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Proanthocyanidin Composition in the Seed Coat of Lentils (*Lens culinaris* L.)

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Lentils (*Lens culinaris* L.) are a popular food in many countries. However, little is known about their phenolic composition. Because polyphenols in lentils are located essentially in their seed coat, the objective of this work was to study the composition of proanthocyanidins, the major group of polyphenols, in this part of the tissue. The use of C_{18} Sep-Pak cartridges permitted the fractionation of lentil seed coat extract into monomer, oligomer, and polymer proanthocyanidin fractions. Subsequent thiolysis of oligomer and polymer fractions followed by HPLC analysis allowed the mean degree of polymerization (mDP) and the structural composition of proanthocyanidins to be determined. A fractionation of lentil seed coat extracts on a polyamide column followed by HPLC and HPLC-DAD-MS analyses was used to identify the individual proanthocyanidins. The results showed that the major monomeric flavan-3-ol was (+) catechin-3-glucose, with lesser amounts of (+)-catechin and (−)-epicatechin. In the oligomer fraction, various dimer, trimer, and tetramer proanthocyanidins constituted of catechin, gallocatechin, and catechin gallate units were identified, and several procyanidins and prodelphinidins from pentamers to nonamers constitute the polymer fraction. The most abundant proanthocyanidins in the seed coat of lentils are the polymers (65−75%), with a mDP of 7−9, followed by the oligomers (20−30%), with a mDP of 4−5.

KEYWORDS: Lentils; seed coat; proanthocyanidins

INTRODUCTION

Proanthocyanidins are a class of phenolic compounds widely distributed in the plant kingdom. These compounds are oligomers and polymers of flavan-3-ol monomer units, linked principally through the C-4 of the flavanol unit, also called the "extension" or "upper" unit, and the C-8 or C-6, the "lower" unit.

The most proanthocyanidins in foods are procyanidins with a 3′,4′-dihydroxy substitution on the B ring and prodelphinidins with a 3′,4′,5′-trihydroxy substitution. Flavan-3-ol units are also found as gallic acid esters and glycosylated in the hydroxyl group at the C-3 position (*1*, *2*) or C5 position (*3*).

Proanthocyanidins play an important role in the sensory properties and biological quality of foods. It has been reported that proanthocyanidins possess various biological activities, in particular their potent antioxidant activity (*4*, *5*) and their free radical scavenging activity (*6*). These compounds inhibit platelet aggregation (*7*, *8*), as the inhibition of the oxidation of low-density lipoproteins, and they present antiulcer activity against stomach mucosa injury (*9*). It was also observed that procyanidins from grape seeds present radioprotective effects against chromosomal damage induced by X-rays (*10*). The majority of these properties depend on the structures and especially on the degree of polymerization (DP).

Proanthocyanidins are present in many plant-derived foods. Tea (*11*), grape seed (*12*, *13*), beer (*14*), red wine (*15*, *16*), apples (*17*, *18*), and cocoa seeds and chocolate (*19*, *20*) have been known to be substantial sources of these compounds. However, there is less information about the presence of proanthocyanidins in legumes. Several authors have studied the DP and structural composition of oligomeric and polymeric proanthocyanidins, overall in grape seeds (*21*, *22*) and apples and cider (*23*), but to our knowledge, there are no such published data about legumes. Lentils (*Lens culinaris* L.), a kind of legume rich in proteins, carbohydrates, and dietary fiber, are a popular food in many countries. Like many other legumes such as soybean, broad beans, faba beans, and peas (*24*−*28*), polyphenols in lentils are also located essentially in their seed coat, including flavonol, flavone glycosides such as quercetin 3-*O*-rhamnoside, myricetin 3-*O*-rhamnoside, luteolin 7-*O*-glucoside, apigenin 7-*O*-apiofuranosyl, *trans*-resveratrol 3-*O*-glucoside, and an important concentration of proanthocyanidin (∼3 mg/g), although the seed coat of lentil represents a small percentage of the entire lentil seed, ranging from 8 to 11% (*29*).

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Thus, the main purpose of this work was to study the proanthocyanidin compositions in seed coats of lentils by fractionation on C_{18} Sep-Pak cartridges and on a polyamide column, followed by thiolysis, HPLC, and HPLC-DAD-MS analysis.

MATERIALS AND METHODS

Standards. (+)-Catechin, (−)-epicatechin, and toluene-α-thiol (benzyl mercaptan) were obtained from Fluka AG (Buchs, Switzerland). Procyanidins B1 and B3 were from Extrasynthese (Genay, France) and B2 from Sigma (Barcelona, Spain). The C_{18} Sep-Pak cartridges were purchased from Waters Associates (Milford, MA). The benzyl thioethers of catechin, epicatechin, and epicatechin 3-*O*-gallate were isolated and purified by semipreparative HPLC using a μBondapak C_{18} column (300 × 7.8 mm, 5 μm particle size) (*22*).

Preparation of Samples and Extraction of Polyphenols. Two varieties of lentils (*L. culinaris*), Pardina and Castellana (Spain), and two samples of each variety purchased from a local market were used. The seed coats of lentils were manually separated from the cotyledon and ground in a little mill (Retsch MM 2000). The powder obtained was put into plastic bags and stored at −20 °C under vacuum until used. The extraction of polyphenols from the seed coat was performed according to the method described by Bourzeix et al. (*30*).

Fractionation of the Phenolic Extract with C_{18} Sep-Pak Cartridges. The dealcoholized seed coat phenolic extract was fractionated through two connected C_{18} Sep-Pak cartridges to obtain monomeric flavan 3-ol (F_I), oligomeric (F_{II}), and polymeric (F_{III}) proanthocyanidin fractions, as described by Sun et al. (*31*).

Vanillin Assay. Quantification of total flavan-3-ols in each fraction (F_I, F_{II}, and F_{III}), obtained by C_{18} Sep-Pak cartridges, was performed by using the modified vanillin assay according to Sun et al. (*32*). Each fraction (F_I, F_{II}, and F_{III}) from C_{18} Sep-Pak cartridges was evaporated to dryness and dissolved in methanol to give a desired concentration (so that the A_{500} of the final vanillin reaction medium is fixed between 0.3 and 0.5). One milliliter of this methanolic solution was added to 2.5 mL of 1% vanillin in methanol and 2.5 mL of 25% H_2SO_4 in methanol. The A_{500} value of the reaction medium was measured, and the total amount of proanthocyanidins in each fraction was expressed as (+)-catechin.

Degradation of Proanthocyanidins with Toluene-α-thiol. Acid-catalyzed degradation on the fractions F_{II} and F_{III} obtained from C_{18} Sep-Pak cartridges was carried out according to the method of Prieur et al. (*22*). The hydrolyzed products were then analyzed by HPLC to determine both mean degree of polymerization (mDP) and structural composition of proanthocyanidins, under the conditions already described (*31*).

Fractionation of the Phenolic Extract by Polyamide Column. The dealcoholized seed coat phenolic extract was also fractionated by a polyamide column to isolate monomeric flavan-3-ol (F_{cat}) and oligomeric proanthocyanidin (F_{pro}) fractions as described by Ricardo da Silva et al. (*33*). Both fractions were analyzed by HPLC and HPLC-DAD-MS under the conditions described below.

HPLC Conditions. The HPLC apparatus used was a Hewlett-Packard 1050, equipped with a quaternary pump, a UV−visible detector coupled to a data processing computer (Millennium 2010), a thermostat controlling the column temperature, and a manual injection valve. The column was a Lichrospher 100RP-18 (5 μm, 250 × 4 mm) (Merck). The detection was at 280 nm, and the temperature of the column was 30 °C. The solvents were (A) water/acetic acid (99:1, v/v) and (B) water/acetic acid (90:10, v/v). Two different elution conditions were used. For monomeric flavanols (catechins), the linear gradient was 0−78% B in 15 min, followed by 78−100% B in 30 min; a flow rate of 0.7 mL/min was used. The linear gradient for proanthocyanidins was 0−67% B in 45 min, 67−83% B in 10 min, and 83−100% B in 20 min; the flow rate was 1.0 mL/min.

HPLC-DAD-MS Conditions. A Hewlet-Packard series 1100 (Palo Alto, CA) chromatograph equipped with DAD and MS detectors and an electrospray ionization (ESI) interface was used. A gradient of solvent A (water/acetic acid, 99:1, v/v) and solvent B (water/acetic acid, 90:10, v/v) was applied to a reversed-phase Nova-Pak C_{18} column [30 × 3.9 mm (inside diameter)] as follows: 67% B from 0 to 45 min, 67−83% B from 45 to 55 min, 83−100% B from 55 to 75 min; the flow rate was 0.7 mL/min. Nitrogen was used as the nebulizing and drying gas. ESI conditions were as follows: nitrogen pressure, 40 psi; drying gas, 10 mL/min at 340 °C; ion spray voltage, 4000 V; and variable fragmentator voltage, 80 V (m/z < 200), 200 V (m/z 200−1000), 200 V (m/z 1000−3000). Mass spectra were recorded from m/z 100 to 3000.

Acid Hydrolysis. The hydrolysis was used as a method of cleavage of glycosylated catechins to remove a sugar from the molecule. The sample was dissolved in ethanol and 1 N HCl on a steam bath for 10 min and extracted three times with ethyl acetate; the organic fractions were evaporated to dryness under vacuum, and the residue was redissolved in methanol/water (1:1, v/v) and analyzed by HPLC-DAD to identify the phenolic compound.

Statistical Analysis. Sampling and analyses were performed in duplicate or triplicate, and the data are presented as mean ± SD. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Statgraphics Plus 5.0 v. (Graphics Software System, Rockville, MD).

RESULTS

Structural Composition of Oligomeric and Polymeric Proanthocyanidins. HPLC analysis of thiolyzed products of oligomeric (F_{II}) and polymeric (F_{III}) fractions obtained by C_{18} Sep-Pak cartridges permitted the identification of free (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-*O*-gallate as terminal units, and the benzyl thioethers of catechin, epicatechin, epicatechin 3-*O*-gallate and epigallocatechin as extension units. The mDP and the data of structural composition of oligomeric and polymeric proanthocyanidins can thus be calculated as described (*22*). These results were presented in **Table 1**.

The mDP was estimated to be 4−5 for the oligomeric fraction and 7−9 for the polymeric fraction. Pardina 2 shows the highest values for both fractions. In both oligomeric and polymeric fractions, (+)-catechin in terminal units is the most abundant, with a small proportion of (−)-epicatechin. Only Pardina 1 shows a small percentage of (−)-epicatechin 3-*O*-gallate in the oligomeric and polymeric fractions.

As extension units, the (+)-catechin is predominant in the oligomeric and polymeric fractions. The (−)-epicatechin in the oligomeric fraction represents a lower percentage (20%) than in the polymeric fraction (30%). The proportions of (+)-catechin as extension units in the polymeric fraction are noticeably variable from one to another variety (50−60%), being the highest in Castellana varieties. De Pascual-Teresa et al. (*34*) reported the presence of prodelphinidins in lentils. Our results show that the prodelphinidins in the seed coat of lentil are mainly presented in polymeric forms, because epigallocatechin units were essentially detected in this fraction (**Table 1**). In the oligomeric fraction, traces of epigallocatechin units were detected in all samples. Furthermore, this fraction could present prodelphinidins but in much lower concentration.

Identification of Individual Flavan-3-ols in Monomeric and Oligomeric Proanthocyanidin Fractions Obtained by a Polyamide Column. Figures 1 and **2** present, respectively, typical HPLC chromatograms of F_{cat} and F_{pro} obtained from a polyamide column.

In both **Figures 1** and 2, all numbered peaks gave positive reaction with vanillin−H_2SO_4 under the conditions as described (*32*), indicating that they were flavanol-type compounds.

In **Figure 1**, peaks 2 and 3 were identified as (+)-catechin and (−) epicatechin and confirmed by the presence of an ion mass $[M - H]^-$ at m/z 289, corresponding to a monomer of flavan-3-ol, in the ESI-MS analysis.

Table 1. Mean Degree of Polymerization and Structural Composition (Relative Percentage of Each Unit)[a] of Oligomeric and Polymeric Proanthocyanidin Fraction from C_{18} Sep-Pak Cartridges

				terminal units			extension units				
fraction	sample		mDP	Cat[c]	Epicat[c]	EpicatG[c]	Epig[c]	Cat (cis)	Cat (trans)	Epicat	EpicatG
oligomers	Pardina 1	mean	3.8a	22.61a	3.58a	0.18	tr[d]	10.62a	42.14a	19.10a	1.78
		SD	0.1	0.53	0.44	0.01		0.36	0.17	1.02	0.13
	Pardina 2	mean	4.7c	18.25b	2.84a	nd[e]	tr	12.85b	49.27b	16.79a	nd
		SD	0.0	0.17	0.20			0.02	0.25	0.25	
	Castellana 1	mean	4.2b	21.11a	2.89a	nd	tr	13.31b	52.00c	10.69b	nd
		SD	0.0	0.66	0.55			0.11	1.37	1.37	
	Castellana 2	mean	4.0ab	21.62a	3.49a	nd	tr	12.82b	49.64bc	12.42b	nd
		SD	0.2	1.00	0.24			0.03	0.97	0.31	
polymers	Pardina 1	mean	6.9a	11.84a	1.69a	0.10	5.67a	11.11a	38.35a	30.47a	0.79
		SD	0.1	0.02	0.19	0.03	0.41	0.40	0.17	0.39	0.36
	Pardina 2	mean	9.4c	8.61b	1.29b	nd	6.72b	10.42b	39.08b	33.87b	nd
		SD	0.1	0.09	0.03		0.24	0.09	0.30	0.09	
	Castellana 1	mean	7.1a	11.89a	1.64a	nd	3.75c	13.49c	48.42c	20.81c	nd
		SD	0.1	0.28	0.04		0.41	0.07	0.35	0.25	
	Castellana 2	mean	7.8b	10.45c	1.65a	nd	6.47ab	12.00d	43.25d	26.18d	nd
		SD	0.1	0.14	0.02		0.07	0.09	0.02	0.26	

[a] The values presented for terminal and extension units are their relative molar concentrations. [b] Mean degree of polymerization. [c] Cat, (+)-catechin; Epicat, (–)-epicatechin; EpicatG, (–)-epicatechin 3-*O*-gallate; Epig, (–)-epigallocatechin. For each fraction, means ($n = 3$) followed by the same letter in a column are not significantly different (LSD, 5%). [d] Trace. [e] Not identified.

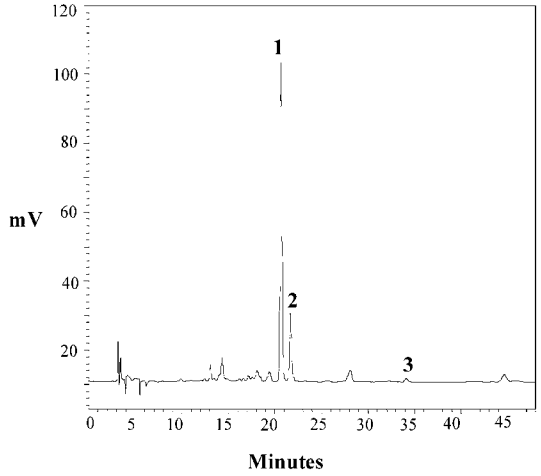

Figure 1. Typical HPLC chromatogram (280 nm) of the monomeric fraction of lentil seed coat (Pardina 2) obtained by fractionation on a polyamide column. Peaks: 1, (+)-catechin 3-*O*-glucoside; 2, (+)-catechin; 3, (–)-epicatechin.

To elucidate the unknown structure of peak 1, it was collected from the HPLC column and analyzed with the ESI-MS system in the negative mode. An ion mass $[M - H]^-$ at *m*/*z* 451.1 and a fragment ion at *m*/*z* 289, which corresponds to the loss of glucose from the structure of (+)-catechin 3-*O*-glucoside, were observed. To obtain additional support that allowed confirmation of the (+)-catechin unit, peak 1 was isolated by HPLC and submitted to acid hydrolysis. So far as the degradation occurred, the original peak disappeared partially and the peak corresponding to (+)-catechin emerged. This compound was also detected in whole lentils by De Pascual-Teresa et al. (*34*).

In **Figure 2**, two different maximum wavelengths (λ_{max}) of DAD-UV spectra can be observed (**Table 2**). The spectral parameters of the peaks with λ_{max} at 278.5 nm correspond to those of procyanidins formed by different units (*12*), whereas others presenting a different shape and a λ_{max} at 277.2 (**Figure 3**) could correspond to prodelphinidins. Furthermore, this fraction was also analyzed by HPLC-DAD-MS. From the negative molecular ions ($[M - H]^-$ *m*/*z*), we have identified dimers, trimers, and tetramers of different procyanidins and prodelphinidins together with some galloylated compounds

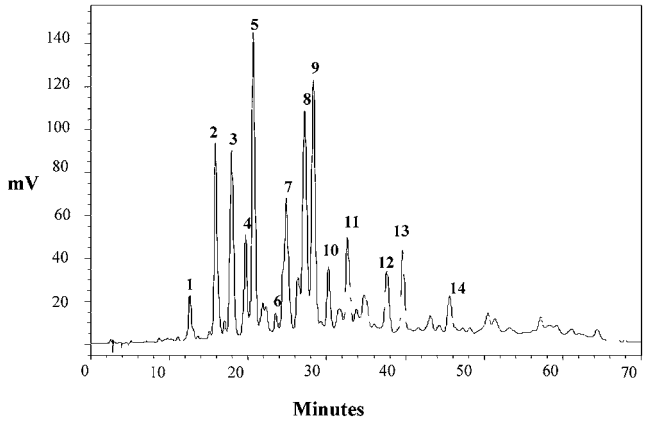

Figure 2. Typical HPLC chromatogram (280 nm) of the oligomeric fraction of lentil seed coat (Pardina 2) obtained by fractionation on a polyamide column. See **Table 2** for peak identification.

(**Table 2**). In this way peaks 2−4 and 7 have been confirmed as prodelphinidins.

ESI-MS Analysis of the Polymeric Fraction Obtained from Sep-Pak C_{18} Cartridges. On the other hand, the polymeric fraction obtained from the fractionation of Sep-Pak C_{18} cartridges was analyzed by HPLC-MS to identify the proanthocyanidin composition in this fraction. The ions observed for the proanthocyanidins are shown in **Table 4**.

Molecular ions $(M - H)^-$ at *m*/*z* 1457, 1745, 1793, and 2369 correspond to prodelphinidins, from pentamer to octamer; at *m*/*z* 1593 to a pentamer gallate, and at 2017 and 2369 to procyanidin octamer and nonamer. Also, double-charged ions $(M - 2H)^{2-}$ were observed, indicating the propensity of the higher polymers to form multiple charges (*36*). In this case the $(M - 2H)^{2-}$ ions at *m*/*z* 1032 and 1368 correspond to heptamer and nonamer prodelphinidins, and those at *m*/*z* 1084 and 1228 correspond to gallate heptamer and octamer (**Table 4**). The formation of multiply charged species as the degree of polymerization increases has been previously discussed by Guyot et al. (*23*).

From these results the presence of procyanidins and prodelphinidins, from pentamer to nonamer in the polymer fraction, seems to be proven.

Table 2. Characterization of Proanthocyanidins Detected in the Oligomeric Fraction from a Polyamide Column

peak	λ_{max}[a] (nm)	molecular ion $(M-H)^-$, *m/z*	no. of units[b]	type
1	278.5	865	trimer (3C)	procyanidin
2	277.2	897	trimer (2GC–C)	prodelphinidin
3	277.2	593	dimer (GC–C)	prodelphinidin
4	277.2	1185	tetramer (2GC–2C)	prodelphinidin
5	278.5	881	dimer (Cgal–Cgal)	procyanidin
6	278.5	1169	trimer (Cgal–Cgal–C)	procyanidin
7	277.2	593	dimer (GC–C)	prodelphinidin
8	278.5	577	dimer (B3) (Cat–Cat)	procyanidin
9	278.5	865	trimer (3C)	procyanidin
10	278.5	577	dimer (B1) (Epic–Cat)	procyanidin
11	278.5	1153	tetramer (4C)	procyanidin
12	278.5	865	trimer (3C)	procyanidin
13	278.5	577	trimer (B2) (Epic–Epic)	procyanidin
14	278.5	1153	tetramer (4C)	procyanidin

[a] Wavelength of maximun absortion in UV; $[M-H]^-$ *m/z*: negative molecular ion. [b] C, (epi)catechin; GC, (epi)gallocatechin; Cgal, (epi)catechin 3-*O*-gallate; Cat, (+)-catechin; Epicat, (–)-epicatechin.

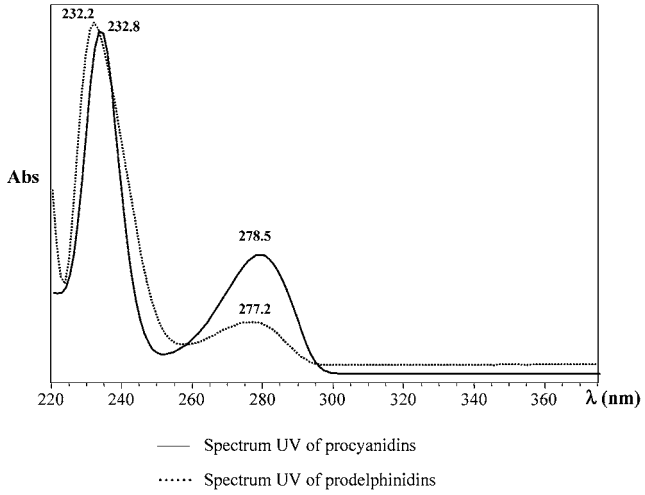

Figure 3. UV spectra of procyanidins and prodelphinidins.

Vanillin Assay for Total Monomeric, Oligomeric, and Polymeric Proanthocyanidins. Figure 4 presents the total amount of flavan-3-ols in each fraction obtained from C_{18} Sep-Pak cartridges (F_I, F_{II}, and F_{III}) quantified by modified vanillin assay. It can be seen that proanthocyanidins in the seed coat are essentially in highly polymerized form (65−75%), to a lesser extent in oligomers (20−30%), and very little is in monomeric form (<2%). The oligomer fraction presents more differences between varieties than monomeric and polymeric ones. The oligomer fraction in Pardina 1 has the lowest percentage (23%), and Pardina 2 shows the highest percentage of all samples (39%). Also, Pardina 2 was significantly different (LSD, 5%) with respect to the oligomeric and polymeric fractions (data not shown).

HPLC Analysis of Individual Catechins and Oligomeric Proanthocyanidins. Individual catechins and oligomeric proanthocyanidins shown in HPLC chromatograms (**Figures 1** and **2**) can be quantitatively determined. These results are given in **Table 3**. In the monomer fraction, (+)-catechin-3-glucose is the most abundant in the four samples, and small differences are found between varieties ($p < 0.05$). In the oligomer fraction, prodelphinidins (peaks 2−4) presented the highest concentrations.

Table 3. Concentration of the Proanthocyanidins (Milligrams per Gram of Dry Seed Coat) from Fractionation on a Polyamide Column[a]

compound		Pardina 1	Pardina 2	Castellana 1	Castellana 2
(+)-catechin	mean	0.209a	0.134a	0.191a	0.174a
	SD	0.006	0.003	0.010	0.074
(+)-catechin-3-*O*-glucose	mean	0.794c	0.643b	0.680b	0.514a
	SD	0.009	0.003	0.014	0.022
peak 1 trimer	mean	0.581c	0.734d	0.216a	0.309b
	SD	0.048	0.026	0.023	0.016
peak 2 (2GC–C)	mean	3.399b	3.814c	2.647a	3.687c
	SD	0.016	0.021	0.130	0.142
peak 3 (GC–C)	mean	4.110b	4.448b	3.058a	3.846ab
	SD	0.405	0.208	0.350	0.105
peak 4 (2GC–2C)	mean	1.302a	2.033c	1.143a	1.592b
	SD	0.034	0.023	0.042	0.166
peak 5 PC(Cgal–Cgal)	mean	0.707a	1.073c	0.739a	0.825b
	SD	0.008	0.029	0.031	0.024
peak 6 PC(Cgal–Cgal–C)	mean	0.141b	0.161b	0.110a	0.148b
	SD	0.002	0.010	0.010	0.011
peak 7 PD (GC–C)	mean	1.301a	3.157c	1.580a	1.883b
	SD	0.055	0.013	0.093	0.181
peak 8 B3	mean	1.436a	2.317b	1.651a	1.673a
	SD	0.145	0.132	0.181	0.079
peak 9 trimer	mean	1.832a	4.585c	2.551b	2.660b
	SD	0.049	0.016	0.059	0.146
peak 10 B1	mean	0.347b	0.611c	0.267a	0.348b
	SD	0.012	0.008	0.008	0.041
peak 11 tetramer	mean	0.501a	1.794c	0.826b	0.815b
	SD	0.028	0.021	0.020	0.186
peak 12 trimer	mean	0.444a	1.162c	0.666b	0.685b
	SD	0.009	0.021	0.013	0.027
peak 13 B2	mean	0.346a	0.608c	0.397b	0.404b
	SD	0.002	0.002	0.015	0.019
peak 14 tetramer	mean	0.151a	0.732c	0.321b	0.332b
	SD	0.004	0.098	0.017	0.014

[a] Means ($n = 3$) followed by the same letter in a row are not significantly different (LSD, 5%). [b] (+)-Catechin 3-*O*-glucoside was quantified as (+)-catechin; peaks 2–4 and 7 were quantified as (–)-epigallocatechin; peaks 5 and 6 were quantified as (–)-epicatechin 3-*O*-gallate; and peaks 1, 9, 11, 12, and 14 were quantified as trimer C_1.

Table 4. Ions of Proanthocyanidins from HPLC-MS Analysis of the Polymeric Fraction

polymer[a]	molecular ion $(M-H)^-$	molecular ion double charge $(M-2H)^{2-}/2$
gallate pentamer	1593	
PD pentamer (1GC–4C)	1457	
PD hexamer (1GC–5C)	1745	
PD hexamer (4GC–2C)	1793	
PD octamer (4GC–4C)	2369	
PC heptamer	2017	
PC octamer	2369	
PD heptamer (3CG–4C)		1032
gallate heptamer		1084
gallate octamer		1228
PD nonamer (9GC)		1368

[a] C, (epi)catechin; GC, (epi)gallocatechin; PC, procyanidin; PD, prodelphinidin.

DISCUSSION

Acid degradation in the presence of a nucleophilic agent such as phloroglucinol or toluene-α-thiol followed by HPLC analysis of the hydrolyzed products is a powerful tool to determine the nature of the flavanol units, DP, and structural characteristics of proanthocyanidins (*22*, *35*). In this work, we applied this method to the oligomeric (F_{II}) and polymeric (F_{III}) fractions obtained by C_{18} Sep-Pak cartridges. As compared with the oligomeric fraction, a much higher amount of epigallocatechin units was found in the polymeric fraction, suggesting that as the DP increases, the percentage of prodelphinidins increase. It should be mentioned that the mDP values measured might be

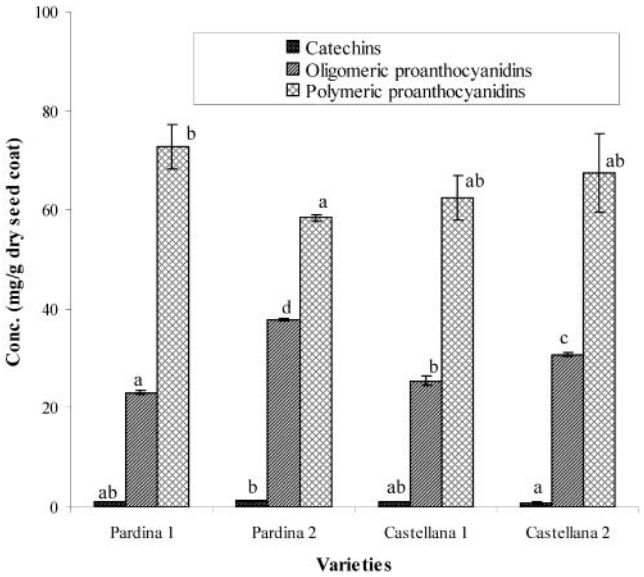

Figure 4. Catechin and proanthocyanidin concentrations in seed coat of lentil varieties. Vertical bars represent the standard deviation ($n = 3$). For the same fraction, means followed by the same letter are not significantly different (LSD, 5%).

slightly overestimated because the epimerization reaction could occur under the conditions of thiolysis (*22*, *36*). Furthermore, the total amount of flavan-3-ols in F_I, F_{II}, and F_{III} can further be quantified by modified vanillin assay (**Figure 4**). To our knowledge, this is the first time that the mDP, structural composition and quantitative amount of oligomeric and polymeric proanthocyanidins in the seed coat of lentils have been reported.

For studying individual flavan-3-ols, fractionation of samples on a polyamide column, as described by Ricardo-da-Silva et al. (*33*), allows catechin and oligomeric proanthocyanidin fractions to be isolated, and these could subsequently be analyzed by HPLC-DAD and ESI-MS. Application of this method to the lentil seed coat extracts permitted the identification and quantification of various individual catechins, oligomeric procyanidins, and prodelphinidins. Although the seed coat of lentil represents only a small percentage of the entire lentil seed on a dry weight basis, ranging from 8 to 11%, phenolic compounds in the seed coat are closely associated with the biological and sensory quality of the legume (*29*, *37*). On the other hand, the presence of a high concentration of these bioactive phenolic compounds in the seed coat of lentils could be of interest in both the food and pharmaceutical industries, where it might be used as a primary source of naturally occurring antioxidants.

ABBREVIATIONS USED

DP, degree of polymerization; mDP, mean degree of polymerization; F_I, F_{II}, and F_{III}, monomeric, oligomeric, and polymeric proanthocyanidin fractions, respectively, obtained from C_{18} Sep-Pak cartridges; F_{cat} and F_{pro}, catechin and oligomeric proanthocyanidin fractions, respectively, obtained from a polyamide column.

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