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Antioxidant capacity and polyphenolic content of high-protein lupin products

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ABSTRACT

In order to produce high protein lupin products, α-galactoside extraction from *Lupinus angustifolius* cv. Troll and cv. Emir and *Lupinus albus* cv. Multolupa, and protein isolation from *L. albus* cv. Multolupa were carried out. Trolox equivalent antioxidant capacity (TEAC), DPPH radical-scavenging activity (DPPH-RSA), peroxyl radical-trapping capacity (PRTC), superoxide dismutase-like activity (SOD-like activity), total phenolic compounds (TPC) and total flavonoids were determined in lupin products. In *L. angustifolius* cv. Troll, *L. angustifolius* cv. Emir and *L. albus* cv. Multolupa α-galactoside-low flours, the TEAC and DPPH-RSA decreased (43%, 38%; 73%, 82%; 77%, 38%, respectively). PRTC decreased in *L. angustifolius* cv. Troll and *L. albus* cv. Multolupa α-galactoside-low flours (13% and 98%, respectively), while in those of *L. angustifolius* cv. Emir, PRTC increased (25%). SOD-like activity and TPC were also affected by α-galactoside extraction and reductions of 30–52% and 38–56%, respectively, were observed. The protein isolate of *L. albus* cv. Multolupa presented lower TEAC (24%), a similar level of PRTC and twice higher level of DPPH-RSA than did α-galactoside-low Multolupa flour. Moreover, the SOD-like activity of lupin protein isolate was sharply reduced but the TPC content was 24% higher. The technological procedures, α-galactoside extraction and protein isolation, provide high protein lupin products but with lower antioxidant capacity and total phenolic compounds than the raw seeds, although the levels of antioxidant capacity of these lupin products resemble those of cereals.

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1. Introduction

The demand for healthy foods is increasing rapidly in developed countries. In this sense, World Health Organizations advise the frequent consumption of vegetable proteins instead of animal proteins with considerable amounts of saturated fat and cholesterol. Leguminous seeds present one of the most promising alternative protein sources for the nutritional supplementation and technological improvement of traditional foods. Thus, soybean is the world's most used protein legume, widely cultivated and economically feasible (Sugano, 2006). Lupin seeds are also a rich protein crop and successful breeding programmes have generated sweet lupin seeds with low contents of alkaloids (Mulayim, Tamkoç, & Babaoglu, 2002). Lupin seed protein has been proposed as an alternative to proteins extracted from soybean due to its similar protein content (30–40% of dry weight) and its satisfactory functionality in a number of food systems (Martínez-Villaluenga, Frías, & Vidal-Valverde, 2006; Pozani, Doxastakis, & Kiosseoglou, 2002). Lupin is a good source of nutrients, not only proteins but lipids, dietary fibre, minerals and vitamins (Martínez-Villaluenga, Frías, et al., 2006; Torres, Frias, & Vidal-Valverde, 2005). Furthermore, lupins contain phytochemicals with antioxidant capacity, such as polyphenols, mainly tannins and flavonoids (Oomah, Tiger, Olson, & Balasubramanian, 2006).

However, although lupin seed is one of the legumes with the lowest levels of non-nutritional compounds (trypsin inhibitors, phytic acid, saponins and lectins) (Martínez-Villaluenga, Frías, et al., 2006; Petterson, 1998; Torres et al., 2005), it contains large amounts of α-galactosides (7–15%) that are associated with negative physiological functions when consumed in high amounts, as recently reviewed by Martínez-Villaluenga, Frías, and Vidal-Valverde (2008). A simple and economic procedure was proposed by Gulewicz et al. (2000) to remove α-galactosides from legume seeds to increase their nutritive value. Recently, it has been shown that this water–ethanol extraction produced functional lupins with higher protein (up to 50%) and lower α-galactosides content than raw seeds (Martínez-Villaluenga, Frías, et al., 2006; Torres et al., 2005).

In addition, lupin seeds are submitted to processes such as the production of protein isolates. Protein isolates (90% protein minimum dry weight basis) are increasingly used as ingredients in various types of prepared foods, meat analogues, dairy, bakery and pasta products. The high nutritional value and excellent functional

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properties make these lupin products very valuable food ingredients (D'Agostina et al., 2006). Martínez-Villaluenga, Urbano, Porres, Frías, and Vidal-Valverde (2007) showed that biological protein utilisation of lupin protein isolates from *Lupinus albus* cv. Multolupa supplemented with ascorbic acid reflected a nutritive utilisation of protein and weight gain and food transformation index similar to those obtained with a casein-methionine control diet.

Our research group has demonstrated that technological processes, such as seed germination and flour fermentation, used to increase the nutritive value of lupin, improved the antioxidant capacity of this legume (Fernandez-Orozco et al., 2006, 2008a). However, there is a lack of information about the effect of α-galactoside alcoholic extraction and protein isolation on the antioxidant properties, total phenolic and flavonoid contents of these high protein products. Therefore, trolox equivalent antioxidant capacity (TEAC), DPPH radical-scavenging activity (DPPH-RSA), peroxyl radical-trapping capacity (PRTC), superoxide dismutase-like activity (SOD-like activity) and total phenolic compounds (TPC), including total flavonoids (TF) were determined in α-galactoside-low lupin flours and lupin protein isolates.

2. Materials and methods

2.1. Reagents

The superoxide dismutase kit (RANSOD) was from Randox Laboratories Ltd., UK. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were obtained from Sigma-Aldrich. 2,2′-Azobis(2-amidopropane) hydrochloride (ABAP) was purchased from Wako Pure Chemical Industries, LTD., Osaka, Japan. Bovine serum albumin, fraction V (BSA), bicinchonic acid (BCA) and potassium persulfate were obtained from Sigma (Sigma Chemicals. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

2.2. Seeds

Sweet lupin seeds of three different cultivars were used. *Lupinus angustifolius* cv. Troll and *L. angustifolius* cv. Emir were kindly provided by Prof. Gulewicz from the Institute of Bioorganic Chemistry (PAS, Poznan, Poland) while *L. albus* cv. Multolupa was obtained from the Agricultural Research and Technology Development Service of the Agriculture and Commerce Council of the Junta de Extremadura, Spain.

2.3. Technological processes

2.3.1. α-Galactoside-low lupin flours

The extraction of α-galactoside in lupin seeds was carried out according to Gulewicz et al. (2000).

2.3.2. Lupin protein isolates

Lupin protein isolates (LPI) were prepared by alkaline water extraction and isoelectric precipitation (Martínez-Villaluenga et al., 2007). Lupin seeds were milled and then sieved (through 60-mesh). One hundred grammes of lupin (*L. albus* cv. Multolupa) flour was suspended in 1 l of distilled water containing 0.025% (w/v) Na_2SO_3, then adjusted to pH 9.0 using 1 M NaOH. The suspension was stirred for 1 h at room temperature, and was then centrifuged at 3000*g* for 30 min. In order to obtain higher yields, the extraction and centrifugation were repeated on the residue. The extracts were combined and acidified to pH 4.5 using 1 M HCl. The precipitate was recovered by centrifugation at 3000*g* for 30 min, then neutralised with 1 M NaOH to pH 7.0 and washed with distilled water several times. The neutralised precipitate was freeze-dried, milled using a household mill (Braun, Germany), sieved (through 60-mesh) and finally stored under vacuum.

2.4. Preparation of PBS extracts for determination of SOD-like activity and TEAC

Raw seeds, α-galactoside-low lupin flours and lupin protein isolate from *L. albus* cv. Multolupa were extracted in triplicate with phosphate buffered saline, pH 7.4 PBS (1 g/10 ml), shaking at 37 °C for 2 h. Further, they were centrifuged at 12,000*g* in a Beckman GS-15 R centrifuge (Beckman Instruments, Inc., Palo Alto, CL, USA) and fresh supernatants were used to determine the ability to scavenge superoxide anion radicals (SOD-like activity) and trolox equivalent antioxidant capacity (TEAC).

2.5. Preparation of 80% methanol extracts for determination of DPPH-RSA, PRTC, total phenolics (TPC) and total flavonoids (TF)

In order to prepare 80% methanolic extracts, the raw seeds, α-galactoside-low flours and lupin protein isolate from *L. albus* cv. Multolupa were extracted with 80% aqueous methanol (1 g/10 ml), shaking at 37 °C for 2 h. The mixture was then centrifuged at 12,000*g* in a Beckman GS-15 R centrifuge (Beckman Instruments, Inc., Palo Alto, CL, USA) and fresh supernatants were used to determine DPPH radical-scavenging activity, peroxyl radical trapping capacity (PRTC), total phenolics (TPC) and total flavonoids (TF).

2.6. Analytical methods

2.6.1. Determination of trolox equivalent antioxidant capacity (TEAC)

This test was based on the reduction of the ABTS radical cations ($ABTS^{\cdot+}$) by antioxidants present in PBS extracts according to the procedure described by Re et al. (1999). The ABTS radical cations were prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture had to remain for 12–24 h until the reaction was completed and the absorbance was stable (0.700 ± 0.020 at 734 nm at 30 °C). For the spectrophotometric assay, 1.48 ml of the $ABTS^{\cdot+}$ solution and 20 μl of the PBS extract, or trolox solutions were mixed and measured immediately after 6 min at 734 nm at 30 °C. Trolox (2.5 mM) was prepared in PBS buffer to use as stock standard. Appropriate solvent blanks were run in each assay. TEAC was expressed as μmol trolox/g d.m. of sample. All samples were analysed in triplicate.

2.6.2. Determination of SOD-like activity

The superoxide scavenging activity of the phosphate buffered saline flour extracts was measured by a superoxide dismutase kit (RANSOD). The test required 50 μl of sample, with a read time of 3 min. The SOD-like activities of the extracts were calculated as SOD units/ml and finally the results were recalculated per milligramme of soluble protein or gramme of flour. The soluble protein content was determined by protein microassay according to Smith et al. (1985). The assays were performed at 37 °C using a spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan). All samples were analysed in triplicate.

2.6.3. DPPH radical-scavenging assay (DPPH-RSA)

The DPPH-RSA assay was based on a modified method of Brand-Williams, Cuvelier, and Berset (1995). In this assay, antioxidants present in the sample reduce the $DPPH^{\cdot}$ radicals, which have an absorption maximum at 515 nm. The $DPPH^{\cdot}$ radical solution was prepared by dissolving 10 mg DPPH in 25 ml of 80% methanol. First, the extinction of the disposable cuvette with 250 μl of the

methanolic DPPH· solution and 2.1 ml of 80% methanol was measured as blank. Then, the 80% methanol extracts (100 μl) were added to 250 μl of the methanolic DPPH· solution and 2 ml of 80% methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 20 min. The decrease in absorbance of the resulting solution was monitored at 517 nm for 20 min using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The trolox standard solution (concentration 0.1–2.0 mM) in 80% methanol was prepared and assayed under the same conditions. DPPH-RSA-scavenging activity was expressed as μmol trolox/g d.m. of sample. All samples were analysed in triplicate.

2.6.4. Peroxyl radical-trapping capacity (PRTC)

The assay was carried out according to Bartosz, Janaszewska, Ertel, and Bartosz (1998). PBS was pre-heated to 37 °C and added to a cuvette to obtain 3 ml of the final reaction volume. Then 90 μl of 5 mM ABTS solution and 80 μl of 80% methanol extracts or trolox solution were added, followed by 300 μl of 200 mM 2,2′-azobis(2-amidopropane) hydrochloride (ABAP) solution. The cuvettes were placed in a thermostatted recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan) adjusted to 37 °C inside the cuvettes and absorbance was measured at 414 nm. Results were expressed as μmol trolox/g d.m. of sample. All samples were analysed in triplicate.

2.6.5. Determination of total phenolics content (TPC)

TPC values from 80% methanol extracts were determined according to the method of Shahidi and Naczk (1995). A 0.25 ml aliquot of the respective extract was mixed with 0.25 ml of Folin-Ciocalteu reagent (previously diluted with water 1:1 v/v) and 0.5 ml saturated sodium carbonate (Na_2CO_3) solution and 4 ml water. The mixture was allowed to stand at room temperature for 25 min and then centrifuged at 5000 rpm for 10 min. Supernatant absorbance was measured at 725 nm, using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The results were expressed as mg ferulic acid equivs./g d.m. of sample. All samples were analysed in triplicate.

2.6.6. Determination of total flavonoids content (TF)

Total flavonoids content was determined, using a colorimetric method described by Jia, Tang, and Wu (1998). Briefly, 0.25 ml of 80% methanolic extract was diluted with 1.25 ml of distilled water. Then 75 μl of a 5% $NaNO_2$ solution were added, and the mixture was allowed to stay at room temperature. After 6 min, 150 μl of a 10% $AlCl_3 \times 6H_2O$ solution were added, and the mixture was allowed to stand for a further 5 min. After that, 0.5 ml of 1 M NaOH was added. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan) in comparison with the standards prepared similarly with known (±) catechin concentrations. All samples were analysed in triplicate.

2.7. Statistical analysis

Data were subjected to multifactor analysis of variance (ANOVA) using the least-squared difference test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, MD, USA) and multiple correlation using the Statistica 5.1 Program (Statsoft, Tulsa, Okla, USA) for Windows using a PC-Pentium.

3. Results and discussion

Tables 1 and 2 collect the antioxidant capacity of raw seeds, α-galactosides-low flours of *L. angustifolius* cv. Troll and cv. Emir

Table 1
Trolox equivalent antioxidant capacity (TEAC) and superoxide dismutase like activity (SOD-like activity) of lupin products

Lupin products	TEAC (μmol trolox/g d.m.)	SOD-like activity (U/g d.m.)	Soluble protein (mg/g d.m.)	SOD-like activity (U/mg protein)
L. angustifolius cv. Troll				
Raw seeds	47.9 ± 0.89[b]	73.5 ± 0.40[b]	99.0 ± 2.91[b]	0.82 ± 0.02[b]
α-Galactoside-low flour	27.1 ± 1.30[a]	40.7 ± 2.90[a]	75.5 ± 2.66[a]	0.58 ± 0.06[a]
L. angustifolius cv. Emir				
Raw seed	47.0 ± 0.93[b]	81.0 ± 2.44[b]	100 ± 9.13[b]	0.89 ± 0.06[b]
α-Galactoside-low flour	12.9 ± 1.53[a]	30.2 ± 1.41[a]	54.4 ± 0.58[a]	0.61 ± 0.02[a]
L. albus cv. Multolupa				
Raw seed	71.4 ± 0.08[c]	58.2 ± 4.94[c]	149 ± 8.14[c]	0.44 ± 0.06[c]
α-Galactoside-low flour	16.2 ± 0.09[b]	17.8 ± 2.86[b]	90.0 ± 3.39[b]	0.21 ± 0.03[b]
Protein isolate	12.3 ± 0.30[a]	2.03 ± 0.15[a]	48.4 ± 0.88[a]	0.04 ± 0.00[a]

[*]Data are the means ± standard deviation of three determinations. Different superscripts for each lupin cultivar within columns indicate significant statistical differences ($P \leqslant 0.05$).

Table 2
DPPH radical-scavenging activity (DPPH-RSA), peroxyl radical-trapping capacity (PRTC), total phenolics (TPC) and flavonoids (TF) content of lupin products

Lupin products	DPPH-RSA (μmol trolox/g d.m.)	PRTC (μmol trolox/g d.m.)	TPC (mg ferrulic acid/g d.m.)	TF (μg catechin/g d.m.)
L. angustifolius cv. Troll				
Raw seeds	3.09 ± 0.17[b]	0.71 ± 0.22[a]	2.23 ± 0.01[b]	133 ± 12.6[b]
α-Galactoside-low flour	1.92 ± 0.07[a]	0.62 ± 0.11[a]	1.39 ± 0.01[a]	445 ± 8.83[a]
L. angustifolius cv. Emir				
Raw seeds	3.06 ± 0.02[b]	1.26 ± 0.03[a]	2.53 ± 0.06[b]	362 ± 9.00[b]
α-Galactoside-low flour	0.56 ± 0.00 [a]	1.58 ± 0.10[b]	1.12 ± 0.02[a]	349 ± 0.00[a]
L. albus cv. Multolupa				
Raw seeds	2.83 ± 0.09[b]	1.26 ± 0.10[b]	1.82 ± 0.01[c]	1100 ± 17.6[c]
α-Galactoside-low flour	1.76 ± 0.05[a]	0.03 ± 0.00[a]	0.90 ± 0.01[a]	153 ± 10.5[b]
Protein isolate	3.19 ± 0.08[c]	0.03 ± 0.00[a]	1.12 ± 0.00[b]	36.9 ± 5.15[a]

[*]Data are the means ± standard deviation of three determinations. Different superscripts for each lupin cultivar within columns indicate significant statistical differences ($P \leqslant 0.05$).

and *L. albus* cv. Multolupa and lupin protein isolate from *L. albus* cv. Multolupa. The antioxidant capacity of raw seeds, measured by TEAC assay, ranged from 71 to 47 μmol trolox/g d.m. (Table 1) while the DPPH radical-scavenging activity and the peroxyl radical-trapping capacity (PRTC) ranged from 2.83–3.09 and 1.26–0.71, respectively (Table 2). TEAC levels were in agreement with results reported in the literature for raw seeds of lupin (Fernandez-Orozco et al., 2006, 2008a) and other legumes, such as cowpea (*Vigna sinensis* L. cv. Carilla) and soybean (*Glycine max* L. cv. Merit) (Doblado et al., 2005; Fernandez-Orozco et al., 2007). However, raw seeds of mung bean and *G. max* L. cv. Jutro exhibited lower TEAC values (Fernandez-Orozco et al., 2008b). DPPH-RSA values observed in *L. angustifolius* cv. Troll and cv. Emir and *L. albus* cv. Multolupa were in agreement with those found recently by Amarowicz and Pegg (in press) in different lupin cultivars. These authors showed that *Lupinus luteus* cv. Idol exhibited the highest ability to scavenge DPPH radicals, followed by *L. angustifolius* cv. Troll, *L. albus* cv. Pikador and *L. luteus* cv. Polo and Teo. Finally, the lowest DPPH RSA was observed for *L. luteus* cv. Markiz. Antiradical activities, e.g. scavenging of DPPH radicals, have been reported for other leguminous extracts (Amarowicz & Troszyńska, 2003; Zhou & Yu, 2006). These studies showed that lupin seeds exhibit a rather moderate ability to scavenge DPPH radicals while adzuki bean presented the highest DPPH-scavenging activity among leguminous plants (Amarowicz, Estrella, Hernandez, & Troszyńska, 2007).

PRTC values, shown in the present work, were lower than those found in raw seeds of lupin (Fernandez-Orozco et al., 2006, 2008a) and in other legumes (Doblado et al., 2005; Fernandez-Orozco et al., 2007, 2008b).

The extraction of α-galactosides from *L. angustifolius* cv. Troll and *L. albus* cv. Multolupa caused reductions of TEAC, DPPH-RSA and PRTC values (43%, 38% and 13% and 77%, 38% and 98%, respectively) (Tables 1 and 2). Also a reduction in TEAC and DPPH-RSA (73% and 82%, respectively) was observed when the process was carried out with *L. angustifolius* cv. Emir but the PRTC increased by 25%. This result indicated different scavenging activities of the compounds extracted by PBS or by 80% methanol present in raw and α-galactoside-low lupin flours of the three studied lupin cultivars against different generated free radicals. The ABTS cation radicals and DPPH radicals can be scavenged, not only by phenolic compounds, but also by proteins present in lupin PBS extracts or by protein-phenolic complexes (Brand-Williams et al., 1995; Miller & Rice-Evans, 1996). It was also reported that small molecular weight fractions, such as peptides, released from high protein legume products, were effective scavengers of DPPH radicals (Aluko & Monu, 2003). In contrast, soluble proteins have no ability to scavenge DPPH? and peroxyl radicals (Bartosz et al., 1998). Thus, the higher values of TEAC in comparison with those of DPPH-RSA and PRTC reported here can be explained by different abilities of flour constituents to scavenge not all but rather selected free radicals.

Table 1 shows the superoxide dismutase activity (SOD-like activity) and soluble protein content in raw and α-galactoside-low lupin flours of three cultivars. SOD-like activity ranged from 58 U/g d.m. in *L. albus* cv. Multolupa to 81 U/g d.m. in *L. angustifolius* cv. Emir, whereas soluble protein ranged from 99 mg/g d.m. in *L. angustifolius* cv. Troll to 149 mg/g d.m. in *L. albus* cv. Multolupa. When SOD-like activity was expressed as U/mg protein, the two cultivars of *L. angustifolius* showed the highest value (0.8–0.9 U/mg protein) whilst *L. albus* cv. Multolupa was twice lower. The SOD-like activity shown by raw lupin seeds was lower than those found in literature for *L. angustifolius* cv. Zapaton (Fernandez-Orozco et al., 2006), *V. sinensis* cv. Carilla (Doblado et al., 2005), soybean (Fernandez-Orozco et al., 2007) and mung bean (Fernandez-Orozco et al., 2008b).

The removal of α-galactosides from raw lupin resulted in their lower ability to scavenge superoxide anion radicals and to decrease the soluble protein content (Table 1). Thus the SOD-like activity of α-galactoside-low flours and the soluble protein decreased from 30–52% and 24–46%, respectively, for the lupin cultivars studied in comparison with the raw seeds. These results agree with those reported in the literature (Martínez-Villaluenga, Sironi, Vidal-Valverde, & Duranti, 2006). The SOD-like activity expresses the cumulative action of antioxidants to scavenge superoxide anion radicals by non-enzymatic and by superoxide dismutase action. The latter catalyzes the dismutation of two superoxide radical anions into hydrogen peroxide and oxygen and its essential role is connected with removing damaging reactive oxygen species (ROS) from the cellular environment (Attar, Keyhani, & Keyhani, 2006).

Table 2 shows the effect of α-galactoside extraction on total phenolic compounds (TPC) and total flavonoids (TF) on three studied lupins. TPC in raw lupin seeds was within the range 1.8–2.5 mg ferrulic acid equiv/g d.m. and TF ranged from 133 to 1100 μg catechin equivs./g d.m. Results for TPC were in accordance with those previously reported for *L. angustifolius* cv. Zapatón (Fernandez-Orozco et al., 2006). TF represented only 6%, 14% and 60% of TPC in *L. angustifolius* cv. Troll, *L. angustifolius* cv. Emir and *L. albus* cv. Multulopa, respectively. After α-galactoside extraction, TPC decreased by 38%, 56% and 51% for *L. angustifolius* cv. Troll, *L. angustifolius* cv. Emir and *L. albus* cv. Multulopa, respectively, (Table 2). The antioxidant capacity, measured as DPPH-RSA and PRTC, of raw and α-galactosides-low lupin flours was correlated with the content of TPC. Positive correlation coefficients between DPPH-RSA and TPC content were found ($r = 0.84$).

In this study a different content of TF was noted when α-galactosides were extracted from lupin flour. For *L. angustifolius* cv. Troll a threefold higher TF content was obtained in the processed flour; however, TF did not statistically change in processed Emir cultivar. In contrast, TF content decreased 86% in α-galactoside-low Multolupa lupins. These findings clearly indicate that α-galactoside extraction from *L. angustifolius* increased the pool of TF in TPC from 6% to 32% (cv. Troll) and from 14% to 31% (cv. Emir), but caused a sharp decrease, from 60% to 17%, in processed flour of *L. albus* cv. Multulopa.

In addition to the α-galactoside content, a potentially detrimental aspect of lupin use in human nutrition is their Mn content. *L. albus* species contains 10 times more Mn (~90 mg/100 g d.m.) (Martínez-Villaluenga et al., 2007) than do other lupin species, such as *L. angustifolius* (~7–8 mg/100 g d.m.) or *L. luteus* (~5–7 mg/100 g d.m.) (Porres, Aranda, Lopez-Jurado, & Urbano, 2007). Several authors have reported the adverse effects of high Mn dietary intake in human and animal studies which can cause loss of appetite and reduce growth of sheep, pigs and poultry (Karunajeewa & Barlett, 1985), as well as neurotoxicity in humans (Stredrick et al., 2004). The α-galactoside-low flour from *L. albus* cv. Multolupa still had a high content in Mn of ~82 mg/100 g d.m. (Porres et al., 2007); therefore, *L. albus* should be restricted to the production of protein isolates, as the Mn, is removed during precipitation of proteins, remaining in the protein isolates in amounts of 9 mg/100 g d.m. (Martínez-Villaluenga et al., 2007). Thus, Tables 1 and 2 show the results of the antioxidant properties and polyphenol contents of protein isolate from *L. albus* cv. Multolupa in comparison to the flour without α-galactosides. It was also found that the protein isolate from *L. albus* cv. Multolupa presented lower TEAC and SOD-like activity was sharply reduced (Table 1) while the DPPH-RSA value was higher (Table 2) than that in the α-galactosides-low flour. At the same time, TPC content in these protein isolates was 24% higher than that in α-galactoside-low flour whilst TF level was negligible since it only represented 3.3% of total TPC (Table 2). When these results, obtained for protein isolates, were compared to those for raw Multolupa seeds, decreases of TPC and TF were 38% and 97%, respectively. In this study the

results of antioxidant capacity provided for lupin protein isolate are in agreement with those reported by Tsaliki, Lagouri, and Doxastakis (1999) for protein isolates from lupin (*L. albus* cv. Graecus) and these authors found that lupin products presented higher antioxidant activity than did soy protein isolates.

In summary, α-galactoside extraction and protein isolation of lupin seeds caused a reduction of antioxidant capacity and polyphenol content, although the remaining total antioxidant capacity of these lupin-derived products, measured as TEAC, was higher than those reported for cereal-based products (Michalska et al., 2007).

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