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Screening of Foods Containing Proanthocyanidins and Their Structural Characterization Using LC-MS/MS and Thiolytic Degradation

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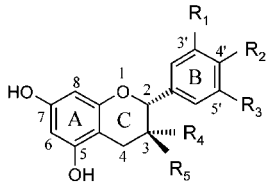
A normal-phase HPLC-MS/MS method was applied to screen for proanthocyanidins in 88 different kinds of foods. Thirty-nine foods were found to contain proanthocyanidins. These foods include 19 kinds of fruits, eight cereals/beans, seven nuts, two beverages, two spices, and one vegetable. Twenty-five kinds of foods were found to contain both oligomeric (DP ≤ 10) and polymeric proanthocyanidins (DP > 10), and the other 14 foods contained only oligomers. Procyanidins with B-type linkages were detected as the only components in 21 foods and also as principal components in the others. Propelargonidins were identified in pinto bean, raspberry, strawberry, and almond, etc. Plum, avocado, peanut, curry, and cinnamon were identified as potential sources of A-type proanthocyanidins in addition to cranberry. Thiolytic degradation and MS/MS analyses indicated that the A-type linkages are present as a terminal unit in plum or between the extension units in curry, cinnamon, and avocado, whereas A-type linkages exist at both positions in cranberry and peanut.

KEYWORDS: Catechin; propelargonidin; procyanidin; proanthocyanidins; tannins; foods

INTRODUCTION

Proanthocyanidins are better known as condensed tannins. They are ubiquitous and present as the second most abundant natural phenolics after lignin. Proanthocyanidins are mixtures of oligomers and polymers composed of flavan-3-ol units linked mainly through C4→C8 bond, but the C4→C6 linkage also exits (both are called B-type). The flavan-3-ol units can also be doubly linked by an additional ether bond between C2→O7 (A-type). The size of the proanthocyanidin molecule is described by the degree of polymerization (DP) (*1*). The common flavan-3-ols in proanthocyanidins are shown in **Scheme 1**. These flavan-3-ols could be esterified with gallic acid to form 3-*O*-gallates. The proanthocyanidins consisting exclusively of (epi)catechin are designated as procyanidins. Proanthocyanidins containing (epi)afzelechin or (epi)gallocatechin as subunits are named propelargonidin or prodelphinidin, respectively. Procyanidins exist most widely in plants. Propelargonidin and prodelphinidin are less common in nature. They are mostly heterogeneous in their constituent units and coexist with the procyanidins (*1*).

Scheme 1. Structures of the Flavan-3-ol Units in Proanthocyanidins



Flavan-3-ols	R_1	R_2	R_3	R_4	R_5
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

The proanthocyanidins in grape and wine have been suggested to contribute to the phenomenon called "French Paradox" (*2*). Many other health-promoting effects, such as antioxidant, anticarcinogenic, and antiinflammatory effects, have also been reported for proanthocyanidins in grape seed and cocoa (*2*−*4*). Surprisingly, only a few foods have been studied for their proanthocyanidin content; thus, the distribution and the structural features of proanthocyanidins in most of the foods are unknown. In previous studies, we have established a normal-phase HPLC-MS method to separate the procyanidin oligomers up to decamers and all the polymers beyond decamers as a distinct

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single peak (*5*). The proportion of the constituent units and the average DP of procyanidins has been determined after thiolytic degradation. In the present studies, these methods were applied to detect and characterize proanthocyanidins in foods collected in the U. S.

MATERIALS AND METHODS

Chemicals. Methanol, methylene chloride and acetic acid (HPLC grade) were purchased from Fisher Scientific (Boston, MA). Sephadex LH-20 and toluene-α-thiol are products of Sigma Chemical Co. (St. Louis, MO).

Reference Compounds. (−)-Epicatechin, (+)-catechin, (−)-epigallocatechin, (−)-gallocatechin, (−)-epigallocatechin 3-*O*-gallate, and (−)-gallocatechin 3-*O*-gallate were purchased from Sigma Chemical Co. (St. Louis, MO). A composite procyanidin oligomer standard containing monomers through decamers was purified from cacao and characterized by Adamson et al. (*6*). A polymeric procyanidin fraction with an average DP of 36.1 was used as a polymer standard. These polymers were fractionated from blueberries on a Sephadex LH-20 column and contained no procyanidins with DP < 10 (*5*).

Food Samples. Foods were sampled by the Department of Biochemistry of the Virginia Technical University (Blacksburg, VA). They were shipped frozen on dry ice to Arkansas Children's Nutrition Center and kept at −70 °C before use. The fruits and vegetables were freeze-dried and ground into powder. Nuts, cereals/beans, and spices were ground samples without freeze-drying. Beverages were in their original liquid form.

Sample Extraction and Purification. The ground food sample (1 g) was extracted in a 15-ml screw-cap tube. The nuts were defatted with 10-mL hexane prior to the proanthocyanidin extraction. The tubes were put into the hood overnight to evaporate the residual hexane. Proanthocyanidins were extracted with 10 mL of mixed solvent (acetone/water/acetic acid, 70:29.5:0.5 v/v/v). After adding the mixed solvent, the tube was vortexed for 30 s followed by sonication at 37 °C for 10 min. The tube was inverted once in the middle of sonication to suspend the samples. Then the tube remained at room temperature for 50 min. The tube was vortexed for 30 s after 25 min. At the end of the extraction, the tube was centrifuged at 3500 rpm for 15 min. Part of the supernatant (7.5 mL) was pipetted out and the acetone was evaporated at 25 °C in a SpeedVac (SC210A, Thermo Savant, Holbrook, NY) under partial vacuum. The residue after evaporation of acetone was dissolved in approximately 6 mL of 30% (v/v) aqueous methanol and loaded onto a Sephadex LH-20 column. The beverage samples (20 mL) were loaded onto the column without extraction. The Sephadex LH-20 columns (6×1.5 cm) were manually packed with 3 g of Sephadex LH-20, which had been equilibrated in 30% (v/v) aqueous methanol for over 4 h. After loading of the sample, the column was washed with 40 mL of 30% methanol/water to remove the sugars and other phenols. Proanthocyanidins were recovered from the column by eluting with 70 mL of 70% (v/v) aqueous acetone. The effluents were evaporated to dryness under vacuum in a SpeedVac at 25 °C. The dried substance was dissolved in the mixed extraction solvent and transferred to a volumetric flask. The final volume was brought up to 5 mL. The solution was centrifuged at 14 000 rpm for 10 min before it was injected for normal-phase HPLC-MS/MS analyses. The overall recovery rates of oligomeric and polymeric proanthocyanidins during extraction and purification were over 80% (*5*).

Normal-Phase HPLC-MS/MS. The analyses were conducted using an Agilent 1100 HPLC system coupled with a Bruker Esquire-LC ion trap mass spectrometer with the same column and detection parameters described before (*7*). A slight modification has been made with the gradient. The mobile phase consisted of (A) methylene chloride, (B) methanol, and (C) acetic acid and water (1:1 v/v). The gradient was 0−20 min, 14.0−23.6% B linear; 20−50 min, 23.6−35.0% B linear; 50−55 min, 35.0−86.0% B linear; 55−65 min 86.0% B isocratic; 65−70 min, 86.0−14.0% B linear followed by 10 min of reequilibration of the column before the next run. A constant 4.0% C was kept throughout the gradient. The mass spectrum data were acquired at negative mode. A fragmentation energy level of 100% was applied to obtain the product ion spectra.

Thiolytic Degradation. The proanthocyanidin extract (50 μL) was put into a 250-μL polypropylene insert (Fisher Scientific, Boston, MA.). The solvent was evaporated at 25 °C in a SpeedVac. Methanol (50 μL) was added to dissolve the residue. Then, it was mixed with 50 μL of methanol acidified with concentrated HCl (3.3%, v/v) and 100 μL of toluene-α-thiol (5% v/v in methanol). The inserts were put into a 1.5-mL vial and sealed with an inert Teflon cap. The reaction was carried out at room temperature for 10 h. At the end of the reaction, the reaction mixtures were kept in the freezer (−18 °C) before 10 μL was injected for reversed phase HPLC-MS/MS analysis.

Reversed-Phase HPLC-MS/MS and DP Calculation. The column and gradient reported before was used (*5*). A different gradient was applied to separate the A-type proanthocyanidins after thiolytic degradation. The binary mobile phases consisted of A (2% acetic acid in water, v/v) and B (methanol). The gradient started with 72% of solvent A and 28% of solvent B and ended with 37% of solvent A and 63% of solvent B in 55 min. The terminal units of the proanthocyanidins are released as free flavan-3-ols after the thiolytic degradation, which cannot be distinguished from the flavan-3-ol monomers in the sample by HPLC. The proanthocyanidin solutions were analyzed without thiolytic degradation to subtract the monomers.

The proportions of constituent flavan-3-ol and average DP were calculated according to a published method (*5*). This method is based on the assumption that flavan-3-ol benzylthioethers have the same molar absorptivities as their respective flavan-3-ol monomers. For the A-type procyanidins, one molecule of A-type procyanidin dimer and its benzylthioether were assumed to have the same UV absorptivity of two molecules of (+)-catechin. The epimerization rates of pure flavan-3-ols in our thiolytic degradation reaction were found to be less than 6%. The proportion of flavan-3-ols in terminal units was not corrected according to them.

RESULTS AND DISCUSSION

Normal-Phase HPLC-MS/MS Analyses. In 88 kinds of foods that have been analyzed, 39 foods were found to contain proanthocyanidins. The constituent flavan-3-ols, type of interflavan linkages, and the range of DP of proanthocyanidins in these foods are listed in **Table 1**. The foods containing no detectable proanthocyanidins are listed in **Table 2**. The existence and the structural features of proanthocyanidins in most of the foods in **Table 1** have not been reported previously. Fruits (detected in 19 out of 29) are found to be the major sources of proanthocyanidins in the diet. Proanthocyanidins are detected in plum and grape, whereas they are absent in prune (dried plum) and raisin, which suggests that the proanthocyanidins were degraded during the drying procession. Vegetables are not an important source of proanthocyanidins. Proanthocyanidins are detected only in Indian squash from the 19 vegetables tested. Most of the minor cereals such as barley, pinto bean, red kidney bean, and sorghum contain proanthocyanidins, whereas they are not detected in the staple crops such as corn, rice, and soybean. However, detection of proanthocyanidins in rice and soybean with a pigmented coat has been reported (*8*, *9*). Most of the nuts (7 out of 10) contain proanthocyanidins. Beer has been identified to be a dietary source of proanthocyanidins, which are thought to derive from barley and hop (*10*).

Most of the foods in **Table 1** (21 out of 39) contain exclusively homogeneous B-type procyanidins. Procyanidin monomers through decamers are identified based on their mass spectra as well as in comparison with the retention time of our external standards. The peak of the polymers was identified according to its retention time and the UV absorption profile of flavan-3-ols. The typical profiles of B-type procyanidins in blueberry, cocoa, sorghum, and apple have been reported by us previously (*5*, *11*).

Table 1. Constituent Flavan-3-ols and DP Range of Proanthocyanidins in Foods[a]

no.	items	A-type	B-type	(epi)afz	(epi)cat	(epi)GC	DP range	(epi)cat glycoside
				fruits				
1	blueberry		√		√		**1-P**	
2	cranberry	√	√		√		**1-P**	
3	blackberry		√		√		**1-P**	
4	raspberry		√	√	√		1–10	
5	strawberry		√	√	√		**1-P**	
6	blackcurrant		√		√	√	**1-P**	
7	cherry		√		√		1–8	√
8	grape		√		√*	√	**1-P**	
9	apple		√		√		**1-P**	
10	apricot		√		√		**1-P**	
11	banana		√		√		1–5	
12	dates, deglet noor		√		√		2–5	
13	kiwi		√		√		1–10	√
14	mango		√		√		1–6	
15	peach		√		√		**1-P**	
16	green pear		√		√		**1-P**	
17	nectarine		√		√		**1-P**	
18	plum	√	√		√		**1-P**	
19	avocado	√	√		√		1–8	
				vegetables				
20	india squash		√		√		**1-P**	
				cereals and beans				
21	sorghum		√		√		**1-P**	
22	barley		√		√		1–4	√
23	blackbean		√		√		1–2	√
24	blackeye pea		√		√		1–4	√
25	pinto bean		√	√	√		**1-P**	√
26	small red bean		√	√	√		**1-P**	√
27	red kidney bean		√	√	√		**1-P**	√
28	cocoa		√		√		**1-P**	
				nuts				
29	almond		√	√	√		**1-P**	√
30	cashew		√		√		1–2	√
31	hazelnut		√		√*	√	**1-P**	√
32	pecan		√		√*	√	**1-P**	√
33	walnut		√		√*		**1–7, P**	
34	pistachios		√		√*	√	**1-P**	√
35	peanut	√	√		√		1–5	√
				beverages				
36	wine		√		√*	√	**1-P**	
37	beer		√		√	√	1–5	√
				spices				
38	curry	√	√		√		1–6	
39	cinnamon	√	√	√	√		**1-P**	

[a] The (epi)afz, (epi)cat, and (epi)GC stand for the constituent units (epi)afzelechin, (epi)catechin, and (epi)gallocatechin, respectively. The "1–5" or "1-P" in column "DP range" indicates that monomers through pentamers or polymers were detected. * denotes the presence of 3-*O*-gallate.

Table 2. Foods Containing No Detectable Proanthocyanidins

no.	fruits	vegetables	cereal/beans	nuts	spices	beverages
1	cantaloupe	agave	blue corn meal	macadamia	basil leaf, dried	lime juice
2	date, medjool	artichoke	oat	pine nut	oregano leaf, dried	
3	grapefruit	asparagus	rice	brazil nut	parsley, dried	
4	honeydew	banana melon	soybean		paprika	
5	navel orange	broccoli	navy bean		pepper, black, ground	
6	pineapple	cabbage			mustard seed, yellow, ground	
7	prune	carrot			poppy seeds	
8	tangerine	celery			garlic powder	
9	watermelon	cucumber			ginger, ground	
10	raisin	fig			onion powder	
11		lettuce			turmeric	
12		mission fig			cloves, ground	
13		onion			chili powder	
14		pepper				
15		potato				
16		radish				
17		sweet potato				
18		tomato				

Heterogeneous proanthocyanidins were detected in 18 foods. According to the structural features of the proanthocyanidins, these foods could be classified into three categories: the propelargonidin group, the prodelphinidin group, and the A-type

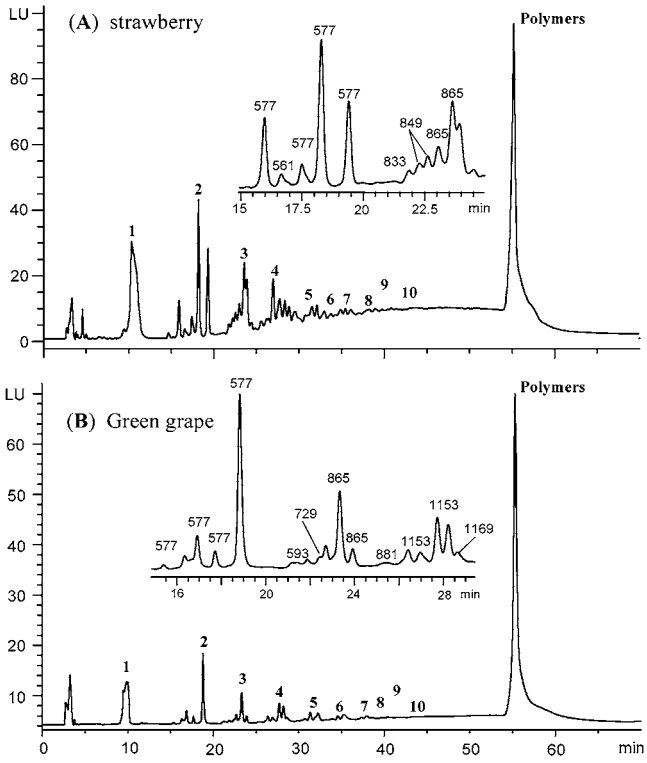


Figure 1. Normal-phase HPLC fluorescence trace of the proanthocyanidins from (A) strawberry and (B) green grape. The labels 1–10 on the peak indicate the degree of polymerization of proanthocyanidins in the peaks. The $[M - H]^-$ (*m*/*z*) of the peaks are labeled in expanded inserts. The chromatogram of cranberry refers to literature (*5*).

proanthocyanidin group. Some foods containing very heterogeneous proanthocyanidins, like cinnamon, can fit into two categories.

Recent studies suggested that the low proanthocyanidin oligomers (DP < 4) could be absorbed in the gastrointestinal tract (*2*). Dimers have been detected in blood after human subjects consumed a proanthocyanidins-rich diet (*12*). Trimers have been shown to be absorbed through the human intestinal cell line Caco-2 (*13*). For this reason, identification of heterogeneous proanthocyanidins, especially the low oligomers, are emphasized.

Identification of Propelargonidins. Strawberry is a typical food containing the propelargonidins. Its proanthocyanidin profile on normal-phase HPLC is shown in **Figure 1A**. Two kinds of dimers were identified in strawberry. The *m*/*z* 577 is indicative of procyanidin dimers. The *m*/*z* 561 is 16 Da less than *m*/*z* 577, which suggests the existence of a subunit with one less hydroxyl group than the (epi)catechin. The product ion spectrum of *m*/*z* 561 is shown in **Figure 2A**. The fragmentation pathway of this dimer is shown in **Figure 3**. A proanthocyanidin dimer consists of an extension unit and a terminal unit. The heterocyclic ring of the flavan-3-ol units fragment through retro-Diels-Alder (RDA) and heterocyclic ring fission (HRF) mechanisms (*14*, *15*). Both fragmentation pathways could take place on the extension unit or the terminal unit. However, fragmentation on the extension unit gives rise to fragment ions with a larger $\pi-\pi$ hyperconjugate system; thus, it is more energetically favorable. The RDA fragmentation of the dimer produced *m*/*z* 425.0. Loss of 136 Da through RDA indicates that ring B of the extension unit has one hydroxyl group. The *m*/*z* of 407.0 results from water elimination of *m*/*z* 425.0, most likely from the 3-OH. The HRF of the dimer produces *m*/*z* 435.1. Loss of 126 Da indicated that ring A of the extension unit had a 1,3,5-trihydroxybenzene structure. The HRF pathway also indicates the existence of a free 4′-OH group on ring B. Thus the extension unit of this dimer was deduced to be (epi)afzelechin. Because the chirality of C3 on the flavan-3-ols cannot be differentiated by mass spectrometry, the (epi)afzelechin stands for either afzelechin or epiafzelechin. The connection sequence of this dimer was identified to be (epi)afzelechin→(epi)catechin. This sequence was confirmed by the fragment ion *m*/*z* 271.0 and 289.0, which were derived from the extension unit and terminal unit, respectively, after the Quinone-Methide (QM) cleavage of the interflavan bond (**Figure 3**) (*16*).

The Quinone-Methide cleavage of the interflavan bond provides the information about the connection sequence of a heterogeneous proanthocyanidin. The QM cleavages of different trimers are shown in **Scheme 2**. Propelargonidin trimers (*m*/*z* 833 and 849 in **Figure 1A**), tetramers, and pentamers have been identified in strawberry as are shown in **Table 3**. Sequencing of the propelargonidins with DP > 6 cannot be done because of the fragmentation complexity. However, their $[M - H]^-$ or $[M - 2H]^{2-}$ indicated that they contain various numbers of (epi)afzelechin as subunits. The propelargonidin oligomers with the similar structures were also identified in pinto bean, small red bean, red kidney bean, strawberry, almond, raspberry, and cinnamon. On the basis of the peak area on the normal-phase HPLC, about 5.4−7.8% of the proanthocyanidins in strawberry contained at least one (epi)afzelechin subunit. A similar proportion was observed for almond. Propelargonidins contribute about 8.2−11.6% of the proanthocyanidins in pinto bean, small red bean, and red kidney bean. The principal proanthocyanidins in these foods are, however, procyanidins. Four peaks of procyanidin dimers ($[M - H]^-$, *m*/*z* 577) were observed in strawberry (**Figure 1A**). They were the different stereoisomers. However, all these dimers gave rise to almost the same mass spectra and fragment pattern (**Figure 2B**). The position and the stereochemistry of the interflavan linkage cannot be elucidated by mass spectrometry, which has been pointed out before (*17*).

Identification of Prodelphinidins. The prodelphinidins were detected in 7 foods (**Table 1**). The profile of proanthocyanidins in green grape on normal-phase HPLC is shown in **Figure 1B**. The proportion of polymers in green grape is 70.0% based on the peak area. A similar proportion had been reported for the proanthocyanidins in grape skin (*18*). The product ion spectrum of the major prodelphinidin dimers ($[M - H]^-$ *m*/*z* 593.0) eluted at 21.6 min is shown in **Figure 2C**. These dimers were identified as (epi)gallocatechin→(epi)catechin based on the *m*/*z* 289.0 and 303.0 derived from the QM cleavage. RDA and HRF cleavage on the extension unit of the dimer gave rise to *m*/*z* 425.0 and 467.1. Other prodelphinidin oligomers identified in grape are listed in **Table 3**. Similar oligomers and their isomers were also detected in beer, wine, blackcurrant, pistachios, hazel nuts, and pecan. On the basis of peak areas of the oligomers, prodelphinidins contribute 13.4−18.7% of the proanthocyanidins in grape. Similar proportions were observed for pecan and wine, whereas over 50% of proanthocyanidins in beer and blackcurrant contain the (epi)gallocatechin units.

Identification of A-Type Proanthocyanidins. The A-type proanthocyanidins have been suggested to be the active components in cranberry that inhibit the adherence of uropathogenic *E. coli* to the uroepithelial-cell surfaces (*19*). Until now, the A-type proanthocyanidins were found in only a few foods (i.e., peanut and cinnamon) (*20*, *21*). Our studies have indicated that plum, avocado, and curry are also potential dietary sources of A-type proanthocyanidins. The proanthocyanidins with one A-type linkage were identified readily on MS by their [M −

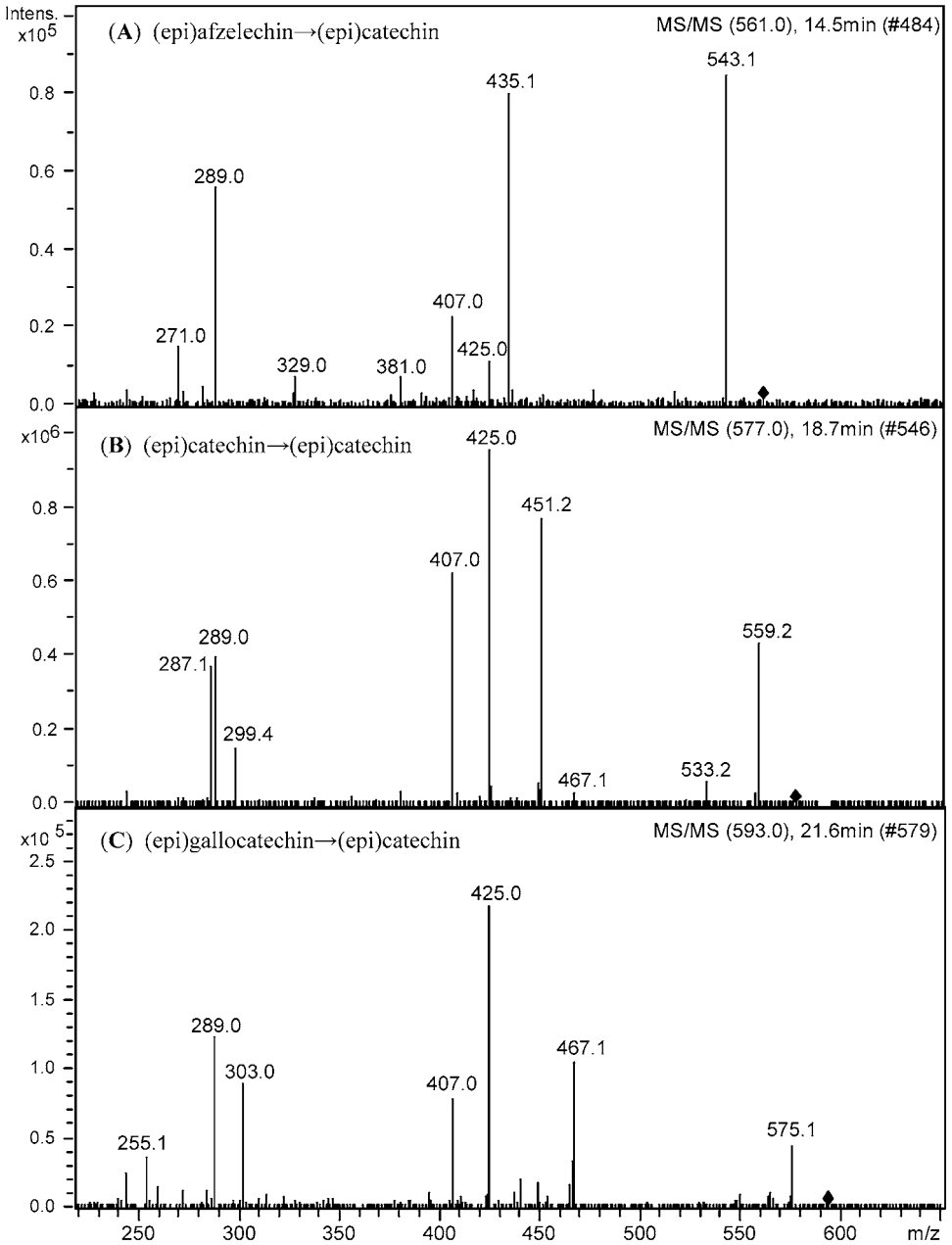


Figure 2. Product ion spectra of different dimers on negative mode ESI-MS.

H]$^-$ being 2 Da less than those of the B-type proanthocyanidins. In the presence of B-type interflavan bond, A-type interflavan bonds in the same molecule do not undergo QM cleavage under our fragmentation energy level of 100%. Thus, the position of an A-type linkage in the proanthocyanidin oligomers could be identified according to their product ion spectra (**Scheme 2**, parts **C** and **D**). Various A-type oligomers in cranberry have been identified and are listed in **Table 3**. Except for A-type dimer and trimers, which have been purified (*19*), most of these oligomers have not been reported before. The majority of the A-type procyanidins in cranberry contain one A-type linkage, whereas tetramers and pentamers containing two A-type interflavan linkages were identified as minor components. Similar A-type procyanidins containing one A-type linkage were also identified in peanut. The A-type linkages in these procyanidins are present between the extension units or as an A-type terminal unit. The A-type procyanidin oligomers identified in the other four foods showed clear differences (A-type dimer excluded). The A-type linkage is present only as an A-type terminal unit in the A-type procyanidin trimers, tetramers, and pentamers in plum. For example, the A-type tetramers identified in plum have an (epi)catechin→(epi)catechin→(epi)catechin→A→(epi)catechin connection. The A-type terminal units are absent in avocado, curry, and cinnamon, where the A-type linkages are between the extension units in the identifiable oligomers. A few trimers, tetramers, and pentamers had been purified from cinnamon before (*21*). The A-type linkages in all of these oligomers were present between the extension units. Our conclusion from cinnamon is in agreement with this previous report. The proportions of A-type procyanidins in the total procyanidins were also different in these foods. On the basis of the peak area, it was found that over 84−90% of procyanidins in curry and cinnamon were A-type procyanidins, while cranberry and peanut contained less (51−65%). About 17−29% of procyanidins in plums were A-type procyanidins, and avocado contained the least A-type procyanidins (<12%).

Most of the flavan-3-ols in plants are aglycones. Glycoside forms are considered to be rare (*1*). We observed an interesting

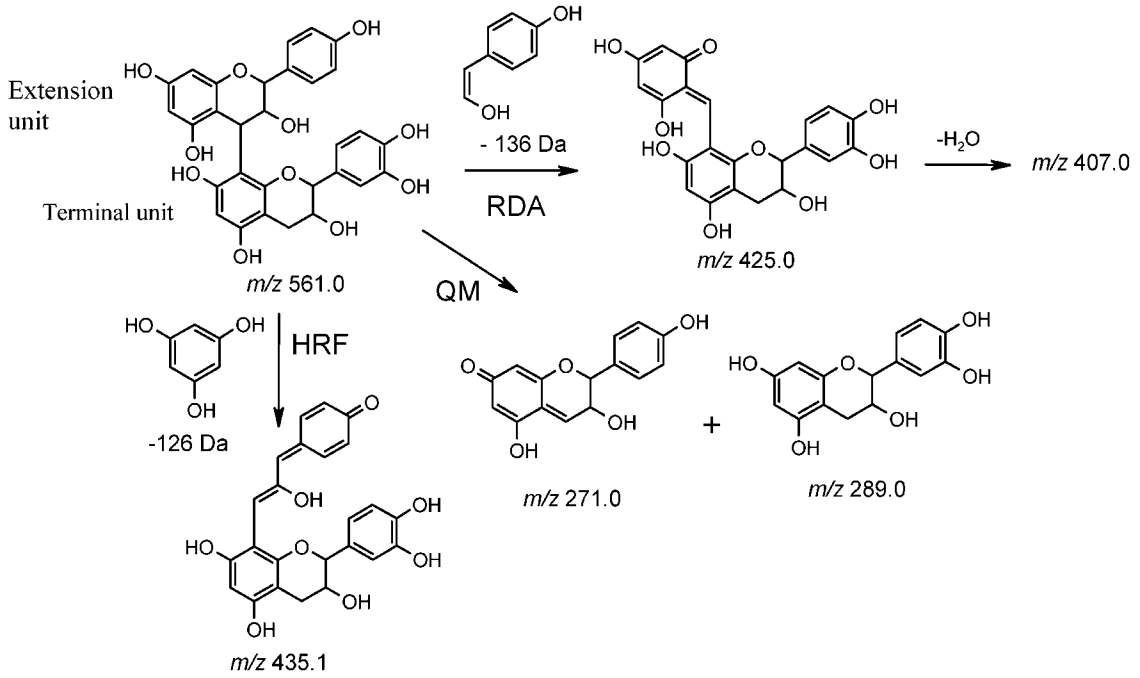
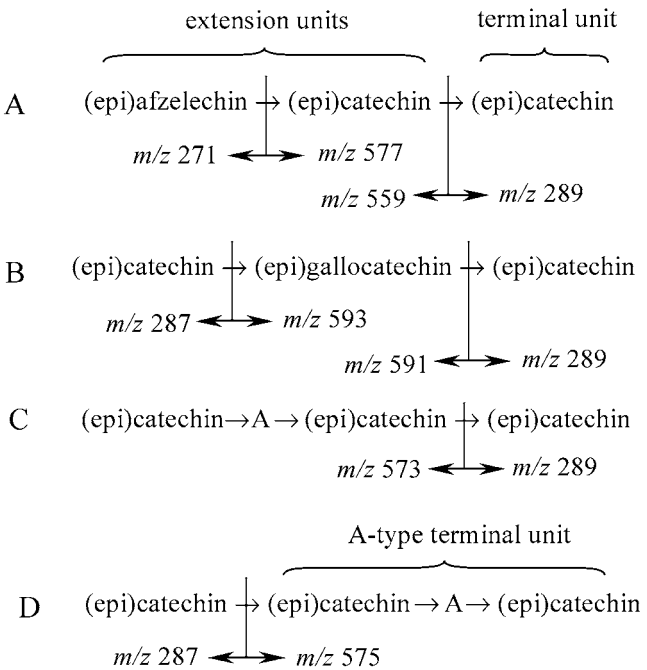


Figure 3. Fragmentation pathway of a propelargonidin dimer detected in strawberry.

Scheme 2. Fragmentation of Interflavan Bond in Trimers through Quinone-Methide Mechanism[a]



[a] The →A→ stands for an A-type interflavan linkage.

component in some foods as (epi)catechin glycoside (**Table 1**), which eluted between procyanidin dimers and trimers. Its mass spectra showed an $[M - H]^-$ at *m/z* 451 and a fragment ion *m/z* 289.

Thiolytic Degradation. Thiolytic degradation coupled with the reverse phase HPLC-MS/MS analysis was applied to determine the proportion of the constituent units and the average DP of the proanthocyanidins in foods. The chromatograms of thiolytic degraded proanthocyanidins in strawberry, green grape, and cranberry are shown in **Figure 4**. The catechin in the extension units of proanthocyanidins is captured by the toluene-α-thiol to form the 3,4-*cis*-catechin benzylthioether and 3,4-*trans*-catechin benzylthioether. The 3,4-*cis*-catechin benzylthioether is the major product (*22*). The epicatechin in the extension yields only 3,4-*trans*-epicatechin benzylthioether (*22*, *23*). The procyanidins in cocoa and sorghum are known to contain exclusively epicatechin as extension units (*24*, *25*). Comparison between their chromatograms in conjunction with the mass spectrometry indicated that Peak 8 in **Figure 4** was 3,4-*trans*-epicatechin benzylthioether. Peaks 5 and 7 showed similar mass spectra to peak 8 (*m/z* 411 with a characteristic fragment of *m/z* 287). They were identified to be 3,4-*trans*-catechin benzylthioether and 3,4-*cis*-catechin benzylthioether, respectively. Peak 10 in **Figure 4B** gave rise to *m/z* 563 in negative ion mode and a fragment ion *m/z* 439. It can be catechin 3-*O*-gallate benzylthioether or epicatechin 3-*O*-gallate benzylthioether. Only epicatechin 3-*O*-gallate has been identified in the extension units of proanthocyanidins in grape (*26*); thus, the possibility of peak 10 being catechin 3-*O*-gallate benzylthioether was excluded. The epicatechin 3-*O*-gallate benzylthioether was deduced to be a 3,4-trans structure due to the steric hindrance of the gallate group. Peaks 6 and 9 were identified as (epi)gallocatechin benzylthioether and (epi)afzelechin benzylthioether, respectively. The chirality of the C-3 of these two flavan-3-ols cannot be discerned based on mass spectra. The A-type linkage remains stable during the thiolytic degradation (*27*). The A-type terminal unit is released as an A-type dimer, whereas the A-type linkage between the extension units yields an A-type dimer benzylthioether. Our early report had revealed that 46.1% of the terminal units in the polymeric procyanidins in cranberry are A-type dimers (*5*). However, the A-type linkages between the extension units were not observed. Modification of our previous elution gradient for the reversed phase HPLC has resulted in an additional peak as peak 11 in **Figure 4C**. Its mass spectrum showed $[M - H]^-$ *m/z* 696 and a fragment ion *m/z* 573. This peak was identified to an A-type dimer benzylthioether, which is indicative of the A-type linkages between the extension units.

The proportions of constituent flavan-3-ol and average DP for foods containing heterogeneous B-type proanthocyanidins and selected foods containing the homogeneous B-type pro-

Table 3. Proanthocyanidins Oligomers in Selected Foods Containing Heterogeneous Subunits and A-type Linkages

source	oligomers	$[M - H]^-$	Product ions
strawberry	(epi)afz→(epi)afz→(epi)cat	833.0	561.1, 543.0
	(epi)afz→(epi)cat→(epi)cat	849.1	577.2, 559.1
	(epi)afz→(epi)afz→(epi)cat→(epi)cat	1121.1	831.2, 577.3, 543.2
	(epi)afz→(epi)cat→(epi)cat→(epi)cat	1137.2	865.2, 847.2, 577.2
	(epi)afz→(epi)afz→(epi)cat→(epi)cat→(epi)cat	1409.3	865.0
	(epi)afz→(epi)cat→(epi)cat→(epi)cat→(epi)cat	1425.4	1153.3, 1135.3, 865.0, 847.0
green grape	(epi)cat→epicatechin gallate	729.2	577.2, 451.0, 441.0, 289.0
	epicatechin gallate→epicatechin gallate	881.2	729.2, 577.1
	(epi)cat→(epi)GC→(epi)cat	881.1	593.2, 591.1, 303.0
	(epi)GC→(epi)cat→(epi)cat	881.1	577.2, 591.2
	(epi)cat→(epi)GC→(epi)cat→(epi)cat	1169.2	881.2, 591.2, 577.1
cranberry	(epi)cat→A→(epi)cat	575.1	449.0
	(epi)cat→(epi)cat→A→(epi)cat	863.1	575.1
	(epi)cat→A→(epi)cat→(epi)cat	863.2	573.1, 289.0
	(epi)cat→ (epi)cat→A→(epi)cat→A→(epi)cat	1149.2	861.3, 575.3
	(epi)cat→(epi)cat→(epi)cat→A→(epi)cat	1151.2	863.5, 575.3
	(epi)cat→(epi)cat→A→(epi)cat→(epi)cat	1151.2	863.3, 573.0
	(epi)cat→A→(epi)cat→(epi)cat→(epi)cat	1151.2	861.2, 573.2
	(epi)cat→(epi)cat→A→(epi)cat→(epi)cat→A→(epi)cat	1437.2	1149.1, 861.2
	(epi)cat→(epi)cat→(epi)cat→(epi)cat→A→(epi)cat	1439.3	1153.3, 863.4, 575.2
	(epi)cat→(epi)cat→A→(epi)cat→(epi)cat→(epi)cat	1439.3	1153.3, 1149.3, 861.2, 573.2

[a] (epi)afz, (epi)cat, and (epi)GC are the abbreviation for (epi)afzelechin, (epi)catechin, and (epi)gallocatechin, respectively. →A→ stands for an A-type interflavan linkage.

Table 4. Molar Proportion of Constituent Flavan-3-ols and Average DP of B-Type Proanthocyanidins in Selected Foods (Mean ± SD of Duplicate Tests)[a]

	terminal units (%)					extension units (%)					
foods	Cat	Ec	EcG	EGC	GC	(Epi)GC	Cat	Ec	EcG	(Epi)afz	average DP[b,c]
green grape	4.2 ± 0.1	0.5 ± 0.0	0.1 ± 0.0			13.0 ± 1.6	9.5 ± 0.2	71.5 ± 1.3	1.2 ± 0.0		20.9 ± 0.3 (16.1)
red grape	4.0 ± 0.2	0.6 ± 0.0	0.5 ± 0.0			12.0 ± 2.3	10.4 ± 0.4	69.1 ± 1.7	3.3 ± 0.1		19.6 ± 0.9 (15.4)
red wine	6.7 ± 0.2	2.1 ± 0.1		4.9 ± 0.0		8.0 ± 0.2	24.2 ± 0.5	52.8 ± 0.3	1.3 ± 0.0		7.3 ± 0.2 (5.2)
hazelnut	5.7 ± 0.4	1.5 ± 0.2				10.8 ± 0.6	39.5 ± 0.2	41.9 ± 0.4	0.5 ± 0.1		14.0 ± 1.0 (10.8)
pistachio	5.2 ± 0.1	0.5 ± 0.0				3.2 ± 0.0	8.8 ± 0.1	77.4 ± 0.4	5.0 ± 0.2		17.7 ± 0.3 (9.1)
pecan	7.0 ± 0.3	1.6 ± 0.1				18.8 ± 1.9	30.6 ± 0.7	36.0 ± 0.7	6.0 ± 0.2		11.6 ± 0.3 (8.2)
beer	14.4 ± 0.5	2.6 ± 0.1			31.3 ± 0.1	20.3 ± 0.5	28.8 ± 0.2	2.7 ± 0.0			2.1 ± 0.0 (1.7)
blackcurrant	1.3 ± 0.1	0.8 ± 0.1				40.2 ± 4.4	4.4 ± 0.4	53.3 ± 3.8			47.9 ± 5.1 (38.7)
walnut	4.2 ± 0.2	2.0 ± 0.1					7.3 ± 0.1	75.6 ± 0.2	11.0 ± 0.2		16.2 ± 0.4 (7.8)
almond	3.6 ± 0.3	4.3 ± 0.2					7.5 ± 1.1	78.9 ± 0.7		5.7 ± 1.1	12.7 ± 0.3 (8.5)
raspberry	9.9 ± 1.4	27.7 ± 2.9					45.1 ± 0.9	2.9 ± 0.4		14.4 ± 0.2	2.7 ± 0.1 (2.1)
strawberry	9.7 ± 0.2	6.2 ± 0.1					21.5 ± 0.2	56.4 ± 0.0		6.2 ± 0.4	6.3 ± 0.1 (5.4)
pinto bean	11.7 ± 0.1	0.4 ± 0.0					33.2 ± 0.3	46.0 ± 0.1		8.7 ± 0.3	8.3 ± 0.0 (6.8)
small red bean	7.3 ± 0.3	1.1 ± 0.2					5.4 ± 0.1	73.9 ± 0.5		12.3 ± 0.6	12.0 ± 0.1 (9.5)
red kidney bean	14.5 ± 0.0	0.4 ± 0.0					27.4 ± 0.1	43.1 ± 0.0		14.6 ± 0.0	6.7 ± 0.0 (5.5)
black berry	11.4 ± 1.9	20.7 ± 3.1					4.4 ± 2.3	63.6 ± 7.4			3.2 ± 0.5 (2.3)
blueberry	4.5 ± 0.1	1.8 ± 0.0					2.6 ± 0.0	91.1 ± 0.1			15.9 ± 0.3 (14.0)
green pear	1.3 ± 0.1	5.5 ± 0.1						93.1 ± 0.1			14.6 ± 0.1 (10.3)
sorghum, early sumac	9.3 ± 0.2	2.5 ± 0.2						88.2 ± 0.2			8.4 ± 0.1 (8.0)
pycnogenol	12.9	0.7					18.5	67.9			7.4 (6.6)

[a] Cat, catechin; Ec, epicatechin; EcG, epicatechin 3-*O*-gallate; EGC, epigallocatechin; GC, Gallocatechin; (epi)GC, (epi)gallocatechin; (epi)afz, (epi)afzelechin. [b] Average DP=(sum of extension units + sum of terminal units)/(sum of terminal units). [c] Average DPs including the flavan-3-ol monomers are shown in the parentheses.

cyanidins are listed in **Table 4**. **Table 4** shows that (epi)-afzelelchin accounts for 6.2 ± 0.4% of the flavan-3-ols units in the proanthocyanidins in the strawberry as extension units. It is in agreement with our MS/MS data that no (epi)afzelechin is found to be terminal units in the propelargonidin oligomers in strawberry (**Table 3**). Catechin and epicatechin contribute 93.8% of constituent units, which is in accordance with the predominance of the procyanidins in strawberry. Proanthocyanidins in red kidney bean have been found to contain the highest proportion of (epi)afzelechin (14.6 ± 0.0%).

The proanthocyanidins in green grape consist of catechin, epicatechin, (epi)gallocatechin, and epicatechin 3-*O*-gallate. The average DP of proanthocyanidins in green grape is 20.9 ± 0.3, which is in agreement with normal-phase HPLC-MS/MS conclusions that polymers are the predominant components. The average DP of red wine was found to be 7.3 ± 0.2. The lower average DP in wine may be due to removal and depolymerization of polymeric proanthocyanidins during the fining and aging process. The proanthocyanidins in blackcurrant contain 40.2% of (epi)gallocatechin. Their average DP (47.9 ± 5.1) has been found to be the highest among all the foods tested. An early paper reported that the molecular weight of proanthocyanidins in blackcurrant was over 4300 Da, beyond the measure range of ^{13}C NMR (*28*). (Epi)catechin 3-*O*-gallate was not detected as a constituent unit in foods containing exclusively B-type procyanidins, except for the procyanidins in walnut, where epicatechin 3-*O*-gallate contributed 11.0 ± 0.2% of constituent units. **Table 4** also shows that the Pycnogenol, a food supplement extracted from maritime pine tree bark, contains B-type procyanidins with an average DP of 7.4.

Proanthocyanidins with A-type linkages were detected in 6 foods, using normal-phase HPLC-MS/MS. The proportion of

Table 5. Molar Proportion of Constituent Flavan-3-ols and Average DP of Proanthocyanidins Containing A-type Linkage (Mean ± SD of Duplicate Tests)[a]

foods	terminal units (%)			extension units (%)				
	Cat	Ec	A-type dimer unit[b]	Cat	Ec	A-type dimer unit	(Epi)afz	
	a	b	c (d)	e	f	g	h	average DP[c,d]
plum	6.5 ± 0.5	0.5 ± 0.1	1.6 ± 0.1 (0.7)	3.2 ± 0.0	87.6 ± 0.5			11.1 ± 0.4 (7.2)
cranberry	2.7 ± 0.2	5.9 ± 0.1	4.3 ± 0.0 (1.3)	0.7 ± 0.0	69.1 ± 0.2	15.9 ± 0.1		8.5 ± 0.1 (8.3)
peanut	23.0 ± 1.5	3.6 ± 0.5	3.7 ± 0.6 (6.1)	12.6 ± 1.7	43.4 ± 0.3	7.6 ± 0.3		3.2 ± 0.2 (2.6)
avocado	3.9 ± 0.5	27.8 ± 1.0	n.d. (n.d.)	4.3 ± 0.3	61.5 ± 1.2	2.4 ± 0.4		3.2 ± 0.1 (2.4)
curry	6.1 ± 0.2	23.4 ± 0.3	n.d. (2.7)		51.9 ± 1.2	16.0 ± 1.7		3.7 ± 0.1 (3.7)
cinnamon	13.4 ± 0.9	4.2 ± 0.3	n.d. (1.2)	3.4 ± 0.7	55.1 ± 0.1	22.2 ± 0.4	0.6 ± 0.0	6.6 ± 0.4 (6.5)

[a] Cat, catechin; Ec, epicatechin; (epi)Af, (epi)afzelechin; n.d., not detected. [b] Proportions of intrinsic free A-type dimers, which coelute with the A-type dimers released from A-type terminals by toluene -α-thiol, are shown in the parentheses. [c] Average DP = (a + b + c + 2c + 2d + e + f + 2g + h)/(a + b + c + d). [d] Average DPs including the flavan-3-ol monomers are shown in the parentheses.

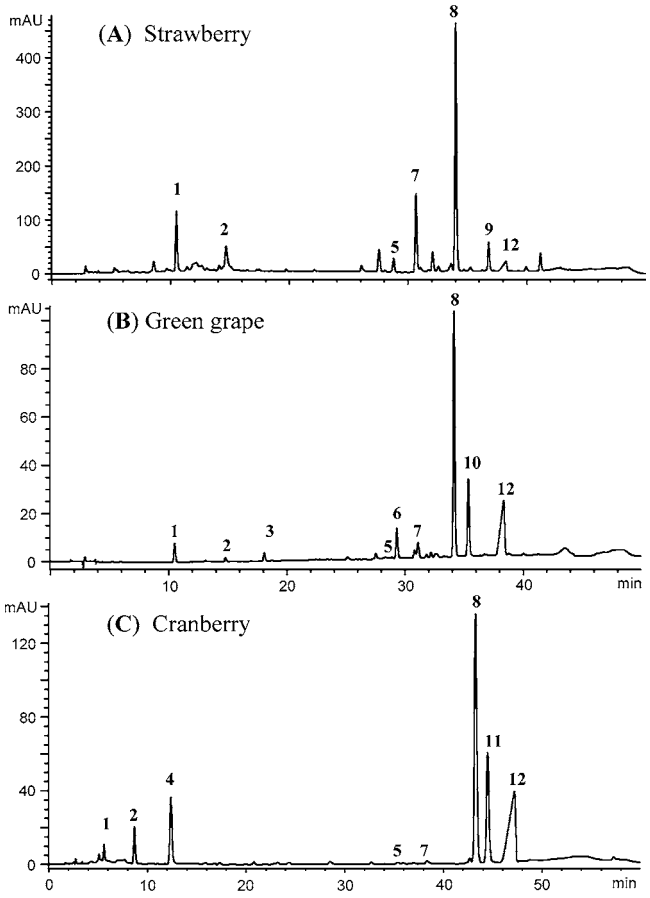


Figure 4. UV trace (280 nm) of proanthocyanidin in (A) strawberry, (B) green grape, and (C) cranberry after thiolytic degradation. Peak 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 are catechin; epicatechin; epicatechin gallate; A-type dimer; 3,4-*trans*-catechin benzylthioether; (epi)gallocatechin benzylthioether; 3,4-*cis*-catechin benzylthioether; 3,4-*trans*-epicatechin benzylthioether; (epi)afzelechin benzylthioether; epicatechin 3-*O*-gallate benzylthioether; A-type dimer benzylthioether; and toluene-α-thiol, respectively. Elution gradient used for (A) and (B) refers to a previous paper (*5*). Gradient used for (C) is described in the text.

their constituent units and average DP after thiolytic degradation is shown in **Table 5**. The intrinsic free A-type dimers before the thiolytic degradation were analyzed and subtracted from the samples after thiolytic degradation so that the A-type dimers released from terminal units could be distinguished. **Table 5** shows that A-type linkages are only present as A-type terminal units in plum, whereas, in avocado, curry, and cinnamon, the A-type linkages are exclusively among the extension units. Cranberry and peanut contain both structures. The thiolytic data agree with the structures of A-type oligomers identified by MS/MS (**Table 3**), which suggests that structure features of the higher oligomers and polymers could be extrapolated from the low oligomers. It is not known whether the A-type procyanidins of different structures differ in their physiological activities, such as the pathogenic bacteria anti-adhesive effects.

In summary, nearly half of the foods derived from plants are dietary sources of proanthocyanidins. Proanthocyanidins in various foods are different in terms of constituent flavan-3-ol units, DP range and average DP value, and the type of interflavan linkage, which may impact the bioavailability, metabolism, and the physiological effects of proanthocyanidins in vivo.

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