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Nutritive Evaluation of the Seed, Germinated Seed, and String Bean of *Erythrina americana* and the Detoxification of the Material by Boiling

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The nutritive and toxicological values of the dry seeds, germinated seeds, and string beans of *Erythrina americana* were studied using raw and boiled samples. Raw germinated seeds had a higher protein content and lower fiber content than dry seeds. The whole string bean had lower protein content and higher fiber content. However, the seeds of the green pod showed the same protein concentration as the dry seeds (dry basis). Boiling and elimination of broth was beneficial in diminishing the alkaloid concentration in all the samples. The trypsin inhibitors, lectins, and tannins were also diminished as was expected. The raw string bean showed the lowest LD_{50}. Although the total essential amino acids content of the boiled germinated seeds was increased, the quality of protein, protein efficiency ratio (PER), was lower than in boiled dry seeds, and in these, the PER was similar to the control (casein). The present results suggest that for the protein quality and low alkaloid content, the boiled dry seeds and string beans could be used for animal feeding. It could be interesting to test the raw string beans in ruminants since in this stage *E. americana* showed the lowest toxicity.

KEYWORDS: ***Erythrina americana*; seed; germinated seed; string bean; alkaloids; nutritive value**

INTRODUCTION

The search for new food sources has been one of man's objectives for his entire history. Plants have played a more important role in human feeding in developing countries. Although so many plant species exist, relatively few are used as food for man due to the presence of toxins or antinutritional factors (ANFs) in many species (*1*−*3*).

Legume seeds are the main protein source in developing countries; therefore, it is important to increase the number of edible legumes for humans or for animals by eliminating the toxic factors in them. At present, technological and biotechnological techniques can be used to destroy or eliminate the harmful components of naturally inedible food, thereby increasing the food supply (*4*−*6*).

The Mexican wild flora have been studied to evaluate the nutritional value of edible and nonedible plants (*7*−*9*).

From the 115 known species of *Erythrina*, 27 species have been located in Mexico (*10*), and in most of them the studies have been concentrated mainly on the content of alkaloids (*11*−*13*).

Erythrina americana is a tree used as an ornament in gardens because of its beautiful red flowers, and in some parts of Mexico the flowers are cooked and eaten after elimination of the broth, to eliminate most of the dissolved alkaloids during cooking. The high alkaloid content in seeds impedes its consumption by humans and animals.

In previous work, the nutritive value was studied in whole and detoxified seeds (*14*) and the protein and nonprotein nitrogen concentrations at different stages of development of this legume were also studied (*15*).

The purpose of this work was to determine the nutritive and toxicological evaluation of the dry seeds, germinated seeds, and string beans of *E. americana* in both raw and boiled samples with elimination of the broth, and to determine if this treatment reduces the toxic effects. If this was the case, this legume could be used for animal feeding.

MATERIAL AND METHODS

The dry seeds and string beans of *E. americana* were collected from a selected tree from the campus of the university.

The procedural diagram is shown in **Figure 1**.

The study was divided in three parts:

One part of the dry seeds was cleaned and milled in a Willey Thomas mill with a 1 mm sieve. Another part of the seeds was boiled in water (1:5 w/v) for 2 h, and after that the broth was removed. The seeds were rinsed with tap water, dried in an oven at 70−75 °C, and milled.

The other part of the dry seeds was used for germination. The testa of seeds was scratched with a steel knife and soaked for 30 min in a 1% sodium hypochlorite solution. After that, the seeds were rinsed several times with distilled water. After the clean seeds were soaked

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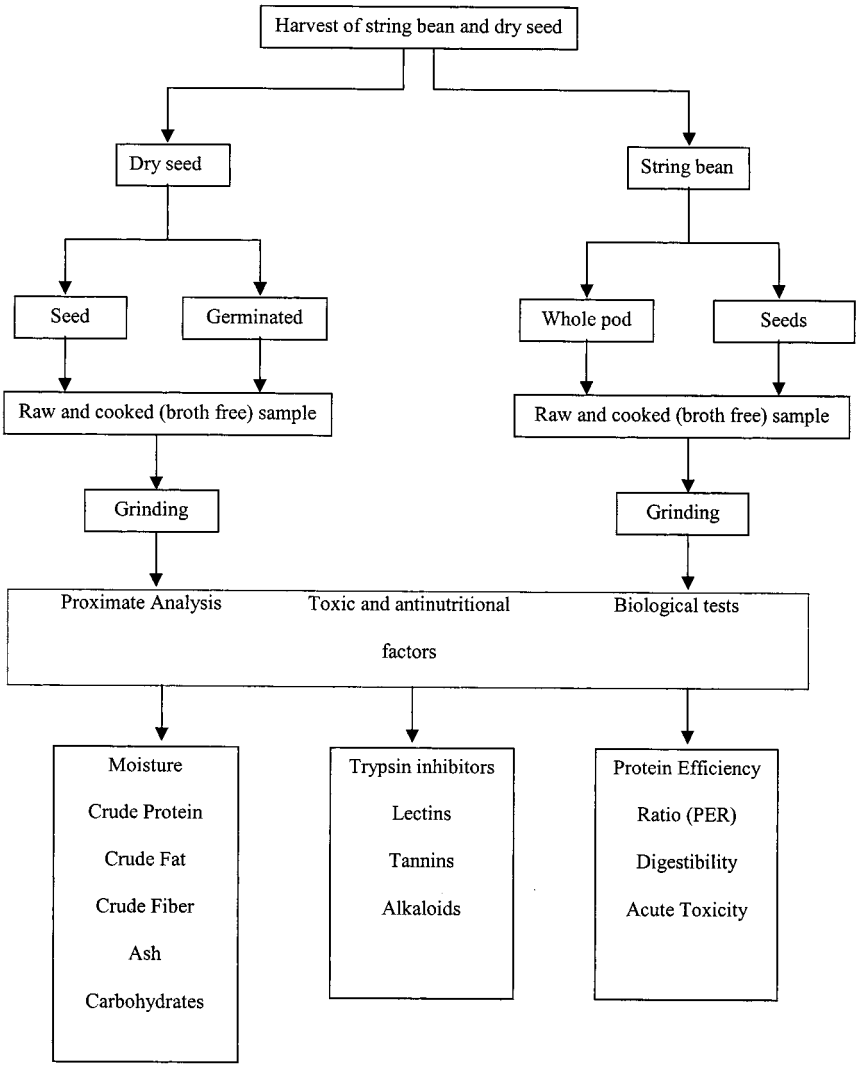

Figure 1. Diagram of the study of the chemical composition and biological tests of the dry seed, germinated seed, and string bean of *E. americana*.

in tap water for 6 h, the seeds were placed in trays lined with filter paper and a layer of wet cotton. The trays were maintained in the dark at room temperature (25 °C) to allow the seeds to sprout.

The germinated seeds were divided in two subparts: One part of the germinated seeds were freeze-dried and milled for the analytical study of nutrients, antinutritional factors, and alkaloids. The other part was boiled in water (1:5 w/v) for 30 min; the broth was eliminated, and the boiled germinated seeds were rinsed with tap water, drained, and dried in an oven at 70−75 °C, and milled.

String bean. The green pods were harvested 6−8 weeks after blooming. The pods were 10 to 20 cm in length, and each of them contained 2−10 uncolored seeds. One part of the whole pods (pod with seeds) was studied intact, and in another lot of string beans, the pod was removed and only the seeds were studied. One part of each raw sample was freeze-dried and the other part was boiled in water (1:5 w/v) for 2 h. This time was chosen to do the same traditional treatment used to cook common beans. After cooking, the material was drained and rinsed with tap water and dried in an oven and milled.

Chemical analysis and biological studies were done on the raw and cooked samples of dry seeds, germinated seeds, and string beans (whole pod and seeds without pod).

Chemical Analyses. The proximate analysis was done according to the AOAC techniques (*16*).

Amino acid determination was done after acid hydrolysis (*17*−*18*), using a Waters HPLC equipment: Pico Tag station, automated gradient controller Mod. 680, HPLC pumps Mod.510, tunable absorbance detector Mod.486, data module Mod.745, and temperature control unit Mod. III.

Tryptophan was measured by a colorimetric method (*19*) after alkaline hydrolysis (*20*). The chemical score of the protein was calculated with the essential amino acids data (*21*).

Antinutritional factors were done in the freeze-dried and boiled material. Trypsin inhibitors were measured according to Kakade method (*22*), and lectins were measured by the Jaffe technique (*23*). Total tannin content was also measured (*24*). Total alkaloids were measured in the raw and boiled samples according to the USP Pharmacopeia, and reported as erythroidine, which is the alkaloid that is in higher concentration (*25*).

Biological Tests. *Protein quality.* The protein efficiency ratio (PER) determination and digestibility were performed (*21*). Isocaloric and isoproteic (10%) diets were prepared. **Table 1** shows the composition of experimental diets. The composition of the Roger-Harper mineral mix and vitamin mix was obtained from ICN Pharmaceuitcal, Cleveland, OH. Roger Harper mix (%): ammonium molybdate 0.003, calcium carbonate 29.290, calcium phoshate·dibasic 0.430, cupric sulfate 0.156, ferric citrate (16−17% Fe) 0.623, magnesium sulfate 9.980, manganese sulfate 0.121, potassium iodide 0.0005, potassium phosphate 34.310, sodium chloride 25.060, sodium selenite 0.002, and zinc chloride 0.020. Vitamin mix (g/Kg): vitamin A acetate (500 000 IU/g) 1.8, vitamin D_2 (850 000 IU/g) 0.125, DL-α-tocopherol acetate 22.0, ascorbic acid 45.0, inositol 5.0, and choline chloride 75.0, in dextrose Q.S. 1 Kg.

The tests were performed using weanling Sprague Dawley male rats 21−23 days old, weight average 40−60 g. The PER determination lasted 21 days. Both food and water were available ad libitum. Food consumption and weight of each of the rats were recorded twice a week throughout the 21 days of the experiment. For digestibility measure-

Table 1. Composition of Experimental Diets with Dry Seed, Germinated Seed, and String Bean of *E. americana*[a]

	diet composition (g/100 g of total diet)								
						string bean			
		dry seed		germinated seed		whole pod		seed	
ingredients	control	R	B	R	B	R	B	R	B
casein (89.19% protein)	11.11								
protein source		39.24	24.29	25.42	26.08	48.08	46.77	33.09	32.76
sucrose	22.00	18.33	19.90	18.90	18.86	15.76	17.20	18.44	19.35
glucose[b]	19.00	15.84	17.20	16.34	16.30	13.61	14.86	15.93	16.71
dextrin[b]	25.00	20.84	22.64	21.50	21.44	17.91	19.55	20.96	21.99
lard	8.00	4.91	6.02	5.80	5.78	6.40	5.50	5.49	4.71
corn oil	6.00	3.68	4.51	4.39	4.34	4.80	4.12	4.12	3.54
Roger-Harper mineral mix[c]	4.00	2.24	0.05	2.82	3.00	0.91	2.16	2.67	3.10
vitamin mix[c]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
fiber cellulose type[b]	2.89		3.39	2.77	2.20				

[a] N × 6.25 g of protein/100 g of total diet. Each diet was calculated to contain 10% protein and 422 kcal/100 g of diet. [b] Sigma, St. Louis Mo. [c] ICN Pharmaceutical, Cleveland, OH. R = raw sample; B = boiled sample.

Table 2. Proximal Analysis of the Dry Seed, Germinated Seed, and String Bean (Whole Pod and Seed without Pod) Raw and Cooked (g/100 g of Dry Basis Sample)[a]

		dry basis				
sample	moisture	protein	lipid	fiber	ash	carbohydrates[b]
dry seed						
raw	9.22 ± 0.07	30.34 ± 0.88[b]	16.40 ± 0.47[a]	14.62 ± 0.29[a]	5.35 ±0.23[a]	33.29
boiled	56.60 ± 1.67	41.43 ± 1.36[a]	14.56 ±0.54[a]	14.64 ±0.30[a]	4.02 ±0.04[b]	24.95
germinated seed						
raw	73.41 ± 2.86	40.01 ± 0.35[a]	14.96 ± 0.36[a]	3.33 ± 0.18[a]	4.73 ± 0.39[a]	36.97
boiled	80.53 ± 2.73	39.49 ± 0.91[a]	15.34 ± 0.48[a]	4.10 ± 0.16[a]	3.97 ± 0.22[b]	37.10
string bean						
raw whole pod	85.09 ± 4.26	24.38 ± 1.01[a]	6.11 ± 0.27[a]	26.14 ± 1.04[b]	6.64 ± 0.09[a]	36.73
boiled whole pod	86.27 ± 2.56	23.12 ± 1.01[a]	6.64 ± 0.38[a]	31.80 ± 2.15[a]	4.27 ± 0.37[b]	34.17
raw seed	83.55 ± 3.22	30.04 ± 0.64[a]	13.77 ± 0.29[b]	17.07 ± 1.43[b]	4.21 ± 0.10[a]	34.91
boiled seed	84.40 ± 1.92	32.44 ± 0.82[a]	16.09 ± 0.60[a]	23.25 ± 0.57[a]	3.00 ± 0.19[b]	25.22

[a] Mean of triplicate sample ± SD; CV < 5%. Values of raw vs boiled samples with different superscripts are significantly different ($p \leq 0.05$). [b] Obtained by difference.

Table 3. Trypsin Inhibitors, Lectins, and Tannins Content in Raw and Cooked Dry Bean, Pods, and Germinated Seeds (dry basis)[a]

sample	trypsin inhibitors (TUI/mg sample)[b]	hemagglutinating activity (titer)[c]	tannins g/100 g sample
dry bean			
raw	85.18 ± 2.42[a]	5	0.14 ± 0.001[d]
boiled	4.75 ± 0.24[c]	2	0.06 ± 0.001[e]
germinated seed			
raw	1.18 ± 0.08[c]	3	0.03 ± 0.005[f]
boiled	ND	1	0.02 ± 0.001[f]
string bean			
raw whole pod	28.67 ± 0.82[b]	5	0.17 ± 0.01[c]
boiled whole pod	1.77 ± 0.18[cd]	3	0.14 ± 0.01[d]
raw seed	88.43 ± 6.85[a]	6	0.37± 0.01[a]
boiled seed	1.90 ± 0.16[cd]	3	0.23 ± 0.01[b]

[a] Mean of triplicate samples ± SD; CV < 10%. Values in each column with different superscripts are significantly different ($p \leq 0.05$). [b] Trypsin unit inhibited. One trypsin unit = 0.01 absorbency units at 410 nm. [c] Titer = maximum dilution where agglutination was observed. ND = not detected.

ments, feces were collected from the 12th to the 21st day of the experiment (10 days) for nitrogen determination.

Acute toxicity test (*26*): 135 CFW female mice 5−6 weeks old, weight average 20−25 g were used. The animals were distributed in three lots of five doses each for the preliminary tests and for the final test of acute toxicity. The temperature of the animal house was 23 ± 1 °C, relative humidity of 45−55%, 18 air changes per hour and periods of 12 h light/dark.

Statistical Analysis. Data on the composition of the samples are presented as mean values of three replicates with a maximum coefficient of variation (CV) of 5%. Biological studies were tested statistically by the one-way analysis of variance (ANOVA), and multiple range analysis (Duncan) using a PC software package (Statsgraphics V.5/1991). The

Table 4. Total Alkaloid Content in Dry Seed, Germinated Seed and String Bean of *E. americana* Raw and Boiled (g/100 g of Dry Sample)[a,b] and LD_{50} in the raw samples

sample	raw	boiled	LD_{50} (mg/kg b.w.)[c]
dry seed	1.056 ± 0.028[a]	0.500 ± 0.014[d]	12000
germinated seed	1.098 ± 0.022[a]	0.313 ± 0.013[e]	28000
string bean whole pod	0.670 ± 0.039[c]	0.301 ± 0.023[e]	78000
seed without pod	0.769 ± 0.054[b]	0.214 ± 0.015[f]	NM

[a] Mean of triplicate samples ± SD; CV < 5%. Values with different superscripts are significantly different ($p \leq 0.05$). [b] g of erythroidine/100 g of sample. [c] The test was done orally, $n = 9$ per doses. NM = not measured.

Table 5. Essential Amino Acid (EAA) Content in the Raw (R) and Boiled (B) Dry Seed, Germinated Seed, and String Bean of *E. americana* (g of amino acid/16 g of N)[a]

	dry seed		germinated seed		string bean				
					whole pod		seed		
amino acid	R	B	R	B	R	B	R	B	FAO/WHO pattern (1973)
Met	1.75	1.70	1.87	1.70	1.15	0.75	1.45	1.44	3.5
Cys	1.00	0.90	0.47	0.50	0.45	0.40	0.70	1.00	
Phe	2.51	2.50	3.00	3.00	1.97	1.83	2.82	2.31	6.0
Tyr	4.39	4.59	5.67	5.70	3.90	2.54	3.21	3.16	
Lys	9.26	9.10	9.15	9.18	8.73	7.70	7.31	7.05	5.5
Ile	3.83	3.43	4.35	4.27	3.27	4.23	5.75	5.68	4.0
Leu	6.55	6.49	7.06	7.10	5.30	6.73	5.91	6.82	7.0
Val	4.05	4.18	4.92	4.75	5.47	5.73	5.43	5.44	5.0
Thr	3.63	3.53	3.40	3.42	3.06	2.17	3.37	3.33	4.0
Trp	1.17	1.27	1.51	1.37	1.31	1.28	1.27	1.07	1.0
total EAA	38.17	37.67	43.40	40.99	34.61	33.36	33.22	37.30	
chemical score[b]	78.57	74.29	66.86	66.00	45.71	32.86	61.43	69.71	
limiting aa	sulfur amino acids in all the samples								

[a] Average of duplicate. [b] Chemical score = (g of amino acid in sample/g of amino acid in FAO/WHO pattern) × 100.

Table 6. Protein Efficiency Ratio (PER) and Digestibility of the Cooked Dry Seeds, Geminated Seeds, and String Bean of *E. americana*[a]

sample	$PER_{(exp)}$	$PER_{(corrected)}$	digestibility
dry seed			
boiled dry seed	2.83 ± 0.31^{a}	2.34	90.42 ± 0.802^{b}
germinated seed			
boiled germinated	2.56 ± 0.25^{b}	2.05	87.19 ± 1.519^{c}
string bean			
boiled pod and seed	2.12 ± 0.11^{c}	1.73	86.55 ± 1.068^{c}
boiled seed	2.48 ± 0.18^{b}	2.02	90.49 ± 1.046^{b}
casein (control)	3.06 ± 0.17^{a}	2.50	98.50 ± 0.345^{a}

[a] Mean ± SD; $n = 6$; CV < 10%. Values in each column with different superscripts are significantly different ($p \leq 0.05$).

LD_{50} was statistically analyzed according to the method of Lichfield and Wilcoxon (*26*). A p value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Chemical Composition. The proximate composition of the samples is shown in **Table 2**. The string beans showed the highest moisture content. The highest protein concentration in the raw samples (dry basis) was found in germinated seeds and the lowest in the string beans; these samples had the highest fiber concentration. Boiling increased the protein concentration in the dry seed. The explanation of these results is that part of the minerals (ash) and sugars were probably dissolved in the broth.

Germination decreased the three ANFs studied (**Table 3**). The most remarkable results were found in the trypsin inhibitors. Similar results had been reported in other work (*8*, *27*). String bean seeds and dry seeds showed the same ANFs profile, and boiling decreased the ANFs as was expected. Germination did not decrease the alkaloid content (**Table 4**). It has been reported by other authors that in lupins the alkaloids increased during germination (*28*). Although the alkaloid content in the dry seed was similar to that in the germinated seed, the toxicity (LD_{50}) was higher in the dry seed. The explanation of these findings is that probably the alkaloids found in the germinated seeds are different and less toxic than those in the dry seeds. In all samples, boiling and removing the broth decreased the alkaloid content. Since the whole string bean in its raw form had a very low toxic effect, it could be tested in future studies for ruminant animal feeding.

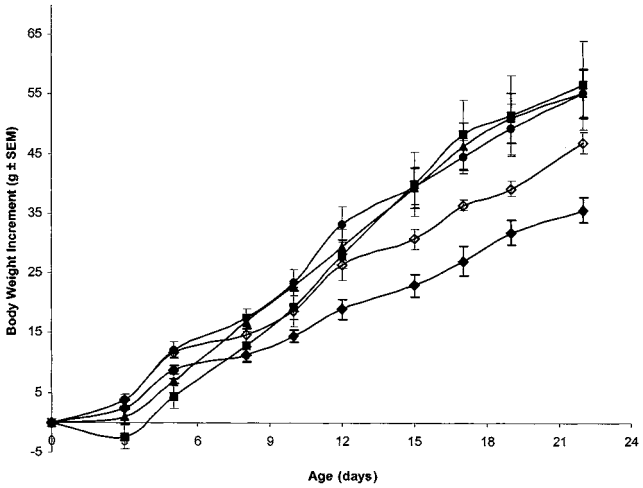

Figure 2. Growth rate of rats fed with boiled *Erythrina* diets: dry seed (■), germinated seed (▲), string bean whole pod (◆), seed without pod (◇), and casein control (●).

Table 5 shows the content of essential amino acids in the raw and boiled samples. It is important to mention the significant content of tryptophan. Sulfur amino acids were in low concentration as in most of the legumes.

Table 6 shows the PER values of the boiled dry seeds, germinated seeds, and string beans. All the rats fed with the raw samples lost weight and died during the second and third week of the experiment. The boiled dry seed had the highest PER value similar to the control, followed by the germinated seed and the seed of the string pod. The whole string bean had the lowest PER. The boiled dry seed had the same PER value obtained in previous study in the detoxified dry seed by methanol extraction (*14*).

These results mean that significant amounts of alkaloids were eliminated in the broth and the remainding alkaloids in the boiled seeds did not affect the growth of the rats. The growing curve of the rats fed with the three different samples is shown in **Figure 2**. It could be concluded that cooking, removing the broth, and rinsing with tap water were enough to remove the most of the alkaloids, obtaining a detoxified dry seed that mixed with cereals might increase the protein quality as well as decrease the alkaloid content and could be tested in other monogastric animals like fowls. The present results suggest that the beneficial effect of heat treatment used in this study was

due to the reduction of the several antinutritional factors, mainly alkaloids. However, one could also consider the possibility that the effectiveness of heat treatment was due to the destruction or elimination of some yet unidentified noxious heat-labile component of this legume. It is necessary also to do some further studies using raw string beans mixed with cereals in ruminants feeding since in this stage it was shown to have the lowest toxicity of the raw samples.

LITERATURE CITED

(1) Aykroyd, W.; Doughty, J. Legumes in human nutrition. In *Food and Nutrition Paper*; Food and Agriculture Organization: Rome, Italy, 1982; pp 152.

(2) 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.

(3) Biological Processes and Their Control, Symposium. In 219th National Meeting of the American Chemical Society. *J. Agric. Food Chem*. **2002**, *50*, 6579−6642.

(4) Reduction of Antinutritional Factors in Legume Seeds and Rapeseed by Process Technology and Biotechnology. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*; Jansman, A. J., Hill, G. D., Huisman, J., van der Poel, A. F., Eds.; European Association for Animal Production: Wageningen, Netherlands, 1998; pp 377−462.

(5) Bressani, R.; Sosa, L. Effect of Processing on the Nutritive Value of Canavalia Jackbeans (*Canavalia ensiformis L.*). *Plants Foods Hum. Nutr*. **1990**, *40*, 207−214.

(6) El-Adawy, T. Nutritional composition and Antinutritional Factors of Chickpeas (*Cicer arietinum*) Undergoing Different Cooking Methods and Germination. *Plant Foods Hum. Nutr*. **2002**, *57*, 83−97.

(7) Sotelo, A. The Nutritive Value of Wild Mexican Legumes with a Potential for Nutritional Use. In *Legumes and Oilseeds for Nutrition*; Nwokolo, E., Smartt, J., Eds.; Chapman and Hall: New York, 1996; pp 33−48.

(8) Sotelo, A. Constituents of Wild Food Plants. In *Functionality of Food Phytochemicals*; Johns, T., Romeo, J., Eds.; Plenum Press: New York, 1997; pp 89−111.

(9) Ruíz, M.; Sotelo, A. Chemical Composition, Nutritive Value, and Toxicology Evaluation of Mexican Wild Lupins. *J. Agric. Food Chem*. **2001**, *49*, 5336−5339.

(10) Neill, D. The Genus *Erythrina*; Taxonomy, Distribution, and Ecological Differentiation. In *Erythrina in the New and Old Worlds*; Westley, S., Powell, M., Eds.; Nitrogen Fixing Tree Association (NFTA): Hawaii, 1993.

(11) Aguilar, M.; Giral, F.; Espejo, O. Alkaloids from the Flowers of *Erythrina Americana*. *Phytochemistry* **1981**, *20*, 2061−2062.

(12) Soto-Hernández, M.; Jackson, A. *Erythrina* Alkaloids: Isolation and Characterization of Alkaloids from seven *Erythrina* species. *Plant. Méd*. **1994**, *60*, 175−177.

(13) García-Mateos, R.; Soto-Hernández, M.; Kelly, D. Alkaloids from six *Erythrina* species endemic to Mexico. *Biochem. Syst. Ecol*. **1998**, *26*, 545−551.

(14) Sotelo, A.; Soto, M.; Lucas, B.; Giral, F. Comparative Studies of the Alkaloidal Composition of Two Mexican Erythrina Species and Nutritive Value of the Detoxified Seeds. *J. Agric. Food. Chem*. **1993**, *41*, 2340−2343.

(15) García-Mateos, R.; Lucas, B.; Zendejas, M.; Soto-Hernández, M.; Martínez, M.; Sotelo, A. Variation of Total Nitrogen, Nonprotein Nitrogen Content, and Types of Alkaloids at Different Stages of Development in *Erythrina americana* Seeds. *J. Agric. Food Chem*. **1996**, *44*, 2988−2991.

(16) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Analytical Chemists: Arlington, VA, 1990.

(17) Cohen, S.; Michaud, D. Synthesis of a Fluorescent Derivatizing Reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its Application for the Analysis of Hydrolysate Amino Acids Via HPLC. *Analytical Biochemistry* **1993**, *211*, 279−287.

(18) Hagen, S.; Frost, B.; Augustin, J. Precolumn phenylisothiocyanate Derivatization and Liquid Chromatography of Amino acids in Food. *J. Assoc. Off. Anal. Chem*. **1989**, *72*, 912−916.

(19) Rama Rao, M.; Tara, M.; Chandra, K. Colorimetric Estimation of Tryptophan Content of Pulses. *J. Food Sci. Technol*. **1974**, *11*, 213-216.

(20) Lucas, B.; Sotelo, A. Effect of Different Alkalies, Temperature, and Hydrolysis Times on Tryptophan Determination of Pure Proteins and Feeds. *Anal. Biochem*. **1980**, *109*, 192−197.

(21) Pellet, P.; Young, V. *Nutritional Evaluation of Protein Foods*; The United Nations University World Hunger Programme, Food and Nutrition Bulletin Supplement 4; The United Nations University: Tokyo, 1980.

(22) Kakade, M.; Rackis, J.; McGhee, J.; Puski, G. Determination of Trypsin Inhibitor Activity of Soy Products: A Collaborative Analysis of an Improved Procedure. *Cereal Chem*. **1974**, *51*, 376−382.

(23) Jaffé, G.; Brucher, O. Toxicity and Specificity of Different Bean Hemagglutins. Arch. *Latinoam. Nutr*. **1972**, *22*, 267−281.

(24) ISO Standard No. 9648. Determination of Tannin Content in Sorghum, first ed., International Organization for Standardization: Geneva, Switzerland, 1988.

(25) USP XX. The United States Pharmacopeia. The National Formulary. NF XV., 15th ed., United states Phamacopeia Convention, Inc. Rockville, MD, pp 913−914.

(26) Litchfield, J.; Wilcoxon, F. Simplied Method of Evaluating Dose−Effect Experiments. *Pharmacol. Exp. Ther*. **1949**, *96*, 99−113.

(27) Godbole, S. A.; Krishna, T. G.; Bhata, C. R. Changes in Protease Inhibitory activity from Pigeon Pea (*Cajanus cajan, L., Mill sp*) during Seed Development and Germination. *J. Sci. Food Agric*. **1994**, *66*, 497−501.

(28) Muzquiz, M.; Pedrosa, M.; Cuadrado, C.; Ayet, G.; Burbano, C.; Brenes, A. Variations of Alkaloids, Alkaloids Esters, Phytic Acid and Phytase Activity in Germinated Seed of *Lupinus albus* and *L. luteus*. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*; Jansman, A. J., Hill, G. D., Huisman, J., van der Poel, A. F., Eds.; European Association for Animal Production: Wageningen, Netherlands, 1998; pp 387−390.

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