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Characterization of Phenolic Compounds in Strawberry (*Fragaria* × *ananassa*) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity

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Phenolic compounds in strawberry (*Fragaria* × *ananassa*) fruits were identified and characterized by using the complementary information from different high-performance liquid chromatography detectors: diode array, mass spectrometer in positive and negative mode, and coulometric array. Electrochemical profiles obtained from the coulometric array detector contributed to the structural elucidation suggested from the UV−vis and mass spectra. About 40 phenolic compounds including glycosides of quercetin, kaempferol, cyanidin, pelargonidin, and ellagic acid, together with flavanols, derivatives of *p*-coumaric acid, and ellagitannins, were described, providing a more complete identification of phenolic compounds in strawberry fruits. Quercetin-3-malonylhexoside and a deoxyhexoside of ellagic acid were reported for the first time. Antioxidative properties of individual components in strawberries were estimated by their electrochemical responses. Ascorbic acid was the single most important contributor to electrochemical response in strawberries (24%), whereas the ellagitannins and the anthocyanins were the groups of polyphenols with the highest contributions, 19 and 13% at 400 mV, respectively.

KEYWORDS: Strawberry; *Fragaria* × *ananassa;* HPLC; MS; coulometric array; antioxidants; phenolic compounds; flavonoids; flavonols; anthocyanins; flavanols; hydroxycinnamic acid; ellagic acid; ellagitannins

INTRODUCTION

Consumption of fruits and vegetables is shown to lower the risk for chronic diseases such as cancer, cardiovascular diseases, and stroke (*1*−*3*). The positive health effects may be due to high contents of certain phenolic compounds in plant-derived foods (*4*, *5*). Strawberries (*Fragaria* × *ananassa*) are consumed in high quantities and can thus be a valuable source of phenolic compounds. The phenolic compounds in strawberries are anthocyanins, responsible for the red color in strawberry flesh, flavonols, flavanols, and derivatives of hydroxycinnamic and ellagic acid (*6*−*8*) (**Figure 1**). Together with raspberries and blackberries, strawberries are the main dietary source of ellagic acid-containing compounds, that is, ellagic acid, ellagic acid glycosides, and ellagitannins (*9*).

There is an increasing awareness that health benefits of dietary polyphenols may be due to their role as modulators of cell signaling and gene expression, in addition to their antioxidative activities (*4*, *10*). In that context, the specific structures of the compounds are crucial. Moreover, as structure is important for bioavailability and metabolism (*4*, *11*), it is principal to analyze the components in their naturally occurring forms, without previous modification such as hydrolysis.

Polyphenols absorb in the UV region and have traditionally been analyzed by DAD after separation by HPLC (*12*). The polyphenolic compounds are then classified on the basis of their retention indices and characteristic UV−vis spectra; for example, flavanols have λ_{max} at 280 nm, hydroxycinnamic acid derivatives at 300−320 nm, anthocyanins at 500−520 nm, etc. Due to their reducing properties, that is, antioxidative activities, polyphenols can be analyzed by electrochemical detection. A coulometric detector, where the analyte flows through the porous electrodes, is highly sensitive (10−100-fold more sensitive than the UV detector) (*13*) and may be the detector of choice when the concentration of the analyte is low, as in bioavailability studies. Besides, information from the coulometric array detector may provide valuable information about chemical structure, because electrochemical response is directly related to the structure of the compound (*13*−*15*). In recent years a powerful tool for characterization, LC-MS, has become more common, and many

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polyphenolic compounds in fruits and berries, including strawberries, have been identified (*7*, *8*, *16*). MS detection provides information about the molecular mass and fragmentation pattern of the analyte. The combined use of ionization in both positive and negative modes gives extra certainty to the determination of the molecular mass. The MS results, however, are hard to interpret without having any additional information about the compound. As mentioned above, the UV−vis spectrum obtained by DAD indicates which phenolic class the compound belongs to, whereas responses from the coulometric array detector reflect differences in the substitution of oxidizable moieties. Thus, the different HPLC detectors provide complementary information about the analytes.

The purpose of the present study was to use the complementary information from different HPLC detectors, DAD, MS in negative and positive modes, and coulometric array, to identify and characterize phenolic compounds in strawberry fruits. Furthermore, electrochemical responses were used to estimate antioxidative properties of individual compounds and their contribution to overall antioxidant activity in strawberries.

MATERIALS AND METHODS

Chemicals. Gallic acid, (+)-catechin, ellagic acid, *p*-coumaric acid, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). Pelargonidin-3-glucoside and cyanidin-3-glucoside were obtained from Polyphenols AS (Sandnes, Norway). Proanthocyanidins B1 (EC-4,8-C) and B2 (EC-4,8-EC) were purchased from Extrasynthese S.A. (Genay, France). Quercetin-3-glucoside was obtained from Carl Roth GmbH (Karlsruhe, Germany). L-(+)-Ascorbic acid, acetic acid, and acetonitrile were obtained from Merck KGAa (Darmstadt, Germany). Water was of Milli-Q quality (Millipore Corp., Cork, Ireland).

Materials. Strawberry (*Fragaria* × *ananassa* Duch., Rosaceae, cultivar Senga Sengana) fruits grown in the western part of Norway were harvested at commercial ripeness in July 2004, single-frozen, and stored at −20 °C for 7 months until extraction.

Extraction of Phenolic Compounds. Whole strawberries (about 150 g, 15 berries) were liquid nitrogen milled, and three subsamples (5 g each) were withdrawn for extraction. Each subsample (5 g) was extracted with acetone (10 mL) by sonication for 10 min. After centrifugation (1500*g* for 10 min at 4 °C, Heraus Multifuge 4 KR, Kendro Laboratory Products GmbH, Hanau, Germany), the supernatant was collected and the insoluble plant material re-extracted twice with 70% acetone (10 mL). Acetone was removed from pooled extracts by a nitrogen flow at 37 °C (Pierce, Reacti-Therm III, Heating/Stirring Module, Rockford, IL). The volume of the extract was made up to 15 mL by water. The three strawberry extracts were stored at −80 °C for 5−7 months before analysis.

Chromatographic Separation. The extract was filtered through a Millex HA 0.45 μm filter (Millipore Corp.) before injection (20 μL). Chromatographic separation was performed on a Betasil C18 column (250 mm × 2.1 mm i.d., 5 μm particle size) equipped with a 5 μm C18 guard column (4.0 mm × 2.1 mm i.d.), both from Thermo Hypersil-Keystone (Bellefonte, PA). Column temperature was 25 °C. The mobile phases were (A) acetic acid/water (2:98, v/v) and (B) acetic acid/acetonitrile/water (2:50:48, v/v/v). The gradient used was 0−5 min, 2% B; 5−59 min, 2−38% B; 59−64 min, 38−100% B; 64−71 min, 100% B; 71−74 min, 100−2% B. The solvent flow rate was 0.25 mL/min. The column was allowed to equilibrate for 6 min between injections.

HPLC with DAD and MS Detection. The analyses were performed on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler cooled to 6 °C, a DAD scanning from 200 to 600 nm, and an MSD XCT ion trap mass spectrometer with an ESI interface. The LC eluate was introduced directly into the ESI interface without splitting. The phenolic compounds were analyzed in negative and positive ion modes. The nebulizer pressure was 40 psi; dry gas flow, 10 L/min; dry temperature, 350 °C; and capillary voltage, 3.5 kV. Analysis was carried out using scan from

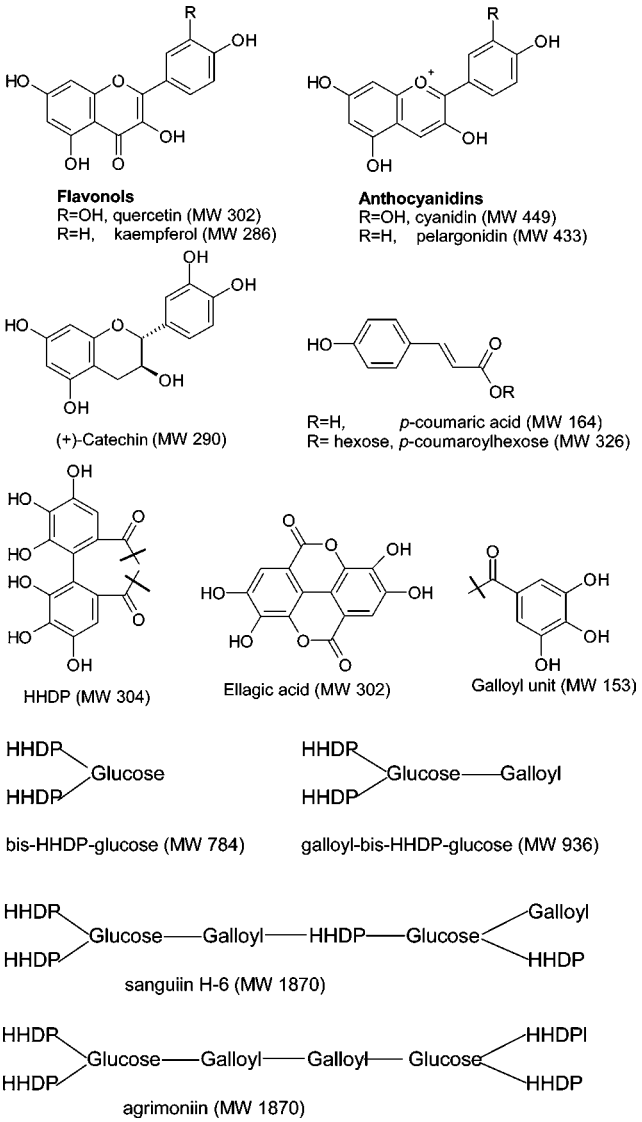

Figure 1. Structures of selected flavonols and anthocyanidins, (+)-catechin, *p*-coumaric acid, and some ellagitannins and their subunits. HHDP, hexahydroxydiphenoyl.

m/*z* 100 to 2200, with a scan speed of 27000 amu/s. Data-dependent fragmentation was performed with 50% energy with helium as collision gas. Enhanced scanning (zoom scan) analysis was performed to determine charge states of ellagitannins.

HPLC with Coulometric Array Detection. The HPLC conditions were as described above, except with 5 μL of sample injected. The coulometric array analyses were performed as previously described (*6*, *14*), using an HP 1050 series HPLC (Hewlett-Packard GmbH, Waldbronn, Germany) interfaced to an ESA coulometric array detector (ESA Inc., Chelmsford, MA) with eight porous graphite working electrodes with associated palladium reference electrodes. The detector array was set from 100 to 800 mV in increments of 100 mV. The ESA CoulArray operating software was used to collect voltammetric data. Raw data were processed using Microsoft Excel. The results were presented as cumulative peak areas at the electrode potentials. For characterization, responses independent of compound concentration (max normalized values) were plotted versus oxidation potential in hydrodynamic voltammograms (HDV).

Identification and Characterization of Phenolic Compounds. The phenolic compounds were characterized by their UV−vis spectra (220−600 nm), retention times relative to external standards, peak spiking, mass spectra and MS^2 and MS^3 fragmentation patterns, and HDVs from coulometric array detections. The characteristics were compared with the literature (*7*, *8*, *16*−*24*).

RESULTS AND DISCUSSION

Preliminary extraction experiments of phenolic compounds from strawberries using 80% methanol in water (v/v) and 70% acetone in water (v/v) showed that acetone extracts contained more polyphenols, especially of the ellagic acid containing compounds, of special interest in strawberries (results not presented). Aqueous acetone was therefore chosen for extraction in the present study. Previously, 70% acetone had been shown to be the most efficient and reproducible extraction solvent for anthocyanins in strawberries (*25*) and cause the highest yield of most classes of polyphenols in berries (*26*).

Characterization of Compounds by UV−Vis and Mass Spectra. The HPLC-DAD chromatograms of strawberry extract recorded at 260, 360, and 500 nm illustrate the increasing selectivity in detection at higher wavelengths (**Figure 2**). Most phenolic compounds absorb at 260 nm, giving a nonspecific chromatogram (**Figure 2A**). Ellagic acid derivatives and flavonols have maximum absorption around 360 nm (**Figure 2B**). However, hydroxycinnamic acids and anthocyanins also have some absorption at this wavelength. In the visible region of the spectra only the pigmented compounds, the anthocyanins, are detected (**Figure 2C**). The chromatogram obtained by MS detection in negative ionization mode (**Figure 3A**) was quite similar to the DAD chromatogram recorded at 260 nm, with the exception that the flavanols had a much higher response in MS than in DAD. The mass spectrum in positive mode (**Figure 3B**) was similar to the DAD chromatogram at 500 nm with the anthocyanins as the dominating compounds. The anthocyanins have maximum sensitivity in positive mode MS, due to their inherent positive charge. For most flavonoids, however, negative ionization mode provides the highest sensitivity (*27*). The chromatogram recorded with electrochemical detection at 300 mV reflected the compounds oxidized at low oxidation potential (**Figure 3C**). L-(+)-Ascorbic acid and the flavanols were prominent peaks at those conditions.

The phenolic compounds in strawberries were classified by comparison of their UV−vis spectra with spectra of available standards and reports from the literature (*6*, *7*, *12*, *18*) into flavonols, anthocyanins, flavanols, hydroxycinnamic and benzoic acid derivatives, ellagic acid glycosides, and ellagitannins (**Figure 1**). Identification of compounds within each class, based on chromatographic behavior, UV−vis and mass spectra, and comparison with literature, is discussed below and summarized in **Table 1**. No effort to identify peaks eluting before acetone (at 6.4 min) was made, except for the large peak eluting at 3.0 min, identified as L-(+)-ascorbic acid (peak 1).

Polyphenols in nature generally occur as conjugates of sugars, usually *O*-glycosides (*12*, *28*). In MS analysis, cleavage of the glycosidic linkage with concomitant H rearrangement leads to elimination of the sugar residue, that is, 162 amu (hexose; glucose or galactose), 146 amu (deoxyhexose; rhamnose), 132 amu (pentose; xylose or arabinose), and 176 amu (glucuronic acid). A useful observation is that the same distribution pattern of sugars and acids usually exists among the flavonoids, that is, flavonols and anthocyanins in strawberries. The most abundant sugar moiety in strawberries is glucose, and the most abundant acids are malonic and *p*-coumaric acid (*6*, *7*, *16*, *23*). The identity of the sugar moiety could not be determined by the methodology used in the present study; thus, designation of sugar moieties is based on literature reports for strawberries.

Flavonols. The flavonols identified in strawberries are derivatives of quercetin and kaempferol (*7*, *8*, *23*). The flavonols were classified on the basis of the shape of the UV−vis spectra with absorption maximum at about 354 nm for quercetin glycosides and about 348 nm for kaempferol glycosides (*12*).

Two peaks had absorption maxima at 354 nm, MS fragmentation ions at *m*/*z* 301 and 303 in negative and positive mode, respectively, and MS^3 fragmentation in negative mode that matched that of authentic standard quercetin (*m*/*z* 179 and 151) and were recognized as quercetin derivatives. Peak 37 with mass 478 and elimination of a glucurone unit (176 amu) during fragmentation was identified as quercetin-3-glucuronide, previously reported to be the major flavonol in strawberries (*7*, *23*). Peak 40, with mass 550, lost malonyl (86 amu) and malonylhexose (248 amu) during fragmentation. The fact that 3-malonylglucoside of pelargonidin was present in the sample and was identified as one of the major anthocyanins in strawberries (*6*) substantiated the identification of peak 40 as quercetin-3-malonylglucoside, not previously identified in strawberries. Malonylation of glycoconjugates is shown to be a pathway in the secondary metabolism of strawberries (*29*). Demalonylation of the thermally labile malonyl glycosides during processing or sample preparation may explain the low or nonexistent content reported in berries and berry products (*18*, *30*).

Three peaks were identified as kaempferol derivatives due to their UV spectra and MS fragmentation ions at *m*/*z* 285 and 287 in negative and positive mode MS, respectively. Peak 41, with mass 448, lost hexose (162 amu) during fragmentation and was probably kaempferol-3-glucoside, previously tentatively identified in strawberries (*7*, *23*). Peak 42 had $[M - H]^-$ at *m*/*z* 533, with fragments at *m*/*z* 489 (loss of CO_2). The fragmentation of isolated *m*/*z* 489 gave ions at *m*/*z* 285 (loss of acetylhexose). This compound could thus be kaempferol-3-malonylglucoside, previously tentatively identified in strawberries (*8*, *21*). A late-eluting compound, peak 44, had maximum absorption at short wavelength (314 nm), indicating that the sugar moiety on this flavonol was acylated with a hydroxycinnamic acid (*12*). MS in negative mode caused ions at *m*/*z* 593 ($[M - H]^-$) and MS^2 fragments at *m*/*z* 531 (loss of 62 amu), 447 (loss of coumaroyl), and 285 (loss of coumaroylhexose). The $[M + H]^+$ at *m*/*z* 595 fragmented to *m*/*z* 309 (loss of kaempferol), 287 (loss of coumaroylhexose), and 165 (*p*-coumaric acid). The MS^3 fragments of the isolated *m*/*z* 309 consisted of *m*/*z* 291 (loss of water) and 147 (coumaroyl, loss of hexose). Thus, this compound was assigned as kaempferol-coumaroylglucoside, previously reported in strawberries (*8*).

Anthocyanins. The red color of strawberries is due to anthocyanins in their flavylium forms. The anthocyanins in strawberries are glycosides of pelargonidin (λ_{max} at 502 nm) and cyanidin (λ_{max} at 516 nm) (*6*−*8*, *16*). The chromatographic conditions in the present study were not optimized for analyses of anthocyanins, and the anthocyanin peaks in the chromatograms were broad (**Figures 2** and **3**). However, identification based on chromatographic retention (including coelution with cyanidin- and pelargondin-3-glucoside), UV−vis and mass spectra, and comparison with previous findings (*6*−*8*, *16*, *19*) was possible. For the anthocyanins it is referred to results from positive mode MS only.

Four peaks had absorption maxima at 502 nm, a shoulder in the 410−450 nm region, and MS fragmentation ions at *m*/*z* 271 and were identified as derivatives of pelargonidin. Peak 24 with $[M]^+$ at *m*/*z* 433 and subsequent loss of 162 amu (hexose) was pelargonidin-3-glucoside, the major anthocyanin in strawberries. Peak 26, with $[M]^+$ at *m*/*z* 579 and loss of 146 amu (deoxyhexose) and 308 amu (deoxyhexose-hexose) upon fragmentation, was assigned as pelargonidin-3-rutinoside. Two less polar

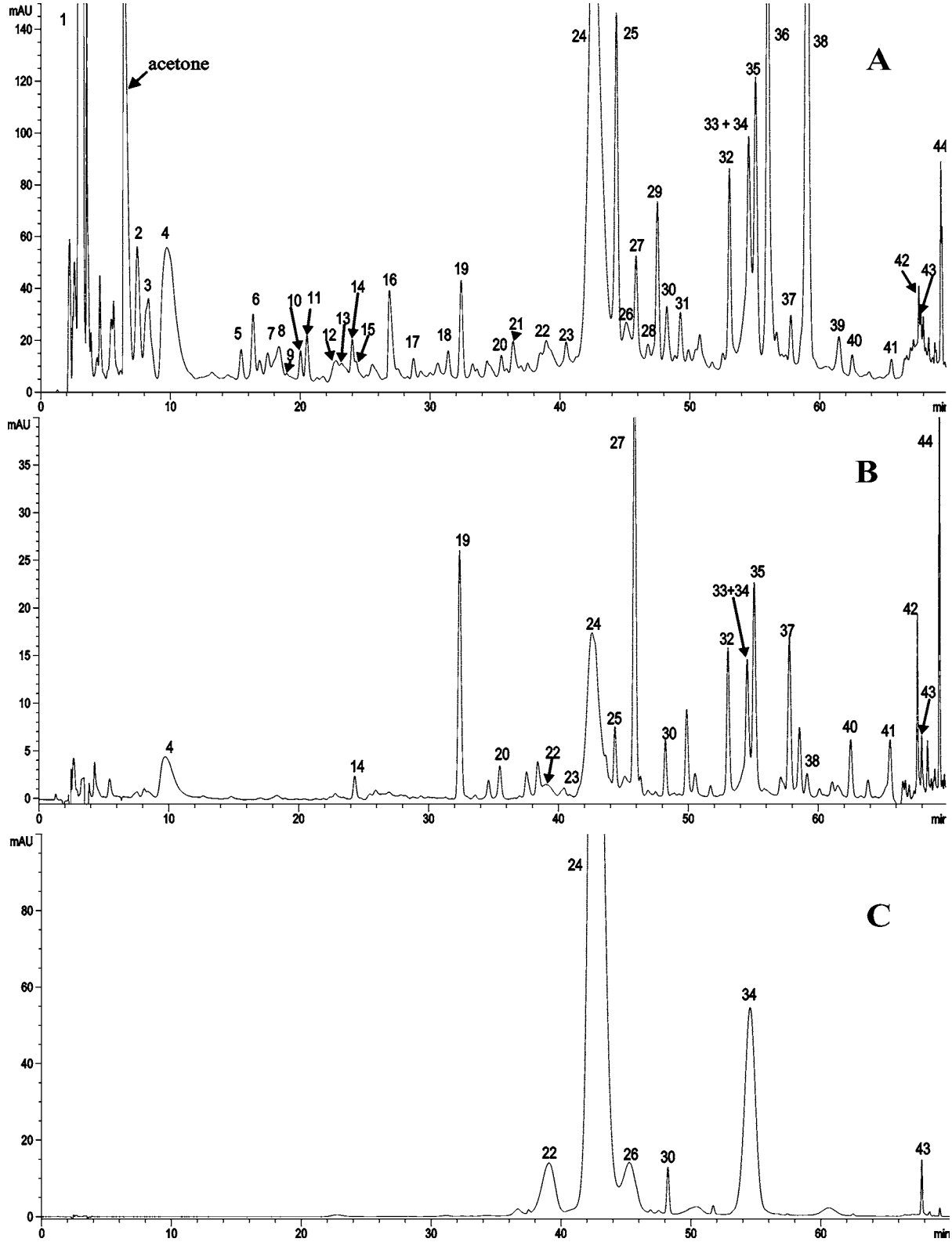

Figure 2. HPLC-DAD chromatograms of polyphenols in strawberry fruits recorded at (**A**) 260 nm, (**B**) 360 nm, and (**C**) 500 nm. Peak numbers refer to **Table 1**.

pelargondin glycosides, peaks 34 and 43, had $[M]^+$ at *m*/*z* 519 and 503, respectively. During fragmentation peak 34 lost 248 amu (malonylhexose) and was identified as pelargonidin-3-malonylglucoside. Peak 43 lost 232 amu (malonyldeoxyhexose or succinylpentose) during fragmentation, suggesting the presence of either pelargonidin-3-malonylrhamnoside or -3-succinylarabinoside, as indicated in a recent publication (*16*). Peak 30, with absorption maximum at low wavelength (492 nm) and $[M]^+$ at *m*/*z* 501, with MS^2 ions at *m*/*z* 339 (loss of hexose) and MS^3 ions at *m*/*z* 295 (loss of CO_2), was assigned as the pyrano form of pelargonidin-3-glucoside, previously isolated from strawberries (*19*) and detected in three strawberry cultivars (*16*). The extraction solvent in the present study was acetone, shown to react with anthocyanins to produce pyranoanthocyanins (*31*). In the present study, however, the pyranopelargonidin was also present in methanol extracts (from the preliminary

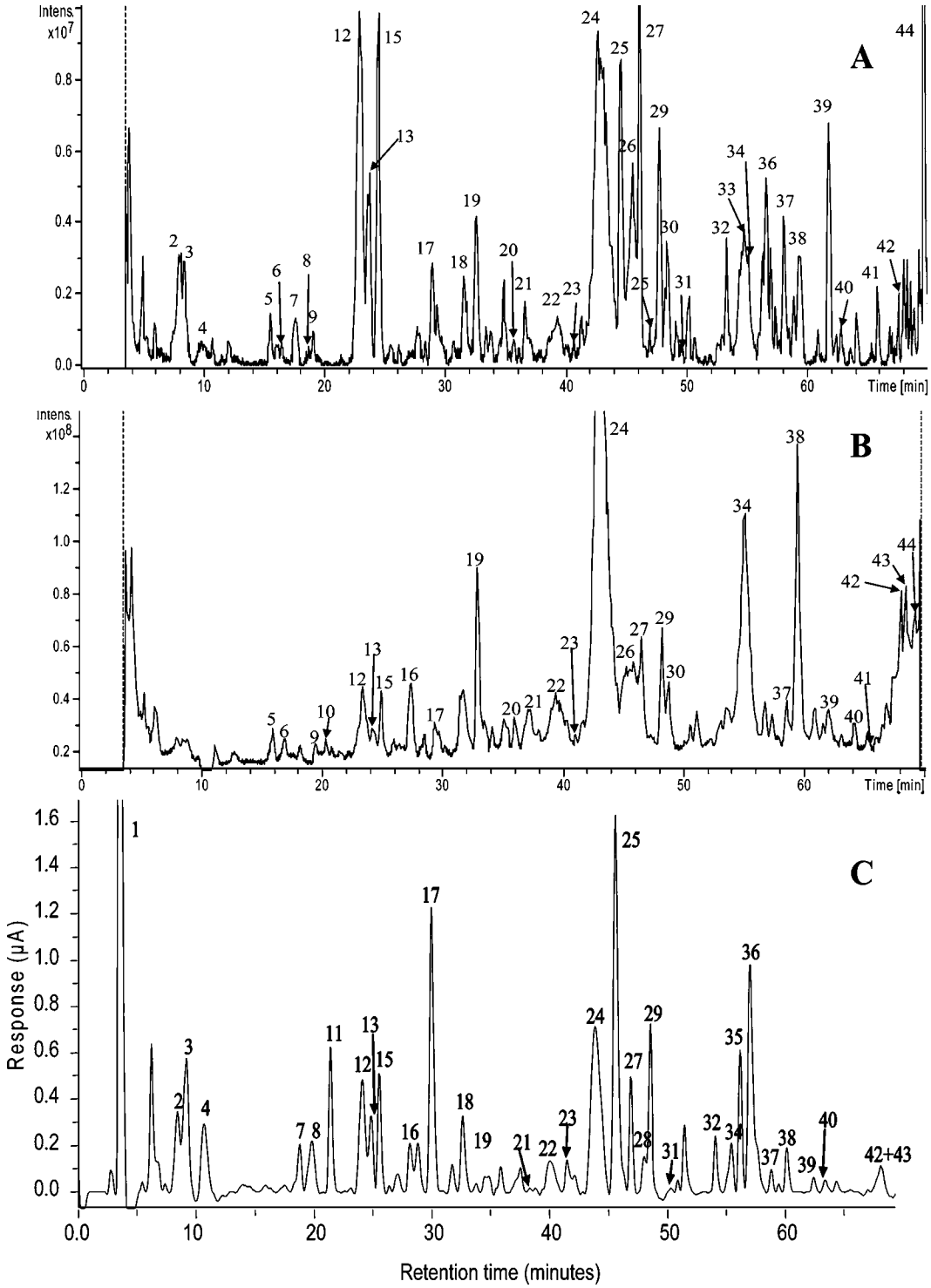

Figure 3. HPLC chromatograms of polyphenols in strawberry fruits detected with (**A**) MS in negative mode, (**B**) MS in positive mode, and (**C**) coulometric array detector at 300 mV. The early-eluting compounds are not depicted in the MS chromatogram. Peak numbers refer to **Table 1**.

extraction experiments) and in juice pressed from the strawberries and was thus believed not to be an artifact from the reaction between the anthocyanin and the extraction solvent. Peak 22 had absorption maximum at 516 nm and molecular ions at *m*/*z* 449, with fragments at *m*/*z* 287 (loss of hexose), and was identified as cyanidin-3-glucoside.

Flavanols. Flavanols have low UV extinctions and maximum absorptions in a region of the UV spectrum (about 280 nm) with many interfering compounds (*12*, *22*). The flavanols, however, have high response in MS. LC-MS analysis revealed that several compounds coeluted in the region of the chromatogram where the flavanols occurred (20−30 min) and that the flavanols were partly hidden in the UV−vis chromatogram by compounds with higher absorbance (**Figures 2A** and **3A**). Mass spectra and fragmentation patterns of the flavanols were compared with those of authentic standards of (+)-catechin and the dimeric proanthocyanidins B1 (EC-4,8-C) and B2 (EC-4,8-EC) (both MW 578).

Peak 17 had $[M - H]^-$ at *m*/*z* 289 and $[M + H]^+$ at *m*/*z* 291 and was identified as (+)-catechin (MW 290) after peak spiking.

Table 1. Characterization of Phenolic Compounds in Acetone Extracts of Strawberry Fruits Using HPLC with Diode Array and Electrospray Ionization MS^n Detection

peak	t_R (min)	λ_{max}[a] (nm)	MW	MS (*m*/*z*); ID	MS^2 ions (*m*/*z*)[b]	MS^3 ions (*m*/*z*)[c]	tentative identification
1	3.0	244	176	175; $[M-H]^-$	115	87	L-(+)-ascorbic acid
2	7.5	232	1580	789; $[M-2H]^{2-}$	1233, 947, **775**, 631	1233, 917, 873, **753**, 633	ellagitannin
3	8.3	232	784	783; $[M-H]^-$	481, **301**	257, 229, 185	bis-HHDP-glucose
				391; $[M-2H]^2$			
4	9.7	232	948	947; $[M-H]^-$	929, **901**	**883**, 871, 599	ellagitannin
5	15.4	302sh	760	759; $[M-H]^-$	**579**, 390, 368	**368**, 270	unknown
		256sh		368; $[M-H-391]^-$	188, **179**, 144	**161**, 143, 119	
		230		392; $[M+H-369]^+$	**230**, 203	212, 168	
6	16.4	262	300	299; $[M-H]^-$	239, 179, **137**		hydroxybenzoylhexose
				323; $[M+Na]^+$	185		
7	17.5	232	784	783; $[M-2H]^{2-}$	481, **301**	257, 229, 185	bis-HHDP-glucose
				391; $[M-H]^-$			
8	18.3	232	634	633; $[M-H]^-$	**481**, 301	**301,** 275	galloyl-HHDP-glucose
9	19.0	312	488	487; $[M-H]^-$	325	187, **163**, 145, 119	*p*-coumaroylhexose-4-*O*-hexoside
				511; $[M+Na]^+$	349	185	
10	20.0	280	290	313; $[M+Na]^+$	285, **185**, 151		unknown
11	20.5	288		ND			unknown
12	22.8	310	578	577; $[M-H]^-$	451, **425**, 407	407	proanthocyanidin B1 (EC-4,8-C)
		286		579; $[M+H]^+$	453, **427**, 409, 301	**407**, 301	
13	23.5	284	866	865; $[M-H]^-$	739, **695**, 577	677, **543**, 451, 405	proanthocyanidin trimer
				867; $[M+H]^+$	715, **579**, 427	**409**	(EC-4,8-EC-4,8-C)
14	24.0	266		ND			unknown
15	24.3	312sh	578	577; $[M-H]^-$	451, **425**, 407	407	proanthocyanidin B3 (C-4,8-C)
		284		579; $[M+H]^+$	453, **427**, 409, 301	**407**, 301	
16	26.9	280 (broad)	–[d]	205; $^+$	188	**146**, 118	unknown
17	28.7	280	290	289; $[M-H]^-$	**245**, 205	227, **203**	(+)-catechin
				291; $[M+H]^+$	**139**, 123		
18	31.4	300sh	634	633; $[M-H]^-$	**301**, 463	257, 229, 185	HHDP-galloyl-glucose
		284					
19	32.4	316	326	325; $[M-H]^-$	265, 187, **163**, 145	119	*p*-coumaroylhexose
				675; $[2M+Na]^+$	349		
20	35.5	316	326	325; $[M-H]^-$	265, 187, **163**, 145	119	*p*-coumaroylhexose
				675; $[2M+Na]^+$	349		
21	36.4	280 (broad)	704	703; $[M-H]^-$	567	549, **473**, 447, 405	unknown
				705; $[M+H]^+$	543	525, 407, 313	
22	39.1	516	449	449; $[M]^+$	287		cyanidin-3-glucoside
		280					
23	40.5	278sh	450	449; $[M-H]^-$	287, **269**		structure similar to cyanidin-3-glucoside[e]
		232		451; $[M+H]^+$			
24	42.6	502	433	433; $[M]^+$	271		pelargonidin-3-glucoside
		428sh					
		328					
		278					
25	44.3	234	936	935; $[M-H]^-$	633	463, **301**	galloyl-bis-HHDP-glucose
				467; $[M-2H]^{2-}$	633, **391**, 301	301	
26	45.3	502	579	579; $[M]^+$	433, **271**		pelargonidin-3-rutinoside
		432sh					
		328					
		278					
27	45.9	338	450	449; $[M-H]^-$	431, **355**, 329, 269, 193	193	ferulic acid hexose derivative[e]
		473		473; $[M+Na]^+$	**455**, 311	293	
		232					
28	46.8	280	936	935; $[M-H]^-$	633	463, **301**	galloyl-bis-HHDP-glucose
		232		467; $[M-2H]^{2-}$	633, **391**, 301	301	
29	47.5	270sh	434	433; $[M-H]^-$	271, 253		structure similar to pelargonidin-3-glucoside[e]
		242		867; $[2M-H]^-$	433	271, 253	
				435; $[M+H]^+$	273, 271		
30	48.2	492	501	501; $[M]^+$	339	295	5-pyranopelargonidin-3-glucoside
		358					
		262sh					
31	49.3	270sh	–[f]	679; $^-$	664	1567, 1265, **935**, 897, 622, 529, 467	ellagitannin
		232					
32	53.1	360	434	433; $[M-H]^-$	301	300, 257, 229, 185	ellagic acid pentoside
		254					
33	54.5	362	448	447; $[M-H]^-$	301	300, 257, 229, 185	ellagic acid deoxyhexoside
		254					

Table 1. (Continued)

peak	t_R (min)	λ_{max}[a] (nm)	MW	MS (*m*/*z*); ID	MS^2 ions (*m*/*z*)[b]	MS^3 ions (*m*/*z*)[c]	tentative identification
34	54.6	502 430sh 268	519	519; $[M]^+$	433, **271**		pelargonidin-3-malonylglucoside
35	55.1	368 254	302	301; $[M-H]^-$	257, 229, 185		ellagic acid
36	56.0	234	1870	934; $[M-2H]^{2-}$	1567, 1265, 1085, **897**, 783, 633	745	dimer of galloyl-bis-HHDP (sanguiin H-6 or agrimoniin)
37	57.8	354 258	478	477; $[M-H]^-$ 479; $[M+H]^+$	301 303	179, 151 229, 165	quercetin-3-glucuronide
38	59.0	284	310	369; $[M+OAc]^-$ 643; $[2M+Na]^+$	309 333	147 **185**, 171	unknown
39	61.4	286	716	715; $[M-H]^-$ 381; $[M+2Na]^{2+}$ 739; $[M+Na]^+$	357 **593**, 378, 185	195	unknown
40	62.5	354 256	550	549; $[M-H]^-$ 551; $[M+H]^+$	463, **301** 303	179, 151 229, 165	quercetin-3-malonylglucoside
41	65.5	348 266	448	447; $[M-H]^-$ 449; $[M+H]^+$	285 287		kaempferol-3-glucoside
42	67.6	346 266	534	533; $[M-H]^-$ 535; $[M+H]^+$	489	285	kaempferol-3-malonylglucoside
43	67.8	506 430sh 280	503	503; $[M]^+$	271		pelargonidin-3-malonylrhamnoside or -3-succinylarabinoside
44	69.3	314 268	594	593; $[M-H]^-$ 595; $[M+H]^+$	531, 447, **285** **309**, 287, 165	 291, 165, **147**	kaempferol-3-coumaroylglucoside

[a] sh, shoulder in the spectrum. [b] The most abundant ions are shown in bold. These ions are isolated for fragmentation. [c] The most abundant ions are shown in bold. [d] It was not possible to determine whether the ions were protonated or sodium adducts. [e] Additional information from the hydrodynamic voltammogram (HDV) made the suggested identification plausible. [f] It was not possible to determine the charge state of the ions.

Peaks 12 and 15 had $[M-H]^-$ at *m*/*z* 577 and $[M+H]^+$ at *m*/*z* 579 and main MS^2 fragmentation with loss of 152 amu, a characteristic loss due to retro Diels−Alder fission (*32*), and were recognized as proanthocyanidin dimers, even if the UV spectra showed interferences with coeluting compounds. Peak 12 was identified as B1 (EC-4,8-C) after peak spiking with authentic standard. Peak 15 probably contained B3 (C-4,8-C), the most abundant flavanol after (+)-catechin in Spanish strawberries (*22*). Peak 13 had $[M-H]^-$ at *m*/*z* 865 and $[M+H]^+$ at *m*/*z* 867, with fragmentation pattern in positive mode in accordance with that of flavanols (*22*, *32*), indicating the presence of a proanthocyanidin trimer (MW 866), most likely EC-4,8-EC-4,8-C previously detected in strawberries (*22*).

Hydroxycinnamic Acid Derivatives. In agreement with previous results, *p*-coumaroyl esters were detected in the strawberries (*7*, *8*). *p*-Coumaric acid (MW 164) has maximum absorption at 311 nm. Esterification with sugar causes a bathochromic shift, whereas glycosylation of the hydroxy group in *p*-coumaric acid causes a hypsochromic shift (*18*).

Peak 9 (λ_{max} at 312 nm) had $[M-H]^-$ at *m*/*z* 487, with MS^2 fragments at *m*/*z* 325 (loss of hexose). The main MS^3 ions were at *m*/*z* 163 (loss of hexose). In positive mode *m*/*z* 511 ($[M+Na]^+$) was found. In MS^2, *m*/*z* at 349 (loss of hexose) gave the major signal. The MS^3 fragmentation spectrum consisted of sodium adducts of hexose (*m*/*z* 185), previously reported for hydroxycinnamic acids (*18*). The compound, consisting of *p*-coumaric acid and two hexose units, was probably *p*-coumaroylhexose-4-*O*-hexoside and not *p*-coumaroyldihexose ester, because the expected fragment from a disaccharide (324 amu) was not observed. The major hydroxycinnamic acid derivative, peak 19 (λ_{max} at 316 nm), had $[M-H]^-$ at *m*/*z* 325. The main MS^2 fragments were at *m*/*z* 163 (loss of hexose). When the instrument was operated in positive mode, a dimer ($[2M+Na]^+$) at *m*/*z* 675 caused the largest signal. Isolation of this ion for further fragmentation gave products at *m*/*z* 349 (loss of 326 amu). The compound was identified as a hexose ester, probably the glucose ester of *p*-coumaric acid. Peak 20 had spectra similar to those of peak 19, indicating the presence of a second *p*-coumaroylhexose.

Hydroxybenzoic Acid Derivatives. Hydroxybenzoic acids have been reported in strawberries (*24*, *26*), but not detected by LC-MS (*7*, *8*, *21*), probably due to the low sensitivity of phenolic acids in LC-MS analysis (*33*).

Peak 6 had $[M-H]^-$ at *m*/*z* 299 with MS^2 ions at *m*/*z* 239 (loss of 60 amu), 179 (loss of 120 amu), and 137 (loss of hexose). $[M+Na]^+$ was at *m*/*z* 323, indicating a molecular mass of 300 of this compound. MS^2 fragments at *m*/*z* 185 (loss of hydroxybenzoic acid) could be sodium adducts of hexose, previously observed in the fragmentation of hexose esters of phenolic acids (*18*). The mass spectral data and UV maxima (262 nm) were consistent with a compound in *Ribes* assumed to be a hexose ester of *p*-hydroxybenzoic acid (*18*), and peak 6 was thus assigned as a hydroxybenzoylhexose.

Ellagic Acid and Ellagic Acid Glycosides. Ellagic acid and its glycosides were distinguished by their characteristic UV−vis spectra with absorption maxima at 254 and 360−368 nm (*6*, *7*).

Peak 35 had $[M-H]^-$ at *m*/*z* 301 and MS^2 fragmentation ions in negative mode at *m*/*z* 257, 229, and 185, typical fragments of ellagic acid (*20*). On the basis of coelution and comparison of UV and mass spectra with authentic standard, this compound was identified as ellagic acid (MW 302). Peaks 32 and 33 had $[M-H]^-$ at *m*/*z* 433 and 447, respectively. MS^2 products for both ions were at *m*/*z* 301, with further fragmentation as for ellagic acid. Peaks 32 and 33 were identified as a pentoside and a deoxyhexoside of ellagic acid, respectively. No ellagic acid glycosides were reported in strawberries by Määttä-Riihinen et al. (*7*), and Seeram et al. (*8*) observed only methyl ellagic acid pentosides in strawberries. In a very recent paper, however, an ellagic acid deoxyhexoside

was detected in strawberry leaves, and an ellagic acid pentoside was detected in the fruits, as well (*21*). In the UV chromatogram, peak 33 was hidden by the coeluting, highly UV−vis absorptive anthocyanin, pelargonidin-3-malonylglucoside (peak 34); however, the MS^n spectra in negative mode were characteristic for an ellagic acid containing compound.

Ellagitannins. Ellagitannins are together with the anthocyanins the most abundant phenolic compounds in strawberries (*6*, *7*, *26*). Ellagitannins are hydrolyzable tannins, consisting of a polyol core, usually glucose, esterified with hexahydroxydiphenic acid(s) (HHDP) and in some cases gallic acid (*9*, *34*). The structures vary from simple monoesters to complex polyesters (**Figure 1**). Ellagitannins have characteristic UV spectra with maxima only below 280 nm (*6*, *7*). In negative ion mode ESI-MS, ellagitannins are shown to give $[M - 2H]^{2-}$ or $[2M - H]^-$ ions in addition to the deprotonated molecule $[M - H]^-$ (*17*, *35*). Typical losses during fragmentation are galloyl (152 amu), HHDP (302 amu), galloylglucose (332 amu), HHDP-glucose (482 amu), and galloyl-HHDP-glucose (634 amu). In our study, as in previous studies, no ionization was obtained in positive mode MS (*17*).

Peak 2 had $[M - 2H]^{2-}$ at *m*/*z* 789, that is, a mass of 1580. The major MS^2 fragments were double-charged ions at *m*/*z* 775 (loss of 28 amu), with minor single-charged fragments at *m*/*z* 1233 (loss of 346 amu), 947 (loss of 632 amu), and 631 (loss of 948 amu). Fragmentation of *m*/*z* 775 caused single-charged fragments at *m*/*z* 1233 (loss of 318 amu), 917 (loss of galloyl-HHDP-glucose), 873 (loss of 678 amu), and 633 (loss of 918 amu). The major signal in MS^3 was caused by the doubled-charged ions at *m*/*z* 753 (loss of 44 amu). A loss of 44 amu from hydrolyzable tannins is characteristic of a free carboxyl, for example, on a trisgalloyl group, as sanguisorboyl or valoneoyl (*35*). It was not possible to further identify this highly hydrophilic compound consisting of one unit with mass 632 (galloyl-HHDP-glucose − 2H) and one unit with mass 948. Peak 4 also had a constituent with mass 948 (*m*/*z* at 947). During fragmentation only small molecules (18, 28, 30, and 46 amu) were lost, with one exception; ions at *m*/*z* 599 were formed during fragmentation of *m*/*z* 947 (loss of 348 amu), 929 (loss of 330 amu), and 901 (loss of 302 amu, possibly HHDP). Peaks 3 and 7 with $[M - H]^-$ at *m*/*z* 783 and $[M - 2H]^{2-}$ at *m*/*z* 391 and MS^2 products of isolated *m*/*z* 783 at *m*/*z* 481 (loss of HHDP) and 301 (loss of HHDP-glucose) were assigned bis-HHDP-glucoses (juglanin or pedunculagin), previously found in strawberry leaves (*34*) and indicated in strawberries (*8*). Peak 8 had $[M - H]^-$ at *m*/*z* 633 and main MS^2 fragments at *m*/*z* 481 (loss of galloyl) and minor signals at *m*/*z* 301 (loss of galloylglucose). Loss of a galloyl unit suggested that the galloyl unit most probably was bonded via a *m*-depside bond, and not attached directly to the glucose core (*17*); thus, the compound could be a *m*-galloyl-HHDP-glucose. Peak 18 had, as peak 8, $[M - H]^-$ at *m*/*z* at 633. The fragmentation, however, was different, with main fragments at *m*/*z* 301 (loss of galloylglucose) and minor signals at *m*/*z* 463 (loss of gallic acid). Loss of gallic acid indicated that the galloyl unit was attached directly to the glucose, because a galloyl bond via a *m*-depside bond is more cleavable and would give loss of a galloyl unit (152 amu) (*17*). Accordingly, peak 18 was probably an HHDP-galloylglucose. Peaks 25 and 28 had $[M - H]^-$ at *m*/*z* 935 and $[M - 2H]^{2-}$ at *m*/*z* 467. The main products of *m*/*z* 935 were ions at *m*/*z* 633 (loss of HHDP), which fragmented further to produce *m*/*z* 301 (loss of galloylglucose). The main products of *m*/*z* 467 were the $[M - 152 - 2H]^{2-}$ ions (*m*/*z* 391). Peaks 25 and 28 were thus identified as galloyl-bis-HHDP-glucoses, probably casuarictin (β-glucoside) and potentillin (α-glucoside), respectively, previously identified in strawberry leaves (*21*, *34*). Peak 36 had $[M - 2H]^{2-}$ at *m*/*z* 934, giving a mass of 1870. Fragmentation of the double-charged ions gave a sequence of single-charged products: *m*/*z* at 1567 (loss of HHDP), 1265 (loss of bis-HHDP), 1085 (loss of bis-HHDP-glucose), 897 (MS^3 at *m*/*z* 745, loss of galloyl), 783 (loss of tri-HHDP-glucose), and 633 (loss of tri-HHDP-galloyl-glucose). The fragmentation pattern matched that of a dimer of galloyl-bis-HHDP-glucose, as agrimoniin, the main peak in the chromatogram of strawberry leaves (*21*) or sanguiin H-6, the major ellagitannin in raspberry fruits (*20*, *36*) (**Figure 1**). Isomers containing galloyl-bis-HHDP-glucose have been detected in strawberry fruits, as well (*7*, *8*). Peak 31 had MS signals at *m*/*z* 679, with MS^2 fragments at *m*/*z* 664. The MS^3 fragmentation ions (*m*/*z* at 1567, 1265, 935, 897, 622, 529, and 467) were similar to the fragmentation obtained for peaks 25, 28, and 36, indicating an ellagitannin with related structure. It was not possible to determine the charge state of the ions at *m*/*z* 679. However, considering the product ions, the parent ions were at least triple-charged, that is, the mass was 2040 or higher.

Partly Identified and Nonidentified Compounds. The compounds described below were characterized as far as possible using information from the UV−vis and mass spectra. Most of the nonidentified compounds had absorption maxima at ca. 280 nm, whereas there were no shared MS characteristics.

Peak 5 was probably labile and was completely decomposed during ionization in positive mode and partly decomposed in negative mode. The mass spectral data indicated that one part of the molecule (MW 391) was ionized in positive mode, whereas the other part (MW 369) and the intact molecule (MW 760) were ionized in negative mode. Ionization of peak 10 in positive mode MS gave ions at *m*/*z* 313, with MS^2 fragments at *m*/*z* 285 (loss of 28 amu), 185 (loss of 128 amu), and 151 (loss of 162 amu). The ions were believed to be sodium adducts; that is, the analyte had mass 290 and consisted probably of hexose and a unit with mass 128. Peak 21 had $[M - H]^-$ at *m*/*z* 703, with products at *m*/*z* 567 (loss of 136 amu). Products of isolated *m*/*z* 567 were at *m*/*z* 549 (loss of water), 473 (loss of 94 amu), 447 (loss of 120 amu), and 405 (loss of 162 amu). In positive mode $[M + H]^+$ was at *m*/*z* 705, with MS^2 fragments at *m*/*z* 543 (loss of 162 amu) and MS^3 fragments at *m*/*z* 525 (loss of water), 407 (loss of 136 amu), and 313 (loss of 230 amu). The compound with mass 704 thus had constituents with masses of 136 and 162 (hexose).

According to mass spectral data, peaks 23 and 29 had structural features similar to those of cyanidin- and pelargondin-3-glucoside, respectively. Peaks 23 and 29, however, had no absorbance in the visible region of the UV−vis spectra, meaning the anthocyanin structure with conjugated double bonds was interrupted. Peak 23 eluted shortly after cyanidin-3-glucoside and had signals in negative mode MS at *m*/*z* 449 ($[M - H]^-$) and in positive mode MS at *m*/*z* 451 ($[M + H]^+$) and 473 ($[M + Na]^+$). Peak 29, being slightly less polar than pelargondin-3-glucoside, had signals in negative mode MS at *m*/*z* 433 ($[M - H]^-$) and 867 ($[2M - H]^-$). In positive mode, ions at *m*/*z* 435 ($[M + H]^+$) were found. Compounds **23** and **29** with masses of 450 and 434, respectively, both had losses of 162 and 180 amu from the deprotonated molecule in negative mode. Cleavage of the glycosidic linkage of a flavonoid 3-*O*-hexoside leads to loss of 162 amu (hexose − H_2O), as observed for anthocyanins and flavonols. The mass loss of 180 amu

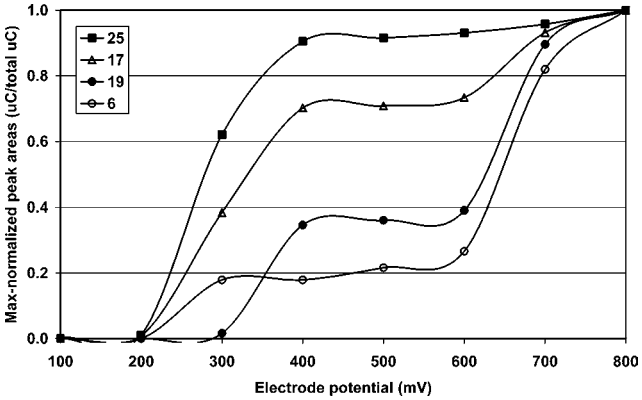

Figure 4. HDVs of selected phenolic compounds in strawberry fruits: galloyl-bis-HHDP-glucose (peak 25) (■); (+)-catechin (peak 17) (△); *p*-coumaroylhexose (peak 19) (●); hydroxybenzoylhexose (peak 6) (○). Peak numbers refer to **Tables 1** and **2**.

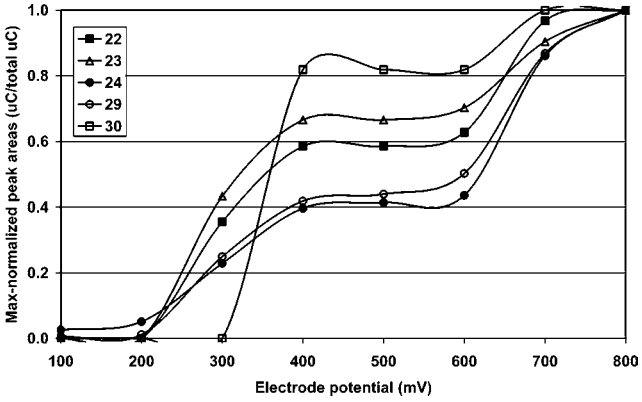

Figure 5. HDVs of anthocyanins and compounds with related structures: 5-pyranopelargonidin-3-glucoside (peak 30) (□); peak 23 (△); cyanidin-3-glucoside (peak 22) (■); peak 29 (○); pelargonidin-3-glucoside (peak 24) (●). Peak numbers refer to **Tables 1** and **2**.

is consistent with that of hexose without concomitant elimination of water. To achieve this, the aglycon must, somehow, have available hydrogen necessary for this mechanism. Mass spectra showed that the molecular weights of compounds **23** and **29** were 1 mass unit higher than that of cyanidin- and pelargondin-3-glucoside, respectively. The compounds could be equilibrium forms of cyanidin- and pelargondin-3-glucoside, although HPLC-DAD-MS analysis of authentic standards did not exhibit new peaks with these mass spectral characteristics.

Peak 27 had $[M - H]^-$ at *m*/*z* 449. Upon MS^2 fragmentation a major loss of 94 amu and also losses of 120 and 180 amu were observed. MS^3 fragmentation of *m*/*z* 355 gave fragments at *m*/*z* 193 (loss of hexose). In positive mode, fragmentation caused loss of water and hexose. The UV spectrum of peak 27 was consistent with a derivative of ferulic acid. In an earlier study traces of ferulic acid glucosides were detected in two strawberry cultivars (*24*). Possible constituents of the compound with mass 450 could be ferulic acid (MW 194), hexose, and an unknown with mass 94, maybe phenol. In *Ribes* species a compound with similar UV−vis and mass spectral data was assigned as a ferulic acid hexose derivate, consisting of an unknown with mass 96 in addition to ferulic acid and hexose (*18*).

Two late-eluting compounds had similar UV−vis spectra. Peak 38 had MS signals at *m*/*z* 369 ($[M + OAc]^-$), with MS^2 fragments at *m*/*z* 309 (loss of acetic acid) and MS^3 fragments

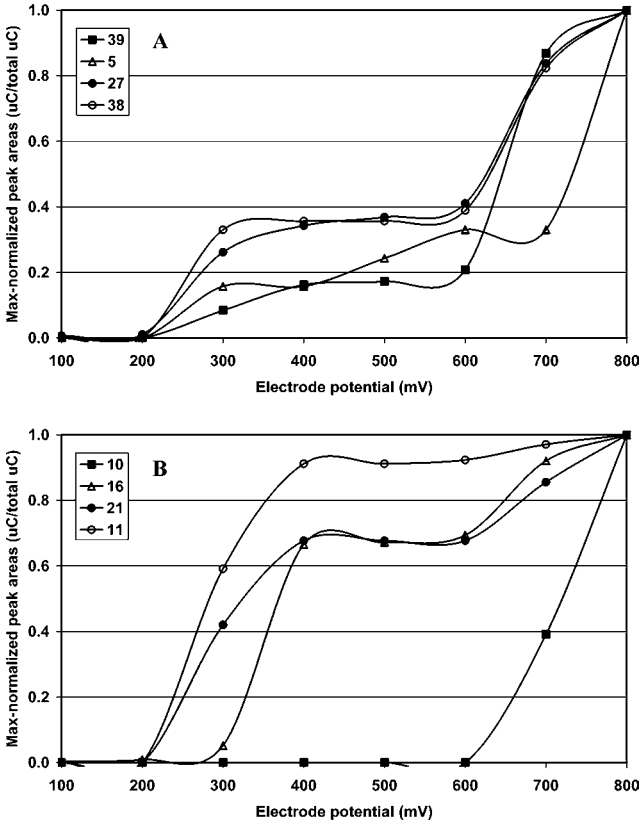

Figure 6. HDVs of partly identified or nonidentified compounds in strawberry fruits: (**A**) peak 39 (■); peak 5 (△); peak 27 (●); peak 38 (○); (**B**) peak 10 (■); peak 16 (△); peak 21 (●); peak 11 (○). Peak numbers refer to **Tables 1** and **2**.

at *m*/*z* 147 (loss of hexose). In MS positive mode, signals at *m*/*z* 643 ($[2M + Na]^+$) occurred. MS^2 fragments ions were at *m*/*z* 333 (loss of 310 amu) and MS^3 fragments at *m*/*z* 185 (loss of 148 amu). This compound with mass 310 probably contained a hexose and an unknown with mass 148. Peak 39 had $[M - H]^-$ at *m*/*z* 715, with MS^2 fragments at *m*/*z* 357 (loss of 358 amu) and MS^3 fragments at *m*/*z* 195 (loss of hexose). In positive mode *m*/*z* 739 ($[M + Na]^+$) and *m*/*z* 381 ($[M + 2Na]^{2+}$) were found. MS^2 of the double-charged ions produced a sequence of single-charged fragments: *m*/*z* 593 (loss of 146 amu), 378 (loss of 361 amu), and 185 (loss of 554 amu). The compound with mass 716 consisted probably of two equal parts, each containing a hexose bond to a compound with mass 196.

Characterization of Compounds by Coulometric Array Responses. When an analyte passes through the cells in the coulometric array, it is oxidized in a stepwise fashion, according to the increasing oxidation potential in the array. The response plotted against oxidation potential, the HDV, reflects oxidizable moieties of the molecule. Electrochemical profiles, the HDVs of maximum normalized responses, of representative compounds from different classes of phenolics in strawberries are shown in **Figure 4**. Adjacent hydroxy groups, as in catechol (1,2-dihydroxy) and pyrogallol (1,2,3-trihydroxy), stabilize the phenoxy radical and lead to lower oxidation potential of the compound (*13*, *14*). The ellagitannin (peak 25), with several pyrogallol groups, was thus oxidized at the lowest oxidation potential, followed by (+)-catechin (peak 17) containing a catechol group. Both *p*-coumaroylhexose (peak 19) and hydroxybenzoylhexose (peak 6) possess a monohydroxy substituent. However, the hydroxybenzoylhexose had a lower first oxidation potential because the carboxylic acid substituent makes resonance delocalization possible. HDVs of the anthocyanins

Table 2. Electrochemical Responses of Peaks in the Chromatogram Given as Cumulative Peak Areas, Proportion of the Compound Oxidized at Certain Oxidation Potentials, and Contribution to Total Electrochemical Response in Strawberry Extract at 300, 400, and 800 mV

		cumulative peak areas (μC) at		proportion of the compound oxidized at		contribution (%) to total electrochemical response at		
compound	peak	400 mV	800 mV	300 mV	400 mV	300 mV	400 mV	800 mV
L-(+)-ascorbic acid	1	264	277	0.8	0.9	33	24	16
ellagitannins								
ellagitannin	2	21	27	0.6	0.8	2	2	1
bis-HHDP-glucose	3	33	50	0.5	0.7	3	3	3
ellagitannin	4	16	19	0.5	0.9	1	1	1
bis-HHDP-glucose	7	7	7	0.9	1.0	1	1	0
galloyl-HHDP-glucose	8	13	17	0.5	0.8	1	1	1
HHDP-galloyl-glucose	18	12	13	0.7	0.9	1	1	1
galloyl-bis-HHDP-glucose	25	67	76	0.7	0.9	8	6	4
galloyl-bis-HHDP-glucose	28	5	5	0.8	1.0	1	0	0
dimer of galloyl-bis-HHDP (sanguiin H-6 or agrimoniin)	36	36	40	0.8	0.9	4	3	2
anthocyanins								
cyanidin-3-glucoside	22	11	17	0.5	0.7	1	1	1
pelargonidin-3-glucoside	24	106	246	0.2	0.4	8	10	14
5-pyranopelargonidin-3-glucoside	30	4	4	0.0	0.9	0	0	0
pelargonidin-3-malonylglucoside	34	18	44	0.4	0.6	1	2	3
flavanols								
proanthocyanidin B1 (EC-4,8-C)	12	25	35	0.5	0.7	2	2	2
proanthocyanidin trimer (EC-4,8-EC-4,8-C)	13	11	12	0.7	0.9	1	1	1
proanthocyanidin B3 (C-4,8-C)	15	19	25	0.5	0.8	2	2	1
(+)-catechin	17	58	77	0.4	0.8	4	5	4
flavonols								
quercetin-3-glucuronide	37	2	9	0.3	0.3	0	0	0
quercetin-3-malonylglucoside	40	2	4	0.4	0.4	0	0	0
kaempferol-3-glucoside	41	1	2	0.0	0.3	0	0	0
kaempferol-3-malonylglucoside	42	25	51	0.3	0.5	2	2	3
p-coumaric acid derivatives								
p-coumaroylhexose	19	26	70	0.0	0.4	0	2	4
p-coumaroylhexose	20	3	8	0.0	0.4	0	0	0
ellagic acid glycosides								
ellagic acid pentoside	32	7	8	0.7	0.9	1	1	0
ellagic acid	35	12	14	0.9	0.9	2	1	1
other compounds given in **Table 1**								
unknown	5	0	3	0.1	0.1	0	0	0
hydroxybenzoylhexose	6	1	8	0.1	0.1	0	0	0
unknown	10	0	1	0.0	0.0	0	0	0
unknown	11	20	21	0.7	0.9	2	2	1
unknown	16	71	118	0.1	0.6	1	6	7
unknown	21	5	6	0.6	0.8	0	0	0
structure similar to cyanidin-3-glucoside	23	5	8	0.4	0.7	0	1	0
ferulic acid hexose derivative	27	14	41	0.3	0.3	2	1	2
structure similar to pelargonidin-3-glucoside	29	30	71	0.2	0.4	3	3	4
unknown	38	5	13	0.4	0.4	1	0	1
unknown	39	3	21	0.1	0.2	0	0	1
total (numbered peaks)		957	1465			90	88	83
early eluting compounds (except ascorbic acid)		52	172			3	5	10
other peaks, not detected by DAD or MS		76	130			7	7	7

[a] From the hydrodynamic voltammogram the compound was identified as mainly pelargonidin-3-malonylglucoside (peak 34), and not ellagic acid deoxyhexoside (peak 33).

pelargonidin-3-glucoside (peak 24), cyanidin-3-glucoside (peak 22), and the pyranoform of pelargonidin-3-glucoside (peak 30), together with the two nonidentified compounds **23** and **29**, are shown in **Figure 5**. Cyanidin-3-glucoside, having a catechol group on ring B, was oxidized at lower potential than pelargonidin-3-glucoside, having a monohydroxy substituent. Oxidation of flavonoids occurs in ring B, and substituents on this ring are vital for electron-donating abilities (*14*, *37*). When a flavonoid has only a monohydroxy on ring B, the structure of the remaining part of the molecule becomes more important. Thus, when the 5-OH is blocked, as in 5-pyranopelargonidin-3-glucoside, the oxidation potential increases. The suggestion that peaks 23 and 29 had structural features similar to those of cyanidin-3-glucoside and pelargonidin-3-glucoside, respectively, were made plausible by the almost identical HDVs with the ordinary anthocyanins.

HDVs of the other partly identified and nonidentified peaks from UV−vis and MS analyses are shown in **Figure 6**. Peaks 39 and 5 were mainly oxidized at high potentials, 700 and 800 mV, respectively (**Figure 6A**), indicating molecules without easily oxidizable moieties. Peak 27, proposed to be a ferulic acid derivative from UV and mass spectra, and the largest unknown peak (38) in the chromatogram recorded at 260 nm (**Figure 2A**), had HDVs similar to those of peak 6 (hydroxybenzoylhexose), however, with a greater part of the molecule oxidized at lower oxidation potential, indicating the presence of a slightly more oxidizable moiety than a monohydroxy, however not as good as a catechol. A methoxy group ortho to

the hydroxy, as in ferulic acid, seems to stabilize the phenoxy radical, resulting in lower oxidation potential than for a monohydroxy-containing compound (*15*). Peak 10 had no electrochemical response at low oxidation potential (**Figure 6B**). Peak 16 had, as *p*-coumaroylhexose (peak 19) and 5-pyranopelargonidin-3-glucoside (peak 30), a first oxidation wave at a relatively high potential (400 mV), indicating only a monohydroxy substituent. Peak 21 had HDVs similar to those of (+)-catechin and cyanidin-3-glucoside, both possessing a catechol group, suggesting that this compound also contains a catechol moiety. Peak 11 had an HDV similar to those of the ellagitannins and might contain a pyrogallol group.

Prediction of Antioxidant Activity from Coulometric Array Response. Whereas the shape of the HDV reflects the chemical structure, the total electrochemical response is a measure of the amount of analyte present. The combination of both attributes, the shape of the HDV and the response at a certain oxidation potential, points toward the antioxidant capacity of the compound. In line with this, electrochemical responses are shown to correlate with antioxidant activities (*6*, *14*, *38*). In a previous work we showed that the highest correlations between antioxidant activities (FRAP and DPPH) and electrochemical responses of selected polyphenols, mainly flavonoids, were obtained with cumulative responses at medium oxidation potentials (400 and 500 mV), whereas the ORAC values were best predicted by the cumulative responses at 800 mV (*14*). Antioxidant activities (FRAP and ORAC) in strawberries correlated well with electrochemical responses at 300 mV and cumulative responses at 400 mV ($r > 0.92$) (*6*). The highest correlations, however, were obtained with the cumulative responses at the end of the coulometric array (at 800 mV) ($r > 0.96$). On the basis of these results (*6*, *14*), cumulative responses at 400 and 800 mV and the proportion of the compound oxidized at 300 and 400 mV were chosen as measures of antioxidant activities of phenolic compounds in strawberries (**Table 2**). In addition, the contribution of each compound to total electrochemical response at 300, 400, and 800 mV was calculated. The single most important contributor to electrochemical response, that is, antioxidant activity, in strawberry extracts was ascorbic acid, contributing 24% to the total cumulative peak area at 400 mV. Ellagitannins and anthocyanins were the groups of polyphenols with the highest contribution, 19 and 13% at 400 mV, respectively. In accordance with our findings in strawberries, the major contributors to antioxidant capacity in raspberries, determined by an HPLC postcolumn antioxidant reaction system or after fractionation by preparative HPLC, were the ellagitannin sanguiin H-6, ascorbic acid, and the anthocyanins (*36*, *39*). The flavanols contributed 10% to antioxidant activity in strawberries in the present study. The partly identified and nonidentified compounds reported in **Table 1** contributed 12 and 14% at 400 and 800 mV, respectively. The main contributor to electrochemical response of these compounds was peak 16. Also, peak 29, with suggested structure similar to that of pelargonidin-3-glucoside, and peak 11 made substantial contributions to the antioxidant activity in strawberries. It is worth noticing that peak 38, the predominant nonidentified peak in the DAD chromatogram recorded at 260 nm (**Figure 2A**), had low electrochemical response and thus was not an important contributor to antioxidative properties in strawberries. Total numbered peaks accounted for about 85% of the summed electrochemical response in strawberry extract. Of the remaining electrochemical activity, about half was attributed to the compounds eluting before acetone, except ascorbic acid.

In conclusion, about 40 phenolic compounds in strawberry fruits were characterized by using the complementary information from the DAD, ESI-MS in negative and positive modes, and coulometric array detectors. This is more than the maximum 26 compounds that has previously been reported in a single study (*7*, *8*, *16*). The information from the coulometric array detector substantiated the structures suggested from DAD-MS detection and was mainly used to provide valuable information about partly identified compounds; for example, the structures of compounds **23**, **27**, and **29** were made plausible by comparison of the HDVs with the HDVs of known compounds. In addition, the electrochemical detector provided a simple method for determining antioxidant activities of compounds in a mixture without prior postcolumn derivatization or isolation of individual compounds. This approach is especially useful when antioxidant activities of unknown compounds are to be revealed.

ABBREVIATIONS USED

amu, atomic mass units; DAD, diode array detector; DPPH, 2,2-diphenyl-1-picrylhydrazyl assay; C, (+)-catechin; EC, (−)-epicatechin; ESI, electrospray ionization; FRAP, ferric reducing activity power; HDV, hydrodynamic voltammogram; HHDP, hexahydroxydiphenic acid; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectroscopy; MW, molecular weight; ORAC, oxygen radical absorbance capacity; UV−vis, ultraviolet−visible light.

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