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Nitrogen Fractions and Mineral Content in Different Lupin Species (*Lupinus albus*, *Lupinus angustifolius*, and *Lupinus luteus*). Changes Induced by the α-Galactoside Extraction Process

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The protein and mineral composition of different varieties of three different lupin species (*Lupinus albus*, *Lupinus angustifolius*, and *Lupinus luteus*) and the effect of α-galactoside removal by means of a hydroalcoholic extraction process on such composition were studied in relationship to nutrient distribution among the different anatomical parts of the seed (embryo, cotyledon, and seed coat). The extent of processing-derived protein insolubilization was assessed by both chemical and electrophoretic techniques and related to the amount of nitrogen soluble in H_2O, NaCl, ethanol, NaOH, and sodium dodecyl sulfate/β-mercaptoethanol (SDS/BME). The α-galactoside extraction process caused a significant increase in the amount of total and insoluble nitrogen and decreased the amount of soluble protein nitrogen, without affecting the content of soluble nonprotein nitrogen. α-Galactoside extraction was not effective at decreasing the levels of Mn present in lupins, and processing caused an increase in the content of this mineral in all of the species studied with the exception of *L. albus* var. *multolupa*. In general, the effect of processing on mineral content varied with the different lupin species, and mineral losses were lower in *L. luteus*.

KEYWORDS: Legumes; lupin; *Lupinus* species; protein; nitrogen fractions; minerals; α-galactoside extraction

INTRODUCTION

Lupins are a valuable source of dietary protein, complex carbohydrates, vitamins, antioxidants, and nutritionally essential minerals for human and animal nutrition (*1*). In addition, lupins are devoid of certain non-nutritional components such as trypsin inhibitors or lectins, although they may exhibit high levels of toxic and bitter-tasting alkaloids. Nevertheless, sweet varieties of *Lupinus albus*, *Lupinus angustifolius*, and *Lupinus luteus* with low alkaloid content have been obtained. Among the major constraints to the wider inclusion of lupins in human or animal diets are their high levels of flatulence-causing α-galactoside oligosaccharides and the accumulation of Mn in *L. albus* seeds (*2*, *3*). With regard to α-galactosides, efficient hydroalcoholic extraction processes that yield a 93% recovery of oligosaccharides have been developed to eliminate these and other non-nutritional components that have a negative effect on the functional properties and the nutritional value of feeds (*4*−*6*). Furthermore, extracted α-galactosides can be used as prebiotics that enhance the functional value of certain food products (*7*). On the other hand, thermal treatments and the use of organic solvents as extractants are known to cause the denaturation of dietary proteins and, consequently, a certain degree of protein insolubilization (*3*, *5*, *8*, *9*). Denaturation may pose either deleterious (*10*, *11*) or beneficial (*4*) effects on the nutritive use of proteins and could be an efficient way to decrease the potential allergenicity of legume proteins, thus further contributing to improving their nutritional value (*6*).

Another consequence of extraction processes are changes in the composition of several nutrients present in the legume seeds; some complex carbohydrates, minerals, and vitamins are liable to be lost as a result of water or hydroalcoholic extraction processes such as α-galactoside removal or protein isolation (*3*, *12*). The degree of compositional changes can be affected by differences in the extraction process (whole seed or seed flour) or extraction solution and by different legume species, seed matrix, or thickness of the seed coat.

The objectives of this study were (1) to assess the distribution of nitrogen fractions in the whole seed and cotyledon of different lupin species and study the effect on such nitrogen fractions of a hydroalcoholic extraction process and (2) to assess the mineral composition of different lupin species and study the changes in ash, P, Ca, Mg, K, Mn, Zn, Cu, and Fe content of whole lupin

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seed caused by a hydroalcoholic extraction process in relation to the distribution of these minerals among the different anatomical parts of the seed.

MATERIALS AND METHODS

Lupins. *Raw Lupin*. Seeds of *L. albus*, vars. *multolupa* and *marta*, and *L. luteus*, vars. *4486* and *4492* were provided by the Agrarian Research and Technology Development Service from the Agriculture and Commerce Council of the Junta de Extremadura (Spain), whereas seeds of *L. angustifolius* vars. *emir* and *troll* were supplied by Dr. Gulewicz from the Institute of Bioorganic Chemistry, PAS, Poznan, Poland. The seeds were ground to a fine powder (0.18 mm sieve) and lyophilized for chemical analysis.

Separation of Different Anatomical Components. Seeds of the three lupin species studied (vars. *multolupa*, *troll*, and *4492*) were manually decorticated to separate the seed coat, embryo, and cotyledons. The different anatomical parts were freeze-dried and ground for further analysis.

α-*Galactoside-Free Flour Preparation.* It was performed according to Gulewicz et al. (*13*). In brief, lupin seeds were imbibed in distilled water at 4 °C for 10−12 h. α-Galactosides were extracted from the imbibed seeds with two consecutive extractions using 50% ethanol at 40 °C overnight. After the extraction process, the extracted seeds were lyophilized and ground to obtain the α-galactoside-free flour.

Analyses. *Chemical Analysis*. The moisture content of raw and α-galactoside-free lupin flours was determined by drying to constant weight in an oven at 105 °C. The total nitrogen was determined according to Kjeldahl's method. Crude protein was calculated as $N \times 6.25$. Soluble protein and nonprotein nitrogen was measured using the methodology described by Periago et al. (*14*) after extraction with 0.2% NaOH and precipitation of soluble protein nitrogen with 30% trichloroacetic acid. The amount of peptidic nitrogen that did not precipitate after treatment with trichloroacetic acid was measured in the soluble nonprotein nitrogen fraction by the method of Lowry (*15*). Insoluble nitrogen was measured in the remaining flour after extraction. Ash content of the raw and α-galactoside-free lupin flours was measured by calcination at 500 °C to a constant weight. Samples of ashed material were dissolved in 6 N HCl before analysis. Calcium, magnesium, iron, zinc, copper, and manganese content were determined by atomic absorption spectrophotometry using a Perkin-Elmer AAnalyst 300 spectrophotometer. Lanthanum chloride was added to calcium and magnesium samples to prevent interferences caused by phosphate ions. Potassium was determined by atomic emission spectrophotometry using a Perkin-Elmer AAnalyst 300 spectrophotometer. Phosphorus was measured spectrophotometrically using the technique described by Chen et al. (*16*). Analytical results were validated by the following standard reference materials: whole-meal flour CRM-189, lyophilized green beans RM-383, and synthetic feed for growing pigs BCR-709 (Community Bureau of Reference, Commission of the European Communities). Mean ± standard deviation (SD) values of five independent CRM-189 replicates were Mn 64.9 ± 0.6, Cu 6.62 ± 0.28, Fe 74.4 ± 1.7, and Zn 57.6 ± 1.0 μg/g versus certified values ± uncertainty range declared by the Community Bureau of Reference for Mn 63.3 ± 1.6, Cu 6.4 ± 0.2, Fe 68.3 ± 1.9, and Zn 56.5 ± 1.7 μg/g; RM-383, ash 2.48 ± 0.01 g/100 g, N 1.03 ± 0.01, Ca 2.78 ± 0.04, and K 7.89 ± 0.05 mg/g versus certified values ± uncertainty range of ash 2.4 ± 0.1, N 1.05 ± 0.02, Ca 2.9 ± 0.2, and K 7.8 ± 0.2; BCR-709, protein 196.6 ± 3.2 g/kg, ash 4.29 ± 0.07 g/100 g, Ca 0.77 ± 0.02, P 5.42 ± 0.01, Mg 1.96 ± 0.01 mg/g, and Cu 17.9 ± 0.34 μg/g versus certified values ± uncertainty range of protein 199 ± 5, ash 4.2 ± 0.4, Ca 1.05 ± 0.16, P 5.4 ± 0.7, Mg 1.89 ± 0.3, and Cu 17.3 ± 2.5. Results for P and Mg were within the range of declared indicative values stated for reference materials CRM-189 and RM-383.

Protein Fractionation. It was carried out using the classic Osborne procedure (*17*) by means of sequential extraction in deionized water (resistivity > 18 Ω), 5% NaCl, 70% (v/v) ethanol, 0.2% (w/v) NaOH, and 100 mM phosphate buffer (pH 7.65) containing 2% (w/v) sodium dodecyl sulfate (SDS) and 2% (v/v) mercaptoethanol (SDS/BME). The additional extraction step using SDS/BME was done to study the degree of protein insolubilization associated to hydrophobic aggregation or disulfide bond formation (*9*) derived from the hydroalcoholic extraction process at 40 °C for 18 h. Lupin flour samples (0.5 g) were extracted with 10 mL of solvent for 2 consecutive times in the case of water, ethanol, and SDS/BME and 3 consecutive times in the case of NaCl and NaOH. All of the supernatants obtained after centrifugation at 2500*g* for 5 min and the final insoluble residue were used for Kjeldahl nitrogen determination. The nitrogen content of each protein fraction was expressed per 100 g of dry matter (DM) and also as the percentage of the sum of nitrogen contained in all of the fractions, including the insoluble residue of the meal. In comparison to the total nitrogen content of the samples (**Table 1**), the recovery of nitrogen contained in several protein fractions (**Table 3**) ranged between 96 and 110%.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). It was carried out in the supernatants of H_2O, NaCl, NaOH, and SDS/BME protein fractionation from *L. albus* var. *marta*, *L. angustifolius* var. *emir*, and *L. luteus* var. *4486* according to the method of Laemmli (*18*). The final concentration of acrylamide in the running gel was 13%. The gels were fixed and stained with 0.2% Coomassie brilliant blue R-250 in methanol/acetic acid/water (5:4:1, v/v/v).

Statistics. Analysis of the data was done using SAS (release 8.02, SAS Institute, Inc., Cary, NC). The effect of lupin species and α-galactoside removal on nitrogen and mineral composition of lupin flours was analyzed by 2×3 factorial analysis of variance (ANOVA) with α-galactoside extraction and lupin species (*L. albus*, *L. angustifolius*, and *L. luteus*) as the main treatments (**Tables 2** and **5**). The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Chemical Composition of Lupins. *Nitrogen.* The amount of total and soluble protein nitrogen was significantly affected by differences in lupin species (**Tables 1** and **2**), with the highest values found in *L. luteus* followed by *L. albus* and *L. angustifolius*. In this respect, no major differences were found between the different varieties within the same lupin species. The content of insoluble nitrogen followed the same trend described for total and soluble protein nitrogen with the exception of *L. angustifolius* var. *emir* for which the insoluble nitrogen content was almost twice the amount found in *L. angustifolius* var. *troll*. With regard to the soluble nonprotein nitrogen content, a significant effect among species was also found, with the highest values corresponding to *L. angustifolius* followed by *L. albus* and *L. luteus*. In all of the lupin species studied, a considerable proportion of the soluble nonprotein nitrogen fraction (67.3−91.5%) was present in the form of peptidic nitrogen detectable by the method of Lowry (*15*) and significant differences were observed in its content between the two different varieties within a single species.

As expected, the content of total and soluble protein nitrogen increased as a result of dehulling in the cotyledons of *L. albus* var. *multolupa*, *L. angustifolius* var. *troll*, and *L. luteus* var. *4492*, whereas the content of soluble nonprotein nitrogen increased in the cotyledons of *L. albus* var. *multolupa* and *L. luteus* var. *4492* but was slightly reduced in the cotyledon of *L. angustifolius* var. *troll* when compared to the whole seed (**Table 1**). The increments in total and soluble protein nitrogen were related to the thickness of the seed coat and its weight with respect to the total seed weight (16.8, 23.9, and 25.7%, respectively, for *L. albus*, *L. angustifolius*, and *L. luteus*, **Table 6**), a finding that was reflected in a higher increase in the content of the above-mentioned nitrogen fractions in the cotyledon of *L. luteus* and *L. angustifolius* than in that of *L. albus*. Regarding the insoluble nitrogen content, an increase in this nitrogen fraction was observed in *L. angustifolius* and *L. albus* as a result of dehulling, whereas no differences were observed in the case of *L. luteus* (**Table 1**). This finding is indicative of the significant influence of the seed coat in the latter lupin species on the

Table 1. Nitrogen Composition of Raw and α-Galactoside-Free Lupin Seed and Cotyledon Flours Expressed in Dry Matter[a]

	total N (g/100 g)	insoluble N (g/100 g)	soluble protein N (g/100 g)	soluble nonprotein N (g/100 g)	peptidic soluble nonprotein N (g/100 g)
L. albus var. *multolupa*					
raw seed flour	5.70 ± 0.03	0.20 ± 0.00 (3.5%)	4.98 ± 0.01 (87.4%)	0.52 ± 0.01 (9.1%)	0.41 ± 0.01
α-galactoside-extracted flour	6.27 ± 0.03	1.14 ± 0.06 (19%)	4.37 ± 0.12 (73%)	0.48 ± 0.00 (8%)	0.41 ± 0.01
cotyledon	6.50 ± 0.03	0.37 ± 0.02 (5.6%)	5.74 ± 0.02 (86.2%)	0.54 ± 0.02 (8.2%)	0.50 ± 0.01
L. albus var. *marta*					
raw seed flour	5.83 ± 0.03	0.23 ± 0.01 (4%)	5.02 ± 0.03 (86%)	0.58 ± 0.04 (10%)	0.49 ± 0.01
α-galactoside-extracted flour	6.02 ± 0.02	0.74 ± 0.01 (12.3%)	4.85 ± 0.01 (80.5%)	0.43 ± 0.02 (7.2%)	0.42 ± 0.00
L. angustifolius var. *troll*					
raw seed flour	5.45 ± 0.02	0.23 ± 0.00 (4.3%)	4.52 ± 0.02 (83.5%)	0.66 ± 0.02 (12.2%)	0.55 ± 0.01
α-galactoside-extracted flour	6.15 ± 0.06	1.16 ± 0.06 (18.8%)	4.33 ± 0.07 (70.2%)	0.68 ± 0.03 (11.0%)	0.54 ± 0.01
cotyledon	6.79 ± 0.02	0.41 ± 0.01 (6.2%)	5.56 ± 0.02 (84.9%)	0.58 ± 0.05 (8.9%)	0.51 ± 0.00
L. angustifolius var. *emir*					
raw seed flour	5.46 ± 0.02	0.48 ± 0.01 (8.6%)	4.47 ± 0.04 (79.3%)	0.68 ± 0.03 (12.1%)	0.50 ± 0.03
α-galactoside-extracted flour	6.05 ± 0.02	2.40 ± 0.04 (37.9%)	3.32 ± 0.05 (52.3%)	0.62 ± 0.01 (9.8%)	0.42 ± 0.00
L. luteus var. *4486*					
raw seed flour	6.44 ± 0.05	0.53 ± 0.02 (8.3%)	5.50 ± 0.03 (83.3%)	0.53 ± 0.02 (8.4%)	0.48 ± 0.01
α-galactoside-extracted flour	7.48 ± 0.04	1.72 ± 0.04 (22.7%)	5.28 ± 0.04 (70%)	0.55 ± 0.02 (7.3%)	0.52 ± 0.01
L. luteus var. *4492*					
raw seed flour	6.72 ± 0.04	0.69 ± 0.02 (10.4%)	5.42 ± 0.10 (81.8%)	0.52 ± 0.03 (7.8%)	0.35 ± 0.01
α-galactoside-extracted flour	7.46 ± 0.02	1.56 ± 0.09 (21.3%)	5.23 ± 0.05 (71.4%)	0.53 ± 0.02 (7.3%)	0.42 ± 0.01
cotyledon	8.46 ± 0.04	0.66 ± 0.04 (7.9%)	7.03 ± 0.06 (83.3%)	0.75 ± 0.02 (8.9%)	0.42 ± 0.00

[a] Results are means ± standard error of the mean (SEM) of three independent replicates. Data in parentheses represent the percentage of total nitrogen content present in each protein fraction.

Table 2. ANOVA of Species, Treatment, and Species–Treatment Interactions on Total, Insoluble, and Soluble Protein and Nonprotein Nitrogen Content from Three Different Lupin Species[a]

	source of variation			
	species effect	treatment effect	species–treatment	CV (%)
		Pr > *F*		
total nitrogen	<0.0001 (65.5%)	<0.0001 (25.6%)	<0.0001 (5.5%)	2.1
insoluble nitrogen	0.0002 (13.6%)	<0.0001 (63.8%)	0.0320 (4.6%)	33.4
soluble protein nitrogen	<0.0001 (66.5%)	0.0001 (11.5%)	0.0435 (4.2%)	5.6
soluble nonprotein nitrogen	<0.0001 (68.4%)	0.0129 (4.5%)	0.0052 (8.0%)	7.0

[a] The level of significance was set at $p < 0.05$. Data in parentheses represent the contribution to total variance of the specific ANOVA component.

content of insoluble nitrogen, because this nitrogen fraction is concentrated in the seed coat and/or because the seed coat interferes with nitrogen extraction from the seed flour during the process of soluble and insoluble nitrogen detection.

The experimental conditions selected for the hydroalcoholic extraction of α-galactosides (50% ethanol at 40 °C for 12 h) led to a significant increase in the total and insoluble nitrogen content of all of the lupin species studied (**Tables 1** and **2**). The highest levels of total nitrogen were obtained for the α-galactoside-free *L. luteus* flour, followed by *L. albus* and *L. angustifolius*, thus resulting in a significant level of interaction ($p < 0.0001$) between the lupin species and the α-galactoside extraction process (**Table 2**). A significant level of interaction between lupin species and the extraction process was also found for insoluble nitrogen ($p = 0.0320$), with the highest values being found for *L. angustifolius*, followed by *L. luteus* and *L. albus*. The observed increments in the concentration of the above-mentioned nitrogen fractions can be attributed to the loss of other seed constituents, such as ash (12−41.9% loss, **Table 4**), α-galactosides and other soluble sugars, and dietary fiber (*3*, *11*, *12*) in the case of total nitrogen, and to protein denaturation caused by the organic solvent and temperature used for the extraction process in the case of insoluble nitrogen (*4*, *5*, *9*, *10*). Regarding the concentration of soluble nonprotein nitrogen, the extraction process decreased the content of this nitrogen fraction (expressed in grams per 100 g of DM) in *L. albus* vars. *multolupa* and *marta* and *L. angustifolius* var. *emir*, whereas no major changes were observed in *L. angustifolius* var. *troll* or *L. luteus* vars. *4486* and *4492*, thus giving rise to a significant species−extraction process interaction ($p = 0.0052$, **Table 2**). Nevertheless, when the results are expressed as a percentage of the total nitrogen content (**Table 1**), the extraction

Table 3. Nitrogen Solubility in Different Extractive Solutions of Raw and α-Galactoside-Free Lupin Flours[a]

lupin	treatment	ddH_2O (g/100 g DM)	5% NaCl (g/100 g DM)	70% EtOH (g/100 g DM)	0.2% NaOH (g/100 g DM)	1% SDS/2% BME (g/100 g DM)	insoluble (g/100 g DM)
	raw	2.44 ± 0.05	2.98 ± 0.06	0.13 ± 0.01	0.51 ± 0.03	0.13 ± 0.01	0.13 ± 0.02
L. albus		(38.6%)	(47.1%)	(2.1%)	(8.0%)	(2.0%)	(2.0%)
var. *multolupa*	α-galactoside free	0.85 ± 0.03	1.20 ± 0.02	0.09 ± 0.02	3.15 ± 0.06	0.53 ± 0.01	0.75 ± 0.03
		(12.9%)	(18.3%)	(1.4%)	(47.9%)	(8.1%)	(11.4%)
	raw	2.37 ± 0.05	2.81 ± 0.07	0.04 ± 0.0	0.45 ± 0.02	0.03 ± 0.01	0.21 ± 0.01
L. albus		(44.1%)	(47.5%)	(0.8%)	(7.6%)	(0.5%)	(3.6%)
var. *marta*	α-galactoside free	0.62 ± 0.03	1.27 ± 0.05	0.03 ± 0.01	3.24 ± 0.03	0.44 ± 0.01	0.43 ± 0.02
		(10.4%)	(21.0%)	(0.5%)	(53.6%)	(7.4%)	(7.2%)
	raw	1.18 ± 0.06	3.45 ± 0.05	0.02 ± 0.01	0.58 ± 0.02	0.04 ± 0.01	0.19 ± 0.02
L. angustifolius		(21.6%)	(63.2%)	(0.5%)	(10.5%)	(0.7%)	(3.5%)
var. *troll*	α-galactoside free	1.06 ± 0.04	1.74 ± 0.05	nd	2.32 ± 0.03	0.58 ± 0.04	0.87 ± 0.02
		(16.2%)	(26.5%)	nd	(35.3%)	(8.8%)	(13.2%)
	raw	1.55 ± 0.05	2.88 ± 0.09	0.09 ± 0.03	0.50 ± 0.05	0.13 ± 0.02	0.36 ± 0.06
L. angustifolius		(28.4%)	(49.7%)	(2.2%)	(9.7%)	(1.9%)	(8.1%)
var. *emir*	α-galactoside free	0.55 ± 0.02	1.22 ± 0.04	0.07 ± 0.02	1.79 ± 0.04	0.71 ± 0.03	1.44 ± 0.04
		(8.96%)	(19.8%)	(1.2%)	(31.8%)	(12.5%)	(25.7%)
	raw	1.07 ± 0.01	4.04 ± 0.03	0.08 ± 0.01	0.61 ± 0.02	0.07 ± 0.01	0.51 ± 0.03
L. luteus		(16.8%)	(63.4%)	(1.2%)	(9.6%)	(1.1%)	(7.8%)
var. *4486*	α-galactoside free	0.63 ± 0.02	2.46 ± 0.04	0.06 ± 0.01	2.30 ± 0.04	0.66 ± 0.02	1.29 ± 0.04
		(8.5%)	(33.3%)	(0.8%)	(31.1%)	(8.9%)	(17.4%)
	raw	1.66 ± 0.09	3.44 ± 0.01	0.06 ± 0.01	0.61 ± 0.02	0.12 ± 0.01	0.60 ± 0.07
L. luteus		(25.3%)	(53.7%)	(1.0%)	(8.9%)	(1.6%)	(9.5%)
var. *4492*	α-galactoside free	0.98 ± 0.07	2.76 ± 0.01	0.04 ± 0.02	2.02 ± 0.09	0.44 ± 0.04	1.17 ± 0.01
		(13.9%)	(36.6%)	(0.7%)	(28.0%)	(5.2%)	(15.5%)

[a] Results are means ± SEM of three independent experiments. Data in parentheses are percentages of total nitrogen soluble in each specific extracting solution. nd = not detected.

Table 4. Mineral Composition of Raw and α-Galactoside-Free Lupin Seed Flours (mg/100 g of Dry Matter)[a]

	ash (%)	Ca	K	Mg	P	Cu	Fe	Mn	Zn
L. albus var. *multolupa*	3.52 ± 0.04	139.0 ± 2.72	1068.0 ± 9.71	145.0 ± 1.65	332.1 ± 5.12	0.72 ± 0.06	3.80 ± 0.05	90.1 ± 1.34	4.30 ± 0.09
α-galactoside-free	2.33 ± 0.02	179.0 ± 1.93	573.0 ± 8.31	70.0 ± 2.59	302.0 ± 1.35	0.78 ± 0.03	3.51 ± 0.08	82.0 ± 0.55	4.20 ± 0.10
L. albus var. *marta*	3.89 ± 0.02	133.6 ± 0.53	1426.0 ± 21.46	193.2 ± 0.86	468.1 ± 1.76	0.75 ± 0.03	6.20 ± 0.06	35.0 ± 0.32	5.24 ± 0.02
α-galactoside-free	2.26 ± 0.04	151.2 ± 1.58	783.8 ± 18.03	98.1 ± 0.28	310.8 ± 1.29	0.69 ± 0.01	3.76 ± 0.12	78.0 ± 0.30	4.54 ± 0.02
L. angustifolius var. *troll*	4.13 ± 0.02	161.8 ± 1.49	1294.4 ± 7.76	191.9 ± 1.00	543.3 ± 2.25	1.02 ± 0.11	4.15 ± 0.03	7.6 ± 0.06	3.65 ± 0.03
α-galactoside-free	3.45 ± 0.02	186.6 ± 2.51	936.5 ± 7.44	163.7 ± 2.96	553.0 ± 5.98	0.92 ± 0.09	3.25 ± 0.10	8.7 ± 0.08	4.05 ± 0.04
L. angustifolius var. *emir*	4.29 ± 0.02	143.0 ± 1.05	1304.9 ± 5.75	219.1 ± 1.25	613.4 ± 2.05	0.95 ± 0.05	4.26 ± 0.03	8.4 ± 0.04	3.79 ± 0.02
α-galactoside-free	3.15 ± 0.02	149.4 ± 0.24	786.4 ± 4.03	171.3 ± 1.37	542.5 ± 1.72	0.58 ± 0.06	2.87 ± 0.03	8.8 ± 0.04	4.20 ± 0.04
L. luteus var. *4486*	4.67 ± 0.02	134.8 ± 0.61	1210.2 ± 12.76	294.0 ± 2.39	715.5 ± 1.39	1.10 ± 0.03	5.84 ± 0.03	5.6 ± 0.03	5.90 ± 0.02
α-galactoside-free	4.05 ± 0.05	163.6 ± 0.31	900.0 ± 32.34	316.1 ± 0.80	757.0 ± 4.57	1.06 ± 0.03	7.61 ± 0.11	7.6 ± 0.03	7.93 ± 0.03
L. luteus var. *4492*	5.17 ± 0.03	110.4 ± 0.66	1424.3 ± 10.26	308.8 ± 1.84	845.7 ± 2.72	1.25 ± 0.04	7.05 ± 0.05	6.8 ± 0.02	6.42 ± 0.02
α-galactoside-free	4.55 ± 0.04	154.6 ± 0.78	1041.7 ± 15.79	330.9 ± 0.70	817.0 ± 3.50	1.11 ± 0.26	7.24 ± 0.03	8.5 ± 0.03	7.66 ± 0.05

[a] Results are expressed as means ± SEM of five independent results.

process tended to decrease the amount of soluble nonprotein nitrogen in all of the lupin species studied. The percentage of Lowry-reactive nitrogen present in the soluble nonprotein nitrogen fraction increased in *L. luteus* as a result of the extraction process, whereas it was not modified or decreased slightly in *L. albus* and *L. angustifolius*. These changes could be attributed to a higher solubility in ethanol of the nonpeptidic nitrogen present in the soluble nonprotein nitrogen fraction of *L. luteus*.

When total variation of the ANOVA treatment (total SS) was partitioned into its various components (species, treatment, and species−treatment interaction), the highest magnitude was found for the species effect in total and soluble protein and nonprotein nitrogen content, whereas the treatment effect had the highest magnitude for the content of insoluble nitrogen (**Table 2**). The above-described results highlight the significant influence of genetic factors on the amount of total nitrogen and the different soluble nitrogen fractions present in the seed, whereas the insoluble nitrogen fraction was preferentially affected by the technological treatment applied under our experimental conditions.

Variations in the content of different nitrogen fractions as a result of processing were reflected in changes in nitrogen extractability in different media. Nearly all of the extractable nitrogen present in the raw lupin flours was soluble in water or NaCl, whereas solubility in 70% ethanol, NaOH, or SDS/BME was considerably lower (**Table 3**). Water-extracted nitrogen consists mainly of the albumin protein and the nonprotein nitrogen fraction, whereas the globulin protein fraction is solubilized by NaCl (*19*, *20*). Nevertheless, as can be seen from

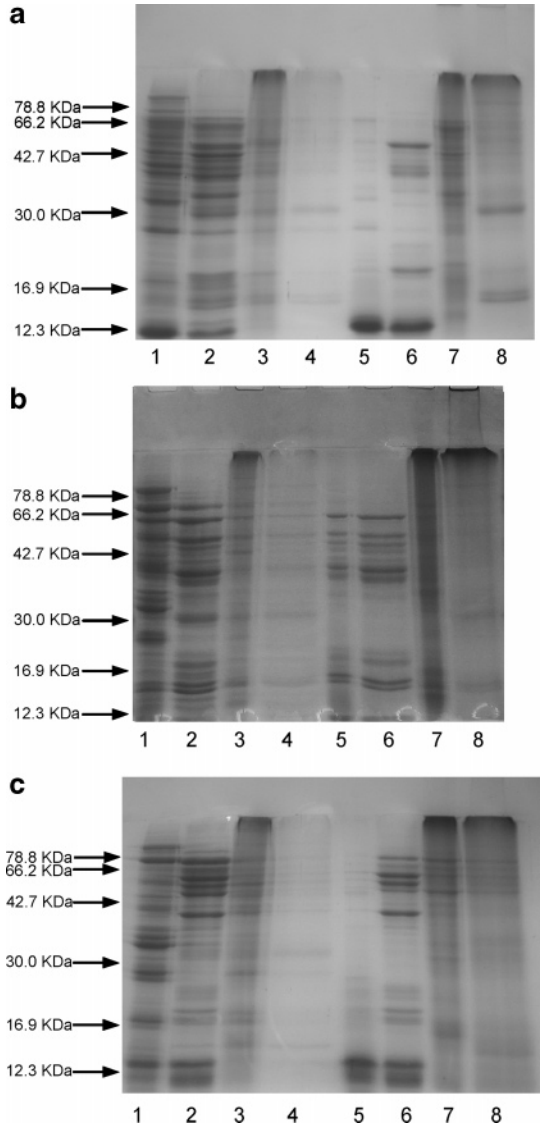

Figure 1. (a–c) Effect of the α-galactoside extraction process on the protein pattern in different extractive solutions. Lane 1, water-soluble N of raw lupin flour; lane 2, NaCl-soluble N of raw lupin flour; lane 3, NaOH-soluble N of raw lupin flour; lane 4, SDS/BME-soluble N of raw lupin flour; lane 5, water-soluble N of α-galactoside-free lupin flour; lane 6, NaCl-soluble N of α-galactoside-free lupin flour; lane 7, NaOH-soluble N of α-galactoside-free lupin flour; lane 8, SDS/BME-soluble N of α-galactoside-free lupin flour. The amount of Kjeldahl-N loaded per lane was (a) lanes 1 and 2, 3.8 μg; lane 3, 1.8 μg; lane 4, 0.24 μg; lanes 5–7, 2.2 μg; lane 8, 1.7 μg; (b) lanes 1 and 2, 4.0 μg; lane 3, 1.7 μg; lane 4, 0.65 μg; lane 5, 2.24 μg; lanes 6–8, 3.2 μg; and (c) lanes 1 and 2, 3.0 μg; lane 3, 2.0 μg; lane 4, 0.48 μg; lanes 5–8, 2.35 μg. Each panel is representative of three independent analyses. The mixture of molecular-weight markers (Merck) consisted of cytochrome *c* (12.3 kDa), myoglobin (16.9 kDa), carboanhydrase (30 kDa), ovoalbumin (42.7 kDa), albumin (66.25 kDa), and ovotransferrin (78 kDa).

the separation of the different protein extracts in SDS−PAGE (parts **a**−**c** of **Figure 1**), minor proportions of globulins are extracted by water.

In a similar way to what has been reported by Nikokyris and Kandylis (*19*) and Zheng et al. (*9*) for legumes, such as the common bean, pea, or lentil, the ethanol-soluble protein fraction ranged from 1 to 2.2% in raw lupin seed flour, thus highlighting the low proportion of prolamins present in legumes compared to cereals. Under our experimental conditions, the amount of proteins extracted by NaOH, usually termed glutelins, contributed 8−10.5% of the total nitrogen content, higher than the proportion of ethanol-soluble proteins but lower than water- or NaCl-soluble proteins and similar to the values reported by Chavan et al. (*20*) for a legume such as *Lathyrus maritimus*.

Table 5. ANOVA of Species, Treatment, and Species–Treatment Interactions on Ash, Ca, K, Mg, P, Cu, Fe, Mn, and Zn Content from Three Different Lupin Species[a]

	source of variation			
	species effect	treatment effect	species–treatment	CV (%)
		Pr > *F*		
ash	<0.0001 (63.1%)	<0.0001 (28.8%)	<0.0001 (3.8%)	5.2
Ca	0.0007 (11.2%)	< 0.0001 (51.2%)	0.0098 (6.7%)	8.7
K	<0.0001 (9.6%)	<0.0001 (69.2%)	0.0124 (3.6%)	11.7
Mg	<0.0001 (86.4%)	<0.0001 (2.4%)	<0.0001 (8.2%)	8.0
P	<0.0001 (66.3%)	<0.0001 (8.3%)	0.0048 (5.2%)	26.8
Cu	<0.0001 (59.7%)	0.0038 (6.0%)	0.0709 (3.6%)	13.7
Fe	<0.0001 (75.1%)	0.0063 (2.2%)	<0.0001 (10.1%)	12.6
Mn	<0.0001 (86.1%)	0.0648 (0.9%)	0.0360 (1.7%)	36.5
Zn	<0.0001 (84.3%)	<0.0001 (3.4%)	<0.0001 (8.5%)	5.7

[a] The level of significance was set at $p < 0.05$. Data in parentheses represent the contribution to total variance of the specific ANOVA component.

The α-galactoside extraction process led to a considerable decrease in the content of water-, NaCl-, and ethanol-extractable nitrogen (10.2−73.8, 20−57.9, and 22−33% reduction, respectively). However, it did not bring about a complete extraction of prolamins, given that the amount of ethanol-soluble nitrogen fluctuated between not determined (nd) and 1.4% of the total nitrogen content present in α-galactoside-free lupin flours. In contrast, the amount of NaOH- and SDS/BME-extractable nitrogen was increased considerably as a result of the hydroalcoholic extraction process (3.3−7.2- and 3.7−14.7-fold increments, respectively), reaching up to 28.0−53.6 and 5.2−12.5% of the total nitrogen content (**Table 3**). When the NaOH- and SDS/BME-extracted proteins were separated by SDS−PAGE, they appeared as high-molecular-weight protein aggregates at the top of the resolving gel or else as a continuous smear of protein residues throughout the resolving gel (lanes 7 and 8 in **a**−**c** of **Figure 1**). The residual insoluble nitrogen content that was left after sequential nitrogen extraction was increased by α-galactoside extraction (**Table 3**). Nevertheless, it was considerably lower than the amount obtained by the soluble protein/nonprotein nitrogen technique (**Table 1**). This difference can be attributed to a higher efficiency of the sequential extraction, and, specifically, to SDS/BME-extractable nitrogen.

Minerals. The mineral content of raw and α-galactoside-free lupins is presented in **Table 4**. The content of total ash, Mg, P, Cu, Fe, and Zn was highest at *L. luteus* seed flour, followed by *L. angustifolius* and *L. albus*, whereas the highest Ca levels were found in *L. angustifolius*, followed by *L. albus* and *L. luteus*. The Mn content of *L. albus*, in which significant differences were found between the two varieties tested, was up to 16-fold higher than that found in *L. angustifolius* and *L. luteus*. In general, all of the lupin species studied exhibited high

Table 6. Distribution of Nitrogen and Minerals in Different Anatomical Parts of *L. albus*, *L. angustifolius*, and *L. luteus*[a]

	L. albus var. *multolupa*			*L. angustifolius* var. *troll*			*L. luteus* var. *4492*		
	embryo	cotyledon	SC	embryo	cotyledon	SC	embryo	cotyledon	SC
weight (mg)		336 ± 9			140 ± 4			100 ± 2	
percent seed weight	3.0	80.2	16.8	3.4	72.6	23.9	3.8	70.5	25.7
N (g/100 g)	12.8 ± 0.05	6.5 ± 0.03	0.6 ± 0.03	12.1 ± 0.05	6.8 ± 0.02	0.4 ± 0.01	6.7 ± 0.04	9.0 ± 0.04	0.6 ± 0.00
N (%)[b]	6.8	91.6	1.6	7.6	90.5	1.9	3.8	94.0	2.3
ash (g/100 g)	5.5 ± 0.04	3.5 ± 0.03	3.3 ± 0.04	5.5 ± 0.05	4.2 ± 0.07	3.6 ± 0.01	6.6 ± 0.05	6.1 ± 0.08	2.6 ± 0.04
ash (%)[b]	4.7	79.7	15.6	4.6	74.6	20.8	4.8	82.2	13.0
Ca (mg/100 g)	77.9 ± 1.24	89.7 ± 0.70	532.8 ± 12.46	284.4 ± 0.94	110.5 ± 0.92	762.1 ± 3.47	126.6 ± 1.13	92.9 ± 1.68	530.5 ± 3.40
Ca (%)[b]	1.4	44.0	54.6	3.6	29.4	67.0	2.3	31.7	66.0
K (mg/100 g)	1307.6 ± 4.35	925.6 ± 5.92	591.6 ± 7.31	1326.8 ± 5.23	1172.9 ± 6.47	833.7 ± 7.35	1365.5 ± 5.81	1355.5 ± 6.74	541.2 ± 5.41
K (%)[b]	4.5	84.3	11.3	4.6	75.7	19.7	4.5	83.3	12.2
Mg (mg/100 g)	328.6 ± 2.43	187.8 ± 1.98	44.3 ± 0.33	406.3 ± 1.32	270.8 ± 0.99	118.1 ± 3.38	644.8 ± 1.81	412.3 ± 1.14	206.3 ± 3.30
Mg (%)[b]	5.9	89.7	4.4	6.5	80.7	12.8	6.6	78.9	14.4
P (mg/100 g)	493.5 ± 0.94	395.0 ± 0.66	3.9 ± 0.26	1050.8 ± 2.48	657.4 ± 5.18	36.3 ± 0.54	1368.1 ± 7.41	1083.1 ± 2.58	21.1 ± 0.16
P (%)[b]	4.5	95.3	0.2	6.9	91.4	1.7	6.3	93.0	0.7
Cu (mg/100 g)	1.4 ± 0.07	0.9 ± 0.06	0.2 ± 0.01	0.8 ± 0.04	0.7 ± 0.03	0.4 ± 0.03	2.0 ± 0.01	1.8 ± 0.01	0.3 ± 0.01
Cu (%)[b]	5.2	89.6	5.2	4.5	79.3	16.2	5.3	89.0	5.7
Fe (mg/100 g)	10.1 ± 0.12	3.1 ± 0.15	2.4 ± 0.05	8.8 ± 0.05	3.2 ± 0.07	6.9 ± 0.09	12.8 ± 0.05	5.3 ± 0.10	13.8 ± 0.03
Fe (%)[b]	9.5	78.1	12.4	7.1	54.5	38.4	6.2	48.0	45.8
Mn (mg/100 g)	196.4 ± 1.10	109.3 ± 1.16	15.0 ± 0.16	20.1 ± 0.21	9.1 ± 0.10	2.0 ± 0.03	14.8 ± 0.14	7.0 ± 0.07	5.9 ± 0.08
Mn (%)[b]	6.2	91.2	2.6	8.9	84.8	6.3	8.0	70.4	21.6
Zn (mg/100 g)	11.9 ± 0.21	5.5 ± 0.10	0.5 ± 0.02	11.1 ± 0.06	4.2 ± 0.04	2.6 ± 0.02	17.9 ± 0.22	8.6 ± 0.23	0.6 ± 0.01
Zn (%)[b]	7.4	90.8	1.8	9.4	75.3	15.2	9.8	88.1	2.1

[a] Results expressed in dry matter are means ± SEM of four independent results. [b] Percentage of the indicated nutrient with respect to the total seed composition.

levels of K, which makes lupin a good dietary source of this mineral, in a similar way to what has been reported for other legumes such as the bean or lentil (*8*, *21*).

The total mineral content of legumes is known to be dependent upon genetic and environmental factors (*22*, *23*). The content of Mn found in each of the two varieties of *L. albus* studied was far above the nutrient recommendations for this mineral in rat, poultry, swine, or human (*24*−*27*). Because of the potential toxicity of high Mn levels in the diet, the excessive amount of this mineral found in *L. albus* could limit the use of this lupin species to the preparation of protein isolates, in which considerable amounts of Mn are lost during processing (*28*), or dietetic products, in which Mn levels would be very low because of the inclusion of lupin flour as a minor food ingredient (*29*). The Fe and Zn content of *L. albus* var. *marta* and *L. luteus* vars. *4486* and *4492* was high and exceeded that of the other lupin species studied, although it was within the range of values found in the literature for other legumes such as beans, peas, lentils, or soybeans (*8*, *21*, *30*−*32*). However, despite the high Fe and Zn levels provided by lupins, the bioavailability of the above-mentioned cations can be affected by the presence of the significant amounts of phytate in all of the lupin species used in the present study (*12*, *33*), which may negatively affect mineral bioavailability (*34*). Furthermore, phytate was present mainly in the form of inositol phosphates with a high degree of phosphorylation that are known to exhibit a higher affinity for nutritionally essential minerals (*35*).

Hydralcoholic extraction of α-galactosides led to considerable changes in the content of total ash and different minerals studied among the different lupin species tested and resulted in a significant treatment effect and a significant level of interaction between lupin species and the extraction process (**Table 5**). In general, losses were observed in total ash and K content of all lupin species (**Table 4**), although to a lesser extent in *L. luteus* and *L. angustifolius* compared to *L. albus*. Likewise, considerable losses were observed in the content of Fe and Mg as a result of the extraction process in *L. albus* and *L. angustifolius* (with smaller losses in the latter lupin species), whereas an increase was found in *L. luteus*. With regard to Ca, Mn, and Zn, the content of these minerals increased in each of the lupin species studied as a result of the extraction process with the exception of Zn in *L. albus* vars. *multolupa* and *marta* and Mn in *L. albus* var. *multolupa*, for which slight reductions were observed.

The effect of α-galactoside extraction on the Cu and P content varied among the different lupin species studied and between the two different varieties within the same lupin species.

From the results of the present experiment, it can be concluded that mineral content of lupin was significantly affected by genetic factors and the hydroalcoholic extraction process. Nevertheless, partitioning the total variation of the ANOVA treatment into its various components (species, treatment, and species−treatment interaction) gave the highest magnitude for ash, Mg, P, Cu, Fe, Mn, and Zn content to the species effect, whereas the treatment effect had the highest magnitude for the content of Ca and K (**Table 5**).

An increase in the mineral content of the lupin seed was expected as a result of the α-galactoside extraction process because of the loss of other seed components that leach out into the soaking solution. However, it appears that a considerable proportion of minerals was also lost during processing in all of the lupin species studied, although the percentage of losses varied considerably among the different minerals and lupin species (**Table 4**). Such differences may be attributed to the different solubility and mineral speciation specific to each particular lupin species, as well as to specific mineral distribution and the association to the different anatomical seed components. With the aim of studying the latter factor in greater detail, we assayed the total N, ash, and specific mineral content of the different anatomical components of the lupin seed (i.e., embryo, cotyledon, and seed coat) in a selected variety of each lupin species studied (*L. albus* var. *multolupa*, *L. angustifolius* var. *troll*, and *L. luteus* var. *4492*; **Table 6**). Mineral distribution in the different anatomical parts of the seed is genetically determined and does not appear to be influenced by differences in environmental or culture conditions (*23*).

Total mineral content was remarkably high in the embryo of the seed (**Table 6**), although, because of the small fraction of

the total seed represented by this anatomical component, it would appear that the corresponding amount of nutrients provided would be equally low. The highest proportion of minerals was located in the cotyledons, with the exception of Ca, which was mainly distributed in the seed coat in a similar way to that reported for other legumes such as lentils or beans (*21*, *23*), and Fe, which was also present in considerable amounts (38.4−45.8% of the total seed content) in the seed coat of *L. angustifolius* and *L. luteus*.

K is located mainly in the cotyledons of lupin seeds, and because of its high solubility, considerable losses were observed as a result of the α-galactoside extraction process. Nevertheless, leaching of this mineral into the extraction solution appeared to be influenced by the thickness of the seed coat, and losses were much lower in *L. angustifolius* and *L. luteus* than *L. albus* (**Table 4**). Similar observations were made for the Mg content that even increased in *L. luteus* as a result of the extraction process, compared to the raw seed.

The content of Mn and Zn in the different lupin species followed a similar pattern of response to the α-galactoside extraction process (**Table 4**). Both minerals are mainly located in the cotyledon, although Mn tends to accumulate more in the seed coat as the thickness of this anatomical component increases (**Table 6**), and the amount of processing-derived mineral losses was inversely related to the thickness of the seed coat, decreasing in the order *L. albus* > *L. angustifolius* > *L. luteus* (**Table 4**). Because the experimental conditions used for the α-galactoside extraction did not have any appreciable effect on Mn content, this methodology of selective extraction does not represent an efficient means of reducing the amount of Mn present in lupins, irrespective of the mineral distribution in the cotyledon and seed coat (**Table 6**). This lack of effect can be attributed to the low intrinsic solubility exhibited by Mn in legumes (*36*) or the protective effect of the lupin seed coat. Other processing methodologies, such as the preparation of protein isolates, can lead to a significant reduction in the amount of Mn present in the final product (*28*), a fact that highlights the low degree of association between Mn and the protein component of the seed. Koplík et al. (*36*) have reported that a considerable proportion of the Mn extractable from lentil and pea flours is associated with low−medium-molecular-weight proteins, whereas only a minor proportion was associated with high-molecular-weight proteins. Similar findings were also observed for Zn by the same authors. This lack of association may be partly responsible for the losses in these minerals observed as a result of protein isolate preparation, compared to the raw seed flour (*28*, *37*).

Although Fe was located mainly in the cotyledon of the lupin seed, a considerable amount was also accumulated in the seed coat of *L. angustifolius* and *L. luteus* (**Table 6**). Therefore, seed coats of these lupin species could be used as a valuable dietary source of bioavailable Fe in a similar way to what has been reported for the soybean seed coat (*38*, *39*), in which the high rate of Fe availability has been related to the low levels of phytic acid in this anatomical component of the seed (*40*); a finding that is corroborated under the experimental conditions of the present study by the presence of only minor quantities of P in the seed coat (0.2−1.7%), whereas the highest proportion of this mineral is present in the cotyledon (91.4−95.3%) followed by the embryo (4.5−6.9%). The inclusion of lupin seed coats in different integral products would be further supported by their high levels of dietary fiber (*41*, *42*) and essential minerals such as Ca (**Table 6**). Nevertheless, under our experimental conditions, Ca appeared to be in a rather insoluble form in the seed coat, because the concentration of this mineral increased significantly in all lupin species after the selective extraction process (**Tables 4** and **5**). The low level of Ca solubility could be attributed to the formation of poorly soluble complexes with oxalate or pectins in the seed coat (*43*, *44*).

Higher Fe levels were found in the seed coat of dark-colored seeds (*L. angustifolius* and *L. luteus*) compared to light-colored ones (*L. albus*). These results are in agreement with what has been observed in the common bean by Moraghan (*45*), who reported a positive correlation between Fe accumulation in the seed coat and the level of antocyanins responsible for its color but not with the levels of condensed tannins.

From the results of the present study, it can be concluded that thickness of the seed coat appeared to play a major role on the leaching of minerals to the hydroalcoholic extractive solution, independent of the anatomical component of the seed where they are located. On the other hand, because of the high content of Ca and potentially bioavailable Fe present in the seed coat, this seed component can be used as a valuable source of the above-mentioned nutrients for the preparation of integral food products.

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