

Montserrat Dueñas · Teresa Hernández
Isabel Estrella

Phenolic composition of the cotyledon and the seed coat of lentils (*Lens culinaris* L.)

Received: 27 May 2002 / Published online: 8 October 2002
© Springer-Verlag 2002

Abstract The phenolic composition of the seed coat and the cotyledon of two varieties of lentils, Pardina and Castellana have been investigated by HPLC-photodiode array detection (PAD) and HPLC-MS. Large quantitative and qualitative differences have been found in the phenolic composition of the two seed parts. In both varieties of lentils the seed coat is very rich in catechins, procyanidins dimers and trimers, and in minor concentration it contains glycosides of quercetin, myricetin, luteolin and apigenin. The cotyledon contains mainly hydroxybenzoic and hydroxycinnamic acids in low concentration. Two esters of the *trans-p*-coumaric acid, *p*-coumaroylmalic acid and *p*-coumaroylglycolic acid have been identified in the cotyledon, and the stilbene *trans*-resveratrol-5-glucoside in the seed coat. These compounds had not previously been reported in lentils. Results presented allow an overview of the distribution of the phenolic compounds in the seed lentils, and contribute to knowledge of the implications in dietary intake of these compounds.

Keywords Lentils · Seed coat · Cotyledon · Phenolics · HPLC-PAD · HPLC-MS

Introduction

Polyphenols constitute a group of compounds from the secondary plants metabolism. Between them hydroxybenzoic and hydroxycinnamic compounds and flavonoids are widely distributed in plants and food of plant origin.

Legumes are one of the most important foodstuffs. They supply both, micro and macronutrients and have a high content of proteins, carbohydrates, vitamins and minerals unevenly distributed in the seed. Three different parts are recognized in the legume seed: the seed coat or

testa, the cotyledon, and the embryonic axe which, in average, represent 10%, 89% and 1%, respectively, of the seed content. The cotyledon contains the main reserve substances, basically proteins and carbohydrates. In the testa, which acts as a protective barrier for the cotyledon, are located the majority of phenolic compounds reported in legumes.

Among legumes, lentils (*Lens culinaris*) constitute an important source of food for humans in many countries. They contain phenolic compounds, but as in other legumes, best reported are total condensed tannins in relation to the nutritional properties of lentils [1, 2, 3, 4, 5]. Other phenolic compounds, flavonoid and non-flavonoid, are not as well characterized in lentils. After hydrolysis of lentil flours the phenolic acids, ferulic, *p*-coumaric and *p*-hydroxybenzoic [6], and the flavonoids, quercetin and apigenin [7] have been identified. In non-hydrolyzed lentil flour were found different contents of procatechuic, *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids and *p*-hydroxybenzoic aldehyde, and higher quantities of catechin and several procyanidins, dimers and trimers [8, 9, 10, 11]. The presence of kaempferol, 4'-methoxykaempferol and 8-hydroxykaempferol, and some benzoic acids was also reported [12]. Also (+)-catechin-3-glucose, (+)-catechin, procyanidin B3 and gallicocatechin have been found [13].

Little is known of the distribution of phenolic compounds in lentil seeds, only the prevalence of condensed tannins in the outside, or seed coat has been reported [14]. Compared with other legumes the seed coat must keep the majority of flavonoid compounds [15, 16, 17, 18].

In order to elucidate the specific phenolic compounds of the seed coat and the cotyledon of lentils and their contribution to the complete phenolic composition, in this work we have investigated the phenolic composition of two different varieties of lentils by different techniques of analysis (HPLC-photodiode array detection (PAD), HPLC-MS and selective cleavage).

M. Dueñas · T. Hernández · I. Estrella (✉)
28006, MadridSpain
e-mail: iestrella@ifi.csic.es
Tel.: +34-915622900; Fax: +34-915644853

Materials and methods

Samples

Two varieties of commercial lentils (*Lens culinaris* L.) were used, Pardina and Castellana, and two samples of both varieties, from different areas of the central region of Spain, were analyzed: Pardina 1 (Palencia), Pardina 2 (Salamanca), Castellana 1 (Salamanca) and Castellana 2 (León). The four samples of seeds were harvested in 1998. The cotyledon was manually separated from the seed coat, and both were ground separately.

Sample extraction

Extractions of phenolic compounds from the cotyledon and from the seed coat, were performed as follows [19]: 1.5 g of seed coat and 10 g of cotyledon were macerated separately (3×10 h) with 80 mL of a solution of methanol-HCl (10/1000)-water (80:20 v/v), in a bath at room temperature and stirring. The supernatant was separated by centrifugation. In both cases the three combined supernatants were taken to a fixed volume (240 mL) with methanol-HCl (10/1000)-water (80:20 v/v). An aliquot of this solution (100 mL) was extracted four times with diethyl ether and four times with ethyl acetate, to more purify phenolic compounds, and the organic fractions combined and dried with anhydrous Na₂SO₄. This extract was evaporated to dryness under vacuum and the residue, redissolved in methanol-water (1:1, v/v), was analysed by high-performance liquid chromatography with photodiode array detection (HPLC-PAD).

HPLC-PAD analysis

The chromatographic system consisted of a photodiode array detector 2001 (Waters, Milford, Mass, USA). The column was a Nova Pack C₁₈ (300×3.9 mm I.D., 4 μm particle size). Two mobile phases were employed for elution, A: water-acetic acid (98:2, v/v) and B: water-acetonitrile-acetic acid (78:20:2, v/v/v). The gradient profile was 0–55 min, 100%–20% A; 55–70 min, 20%–10% A; 70–80 min, 10%–5% A; 80–90 min, 100% B, with a flow rate of 1 mL/min. Detection was performed by scanning from 210 to 400 nm with an acquisition speed of 1 s. Samples were analysed in triplicate.

Alkaline hydrolysis

The hydrolysis was used as a method of cleavage of esters of phenolic compounds, to remove an organic acid from the molecule. The method used was based on that of Markham [20]. The sample dissolved in 2 M NaOH on a steam bath for 2 h. Neutralized with 1 M HCl and extracted with diethyl ether and ethyl acetate, following the method described above. The organic fractions were chromatographed by HPLC-PAD to identify the phenolic compounds.

Identification and quantification of compounds

Chromatographic peaks were identified by comparing retention times, UV spectra and data of UV spectral parameters [21, 22], recorded with the photodiode array detector (PAD), with those of standards. The standards (+)-catechin, (–)-epicatechin, the benzoic acids, gallic, protocatechuic, *p*-hydroxybenzoic, the cinnamic acids, *trans-p*-hydroxycinnamic, *trans*-ferulic, *trans*-cinnamic and the *trans*-resveratrol are from Aldrich Chimie (Germany); the procyanidins B₁, B₂ and B₃, and the flavonols (myricetin-3-ramnoside and quercetin-3-ramnoside) and flavones (luteolin-7-glucoside, luteolin, apigenin-7-apioglucoside and apigenin-7-glucoside) are from Extrasynthèse (France).

Compounds with the UV spectra corresponding to that of the procyanidins, for which standards were not available, were identified as procyanidins, dimers and trimers, based on the study of data of UV spectral parameters, for the analysis of procyanidins by HPLC-PAD [22].

Compounds with the same shape and wavelength maxima of the UV spectrum of cinnamic acids, but with different retention times were identified as derivatives, esters or glycosides of these free acids [23].

Quantifications were made using the external standard method, with commercial standards. The calibration curves were obtained by injection of different volumes of the standard solution under the same conditions as for the samples analyzed. Hydroxycinnamic acid derivatives were quantified using the calibration curves of the corresponding free acid, and procyanidins with the calibration curve of (+)-catechin. The unknown flavonoid glycosides were quantified with the calibration curves of the corresponding aglycones.

HPLC-MS analysis

Mass spectra were obtained using a Hewlett-Packard 110MS system equipped with an API source, working in negative mode. The interface settings were: nebulizer pressure, 40 psi; drying gas temperature and flow rate, 320 °C and 10 L/min respectively; voltage at capillary entrance, 4000 V; fragmentation voltage, variable. A Nova-pak column (3.9×150 mm, 4 μm) was used. The sample, diluted in acetonitrile-water (4:1 v/v) was injected, with a flow rate of 0.5 mL/min, using as eluent a 50:50 (v/v) mixture of 1% acetic acid in acetonitrile and 1% acetic acid in water. The injection volume was 25 μL. The scan range was set at *m/z* 100–3000. Data were collected using software MHP-1100.

Results

Figures 1 and 2 show the chromatograms of the cotyledon and the seed coat of one of the lentil samples (Pardina 2). In the investigated samples there are important qualitative and quantitative differences in the phenolic composition of the seed coat and the cotyledon.

In the cotyledon the acids, protocatechuic, *p*-hydroxybenzoic, *trans*-ferulic, and *cis* and *trans-p*-coumaric, in addition to (+)-catechin have been identified (Fig. 1).

Peaks 4 and 5 (Fig. 1) with UV spectra similar to that of *p*-coumaric acid, under HPLC-PAD conditions, were collected separately from the HPLC column.

The isolated peaks were hydrolysed by 2 M NaOH, as explained above. The organic fractions from the hydrolysis were chromatographed by HPLC-PAD in the conditions of analysis of phenolic compounds. In both cases free *trans-p*-coumaric acid is identified, therefore the peaks 4 and 5 should be derivatives of this acid.

These peaks were also submitted to the analysis by HPLC-MS. From the electrospray mass spectra of the peak 4, the main ion observed, in the negative ion mode, is [M–H][–] at *m/z* 278.1, corresponding to *trans-p*-coumaroylmalic acid (Fig 3). In the case of peak 5 the main ion observed is [M–H][–] at *m/z* 220.1, corresponding to *trans-p*-coumaroylglycolic acid (Fig 3).

By that way we have identified the two esters in the cotyledon of the four samples of lentils. We have not found references about the presence of *p*-coumaroylmalic acid and *p*-coumaroylglycolic acid in lentils. The pres-

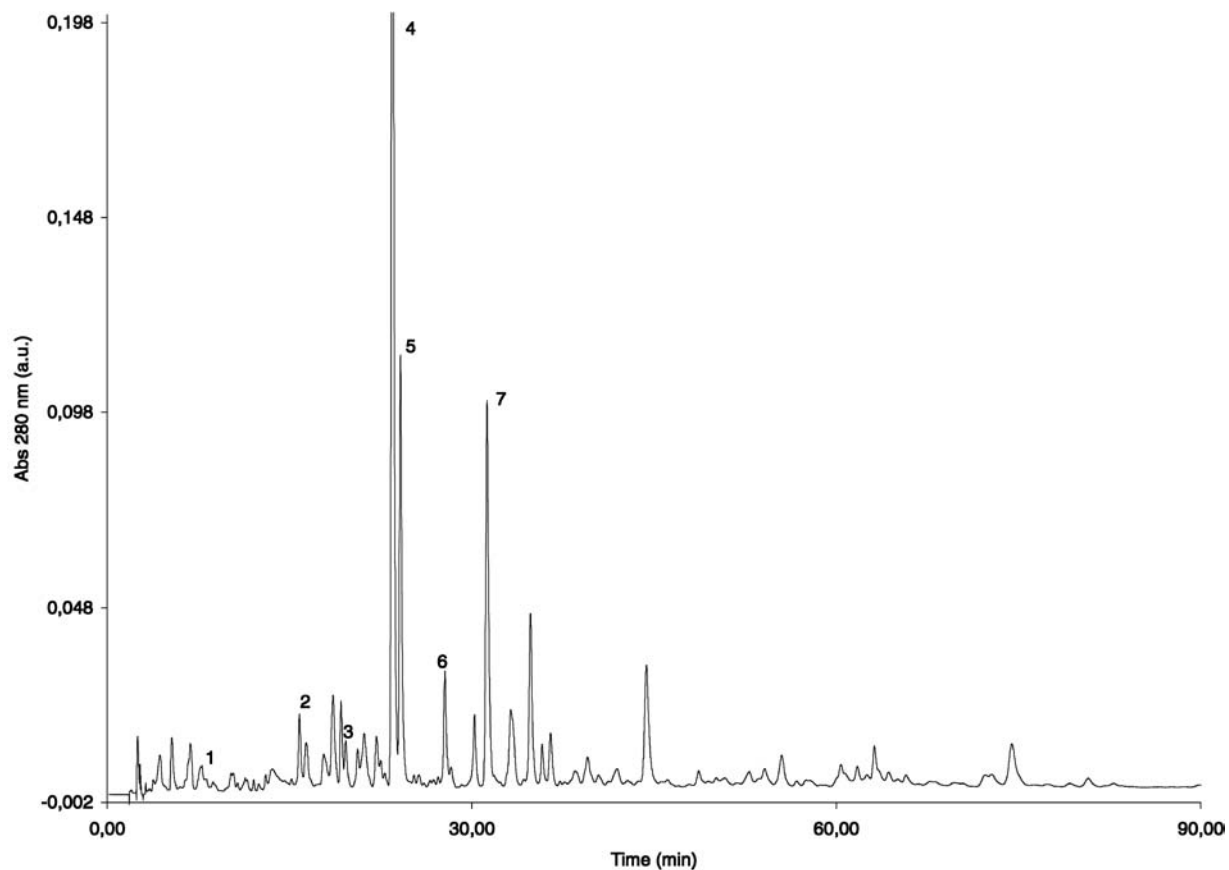
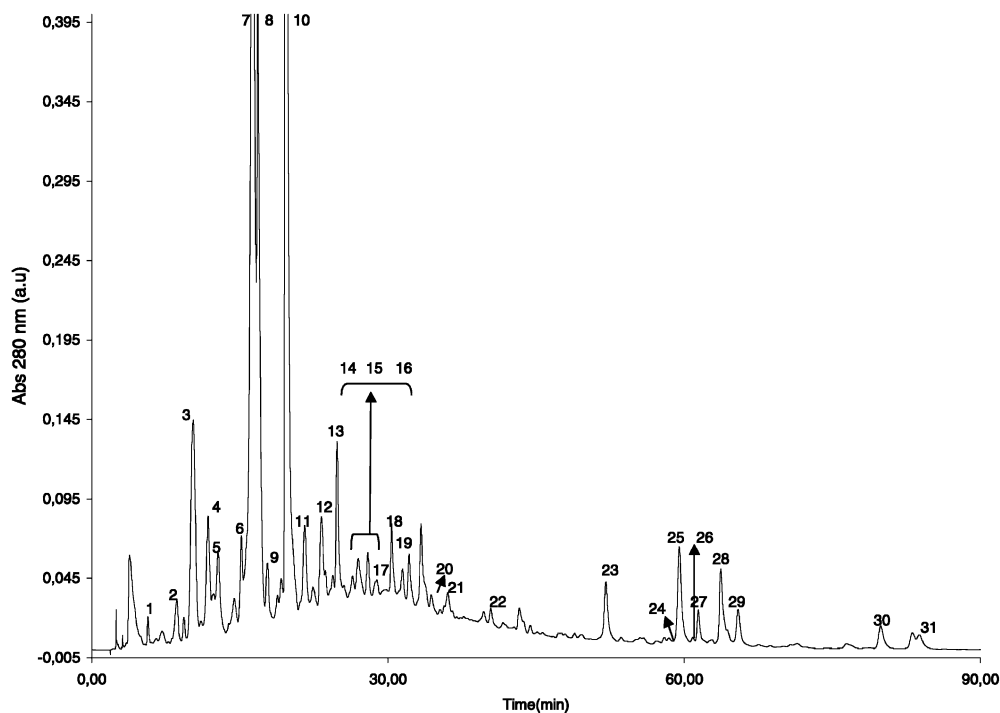


Fig. 1 Chromatogram at 280 nm of the cotyledon (Pardina 2). 1, *trans-p-coumaroylmalic acid*; 2, *protocatechuic acid*; 3, *p-hydroxybenzoic acid*; 4, *(+)-catechin*; 5, *trans-p-coumaroylglycolic acid*; 6, *trans p-coumaric acid*; 7, *trans-ferulic acid*

Fig. 2 Chromatogram at 280 nm of the seed coat (Pardina 2). 1, gallic acid; 2, 3,4,5-trihydroxybenzaldehyde; 3, 4, 5, 6, 9, 11, 12, 19, 20, 21, procyanidin dimers; 14, 15, 16, procyanidin trimers; 7, B₃; 8, B₁; 10, (+)-catechin; 13, B₂; 17, (-)-epicatechin; 18, *trans-p-coumaric acid*; 22, *trans-resveratrol-5-glucoside*; 23, *myricetin-3-ramnoside*; 24, *luteolin-7-glucoside*; 25, *apigenin-7-apioglucoside*; 26, *apigenin-7-glucoside*; 27, 29, *luteolin glycosides*; 28, *quercetin-3-ramnoside*; 30, *apigenin glycoside*; 31, *luteolin*



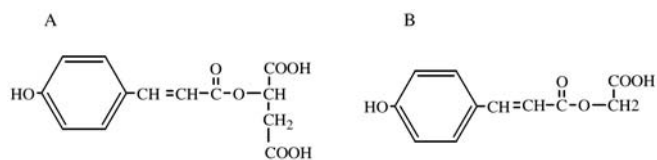


Fig. 3 **A** *trans-p*-coumaroylmalic acid; **B** *trans-p*-coumaroylglycolic acid

ence of these conjugate cinnamates has been reported in leaves of Faba (*Phaseolus* spp.) [24].

In the seed coat (Fig 2) the acids, gallic, protocatechuic, *trans-p*-coumaric; the aldehyde, 3,4,5, trihydroxybenzoic; (+)-catechin and (–)-epicatechin; the procyanidin dimers, B₃ [(+)-catechin-(4β→8)-(+)-catechin], B₁ [(–)-epicatechin-(4β→8)-(+)-catechin] and B₂ [(–)-epicatechin-(4β→8)-(–)-epicatechin]; the flavonols, myricetin-3-ramnoside, quercetin-3-ramnoside and the flavones, luteolin-7-glucoside, apigenin-7-glucoside, apigenin-7-apioglucoside and luteolin, have been identified by comparison of their retention time, UV spectra and data of UV spectral parameters with those of standards. Additional compounds with the same shape and wavelength maxima of UV spectrum to the procyanidins have been characterized, as procyanidin dimers (peaks 3, 4, 5, 6, 9, 11, 12, 19, 20 and 21) and trimers (peaks 14, 15 and 16) based on the data of their UV spectral parameters [21]. Peaks 24, 27, 29 and 30 have been characterized as luteolin or apigenin glycosides by their maxima in the UV spectra, which spectral characteristics are similar to that of corresponding aglycons, but different retention times.

Under HPLC-PAD conditions, the retention time and the UV spectrum of peak 22 (Fig 2) corresponds to *trans*-resveratrol-5-glucoside [25]. The presence of this compound in the samples has been confirmed by HPLC-MS. From the electrospray mass spectra of this peak, the main ions observed in the negative ion mode are [M–H][–] at *m/z* 227 and [M–H][–] at *m/z* 389, corresponding to resveratrol and resveratrol glucoside. The *trans*-resveratrol-5-glucoside is found in the seed coat of every

lentil samples. To our knowledge the stilbene, *trans*-resveratrol-5-glucoside, has not yet been identified in lentils. Compounds belonging to this family of stilbenes are present mainly in grape skins and wine [25, 26].

In the cotyledon (Table 1) we have found mainly hydroxybenzoic and hydroxycinnamic compounds, the latter in greater concentration than the former. However there are few data about the phenolic composition of legumes, López-Amorós [11] and Bartolomé [9] have been observed similar relation in the whole seed of peas and lentils.

The concentration of the hydroxybenzoic compounds is not too different between both varieties (Table 1), but the percentages of total phenolics are different, in Pardina lentils are 8.64% and 8.60%, while in the Castellana these percentages are larger, 34.89% and 20.18%, respectively. In both varieties *p*-hydroxybenzoic acid is the main benzoic component.

On the other hand, the hydroxycinnamic compounds of the cotyledon show different concentrations in the Pardina and Castellana lentils analysed. The major compound in Pardina is *trans-p*-coumaroylmalic acid, followed by *trans-p*-coumaric acid and *trans-p*-coumaroylglycolic acid. In Castellana lentils the major compound is *trans-p*-coumaric acid. Considering the percentages of hydroxycinnamic compounds with respect to the total phenolics of the cotyledon, the differences between the varieties are small, 78.81% and 87.10% in Pardina samples and 61.90% and 68.65%, in Castellana.

As would be expected, the seed coat is richer in phenolic compounds than the cotyledon, and they are mainly flavonoid compounds. It is remarkable (Table 2), the high concentration of flavonoid compounds, catechins (917.55–1530.11 µg/g), procyanidin dimers and trimers (1281.21–2386.04 µg/g), flavonols glycosides (9.60–323.83 µg/g) and flavone glycosides (33.12–224.83 µg/g), in comparison to the non-flavonoids, hydroxybenzoic (28.39–37.70 µg/g) and hydroxycinnamic (11.78–29.45 µg/g) compounds.

The *trans-p*-coumaric acid, which is also detected in the cotyledon, is the only phenolic acid detected in the testa. The main component of the seed coat in both vari-

Table 1 Concentration of phenolic compounds (µg/g) in the cotyledon of two varieties of lentils Pardina and Castellana

Peak no.	Compounds	Pardina 1	Pardina 2	Castellana 1	Castellana 2
1	Protocatechuic acid	0.76±0.01	0.81±0.02	0.73±0.01	0.84±0.03
2	<i>p</i> -Hydroxybenzoic acid	1.16±0.04	1.03±0.03	1.43±0.04	0.93±0.02
3	(+)-Catechin	2.89±0.05	0.92±0.01	0.24±0.01	0.98±0.03
4	<i>trans-p</i> -Coumaroylmalic acid	8.71±0.02	9.22±0.33	0.36±0.02	0.62±0.04
5	<i>trans-p</i> -Coumaroylglycolic acid	2.84±0.03	2.82±0.15	1.03±0.11	1.14±0.10
6	<i>trans-p</i> -Coumaric acid	4.55±0.03	3.25±0.02	2.65±0.01	3.44±0.02
	<i>cis-p</i> -Coumaric acid	0.49±0.01	nd	nd	0.31±0.01
7	<i>trans</i> -Ferulic acid	0.69±0.00	0.90±0.01	0.59±0.02	0.51±0.01
	Total benzoics I(%)	1.92/(8.64)	1.84/(8.60)	2.66/(34.89)	1.77/(20.18)
	Total cinnamics I(%)	17.89/(78.81)	18.62/(87.10)	4.63/(61.90)	6.02/(68.65)
	% (+)-Catechin	12.73	4.30	3.21	11.17

nd, Not detected; (%), the percentage of the total of phenolics. The numbers of compounds correspond to those of the peak chromatogram.

Table 2 Concentration of phenolic compounds ($\mu\text{g/g}$) in the seed coat of two varieties of lentils Pardina and Castellana

Peak no.	Compounds	Pardina 1	Pardina 2	Castellana 1	Castellana 2
1	Gallic acid	4.49 \pm 0.44	3.34 \pm 0.92	4.94 \pm 1.00	1.22 \pm 0.64
	Protocatechuic acid	nd	nd	11.11 \pm 1.50	10.53 \pm 0.97
2	3,4,5-Trihydroxybenzaldehyde	23.90 \pm 0.58	29.58 \pm 1.30	18.65 \pm 0.99	25.78 \pm 2.59
3	Procyanidin D	507.30 \pm 4.16	372.49 \pm 5.45	223.36 \pm 4.15	282.39 \pm 6.10
4	Procyanidin D	121.15 \pm 1.41	106.39 \pm 2.97	55.32 \pm 0.89	74.38 \pm 3.25
5	Procyanidin D	102.11 \pm 4.73	62.47 \pm 1.00	47.84 \pm 2.85	59.45 \pm 5.20
6	Procyanidin D	76.53 \pm 5.55	41.89 \pm 0.93	85.97 \pm 3.87	29.01 \pm 2.15
7	Procyanidin B3	745.25 \pm 7.04	869.88 \pm 23.07	754.42 \pm 11.53	445.31 \pm 10.50
8	Procyanidin B1	305.94 \pm 5.06	324.65 \pm 12.75	88.34 \pm 3.18	89.60 \pm 7.81
9	Procyanidin B	65.11 \pm 2.15	45.15 \pm 3.43	38.77 \pm 3.52	41.73 \pm 5.65
10	(+)-Catechin	1475.65 \pm 7.07	1471.04 \pm 33.55	1281.27 \pm 26.44	899.31 \pm 24.84
11	Procyanidin D	25.82 \pm 0.70	103.64 \pm 7.47	8.74 \pm 2.00	1.88 \pm 0.20
12	Procyanidin D	74.98 \pm 1.80	108.56 \pm 9.43	78.83 \pm 5.33	58.61 \pm 4.04
13	Procyanidin B2	158.04 \pm 3.72	191.34 \pm 8.45	209.68 \pm 14.46	131.67 \pm 5.80
14	Procyanidin T	13.80 \pm 1.28	14.69 \pm 2.55	12.74 \pm 2.37	8.27 \pm 1.37
15	Procyanidin T	5.18 \pm 0.32	45.51 \pm 2.09	27.30 \pm 1.63	19.06 \pm 1.77
16	Procyanidin T	7.95 \pm 0.30	20.17 \pm 1.20	9.54 \pm 1.61	14.83 \pm 0.81
17	(-)-Epicatechin	21.99 \pm 0.83	59.07 \pm 0.48	19.72 \pm 1.87	18.25 \pm 1.59
18	<i>trans-p</i> -Coumaric acid	19.51 \pm 1.22	11.78 \pm 0.71	20.29 \pm 2.45	29.45 \pm 2.87
19	Procyanidin D	28.64 \pm 0.62	49.48 \pm 1.07	20.12 \pm 1.42	14.16 \pm 1.32
20	Procyanidin D	11.32 \pm 1.49	14.00 \pm 1.45	24.38 \pm 2.57	10.86 \pm 1.13
21	Procyanidin D	t	15.73 \pm 1.79	t	t
22	<i>trans</i> -Resveratrol-5-glucoside	9.34 \pm 0.40	6.39 \pm 0.01	5.75 \pm 0.64	5.50 \pm 1.00
23	Myricetin 3- ramnoside	57.91 \pm 1.72	120.53 \pm 4.66	13.36 \pm 0.85	9.60 \pm 1.21
24	Luteolin 7-glucoside	10.47 \pm 1.08	3.95 \pm 0.20	5.25 \pm 0.68	5.18 \pm 1.13
25	Apigenin 7-apioglucoside	75.50 \pm 1.28	94.20 \pm 2.51	13.68 \pm 0.45	5.58 \pm 0.65
26	Apigenin 7-glucoside	17.53 \pm 1.58	4.07 \pm 0.12	6.44 \pm 0.85	11.45 \pm 0.29
27	Luteolin glycoside	11.66 \pm 1.54	39.90 \pm 1.50	t	11.68 \pm 1.38
28	Quercetin 3-ramnoside	59.77 \pm 1.32	203.30 \pm 4.15	9.67 \pm 1.93	t
29	Luteolin glycoside	18.00 \pm 1.08	33.94 \pm 1.82	4.14 \pm 0.55	4.15 \pm 1.12
30	Apigenin glycoside	t	39.51 \pm 2.44	t	t
31	Luteolin	3.47 \pm 0.78	9.26 \pm 0.39	3.61 \pm 0.44	3.25 \pm 0.58
	Total benzoic acids/(%)	28.39/(0.70)	32.92/(0.73)	34.70/(1.12)	37.53/(1.62)
	Total cinnamic acids/(%)	19.51/(0.48)	11.78/(0.26)	20.29/(0.66)	29.45/(1.27)
	Total catechins/(%)	1497.64/(36.98)	1530.11/(33.93)	1300.99/(42.00)	917.55/(39.61)
	Total procyanidin dimers/(%)	2222.19/(54.88)	2305.67/(51.13)	1635.77/(52.81)	1239.05/(53.49)
	Total procyanidin trimers/(%)	26.93/(0.66)	80.37/(1.78)	49.50/(1.60)	42.16/(1.82)
	Total flavonols/(%)	117.68/(2.91)	323.83/(7.18)	23.03/(0.74)	9.60/(0.41)
	Total flavones/(%)	136.63/(3.37)	224.83/(4.90)	33.12/(1.07)	41.29/(1.78)
	% Glucoside resveratrol	0.23	0.14	0.19	0.24

nd, Not detected; t, traces; %, the percentage of the total of phenolics. The numbers of compounds correspond to those of the peak chromatogram.

eties is (+)-catechin, present in much larger concentration than (-)-epicatechin.

In relation to the major procyanidins, we can remark on the high concentration of the B₃ in all the samples, together with smaller amounts of B₁, B₂ and a procyanidin dimer (peak 3). The greatest differences between both varieties are observed with B₁. As to the total content of dimers and trimers, some differences are observed between the Castellana and the Pardina, the procyanidin concentrations are greater in the Pardina (2249.12 and 2386.04 $\mu\text{g/g}$) than in the Castellana (1281.21 and 1685.27 $\mu\text{g/g}$), although there are similar percentages of dimers.

The greatest differences observed between the Pardina and the Castellana are related to the flavonol and flavone glycosides content (Table 2). The samples from the Castellana variety are the poorer in this type of compounds. Concentrations of the flavonol glycosides, and flavone glycosides are higher in the Pardina than the

Table 3 Proportions (%) of cotyledon and seed coat of the weight of different lentils

Samples	Cotyledon	Seed coat
Pardina 1	88.6	11.4
Pardina 2	90.3	9.7
Castellana 1	91.8	8.2
Castellana 2	91.7	8.3

Castellana variety. There are a higher percentage of these compounds in the Pardina (7% and 11%) than in the Castellana (~2%), in relation to total of the phenolics of the seed coat.

The proportion of the seed coat and the cotyledon in the weight of the whole seed are shown in Table 3. The cotyledon represents between 88.6 to 91.8% of the seed. Little differences are found between Castellana lentils. In

Pardina the higher weight of seed coat of Pardina 1 could be attributed to the size of the seed, which was smaller than Pardina 2.

From the results tabulated in Tables 1, 2 and 3 it can also be deduced that although the seed coat represents only from 8.2% to 11.4% of the total weight of these lentils, the seed coat catechins and procyanidins and, in a smaller proportion, flavonols and flavones are the main contributors to the total content of phenolic compounds in the lentil seeds of the samples investigated. The cotyledon, representing from 88.6%–91.8% of the total weight, would provide a very low concentration of phenolic compounds, mainly cinnamic and benzoic compounds.

Conclusions

Altogether, from these results, we can conclude that hydroxycinnamic and hydroxybenzoic compounds can be found in the external or the internal tissue of the seed lentils, albeit in different concentrations. Flavonol and flavone glycosides were found in the outside of the seed and they are in different concentrations in the samples from the different varieties analyzed. Catechins and procyanidins are also much more abundant in the external tissues (catechins, 34%–42% and procyanidins, 53%–56%). In short, the flavonoid compounds are found almost exclusively in the lentil seed coat while the non-flavonoid can be found in the seed coat and the cotyledon. A similar distribution has been previously reported for fruits between the skin and the pulp [27, 28].

The present study is a part of extensive research about phenolic composition of lentils being developed in our laboratory.

Acknowledgements This work is a part of the Project ALI97–0636 supported by Spanish “Comisión Interministerial de Ciencia y Tecnología”. The authors are grateful to Mrs Isabel Jimenez for technical assistance in the HPLC-MS analysis and Mrs Soledad Campos for technical assistance in the translation.

References

- Vidal-Valverde C, Frías J, Estrella I, Gorospe MJ, Ruiz R, Bacon J (1994) *J Agric Food Chem* 41:2291
- Tabera J, Frías J, Estrella I, Villa R, Vidal-Valverde C (1995) *Z Lebensm Unters Forsch* 201:587
- Sharma A, Jood S, Sehgal S (1996) *Nahrung* 40:182
- Troszynska A, Kozłowska H, Estrella I, López-Amorós ML, Hernández T (1998) Third European Conference on Grain Legumes. (AEP) Valladolid, Spain
- Solanki IS, Kapoor AC, Singh U (1999) *Plant Food for Human Nutr* 54:79
- Sosulski F, Dabrowski KJ (1984) *J Agric Food Chem* 38:817
- Pietta PG, Mauri PL, Simonetti P, Testolin G (1995) *Fresenius' J Anal Chem* 352:788
- Bartolomé B, Hernández T, Estrella I (1997) *Z Lebensm Unters Forsch* 205:290
- Bartolomé B, Estrella I, Hernández MT (2000) *J Food Chem* 65:617
- López-Amorós ML, Lomas I, Estrella I, Hernández T (1998) In: Carnovale E (ed) *Effects of antinutrients on the nutritional value of legume diets*, vol 7. European Communities, Luxembourg, p 129
- López-Amorós ML, Estrella I, Hernández T (1998) Third European Conference on Grain Legumes (AEP). Valladolid, Spain
- Latha S, Daniel M (2001) *J Food Sci Technol India* 38:272
- De Pascual Teresa S, Santos-Buelga C, Rivas-Gonzalo JC (2000) *J Agric Food Chem* 48:5331
- Troszynska A, Bednarska A, Latosz A, Kozłowska H (1997) *Polish J Food and Nutr Sci* 6/47:37
- Shahidi F, Chavan UD, Naczek M, Amarowicz R (2001) *J Agric Food Chem* 49:926
- Amarowicz R, Naczek M, Zadernowski R, Shahidi F (2000) *J Food Lipid* 7:195
- Duh PD, Yeh DB, Yen GC (1992) *J Am Oil Chem Soc* 69:814
- Beninger CW, Hosfield GL, Bassett MJ (1999) *J Am Soc Hortic Sci* 124:514
- Dueñas M, Hernández T, Estrella I (2001) European conference: *Bioactive Compounds in Plant Foods. Health Effects and Perspectives for the Food Industry*. Tenerife, Spain
- Markham KR (1982) In: *Techniques of flavonoid identification*. Academic Press, New York
- Bartolomé B, Bengoechea ML, Galvez MC, Pérez-Illarbe FJ, Hernández T, Estrella I, Gómez-Cordovés C (1993) *J Chromatogr A* 655:119
- Bartolomé B, Hernández T, Bengoechea ML, Quesada C, Gómez-Cordovés C, Estrella I (1996) *J Chromatogr A* 723:19
- Bengoechea ML, Hernández T, Quesada C, Bartolomé B, Estrella I, Gómez-Cordovés C (1995) *Chromatographia* 41:94
- Clifford MN (1999) *J Sci Food Agric* 79:326
- Peña A, Hernández T, García-Vallejo C, Estrella I, Suarez JA (2000) *Eur Food Res Technol* 210:445
- Mattivi F, Reniero F (1996) In: Vercauteren J, Chèza C, Dumon MC, Weber JF (eds) *Polyphenols communications* 96. Bordeaux, France p 125
- Pérez-Illarbe J, Hernández T, Estrella I (1991) *Z Lebensm Unters Forsch* 192:551
- Awad MA, de Jager A (2000) *Postharvest Biol Technol* 20:15