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Distribution and Quantification of Flavan-3-ols and Procyanidins with Low Degree of Polymerization in Nuts, Cereals, and Legumes

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ABSTRACT: The monomeric flavan-3-ols catechin and epicatechin as well as procyanidins are of great interest due to their potential beneficial health effects observed in epidemiological studies. However, the occurrence and concentration of these compounds is not well-known due to the fact that reference compounds are not commercially available. In this study we determined the pattern and concentration of catechin, epicatechin, and different dimeric and trimeric procyanidins in 38 food samples (nuts, cereals, legumes) using a reversed phase high-performance liquid chromatography electrospray ionization tandem mass spectrometry (RP-HPLC-ESI-MS/MS) approach based on isolated authentic reference compounds. Of the analyzed food samples 21 were found to contain dimeric and trimeric procyanidins and their monomeric building units catechin and epicatechin. Mainly the monitored nut samples contained the analyzed procyanidins as well as catechin and epicatechin whereas only 3 cereals were identified as sources of the analyzed compounds. The concentration ranged from 148 μg/100 g in macadamia nut to 55 mg/100 g in pinto bean. Catechin and procyanidin B3 were found to be the most abundant analytes. The only A-type procyanidin that could be identified was procyanidin A2, which was found in peanut. The achieved data could be used for authenticity control and furthermore in combination with dietary studies to calculate the daily intake of monomeric flavan-3-ols and procyanidins. To our knowledge this is the first detailed study quantifying monomeric flavan-3-ols and dimeric and trimeric procyanidins in various nuts, cereals, and legumes.

KEYWORDS: *procyanidins, flavan-3-ols, HPLC-MS/MS, quantification, nuts, cereals, legumes*

INTRODUCTION

Polyphenols are aromatic secondary plant metabolites comprising a broad spectrum of compounds with highly diverse structures. They include flavonoids consisting of a C6−C3−C6 backbone known as 2-phenylbenzopyran structure (Figure 1).[1] According to the substitution pattern and the degree of oxidation flavonoids can be divided into different subclasses. An example are proanthocyanidins (PAs), representing oligomers and polymers composed of different flavan-3-ol monomers. Depending on the type of monomeric flavan-3-ols, proanthocyanidins can be devided into propelargonidins, prodelphinidins, and procyanidins. Of which the most abundant group are procyanidins (PCs) composed exclusively of the monomeric flavan-3-ol-constituents epicatechin (EC) and catechin (CT). Commonly those monomeric constituents are connected through a C4→C8 linkage, but C4→C6 linkages also occur. In both cases they are named B-type PCs. A-type PCs are characterized by an additional ether linkage between C2→O7.[2] The molecular size is specified by the degree of polymerization (DP). Structures of the most important dimeric and trimeric PCs are shown in Figure 1.

The interest in PAs was aroused by the recognition of their potential beneficial health effects observed in epidemiological studies. They are reported to act as antioxidative, cardiopreventive, and anticarcinogenic agents.[3−6]

However, even today, the knowledge about the actual effects caused by alimentary PAs is rather limited. To explore the correlation described in the literature it is necessary not only to investigate the health effects related to PAs but also to take a closer look at the concentration and the distribution of PAs in our daily diet. Former studies already dealt with the distribution of PAs in different food products and with the estimated daily intake. Gu et al. analyzed the concentrations of PAs in different food samples and estimated the mean daily intake to be 57.7 mg/person in the U.S. population,[7] while Wang et al. proposed a total PA intake of 95 mg/d for U.S. adults older than 19 years.[8] Knaze et al. suggest an even higher daily intake for the population across several European countries, combining a dietary study with a flavonoid composition database.[9] The distribution of PAs in food products of plant origin available in Finland was determined by Hellström et al.[10] All mentioned studies have in common, that the estimated intake and determination of contents was based on the DP, meaning the concentrations of monomers, dimers, trimers, and PAs with higher DP were calculated as the sum of each group. However, distribution and intake of individual compounds have not been characterized. This is caused by the fact that the majority of the studies dealing with the distribution of PAs are based on NP (normal phase)-HPLC.[10−12] With use of this technique analyzed PAs are separated into clusters according to their DP, but not individually. As different PAs might be responsible for distinct biological effects, it is of scientific interest not only to monitor the total intake of monomeric flavan-3-ols and PAs according to their DP, but also to take a closer look at the pattern and the concentration of single compounds. For instance da Silva Porto et al. pointed out that the distinct radical-trapping antioxidant activity of procyanidins is dependent on the

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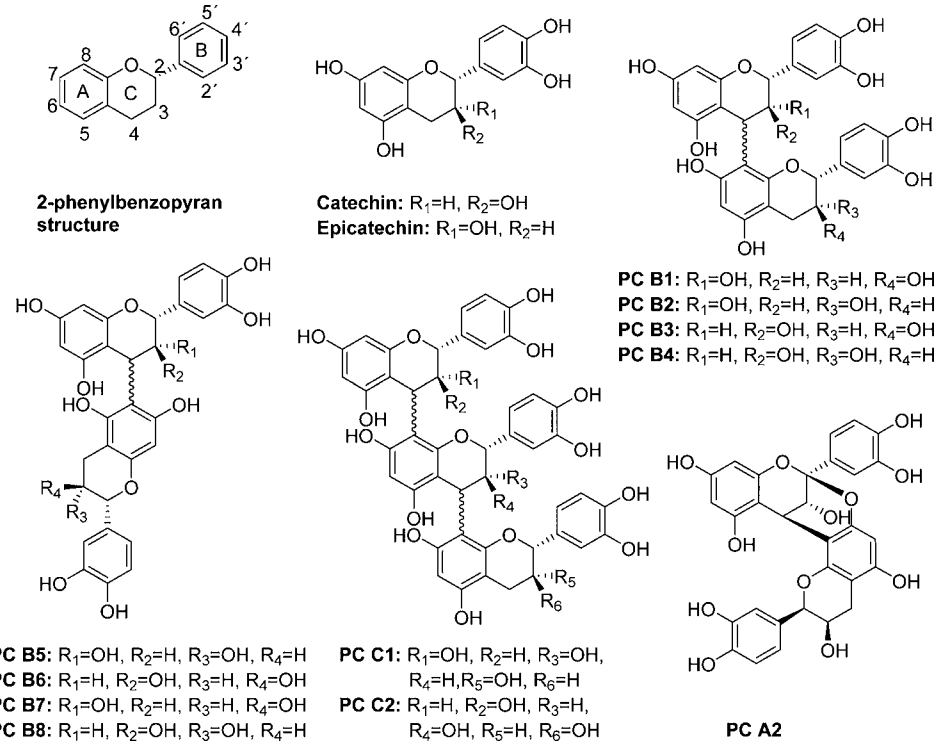

Figure 1. Sructures of 2-phenylbenzopyran, flavan-3-ols, and procyanidins.

interflavan linkage type (C4→C6 or C4→C8).[13] Besides especially procyanidins bearing an A-type linkage are supposed to be involved in the prevention of type 2 diabetes mellitus or of urinary tract infections.[14,15]

Mainly the analysis and quantification of monomers, dimers, and trimers is of interest as these low molecular weight analytes are supposed to be absorbed in the small intestine, while PCs with a higher DP are likely not to be absorbed before they are metabolized through the colonic microflora, for example, to phenolic acids.[16−19] For this purpose it is reasonable to apply RP-HPLC for the analysis. Compared to NP-HPLC this technique offers the advantage to separate compounds individually and not according to their DP, thus allowing the distribution of individual compounds to be monitored. However, separation is limited to DP of up to 4, while substances with a higher DP result in a baseline drift or an unresolved hump at the end of the chromatogram.[20] RP-HPLC was, for example, used for the quantification of monomeric flavan-3-ols and PA dimers and trimers in different kinds of Spanish food products by de Pascual-Teresa et al. allowing the characterization of individual compounds.[21] However, the occurrence and concentration of monomeric flavan-3-ols as well as procyanidins is not well studied due to the fact that reference compounds are not commercially available. Therefore in many studies quantification is based on equivalent concentrations, meaning the required PAs are calculated as equivalents of monomeric flavan-3-ols or specific dimeric or trimeric PCs.[22,23] For example, for the quantification of flavan-3-ols in hazelnut skin Del Rio et al. used PC B2 as equivalent for all quantified PAs, while monomers were quantified by using CT and EC 3-*O*-gallate as standards.[24] In a previous study a method using RP-HPLC-ESI-MS/MS has been developed for quantification of the monomeric flavan-3-ols CT, EC, and of 12 PCs in food samples.[25] The main advantage of this method is the compensation of matrix effects by using the echo-peak technique.[26] In the current study this method has been applied to the analysis of nuts, cereals, and legumes.

The aim of the present study was to determine the concentration and distribution of monomeric flavan-3-ols and dimeric and trimeric PCs to obtain more qualitative and quantitative information about the intake of flavan-3-ols and especially PCs with a low degree of polymerization. Identification and quantification was based on isolated authentic reference compounds as most of these are not commercially available.

MATERIALS AND METHODS

Chemicals and Reagents. Solvents for sample extraction as well as for chromatography were purchased from Carl Roth (Karlsruhe, Germany), Sigma-Aldrich (Steinheim, Germany), and Grüssing (Filsum, Germany) in gradient or analytical grade. Water for extraction and HPLC was purified with a Milli-Q Gradient A10 system (Millipore, Schwalbach, Germany). (+)-CT was obtained from Applichem (Darmstadt, Germany) and (−)-EC from Sigma-Aldrich. PCs B1, B2, B3, B4, B5, B6, B7, C1, C2, A2 and cinnamtannin B1 have been previously isolated from plant material or synthesized according to Rzeppa et al.[25]

Sample Preparation. Analyzed samples were obtained from local food stores. Cereal and legume samples were ground into powder with use of an IKA A10 analytical grinder (IKA Labortechnik, Staufen, Germany) and sieved (100 μm) afterward. Nut samples were ground with a Fritsch Pulverisette 14 impact mill with a round hole sieving ring (400 μm) (Fritsch, Idar-Oberstein, Germany). All samples were extracted according to a published method with slight modifications.[25] For nut samples 2 g of each sample were extracted. Instead of *n*-hexane, *n*-pentane was used to remove fat. Nut samples were extracted with 30 mL of *n*-pentane, all other samples using 15 mL of *n*-pentane. The PC extraction was carried out two times with 20 mL of acetone/water (70:30, v/v). Different aliquots of the extract were evaporated to dryness under a nitrogen stream and dissolved in 0.1% formic acid/acetonitrile (90:10, v/v) afterward. In addition to previously used aliquots of 100, 250, 500, and 1000 μL dissolved in 1 mL, aliquots of 1000 and 2000 μL were also evaporated to dryness

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and dissolved in 500 μL. Quantification was carried out by using the sample most suitable to the range of the calibration curve.

RP-HPLC-ESI-MS/MS. PCs and monomeric flavan-3-ols were analyzed by using the same conditions of separation, detection, and quantification as described by Rzeppa et al.[25] In brief analysis was carried out by using RP-HPLC-ESI-MS/MS in Multiple Reaction Monitoring (MRM) mode. The system was equipped with an additional valve between HPLC and MS for the removal of sugars and other polar compounds. Quantification was performed with the echo-peak technique, using CT and EC as echo-peaks. Calibration curves were obtained by calculating the peak ratios of each analyte to one of the two echo-peak areas and plotting it against the concentration. The range of the calibration curve was from 50 to 1000 ng/mL for all analytes. For the assured identification of analytes the relative ion intensities of the different MRM transitions where monitored. As demanded in the Commission Decision 2002/657/EC of the European Commission, for a reliable identification of an analyte two different mass transitions shall be observed. The one with the higher intensity was selected as quantifier while the second one was used as qualifier. Depending on the relative intensity, expressed as a percentage of the intensity of the most intense transition, there are maximum permitted tolerances which should be adhered to.[27] For the appropriate identification of each analyte it was required, that these guidelines were accomplished.

To prove the suitability of the method recovery rates were determined. Therefore for each food category (nuts, cereals, and legumes) a blind matrix containing neither monomeric flavan-3-ols nor PCs was chosen. The selected blind matrices were brazil nut, wheat, and white bean. In the case of brazil nut and wheat CT, EC, PCs B2, B3, B5, C1, C2, and A2 were added in concentrations of 0.5, 1, or 10 mg/100 g fresh weight. As concentrations were higher in legume samples, white bean was spiked with CT, PCs B3, B6, and C2 in concentrations of 0.5, 1, or 20 mg/100 g fresh weight. Determination of LOD (limit of detection) and LOQ (limit of quantification) was based on S/N (signal-to-noise ratio). For LOD a S/N of 3 and for LOQ a S/N of 10 was required and screened individually for each sample and analyte.

RESULTS AND DISCUSSION

The extraction method used in this study is based on previous investigations dealing with the analysis of CT, EC, and PCs in fruit samples[18] with slight modifications. Due to the higher fat content of nut samples the amount of *n*-pentane for the defatting had to be increased, while for cereals and legumes the previously used amount was sufficient. In neither of the *n*-pentane fractions were monomeric flavan-3-ols or PCs detectable (data not shown). Apart from that, two consecutive extractions with acetone/water (70:30, v/v) were sufficient to extract >95% of the expected analytes.

The suitability of the method was proven by the determination of recovery rates. As representative matrices brazil nut, wheat, and white bean were chosen. The absence of monomeric flavan-3-ols and PCs was tested in advance. As described by Rzeppa et al. CT, EC, PCs B2, B3, B5, C1, C2, and A2 were chosen as representative compounds for the determination of recovery rates in brazil nut and wheat.[25] The recovery rates for brazil nut ranged from 85% to 130% whereas those for wheat ranged from 85% to 118% with slightly lower recovery rates for PC B5 from 71% to 85%. Determination of recovery rates for legumes was carried out by using white bean as exemplary matrix. In this case CT, PCs B3, B6, and C2 were added in a higher concentration to cover the higher contents occurring in some legume samples. The obtained recovery rates varied between 86% and 126%.

The method was applied to the analysis of nuts, cereals, and legumes. Of the 38 analyzed food samples 21 contained monomeric flavan-3-ols and PCs, whereas in 17 samples none of the investigated analytes were detectable. In Figure 2 an exemplary

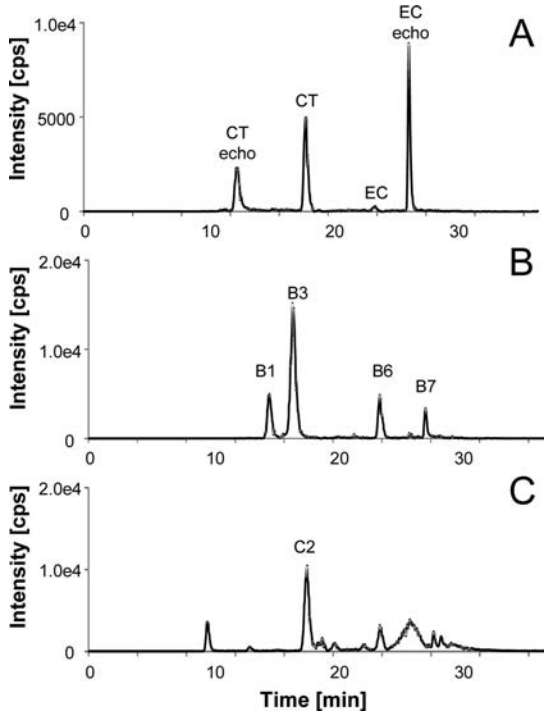

Figure 2. RP-HPLC-ESI-MS/MS chromatograms of an extract of green lentil: (A) monomeric flavan-3-ols with MRM *m/z* 289.0 → 124.9 $[M - H]^-$, (B) dimeric procyanidins with MRM *m/z* 577.1 → 124.9 $[M - H]^-$, (C) trimeric procyanidins with MRM *m/z* 865.3 → 125.1 $[M - H]^-$. Peak labels: CT echo (catechin-echo); CT (catechin); EC (epicatechin); EC echo (epicatechin-echo); B1 (procyanidin B1); B3 (PC B3); B6 (PC B6); B7 (PC B7); C2 (PC C2).

chromatogram of green lentil is shown. The concentrations of the detected monomeric flavan-3-ols and PCs are listed in Table 1. In the food samples listed in Table 2 CT, EC, and PCs with a low degree of polymerization were absent. It is remarkable that most of the nuts contain monomeric flavan-3-ols or PCs. The only exception of the analyzed nuts was brazil nut. In contrast to that only a few cereals contain the analyzed compounds. Whereas they were absent in most of the analyzed samples naked barley and buckwheat showed comparatively high concentrations. The most abundant analyte was CT, which was detectable in all of the analyzed samples while PC B4 and PC B8 as well as Cin B1 could not be detected in any samples. The contents ranged from only a few hundred micrograms per 100 g for macadamia nut (148 μg/100 g) or mung bean (665 μg/100 g) to 55 mg/100 g in the case of pinto bean.

Nuts. As mentioned before in all of the analyzed nut samples, except brazil nut, monomeric flavan-3-ols and low molecular weight PCs were detectable. All nut samples contained CT and almost all EC except pecan nut and walnut. In the case of trimeric PCs larger amounts of PC C1 were detectable in almonds while pecan nut exhibited higher amounts of PC C2. Moreover peanut was the only sample found to contain A-Type PCs. This observation is consistent with the findings of Gu et al., who also identified A-type PCs in peanut. Additionally they detected A-type PCs in some fruit and spice samples which were not monitored in the present work.[12] Apart from that Lou et al. could also identify 6 different A-Type PAs in peanut skin among which was also PC A2.[28]

Comparing the results for the two different analyzed almond samples it is notable, that they show an equal pattern of monomeric flavan-3-ols and PCs although the absolute

Table 1. Proanthocyanidin Contents in Samples[a]

	concentration (mg/100 g)														
sample	CT	EC	B1	B2	B3	B4	B5	B6	B7	B8	C1	C2	A2	Cin B1	total
nuts															
almond 1	10.307 ± 0.550	1.612 ± 0.025	7.282 ± 0.378	3.669 ± 0.240	0.451 ± 0.031	n.d.	0.431 ± 0.024	n.d.	1.427 ± 0.013	n.d.	5.197 ± 0.379	n.d.	n.d.	n.d.	30.376 ± 1.639
almond 2	2.270 ± 0.073	0.650 ± 0.031	1.691 ± 0.141	0.866 ± 0.035	0.189 ± 0.015	n.d.	<LOQ	n.d.	0.276 ± 0.018	n.d.	1.148 ± 0.005	n.d.	n.d.	n.d.	7.090 ± 0.319
cashew nut	6.881 ± 0.417	3.425 ± 0.041	0.165 ± 0.007	0.151 ± 0.008	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	10.623 ± 0.473
hazelnut	1.536 ± 0.064	<LOQ	1.850 ± 0.097	0.198 ± 0.011	2.079 ± 0.070	n.d.	n.d.	0.170 ± 0.011	0.281 ± 0.10	n.d.	n.d.	2.390 ± 0.046	n.d.	n.d.	8.497 ± 0.311
macadamia nut	0.037 ± 0.001	0.110 ± 0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.148 ± 0.004
peanut	2.495 ± 0.019	1.073 ± 0.061	n.d.	n.d.	0.195 ± 0.019	n.d.	n.d.	n.d.	0.086 ± 0.006	n.d.	n.d.	n.d.	0.626 ± 0.045	n.d.	4.474 ± 0.149
pecan nut	2.418 ± 0.160	n.d.	1.761 ± 0.152	0.178 ± 0.013	4.865 ± 0.129	n.d.	n.d.	0.585 ± 0.017	0.488 ± 0.019	n.d.	n.d.	5.020 ± 0.064	n.d.	n.d.	15.316 ± 0.554
pistachio	3.111 ± 0.201	n.d.	0.826 ± 0.055	n.d.	0.454 ± 0.032	n.d.	n.d.	n.d.	0.137 ± 0.012	n.d.	0.190 ± 0.008	0.187 ± 0.013	n.d.	n.d.	4.906 ± 0.321
walnut	6.713 ± 0.554	n.d.	2.190 ± 0.108	n.d.	0.580 ± 0.028	n.d.	n.d.	0.164 ± 0.015	1.204 ± 0.034	n.d.	1.228	0.454 ± 0.024	n.d.	n.d.	12.533 ± 0.779
cereals															
buckwheat	5.933 ± 0.056	11.868 ± 0.289	0.301 ± 0.027	2.540 ± 0.152	0.409 ± 0.020	n.d.	0.393 ± 0.023	<LOQ	<LOQ	n.d.	0.881 ± 0.049	n.d.	n.d.	n.d.	22.324 ± 0.616
naked barley	4.112 ± 0.182	n.d.	n.d.	n.d.	19.807 ± 1.486	n.d.	n.d.	0.986 ± 0.032	n.d.	n.d.	n.d.	9.978 ± 0.558	n.d.	n.d.	34.882 ± 2.257
unripe spelt grain	0.253 ± 0.009	n.d.	n.d.	n.d.	0.290 ± 0.006	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	0.276 ± 0.003	n.d.	n.d.	0.738 ± 0.019
legumes															
azuki bean	6.296 ± 0.171	n.d.	11.876 ± 0.673	<LOQ	3.392 ± 0.076	n.d.	n.d.	0.271 ± 0.015	1.779 ± 0.079	n.d.	n.d.	0.422 ± 0.043	n.d.	n.d.	24.036 ± 1.058
black bean	2.164 ± 0.138	n.d.	2.251 ± 0.133	n.d.	0.354 ± 0.018	n.d.	n.d.	n.d.	0.259 ± 0.032	n.d.	n.d.	n.d.	n.d.	n.d.	5.029 ± 0.321
kidney bean	5.873 ± 0.507	<LOQ	7.428 ± 0.400	n.d.	1.454 ± 0.117	n.d.	n.d.	<LOQ	1.176 ± 0.039	n.d.	1.751 ± 0.148	n.d.	n.d.	n.d.	17.681 ± 1.211
lentil (beluga)	3.258 ± 0.068	<LOQ	3.128 ± 0.286	n.d.	15.023 ± 1.414	n.d.	n.d.	3.353 ± 0.142	0.510 ± 0.032	n.d.	n.d.	13.175 ± 0.605	n.d.	n.d.	38.448 ± 2.545
lentil (brown)	3.070 ± 0.156	n.d.	3.085 ± 0.205	n.d.	15.689 ± 0.691	n.d.	n.d.	3.287 ± 0.165	0.286 ± 0.004	n.d.	n.d.	21.272 ± 0.347	n.d.	n.d.	46.689 ± 1.568
lentil (green)	4.255 ± 0.064	<LOQ	4.024 ± 0.388	n.d.	10.481 ± 0.795	n.d.	n.d.	2.139 ± 0.088	1.000 ± 0.013	n.d.	n.d.	8.027 ± 0.654	n.d.	n.d.	29.926 ± 2.002
mung bean	0.132 ± 0.003	<LOQ	0.325 ± 0.031	n.d.	0.126 ± 0.008	n.d.	n.d.	n.d.	0.082 ± 0.007	n.d.	n.d.	n.d.	n.d.	n.d.	0.665 ± 0.050
pinto bean	20.181 ± 0.708	0.540 ± 0.032	19.201 ± 1.359	<LOQ	4.891 ± 0.541	n.d.	n.d.	<LOQ	3.353 ± 0.106	n.d.	4.886 ± 0.460	1.665 ± 0.033	n.d.	n.d.	54.718 ± 3.240
runner bean	7.041 ± 0.631	0.550 ± 0.021	10.415 ± 0.465	0.553 ± 0.035	0.529 ± 0.033	n.d.	n.d.	n.d.	1.087 ± 0.068	n.d.	n.d.	n.d.	n.d.	n.d.	20.174 ± 1.254

[a]Milligrams per 100 g of fresh weight, mean ± standard deviation, $n = 3$, n.d. = not detectable.

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Table 2. Samples with No Detectable Proanthocyanidins

nuts	cereals	legumes
brazil nut	amaranth	chickpea
	corn	pea
	kamut	soy bean
	millet	white bean
	oat/naked oat	
	quinoa	
	rice	
	rye	
	spelt	
	wheat/einkorn wheat	

concentration differs (Figure 3). This aspect has also been observed by de Pascual-Teresa et al. comparing different samples of the same food. They pointed out that the relative composition of monomeric flavan-3-ols and PCs might be characteristic for the different sample types while the absolute concentration could be subject to external factors as origin of the sample or stage of ripeness.[21] As the relative distribution of PCs revealed a characteristic pattern for different samples it can be used for authenticity control. However, in further investigations additional different samples of the same product need to be analyzed.

Cereals. The only cereals found to contain monomeric flavan-3-ols and PCs with a low degree of polymerization are buckwheat, naked barley, and unripe spelt grain. This is in accordance with the results of Hellström et al,. who detected buckwheat grits, barley flour, and beer to be the only cereal sources for PCs.[10] Concentrations of monomeric flavan-3-ols and PCs with 22 mg/100 g and 34 mg/100 g, respectively, are comparatively high in buckwheat and naked barley. PCs in naked barley are exclusively composed of CT as monomeric unit in contrast to buckwheat which exhibits a broader spectrum of PCs. An interesting observation can be made considering the results for spelt and unripe spelt grain. Whereas there were no monomeric flavan-3-ols and low molecular weight PCs detectable for the spelt sample, for its analogue harvested in an unripened state we could find minor concentrations of CT and PCs.

Legumes. Among the analyzed types of various food samples, legumes were found to be the major source of monomeric flavan-3-ols and PCs. With mung bean and black bean as the only exceptions all samples show comparatively high contents. Concentrations vary between 0.7 mg/100 g and 54 mg/100 g. All samples contain beside CT as monomeric flavan-3-ol, PC B1, B3, and notably B7 as C4→C6 linked PC. In contrast PC B5 appears in none of the samples although it

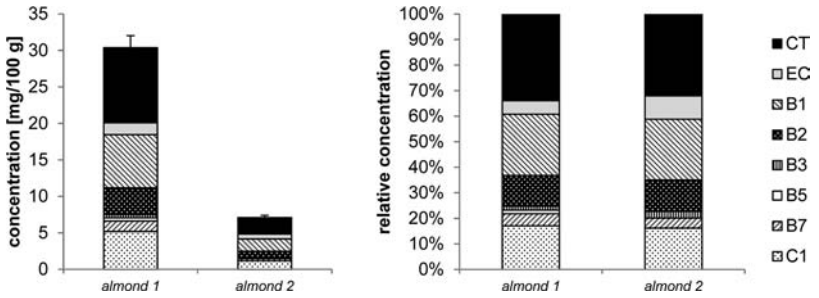

Figure 3. Absolute concentrations ± SD (left) and relative concentrations (right) of flavan-3-ols and procyanidins in almonds (n = 3).

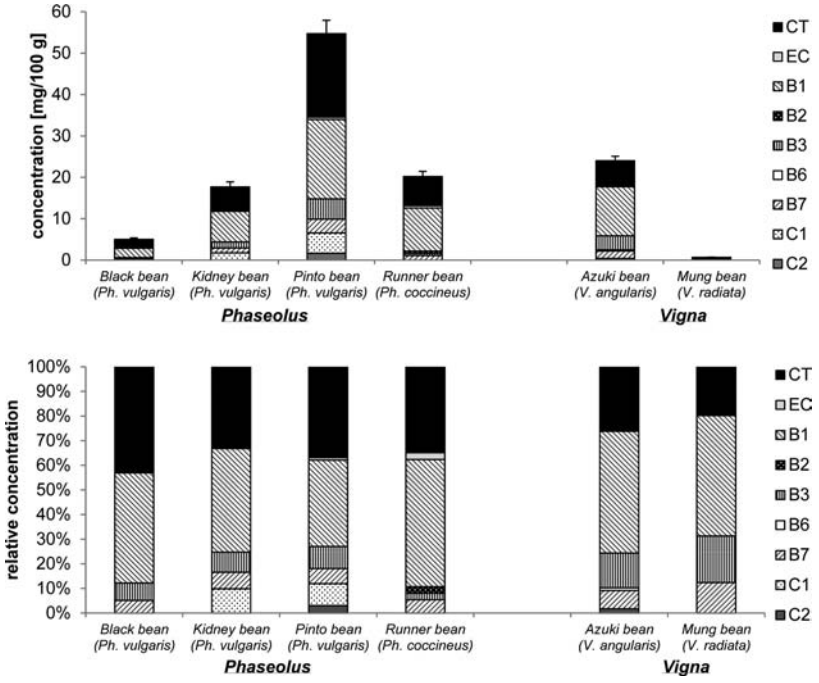

Figure 4. Absolute concentrations ± SD (top) and relative concentrations (bottom) of flavan-3-ols and procyanidins in legumes (genus phaseolus and vigna) (n = 3).

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occurs relatively frequently in fruits.[25] In addition EC, PC B2, B6, and C1 can be found in some samples. Taking a closer look at the pattern of the analytes interesting similarities appear. Comparing species belonging to the same genus and their varieties, similar patterns of monomeric flavan-3-ols and PCs were observed. For example, this can be seen for beans belonging to the genus *Phaseolus*. Black bean, kidney bean, and pinto bean, all varieties of the species *Phaseolus vulgaris*, show different concentrations for the occurring monomeric flavan-3-ols and PCs as well as for the total amounts (5−55 mg/100 g). However, relative concentrations show a similar distribution (Figure 4). Additionally for runner bean belonging to the species *Phaseolus coccineus* similarities in the pattern can be observed. Second examples are beans of the *Vigna* genus. Although the total content for azuki bean and mung bean differs, the percentaged distribution is comparable. A similar observation was made in the previous study by Rzeppa et al. comparing closely related fruits or different fruit cultivars.[25]

In summary the applied method based on RP-HPLC-ESI-MS/MS analysis and the use of isolated authentic reference compounds allows the quantification of monomeric flavan-3-ols and the main dimeric and trimeric PCs individually in different food samples. By this a monitoring of the distribution of specific compounds is possible. The method has been used to analyze 38 different food samples including nuts, cereals, and legumes. Twenty-one samples contain monomeric flavan-3-ols and PCs in a concentration range from a few micrograms up to 55 mg/100 g. Especially in legumes high concentrations were observed, whereas the concentrations in nuts are comparatively lower. Only a few cereals contain monomeric flavan-3-ols and PCs. Additionally for legumes belonging to the same genus and their varieties, similar patterns of monomeric flavan-3-ols and PCs were observed. The obtained data combined with dietary studies allow the calculation of the daily intake of monomeric flavan-3-ols and PCs with a low degree of polymerization which are likely to be absorbed in the small intestine without metabolism through the colonic microflora. Regarding the possible differences in the content due to external influences further investigations including additional different samples of the same product might be interesting.

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Notes

The authors declare no competing financial interest.

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