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ARTICLE

HPLC-MS Analysis of Proanthocyanidin Oligomers and Other Phenolics in 15 Strawberry Cultivars[†]

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The phenolic compounds of 15 strawberry cultivars grown in Spain were analyzed and quantified: anthocyanins (20.2−47.4 mg/100 g of fw) (cyanidin 3-glucoside and pelargonidin 3-glucoside, 3-rutinoside, and 3-malonyl glucoside), flavonols (1.5−3.4 mg/100 g of fw) (quercetin 3-glucuronide and kaempferol 3-glucoside and 3-*p*-coumaroyl-glucoside), proanthocyanidins (53.9−163.2 mg/100 g), *p*-coumaroyl-glucose (0.84−6.70 mg/100 g), ellagitannins (9.67−22.86 mg/100 g) (sanguiin H-6, lambertianin C, and galloyl bis-HHDP-glucose), and ellagic acid glycosides (0.88−2.06 mg/100 g of fw) (two ellagic acid deoxyhexosides). Proanthocyanidins, the main phenolic compounds, were characterized by phloroglucinol degradation. Their mean degree of polymerization ranged from 3.4 for cv. Chiflon to 5.8 for cv. Ventana, the average value being 4.3. The terminal unit of proanthocyanidin oligomers was always (epi)catechin (17.36−29.93%) and (epi)catechin (61.66−75.39%) or (epi)afzelechin (4.50−10.54%) as extension units. Different combinations of (epi)-catechin and (epi)afzelechin were detected, and their sequence of linkage was characterized by HPLC-MS-MS. Relative percentages of dimers, trimers, tetramers, and pentamers were evaluated by the extracted ion chromatogram (EIC) analysis.

KEYWORDS: ***Fragaria* × *ananassa*; flavonoids; anthocyanins; ellagitannins; ellagic acid conjugates; hydroxycinnamates; proanthocyanidins**

INTRODUCTION

In recent years, in addition to quality attributes, the selection of strawberry cultivars has focused on their health-promoting compounds as these may play a significant role in the prevention of chronic diseases. Strawberries are an important source of vitamin C and other bioactive compounds that have physiological effects (*1*, *2*). Among the bioactive compounds, phenolics are one of the main groups of phytochemicals present in strawberry that strongly influence its quality, contributing to both the sensorial−organoleptic attributes and the nutritional value (*3*). The phenolic profile of strawberries from diverse origins has previously been studied. In general, the studies of different strawberry cultivars often evaluate hydrolysis products for quantification purposes (*4*−*11*), whereas the studies including a detailed characterization of different phenolics, mainly using HPLC-MS-MS techniques but without quantitative determinations, are based on a single cultivar (*12*, *13*). Investigations combining both individual phenolics characterization and quantitative studies in different cultivars have not been carried out yet. In addition, the study of proanthocyanidins in strawberries has been neglected so far, probably due to the complexity of the proanthocyanidin profiles in strawberries and the difficulties inherent in the HPLC analysis of these flavonoids. The characterization of individual phenolics is essential as they have different bioavailabilities and, therefore, health beneficial effects (*14*). Flavan 3-ols and proanthocyanidin oligomers and polymers have recently been reported in strawberries (*15*). Their quantification and characterization are, however, difficult due to analytical problems and the complexity of the chemical structures of the proanthocyanidins present in strawberry as they combine (epi)catechin and (epi)afzelechin units (*15*). Another explanation for the scarce information related to strawberry proanthocyanidins is the low extractability of proanthocyanidin polymers in the aqueous−organic solvents generally used for polyphenol analysis. This has recently been recognized as a key factor for the underestimation of polyphenols in foods (*16*). Most literature data on strawberry proanthocyanidin contents come from HPLC analyses of aqueous−organic extracts of foods, assuming that all or most of the proanthocyanins are extracted by aqueous−organic solvents. However, an important fraction of oligomeric and polymeric proanthocyanidins remains as nonextractable proanthocyanidins that may escape analysis and are usually not taken into account in chemical and nutritional studies (*16*). Procedures for proanthocyanidin quantification, such as that analyzing proanthocyanidin cleavage

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products following acid catalysis in the presence of excess phloroglucinol, evaluate quantitatively the proanthocyanidins present in the food matrix without a previous extraction (*17*) and can be very appropriate for the estimation of these phytochemicals.

The aim of this study was the characterization of 15 strawberry cultivars by the individual and total phenolic compounds with special emphasis on the quantification of proanthocyanidins. The phenolic composition of strawberries and its influence of genetic differences among 15 cultivars were studied. The information provided is relevant for food, breeding, and nutraceutical purposes.

MATERIALS AND METHODS

Plant Material. Strawberries (*Fragaria* × *ananassa* Duchesne) of the cultivars 'Aguedilla', 'Albión', 'Camarosa', 'Candonga', 'Carmela', 'Chiflón', 'Cisco', 'Coral', 'Festival', 'Galexia', 'Macarena', 'Marina', 'Medina', 'Rubygem', and 'Ventana' were grown in conventional culture in Moguer (Huelva, Spain). All of the cultivars were grown in the same field and under the same conditions to minimize the effect of environmental and agronomic factors. Strawberry plants were planted on October 21, 2005, on raised beds mulched with a black 35 μm polyethylene (PE) film under macrotunnels with an average height of 2.4 m made of PE 175 μm. For each bed, two rows of plants separated by 25 cm were set. The soil was classified as a sandy texture, with a pH of 6.5, an electrical conductivity of 0.019 $S \cdot m^{-1}$, and a total organic matter content of 0.26%. During the vegetation period, the average temperature and solar radiation outside the macrotunnel were 13 °C and 12.48 $MJ \cdot m^{-2} \cdot day^{-1}$, respectively, and a total water dosage of 0.45 $m^3 \cdot m^{-2}$ was applied. First-grade standard fruits were harvested on April 24, 2006, early in the morning at commercial maturity (full size and at least 75% red surface). Strawberries were transported to the laboratory on the day of harvest in a refrigerated vehicle at 4 °C. The next morning, strawberries were sorted, and those showing defects, overripeness, or small size were eliminated. Three replicates of approximately 1000 g of fruit per cultivar were analyzed. The fruits were frozen at −20 °C, freeze-dried, and stored in a desiccator until analysis.

Extraction of Phenolic Compounds. The freeze-dried samples were powdered, and ca. 0.8 g were homogenized with 25 mL of extraction solution (acetone/water/acetic acid; 70:29.5:0.5 v/v/v) using an Ultra Turrax (Ika, Staufen, Germany) for 1 min on ice followed by sonication in an ultrasound bath for 15 min (*18*). The homogenates were then centrifuged at 1765*g* for 10 min (JP Selecta Centronic Centrifuge, Barcelona, Spain). The acetone was evaporated under reduced pressure at 35 °C. The aqueous residue was filtered through an activated Sep-Pack C-18 solid phase extraction cartridge (Waters, Milford, MA), washed with water, and the retained phenolic compounds were eluted with 8 mL of methanol. The methanol was evaporated at 35 °C under reduced pressure and the residue redissolved in 1 mL of extraction solution, filtered through a 0.45 μm nylon filter, and directly analyzed by HPLC. For cocoa procyanidin analyses the same extraction solvent and method were used for the extraction of 0.8 g of cocoa powder provided by Natraceutical (Valencia, Spain).

Analysis of Phenolic Compounds by Reversed-Phase HPLC-DAD and HPLC-MS-MS. Samples of 20 μL of the different extracts were analyzed using an HPLC system (Merck Hitachi, Tokyo, Japan) equipped with a model L-7100 pump and a model L-7455 photodiode array UV−vis detector. The samples were injected using a model L-7200 autosampler. The separation was achieved on a 250 mm × 4 mm i.d., 5 μm, reversed phase LiChrocart C18 column (Merck, Darmstadt, Germany), with water/formic acid (95:5 v:v) (A) and methanol (B) as mobile phases. A linear gradient was used starting with 3% B to reach 5% B at 5 min, 8% B at 10 min, 13% B at 15 min, 15% B at 19 min, 40% B at 47 min, 65% B at 64 min, 98% B at 66 min, 98% B at 69 min, and 3% B at 70 min. The flow rate was 1 mL/min, and chromatograms were recorded at 280, 320, 360, and 510 nm. Anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside at 510 nm, flavonols as quercetin 3-rutinoside at 360 nm, hydroxycinnamic acid derivatives as chlorogenic acid at 320 nm, ellagic acid conjugates as ellagic acid at 360 nm, and flavan-3-ols as catechin at 280 nm (all of these standards were from Sigma, St. Louis, MO). Ellagitannins were quantified as an authentic standard of castalagin provided by Dr. S. Quideau (Bordeaux). The results were expressed as milligrams per 100 g of fresh weight. The analytical conditions for the HPLC-MS-MS analyses were the same as those described above for the HPLC-DAD analysis, but using water with 1% formic acid as solvent A for mobile phase.

The HPLC system equipped with a UV−vis DAD and a MS detector in series consisted of a G1322A binary pump, a G1313A autosampler, a G1322 degasser, a G1315 B photodiode array detector, and an ion trap mass spectrometer equipped with electrospray ionization (ESI) and operated in the negative ion mode controlled by software (v.4.0.025) from Agilent Technologies (Waldbronn, Germany). The capillary was maintained at 350 °C and at a voltage of 4 kV. Mass scan (MS) and daughter (MS-MS) spectra were measured from *m/z* 100 to 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative mode for all phenolic compounds but anthocyanins, which were in the positive mode. The flavonoid characterization in strawberry was carried out by means of their UV spectra, molecular weight, their MS-MS fragments, and, whenever possible, chromatographic comparisons with authentic standards. Proanthocyanidin oligomers were evaluated in different cultivars using the extracted ion chromatogram utility.

Normal Phase HPLC-MS of Proanthocyanidin Extracts. To separate proanthocyanidins, normal phase analysis was carried out as reported previously (*19*). The same HPLC-MS equipment described above was used. The column used was a Develosil diol column (250 × 4.6 mm i.d., 5 μm) (Phenomenex, Torrance CA). The solvents used as mobile phase were CH_3CN/HOAc (98:2, v/v) (A) and CH_3OH/H_2O:HOAc (95:3:2, v/v/v) (B). A linear gradient elution with a flow rate of 1 mL/min and an injection volume of 20 μL was used. The gradient started with 0% B to reach 40% B after 35 min. The elution became isocratic between 35 and 45 min. UV detection was set at 280 nm, and fluorescence detection used excitation at 276 nm and emission at 316 nm (*19*, *20*). The system was also coupled to the MS detector under the conditions described above for the HPLC analyses.

Procedure Using Phloroglucinol for Proanthocyanidin Analysis. Freeze-dried strawberries (0.8 g) were treated with a solution of 0.1 N HCl in MeOH containing 5 g/L phloroglucinol and 10 g/L ascorbic acid at 50 °C for 10 min and then combined with 1.2 mL of aqueous sodium acetate to stop the reaction (*17*). Phloroglucinol adducts were analyzed by reversed-phase HPLC. The column was the same as described above protected by a guard column containing the same stationary phase. The method utilized a binary gradient with a mobile phase containing 1% v/v aqueous acetic acid (mobile phase A) and MeOH (mobile phase B). Eluting peaks were monitored at 280 nm. The elution was preformed with 5% B isocratic for 10 min; a linear gradient was then installed to reach 20% B at 20 min and 40% B at 25 min. The flow rate was 1.0 mL/min, the injection volume was 20 μL, and the column was washed with 90% B for 10 min and then re-equilibrated with 5% B for 5 min before the next injection.

Statistical Analysis. The analysis of variance (ANOVA) was performed with SPSS version 17.0 for Windows (2005; SPSS Inc., Chicago, IL). When the significance of error variances was $< 5\%$, a Duncan's test was applied.

RESULTS AND DISCUSSION

Characterization of Phenolic Compounds. Preliminary extraction with two different solvents, methanol 80% and acetone 70%, both in water (v/v), showed that acetone extracted more phenolic compounds, especially anthocyanins and proanthocyanidins, of special interest in strawberry in agreement with previous results (*18*). **Figure 1** shows the HPLC-DAD profiles at 280, 320, 360, and 520 nm of a Camarosa strawberry extract. The HPLC-DAD analysis of the different strawberry cultivars allowed the characterization of 16 compounds, labeled as peaks 1−16 following the elution order in the HPLC-DAD chromatograms. These can be classified into six groups of phenolic compounds:

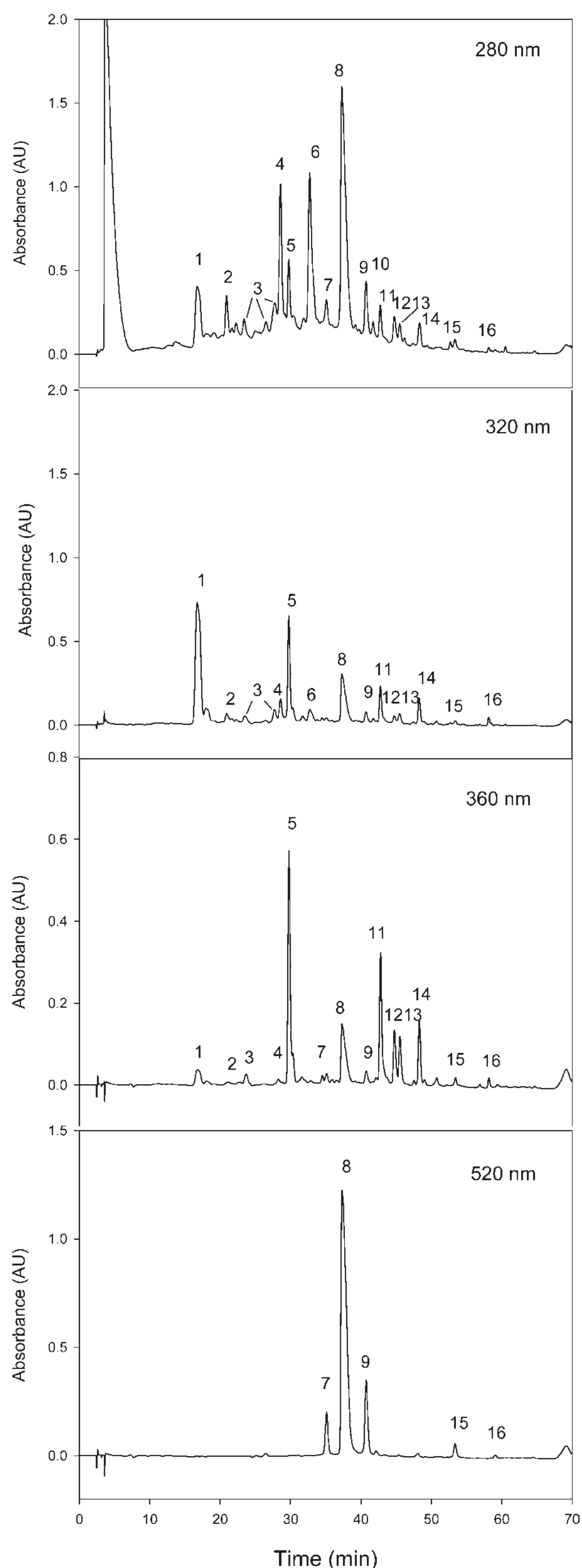

Figure 1. HPLC chromatograms obtained at 280, 320, 360, and 520 nm of strawberry (cv. Camarosa) phenolic compounds. Peaks: 1, *p*-coumaroyl-glucose; 2, galloyl bis-HHDP-glucose; 3, proanthocyanidin; 4, sanguiin H-6; 5, ferulic acid; 6, lambertianin C; 7, cyanidin 3-glucoside; 8, pelargonidin 3-glucoside; 9, pelargonidin 3-rutinoside; 10, unknown; 11, quercetin 3-glucuronide; 12, ellagic acid rhamnoside; 13, ellagic acid rhamnoside; 14, kaempferol 3-glucoside; 15, pelargonidin 3-acetyl-glucoside; 16, kaempferol 3-*p*-coumaroyl-glucoside.

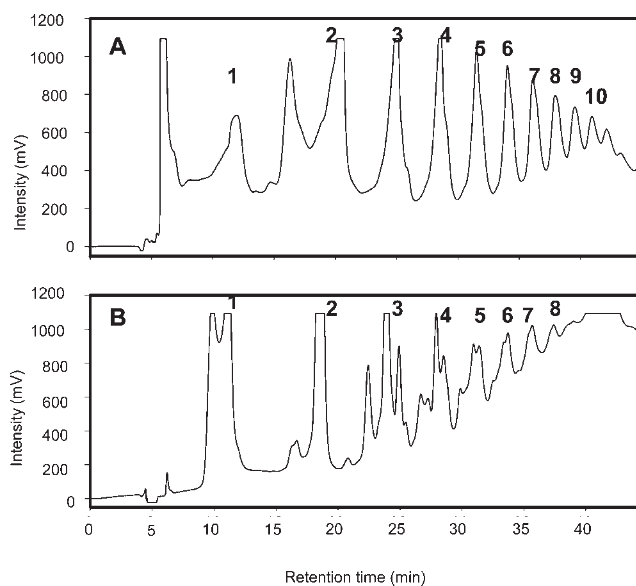

Figure 2. HPLC–fluorescence chromatograms of proanthocyanidins on normal phase diol columns: (**A**) cocoa extract; (**B**) strawberry extract (cv. Camarosa). The numbers denote the polymerization degree (1, monomers; 2, dimers; 3, trimers, etc.).

ellagitannins, ellagic acid conjugates, hydroxycinnamic acids (*p*-coumaric and ferulic acid derivatives), flavonols (quercetin and kaempferol conjugates), anthocyanins (pelargonidin and cyanidin derivatives), and flavan-3-ols [(+)-catechin + proanthocyanidins].

Ellagitannins. These compounds were better analyzed and quantified at 280 nm (**Figure 1**). Ellagitannins are hydrolyzable tannins, consisting of a polyol core, usually glucose, esterified with hexahydroxydiphenic acid(s) (HHDP) and in some cases gallic acid as well. In all of the extracts of the strawberry cultivars analyzed, three ellagitannin peaks (2, 4, and 6) were detected and quantified, with the exception of cv. Cisco, in which only two ellagitannin peaks were present at significant amounts. These ellagitannins showed characteristic UV spectra and produced fragments at m/z 301 corresponding to the released ellagic acid. Compound **2** showed a $[M - H]^-$ ion at m/z 935 and main MS^2 fragments at m/z 633 and 301 consistent with galloyl bis-HHDP-glucose (galloyl bis-hexahydroxydiphenoyl-glucose). Compound **4** showed a double-charge ion $[M - 2H]^{2-}$ at m/z 935 indicative of a mass of 1870. Fragmentation of the double-charged ion gave a sequence of single-charged products at m/z 1697, 1567, 1407, 897, 783, 633, and 301 consistent with sanguiin H-6 (*12*, *13*). Compound **6** had a $[M - 2H]^{2-}$ at m/z 1401 and daughter ions at m/z 2019, 1869, 1567 (loss of HHDP), 1402, 935, 897, 633 (loss of tri-HHDP-galloyl-glucose). This compound was tentatively identified as lambertianin C (*12*, *13*).

Ellagic Acid Conjugates. Ellagic acid and its glycosides were distinguished by their characteristic UV–visible spectra with absorption maxima at 254 and 360–368 nm. In the strawberry varieties analyzed two ellagic acid conjugates were detected at 45.0 and 45.4 min (compounds **12** and **13**, **Figure 1**). Both showed a $[M - H]^-$ ion at m/z 447 consistent with an ellagic acid deoxyhexoside. MS^2 fragmentation produced ions at m/z 301 (ellagic acid), with further fragmentation confirming the ellagic acid structure. Both compounds were then identified as two deoxyhexosides of ellagic acid. Seeram et al. (*12*) reported only methyl ellagic acid pentosides in strawberries, but Aaby et al. (*13*) found ellagic acid deoxyhexoside. With regard to the ellagic acid structure, the two feasible rhamnosyl conjugates were detected in the HPLC-MS analyses.

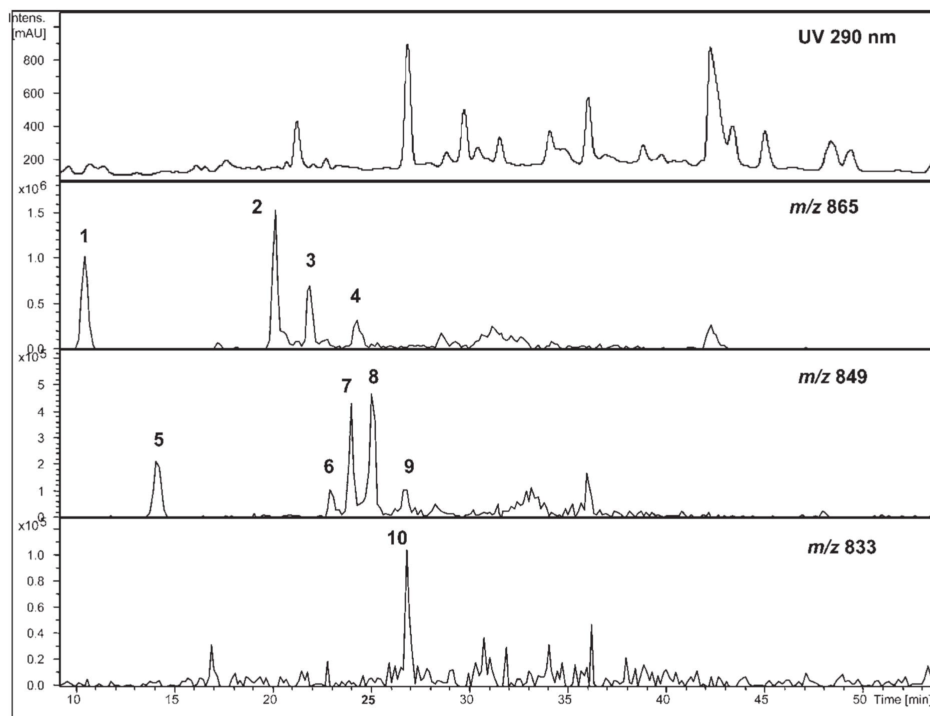

Figure 3. HPLC-DAD-MS extracted ion chromatograms in negative ionization mode of strawberry (cv. Camarosa): proanthocyanidin trimers on a reversed-phase column. Peaks 1−4 are isomers of (epi)catechin−(epi)catechin−(epi)catechin trimers (*m/z* 856); peaks 5−9 are isomers of (epi)-afzelechin−(epi)catechin−(epi)catechin trimers (*m/z* 849); peak 10 is (epi)afzelechin−(epi)afzelechin−(epi)catechin trimer (*m/z* 833). Axes: *x*, time (min); *y*, intensity.

Hydroxycinnamic Acids. In the UV chromatogram registered at 320 nm the most important peaks corresponded with hydroxycinnamic acids: compound **1** was identified as a *p*-coumaric acid ester (absorption maximum at 310 nm) (t_R 17 min). This peak had a $[M - H]^-$ at *m/z* 487 with a MS^2 fragment at *m/z* 325 (loss of hexose). This compound was identified as a hexose ester, probably the glucose ester of *p*-coumaric acid previously reported in strawberries. Its UV spectrum had experienced a bathochromic shift of the maximum compared to that of the corresponding aglycone. This shift suggests that the hexose forms an ester with the carboxylic residue of the hydroxycinnamic acid. This is confirmed by its UV spectrum as the esterification with sugar caused a bathochromic shift (shift to longer walelength) in the UV spectrum of *p*-coumaric acid, whereas glycosylation of the phenolic hydroxy group produced a hypsochromic shift (unpublished observations), and this was not the case. Compound **5** was identified as a ferulic acid derivative (absorption maximum at 326 nm) (t_R 30 min). Free hydroxycinnamic acids are uncommon in fruits, and they are found more likely in their conjugated forms. However, it has also been reported that *p*-coumaric acid is the common hydroxycinnamic acid aglycone in strawberry and raspberry (*21*).

Flavonols. Quercetin and kaempferol conjugates were detected. These compounds were characterized on the basis of the shape of their UV−visible spectra, with an absorption maximum at 352 nm for quercetin 3-*O*-glycosides and at 344 nm for kaempferol 3-*O*-glycosides, and by their MS fragments, as the former produced an aglycone fragment at *m/z* 301 whereas the second produced a fragment at *m/z* 285. Three flavonol peaks were detected in the UV chromatograms recorded at 360 nm, with t_R 42, 48, and 50 min. Compound **11** had a MS fragmentation ion at *m/z* 301 in negative mode consistent with a quercetin derivative, particularly a quercetin glucuronide, previously reported to be the main flavonol in strawberry (*12*, *13*). Compounds **14** and **16** were identified as kaempferol derivatives due to their UV spectra and MS^2 fragmentation producing ions at *m/z* 285 in negative mode consistent with 3,5,7,4′-tetrahydroxyflavone (kaempferol). Compound **14** showed a $[M - H]^-$ ion at *m/z* 447 and the loss of a hexosyl residue (162 mass units) during fragmentation. This was characterized as kaempferol 3-glucoside. Compound **16** eluted later, showing that it was quite lipophilic, and showed a characteristic absorption maximum at a shorter wavelength (312 nm) than the kaempferol glucosides and a UV spectrum characteristic of kaempferol *p*-coumaroyl-glucosides, indicating that the sugar moiety on this flavonol was acylated with a hydroxycinnamic derivative (most probably *p*-coumaric acid). The MS analysis in negative mode showed ions at *m/z* 593 ($[M - H]^-$) and MS^2 fragments at *m/z* 447 (loss of coumaroyl residue) and 285 (loss of coumaroyl + hexosyl residues). This peak was tentatively identified as kaempferol *p*-coumaroyl-glucoside, previously reported in strawberries (*12*).

Anthocyanins. The red color of strawberry is mainly due to anthocyanins. The anthocyanins in strawberry are glycosides of pelargonidin (λ_{max} at 495 nm) and cyanidin (λ_{max} at 512 nm). In the DAD chromatogram, compound **7** had an absorption maximum at 512 nm and a $[M - H]^-$ ion at *m/z* 447, with fragments at *m/z* 285 (loss of hexosyl residue), and this was identified as cyanidin 3-*O*-glucoside. Three chromatographic peaks had absorption maxima at 495 nm and MS fragmentation ions at *m/z* 269 and were identified as pelargonidin derivatives. Compound **8** with $[M - H]^-$ at *m/z* 431 and subsequent loss of a hexosyl residue (162 mu) was identified as pelargonidin 3-glucoside. This was the main anthocyanin in strawberry in accordance with previous results (*22*, *23*). Compound **9** with $[M - H]^-$ at *m/z* 577 and a loss of 308 mass units (deoxyhexosyl−hexosyl residue) was identified as pelargonidin 3-rutinoside. Compound **15**, a less polar pelargonidin derivative, showed a $[M - H]^-$ at *m/z* 473 and loss of 204, consistent with pelargonidin acetyl-hexoside.

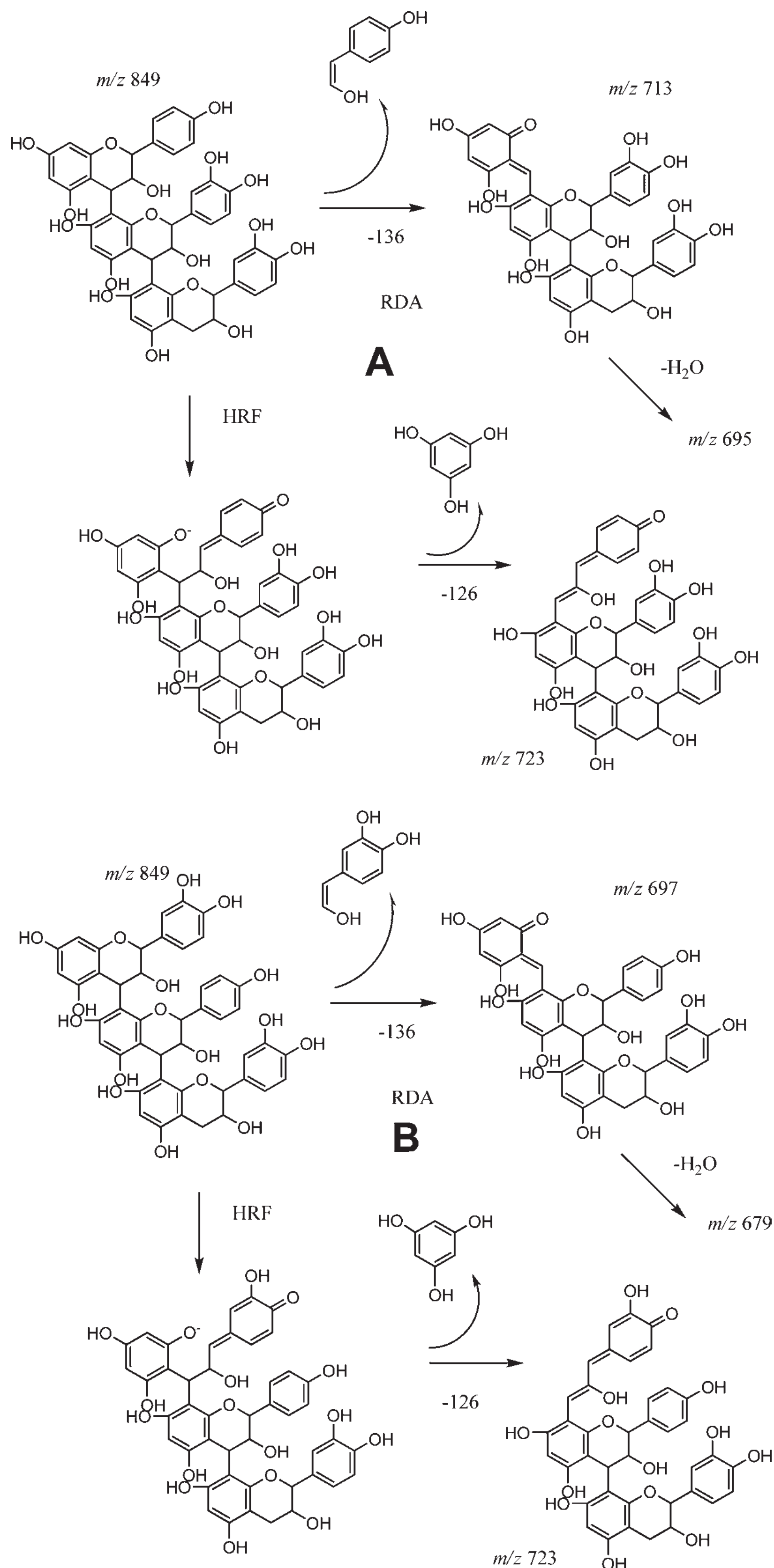

Figure 4. HPLC-MS-MS fragmentation of two proanthocyanidin trimer isomers (*m/z* 849): (**A**) peak 5 in **Figure 3**; (**B**) peak 6 in **Figure 3**. RDA, retro Diels–Alder fragmentation; HRF, heterocyclic ring fission fragmentation.

Flavan-3-ols. These compounds were difficult to detect in the HPLC chromatograms (**Figure 1**). They have low UV extinction coefficients and absorption maxima in a region of the UV spectrum (around 280 nm) with many interfering compounds. In addition, the HPLC-DAD chromatograms revealed that several compounds coeluted at the same time as the flavan-3-ols, and these were partly hidden in the UV chromatogram by compounds with higher UV response due to higher extinction

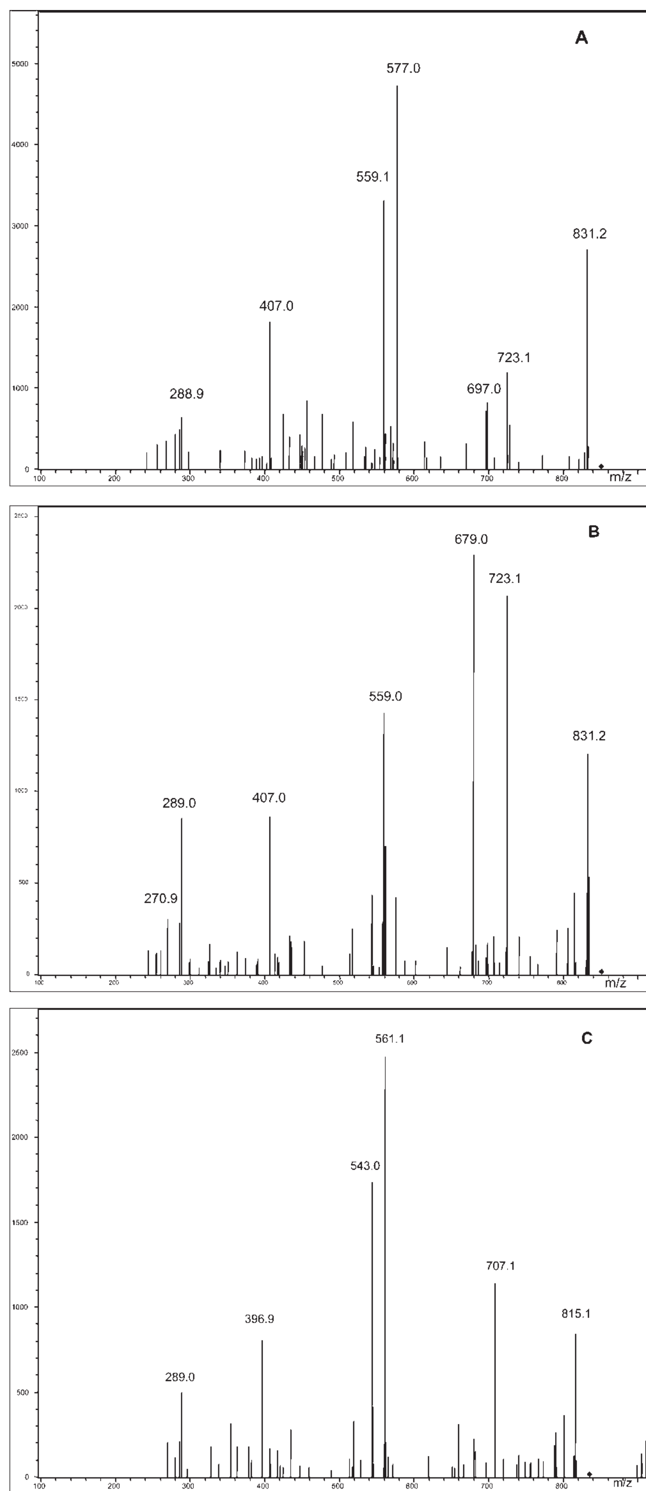

Figure 5. HPLC-MS-MS spectra in negative ionization mode of strawberry (cv. Camarosa), proanthocyanidin trimers: (**A**) peak 5 in **Figure 3** tentatively identified as (epi)afzelechin−(epi)catechin−(epi)catechin; (**B**) peak 6 in **Figure 3** tentatively identified as (epi)catechin−(epi)afzelechin−(epi)catechin; (**C**) peak 10 in **Figure 3** tentatively identified as (epi)afzelechin−(epi)afzelechin−(epi)catechin. Axes, *x*, *m*/*z*; *y*, intensity.

coefficients. The MS analyses showed the presence of relevant peaks at *m*/*z* 577, 865, 1153, and 1441 corresponding to (epi)catechin-based proanthocyanidin dimers, trimers, tetramers, and pentamers, respectively. For this reason, the HPLC-DAD analysis was considered to be an inaccurate method for flavanol quantification. However, other detection methods such as MS and fluorescence were also tested as flavanols have a higher response in MS and in fluorescence than in UV.

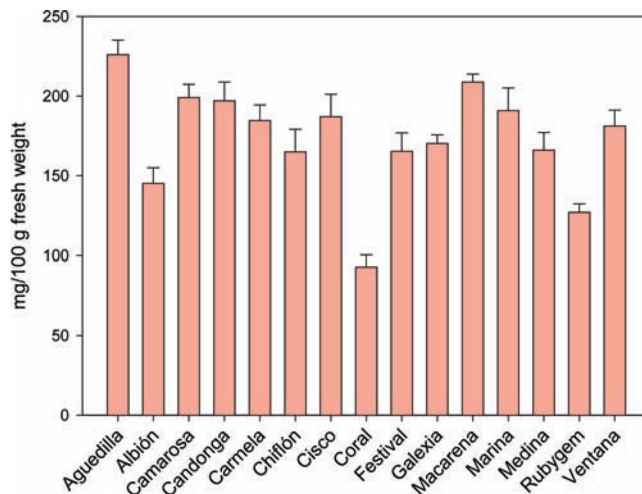

Figure 6. Total phenolic content of the different strawberry cultivars. Values were calculated by addition of individual phenolics quantified by reversed-phase HPLC-DAD and proanthocyanidins by HPLC-DAD after phlorogluconolysis. Axes: *x*, strawberry cultivars; *y*, mg/100 g of fresh weight. Bars represent means ($n = 3$) ± SD.

HPLC analysis using normal phase columns has been used successfully for the analysis of proanthocyanidin oligomers in cocoa, generally in combination with fluorescence detection (*19*). This method allows the clear separation of oligomers with different polymerization degrees (**Figure 2A**). Strawberry extracts were also analyzed using a normal phase Develosil diol column and compared with the analysis of a cocoa extract produced with the same solvent and conditions as those described above for strawberry (**Figure 2B**). The MS analysis allowed the tentative identification of the different chromatographic peaks. Cocoa is characterized by a clean chromatogram, in which (epi)catechin monomers, dimers, trimers, tetramers, etc. (1, 2, 3, and 4 in the chromatogram) show a single peak with a consistent MS spectrum for each peak. In the case of strawberry, the chromatograms were much more complex, with several peaks for the dimer, trimer, tetramer, etc., fractions, showing that in this case (epi)catechin and propelargonidin [(epi)afzelechin] were combined, producing different oligomers (*15*). The normal phase HPLC analysis with MS detection, and EIC function (chromatograms for single ions) show that flavan-3-ol monomers ionized very poorly in the HPLC-MS-MS system, and their detection and characterization by HPLC-MS were not possible. EIC analysis of a single oligomer, for instance, the trimer (epi)catechin−(epi)catechin−(epi)catechin with *m*/*z* 865, shows single- and double-charged ions in the chromatograms (the first corresponding to the trimer and the second to the hexamer). The retention times in EIC analyses of strawberry trimers [(epi)afzelechin−(epi)catechin−(epi)catechin (*m*/*z* 849) and (epi)afzelechin−(epi)afzelechin−(epi)catechin (*m*/*z* 833)] decreased when the number of hydroxyls in the molecule was decreased, consistent with a normal phase separation. The presence of double-charged ions, corresponding to the hexamers, was detected in all cases. Proanthocyanidin oligomers were also HPLC analyzed using a reversed-phase column coupled to a MS detector. Proanthocyanidin trimers were analyzed by extracting ions at *m*/*z* 865 [trimers of (epi)catechin], *m*/*z* 849 [trimers of (epi)afzelechin−(epi)catechin−(epi)catechin], and *m*/*z* 833 [trimers with two (epi)afzelechin and one (epi)catechin residue]

Table 1. Individual and Total Anthocyanin Contents (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a]

	Cy 3-Glu	Pg 3-Glu	Pg 3-Ru	Pg 3-Acetylglu	total anthocyanins
Aguedilla	3.8 ± 0.3 a	36.3 ± 2.3 a	5.6 ± 0.4 b	0.9 ± 0.1 b	46.6 ± 2.9 a
Albión	1.5 ± 0.1 fg	20.2 ± 1.6 d	1.6 ± 0.1 ef	0.3 ± 0.0 hi	23.5 ± 1.9 ef
Camarosa	4.2 ± 0.1 a	35.5 ± 0.3 a	7.0 ± 0.0 a	0.7 ± 0.1 c	47.4 ± 0.4 a
Candonga	2.5 ± 0.2 bc	23.9 ± 1.9 bcd	2.8 ± 0.3 c	0.4 ± 0.1 fg	29.7 ± 2.5 cde
Carmela	1.9 ± 0.3 cdef	24.2 ± 1.7 bcd	2.2 ± 0.2 de	0.7 ± 0.0 c	29.0 ± 2.2 cde
Chiflón	0.9 ± 0.1 gh	32.9 ± 0.2 a	1.9 ± 0.2 ef	0.5 ± 0.0 efg	36.1 ± 0.4 b
Cisco	1.5 ± 0.2 f	25.7 ± 2.2 bc	3.0 ± 0.4 c	1.8 ± 0.1 a	32.1 ± 2.9 bc
Coral	1.6 ± 0.1 f	16.2 ± 0.3 e	2.0 ± 0.0 ef	0.4 ± 0.0 gh	20.2 ± 0.4 f
Festival	2.2 ± 0.1 cde	26.8 ± 1.5 bc	2.7 ± 0.2 cd	0.2 ± 0.0 i	31.9 ± 1.8 bc
Galexia	1.7 ± 0.1 def	24.0 ± 1.1 bcd	3.0 ± 0.1 c	0.5 ± 0.0 defg	29.2 ± 1.4 cde
Macarena	2.3 ± 0.2 cd	20.6 ± 0.8 d	1.7 ± 0.1 ef	0.4 ± 0.0 g	25.0 ± 1.1 cde
Marina	3.4 ± 0.0 b	35.5 ± 1.4 a	5.3 ± 0.3 b	0.7 ± 0.1 cd	44.8 ± 1.8 a
Medina	1.6 ± 0.1 ef	27.0 ± 0.6 bc	1.5 ± 0.0 f	0.6 ± 0.0 cdef	30.7 ± 0.7 cd
Rubygem	0.5 ± 0.0 h	28.0 ± 0.8 b	3.0 ± 0.2 c	0.6 ± 0.0 cde	32.0 ± 1.0 bc
Ventana	0.6 ± 0.1 h	23.2 ± 1.7 cd	2.0 ± 0.2 ef	0.2 ± 0.0 hi	26.0 ± 1.9 cde

[a] Means ($n = 3$) ± SD in the same column followed by different letters are significantly different by Duncan's test ($p < 0.01$).

Table 2. Individual and Total Ellagitannins Contents (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a]

	galloyl bis-HHDP Glu	sanguiin-H6	lambertianin C	total ellagitannins
Aguedilla	3.6 ± 0.2 a	4.1 ± 0.2 fg	4.0 ± 0.1 g	11.7 ± 0.5 ef
Albión	1.3 ± 0.3 ef	3.9 ± 0.3 fg	5.5 ± 0.5 efg	10.7 ± 1.0 ef
Camarosa	2.4 ± 0.0 bc	3.4 ± 0.1 fg	7.3 ± 0.2 e	13.2 ± 0.3 de
Candonga	4.0 ± 0.3 a	2.9 ± 0.2 g	12.0 ± 0.5 d	18.9 ± 1.0 c
Chiflón	2.7 ± 0.2 b	3.0 ± 0.1 fg	14.4 ± 0.9 c	20.1 ± 1.1 abc
Cisco	1.9 ± 0.1 cd	6.5 ± 0.1 a	6.5 ± 0.1 ef	22.3 ± 1.0 ab
Carmela	2.6 ± 0.1 b	3.4 ± 1.2 fg	16.4 ± 2.0 b	22.9 ± 3.2 a
Coral	1.1 ± 0.1 f	9.5 ± 0.3 c	4.3 ± 0.2 fg	14.8 ± 0.6 d
Festival	2.7 ± 0.3 b	7.0 ± 0.1 de	10.6 ± 0.9 d	20.3 ± 1.2 abc
Galexia	1.96 ± 0.1 cd	8.0 ± 0.8 d	5.1 ± 0.0 fg	15.0 ± 0.9 d
Macarena	1.9 ± 0.2 cd	3.5 ± 0.0 fg	4.3 ± 0.3 fg	9.7 ± 0.5 f
Marina	2.4 ± 0.2 bc	6.2 ± 0.1 e	11.3 ± 0.7 d	19.8 ± 1.1 bc
Medina	2.0 ± 0.1 cd	4.3 ± 0.3 b	4.3 ± 0.3 fg	18.3 ± 1.1 c
Rubygem	1.6 ± 0.1 de	4.3 ± 0.3 f	5.8 ± 0.3 efg	11.7 ± 0.7 ef
Ventana	nd	0.3 ± 0.0 h	20.6 ± 1.0 a	21.0 ± 1.1 abc

[a] Means ($n = 3$) ± SD in the same column followed by different letters are significantly different by Duncan's test ($p < 0.01$). nd, not detected.

Table 3. Individual and Total Ellagic Acid Conjugates (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a]

	ellagic rhamnoside	ellagic rhamnoside	total ellagic acid conjugates
Aguedilla	0.5 ± 0.0 ef	0.5 ± 0.0 gh	0.9 ± 0.0 fg
Albión	0.5 ± 0.1 de	0.7 ± 0.0 ef	1.1 ± 0.1 e
Camarosa	0.5 ± 0.0 ef	0.5 ± 0.0 gh	0.9 ± 0.0 fg
Candonga	0.5 ± 0.0 ef	0.5 ± 0.0 gh	1.0 ± 0.1 fg
Chiflón	0.4 ± 0.0 fg	0.5 ± 0.0 gh	0.9 ± 0.0 g
Cisco	0.9 ± 0.0 a	1.2 ± 0.1 a	2.1 ± 0.1 a
Carmela	0.7 ± 0.0 bc	0.8 ± 0.1 de	1.5 ± 0.1 c
Coral	0.5 ± 0.1 de	0.5 ± 0.1 gh	1.0 ± 0.1 fg
Festival	0.8 ± 0.1 ab	1.0 ± 0.1 bc	1.8 ± 0.1 b
Galexia	0.7 ± 0.0 c	0.6 ± 0.0 gh	1.3 ± 0.1 de
Macarena	0.4 ± 0.0 g	0.6 ± 0.0 fg	1.0 ± 0.1 fg
Marina	0.6 ± 0.0 d	0.4 ± 0.1 h	1.0 ± 0.1 fg
Medina	0.8 ± 0.0 ab	1.0 ± 0.0 b	1.8 ± 0.0 b
Rubygem	0.5 ± 0.0 de	0.9 ± 0.0 cd	1.5 ± 0.0 cd
Ventana	0.5 ± 0.0 de	0.7 ± 0.0 f	1.1 ± 0.1 ef

[a] Means ($n = 3$) ± SD in the same column followed by different letters are significantly different by Duncan's test ($p < 0.01$).

(**Figure 3**). Four peaks were observed for trimers of (epi)catechin (m/z 865), five peaks for trimers combining one (epi)afzelechin and two (epi)catechin residues (m/z 849), and just one peak for trimers having two (epi)afzelechins and one (epi)catechin residue (m/z 833). Despite the clear EICs, no corresponding peaks were observed in the HPLC-DAD chromatogram at the optimal wavelength for proanthocyanidin oligomers (280 nm). The fluorescence detection chromatograms were not better than the UV chromatograms due to multiple interferences (results not shown). The MS-MS analysis of proanthocyanidin oligomers has been previously used to characterize these compounds following the fragmentations produced by retro-Diels−Alder (RDA), heterocyclic ring fission (HRF), and quinone−methide (QM) fragmentations (*15*). In this case we used these fragmentations to study the different proanthocyanidin trimers with m/z 849. In **Figure 4**, the different fragments potentially produced by the RDA and HRF fragmentations of (epi)afzelechin−(epi)catechin−(epi)catechin (**A**) and the isomeric (epi)catechin−(epi)afzelechin−(epi)catechin (**B**) are shown. The MS-MS fragments of the m/z 849 of peaks 5 and 6 in **Figure 3** are shown in **Figure 5** (**A** and **B**, respectively). The MS fragmentation shows that the first (5) is the (epi)afzelechin−(epi)catechin−(epi)catechin isomer (a main fragment at m/z 577 corresponding to the quinone methide fragmentation) and the second (6) is (epi)catechin−(epi)afzelechin−(epi)catechin (with a main RDA fragment ion at m/z 679). The MS-MS fragmentation (**Figure 5C**) of the only isomer with two afzelechins and one catechin (peak 10 in **Figure 3**, m/z 833) shows a fragmentation consistent with (epi)afzelechin−(epi)afzelechin−(epi)catechin, with a characteristic RDA fragment at m/z 707 and QM fragment at m/z 561.

Influence of Cultivar on Phenolic Composition. All of the cultivars assayed were grown under the same agronomic conditions and harvested at the same time and at commercial maturity to avoid environmental and agronomic effects on phenolic composition (*10*, *25*).

The total phenolic content of the 15 strawberry cultivars showed significant differences ($p < 0.001$) among them (**Figure 6**). There was a 50% difference between the highest and lowest contents of total phenolics. Cultivars Aguedilla and Macarena showed the highest total phenolics content (addition of all the individual phenolics quantified by the HPLC analyses), whereas cv. Coral had the lowest (**Figure 6**). The range of concentration was from 92.9 mg/100 g of fresh fruit in cv. Coral to 226.1 mg/100 g of fresh fruit in cv. Aguedilla.

Cultivars Aguedilla, Camarosa, and Marina also showed the highest content of anthocyanins, whereas cultivars Coral and Albion had the lowest (**Table 1**). Total anthocyanins ranged between 20.2 mg/100 g of fresh weight in cv. Coral and 47.4 mg/100 g of fresh weight in cv. Camarosa. In general, these values are similar to those reported by other authors (*25*). The anthocyanin

content of cv. Camarosa was also determined by Castro et al. (*26*), who reported values close to those found in our samples (482 ± 14 mg/kg), whereas Hernanz et al. (*10*) reported lower values for cultivars Camarosa, Medina, and Ventana. These differences could be explained by the different extraction solvents and conditions used. Kosar et al. (*27*) also found lower anthocyanin values for cv. Camarosa, but they acknowledged that during maturation the amount of phenolic compounds changed significantly.

With regard to anthocyanin distribution, there were significant differences ($p < 0.001$) in the contents of individual anthocyanins among the 15 cultivars (**Table 1**). In general, Pg 3-glucoside was the predominant compound in the strawberry extracts with a range of 74.9% of the total anthocyanins in cv. Camarosa to 90.9% in cv. Cisco, followed by Pg 3-rutinoside with a range of 4.8% in cv. Medina to 14.9% in cv. Camarosa and Cy 3-glucoside with a range of 1.5% in cv. Rubygem to 9.3% in cv. Macarena. Pg 3-acetylglucoside was always a minor pigment, the content of which averaged 2% of the total pigments. Hernanz et al. (*10*) found similar percentages for cv. Camarosa. In general, the content was similar to that reported by Määttä et al. (*11*), who analyzed varieties grown in Finland where climatic conditions are very different from those found in southern Spain.

The content of ellagitannins in cv. Carmela was 22.9 mg/100 g of fresh weight, the highest among the analyzed cultivars, whereas the lowest was found in cv. Macarena (9.7 mg/100 g of fresh weight) (**Table 2**). Within ellagitannins, lambertianin C was the main ellagitannin, which reached 70% of total ellagitannins for cv. Carmela and almost 100% for cv. Ventana. Galloyl bis-HHDP glucose and sanguiin-H6 were the other two ellagitannins quantified, and these showed large quantitative differences ($p < 0.001$) among the studied cultivars. Cultivars Aguedilla and Candonga had the highest content of galloyl bis-HHDP glucose (3.6−4.0 mg/100 g of fresh weight), whereas it was not detected in cv. Ventana (**Table 2**). Additionally, cv. Cisco had the highest amount of sanguiin-H6, whereas cv. Candonga showed the lowest (**Table 2**).

Ellagic acid is mainly present in strawberries in bound form as part of the ellagitannins (in the form of hexahydroxydiphenic acid), but it also occurs as conjugated derivatives with rhamnose in two different ellagic acid conjugates. The relative amount of ellagic acid and its conjugates was < 5% of total phenolics. In our study, the content of free ellagic acid in the strawberry cultivars studied was similar to the values reported by Aaby et al. (*28*) but lower than the values obtained by Koponen et al. (*25*). This could be due to the extraction method used, which was similar to that used by Aaby et al., whereas Koponen et al. used acid hydrolysis, and therefore these authors quantified bound ellagic acid as well. The content of ellagic acid conjugates varied between 0.9 mg/100 g of fresh weight in cv. Chiflon and 2.1 mg/100 g of fresh weight in cv. Cisco (**Table 3**).

Table 4. Individual and Total Flavonols (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a]

	quercetin 3-glucuronide	kaempferol 3-glucuronide	kaempferol acetyl-glucoside	total flavonols
Aguedilla	1.2 ± 0.1 efgh	0.5 ± 0.0 de	0.2 ± 0.0 abc	1.9 ± 0.1 defg
Albión	1.9 ± 0.1 b	1.0 ± 0.1 a	0.1 ± 0.0 g	2.9 ± 0.2 b
Camarosa	0.9 ± 0.0 h	0.4 ± 0.0 de	0.2 ± 0.0 abcde	1.6 ± 0.1 fg
Candonga	1.4 ± 0.1 def	0.3 ± 0.0 e	0.2 ± 0.0 cde	1.9 ± 0.1 defg
Chiflón	1.5 ± 0.1 cde	0.7 ± 0.0 cd	0.2 ± 0.0 ef	2.3 ± 0.1 cd
Cisco	0.9 ± 0.0 h	0.4 ± 0.0 de	0.1 ± 0.0 fg	1.5 ± 0.0 g
Carmela	1.2 ± 0.1 defgh	0.5 ± 0.0 de	0.3 ± 0.0 a	2.1 ± 0.2 cdef
Festival	1.7 ± 0.1 bc	0.9 ± 0.1 a	0.3 ± 0.0 ab	2.9 ± 0.2 b
Galexia	1.1 ± 0.1 fgh	0.5 ± 0.0 de	0.2 ± 0.0 ef	1.7 ± 0.1 efg
Macarena	2.3 ± 0.2 a	0.9 ± 0.1 ab	0.3 ± 0.0 ab	3.4 ± 0.3 a
Marina	1.3 ± 0.1 defg	0.6 ± 0.1 cd	0.1 ± 0.0 fg	2.0 ± 0.2 cdef
Medina	1.0 ± 0.1 gh	0.7 ± 0.0 bc	0.2 ± 0.0 abcd	1.9 ± 0.2 defg
Coral	1.5 ± 0.2 cd	0.4 ± 0.1 de	0.2 ± 0.0 cde	2.1 ± 0.2 cde
Rubygem	1.4 ± 0.1 def	0.9 ± 0.1 a	0.2 ± 0.0 def	2.5 ± 0.2 bc
Ventana	1.1 ± 0.1 fgh	0.8 ± 0.1 abc	0.2 ± 0.0 bcde	2.1 ± 0.2 cde

[a] Means (n = 3) ± SD in the same column followed by different letters are significantly different by Duncan's test (p < 0.01).

Table 5. Individual and Total Phenolic Acids (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a]

	p-coumaric acid glucoside	ferulic acid derivative	total phenolic acids
Aguedilla	0.3 ± 0.0 i	1.5 ± 0.2 b	1.8 ± 0.2 fg
Albión	0.5 ± 0.0 ij	0.9 ± 0.0 cd	1.5 ± 0.1 g
Camarosa	2.5 ± 0.1 d	nd	2.5 ± 0.1 de
Candonga	3.1 ± 0.1 c	1.1 ± 0.2 bc	4.2 ± 0.3 c
Chiflón	1.6 ± 0.0 f	1.5 ± 0.0 b	3.1 ± 0.1 d
Cisco	1.6 ± 0.1 f	1.2 ± 0.2 bc	2.8 ± 0.3 de
Carmela	3.0 ± 0.1 c	1.0 ± 0.3 bcd	4.0 ± 0.4 c
Coral	0.3 ± 0.0 i	0.6 ± 0.1 d	0.8 ± 0.1 h
Festival	1.1 ± 0.1 gh	1.2 ± 0.1 bc	2.3 ± 0.2 ef
Galexia	1.5 ± 0.2 f	0.9 ± 0.1 cd	2.4 ± 0.3 e
Macarena	0.8 ± 0.1 hi	0.9 ± 0.1 cd	1.7 ± 0.2 g
Marina	4.6 ± 0.2 a	2.1 ± 0.3 a	6.7 ± 0.5 a
Medina	2.0 ± 0.1 e	1.1 ± 0.2 bc	3.1 ± 0.3 d
Rubygem	1.4 ± 0.2 fg	1.3 ± 0.3 bc	2.7 ± 0.5 de
Ventana	4.2 ± 0.3 b	1.0 ± 0.1 bcd	5.2 ± 0.4 b

[a] Means (n = 3) ± SD in the same column followed by different letters are significantly different by Duncan's test (p < 0.01). nd, not detected.

Table 6. Proanthocyanidin Characterization and Content (Milligrams per 100 g of Fresh Weight) of 15 Strawberry Cultivars[a].

	DPn	total proanthocyanidin	CTe (%)	AFZe (%)	CTt (%)
Aguedilla	4.4 ± 0.5 bcde	163.2 ± 5.3 a	65.1 ± 3.8 bcde	10.5 ± 1.1 a	24.4 ± 2.8 abcd
Albión	3.9 ± 0.4 bcde	105.4 ± 6.7 ef	67.1 ± 2.2 bcde	6.8 ± 0.9 bcd	26.0 ± 2.9 abcd
Camarosa	4.6 ± 0.2 bc	133.6 ± 7.4 bc	69.9 ± 1.0 abcd	8.39 ± 0.3 abc	21.8 ± 0.9 cde
Candonga	4.4 ± 0.2 bcd	141.5 ± 7.8 b	70.5 ± 0.4 abc	6.6 ± 1.3 bcd	22.9 ± 1.1 bcde
Chiflón	3.4 ± 0.3 e	102.4 ± 12.5 def	61.7 ± 1.65 e	8.4 ± 0.9 abc	29.9 ± 2.4 a
Cisco	3.6 ± 0.2 de	126.3 ± 9.8 cd	64.6 ± 1.6 bcde	7.4 ± 0.7 abcd	28.0 ± 1.8 ab
Coral	3.6 ± 0.4 de	53.9 ± 6.1 h	63.5 ± 5.1 cde	8.5 ± 2.4 abc	28.2 ± 3.1 ab
Festival	3.7 ± 0.2 cde	106.2 ± 6.6 f	62.9 ± 0.4 de	9.9 ± 0.9 ab	27.2 ± 1.2 abc
Galexia	4.2 ± 0.2 bcde	120.7 ± 2.6 cde	68.6 ± 1.0 abcde	7.4 ± 1.8 abcd	24.0 ± 1.2 abcd
Macarena	4.2 ± 0.2 bcde	168.1 ± 2.9 a	70.9 ± 1.6 ab	4.5 ± 0.3 d	24.6 ± 1.4 abcd
Marina	4.8 ± 0.2 b	116.6 ± 10.4 def	71.2 ± 2.0 ab	7.9 ± 1.0 abcd	20.9 ± 1.0 de
Medina	4.6 ± 0.2 bcd	110.2 ± 8.8 ef	71.6 ± 1.2 ab	6.1 ± 0.1 cd	22.3 ± 1.1 bcde
Rubygem	4.7 ± 0.1 b	76.7 ± 2.9 g	70.1 ± 1.1 abc	8.4 ± 0.4 abc	21.5 ± 0.6 cde
Ventana	5.8 ± 0.4 a	125.9 ± 6.2 cde	75.4 ± 1.0 a	7.3 ± 0.1 abcd	17.4 ± 1.2 e

[a] Means (n = 3) ± SD in the same column followed by different letters are significantly different by Duncan's test (p < 0.01). DPn, mean degree of polymerization; CTe, catechin extension units; AFZe, afzelechin extension units; CTt, catechin terminal units.

The flavonol content was also significantly influenced by the cultivar ($p < 0.001$). Macarena strawberries had the highest flavonol content (3.4 mg/100 g of fresh weight), whereas cv. Cisco had the lowest (1.5 mg/100 g of fresh weight) (**Table 4**). Quantitatively, quercetin 3-glucuronide was the predominant flavonol and ranged from 0.9 mg/100 g of fresh weight (cv. Cisco and cv. Camarosa) to 2.3 mg/100 g of fresh weight (cv. Macarena). Kaempferol 3-glucoside was present in smaller amounts than quercetin 3-glucuronide, whereas kaempferol *p*-coumaroyl glucoside was the minor flavonol (**Table 4**). The flavonoid content in strawberries, as in any source, depends on a series of factors, such as the stage of maturity, cultivar, storage conditions, and analytical methods. However, in this case, similar quantitative results were previously reported by other authors (*29*).

The hydroxycinnamic acid content varied from 0.8 mg/100 g of fresh weight in cv. Coral to 6.7 mg/100 g of fresh weight in cv. Marina (**Table 5**). This group of compounds comprised >6% of the total content of phenolics, and their levels were statistically different ($p < 0.001$). *p*-Coumaric acid derivatives were the main phenolic acids in the samples studied, which was in consonance with the findings of other authors (*6*).

Proanthocyanidins were the main strawberry phenolics. Their concentration ranged from 53.9 mg/100 g of fresh weight in cv. Coral to 168.1 mg/100 g of fresh weight in cv. Macarena (**Table 6**). In previous studies 4.9 mg/100 g of fresh weight (*30*) and 10 mg/100 g of fresh weight (cv. Totem and Puget Reliance) (*28*) were reported. The proanthocyanidin content evaluated using the phloroglucinolysis method was generally higher than those previously found using other methods (*28*, *30*), and this could be explained by the analytical method used in addition to differences among cultivars. We analyzed proanthocyanidins following acid catalysis in the presence of excess phloroglucinol coupled with the reversed-phase HPLC-MS-MS (**Figure 7**) (*17*). The mean degree of polymerization (DPn) of proanthocyanidins averaged 4.3; the highest was for cv. Ventana (5.8) and the lowest for cv. Chiflon (3.4). This means that cv. Ventana had larger oligomers than cv. Chiflon. This fact could have biological significance as the monomers and dimers can be better absorbed than the oligomers with larger size, and this can also influence their biological activity (*32*). Therefore, cv. Chiflon contains proanthocyanidins that are potentially better absorbed than cv. Ventana. This hypothesis should be confirmed by bioavailability studies in humans. As extension units (epi)catechin and (epi)afzelechin were detected, and as terminal unit only (epi)catechin was found, in agreement with previous reports on the analysis of proanthocyanidins

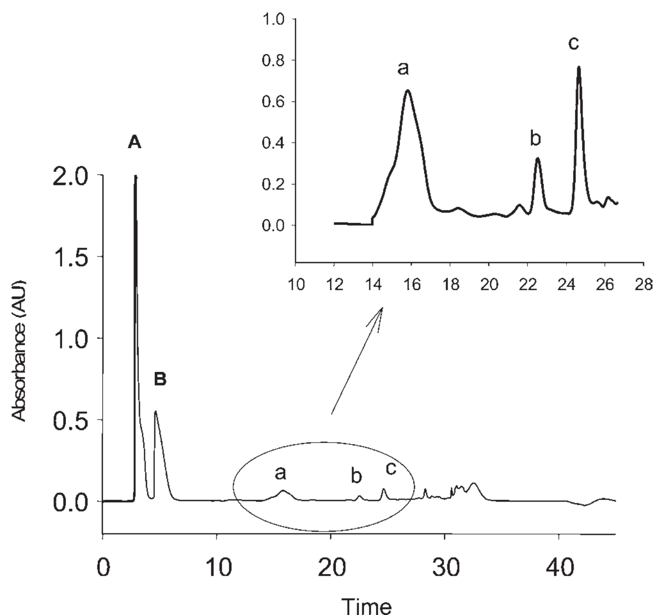

Figure 7. HPLC-DAD chromatogram (280 nm) of strawberry proanthocyanidin phloroglucinolysis breakdown products: (a) (epi)catechin adduct; (b) (epi)afzelechin adduct; (c) (epi)catechin; (B) excess phloroglucinol; (A) excess ascorbic acid.

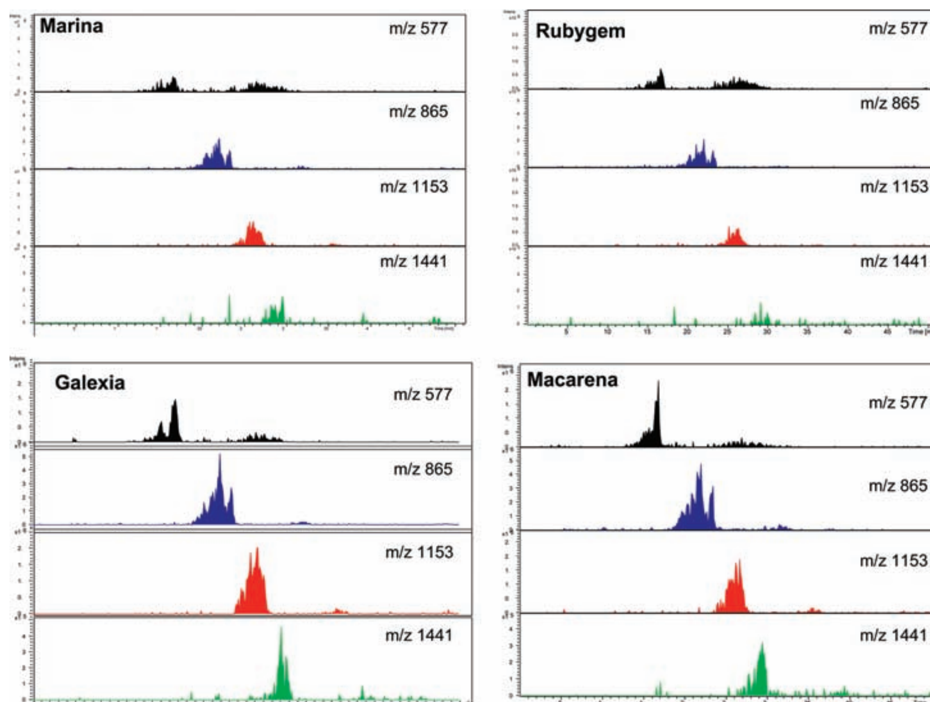

Figure 8. HPLC-MS extracted ion chromatogram analysis of proanthocyanidin oligomers from selected strawberry cultivars. Separations were performed on normal phase column (diol). In most cases a double charged ion is also observed. *m/z* 577, (epi)catechin dimers; *m/z* 865, (epi)catechin trimers; *m/z* 1153, (epi)catechin tetramers; *m/z* 1441, (epi)catechin pentamers.

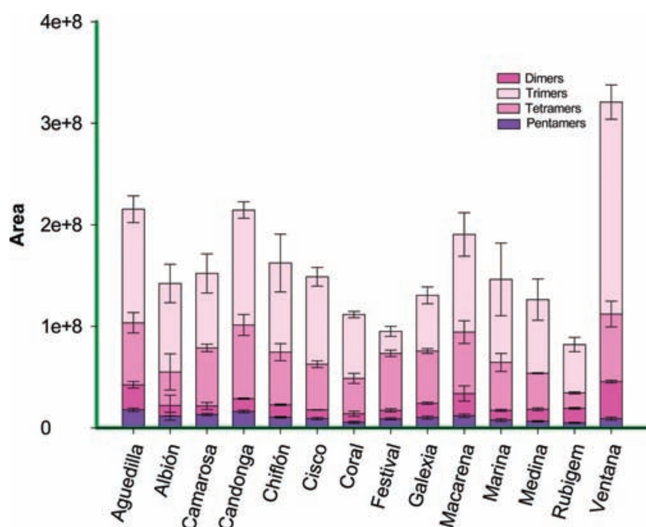

Figure 9. Relative HPLC-MS-MS EIC quantification of (epi)catechin dimers, trimers, tetramers, and pentamers in strawberry cultivars. Bars represent means (n = 3) ± SD for each oligomer.

in different food products (*15*). DPn values between 4.9 and 5.9 were previously reported for cultivars Kent and Elsanta, respectively (*31*). Degradation by phloroglucinolysis coupled to HPLC-DAD-MS-MS allowed a nice quantification method. Thus, these analyses revealed that proanthocyanidins were the main phenolics in strawberries, as they were present in larger amounts than those of anthocyanins. In all cases (epi)catechin was the terminal flavan 3-ol in oligomers, and accounted from 17 to 28% of total proanthocyanidins. The extension units were both (epi)catechin (60−70% of the proanthocyanidins) and (epi)afzelechin in smaller proportions (6−10% of the proanthocyanidins). These data show that although (epi)catechin is the main building block for strawberry proanthocyanidins, propelargonidin [(epi)afzelechin] was also present, making the proanthocyanidin profile complex.

HPLC-MS-MS studies with EICs for the main proanthocyanidin oligomer ions were used to carry out a relative quantification of the different oligomers in the different cultivars. In **Figure 8** an example of the EIC of the dimer to pentamer ions based on (epi)catechin evaluated in four selected cultivars is shown. Cultivars Galexia and Macarena had a higher response in the EIC chromatogram, and therefore a higher proanthocyanidin oligomer content, than cultivars Rubigem and Marina. These relative quantification values were generally consistent with the values obtained after phloroglucinolysis (**Table 6**), as cv. Rubigem had substantially less proanthocyanidins than cv. Macarena. However, the same correlation was not observed for cv. Galexia. The relative quantifications were calculated for the different cultivars, and the results are shown in **Figure 9**. Clear differences were observed between cultivars. The cultivars with a high response in the HPLC-MS EIC study (cultivars Ventana, Macarena, Candonga, and Aguedilla) also had high proanthocyanidin content evaluated by phloroglucinolysis (**Table 6**), whereas cultivars with lower HPLC-MS response (Rubigem, Festival, and Coral) also had a lower proanthocyanidin content evaluated by phloroglucinolysis. The correlations between proanthocyanidins quantified using the phloroglucinolysis methods and the quantification of (epi)catechin-based oligomers by EI chromatogram were evaluated. This correlation was rather low (R^2 = 0.45) when all cultivars were evaluated together, showing that cv. Ventana was completely outside the linear trend. The correlation calculated excluding cv. Ventana was much better (R^2=0.73), showing that, in general, the relative quantification by HPLC-MS EIC correlates with the phloroglucinolysis quantification. The differences observed between both methods can be related to the different polymerization degrees and the occurrence (percentage) of (epi)afzelechin in the cultivar assayed, as the HPLC-MS-MS EIC quantification used only evaluated the (epi)catechin oligomers from dimers to pentamers. It is interesting that cv. Ventana behaved quite differently from the rest of cultivars, as it has the highest degree of polymerization (5.8) and showed the highest relative quantification of (epi)catechin oligomers by HPLC-MS. This cultivar is a very characteristic one that produces fruits very early (December−January) and that is quite different from the others from the agronomic and postharvest points of view.

This study shows that strawberries are a very good source of phenolics, in which proanthocyanidins are the main phenolics. Quantitative and qualitative differences are observed among cultivars, and this opens the opportunity for selecting cultivars with characteristic phenolic profiles that potentially can be associated with their health-promoting properties.

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