JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 10101–10108 10101
DOI:10.1021/jf102222t

Evaluation of Phenolic Profile and Antioxidant Properties of Pardina Lentil As Affected by Industrial Dehydration

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This study presents the effects of soaking, cooking, and industrial dehydration treatments on phenolic profile and also on antioxidant properties in Pardina lentil. HPLC-PAC and HPLC-MS (ESI) analysis identified a total of 35 phenolic compounds in raw and processed lentil flours, corresponding to catechins and procyanidins (69% of the total of identified phenolic compounds), flavonols (17%), flavones, and flavanones (5%), and hydroxybenzoic and hydroxycinnamic compounds (5 and 4%, respectively). During the industrial process, catechins and procyanidins, flavonols, flavones, and flavanones decreased, while hydroxybenzoic compounds exhibited an important increase. In addition, raw lentils showed high values of the antioxidant activity (66.97 μmol Trolox/g); although the thermal processing promotes decreased, the levels of antioxidant activity were still relevant. Thus, the significant occurrence of bioactive phenolic compounds along with the interesting antioxidant capacity of dehydrated lentil flours make them useful for daily inclusion in the human diet as ready-to-use for special meals to specific populations.

KEYWORDS: Pardina lentil; industrial dehydration; polyphenols; antioxidant properties

INTRODUCTION

The nutritional value of legumes is gaining considerable interest in developed countries because of the demand for healthy foods. Attitudes and perceptions toward legumes have changed, bringing about a revival of consumer interest. The consumption has increased due to food industry and professional organizations that have incorporated legumes in novel, convenient, and healthy food products (*1*). Foods based on legumes are prepared by a wide range of recipes and preparation methods. To improve their palatability and nutritional quality, heat processing is a well-established method to obtain legume-based products with added value for manufacturing functional foods. In this sense, dehydration is a technology classified as a high temperature process to produce a variety of foods and ingredients (*2*, *3*) and offers numerous advantages, including prolonged preservation time, high productivity, and quality of resulting products (*4*). Moreover, legumes have been nutritionally enhanced by dehydration process, showing increases of available starch, dietary fiber, and protein digestibility (*5*, *6*) or important decreases of antinutritional factors such as enzyme inhibitors, lectins, and phytic acid (*7*).

Lentils are often recommended in Western diets because of their beneficial effects, they are considered to be good sources of nutrients and calories. Lentil food components like proteins, starch, fiber, phenolic compounds, or antinutritional factors are not only a source of constructive and energetic compounds, but also they may play bioactive roles by themselves. Epidemiological and intervention studies indicate that legume consumption is inversely associated with the risk of coronary heart disease, type II diabetes mellitus, obesity, and lower LDL cholesterol and higher HDL cholesterol levels (*8*). These potential health benefits of lentils have been attributed to the presence of secondary metabolites such as phenolic compounds (*9*). These components exhibit antioxidant properties that protect the human body from the damage of reactive oxygen species reducing their activity by scavenging the free radicals generated, complexing pro-oxidant metals and quenching singlet oxygen (*10*, *11*).

There is information about polyphenols and their properties in lentil, but scarce knowledge is available regarding to the effect of processing on the phenolic compounds. Hence, the aim of this study was to investigate the impact of industrial dehydration process on the phenolic profile and antioxidant activity of lentils (*Lens culinaris* var. Pardina) in order to provide useful information on the effective development of functional food products containing bioactive polyphenolic constituents.

MATERIALS AND METHODS

Samples. Lentils (*Lens culinaris* var. Pardina) used in the present study were obtained from agri-food industry Vegenat SA (Badajoz, Spain). There were three batches of 250 g of raw and processed samples. The seeds were freeze-dried, milled to flour, and passed through a 250 μm sieve.

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Processing Conditions. Lentils were subjected to an industrial dehydration process carried out in Vegenat SA. The processing was the following: raw material was soaked (S) in tap water (1:10 w/v) for 16 h at 20 °C. After draining the soaking water, the soaked legumes were cooked (S + C) by boiling for 30 min. The soaked−cooked seeds were dehydrated (S + C + D) in a forced-air tunnel at 75 ± 3 °C for 6 h. Samples were taken at each step.

Chemical Composition Analysis. Proximate chemical composition analysis of the seed flours including total ash, crude fat, and crude protein were performed according to official AOAC procedures (*12*). Ash was calculated from the weight remaining after heating the sample at 550 °C for 2 h. Soxhlet extraction was employed to determinate crude fat. Crude protein was analyzed using Kjeldahl block digestion. Total carbohydrates were determined by difference. Gross energy was calculated based on the following formula (*13*):

$$\text{gross energy(kJ/100g dry matter(DM))} = (\text{protein} \times 16.7) + (\text{lipid} \times 37.7) + (\text{carbohydrates} \times 16.7)$$

Preparation of Samples and Extraction of Phenolic Compounds. Legume flours (5.0 g) were macerated with 3 × 50 mL of a solution of methanol−HCl (1°/$_{00}$)/water (80:20 v/v) using an orbital shaker at room temperature, separating the supernatants by centrifugation (3024*g*, 10 min, 5 °C). The three combined supernatants were taken to a fixed volume (150 mL) of the methanol solution, yielding a methanol extract where the radical scavenging activity of the extract was determined. An aliquot of the methanolic solutions (50 mL) was extracted three times with ethyl ether (3 × 15 mL) and ethyl acetate (3 × 15 mL). The organic phases were combined and dried with anhydrous Na_2SO_4 for 20 min and evaporated to dryness under vacuum. The residue was dissolved in 1 mL of methanol/H_2O (50:50, v/v) and finally filtered (0.45 μm) for HPLC analysis. The extractions were performed in duplicate.

HPLC-PAD and HPLC-MS Analysis. The chromatographic system was equipped with a 717Plus autosampler, a quaternary pump, a photodiode-array detector (PAD) 2001, Millennium 32 chromatography manager software (Waters, Milford, MA). Separation was performed on a 250 mm × 4.6 mm rd, 4 μm reversed-phase Nova-Pak C18 (Waters) column at room temperature. A gradient consisting of solvent A (water/acetic acid, 98/2, v/v) and solvent B (water/acetonitrile/acetic acid, 78/20/2, v/v/v) was applied at a flow rate of 1.0 mL/min as follows: 0−80% B linear from 0 to 55 min, 80−90% B linear from 55 to 57 min, 90% B isocratic from 57 to 70 min, 90−95% B linear from 70 to 80 min, 95−100% B from 80 to 90 min, followed by washing (methanol) and re-equilibration of the column from 90 to 120 min. Detection was performed by scanning from 210 to 400 nm with an acquisition speed of 1 s. A volume of 25 μL was injected. The samples were analyzed in duplicate.

In addition to their UV spectra, the identification of phenolic compounds was carried out by mass spectrometry coupled to HPLC. Mass spectra were obtained using a Hewlett-Packard 1100 MS (Palo Alto, CA) chromatograph equipped with an electrospray ionization (ESI) interface. Separation conditions were the same as described previously with the exception of the flow rate, which was set to 0.7 mL/min. The ESI parameters were drying gas (N_2) flow and temperature, 10 L/min, and 350 °C, respectively; nebulizer pressure, 55 psi, and capillary voltage, 4000 V. The ESI was operated in negative mode scanning from m/z 100 to m/z 2000 using the following fragmentation program: from m/z 0 to 200 (150 V) and from m/z 200 to 3000 (300 V).

Chromatographic peaks were identified by comparison to retention times, UV spectra and data of UV spectral parameters with those of standards and confirmed by analysis of HPLC-MS spectra recorded for each peak. The standards, *p*-hydroxybenzoic *trans-p*-coumaric acids, protocatechuic aldehyde, and tryptophan; flavan-3-ols (+)-catechin, (−)-epicatechin, and procyanidin B2; flavones apigenin methylether, luteolin 3′-7-*O*-diglucoside, and 5,7-dimethoxyflavone; flavonols myricetin 3-*O*-rhamnoside, kaempferol 3-*O*-rutinoside, and kaempferol 3-*O*-glucoside; kaempferol 3-*O*-robinoside-7-*O*-rhamnoside; flavanones eridictyol 7-*O*-rutinoside and eriodictyol were obtained from Extrasynthèse (France). Other compounds, for which no standards were available, were identified based on the study of data of UV spectral parameters and by HPLC-MS (ESI) (*14*). Most of the kaempferol derivatives were identified agree with data of Abad-García et al. (*15*).

Quantification was carried out using the external standard method with commercial standards. The calibration curves were made by injection of different volumes from the stock solutions over the range of concentration observed for each of the compounds, using a linear regression for the relationship of area sum versus concentration, under the same conditions as for the samples analyzed. The unknown nonflavonoid and flavonoid derivatives were quantified with the calibration curves of the most similar compounds.

Oxygen Radical Absorbing Capacity Assay. The radical scavenging activity of the extracts was determined in the methanol extract by the ORAC method using fluorescein as a fluorescence probe (*16*). Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) and the final assay mixture (200 μL) contained fluorescein (70 nM), 2,2-azobis(2-methyl-propionamidine)-dihydrochloride (12 mM), and antioxidant (Trolox [1−8 μM] or sample [at different concentrations]). A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters was used. The equipment was controlled by the Fluostar Galaxy software version (4.11−0) for fluorescence measurement. Black 96-well untreated microplates (Nunc, Denmark) were used. The plate was automatically shaken before the first reading and the fluorescence was recorded every minute for 98 min. 2,2-Azobis(2-methyl-propionamidine)-dihydrochloride and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). All reaction mixtures were prepared in duplicate, and at least three independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\text{AUC} = 1 + \sum_{i=1}^{i=98} f_i/f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time *i*. The net AUC corresponding to a sample was calculated as follows:

$$\text{net AUC} = \text{AUC antioxidant} - \text{AUC blank}$$

The net AUC was plotted against the antioxidant concentration and the regression equation of the curve was calculated. The ORAC value was obtained by dividing the slope of the latter curve between the slopes of the Trolox curve obtained in the same assay. Final ORAC values were expressed as μmol of Trolox equivalents/g of legume.

Statistical Analysis. Results were analyzed using Duncan's multiple range test (DMRT) (*17*), and principal components were performed using Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Proximate Composition. Table 1 shows the proximate composition of raw and processed lentil flours. Protein content of raw legume (19.5 g/100 g DM) is slightly lower than those reported by literature (*18*). It should be stressed that protein content in edible legumes may vary markedly by cultivation conditions, maturity of the grain, and the species variety. The concentration of protein tends to rise during processing, probably due to solubilization of components and, consequently, as a concentration effect (*19*). However, the ash levels show lower values in processed flours than in raw lentil due to mineral losses during thermal processing. Total carbohydrates are the major component of lentil (74.3 g/100 g), and they are solubilized during heat processing. These data are similar to those found in the literature (*20*, *21*) but are lower when soaking process is carried out in different medium to distilled water (*22*). Nevertheless, the processed flours reveal high energy levels due to the high protein and carbohydrate contents.

Table 1. Proximate Composition of Raw and Processed Pardina Lentil (g/100 g DM)[a]

legume	ash	fat	protein	total carbohydrates[b]	energy value (kJ/100 g)
raw	3.4 ± 0.3^{b}	2.8 ± 0.3^{a}	19.5 ± 0.3^{a}	74.3 ± 0.4^{b}	1672.0 ± 7.6^{a}
S	1.4 ± 0.1^{a}	2.9 ± 0.1^{a}	20.6 ± 0.3^{b}	75.1 ± 0.3^{b}	1707.5 ± 6.4^{b}
S + C	1.6 ± 0.2^{a}	3.0 ± 0.4^{a}	25.2 ± 0.5^{c}	70.2 ± 0.5^{a}	1706.3 ± 6.8^{b}
S + C + D	1.4 ± 0.4^{a}	3.1 ± 0.3^{a}	25.9 ± 0.2^{c}	69.6 ± 0.4^{a}	1711.7 ± 7.1^{b}

[a] Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test ($p < 0.05$). Mean ± SD ($n = 6$). [b] Total carbohydrates are calculated by difference.

Identification of Phenolic Compounds on Raw and Processed Lentil Flours. Figure 1 shows the chromatograms of the raw (**A**), soaked (**B**), cooked (**C**) and dehydrated (**D**) lentil flours, identifying a total of 35 phenolic compounds. Data (retention time, λ_{max} in the visible region, molecular ion and main fragment ions observed in MS^2) are presented in **Table 2**. Analysis of MS spectra recorded for each peak, together with comparison of MS^2, UV-spectra, and retention times, led to identification of some of the compounds from the chromatographic conditions. Among the analyzed sample extracts several phenolic compounds, nonflavonoids as hydroxybenzoic and hydroxycinnamic acids, and flavonoids, such as flavones, flavonols, flavan-3-ols, dihydroflavonols, and flavonones, were identifed.

Hydroxybenzoic and Hydroxycinnamic Compounds. Peaks 1, 3, 4, 5, 8, 9, 12, 14, and 15 presented UV spectra that correspond to hydroxybenzoic acids and aldehydes and hydroxycinnamic acids. Some of them, peaks 4, 5, 14, and 15, were identified as protocatechuic aldehyde, *p*-hydroxybenzoic acid, *trans-p*-coumaric, and *cis-p*-coumaric acids, respectively, by comparison of retention times and UV spectra with those of corresponding standards.

Peak 1 showed a UV spectrum similar to that of *p*-hydroxybenzoic acid, and it presented an $[M - H]^-$ at m/z 153 corresponding to dihydroxibenzoic acid. Peak 3 showed a UV spectrum similar to that of vanillic acid and it presented an $[M - H]^-$ at m/z 329 corresponding to vanilllic acid linked to a hexose and one fragment ion $[M - H]^-$ at m/z 167 from vanillic acid. This compound is identified as vanillic acid hexoside.

The UV spectra of peaks 8 and 9 are similar to that of *trans-p*-coumaric acid. In the HPLC-MS (ESI) analysis they presented an $[M - H]^-$ at m/z 279 and 221, respectively, which correspond to *p*-coumaric acid linked to a malic and glycolic acid, respectively, and a fragment $[M - H]^-$ at m/z 163 from *p*-coumaric acid. These compounds are identified as *trans-p*-coumaroyl-malic and *trans-p*-coumaroyl-glycolic acids, respectively. These peaks are also identified in the lentil and pea cotyledon (*14*, *23*).

Peak 12 presented UV spectra similar to *trans-p*-coumaric acid but with different retention times and confirmed with the fragment $[M - H]^-$ at m/z 163 from *p*-coumaric acid. This compound is identified as a *trans-p*-coumaric acid derivative because data of its MS spectra could not be obtained.

Catechins and Procyanidins. Peaks 2, 6, 10, 13, 21, 22, 23, 24, 30, and 31 presented UV spectra which correspond to procyanidin. Some of them, peaks 10, 30, and 31, are identified by comparison of retention times and UV spectra with those of corresponding standards.

Peak 2 showed at λ_{max} 279 nm, characteristic of procyanidin oligomers, and an $[M - H]^-$ at m/z 865, corresponding to procyanidin trimer. Peaks 16 and 21−24 presented an $[M - H]^-$ at m/z 577, correspondig to procyanidin dimer.

Peak 6 presented a molecular ion $[M - H]^-$ at m/z 451 and a fragment ion at m/z 289, which corresponds to the loss of glucose from the structure of (+)-catechin 3-glucoside. This compound is also detected in seed coat and whole lentils by Dueñas et al. (*14*, *24*).

Flavonols. The UV spectra of peaks 18, 20, 25−28, 32, and 33 are considered flavonols, taking into account their UV characteristics.

Peak 18 presented a UV spectrum (λ_{max} 346 nm) corresponding to the flavonol kaempferol, it had a molecular ion $[M - H]^-$ at m/z 901, which corresponds to the kaempferol linked to two molecules of rutinoside and a majority fragment ion $[M - H]^-$ at m/z 285 ($[M-616]^-$ loss of two rhamnosideglucoside molecules), corresponding to kaempferol. This compound is identified as kaempferol dirutinoside.

The following compounds, kaempferol 3-robinoside-7-rhamnoside (peak 20), myricetin 3-rhamnoside (peak 26), kaempferol 3-rutinoside (peak 27), and kaempferol 3-glucoside (peak 28) are identified by comparison of retention times and UV spectra to those of standards and confirmed by HPLC-MS (ESI) analysis.

Peak 25 showed a λ_{max} 346 nm characteristic of kaempferol; it is considered a kaempferol derivative based on the fragment ion $[M - H]^-$ at m/z 285.

Peak 32 showed a UV spectrum characteristic of kaempferol derivatives. In the analysis by HPLC-MS, it showed a negative molecular ion $[M - H]^-$ at m/z 755, which corresponded to the kaempferol linked to two hexoses and a rhamnose and a fragment ion $[M - H]^-$ at m/z 285 ($[M - 308-162]^-$, loss of rhamnosidehexose + hexose residue), corresponding to kaempferol. It is identified as kaempferol rhamnosidehexose−hexose.

Peak 33 showed a negative molecular ion $[M - H]^-$ at m/z 545, which corresponded to the kaempferol glycoside acetylate and a fragment ion $[M - H]^-$ at m/z 285 ($[M - 204]^-$, loss of acetylglycoside residue). This compound is identified as kaempferol acetylglycoside.

Flavones and Dihydroflavonol. The following compounds, apigenin methyl ether (peak 17), luteolin 3′-7-diglucoside (peak 19), and 5,7-dimethoxyflavone (peak 29), are identified by comparison of retention times and UV spectra to those of standards and confirmed by HPLC-MS (ESI) analysis (**Table 2**).

Peak 11 presented λ_{max} at 292 characteristics of dihydrokaempferol derivatives. It showed a negative molecular ion $[M - H]^-$ at m/z 449. Thus, this compound is tentatively identified as dihydrokaempferol glycoside because data of its MS^2 spectra could not be obtained.

Peaks 34 and 35 are identified as eriodictyol 7-rutinoside and eriodictyol, respectively, by comparison with the standards and confirmed by HPLC-MS (ESI) analysis.

Other Components. Peak 7 is identified as trytophan by comparison of retention time and UV spectra to that of standard amino acid and confirmed by HPLC-MS (ESI) analysis. This is an aromatic amino acid that was extracted in the conditions of the analysis of phenolic compounds.

Influence of Processing on the Individual Phenolic Compounds of Lentil Flours. Relevant qualitative and quantitative differences in the identified phenolic compounds are observed between raw and processed lentil flours (**Table 3**). Low molecular weight secondary plant metabolites such as hydroxybenzoics, hydroxycinnamics, catechins, and procyanidins, flavonols, flavones, dihydroflavonols, and flavonones (**Table 4**) are the main components of the methanol extracts.

Hydroxybenzoic Compounds. The content of hydroxybenzoic compounds in raw lentils represented 5% of identified

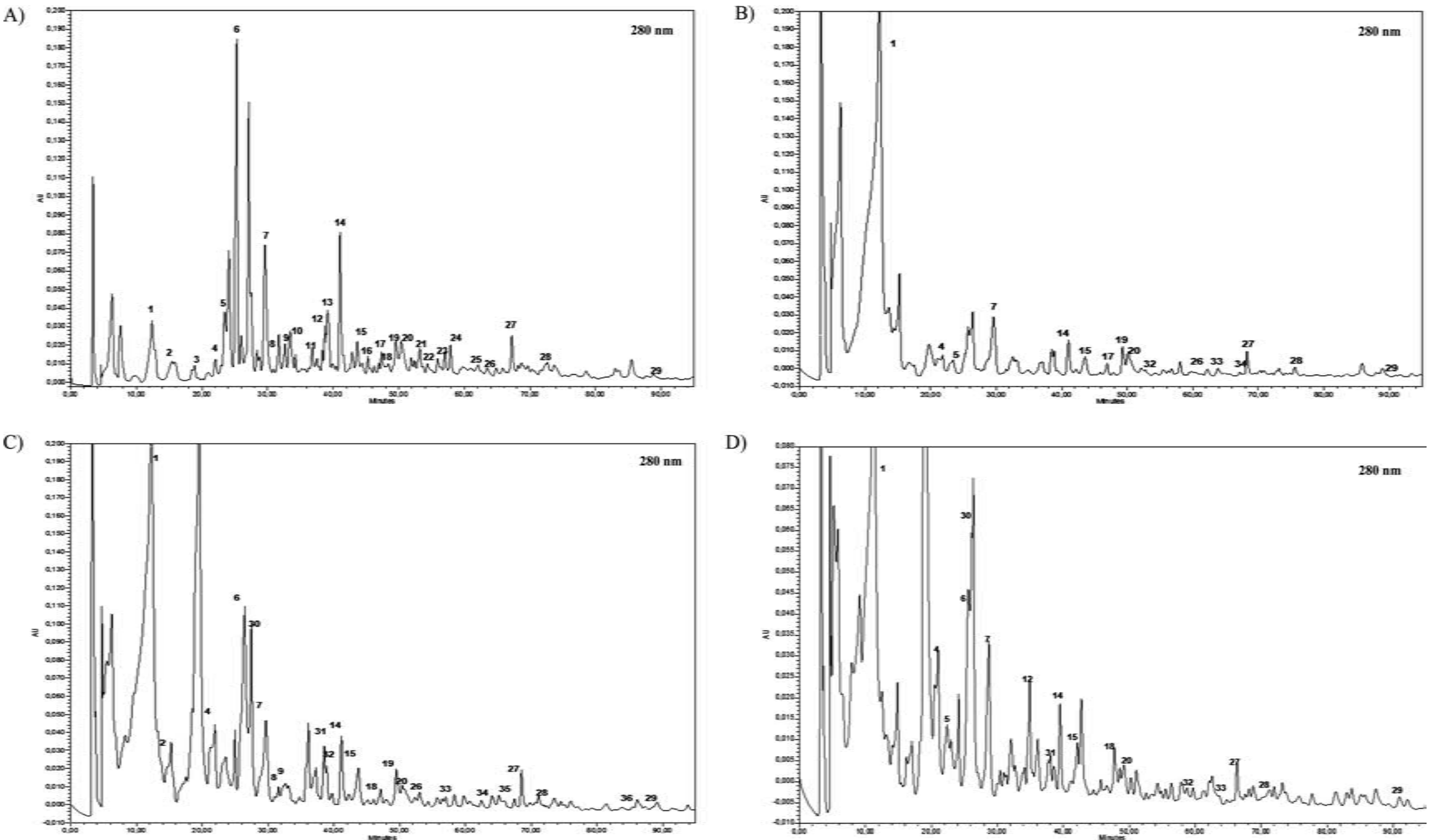

Figure 1. HPLC chromatogram of raw (**A**), soaked (**B**), cooked (**C**), and dehydrated (**D**) Pardina lentil (280 nm).

Table 2. Wavelength of Maximum UV Absorption and Molecular Ions of Identified Compounds in Raw and Processed Pardina Lentil[a]

peak no.	identified compds	λ_{max} (nm)	$[M - H]^-$ (m/z)	MS^2 (m/z)
1	dihydroxybenzoic acid	257	153	
2	procyanidin trimer	279	865	289
3	vanillic acid glycoside	254, 293	329	167
4	protocatechuic aldehyde	233, 280, 314	137	
5	*p*-hydroxybenzoic acid	223, 256	137	
6	(+)-catechin 3-glucoside	279	451	289
7	tryptophan	279	203	
8	*trans-p*-coumaroyl malic acid	308	279	163
9	*trans-p*-coumaroyl glycolic acid	307	221	163
10	procyanidin B2	232, 279	577	289
11	dihydrokaempferol glycoside	292	449	
12	*trans- p*-coumaric acid derivative	232, 310		163
13	procyanidin dimer 1	232, 279	577	289
14	*trans-p*-coumaric acid	231, 309	163	
15	*cis-p*-coumaric acid	232, 298	163	
16	procyanidin dimer 6	232, 279	577	289
17	apigenin methyl ether	242, 264sh, 330	283	
18	kaempferol dirutinoside	266, 346	901	755, 285
19	luteolin 3′-7-diglucoside	264, 350	609	285
20	kf 3-robinoside-7-rhamnoside	264, 299sh, 353	739	285
21	procyanidin dimer 2	232, 279	577	289
22	procyanidin dimer 3	232, 279	577	289
23	procyanidin dimer 4	232, 279	577	289
24	procyanidin dimer 5	232, 279	577	289
25	kaempferol derivative	264, 346		285
26	myricetin 3-rhamnoside	247, 348	463	317
27	kaempferol 3-rutinoside	266, 338	593	447, 285
28	kaempferol 3-glucoside	266, 340	447	285
29	5,7-dimethoxyflavone	310	281	
30	(+)-catechin	232, 279	289	
31	(−)-epicatechin	232, 279	289	
32	kf rhamnosidehexose−hexose	266, 348	755	285
33	kaempferol acetylglycoside	268, 298sh, 346	545	285
34	eriodictyol 7-rutinoside	285	595	287
35	eriodictyol	285	287	

[a] Kf: kaempferol; sh: shoulder.

phenolics (5.69 μg/g) (**Table 4**). Similar contents are also found by Dueñas et al. (*24*) in Pardina lentil. Dihydroxybenzoic acid is the main phenolic compound in raw lentil (3.68 μg/g), and an important increment is observed once the dehydration process took place (31.69 μg/g) (**Table 3**). This increase might be originated from the disruption of cell walls during processing or the breakdown of insoluble phenolic compounds because it could have led to better extractability of these compounds.

Hydroxycinnamic Compounds. This phenolic group only represented 4% of the identified phenolic compounds in raw lentils (**Table 4**). The hydroxycinnamic compounds are mainly constituents of the cell wall, in various bonds and esterified forms, linked to arabinoxylans and lignin. This group included hydroxycinnamic compounds in free forms (*trans-p*-coumaric and *cis-p*-coumaric acid) and those linked to other compounds (hydroxyacids) (**Table 3**). Most of these phenolic acids have been detected in raw leguminous seeds and their extracts, as reviewed Amarowicz and Pegg (*25*). In general terms, these compounds decrease with thermal treatment, except the *trans-p*-coumaric acid derivative. The loss of these components could be either due to leaching or diffusion of these phenols into soaking and cooking water or due to the breakdown of phenolics during processing (*10*). These decreases are also reported in several legumes such as beans and lentils together with carbohydrate and antinutritional factor reductions (*7*, *26*).

Catechins and Proanthocyanidins. The content of catechin and proanthocyanidin compounds represented the highest percentage (69%) of identified phenolics (74.48 μg/g) in raw lentils (**Table 4**). The main compound is (+)-catechin 3-glucoside (39.89 μg/g), followed by a procyanidin trimer and procyanidin B2 (**Table 3**). These results are in agreement with the findings of Escarpa and González (*27*), Amarowicz and Karamac (*28*), and Martín-Cabrejas et al. (*7*). However, prodelphinidins are not detected in raw lentils, in contrast to the work of Dueñas et al. (*24*). Important reductions are observed in the content of these phenols as affected by the industrial dehydration. (+)-Catechin 3-glucoside exhibited the most pronounced decrease (95%), as well as most of procyanidins that were not detected at the end of the industrial process of dehydration. The thermal treatments (cooking and dehydration) would allow to degradation reactions of the more polymerized proanthocyanidins, hydrolysis of glycosylated flavonoids, and decomposition of aglycones (*10*, *29*). The data agree with those found by Alonso et al. (*30*) and El-Hady and Habiba (*31*) in other legumes using extrusion as processing. In general, air-drying at temperatures >60 °C is regarded as unfavorable to efficiency extraction of phenolic compounds due to oxidative condensation or decomposition of thermolabile compounds such as (+)-catechins (*32*).

Flavonols and Dihydroflavonols. This group represented 17% of total identified phenols in raw lentil (**Table 4**), kaempferol glycosides being the most abundant flavonols (**Table 3**). Similar contents are reported by Escarpa and González (*27*) in common legumes. Flavonoid compounds together with procyanidins show high antioxidant capacity due to their structure (*33*, *34*). However, this phenolic group presents a drastic reduction (67%) when processing was carried out. After cooking, three compounds identified as kaempferol dirutinoside, kaempferol acetylglycoside, and kaempferol 3-glucoside significantly increased compared to soaked samples. This indicated that thermal processing released these compounds from bonded forms. In addition, dehydration process gave rise to an increase of some compounds as compared to soaked lentils (kaempferol dirutinoside, kaempferol 3-rutinoside, kaempferol 3-robinoside-7-rhamnoside, and kaempferol acetylglycoside), in contrast to dihydrokaempferol glycoside, kaempferol derivative, and myricetin 3-rhamnoside, which became undetectable at the end of the industrial process (**Table 3**).

Flavones and Flavanones. The flavones and flavanones represented only 5% of the total phenols in raw sample (**Table 4**). Luteolin diglucoside was the main flavone compound (4.55 μg/g) (**Table 3**) as reported in other legumes such as lupin and Castellana and Pardina lentils (*14*, *27*, *35*). In general terms, flavones and flavanones decreased during industrial processing, only being detected 5,7-dimethoxyflavone (0.50 μg/g) in the dehydrated flour (**Table 4**). A partial leaching and thermal/oxidative deterioration of flavones and flavanones occurred, in agreement with Xu and Chang (*9*, *36*).

Other Components. Tryptophan, which is initially found in raw lentils (0.91 μg/g), was increasing during the industrial dehydration process (**Table 3**). The amino compounds were also identified in literature (*25*).

Antioxidant Activity of Raw and Processed Lentil Flours. The oxygen radical absorbance capacity (ORAC) is the only method so far that combined both inhibition time and degree of inhibition into a single quantity (*37*). **Table 4** shows the ORAC values of raw, soaked, cooked, and dehydrated lentil flours. The antioxidant activity is high in raw lentils (66.97 μmol Trolox/g) as compared to other common legumes such as chickpeas, green peas,

Table 3. Influence of Industrial Dehydration Process on the Content (μg/g) of Phenolic Compounds in Raw and Processed Pardina Lentil[a]

peak no.	identified compounds (μg/g)	raw	S	S + C	S + C + D
	Hydroxybenzoics				
1	dihydroxybenzoic acid	3.68 ± 0.31	39.20 ± 1.91	25.75 ± 1.12	31.69 ± 1.15
3	vanillic acid glycoside	0.04 ± 0.01	nd	nd	nd
4	protocatechuic aldehyde	0.07 ± 0.02	0.76 ± 0.08	3.44 ± 0.28	3.61 ± 0.52
5	*p*-hydroxybenzoic acid	1.90 ± 0.33	0.48 ± 0.02	nd	0.64 ± 0.08
	Hydroxycinnamics				
8	*trans- p*-coumaroyl malic acid	0.66 ± 0.06	nd	0.04 ± 0.01	nd
9	*trans-p*-coumaroyl glycolic acid	0.42 ± 0.07	nd	0.05 ± 0.01	nd
12	*trans-p*-coumaric acid derivative	0.12 ± 0.01	nd	nd	0.73 ± 0.08
14	*trans-p*-coumaric acid	2.14 ± 0.12	0.30 ± 0.01	0.58 ± 0.05	0.36 ± 0.05
15	*cis-p*-coumaric acid	0.42 ± 0.05	0.24 ± 0.04	0.43 ± 0.02	0.12 ± 0.02
	Catechins and Procyanidins				
6	(+)-catechin 3-glucoside	39.89 ± 2.06	nd	1.84 ± 0.08	1.86 ± 0.06
10	procyanidin B2	8.92 ± 0.63	nd	nd	nd
30	(+)-catechin	nd	nd	5.10 ± 0.21	1.64 ± 0.22
31	(−)-epicatechin	nd	nd	0.68 ± 0.05	1.04 ± 0.14
2	procyanidin trimer	9.30 ± 0.54	nd	0.53 ± 0.07	nd
13	procyanidin dimer 1	3.82 ± 0.22	nd	nd	nd
21	procyanidin dimer 2	1.81 ± 0.12	nd	nd	nd
22	procyanidin dimer 3	0.93 ± 0.08	nd	nd	nd
23	procyanidin dimer 4	2.98 ± 0.21	nd	nd	nd
24	procyanidin dimer 5	4.45 ± 0.33	nd	nd	nd
16	procyanidin dimer 6	2.38 ± 0.21	nd	nd	nd
	Flavonols and Dihydroflavonols				
11	dihydrokaempferol glycoside	0.66 ± 0.08	nd	nd	nd
18	kf-dirutinoside	2.09 ± 0.19	nd	0.96 ± 0.07	1.47 ± 0.13
32	kf-rhamnosidehexose−hexose	nd	1.87 ± 0.13	1.41 ± 0.11	0.49 ± 0.04
28	kaempferol 3-glucoside	3.66 ± 0.21	1.21 ± 0.11	1.47 ± 0.14	0.43 ± 0.04
20	kf 3-robinoside-7-rhamnoside	2.94 ± 0.23	0.53 ± 0.07	0.44 ± 0.08	0.78 ± 0.11
26	myricetin 3-rhamnoside	1.83 ± 0.13	0.50 ± 0.07	0.54 ± 0.05	nd
33	kaempferol acetylglycoside	nd	0.51 ± 0.05	0.73 ± 0.10	0.83 ± 0.07
27	kaempferol 3-rutinoside	5.95 ± 0.28	1.23 ± 0.11	0.78 ± 0.04	1.53 ± 0.12
25	kaempferol derivative	0.76 ± 0.05	nd	nd	nd
	Flavones and Flavanones				
17	apigenin methyl ether	0.15 ± 0.04	0.65 ± 0.07	nd	nd
19	luteolin 3′-7-diglucoside	4.55 ± 0.15	1.20 ± 0.09	1.78 ± 0.12	nd
34	eriodictyol 7-rutinoside	nd	nd	0.17 ± 0.06	nd
35	eriodictyol	nd	0.16 ± 0.05	0.20 ± 0.04	nd
29	5,7-dimethoxyflavone	0.19 ± 0.03	0.24 ± 0.03	0.06 ± 0.01	0.50 ± 0.03
	Other Compounds				
7	tryptophan	0.91 ± 0.08	1.68 ± 0.14	1.99 ± 0.12	2.86 ± 0.11

[a] Kf, kaempferol; nd: not detected; mean ± SD (n = 4).

Table 4. Concentrations (μg/g) of Identified Phenolic Compounds, Grouped in Hydroxybenzoics, Hydroxycinnamics, Catechins, and Proyanidins, Flavonols, Flavones, and Flavanones and Others in Raw and Processed Pardina Lentil[a]

legume	hydroxybenzoics	hydroxycinnamics	catechins and procyanidins	flavonols and dihydroflavonols	flavones and flavanones	other components	ORAC values[b] (μmol Trolox eq/g DM)
raw	5.69 ± 0.67[a]	3.76 ± 0.31[c]	74.48 ± 4.19[c]	17.89 ± 1.18[b]	4.89 ± 0.18[c]	0.91 ± 0.08[a]	66.97 ± 4.90[c]
S	40.44 ± 2.41[b]	0.54 ± 0.05[a]	n.d.	5.85 ± 0.45[a]	2.25 ± 0.21[b]	1.68 ± 0.14[b]	17.36 ± 1.60[a]
S + C	29.19 ± 4.47[b]	1.10 ± 0.08[b]	13.75 ± 0.69[b]	6.33 ± 0.58[a]	2.21 ± 0.19[b]	1.99 ± 0.13[c]	24.73 ± 1.30[b]
S + C + D	35.94 ± 2.71[b]	1.21 ± 0.14[b]	8.63 ± 0.58[a]	5.53 ± 0.49[a]	0.50 ± 0.03[a]	2.86 ± 0.11[d]	21.95 ± 2.10[b]

[a] ORAC values were also determined. Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test ($p < 0.05$). Mean ± SD (n = 4). nd: not detected. [b] Mean ± SD (n = 6).

and yellow peas (9.57−18.66 μmol Trolox/g) (*38*). However, Xu and Chang (*36*) detected higher levels of antioxidant activity in medium green lentils (94.9 μmol Trolox/g). This relevant antioxidant activity could be due to the presence of the high concentrations of phenolic compounds, especially flavonoids, present in the seed coat as reported Dueñas et al. (*39*).

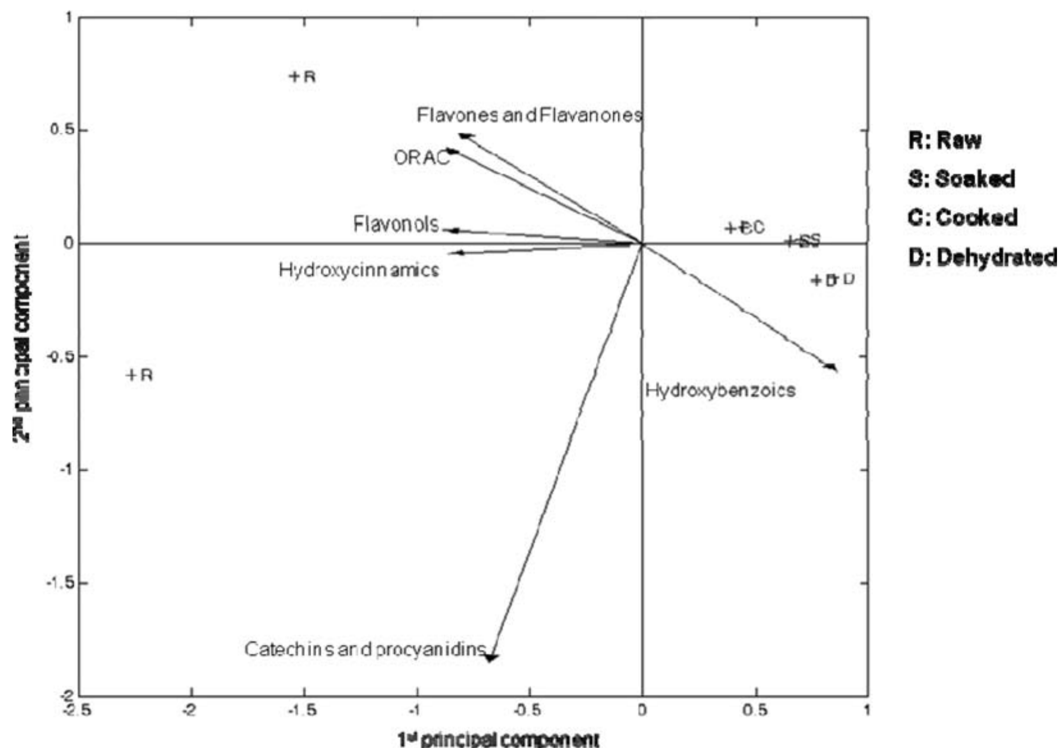

Figure 2. Plot of principal components of the phenolic compounds and antioxidant activity (ORAC) of raw (R), soaked (S), cooked (C), and dehydrated (D) Pardina lentil.

The process of soaking, cooking, and dehydration of lentil caused significant decreases in ORAC values in comparison to the raw sample. During soaking treatment, it showed a significant decrease of ORAC value (17.36 μmol Trolox/g) that could be attributed to elimination of catechins and procyanidins and low content of flavonols. These decreases are in agreement with Ranilla et al. (*10*) and Xu and Chang (*36*) in the soaking period.

As compared to soaking treatment, cooking processing caused a significant increase in ORAC value (42%). Xu and Chang (*36*) reported similar behavior in cooked chickpeas and lentils; however the contrary was observed in cooked black beans (*40*). There was no significant difference between cooked and dehydrated lentil flours. Thus, the changes of ORAC values could be attributed to the leaching of phenolic compounds into the soaking and cooking water. However, other phenomena such as better solubility of antioxidant nonphenolic compounds following thermal treatment and the formation of Maillard products with increased free radical scavenging properties may be involved (*41*).

The antioxidant capacity of phenolic compounds is related to their chemical structure (*33*, *34*); thus, to relate the antioxidant activity to the identified phenols in the lentil processed flours, an analysis of principal components was carried out. Principal component analysis is performed on the values of the antioxidant activity and the concentrations of the identified phenolic groups in raw, soaked, cooked, and dehydrated flours (**Table 4**). Six components were obtained of which the first two accounted for 96% of the total variance. **Figure 2** illustrates the graphic representation of the first two components. In the graphic four groups of phenolic compounds (hydroxycinnamics, flavonols, flavones, and flavanones) demonstrate a higher correlation with the antioxidant activity and the raw sample. In this study, flavones and flavanones show the most influence on the antioxidant activity, whereas catechins and procyanidins appeared less associated to this activity; contrary to that reported by Dueñas et al. (*39*) in raw lentils. These differences could be due to the drastic decreases of these compounds during the industrial processing.

It is well-known that nonflavonoid compounds show less antioxidant activity than flavonoids (*42*). However, hydroxycinnamic compounds reveal a relationship with the antioxidant activity; this finding is agreed with previous works (*26*, *39*). In addition, hydroxybenzoic compounds are the only group that showed a relevant relationship with the processed samples (soaked, cooked, and dehydrated) although they are not related to antioxidant activity.

The results obtained in this study provide the first documentation on the phenolic profile and antioxidant properties of Pardina lentil as affected by the industrial dehydration that may be useful to produce legume-based products with added value for functional food market. HPLC-PAD and HPLC-MS (ESI) data reveal 35 phenolic compounds in raw and processed lentil flours, being (+)-catechin 3-glucoside and procyanidin trimer and procyanidin B2, the predominant phenolic compounds. The results demonstrate qualitative and quantitative differences between the phenolic compounds during processing. The dehydration did not cause any further effects than ordinary cooking on reducing phenolic compounds of lentil flours. In addition, the important antioxidant activity of raw lentil flours decrease during the processing, although the ORAC values are still relevant in processed lentil flours. Thus, the significant occurrence of bioactive phenolic compounds along with the interesting antioxidant capacity of dehydrated lentil flours make them useful for daily inclusion in the human diet as ready-to-use for special meals to specific populations.

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Received for review June 9, 2010. Revised manuscript received August 3, 2010. Accepted August 10, 2010.