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Analysis of Flavan-3-ols and Procyanidins in Food Samples by Reversed Phase High-Performance Liquid Chromatography Coupled to Electrospray Ionization Tandem Mass Spectrometry (RP-HPLC-ESI-MS/MS)

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ABSTRACT: Concentrations of the main dimeric and trimeric procyanidins (PC) and their monomeric constitutive units catechin (CT) and epicatechin (EC) were determined in food samples by using reversed phase high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (RP-HPLC-ESI-MS/MS). In a first step, 12 PCs (PC B1, B2, B3, B4, B5, B6, B7, B8, C1, C2, and A2 and cinnamtannin B1), of which most are not commercially available, were isolated from plant materials or synthesized and purified by a combination of column chromatographic separation techniques with different stationary phases. These PCs in combination with CT and EC were used as standard substances for identification and quantification during the following screening of food samples by RP-HPLC-ESI-MS/MS analysis. The main focus of the newly developed RP-HPLC-ESI-MS/MS method is the compensation of matrix effects by using the echo-peak technique simulating internal standard injection. The suitability of this new method was demonstrated by the determination of recovery rates being 90% or higher. Use of this method allowed the determination of patterns and concentrations of PCs in 55 food samples.

KEYWORDS: procyanidins, flavan-3-ols, HPLC-MS/MS, foods, isolation, quantification

INTRODUCTION

Proanthocyanidins (PA) as secondary plant metabolites are present in a large number of foods. In the human diet fruits, cereals, nuts, beverages, and chocolate are the main sources.[1] On the basis of hydroxylation pattern and linkage of constitutive units PAs can be divided into several classes. The most common PAs are procyanidins (PC) with catechin (CT) and epicatechin (EC) as constitutive units (see Figure 1). Further important classes of PAs are prodelphinidins and propelargonidins with (epi)gallocatechin and (epi)afzelechin, respectively, as constitutive units. In the case of linkage between units, B-type PAs with a C4−C8 or C4−C6 linkage are the most dominant. A-type PAs bear in addition to the C−C linkage an ether bond between C2 and O7 or between C2 and O5.[2] PAs are bitter and astringent and contribute by this to the taste of foods. Besides their sensory properties, a large number of beneficial health effects are described for PAs in the literature. Several studies demonstrate, for example, their antioxidative[3,4] and anticarcinogenic[5] effects. A reduction of the incidence of cardiovascular diseases is also described in relationship to the so-called "French paradox".[6,7]

Data concerning concentrations of PCs, the most common group among PAs, are essential for a better understanding of whether naturally occurring concentrations in foods are sufficient for possible health effects. Therefore, different attempts were made to quantify PCs in food samples in recent years.[8] Simple colorimetric assays are the acid−butanol and vanillin−HCl assays. These assays allow only determination of total PCs as a sum without a specification of individual compounds. By using normal phase chromatography, a separation according to the degree of polymerization (DP) in clusters up to a DP of 10 is possible. Gu et al. used silica as stationary phase, and the mobile phase consisted of methylene chloride, methanol, and a constant concentration of acetic acid/water (1:1, v/v).[9] A method developed by Kelm et al. avoided the use of halogenated solvents. Instead, separation was performed on a diol phase with a gradient of acidified acetonitrile and methanol/water.[10] In contrast to normal phase chromatography, RP chromatography enables separation of single compounds. Resolution is possible up to a DP of 4. Higher oligomeric and polymeric PCs elute in an unresolved hump. The elution order of PCs depends on the stereochemistry and overall polarity of single compounds; for example, C4−C8 linked dimeric procyanidins elute prior to the C4−C6 linked compounds. RP chromatography for quantification was used, for example, by de Pascual-Teresa et al.[11] After HPLC separation of PCs, UV, FLD, or electrochemical detection as well as mass spectrometry can be used. In the case of UV detection absorbance at 280 or 210 nm can be performed. Also, a postcolumn derivatization with *p*-dimethylaminocinnamaldehyde (DMACA) before UV−vis detection is possible.[11] Enhanced selectivity can be reached by using FLD or electrochemical detection. Kelm et al. used as excitation wavelength 276 nm, and emission was measured at 316 nm.[10] Mass spectrometry offers high selectivity by measuring the characteristic transitions of the different DPs.

The aim of this study was the development of a RP-HPLC-ESI-MS/MS method for the quantification of 12 PCs and the flavan-3-ols CT and EC (see Figure 1) in different food samples. In comparison to other studies using equivalent concentrations for quantification, authentic standard substances were used for quantification in this study. Therefore, PC standard substances

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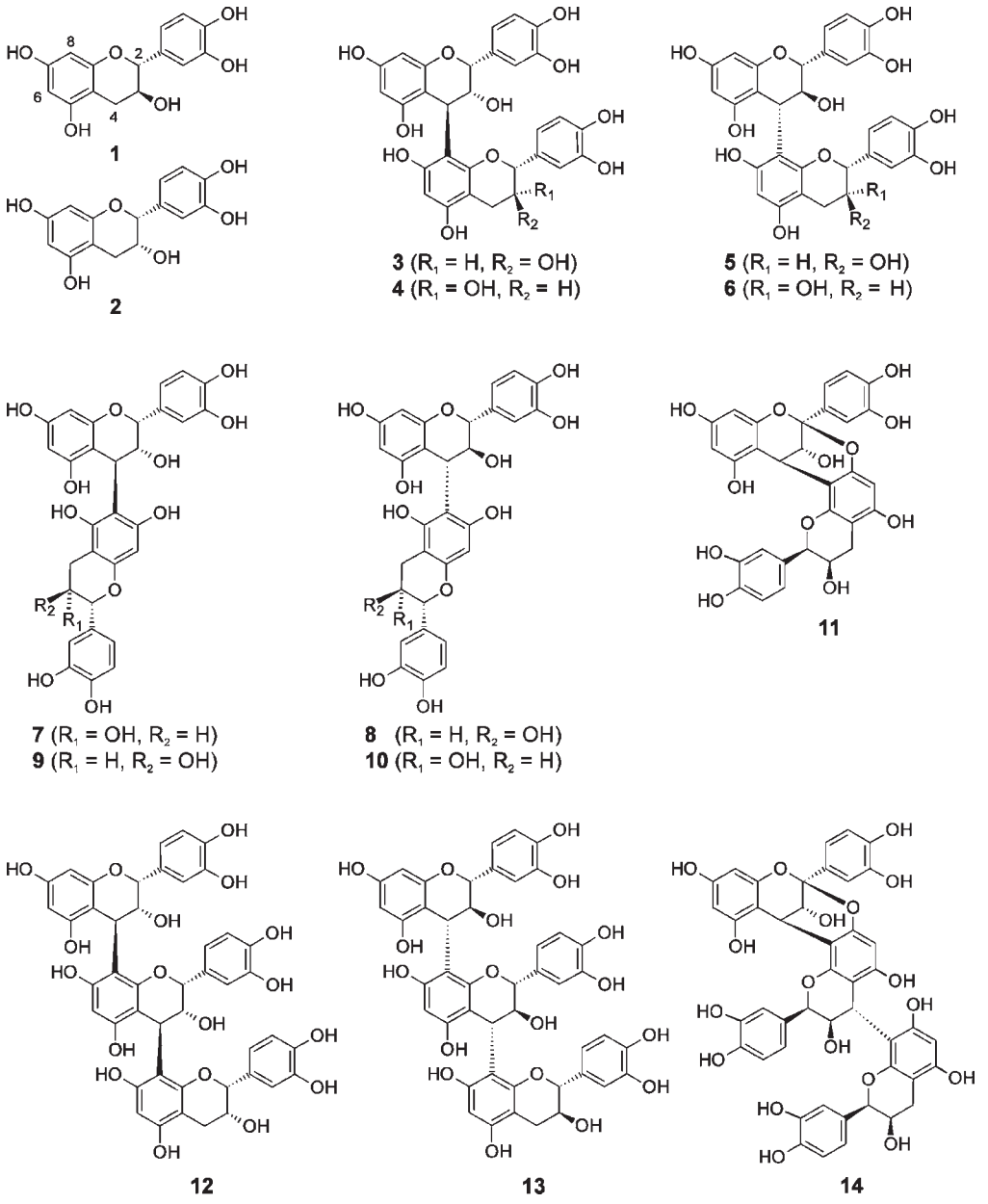

Figure 1. Structures of analyzed flavan-3-ols and procyanidins: (+)-catechin (**1**); (−)-epicatechin (**2**); procyanidins B1 (**3**), B2 (**4**), B3 (**5**), B4 (**6**), B5 (**7**), B6 (**8**), B7 (**9**), B8 (**10**), A2 (**11**), C1 (**12**), and C2 (**13**); cinnamtannin B1 (**14**). Numbering of important C atoms is given for catechin (**1**).

were isolated from plant materials or synthesized. Purification was performed by using column chromatography with different stationary phases. With regard to quantification, the most important task was the consideration of matrix effects. Therefore, the so-called echo-peak technique with a time-shifted injection of CT and EC solutions of known concentrations was applied.[12] To show the suitability of this method, recovery rates in two food samples containing no PCs were investigated and calculated. In 55 food samples, especially fruits, patterns and concentrations of PCs were determined.

MATERIALS AND METHODS

Chemicals. Solvents used for sample extraction and chromatography were obtained from VWR (Darmstadt, Germany) and Carl Roth (Karlsruhe, Germany). Water was purified with a Milli-Q Gradient A10 system (Millipore, Schwalbach, Germany). Grape seed extract (GSE) was kindly provided by Kaden Biochemicals (Hamburg, Germany). (+)-CT was obtained from Applichem (Darmstadt, Germany) and from Carl Roth. (−)-EC was purchased from Sigma-Aldrich (Steinheim, Germany) and (+)-taxifolin from Foxtank GmbH (Berlin, Germany). Sephadex LH20 was obtained from GE Healthcare (Freiburg, Germany). MCI CHP20P was obtained from Sigma and diol material from VWR.

Isolation of PCs. The following PCs were isolated from the plant materials given in parentheses: PC B1 (apple peel, variety Cox Orange); PCs B2, B5, and C1 (cocoa); PC B3 (barley); PCs B4 and B7 (GSE); and PC A2 as well as cinnamtannin B1 (both litchi pericarp). With the exception of grape seed extract, the plant materials were ground and defatted with *n*-pentane in the case of cocoa and barley. Procyanidins were extracted with acetone/water (70:30, v/v). Acetone was removed, and the aqueous residue was extracted three times with the same volume

of ethyl acetate. The ethyl acetate extracts were reduced to dryness. The ethyl acetate extract of cocoa was additionally macerated with a small amount of cold methanol to remove theobromine. The supernatant containing PCs was evaporated to dryness.

Synthesis of PCs. Procyanidins B6 and C2 were obtained by synthesis according to the method of Delcour et al.[13] In brief, (+)-taxifolin (10 mmol) and (+)-CT (3.5 mmol) were dissolved in 30 mL of absolute ethanol. A solution of sodium borhydride (6.6 mmol) dissolved in 7 mL of absolute ethanol was added dropwise under nitrogen during 30 min. After the addition of water (35 mL), the pH was adjusted to 5.0 with acetic acid solution (0.15 M). After 24 h of stirring, ethanol was evaporated. PCs were extracted three times with the same volume of ethyl acetate from the aqueous residue. Ethyl acetate extracts were combined and reduced to dryness. PC B3 can be also obtained by the same synthesis. In the case of PC B8 (−)-EC was used as educt instead of (+)-CT. As second reaction product, PC B4, can be also purified out of the same reaction mixture.

Column Chromatography with Sephadex LH20. A column filled with Sephadex LH 20 (5.5 × 90 cm (fill height)) was used for the separation of PCs in the obtained dry ethyl acetate extracts or, respectively, the grape seed extract. The column was equilibrated with ethanol for 96 h at a flow rate of 1 mL/min. Plant extracts (maximal 15 g for described column dimension) were dissolved in a small amount of ethanol and loaded onto the column. Elution was performed with ethanol as eluent at a flow rate of 1 mL/min. Fractions (20 mL) were collected with a fraction collector. Elution was monitored by combination of UV detection and thin-layer chromatography on silica with ethyl acetate/formic acid/water (90:5:5, v/v/v) as mobile phase. For detection, vanillin−HCl spray reagent was used. The eluates were combined to the following fractions: monomeric compounds (fraction 1), C4−C8-linked dimeric PCs (fraction 2), and C4−C6-linked dimeric and trimeric PCs (fraction 3) . Separation of A-type PCs was analogue. Depending on separation, fractions were in some cases combined to subgroups, which simplified further purification. The combined fractions were reduced to dryness.

Column Chromatography with MCI CHP20P. The system consisted of a Degasys DG-1310 (Uniflows, Japan), an L-6200 pump, and an L-7420 UV detector (Merck). As stationary phase a self-packed column (25 × 390 mm, YMC Europe, Dinslaken, Germany) filled with MCI CHP20P was used. The mobile phase consisted of a linear gradient of water/methanol (80:20, v/v) (A) and water/methanol (20:80, v/v) (B). Before loading, the column was equilibrated with 100% A for 1 h. Afterward, the sample dissolved in a small amount of mobile phase A was loaded onto the column via an injection valve 7725i (Rheodyne, USA). The following gradients were used: cleanup of PC B2 from fraction 2 of cocoa, 0% B (0 min), 50% B (120 min), 50% B (150 min), 75% B (210 min); separation of PCs B5 and C1 from fraction 3 of cocoa, 0% B (0 min), 100% B (240 min); separation of dimeric C4−C8-linked PCs from fraction 2 of the grape seed extract, 0% B (0 min), 0% B (60 min), 50% B (300 min), 50% B (360 min); cleanup of cinnamtannin B1 from fraction 3 of litchi pericarp, 0% B (0 min), 0% B (60 min), 50% B (240 min), 50% B (300 min). The flow rate was 6 mL/min in all cases, and detection was performed at 280 nm. Fractions (15 mL) were collected with a fraction collector and combined according to the results of UV detection with the exception of the fractions obtained from the grape seed extract (fraction 2). In this case fractions were monitored by analytical RP-HPLC (system see HPLC-DAD, -FLD, and -ELSD) and combined according to these results. Solvent was removed from the combined fractions, and the aqueous residue was freeze-dried. After every separation, the MCI CHP20P column was washed with pure methanol for 1 h.

Column Chromatography with Diol Material. For the separation of fraction 3 containing C4−C6-linked dimeric and C4−C8-linked trimeric PCs from the grape seed extract obtained with Sephadex LH20, column chromatography with diol material as stationary phase was used. The column (15 × 200 mm) was equilibrated with acetonitrile. The fraction containing the PCs described above was dissolved in a small amount of acetonitrile and loaded onto the column. Elution was performed by the following mixtures of acetonitrile (A) and methanol/water (95:5, v/v) (B): 100% A (20 mL), 97.5% A (20 mL), 95% A (30 mL), and 92.5% A (20 mL). The flow rate was about 1 mL/min. Fractions (10 mL) were monitored by thin-layer chromatography with the described system and combined appropriately.

Preparative RP-HPLC. Final purification, if necessary, was performed by preparative RP-HPLC. System A consisted of two PU-2080 pumps, a PU-2070 UV−vis detector (both Jasco, Gross-Umstadt, Germany), and a 7725i injector (Rheodyne), and system B consisted of two Pro Star M-210 pumps, a Pro Star 325 UV−vis detector (both Varian, Waldbronn, Germany), and a 7725i injector (Rheodyne). As stationary phases Eclipse XDB-C18, 250 × 9.4 mm with 5 μm particle size (Agilent, Waldbronn, Germany) and Microsorb 100-5 C18, 250 × 10 mm, with 5 μm particle size (Varian) were used. As eluents 0.01% formic acid and methanol or acetonitrile were used. The flow rate was 4 mL/min in all cases. Detection was monitored at 280 nm.

MS, NMR, and CD. The identity of standard substances was checked by mass spectrometry and nuclear resonance spectroscopy. Data are in accordance with the literature.[14−20] Stereochemistry was investigated by circular dichroism spectroscopy.

RP-HPLC-DAD, -FLD, and -ELSD. The purity of isolated PCs was investigated by RP-HPLC coupled to a diode array (DAD), fluorescence (FLD), or evaporation light scattering (ELSD) detector. The system consisted of a DGU-20A3 degasser, two LC-20AT pumps, an SIL-20A autosampler, an SPD-M20A DAD, an RF-10AXL fluorescence detector, and an ELSD-LT evaporation light scattering detector (all from Shimadzu, Duisburg, Germany). Separation was performed on a LiChrospher RP18 column, 250 × 2 mm, with 5 μm particle size (Merck) as stationary phase. The mobile phase consisted of the following linear gradient of water/formic acid (99.9:0.1 v/v) (A) and acetonitrile (B): 10% B (0 min), 10% B (5 min), 15% B (15 min), and 40% B (30 min). The flow rate was set at 300 μL/min. The injection volume was 10 μL (dissolved in 0.1% formic acid/acetonitrile 10:90, v/v). In the case of the DAD absorbance was recorded at 280 nm. The excitation wavelength of the FLD was 276 nm, the emissions were monitored at 316 nm. The ELSD parameters were the following: temperature, 40 °C; and pressure, 2.5 bar (air).

Fractions of the separation on MCI CHP20P were monitored with the same HPLC system. An Eclipse XDB-C18 column, 150 × 4.6 mm, with 5 μm particle size (Agilent) as stationary phase and at a flow rate of 1 mL/min was used. The mobile phase consisted of the following linear gradient of water/formic acid (99:1 v/v) (A) and methanol (B): 15% B (0 min), 21% B (14 min), 100% B (15 min). Detection was performed by UV and fluorescence.

RP-HPLC-ESI-MS/MS. PCs and flavan-3-ols were analyzed on an Agilent 1200 series HPLC (Agilent) linked to an API 3200 mass spectrometer (Applied Biosystems, Darmstadt, Germany). A LiChrospher RP 18 column, 250 × 2 mm, with 5 μm particle size (Merck) was used as stationary phase. Chromatographic separation was performed by using the following binary gradient of water/formic acid (99.9:0.1, v/v) and acetonitrile/formic acid (99.9:0.1, v/v) at a flow rate of 300 μL/min: 10% B (0 min), 10% B (10 min), 15% (20 min), 40% B (35 min). Column temperature was set at 25 °C. For the echo-peak technique the following injector program was used: inject CT-echo, wait 5 min, inject calibration standard/sample, wait 9 min, inject EC-echo. The injection volume was in all cases 20 μL, and the concentration for CT- and EC-echo solutions was 500 ng/mL in both cases. For the removal of sugars and other polar compounds a valve was used. At the beginning of each run the column eluate was delivered into waste. The valve was switched 9 min after the start of a run and the column eluate delivered into the ion source. The mass spectrometer was operated in the MRM mode detecting negative ions. The ion spray voltage was set at −4500 V.

Table 1. Sources and Combinations of Different Chromatographic Techniques for Purification of Procyanidins B1, B2, B3, B4, B5, B6, B7, B8, C1, C2, and A2 and Cinnamtannin B1 (Cin B1)

	B1	B2	B3	B4	B5	B6	B7	B8	C1	C2	A2	CinB1
source	apple peel	cocoa	barley	GSE	cocoa	synthesis	GSE	synthesis	cocoa	synthesis	litchi pericarp	
Sephadex LH20	X	X	X	X	X	X	X	X	X	X	X	X
MCI CHP20P		X		X	X				X			X
diol							X					
RP	X		X	X		X	X	X		X		

Zero-grade air served as the nebulizer gas (30 psi) and was heated at 350 °C, as turbo gas for solvent drying (45 psi). For fragmentation of the molecular ions into the specific fragment ions, nitrogen was used as collision gas (12 psi). The following transition reactions monitored (each 150 ms) in the negative mode were used for quantification (declustering potential (DcP), collision energy (CE), and collision exit potential (CXP) are given in parentheses): monomeric compounds, 289.0−124.9 (DcP, −40 V; CE, −34 V; CXP, 0 V); dimeric compounds, 577.1−124.9 (DcP, −10 V; CE, −46 V; CXP, −2 V); dimeric compounds A-type, 575.0−284.9 (DcP, −95 V; CE, −38 V; CXP, −20 V); trimeric compounds, 865.3−125.1 (DcP, −75 V; CE, −74 V; CXP, 0 V); and trimeric compounds A-type, 863.1−284.9 (DcP, −75 V; CE, −60 V; CXP, −24 V). The following transitions were measured additionally: monomeric compounds, 289.0−245.0 and 289.0−109.1; dimeric compounds, 577.1−407.0 and 577.1−289.0; dimeric compounds A-type, 575.0−124.8 and 575.0−108.9; trimeric compounds, 865.3−407.0 and 865.3−289.1; trimeric compounds A-type, 863.1−288.9 and 863.1−124.9. For the calculation of calibration curves the peak area ratios of the analytes to one of the echo-peaks were plotted against the concentration of the analyte for each point of the calibration curve. For CT and PCs B1, B3, and C2 the CT echo-peak was used and for EC, PCs B2, B4, B5, B6, B7, B8, C1, and A2, and cinnamtannin B1 the EC-echo-peak was used.

Sample Preparation. Samples were collected from local markets. Peels, skins, pips, and/or stones, which are normally not ingested by humans, were removed from at least 500 g of sample. Samples were cut into slices and freeze-dried. Water content was calculated on the basis of the weight before and after freeze-drying. Dry samples were ground into powder and sieved for homogenization (100 μm). One gram of sample was extracted in a 50 mL screw-cap tube with 15 mL of *n*-hexane to remove fat and chlorophyll. Therefore, samples were vortexed for 30 s and mechanically shaken for 10 min. After centrifugation (10 min, 8000*g*, 10 °C) the *n*-hexane phase was decanted. The remaining organic solvent was removed under reduced pressure. PCs were extracted with 15 mL of acetone/water (70:30, v/v). After vortexing (1 min) and sonification (10 min), samples were shaken mechanically for 15 min. Samples were centrifuged (10 min, 8000*g*, 25 °C), and the supernatant was transferred into a 50 mL volumetric flask. The residue was extracted for a second time according to the above-mentioned procedure, and the supernatant was combined with the first one. The volumetric flask was filled with acetone/water (70:30, v/v); 100, 250, 500, and 1000 μL were evaporated under a nitrogen stream to dryness and dissolved in 1 mL of 0.1% formic acid/acetonitrile (90:10, v/v). The sample in the concentration range of the calibration curve was used for quantification. Results were given as milligrams per 100 g of fresh weight of the edible part under consideration of the water content.

For determination of recovery rates, melon and pineapple containing no flavan-3-ols and procyanidins were used as matrix. CT, EC, and PCs B2, B3, B5, C1, C2, and A2 were added to melon at a concentration of 0.5, 1, or 10 mg/100 g dry weight (corresponding to 0.05, 0.1, and 1 mg/100 g fresh weight in the case of a water content of 90%). In the case of pineapple CT, PCs B2 and C1 were added in higher concentrations of 5 and 50 mg/100 g dry weight (corresponding 0.5 and 5 mg/100 g fresh weight in the case of a water content of 90%).

RESULTS AND DISCUSSION

A large number of beneficial health effects are described for PCs, but the knowledge of occurrence and concentrations in food samples is rather limited. For a reliable identification and quantification of PCs authentic standard substances are essential. With only a few exceptions PCs are not commercially available. For this reason often equivalent concentrations were used for quantification. PCs were mostly determined as CT or EC equivalents. Therefore, one focus of this study was the use of authentic PC standard substances for identification as well as for quantification. PCs were isolated from plant materials and/or synthesized by reaction of (+)-taxifolin with (+)-CT or (−)-EC. In the case of PCs B3 and B4, besides isolation from plant material, synthesis is also possible. In the following the method for isolation from plant materials is described. For isolation from plant materials PCs were extracted with acetone/water (70:30, v/v). The aqueous residue after evaporation of acetone was extracted with ethyl acetate. Thereby, an effective enrichment of dimeric and trimeric PCs besides monomeric flavan-3-ols is possible. For purification different column chromatographic systems were used. An overview about sources for isolation and used techniques for purification is shown in Table 1 (for further details see Materials and Methods). The most important and essential step during purification is the column chromatography with Sephadex LH20 using ethanol as eluent.[21] PCs elute nearly according to the degree of polymerization under these conditions. The desired PCs were further purified by column chromatography with RP, MCI CHP20P, and diol as stationary materials with the exception of PC A2. Purity was sufficient after the Sephadex LH20 column in the case of PC A2. Procyanidins B1, B3, B6, and B8 were purified only by using preparative RP-HPLC. For purification of PCs B2, B5, and C1 and cinnamtannin B1 MCI CHP20P was used. MCI CHP20P (styrene−divinylbenzene copolymer) separates PCs like RP material, but this material is useful especially for large-scale separation.[22] Over 100 mg of pure substance can be obtained in one run. PC B4 was isolated by a combination of a separation with MCI CHP20P and RP material. The fraction from the separation on Sephadex LH20 contains PCs B1, B2 and B3 besides B4. Therefore, a cleanup step on MCI CHP20P was used for the enrichment of B4. Final purification by preparative RP-HPLC was clearly simplified. Using first diol material as stationary phase and then preparative RP-HPLC led to the purified standard of PC B7. The use of column chromatography on diol material was necessary for the separation of PC B7 and the trimeric PC C1, which was also present in the Sephadex LH20 fraction. Diol material under the used conditions enables separation according to the degree of polymerization.[10] A direct separation between PCs B7 and C1 was not possible on RP material, because they elute too close together. As second step preparative RP-HPLC was used only for final purification.

Table 2. Purity (Percent) of Isolated/Synthesized Procyanidins Determined by RP-HPLC with UV and FLD

analyte	UV (280 nm)	FLD (ex, 276 nm; em, 316 nm)	analyte	UV (280 nm)	FLD (ex, 276 nm; em, 316 nm)
B1	≥100.0	≥100.0	B7	95.9	97.1
B2	98.7	99.2	B8	92.3	95.3
B3	95.3	99.8	C1	96.9	95.8
B4	95.1	98.9	C2	93.3	95.0
B5	88.5	90.8	A2	99.0	97.7
B6	96.0	99.1	Cin B1[a]	97.8	96.8

[a] Cin B1, cinnamtannin B1.

Purities of standard substances were determined by RP-HPLC-ELSD, -UV, and -FLD. By using an ELSD no impurities, which are non-UV, respectively, nonfluorescence active, were detected. Table 2 shows the results for the detection by UV (280 nm) and FLD (ex, 276 nm; em, 316 nm). Purities were about 90% or higher.

For the analysis of flavan-3-ols and PCs food samples, mainly fruits, were first freeze-dried, ground, and homogenized. Only the edible parts of samples were used for the investigation. Fat and chlorophyll, which can interfere during further analysis, were removed by extraction with *n*-hexane. PCs were not detected in the *n*-hexane phase (data not shown). Acetone/water (70:30, v/v) was used for the extraction of PCs. An acidification of the acetone/water mixture by acetic acid as described in the literature did not improve the efficiency of the extraction.[23] Two extractions of samples are sufficient to extract >98% of flavan-3-ols and dimeric and trimeric PCs. After acetone/water extracts had been diluted to a definite volume, aliquots were reduced to dryness. These aliquots were directly measured by RP-HPLC-ESI-MS/MS after dissolving. Gu et al. used an additional cleanup step with Sephadex LH20 before measurement to remove sugars and other polar compounds.[9] This step can be skipped and replaced by the use of a valve that allows delivery of the HPLC eluate during the first minutes into waste and not into the MS/MS system. The HPLC eluate during the first minutes contained mainly sugars and other polar compounds. The valve was switched 9 min after the start of each run that the eluate can reach the ion source before PCs elute.

Identification and quantification was performed by using mass spectrometry in the MRM mode, which offers the advantage of an unambiguous identification of PCs by measuring the characteristic mass spectrometric transitions of the different DP in contrast to other detection methods, such as UV or fluorescence detection. In extracts of plant materials a large number of other substances can occur, which can, for example, interfere during UV detection. If UV or fluorescence detection is used, often a baseline shift during the HPLC run can be observed caused by higher oligomeric procyanidins with their large structural diversity. These two effects can make peak integration much more difficult. Besides the above-mentioned unambiguous assignment, another important advantage of using mass spectrometry is the shortening of run times because analytes with different m/z can elute at the same time. A run time of 30 min is sufficient for the separation of all analytes (see Figure 2). Examples of simultaneous elution of analytes with different m/z at the same time are PC B6 and EC and PCs B8 and C1. In contrast, the use of a UV, FLD, or electrochemical detector requires baseline separation of the analytes, leading to an extended run time. De Pascual Teresa et al. needed run times of 180 min using UV−vis detection after derivatization with DMACA.[11]

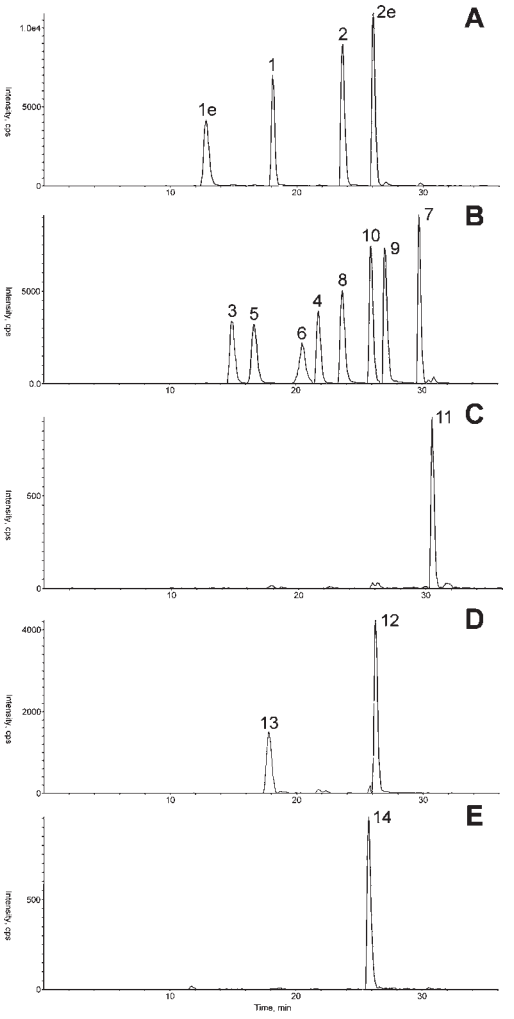

Figure 2. RP-HPLC-ESI-MS/MS chromatograms of a mixture of standard substances (500 ng/mL): (A) monomeric compounds with MRM m/z 289.0 − 124.9 $[M - H]^-$; (B) dimeric procyanidins with MRM m/z 577.1 − 124.9 $[M - H]^-$; (C) dimeric procyanidins A-type with MRM m/z 575.0 − 284.9 $[M - H]^-$; (D) trimeric procyanidins with MRM m/z 865.3 − 125.1 $[M - H]^-$; (E) trimeric procyanidins A-type with MRM m/z 863.1 − 284.9 $[M - H]^-$. Peaks: catechin-echo (1e); catechin (1); epicatechin (2); epicatechin-echo (2e); procyanidins B1 (3), B3 (5), B4 (6), B2 (4), B6 (8), B8 (10), B7 (9), B5 (7), A2 (11), C2 (13), and C1 (12); cinnamtannin B1 (14).

A general problem of mass spectrometry in comparison to UV or fluorescence detection is the appearance of matrix effects. Especially in the quantitative analysis of complex biological or food samples, analyte signals can be suppressed or enhanced in the presence of matrix compounds. Because of the significant difference in the response of an analyte in pure solvent in comparison to that in matrix, it is difficult to obtain accurate results. Different attempts can be made for the compensation of matrix effects. The most usual solution is the use of stable isotope labeled substances as internal standards. A total synthesis of all PC standard substances as isotopically labeled standards would be time-consuming and very complex. Multistep synthesis and the use of protecting groups are necessary to obtain the desired stereochemistry. For that reason only a few attempts were done

for total synthesis of PCs until now.[24] Besides this, internal standards with a similar chemical structure are also quite often used for quantification. Ideally, the internal standard and the investigated compound should coelute or elute very close to each other from the HPLC columns. Only in this case will the altered ionization efficiency caused by matrix effects have the same impact on internal standard and analyte. An interesting, but until now rarely used, solution for the above-mentioned problems is the so-called echo-peak technique. In the literature the use of this technique is described first for the quantification of pesticide residues in plant materials.[12] Afterward, Bartke et al. quantified sphingolipids in potatoes and sweet potatoes by using the echo-peak technique.[25] The principle of the echo-peak technique in the case of the analysis of PCs is demonstrated in Figures 2 and 3,

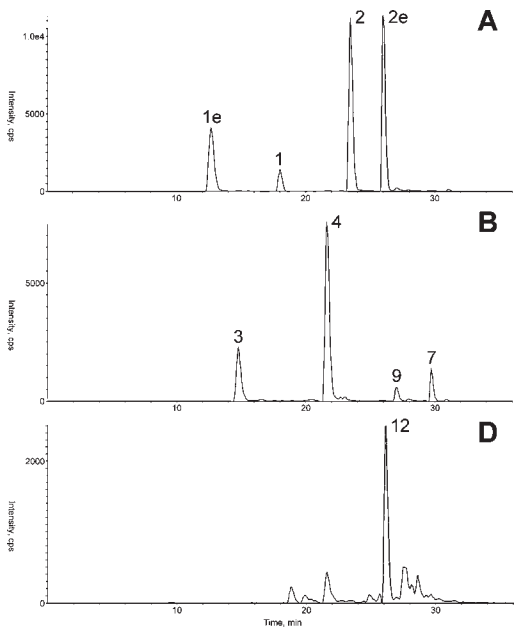

Figure 3. RP-HPLC-ESI-MS/MS chromatograms of apple sample (Braeburn): (A) monomeric compounds with MRM m/z 289.0 → 124.9 $[M - H]^-$; (B) dimeric procyanidins with MRM m/z 577.1 → 124.9 $[M - H]^-$; (D) trimeric procyanidins with MRM m/z 865.3 → 125.1 $[M - H]^-$. Peaks: catechin-echo (1e); catechin (1); epicatechin (2); epicatechin-echo (2e); procyanidins B1 (3), B2 (4), B7 (9), B5 (7), and C1 (12).

showing the RP-HPLC-MS/MS analysis of a standard solution and of an apple sample. CT and EC were used for the generation of echo-peaks. Therefore, solutions of CT and EC in a constant concentration were injected time-shifted to the calibration standard/sample. In the case of the CT echo-peak the solution is injected before injection of the calibration standard or sample, and in the case of the EC echo-peak it is injected after injection of the calibration standard or sample. In sum, each analysis comprises three injections, two for the echo-peaks and one for the calibration standard or sample. The resulting two echo-peaks (CT-echo and EC-echo, see peaks 1e and 2e in Figure 3) were used as internal standards to calculate the analyte concentrations and to compensate matrix effects in the analyzed food samples. The CT echo-peak (1e in Figure 3) was used as internal standard for CT and PCs B1, B3, C2 and the EC echo-peak (2e in Figure 3) as internal standard for EC, PCs B2, B4, B5, B6, B7, B8, C1, and A2, and cinnamtannin B1. For the calibration curves the peak area ratios of the corresponding analyte to one of the echo-peaks were plotted against the concentration of the analyte for each point of the calibration curve. For example, to quantify B1, a calibration curve was generated by plotting the peak area ratios of B1 to CT-echo against the concentration of B1. In the same way calibration curves were generated for all other analytes.

A quantification of every analyte by using its own echo-peak according to the method of Zrostlikova et al.[12] was not possible because of the complexity of the separation as well as overlapping MS transitions.

The suitability of the methodology was evaluated by the determination of recovery rates of selected and representative analytes in melon and pineapple. These matrices were chosen because they contain no flavan-3-ols and no PCs, which was demonstrated in preliminary tests. CT and EC were chosen as monomeric compounds for determination of recovery rates in melon, and PCs B2 and B3 were chosen as C4−C8-linked dimeric compounds. B3 represents a polar dimeric PC eluting early from the column, whereas PC B2 represents a less polar compound. Procyanidin B5 was selected as C4−C6-linked dimeric procyanidin and PC A2 as A-type PC. Furthermore, the polar trimeric PC C2 and the less polar trimeric PC C1 were used for the determination of recovery rates. In the case of melon as matrix obtained recovery rates vary between 90 and 110% with the exception of EC, showing a bit lower recovery rate (see Figure 4). To pineapple as second tested matrix, CT, dimeric PC B2, and trimeric PC C1 were added in higher concentrations

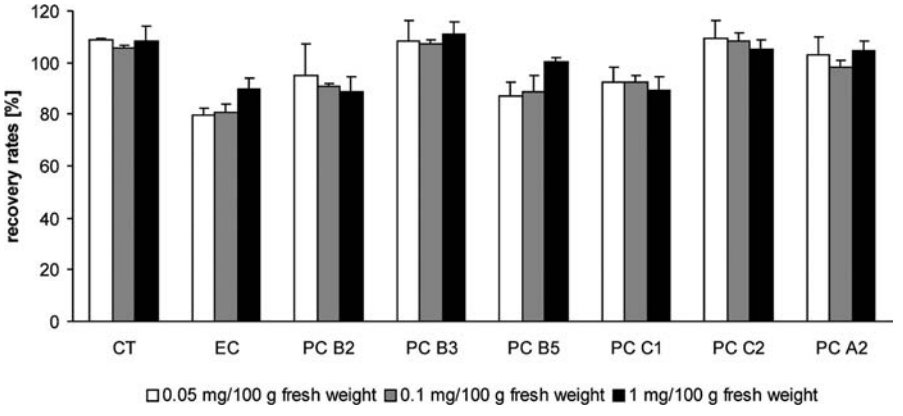

Figure 4. Recovery rates of catechin(CT), epicatechin(EC), and procyanidins B2, B3, B5, C1, C2, and A2 in melon as matrix. Added concentrations are 0.5, 1, and 10 mg/100 g dry weight, respectively, 0.05, 0.1, and 1 mg/100 g fresh weight, in the case of 90% water content (n = 3).

Table 3. Concentrations of Catechin (CT), Epicatechin (EC), and Procyanidins[a]

	concentration (mg/100 g fresh weight (edible part))															
	CT	EC	B1	B2	B3	B4	B5	B6	B7	B8	C1	C2	A2	Cin B1	**sum**	water (%)
apple																
Boskop	1.866 ± 0.082	9.134 ± 0.127	4.226 ± 0.206	12.548 ± 1.037	0	0	1.067 ± 0.027	0	0.831 ± 0.043	0	6.918 ± 0.054	0	0	0	**36.590 ± 1.576**	82.0
Braeburn	0.639 ± 0.048	5.804 ± 0.175	2.135 ± 0.155	7.314 ± 0.158	0	0	0.631 ± 0.014	0	0.363 ± 0.017	0	3.865 ± 0.060	0	0	0	**20.751 ± 0.627**	82.8
Elstar	1.398 ± 0.168	8.903 ± 0.257	3.469 ± 0.317	14.871 ± 0.970	0	0	1.004 ± 0.069	0	0.705 ± 0.055	0	7.751 ± 0.428	0	0	0	**38.101 ± 2.264**	82.9
Elstar without peel	1.288 ± 0.106	7.325 ± 0.480	2.645 ± 0.167	11.069 ± 1.121	0	0	0.794 ± 0.049	0	0.544 ± 0.044	0	4.176 ± 0.194	0	0	0	**27.841 ± 2.161**	83.5
Granny Smith	1.738 ± 0.136	7.524 ± 0.130	4.530 ± 0.220	11.848 ± 0.242	0	0	0.970 ± 0.026	0	0.701 ± 0.006	0	6.299 ± 0.035	0	0	0	**33.610 ± 0.795**	86.1
apricot	2.513 ± 0.007	4.624 ± 0.243	1.086 ± 0.038	3.047 ± 0.198	0.189 ± 0.011	0.411 ± 0.012	0.409 ± 0.009	<LOQ	0.190 ± 0.009	<LOQ	1.609 ± 0.109	<LOQ	0.054 ± 0.001	0.020 ± 0.000	**14.152 ± 0.637**	88.1
avocado	0.059 ± 0.002	1.343 ± 0.047	0.312 ± 0.003	2.131 ± 0.081	<LOQ	0.222 ± 0.015	0.200 ± 0.006	0	0.090 ± 0.002	0	1.178 ± 0.102	0	0	0.237 ± 0.004	**5.772 ± 0.262**	69.1
banana	0	0.078 ± 0.004	0	0.094 ± 0.005	0	0	0.017 ± 0.001	0	0	0	0.059 ± 0.003	0	0	0	**0.247 ± 0.013**	74.4
blackberry	0.908 ± 0.079	20.675 ± 0.303	0.192 ± 0.007	5.028 ± 0.308	1.345 ± 0.031	8.846 ± 0.270	0.888 ± 0.056	0.285 ± 0.006	0	0	1.295 ± 0.133	1.402 ± 0.038	0	0	**40.863 ± 1.231**	86.4
blueberry																
wild	0.786 ± 0.019	5.949 ± 0.223	0.207 ± 0.008	5.198 ± 0.286	0.030 ± 0.002	0	0.592 ± 0.050	0	0.102 ± 0.013	0	2.187 ± 0.054	0	0.543 ± 0.059	5.169 ± 0.138	**20.763 ± 0.852**	85.1
cultivated	0.758 ± 0.049	0.603 ± 0.040	3.501 ± 0.126	1.147 ± 0.073	<LOQ	0	0.093 ± 0.002	0	0.327 ± 0.001	0	0.985 ± 0.048	0	<LOQ	<LOQ	**7.415 ± 0.339**	84.7
cherry	6.794 ± 0.372	7.105 ± 0.125	3.963 ± 0.047	3.963 ± 0.075	0.154 ± 0.011	0.411 ± 0.020	0.486 ± 0.009	0	0.601 ± 0.002	<LOQ	3.217 ± 0.098	0	<LOQ	<LOQ	**26.692 ± 0.759**	80.6
Chinese gooseberry	0.033 ± 0.005	0.523 ± 0.012	0	0.702 ± 0.026	0	0	0.052 ± 0.000	0	0.016 ± 0.001	0	0.309 ± 0.009	0	0	0	**1.634 ± 0.053**	81.1
cranberry	0.487 ± 0.010	3.831 ± 0.132	0.298 ± 0.005	2.945 ± 0.132	0.041 ± 0.001	0.409 ± 0.009	0.362 ± 0.012	0	0.093 ± 0.003	<LOQ	1.501 ± 0.034	0	6.898 ± 0.250	2.115 ± 0.052	**18.979 ± 0.640**	88.0
currant																
black	1.386 ± 0.079	0.723 ± 0.004	0.139 ± 0.003	0.191 ± 0.003	0.126 ± 0.003	0.424 ± 0.016	0.029 ± 0.001	0.045 ± 0.002	0.035 ± 0.002	0	0.062 ± 0.006	<LOQ	0	0	**3.160 ± 0.119**	75.6
red	1.374 ± 0.077	0.294 ± 0.008	0.270 ± 0.007	0.061 ± 0.003	0.244 ± 0.010	0.091 ± 0.002	0.013 ± 0.001	0.028 ± 0.001	0.031 ± 0.002	0	0.031 ± 0.001	0.098 ± 0.004	0	0	**2.534 ± 0.116**	91.9
fig	0.673 ± 0.044	0.133 ± 0.005	0.232 ± 0.014	0.026 ± 0.001	0.129 ± 0.004	0.060 ± 0.003	0	0.018 ± 0.001	0.048 ± 0.002	0	0.102 ± 0.006	0.041 ± 0.004	0	0	**1.461 ± 0.084**	81.1
gooseberry																
green	3.069 ± 0.123	0.296 ± 0.005	1.915 ± 0.185	0.316 ± 0.019	0.535 ± 0.024	0.148 ± 0.006	0.034 ± 0.002	0.034 ± 0.001	0.161 ± 0.004	<LOQ	0.351 ± 0.011	0.145 ± 0.007	0	0	**7.003 ± 0.387**	89.8
red	1.906 ± 0.223	0.841 ± 0.016	1.393 ± 0.016	0.357 ± 0.014	0.392 ± 0.017	0.264 ± 0.003	0.056 ± 0.002	0.045 ± 0.002	0.137 ± 0.004	0	0.351 ± 0.002	0.162 ± 0.007	0	0	**5.905 ± 0.306**	83.3
grape																
blue	1.008 ± 0.098	0.361 ± 0.019	1.164 ± 0.038	0.132 ± 0.003	0.106 ± 0.002	0	<LOQ	0	0.106 ± 0.004	0	0	<LOQ	0	0	**2.877 ± 0.164**	86.3
white A	1.173 ± 0.093	0.137 ± 0.004	0.888 ± 0.010	0.049 ± 0.001	0.116 ± 0.005	0	<LOQ	0	0.100 ± 0.002	0	0	<LOQ	0	0	**2.463 ± 0.115**	87.3
white B	1.733 ± 0.032	0.315 ± 0.013	1.904 ± 0.093	0.141 ± 0.005	0.172 ± 0.002	0	<LOQ	0	0.193 ± 0.005	0	0	<LOQ	0	0	**4.456 ± 0.150**	86.1
kaki	0.167 ± 0.018	<LOQ	0.035 ± 0.002	0	0	0	0	0	0	0	0	0	0	0	**0.202 ± 0.020**	79.8
litchi	0	1.216 ± 0.015	0	1.767 ± 0.053	0	0	0.256 ± 0.008	0	0	0	0.947 ± 0.029	0	0.742 ± 0.042	1.489 ± 0.125	**6.416 ± 0.273**	82.2
mango	1.443 ± 0.103	0.009 ± 0.001	0.200 ± 0.000	0	<LOQ	0	0	0	0.063 ± 0.006	0	0	0	0	0	**1.715 ± 0.110**	86.3
mirabelle	3.591 ± 0.481	1.044 ± 0.148	1.521 ± 0.235	0.636 ± 0.085	0.090 ± 0.008	0.081 ± 0.001	0.064 ± 0.004	0	0.279 ± 0.018	0	0.329 ± 0.047	0	0.591 ± 0.019	0.168 ± 0.002	**8.393 ± 1.048**	78.4
nectarine	0.381 ± 0.014	0.030 ± 0.002	0.393 ± 0.016	0.011 ± 0.001	0.057 ± 0.002	<LOQ	<LOQ	<LOQ	0.050 ± 0.003	0	0	0.010 ± 0.001	0	0	**0.932 ± 0.039**	89.2
papaya	0	0.019 ± 0.005	0	0.005 ± 0.001	0	0	0	0	0	0	0	0	0	0	**0.024 ± 0.006**	85.9
passion fruit	0.018 ± 0.003	1.124 ± 0.117	0	0.042 ± 0.000	0	0	0	0	0	0	0	0	0	0	**1.185 ± 0.120**	72.0
peach																
A	1.247 ± 0.051	0.112 ± 0.010	1.302 ± 0.047	0.052 ± 0.003	0.114 ± 0.010	0	0.011 ± 0.001	0	0.129 ± 0.008	0	0	0.043 ± 0.001	0	0	**3.009 ± 0.131**	86.3
B	3.000 ± 0.180	0.095 ± 0.004	3.052 ± 0.251	0.082 ± 0.001	0.338 ± 0.016	0	0.013 ± 0.000	0	0.284 ± 0.011	0	0	0.057 ± 0.002	0	0	**6.921 ± 0.465**	84.2
pear																
A	1.016 ± 0.012	3.668 ± 0.027	0.735 ± 0.019	2.030 ± 0.031	0.036 ± 0.001	0.072 ± 0.006	0.286 ± 0.002	0	0.169 ± 0.006	0	1.424 ± 0.016	0	0.186 ± 0.027	0.066 ± 0.009	**9.687 ± 0.156**	85.5
B	0.150 ± 0.003	8.620 ± 0.319	0.255 ± 0.023	3.204 ± 0.331	<LOQ	0.092 ± 0.013	0.559 ± 0.023	0	0.145 ± 0.004	0	2.371 ± 0.214	0	2.089 ± 0.220	0.613 ± 0.022	**18.096 ± 1.172**	82.5
plum																
A	3.208 ± 0.155	1.596 ± 0.015	3.800 ± 0.346	3.230 ± 0.240	0.324 ± 0.021	<LOQ	0.281 ± 0.014	0	0.405 ± 0.011	0	2.173 ± 0.038	0.097 ± 0.003	0.234 ± 0.010	0	**15.348 ± 0.853**	83.5
B	2.832 ± 0.128	1.002 ± 0.064	2.261 ± 0.149	1.108 ± 0.088	0.208 ± 0.018	<LOQ	0.154 ± 0.009	0	0.293 ± 0.024	0	0.632 ± 0.067	<LOQ	0.801 ± 0.042	0	**9.291 ± 0.586**	84.6
blue	4.967 ± 0.838	7.448 ± 0.233	7.004 ± 1.190	5.791 ± 0.664	2.406 ± 0.338	2.968 ± 0.232	0.508 ± 0.053	<LOQ	0.626 ± 0.086	0	1.886 ± 0.292	0.715 ± 0.067	3.970 ± 0.207	0	**38.287 ± 4.200**	80.5
red	1.096 ± 0.014	9.461 ± 0.348	0.843 ± 0.012	11.490 ± 0.994	0.254 ± 0.015	3.034 ± 0.028	1.190 ± 0.032	0	0.350 ± 0.015	<LOQ	4.315 ± 0.109	<LOQ	8.619 ± 0.384	0	**40.653 ± 1.951**	83.5
yellow	1.939 ± 0.077	2.069 ± 0.066	2.730 ± 0.077	2.105 ± 0.064	0.581 ± 0.033	0.603 ± 0.034	0.216 ± 0.008	<LOQ	0.240 ± 0.009	0	1.252 ± 0.030	0.137 ± 0.008	2.089 ± 0.122	0	**13.962 ± 0.528**	85.6
pomegranate	1.966 ± 0.083	0.099 ± 0.006	0.454 ± 0.006	0.046 ± 0.003	0.363 ± 0.011	0.065 ± 0.008	<LOQ	0.089 ± 0.004	0.108 ± 0.002	0	0	0.205 ± 0.006	0	0	**3.393 ± 0.129**	80.4
quince	0.030 ± 0.001	2.058 ± 0.098	0.267 ± 0.019	2.999 ± 0.121	0.036 ± 0.002	0.354 ± 0.015	0.274 ± 0.003	0	0.091 ± 0.002	0	2.037 ± 0.037	0	0	0	**8.146 ± 0.298**	82.7

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Table 3. Continued

	concentration (mg/100 g fresh weight (edible part))																water
	CT	EC	B1	B2	B3	B4	B5	B6	B7	B8	C1	C2	A2	Cin B1	sum		(%)
raspberry	0.753 ± 0.050	8.372 ± 0.305	0	0	0.632 ± 0.024	12.132 ± 0.515	0.058 ± 0.003	0.132 ± 0.005	0	0.201 ± 0.009	0	0.350 ± 0.003	0	0	**22.630 ± 0.914**		83.9
rhubarb	5.892 ± 0.307	0.566 ± 0.013	0.721 ± 0.047	1.012 ± 0.040	0.113 ± 0.002	0	0.058 ± 0.002	<LOQ	0.185 ± 0.012	0	0.785 ± 0.028	0	0	0	**9.331 ± 0.451**		93.4
strawberry																	
A	5.553 ± 0.146	0.209 ± 0.002	2.175 ± 0.033	0	3.920 ± 0.089	0	0	0.408 ± 0.023	0.394 ± 0.004	0	0.954 ± 0.110	4.085 ± 0.063	0	0	**17.697 ± 0.470**		87.6
B	2.761 ± 0.419	0.078 ± 0.005	1.357 ± 0.081	0	1.942 ± 0.151	0	0	0.226 ± 0.021	0.268 ± 0.016	0	0.408 ± 0.031	1.457 ± 0.062	0	0	**8.496 ± 0.786**		89.8
C	4.501 ± 0.201	0.132 ± 0.009	1.324 ± 0.118	0	3.600 ± 0.182	0	0	0.452 ± 0.033	0.319 ± 0.034	0	0.806 ± 0.040	4.388 ± 0.360	0	0	**15.522 ± 0.977**		89.5

[a] Values given as the mean ± SD (n = 3). LOQ, limit of quantification (S/N 10); Cin B1, cinnamtannin B1. CT quantified as (+)-CT, and EC quantified as (−)-EC.

of 5 and 50 mg/100 g dry weight (corresponding to 0.5 and 5 mg/100 g fresh weight in the case of an assumed water content of 90%). Similar recovery rates were obtained, in detail being CT, 104.5 ± 6.5% (0.5 mg/100 g fresh weight added) and 100.3 ± 7.0% (5 mg/100 g fresh weight added); PC B2, 102.1 ± 3.0 and 100.4 ± 0.8%; and PC C1, 86.8 ± 4.1 and 92.3 ± 4.9%.

Table 3 presents analysis results of investigated food samples, mainly fruits. Nearly all samples contain flavan-3-ols and PCs with only some exceptions. Only in melon fruits (melon, honeydew melon, red and yellow watermelon, and cantaloupe melon), citrus fruits (clementine, lemon, and orange), pineapple, and cape gooseberry none of the investigated analytes were identified. The sum of PCs varied between several hundred micrograms, as in the case of banana or papaya, and 40 mg/100 g fresh weight (edible part) in the case of blackberry. High concentrations were quantified especially in apples, pears, berry fruits, and plums. The monomers CT and EC occur in nearly all samples. Only a few samples contain only one of the flavan-3-ols such as litchi. In the case of dimeric procyanidins C4−C8-linked PCs are more frequently present as C4−C6-linked PCs. Within the groups frequency of occurrence also differs. PCs B1 and B2 were identified and quantified in more samples in comparison to PCs B3 and B4. In the case of C4−C6-linked PCs the difference is clearer. PC B6 and especially PC B8 occur only in a very few samples. A relevant concentration of B8 was determined only in raspberry. A-type procyanidins were identified in higher concentrations in apricot, avocado, blueberry, cranberry, litchi, mirabelle, pear, and plum. The obtained results concerning the occurrence of PCs are in accordance with literature data. The study of Gu et al. demonstrated the occurrence and absence of PCs for the same fruits, which are mentioned here.[9] In our study more analytes were investigated, and also slight concentration differences were detected in comparison to other studies quantifying procyanidins as single compounds, for example, that of de Pascual-Teresa et al.[11]

Considering these data, interesting observations concerning the patterns of PCs can be made. A comparison of the patterns of PCs of different fruits shows great variations. However, if one considers fruits that are more closely related to each other, similarities can be observed in the patterns of PCs. An example is the comparison between apple, pear, and quince. Pear and quince contain additionally small concentrations of PCs B3 and B4 in comparison to apple. Pear differs from both of the others in the occurrence of A-type PCs. Another example is blackberry and raspberry. In this case the high concentrations of PC B4 are also of interest, because they are characteristic in comparison to other fruits. Different cultivars show nearly the same pattern of PCs, as in the case of different apples. The sum (25−40 mg/100 g fresh weight (edible part)) and also the single concentrations of occurring flavan-3-ols and PCs differ between the cultivars, but, interestingly, the relative concentrations of all five apple samples have nearly the same percentage distribution (see Figure 5). Further investigations are necessary to confirm these observations concerning relative concentrations because in this study only a small number of samples were investigated. The pattern of PCs in foods as well as relative concentrations are also useful to control the authenticity of fruits or fruit products such as fruit pulps.

Data generated by this method in combination with consumption data can contribute to a better understanding of any biological effects of PCs in humans. Under consideration of daily ingested amounts of different fruits, an estimation of the daily intake of flavan-3-ols and of the most important dimeric and

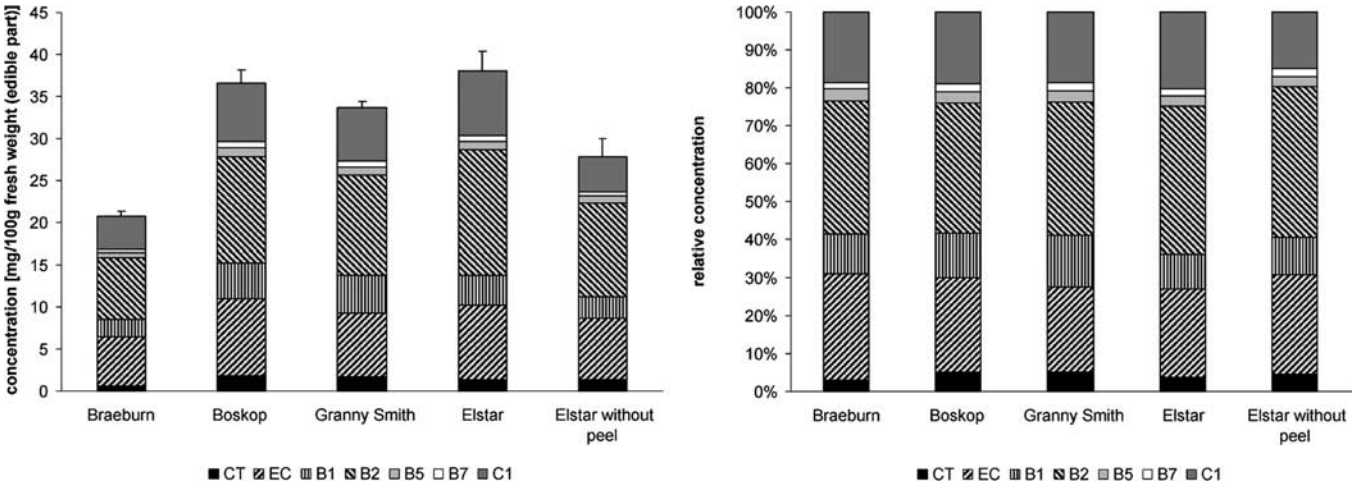

Figure 5. Absolute concentrations ± SD (left) and relative concentrations (right) of procyanidins in different apples cultivars: Braeburn, Boskop, Granny Smith, Elstar, and Elstar without peel (n = 3).

trimeric PC is possible. For a reliable estimation of the daily intake, more samples of the same fruit have to be screened. Especially samples of different years, different cultivars, and different times of harvest are of interest. The presented data show distinct differences in contents of PCs for the same fruit, for example, for strawberry. The total content varies between 8 and 18 mg/100 g fresh weight (edible part). Similar variations were observed for different varieties of apples, berries, and grapes.[26−28] Besides the above-mentioned effects, also storage and processing can have an impact on the content of PCs.[29,30] By taking the estimated daily intake in relationship to the ingested concentrations of PC in bioavailability studies, an evaluation is possible if naturally occurring concentrations are sufficient for any systemic effect in humans. According to the present knowledge only flavan-3-ols and low molecular weight PCs, especially dimeric and trimeric PCs, can be absorbed.[31−34] Holt et al., for example, could detect PC B2 in human plasma after consumption of cocoa.[35]

In conclusion, the newly developed method based on RP-HPLC-ESI-MS/MS offers a powerful tool for the identification and especially quantification of flavan-3-ols and the main dimeric and trimeric PCs in foods. For quantification the use of the echo-peak technique is the crucial step. In consideration of the obtained data of 55 food samples and further investigations taking into account the naturally occurring variability, an estimation of the daily intake of flavan-3-ols and the main dimeric and trimeric PCs in the human diet is possible. A transfer of the presented method to other foods such as cereals or nuts seems to be very interesting.

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ABBREVIATIONS USED

CE, collision energy; CT, catechin; CXP, collision exit potential; DcP, declustering potential; DMACA, *p*-dimethylaminocinnamaldehyde; DP, degree of polymerization; EC, epicatechin; GSE, grape seed extract; HCl, hydrochloric acid; PA, proanthocyanidin; PC, procyanidin; CXP, collision exit potential.

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