Chemical Composition, Nutritive Value, and Toxicology Evaluation of Mexican Wild Lupins[†]

Mario Alberto Ruiz[‡] and Angela Sotelo*

Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México,
Cd. Universitaria, México, D.F. 04510, México

The nutrient composition, toxic factors content, and nutritional and toxicological value of *Lupinus splendens, L. rotundiflorus, L. elegans, L. simulans, L. exaltatus, L. reflexus,* and *L. madrensis* species from Mexico were analyzed. The seeds of these species were a good source of protein. All the species showed a high lysine and tryptophan content, though sulfur amino acids were limiting. Cyanogenic glycosides were absent, and lectins, trypsin inhibitors, and tannins were present in low concentrations. Lupanine was the major alkaloid in almost all the samples, although sparteine was the major alkaloid in *Lupinus reflexus* (26.63 mg/g of sample). Cytisine was not found in any of the studied lupins. *L. reflexus* showed the highest acute toxicity, and *L. elegans* exhibited no toxicity as evaluated using a mice model. The alkaloid was reduced by hot-water extraction. The protein efficiency ratio in water-debittered seeds was relatively poor (1.1–1.5). These results suggest that the wild lupins studied represent a potential protein supply, and they could be domesticated and used for animal feed if the alkaloids were eliminated and the protein was supplemented with methionine, or if the lupins were used in mixture with cereals.

INTRODUCTION

In developing countries the major source of protein in the human diet is from cultivated or wild plants which are also used for feeding domestic animals. Soybeans (G*lycine max)* have been used for many decades as the main grain legume crop, and significant research has been carried out on this grain (*1*). However, at present an increasing importance is being given to the genus *Lupinus* as a possible substitute for soybeans for both human and animal feeding (*2*). Lupin seeds, which have a high protein content and good nutritive value, can be grown even in poor soils where soybeans are unable to grow. The limitation of a wider use of lupins is due to their high content of quinolizidine alkaloids, which make the seeds bitter; in addition, most wild lupin species are considered toxic (*3*). Over 100 quinolizidinic alkaloids have been reported in the literature in the genus *Lupinus* (*4*). The main role of these alkaloids is to provide a chemical defense of the plants against herbivores (*5*). Although sweet lupin varieties have been selected by breeding, these varieties are less resistant to diseases and herbivore attacks. An alternative could be to utilize these bitter lupin species and select a technological process to debitter the seeds by extraction after harvest and take advantage of the extract as a byproduct that would be useful in medicine and agriculture (*6*).

More than 400 lupin species have been described in the world; only 12 species occur in Europe and Africa,

but more than 300 species inhabit the Americas (*7*). In Mexico ca. 100 wild species have been detected, over 15 of which are native to Jalisco State (*8*). However, there is scant information about the toxicological and nutritive characteristics of these wild lupins from Mexico. Recently, Garcia et al. (*9*) obtained a protein isolated from *Lupinus exaltatus* (Zucc.). The present paper attempts to show the chemical composition, protein quality, and toxicology of some wild Mexican lupins species that grow in Jalisco State.

MATERIALS AND METHODS

Lupin Samples. The seeds of seven wild lupin species were collected in Jalisco State, Mexico. The species were *Lupinus splendens, L. rotundiflorus, L. elegans, L. simulans, L. exaltatus, L. reflexus,* and *L. madrensis.* All specimens were botanically classified in the Instituto de Botánica of the Universidad de Guadalajara (IBUG), Jalisco. Voucher specimens were deposited at the IBUG Herbarium.

Chemical Analysis. After the seeds were ground to pass through a 0.5-mm mesh sieve, analyses were carried out on the flour. A proximal analysis was determined according to AOAC (*10*), and the true protein content was determined as recommended by Lucas et al. (*11*). For amino acid determination the samples were hydrolyzed with 6 N HCL at 145 °C for 4 h (*12*). A Technicon amino acid analyzer model NC-2P was used. Tryptophan content was measured by the colorimetric method after enzymatic hydrolysis (*13*). The chemical score was calculated by using the FAO/WHO amino acids pattern (*14*).

Antinutritional Factors and Alkaloid Content. The trypsin inhibitor activity was measured following the technique of Kakade et al. (*15*), by using benzoyl-D-L-arginine ρ-nitroanilide (BAPNA; Sigma, St. Louis, MO) as a substrate. The hemagglutinin determination in seed extract was carried out according to Jaffe et al. (*16*) by using hamster red blood cells. Cyanogenic glycosides were determined by the method of Lucas and Sotelo (*17*). β-Glucosidase was used for the

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Table 1. Composition of Experimental Diets[a]

	diet composition (g/100 g of total diet)				
ingredients	1	2	3	4	5
casein (87% protein)[b]	11.1				
L. elegans		22.9			
L. exaltatus			25.8		
L. reflexus				27.7	
L. rotundiflorus					24.2
sucrose	20.0	18.2	18.2	18.2	18.2
glucose[2]	20.0	18.2	18.2	18.2	18.2
dextrin[2]	20.0	18.2	18.2	18.2	18.2
lard	8.0	8.0	8.0	8.0	8.0
corn oil	7.0	6.7	5.9	5.9	6.7
mineral mix[c]	4.9	4.1	4.1	4.0	4.4
vitamin mix[d]	1.0	1.0	1.0	1.0	1.0
fiber cellulose type[d]	7.9				

[a] $N \times 6.25$, 10 g protein/100 g of total diet. Each diet was calculated to contain 422 kcal/100 g of diet. [b] Sigma, St. Louis, MO. [c] Roger Harper mineral mix, ICN Pharmaceutical, Cleveland, OH. [d] ICN Pharmaceutical.

liberation of HCN from the glycoside. A quantitative analysis of tannin was caried out by a spectrophotometric method, using dimetilformamide to extract the tannins. Tannic acid was used to prepare the standard curve (*18*).

The alkaloids analyzed were sparteine, lupanine, 3-hydroxy-lupanine, 13-hydroxylupanine, and cytisine; sparteine was the only one of these alkaloids commercially available. Pure samples of the other alkaloids were donated by Professor Michael Wink of the Institute for Pharmaceutical Biology, University of Heidelberg. The alkaloids were analyzed by the method of Muzquiz et al (*19*).

Alkaloid Extraction and Measurement. A sub-sample of 0.5 g of lupin flour was homogenized in a vortex with 5 mL of 5% trichloroacetic acid for 1 min and was centrifuged at 784g for 5 min. The extraction was done twice. The supernatant was made basic with 1 mL of 10 M NaOH. The alkaloids were extracted with dichloromethane (3 times with 5-mL portions). The dichloromethane fraction was separated and

evaporated to dryness, and the alkaloids were dissolved in 5 mL of methanol. Caffeine (200 μg) was added as an internal standard. The extract was filtered by Millipore Sep Pack FH 0.5 μm. The measurement was done by gas chromatography (Perkin-Elmer autosystem equipped with a flame ionization detector); the column used was PE-2 (5% phenyl, 95% methyl-polysiloxane), 25 m × 0.53 mm id; and helium was the carrier gas. The temperature of the injector and detector were 240 and 300 °C, respectively. The alkaloids were identified by comparison with authentic samples (sparteine, lupanine, 3-hydroxylupanine, 13-hydroxilupanine, and cytisine). Calibration curves were prepared for lupanine and sparteine. The response was linear over the range 0.0075−2.7 mg mL^{-1}, and the accuracy ranges were 0.030−1.0 mg mL^{-1} and 0.0303− 15.14 mg mL^{-1} for lupanine and sparteine, respectively.

Toxicological Evaluation. The acute toxicity was measured with raw seed of *L. elegans, L. reflexus, L. exaltatus, L. rotundiflorus, L. splendens*, and *L. simulans* according to the Litchfield and Wilcoxon method (*20*). *Lupinus mutabilis* was used as a positive control. A preliminary assay was performed with one of the wild lupins by using 3 mice with each of the following doses: 0.05, 0.15, 1.5, and 15 g/kg body weight (BW). The lupin flour was administrated to the mice by using carboxymethylcellulose at 0.2% in water as a vehicle. The suspension was administered orally (0.8 mL/20 g BW). A final assay was performed with six mice for each range selected after the preliminary assay, using only the following doses: 1.5, 3.0, 6.0, 12.0, and 15.0 g/kg BW. The animals were observed for 72 h.

Protein Quality Evaluation. Four lupin species were selected for the protein efficiency ratio determination (PER) and in vivo digestibility (*10*).Selection of samples for this assay was based on the abundance of the legumes. For the assay the seeds were boiled for 90 min in water (1:4 wt:v) and washed 5 times with hot water. In a preliminary debittering assay it was confirmed that 90−95% of the alkaloids were eleminated. The seeds were dried at 70 °C and ground (0.5-mm mesh).

Thirty five Wistar male rats 21−23 days old were used; each one in a separate cage. Five lots of seven animals each were

Table 2. Proximate Composition of Seven Species of Mexican Wild Lupins (g/100 g DM[a])

	L. elegans	L. exaltatus	L. reflexus	L. rotundiflorus	L. simulans	L. splendens	L. madrensis	mean ± SEM[b]
ash	4.20	3.59	3.61	4.01	3.59	3.30	3.51	3.69 ± 0.08
total lipids	5.79	8.50	7.90	5.50	6.29	8.89	6.80	7.09 ± 0.28
crude fiber	12.91	14.61	16.58	15.11	14.42	12.70	15.40	14.44 ± 0.37
crude protein (N × 6.25)	45.41	40.50	37.31	42.82	40.70	37.20	41.50	40.73 ± 0.61
CHO$_S$[c]	31.69	32.80	34.60	32.56	35.00	38.10	32.80	

[a] DM, dry matter. [b] Mean ± standard error of the mean, $n = 3$. [c] Carbohydrates calculated by difference.

Table 3. Amino Acids Composition of Mexican Wild Lupins (g/16 g of N)

amino acid	*L. elegans*	*L. exaltatus*	*L. madrensis*	*L. reflexus*	*L. rotundiflorus*	*L. simulans*	*L. splendens*	mean ± SEM[a]	FAO/WHO pattern[b]
aromatic AA[c]	6.05	6.09	5.99	5.62	5.99	5.47	5.35	5.79 ± 0.12	6.3
isoleucine	4.48	3.30	3.86	3.96	3.67	3.60	3.92	3.83 ± 0.10	2.8
leucine	7.69	6.01	6.71	6.20	6.58	6.64	6.25	6.58 ± 0.14	6.6
lysine	5.72	5.60	4.90	5.31	4.83	5.04	4.77	5.17 ± 0.10	5.8
sulfur AA[d]	1.84	2.10	2.22	2.05	1.90	2.13	1.74	1.97 ± 0.05	2.5
threonine	4.07	3.69	3.70	3.64	3.77	3.81	3.20	3.69 ± 0.08	3.4
tryptophan	1.14	1.24	1.38	1.38	1.24	1.20	1.24	1.26 ± 0.03	1.1
valine	3.79	3.78	3.83	3.34	4.02	3.58	3.16	3.64 ± 0.08	3.5
chemical score[e]	73	84	80	82	76	85	70		
limiting AA				sulfur amino acid in all studied species					
aspartic acid	11.44	9.00	10.26	9.89	10.03	10.17	9.07	9.97 ± 0.23	
glutamic acid	28.48	21.69	23.85	22.37	24.15	24.03	22.65	23.91 ± 0.60	
alanine	2.57	1.46	2.32	2.50	2.32	2.34	3.08	2.42 ± 0.12	
arginine	12.22	10.51	11.62	10.98	11.15	13.02	11.40	11.56 ± 0.23	
glycine	4.19	2.50	3.55	3.70	3.41	3.38	3.49	3.46 ± 0.12	
histidine	3.71	1.71	3.91	3.37	3.54	3.62	3.26	3.30 ± 0.16	
proline	3.10	2.88	3.35	2.53	3.13	2.11	1.72	2.69 ± 0.13	
serine	9.10	8.90	5.07	5.30	5.65	5.76	4.83	6.37 ± 0.40	

[a] Mean ± standard error of the mean, $n = 21$ (3 per sample). [b] FAO/WHO pattern (1985), preschool age. [c] Phenylalanine + tyrosine. [d] methionine + cysteine. [e] Chemical score = (g of amino acids in sample/g of amino acids in FAO pattern) × 100.

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Table 4. Antinutritional Factor Content in Seeds of Wild Mexican Lupins (Dry Matter)

Lupin	lectins titer[a]	trypsin inhibitors TUI/mg of sample[b]	tannins (g of tannic acid/ 100 g of sample)
L. elegans	5	1.09	0.27
L. exaltatus	4	0.87	0.22
L. reflexus	6	0.93	0.08
L. rotundiflorus	6	0.78	0.07
L. simulans	6	1.06	0.08
L. splendens	6	1.19	0.02
L. madrensis	4	1.16	0.16
mean ± SEM[c]		1.012 ± 0.032	0.12 ± 0.02

[a] Titer = maximal dilution where agglutination was observed. [b] TUI = tripsin units inhibited. One trypsin unit = 0.01 absorbance units. [c] Mean ± standard error of the mean; *n* = 21 (3 per sample).

fed for four weeks with one of the following diets: (1) control diet (casein); (2) *L. elegans* diet; (3) *L. exaltatus* diet; (4) *L. reflexus* diet, and (5) *L. rotundiflorus* diet. All the diets were isocaloric and isoproteic. Diet composition and ingredients used are described in Table 1. During the last week of treatment, feces were collected and dried at 60 °C for nitrogen determination to calculate apparent digestibility. Food and water were offered ad libitum; intake and body weight were recorded every 2 days for 28 days.

RESULTS AND DISCUSSION

Chemical Composition. Table 2 shows the proximate analysis of the samples. The moisture content at harvest ranged from 2.89 to 10.10% with a mean value of 4.48%. In all the lupins evaluated the amount of crude protein ($N \times 6.25$) varied between 37.20 and 45.41%. The nonprotein nitrogen ranged between 14.7 and 32.0% of the total seed nitrogen.

The amino acid composition of the samples is shown in Table 3. All samples showed essential amino acid profiles with a high content of lysine and tryptophan, which is similar to the profiles of other legumes (soy, beans, and peas). The limiting amino acids in all of the lupins, like other legumes, were the sulfur amino acids. The chemical score ranged between 70 in *L. splendens* and 80 in *L. madrensis*.

Antinutritional Factors and Alkaloid Content. As can be seen in Table 4, lectins, trypsin inhibitors, and tannins in all the lupins studied were present in small amounts and were lower in these lupins than in other legumes and in domesticated lupins (*22*). There were no cyanogenic glycosides in the lupins analyzed.

Sparteine, lupanine, 3-hydroxylupanine, and 13-hydroxylupanine content in the wild Mexican lupins is shown in Table 5. Lupanine was the major alkaloid in almost all the lupins analyzed, with its content ranging from 0.03 to 11.5 mg/100 g of sample in *L. elegans* and *L. rotundiflorus,* respectively. This was higher than in other species such as *L. albus*, *L. angustifolius,* and *L.*

mutabilis (*4*). On the other hand, *L. reflexus* contained more sparteine (26.6 mg/100 g of sample). This level is similar to that in a Canadian wild lupin (*23*). *L. elegans* did not contain sparteine. Cytisine was not found in any of the lupins. Wink (*5*) stated that this alkaloid is usually absent in lupins; however, a few species have been found to contain a trace, such as *L. microcarpus, L. densiflorus, L. ruber, L. arbustus, L. argenteus, L. bicolor, L. caudatus,* and *L. nanus.*

Alkaloids have limited lupin incorporation in human and animal nutrition (*14, 25*). In countries that have a long history of lupin use (particularly in South America), lupin grain is of considerable importance in the provision of human food. These foods are prepared either by following traditional debittering of *L. mutabilis* in Ecuador, Bolivia, and Peru (*26, 27*) or by using sweet varieties of *L. albus* in Chile (*28*). The preliminary detoxification trial with *L. elegans* in the present study with boiling water extraction increased the protein content to 45 g/100 g of sample, mainly because water removed the soluble carbohydrates. The alkaloid concentration decreased 95% (not presented). These results were similar to those of Torres et al. (*29*), which were obtained by heat extraction.

Nutritional and Toxicological Study. *L. elegans* and *L. exaltatus* (with no or negible amounts of sparteine) were nontoxic over the dose range studied. *L. rotundiflorus* induced a 33.3% and 50% mortality only at 12 and 15 g/kg BW, respectively, whereas *L. splendens* and *L. simulans* caused a 33.3% mortality only at the highest dose (15 g/kg BW. *L. reflexus* was the most toxic as mortality increased from 33.3% (6 and 12 g/kg BW) up to 100% (15 g/kg BW), and this might be due to its high sparteine content. *Lupinus mutabilis,* used as a positive toxic control, contains both lupanine and sparteine. It was utilized to confirm the toxicology of these alkaloids. The seeds of *L. splendens* and *L. simulans* were toxic at the higher concentration of 15 g of sample/kg of BW. Lower toxicity was observed with the seed of *L. rotundiflorus.*

The protein quality of all debittered lupin seeds gave relatively low protein efficiency ratios (PERs; Table 6). The highest value (1.5) was in *L. elegans* and *L. rotundiflorus*, and the lowest value was in *L. reflexus* (1.06). The low PER value in the lupin meal might be due to the low methionine content. Similar conclusions were reached by Schoeneberger et al. (*30*) with water-debittered seed of *L. mutabilis* (1.53). He stated that lupins, when supplemented with methionine, provide protein of a high PER value similar to that of casein.

In general, the lupin species studied were a good source of protein. *Lupinus elegans* could be taken into consideration for future studies because of the absence of the sparteine and its high true protein content. The debittered seeds of the wild lupin species studied

Table 5. Quinolizidine Alkaloid Content in 7 Wild Mexican Lupins (mg of alkaloids/g DM)

Lupin	sparteine	lupanine	3-hydroxylupanine	13-hydroxylupanine
L. exaltatus	0.03	5.83	1.53	nd
L. elegans	nd	0.03	3.73	nd
L. splendens	0.29	0.89	1.05	1.00
L. reflexus	26.63	2.91	0.16	0.08
L. rotundiflorus	0.11	11.50	4.19	nd
L. simulans	0.40	8.87	2.76	0.09
L. madrensis	0.02	10.63	2.08	0.03
mean ± SEM[a]	3.70 ± 2.16	4.68 ± 0.99	1.81 ± 0.32	0.25 ± 0.11
	n = 18	*n* = 21	*n* = 21	*n* = 12

[a] Mean ± standard error of the mean.

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Table 6. Protein Efficiency Ratios of Four Wild Mexican Lupins[a]

diets	PER	% digestibility
casein (control)	2.34[a]	87.50[a]
L. elegans	1.50[b]	65.50[b]
L. exaltatus	1.14[c]	58.00[c]
L. rotundiflorus	1.51[b]	62.04[d]
L. reflexus	1.06[c]	57.70[c]
mean ± SEM[a]	1.51 ± 0.08	66.13 ± 1.91

[a] Mean ± standard error of the mean, $n = 35$ (7 per sample). Different superscripts are significantly different ($p \leq 0.05$).

represent a valuable source of protein and perhaps, if supplemented with methionine or with cereal, could even be utilized as animal feed.

LITERATURE CITED

(1) Gueguen, J.; Cerletti, P. Proteins of some legume seeds: soybean, pea, fababean and lupin. In *New and Developing Sources of Food Proteins*; Hudson, B. J. F., Ed.; Chapman and Hall: London, 1994; pp 145−183.

(2) Lopez-Bellido, L.; Fuentes, M. Lupin crop as an alternative source of protein. *Adv. Agron.* **1986**, *40*, 239−289.

(3) Keeler, R. F. Quinolizidine alkaloids in range and grain lupin. In *Toxicants of Plant Origin*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 134−159.

(4) Wink, M.; Meiβner, C.; Witte, L. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. *Phytochemistry* **1995**, *38*, 139−153.

(5) Wink M. Modes of action of alkaloids. In *Alkaloids. Biochemistry, ecology and medicinal applications*. Roberts M. F.; Wink M. Eds.; Plenum Press: New York. 1998.

(6) Wink M. Biochemistry and chemical and chemical ecology of lupin alkaloids in: International Lupin Conference Proceedings. ILA, La Rochelle, France, **1984** pp 325−343.

(7) Planchuelo, A. M. Wild lupins distribution and its implication as germoplasm resources. In: *Advances in Lupin Research. Volume of the proceedings of the VIIth International Lupin Conference*. Evora, Portugal 1994.

(8) McVaugh, R., Flora novogaliciana. V. Leguminosae. *A descriptive account of the vascular plants of western Mexico*. Ann Arbor the University of Michigan Press. U. S. A 1987.

(9) Garcia, L. P. M.; Caro, O. A.; Ruiz, L. M..A.; Bañuelos, P. J.; Zamora, N. F. Ruiz, M. J. Functional properties of a protein isolated obtained from *Lupinus exaltatus* Zucc. In *Proceedings of the 3rd European Conference on Grain Legumes*. Ed. By European Association from Grain Legume Research. Valladolid, Spain. 1998.

(10) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; pp 17, 18, 40−62, 69−83.

(11) Lucas, B.; Guerrero, A.; Sigales, L.; Sotelo, A. True protein content and non protein amino acids present in legumes seeds. *Nutr. Rep. Int.* **1988**, 37, 545−553.

(12) Lucas, B.; Sotelo, A. Amino acid determination in pure protein, foods and feeds using two diferent acid hydrolysis methods. *Anal. Biochem.* **1982**, *123*, 349−356.

(13) Ras, R. M. V.; Tara, M. R.; Krishnan, C. K. Colorimetric estimation of tryptophan content of pulses. *J. Food Sci. Technol.* **1974**, *11*, 213−216.

(14) FAO/WHO. Energy and Protein Requirements: Report of a joint FAO/WHO meeting. Expert consultation. Technical report series 724; World Health Organization: Geneva, Switzerland, 1985.

(15) Kakade, M. L.; Rackis, J. J.; McGhee, J. E.; Puski, G. Determination of trypsin inhibitor activity of soy products. A collaborative analysis of an improved procedure. *Cereal Chem.* **1974,** *51*, 376−382.

(16) Jaffe, W. G.; Levy, A.; Gonzalez, D. I. Isolation and partial characterization of bean phytohemagglutinins. *Phytochemistry* **1974***, 13*, 2685−2693.

(17) Lucas, B.; Sotelo, A. A simplified test for the quantitation of cyanogenic glycosides in wild and cultivated seeds. *Nutr. Rep. Int.* **1984,** *29*, 711.

(18) ISO Standard 9648. Determination of tannin content in sorghum. International Organization for Standardization: Geneva, Switzerland, 1988.

(19) Muzquiz, M.; Cuadrado, C.; Ayet, G.; De la Cuadra, C.; Burbano, C.; Osagie, A. Variation of alkaloid components of lupin seeds in 49 genotypes of *Lupinus albus* L. from different countries and locations. *J. Agric. Food Chem.* **1994**, *42*, 1447−1450.

(20) Litchfield, J. T.; Wilcoxon, F. A simplied method of evaluating dose−effect experiments. *Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

(21) Ali, R.; Muzquiz, M. ANFs in tropical legume seeds for human nutrition. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed: Proceedings of the third international workshop on "Antinutritional factors in legume seeds and rapeseed".* Jansman, A. J. M., Hill, G. D., Huisman, J., van der Poel, A. F. B., Eds.; Wageningen: The Netherlands, 1998.

(22) Schoeneberger, H.; Ildefonso, C.; Cremer, H. D.; Gross, R.; Elmadfa, I. Some antinutritive substances in lupines compared with other legumes. In *Agricultural and Nutritional Aspects of Lupines; Proceedings of the First International Lupine Workshop*, Lima, Perú, 1980**.**

(23) Majak, W.; Keller, W. J.; Duan, Z.; Munro, D.; Smith, R. A.; Davis, A. M.; Ogilvie, T. R. Alkaloid distribution in two species of *Lupinus* in Central British Columbia. *Phytochemistry* **1994**, *36*, 883−885.

(24) Yañez, E. Lupin as a source of protein in human nutrition. In *Proceedings of the 6th International Lupin Conference,* Temuco, Pucon, Chile, 1990.

(25) Hill, G. D. The utilization of lupin in animal nutrition. In *Proceedings of the 6th International Lupin Conference*, Temuco, Pucon, Chile, 1990.

(26) Gross, R. El cultivo y utilización del tarwi *Lupinus mutabilis* Sweet. *Estudio FAO: Producciòn y Protección vegetal*; Food and Agriculture Organization: Rome, 1982.

(27) Hinojosa, G.; Torrico, A. Utilization and commercialization of "Tarwi". In *Proceedings of the 6th International Lupin Conference*, Temuco, Pucon, Chile, 1990.

(28) Egaña, J. I.; Uauy, R.; Cassorla, X.; Barrera, G.; Yañez, E. Sweet lupin protein quality in young men. *J. Nutr.* **1992**, *122*, 2341−2347.

(29) Torres, T. F.; Nagata, A.; Dreifuss, S. W. Métodos de eliminación de alcaloides en la semilla de *Lupinus mutabilis*, Sweet. *Arch. Latinoam. Nutr.* **1980**, *30*, 200−209.

(30) Schoeneberger, H.; Gross, R.; Cremer, H. D.; Elmadfa, I. Composition and protein quality of *Lupinus mutabilis*. *J. Nutr.* **1982**, *112*, 70−76.