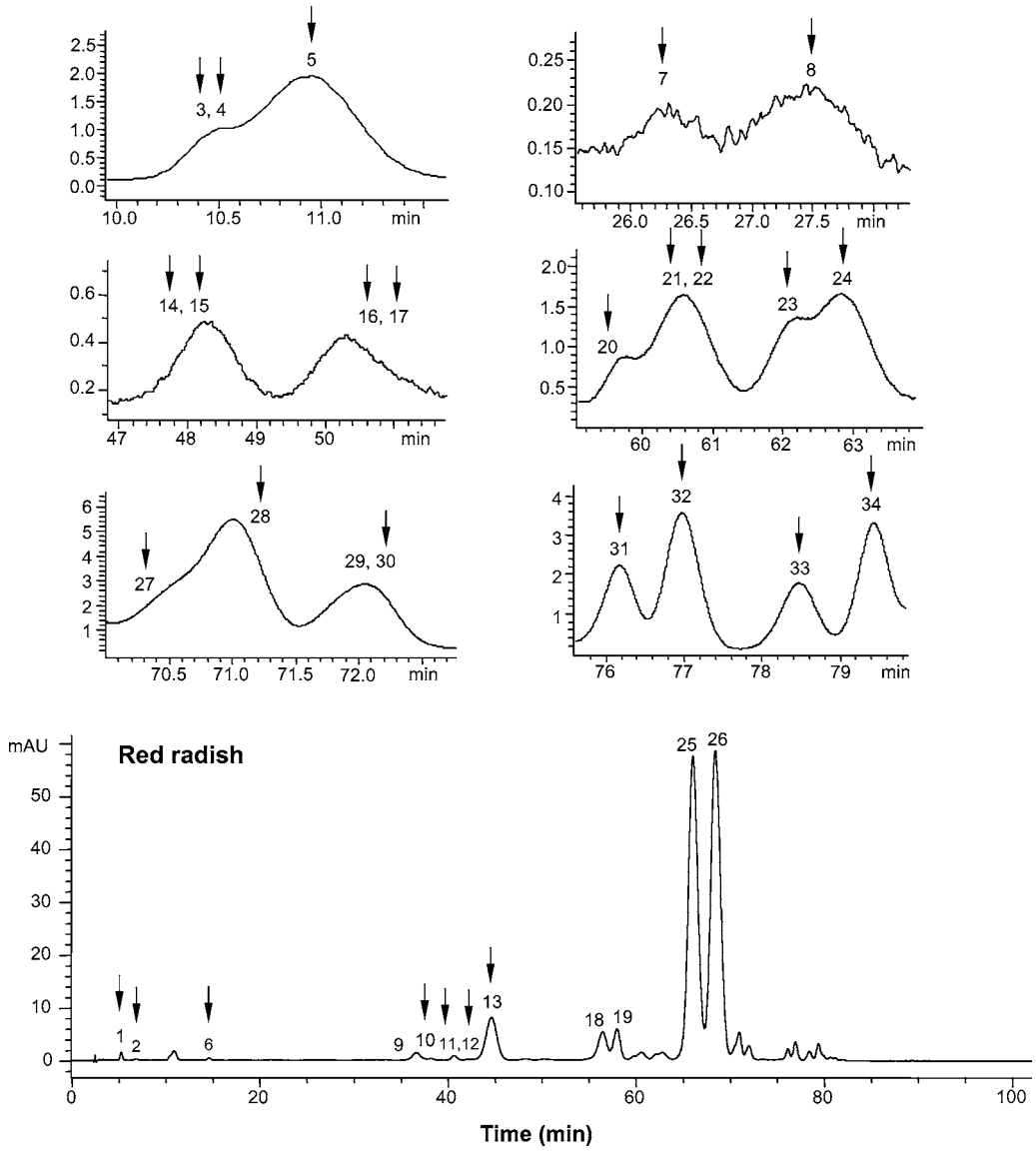


Figure 4. Reverse-phase HPLC chromatograms of anthocyanin profiles of red radish detected at 520 nm. Elution gradient III was used to separate the anthocyanins. Refer to **Table 4** for the identification of each numbered peak. Anthocyanin peaks detected for the first time in red radish were highlighted with arrows.

anthocyanin has been found in red cabbage. Considering that peak 7 had a much longer retention time (23.6 vs 15.4 min) than peak 1 (cyanidin 3-diglucoside-5-glucoside), this anthocyanin was identified as cyanidin 3-diglucoside-5-xyloside because with the similar substitution pattern, the retention time of arabinoside is slightly longer than that of the glucoside, whereas the xyloside has a much longer retention time (*5*). Peak 8 was identified as cyanidin 3-diglucoside based on its MS pattern ($[M]^+$ *m*/*z* 611, MS/MS *m*/*z* 287 $[cyanidin]^+$) (*11*, *5*). MS data of peak 9 indicated a highly acylated derivative of cyanidin 3-diglucoside-5-glucoside ($[M]^+$ *m*/*z* 1081, MS/MS *m*/*z* 919, $[M - glucose]^+$, 449 $[M - diglucose - 308]^+$, and 287 $[cyanidin]^+$), where the 308 fragment accounted for more than one acylated group based on the molecular weights of all possible aliphatic and aromatic acids appearing in anthocyanins known thus far (**Table 1**). It was identified as cyanidin 3-(caffeoyl)(*p*-coumaroyl)diglucoside-5-glucoside by comparison to published data (*24*). Peaks 10, 11, 13, and 19−21 shared very similar structural patterns to peak 9 (**Table 3**); all had molecular weights larger than 1000, which meant they had more than one acylated group in their cyanidin 3-diglucoside-5-glucoside structure. By comparing to published data (*24*, *27*) and calculating the possible combinations, these six anthocyanins were tentatively identified as the anthocyanins presented in **Table 3**. Peak 16 had similar MS fragmentation patterns ($[M]^+$ *m*/*z* 919, MS/MS *m*/*z* 757, $[M - glucose]^+$, 449 $[M - diglucose - 146]^+$, and 287 $[cyanidin]^+$) as peaks 5 or 6 and, thus, was identified as cyanidin 3-(*p*-coumaroyl)diglucoside-5-glucoside. MS data of peak 18 showed a different fragmentation pattern than all other acylated anthocyanins. This is the first time that this anthocyanin has been observed in red cabbage. It contained only one glucose at the 3-position and one glucose at the 5-position ($[M]^+$ *m*/*z* 817, MS/MS *m*/*z* 655, $[M - glucose]^+$, 449 $[M - glucose - 206]^+$, and 287 $[cyanidin]^+$), suggesting that this anthocyanin was cyanidin 3-(sinapoyl)glucoside-5-glucoside. Peak 22 had the same molecular weight as peak 6 and showed a similar MS pattern but contained a pentose rather than glucose at the 5-position based upon its MS data ($[M]^+$ *m*/*z* 949, MS/MS *m*/*z* 817, $[M - pentose(132)]^+$, 419 $[M - diglucose - 206]^+$, and 287 $[cyanidin]^+$). Considering that a xylose-substituted anthocyanin was found in red cabbage as discussed above (peak 7), this anthocyanin was identified as

Table 4. Identification of Anthocyanins in Red Radish (Gradient III)

peak no.	t_R (min)	$[M]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	anthocyanin
1[a]	**5.3**	**757**	**595/433/271**	**pelargonidin 3-diglucoside-5-glucoside**
2[a]	**6.6**	**595**	**433/271**	**pelargonidin 3-glucoside-5-glucoside**
3[a]	**10.0**	**919**	**757/579/433/271**	**pelargonidin 3-(acyl)rutinoside-5-glucoside**[c]
4[a]	**10.1**	**579**	**433/271**	**pelargonidin 3-rutinoside**
5[a]	**10.6**	**843**	**595/519/271**	**pelargonidin 3-diglucoside-5-(malonoyl)glucoside**
6[a]	**14.3**	**609**	**271**	**pelargonidin 3-(feruloyl)glucoside**
7[a]	**26.0**	**827**	**579/519/271**	**pelargonidin 3-rutinoside-5-(malonoyl)glucoside**
8[a]	**27.3**	**595**	**433/287**	**cyanidin 3-(glucosyl)rhamnoside**
9	**36.3**	**919**	**757/433/271**	**pelargonidin 3-(caffeoyl)glucoside-5-glucoside**
10[a]	37.8	1019	771/519	pelargonidin 3-(feruloyl)diglucoside-5-(malonoyl)glucoside
11[a]	**40.2**	**989**	**741/519**	**pelargonidin 3-(p-coumaroyl)diglucoside-5-(malonoyl)glucoside**
12[a]	**40.5**	**905**	**743/433/271**	**pelargonidin 3-(acyl)diglucoside-5-glucoside**[c]
13[a]	**43.9**	**1005**	**757/519/433**	**pelargonidin 3-(caffeoyl)diglucoside-5-(malonoyl)glucoside**
14[a]	**47.6**	**771**	**271**	**pelargonidin 3-(feruloyl)diglucoside**
15[a]	**48.9**	**1125**	**877/519**	**pelargonidin 3-(p-hydroxybenzoyl)(caffeoyl)diglucoside-5-(malonoyl)glucoside**
16[a]	**49.4**	**1019**	**771/519**	**pelargonidin 3-(feruloyl)diglucoside-5-(malonoyl)glucoside**
17[a]	**49.9**	**857**	**609/519/271**	**pelargonidin 3-(feruloyl)glucoside-5-(malonly)glucoside**
18	55.6	903	741/433/271	pelargonidin 3-(p-coumaroyl)diglucoside-5-glucoside
19	57.5	933	771/433/271	pelargonidin 3-(feruloyl)diglucoside-5-glucoside
20[a]	**60.0**	**989**	**741/519/271**	**pelargonidin 3-(p-coumaroyl)diglucoside-5-(malonoyl)glucoside**
21[a]	**61.3**	**1035**	**787/535/287**	**cyanidin 3-(feruloyl)diglucoside-5-(malonoyl)glucoside**
22[a]	**61.6**	**521**	**433/271**	**pelargonidin 3-(acyl)glucoside**[c]
23[a]	**62.2**	**771**	**271**	**pelargonidin 3-(feruloyl)diglucoside**
24[a]	**62.3**	**1019**	**771/519**	**pelargonidin 3-(feruloyl)diglucoside-5-(malonoyl)glucoside**
25[b]	65.1	989	741/519	pelargonidin 3-(*p*-coumaroyl)diglucoside-5-(malonoyl)glucoside
26[b]	67.9	1019	975/771/519/433	pelargonidin 3-(feruloyl)diglucoside-5-(malonoyl)glucoside
27[a]	**70.1**	**1151**	**903/519/433**	**pelargonidin 3-(p-coumaroyl)(caffeoyl)diglucoside-5-(malonoyl)glucoside**
28[a]	**70.7**	**1181**	**933/519**	**pelargonidin 3-(feruloyl)(caffeoyl)diglucoside-5-(malonoyl)glucoside**
29	71.3	1109	861/519	pelargonidin 3-(*p*-hydroxybenzoyl)(*p*-coumaroyl)diglucoside-5-(malonoyl)glucoside
30[a]	**71.7**	**1139**	**891/519**	**pelargonidin 3-(p-hydroxybenzoyl)(feruloyl)diglucoside-5-(malonoyl)glucoside**
31[a]	**75.9**	**771**	**271**	**pelargonidin 3-(feruloyl)diglucoside**
32[a]	**76.7**	**1195**	**947/519**	**pelargonidin 3-(feruloyl)(feruloyl)diglucoside-5-(malonoyl)glucoside**
33[a]	**77.8**	**1135**	**887/519**	**pelargonidin 3-(p-coumaroyl)(p-coumaroyl)diglucoside-5-(malonoyl)glucoside**
34[a]	**79.2**	**1165**	**917/519**	**pelargonidin 3-(p-coumaroyl)(feruloyl)diglucoside-5-(malonoyl)glucoside**

[a] Anthocyanins identified in red radish for the first time (bold). [b] The two anthocyanins underlined are the predominant anthocyanins in red radish. [c] Sufficient information was not available to identify the particular acyl group.

cyanidin 3-(sinapoyl)diglucoside-5-xyloside. Peak 23 had the same molecular weight as peak 21 but showed a different MS pattern from that of peak 21. Its MS data ($[M]^+$ *m*/*z* 1185, MS/MS *m*/*z* 817, [M − glucose − 206]$^+$, 655 [M − diglucose − 206]$^+$, and 287 [cyanidin]$^+$) revealed that even though this anthocyanin had the same backbone cyanidin 3-diglucoside-5-glucoside, its two sinapoyl groups were connected to sugar moieties at different positions on the flavylium cation. This unique structural pattern was found in red cabbage for the first time. Peak 23 was thus tentatively identified as cyanidin 3-(sinapoyl)diglucoside-5-(sinapoyl)glucoside. Among all anthocyanins detected in red cabbage, at least 11 of them are newly identified in this food (**Table 3**).

Red Radish. Pelargonidin was found to be the major anthocyanidin in red radish with trace amounts of cyanidin (*28*, *29*). A total of 34 anthocyanins were detected in red radish by mass spectrometry (**Figure 4** and **Table 4**), which is considerably more than has been previously reported. MS data indicated that red radish contained acylated pelargonidin 3-diglucoside-5-glucoside derivatives as the major anthocyanins and acylated pelargonidin 3-rutinoside-5-glucoside as well as other anthocyanins as minor anthocyanins.

Peaks 1, 2, and 4 were identified as pelargonidin 3-diglucoside-5-glucoside, pelargonidin 3,5-diglucoside, and pelargonidin 3-rutinoside (*5*, *11*). The mass spectra of peak 3 gave a molecular ion $[M]^+$ *m*/*z* 919 and four fragment ions (*m*/*z* 757, 579, 433, and 271) from MS/MS. The four fragment ions corresponded to [M − glucose]$^+$ (*m*/*z* 757), pelargonidin rutinoside ion (*m*/*z* 579), pelargonidin glucoside ion (*m*/*z* 433), and pelargonidin ion (*m*/*z* 271). This anthocyanin was composed of pelargonidin, one rutinose, one glucose, and unknown acylated group(s). The molecular weight(s) of the unknown acylated group(s) was 196 [(919 − 271 − 162 − 308) + 18], where 919, 271, 162, 308, and 18 were the molecular weights of peak 3, pelargonidin, glucose residue, rutinose residue, and water. Unfortunately, we were not able to determine the unknown acylated group(s) based on current information. Peak 5 was shown to be an anthocyanin with a pelargonidin 3-diglucoside-5-glucoside backbone, but the acylated group connected to glucose at the 5-position based upon its MS data ($[M]^+$ *m*/*z* 843, MS/MS *m*/*z* 595, [M − glucose − 86]$^+$, 519 [M − diglucose]$^+$, and 271 [pelargonidin]$^+$) was indicative of a malonoyl group; thus, peak 5 was tentatively identified as pelargonidin 3-diglucoside-5-(malonoyl)glucoside. Peak 6 had only a molecular ion ($[M]^+$ *m*/*z* 609) and one fragment ion at *m*/*z* 271, which suggested that this anthocyanin had only one substitution at the 3-position (*5*, *11*) and was identified as pelargonidin 3-(feruloyl)glucoside. MS spectra of peak 7 were similar to that of peak 5, but rutinose was found to replace the diglucose at the 3-position ($[M]^+$ *m*/*z* 827, MS/MS *m*/*z* 579, [M − glucose − 86]$^+$, 519 [M − rutinose]$^+$, and 271 [pelargonidin]$^+$). Thus, this anthocyanin was identified as pelargonidin 3-rutinoside-5-(malonoyl)glucoside. Peak 8 had the same molecular weight as cyanidin 3-rutinoside, a widely distributed anthocyanin (*5*). However, an unusual fragment ion of *m*/*z* 433 along with the cyanidin ion *m*/*z* 287 was observed in its MS. In the usual rutinoside, glucose was directly linked to cyanidin, rhamnose was linked to glucose, and only the fragment ion of *m*/*z* 449 ([M − 146]$^+$) was observed in addition to the cyanidin ion (*5*). However, from the MS data, it was

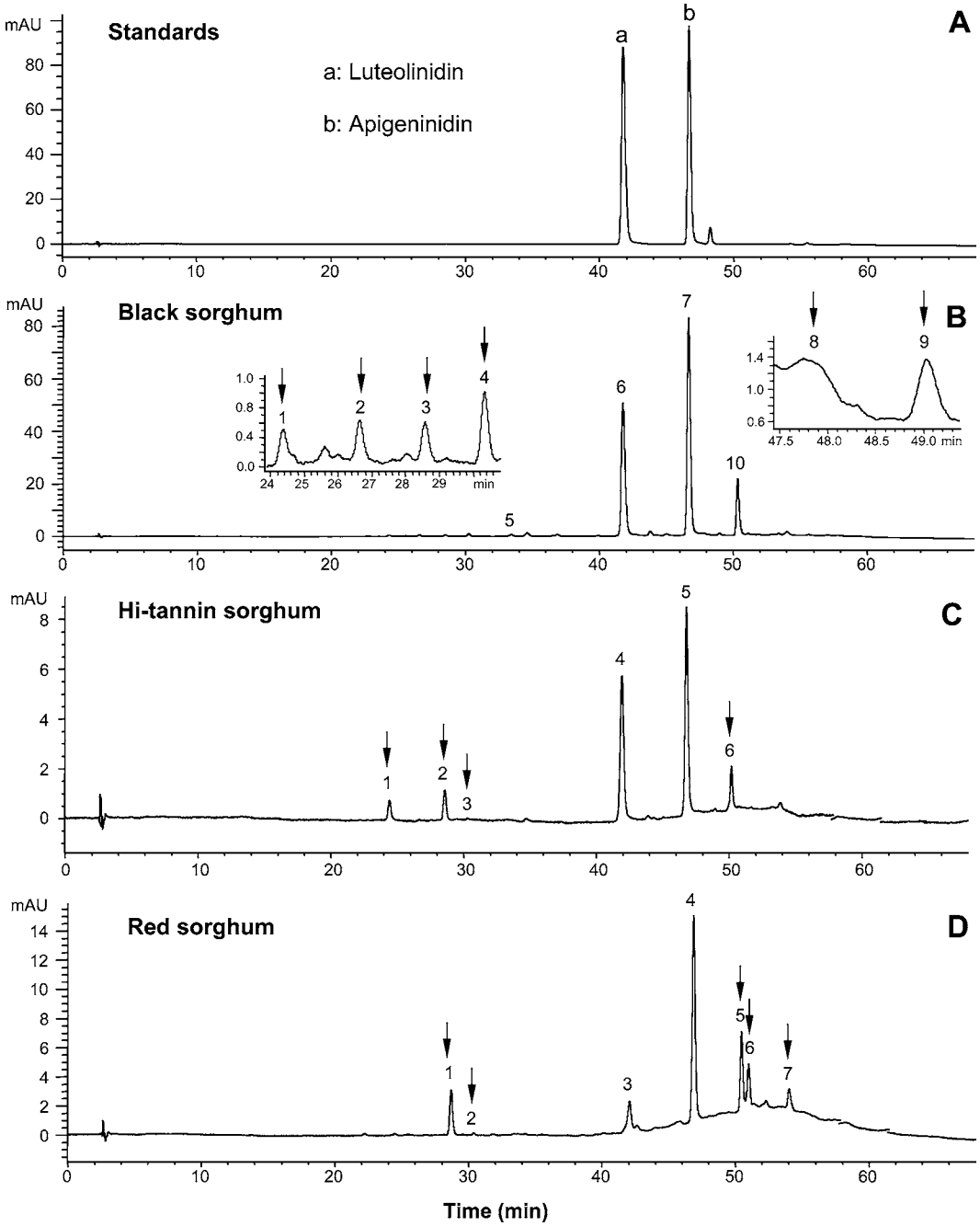


Figure 5. Reverse-phase HPLC chromatograms of anthocyanin profiles of three varieties of sorghum (black sorghum, hi-tannin sorghum, and red sorghum) detected at 480 nm. Elution gradient I was used to separate anthocyanins. Refer to **Table 5** for the identification of each numbered peak. Anthocyanin peaks detected for the first time in each variety of sorghums were highlighted with arrows.

reasonable to identify the anthocyanin in peak 8 as cyanidin directly linked to rhamnose with the rhamnose linked to glucose. This is an unusual structural pattern that, to our knowledge, has never been observed before in anthocyanins. Peaks 9, 12, 18, and 19 shared similar structural patterns. The MS/MS spectra of all four of these anthocyanins indicated a pelargonidin ion (*m*/*z* 271), a pelargonidin glucoside ion (*m*/*z* 433), and an acylated pelargonidin diglucoside ion (*m*/*z* 271 + 324 + acylated group), indicating that they were pelargonidin 3-(acyl)diglucoside-5-glucosides. By comparing to published data and calculating the possible molecular weights of the acylated groups, three of them were identified as pelargonidin 3-(caffeoyl)diglucoside-5-glucoside (peak 9), pelargonidin 3-(*p*-coumaroyl)diglucoside-5-glucoside (peak 18), and pelargonidin 3-(feruloyl)diglucoside-5-glucoside. The acylated group of peak 12 was not identified due to its unusual molecular weight. There were 16 anthocyanins, including peaks 10, 11, 13, 15, 16, 20, 24−30, and 32−34, which shared a similar structural pattern. They were characterized as containing the fragments of the pelargonidin ion (*m*/*z* 271), malonoyl pelargonidin glucoside ion (*m*/*z* 519), and an acylated pelargonidin diglucoside ion (*m*/*z* 271 + 324 + acylated group). Thus, they are all pelargonidin 3-diglucoside-5-(malonoyl)glucosides derivatives with various acylated group(s) connected to the diglucoside at the 3-position. Similar to the previous discussion above, they were identified as the anthocyanins presented in **Table 4**. Peaks 14, 23, and 31 had the same molecular weight and MS data. They were tentatively identified as isomers of pelargonidin 3-(feruloyl)diglucoside from their MS data ($[M]^+$ *m*/*z* 771, MS/MS *m*/*z* 271 [pelargonidin]$^+$). Peak 17 had a similar fragmentation pattern to peak

10, but it has glucose instead of the diglucose connected to the 3-position ($[M]^+$ *m/z* 857, MS/MS *m/z* 609, $[M - \text{glucose} - 86]^+$, 519 $[M - \text{glucose} - 176]^+$, and 271 $[\text{pelargonidin}]^+$) and was identified as pelargonidin 3-(feruloyl)glucoside-5-(malonoyl)glucoside. Peak 21 also had a similar mass spectral pattern as peak 10 except for the difference in the aglycone ($[M]^+$ *m/z* 1035, MS/MS *m/z* 787, $[M - \text{glucose} - 86]^+$, 535 $[M - \text{diglucose} - 176]^+$, *m/z* 287 $[\text{cyanidin}]^+$) and thus was identified as cyanidin 3-(feruloyl)diglucoside-5-(malonoyl)-glucoside. Peak 22 had an unusual fragmentation pattern with a molecular ion of *m/z* 521 and two fragment ions of *m/z* 433 and 271, which indicates that this anthocyanin contained pelargonidin, one glucose, and an acylated group(s). However, the acylated group(s) could not be identified due to limited information. Of all 34 anthocyanins detected in red radish, at least 28 were found for the first time (**Table 4**).

Group 3: Identification of 3-Deoxyanthocyanins and Their Derivatives in Sorghums. Grains and cereals are other important sources of anthocyanins (*1*, *30*). Sorghums were found to contain 3-deoxyanthocyanins and their derivatives as major anthocyanins. This group of anthocyanins is uncommon and specifically distributed in certain plants; sorghum is considered to be the only dietary plant source.

3-Deoxyanthocyanins in sorghum have been known to play an important role in plant physiology as phytoalexins and represent a significant defense mechanism (*31*−*33*). With increasing interest in the possible health effects of sorghum, 3-deoxyanthocyanidins may be active components that exert certain health effects through antioxidant and/or other mechanisms (*34*). The lack of the 3-hydroxyl group makes the 3-deoxyanthocyanidins very different from the other anthocyanins. Unlike other anthocyanidins with a 3-hydroxyl group, they can occur naturally in the aglycone form. They were also reported to be very stable in acidic solutions as compared to other anthocyanidins (*35*). All of these properties may confer unique biological effects.

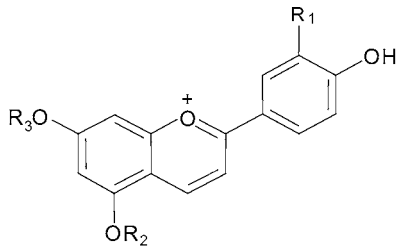
Several advanced methods have been used for the identification and characterization of 3-deoxyanthocyanidins and their derivatives in sorghum, which included HPLC/PDA, plasma desorption mass spectrometry (*36*), and matrix-assisted laser desorption ionization mass spectrometry (*37*). 3-Deoxyanthocyanidins and methylated 3-deoxyanthocyanidins have been found to be the main anthocyanins in sorghum; however, complete information about the anthocyanin composition in sorghum is lacking (*38*). Using reverse-phase HPLC/PDA in tandem with ESI-MS/MS, the deoxyanthocyanidins in three different varieties of sorghum have been identified and characterized.

Black Sorghum. Ten anthocyanins, including 3-deoxyanthocyanins, their derivatives, and one other anthocyanin, were identified in black sorghum (**Figure 5B** and **Table 5**). Peak 1 had a molecular weight of 433 ($[M]^+$, *m/z*) and a fragment ion *m/z* 271 from MS/MS, which indicated that it was a glucoside of luteolinidin and was identified as luteolinidin 5-glucoside (*38*, *39*) (**Figure 6**). Peak 2 was found to be the glucoside of methylated luteolinidin from its MS data ($[M]^+$, *m/z* 447, MS/MS, *m/z* 285). Because 5-methoxyluteolinidin was the only methylated luteolinidin found in sorghum thus far (*38*, *39*), peak 2 was identified as 5-methoxyluteolinidin 7-glucoside). Peak 3 was identified as apigeninidin 5-glucoside by comparing its MS data ($[M]^+$, *m/z* 417, MS/MS, *m/z* 255) with published data (*38*, *39*). MS data of peak 4 indicated that it was the glucoside of methylated apigeninidin ($[M]^+$, *m/z* 431, MS/MS, *m/z* 269). Similar to peak 2, this anthocyanin was identified as 7-methoxyapigeninidin 5-glucoside. Peak 5 had unusual MS data, which indicated a molecular ion of *m/z* 573 and two fragment ions *m/z* 447 and 271 from MS/MS and was most likely an acylated luteolinidin, but its structure was not able to be determined based on the limited information. Peaks 6 and 7 were identified as luteolinidin and apigeninidin, respectively, by comparing their MS data and retention times with those of standards (**Figure 5A**). Peaks 8 and 10 were shown to be methylated luteolinidin and apigeninidin from their MS data and were identified as 5-methoxyluteolinidin and 7-methoxyapigeninidin (*39*, *39*). Peak 9 had the same MS data as peonidin 3-rutinoside, which was found in marionberry (*5*), but its

Table 5. Identification of Anthocyanins in Three Varieties of Sorghum (Gradient I)

peak no.	t_R (min)	$[M]^+$ (*m/z*)	MS/MS (*m/z*)	anthocyanin
		black sorghum		
1[a]	**24.5**	**433**	**271**	**luteolinidin 5-glucoside**
2[a]	**26.6**	**447**	**285**	**5-methoxyluteolinidin 7-glucoside**
3[a]	**28.5**	**417**	**255**	**apigeninidin 5-glucoside**
4[a]	**30.2**	**431**	**269**	**7-methoxyapigeninidin 5-glucoside**
5[a]	**33.6**	**573**	**447/271**	**luteolinidin anchocyanin**
6[b]	41.7	271		luteolinidin
7[b]	46.6	255		apigeninidin
8[a]	**47.3**	**285**		**5-methoxyluteolinidin**
9[a]	**48.9**	**609**	**463/301**	**unknown**
10	**50.5**	**269**		**7-methoxyapigeninidin**
		hi-tannin sorghum		
1[a]	**24.6**	**433**	**271**	**luteolinidin 5-glucoside**
2[a]	**28.6**	**417**	**255**	**apigeninidin 5-glucoside**
3[a]	**30.3**	**431**	**269**	**7-methoxyapigeninidin 5-glucoside**
4[b]	41.8	271		luteolinidin
5[b]	46.7	255		apigeninidin
6[a,b]	**50.6**	**269**		**7-methoxyapigeninidin**
		red sorghum		
1[a]	**28.7**	**417**	**255**	**apigeninidin 5-glucoside**
2[a]	**30.5**	**431**	**269**	**7-methoxyapigeninidin 5-glucoside**
3	42.0	271		luteolinidin
4	46.9	255		apigeninidin
5[a]	**50.7**	**269**		**7-methoxyapigeninidin**
6[a]	**51.1**	**265**		**unknown anthocyanin**
7[a]	**54.1**	**331**		**unknown**

[a] Anthocyanins identified in this sorghum variety for the first time (bold). [b] The anthocyanins underlined are the predominant anthocyanins in the sorghum samples.



1. R_1 = OH, R_2 = Glc, R_3 = H: luteolinidin 5-glucoside (MW = 433)
2. R_1 = OH, R_2 = CH_3, R_3 = Glc: 5-methoxyluteolinidin 5-glucoside (MW = 447)
3. R_1 = H, R_2 = Glc, R_3 = H: apigeninidin 5-glucoside (MW = 417)
4. R_1 = H, R_2 = Glc, R_3 = CH_3: 7-methoxyapigeninidin 5-glucoside (MW = 431)
6. R_1 = OH, R_2 = H, R_3 = H: luteolinidin (MW = 271)
7. R_1 = H, R_2 = H, R_3 = H: apigeninidin (MW = 255)
8. R_1 = OH, R_2 = CH_3, R_3 = H: 5-methoxyluteolinidin 5-glucoside (MW = 285)
10. R_1 = H, R_2 = H, R_3 = CH_3: 5-methoxyapigeninidin (MW = 269)

Figure 6. Chemical structures and molecular weights (MW) of 3-deoxyanthocyanidins and their derivatives in black sorghum.

Table 6. Distribution of Anthocyanins in Selected Vegetables and Pistachio Nuts[a]

	anthocyanidin						sugar moiety							acylated groups							
foods	Dp	Cy	Pt	Pg	Pn	Mv	Glc	Gal	Xyl	Rha	Rut	Sam	Lam	Ac	Cou	Mal	Hyd	Fer	Caf	Sin	Oxa
eggplant	+						+	+			+										
red leaf lettuce		+					+							+		+					
red onion		+			+		+						+	+		+					
black bean	+		+			+	+	+													
small red bean		+		+			+					+									
pistachio		+					+	+													
red cabbage		+					+		+						+		+	+	+	+	+
red radish		+		+			+		+	+	+				+	+	+	+	+		

[a] Abbreviations used in this table were defined as follows. For anthocyanidins: Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pg, pelargonidin; Pn, peonidin; Mv, malvidin. For sugar moieties: Glc, glucose; Gal, galactose; Xyl, xylose; Rha, rhamnose; Rut, rutinose; Sam, sambubiose; Lam, laminaribiose. For acylated groups: Ac, acetoyl; Cou, coumaroyl; Mal, malonoyl; Hyd, *p*-hydroxybenzoyl; Fer, feruloyl; Caf, caffeoyl; Sin, sinapoyl; Oxa, oxaloyl.

retention time was longer than that of peonidin 3-rutinoside (*5*). Thus, it could not be identified with certainty. The chemical structures of all anthocyanins identified in black sorghum are shown in **Figure 6**.

Hi-Tannin Sorghum. Hi-tannin sorghum exhibited a similar anthocyanin profile to that of black sorghum (**Figure 5C**). Six anthocyanins were identified as follows: peak 1, luteolinidin 5-glucoside; peak 2, apigeninidin 5-glucoside; peak 3, 7-methoxyapigeninidin 5-glucoside; peak 4, luteolinidin; peak 5, apigeninidin; and peak 6, 7-methoxyapigeninidin (**Table 5**). All of these anthocyanins were also identified in black sorghum.

Red Sorghum. In red sorghum, five anthocyanins and two other peaks with maximum absorbance at 480 nm were detected (**Figure 5D**). The five anthocyanins were identified as follows: peak 1, apigeninidin 5-glucoside; peak 2, 7-methoxyapigeninidin 5-glucoside; peak 3, luteolinidin; peak 4, apigeninidin; and peak 5, 7-methoxyapigeninidin. Of the two other peaks, peak 6 had a molecular ion *m*/*z* 265; whether it was an anthocyanin is unknown. Peak 7 had a molecular ion of *m*/*z* 331, which was identical to the molecular weight of malvidin. Normally, anthocyanidins with a 3-hydroxyl group do not exist naturally in their aglycone form. Thus, we are suspicious of any identification of this peak as malvidin; further work will be needed to actually identify it (**Table 5**).

Distribution of Anthocyanins in Vegetables and Nuts. The distributions of anthocyanins in vegetables and nut are shown in **Tables 2**−**4** and are summarized in **Table 6**. In our previous study, we categorized common fruits and berried into two anthocyanin distribution groups, namely, "sugar-determined group" and "anthocyanidin-determined group", respectively (*5*). Of all foods studied in this paper, black bean was found to fit into the sugar-determined group and eggplant, red leaf lettuce, red onion, small red bean, and pistachio fit into the anthocyanidin-determined group. Red cabbage and red radish were demonstrated to have very complicated anthocyanin structural patterns. This characterization distinguished them from other foods containing simple anthocyanins. Hence, we designated a new group for these two foods, namely, "highly conjugated anthocyanin group". Food samples in this group contained primarily one anthocyanidin, which was highly conjugated with sugars and various acylated groups. Most of the highly conjugated anthocyanins shared similar structural patterns.

The differential distribution patterns of anthocyanins might indicate different biosynthetic pathways. Determination of distribution pattern could help us identify anthocyanins in a given sample, especially a sample with a complicated anthocyanin composition.

Regularities in Anthocyanin Identification. *General Structural Patterns.* Anthocyanins are composed of two essential components, the anthocyanidin (aglycone) and sugar(s), and one optional component, the acylated group(s). **Table 1** illustrates the possible "units" that could be used to build anthocyanins. Generally speaking, if a sugar was found to be connected to one position of the anthocyanidin, it was connected to the 3-position. If sugars were found in two different positions, in most cases, one was at the 3- and the other at the 5-position of the flavylium ring. The position of the sugar can be determined by comparing to the appropriate anthocyanidin-3-glycoside standard, the MS information, and the retention times. The retention time will be different if the sugar moiety is at the 3- or 5-position, but the mass will be the same if it is the same sugar. If sugar moieties are shown to be different at the 3- and 5-positions, the larger molecular weight sugar moiety tends to link to the 3-position (*1*). For instance, the disaccharides including diglucose and rutinose were found exclusively at the 3-position, whereas glucose was found at the 5-postion, in anthocyanins in red radish. This conclusion was also verified by a number of identified anthocyanins in foods (*1*).

Mass Spectrum. The mass spectrum provides the most valuable information in anthocyanin identification. By using HPLC in tandem with ESI-MS/MS, we were able to separate complicated anthocyanins and obtain the molecular weight and useful fragmentation ions in one single run. Giusti et al. (*11*) proposed that with only one exception (i.e., rutinoside) that fragmentation only occurred between the flavylium ring and the sugar moieties. In our previous study, we found an additional exception to that rule in that there is also fragmentation of the malonoyl group from the sugar (*5*). This finding was also observed in these studies (e.g., peak 26 in red radish or peak 3 in red leaf lettuce). No other exceptions were observed. We found that the fragment ions from the direct cleavage between the anthocyanidin and the glycosides are major peak(s) in the MS/MS spectrum and easily distinguished. Moreover, the intensity of fragment ions under certain experimental condition may help determine the position(s) at which sugars are connected to the flavylium ring. For a 3,5-diglycoside without acylated groups, the intensity of the fragment ion of the aglycone was found to be the highest or among the highest ones. The anthocyanidin 3-glycoside residue was found not to be different from that of the anthocyanidin 5-glycoside residue (**Figure 7A**). This meant that the ease of fragmentation between the anthocyanidin and the sugar moieties without acylated groups was fairly easy. For a 3,5-diglycoside with acylated groups at one position, the fragmentation ion of the residue of anthocyanidin (acyl)glycoside was always higher in intensity, regardless of whether the acylated group was in the 3- or 5-position (**Figure 7B**,**C**). If more than two acylated groups existed at two different positions, the intensity of the fragmentation ion of the aglycone

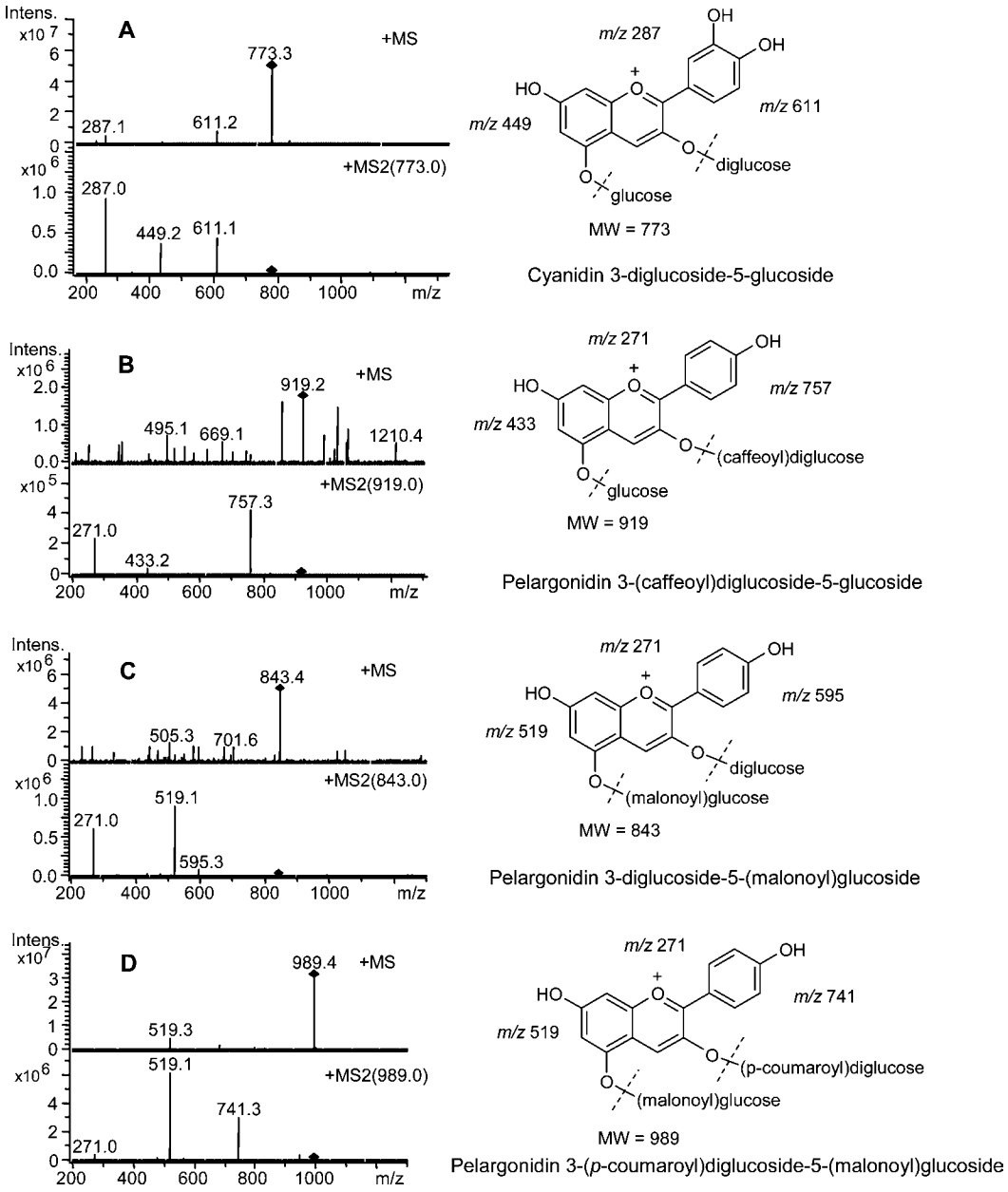


Figure 7. MS and MS/MS spectra of (**A**) cyanidin 3-diglucoside-5-glucoside from red cabbage, (**B**) pelargonidin 3-(caffeoyl)diglucoside-5-glucoside, (**C**) pelargonidin 3-diglucoside-5-(malonoyl)glucoside, and (**D**) pelargonidin 3-(*p*-coumaroyl)diglucoside-5-(malonoyl)glucoside from red radish. MS spectra showed that intensities of fragment ions were related to the structure of anthocyanin.

was very low, sometimes even undetectable. The peak intensity would be determined by the type of acylated groups. From the data that we have, the (malonoyl)glycoside residue ion seems always to be the highest intensity peak in MS/MS, regardless of the connection of the sugars at the 3- or 5-position (**Figure 7D**). Recognition of this MS pattern can help to locate the position of different sugars and/or acylated group(s).

It is important to remember that the regularities that we discussed here and previously (*5*) were general conclusions deduced from our limited data. They may provide some assistance in identifying anthocyanins, but unless standards or standard references are available to make comparisons, it is difficult to know the exact chemical structures. One should be cautious to expand these conclusions to samples containing totally unknown or complicated anthocyanins. Attempts to determine the exact chemical structures may need to resort to NMR or other chemical/analytical methods.

ACKNOWLEDGMENT

The assistance of Joanne Holden and her staff of the Nutrient Database Laboratory in the Beltsville Human Nutrition Center in obtaining the food samples is acknowledged. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

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Received for review December 15, 2004. Revised manuscript received February 24, 2005. Accepted February 25, 2005.

JF0478861