PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 11 (8): 774-780, 2012
ISSN 1680-5194

Comparison of Chemical Composition and Protein Digestibility, Carotenoids, Tanins and Alkaloids Content of Wild Lupinus Varieties Flour

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Abstract: Proximate composition, carotenoids, tannins, quinolizidine alkaloids and *in vitro* protein digestibility were determined in flours of two wild lupines seeds recollected at central region of Mexico. Varieties identified as *Lupinus barkeri* and *Lupinus montanus* were compared with a domesticated cultivated *Lupinus albus* crop. Although total protein content resulted significantly ($p<0.05$) higher for both *L. barkeri* and *L. montanus*, no significantly ($p>0.05$) difference were found in *in vitro* protein digestibility. Ash and crude fiber contents were significantly ($p<0.05$) higher for *L. barkeri* and *L. montanus* ether extract was significantly ($p<0.05$) higher than the other lupin samples. In general, chemical composition related to ash, fiber and ether extract contents are close to the reported range for other *Lupinus* species. Wild varieties of *Lupinus* could represent a viable alternative looking for new protein resources, from the techno-functional and nutritional point of view. Lupin flour is a good source of minerals and functional compounds, like carotenoids as antioxidant or dietary fiber, with health-promoting properties. Antinutritional factors associated to lupin can be minimized or eliminated by processing (soaking, dehulling and cooking). These characteristics of wild *Lupinus* varieties result in a revalorization of these crops as a protein and other healthy promoting compounds for human or animal consumption.

Key words: Wild *Lupinus*, chemical composition, carotenoids, tannins, alkaloids, *in vitro* protein digestibility

INTRODUCTION

Lupinus has been a relatively large genus and one of the most geographically widespread with a wide diversity in both Old World (Mediterranean and North-East Africa) and the New World (North-South America) species. The *Lupinus* species mostly have habitats range from desert valleys to tropical highlands; from high mountain regions to coastal plains and in general the species seems well adapted to a number of climatic environments and there is considerable variation within this species (Wolko *et al*., 2011).

The use of *Lupinus* in food process applications requires reduction of content of non-nutritional ingredients such as tannins, alkaloids and oligosaccharides, undesirable compounds that must be removed before consumption (Ballester *et al*., 1980; Jimenez *et al*., 2001). *Lupinus* contain a lower concentration of lectins (Petterson *et al*., 1997), phytates (Fudiyansyah *et al*., 1995) and saponins (Gurfinkel and Rao, 2002) than soybean and need no heat treatment to deactivate substances such as the lectins and protease inhibitors that reduce protein digestion and availability (Wolko *et al*., 2011). Lupine is free of haemagglutinins, isoflavones and other components typical in legumes, with a relatively low content of flatus-inducing oligosaccharides (Cerletti and Duranti, 1979). Detoxified *Lupinus* seeds are mainly used to fortify foods and counteract nutrient deficiencies. *Lupinus* seed flour is increasingly used in cereal-based foods (Witting de Penna *et al*., 1989; Mohamed and Rayas, 1995; Dervas *et al*., 1999; Clark and Johnson, 2003; Guemes-Vera *et al*., 2008). The main interest in lupin for foods is related to its high content of protein content (Sgarbieri and Galeazzi, 1978; Ruiz and Sotelo, 2001). *Lupinus* seed flours have been used for the production of protein isolates with good functional and nutritional properties (Lqari *et al*., 2002). In same way, protein digestibility of lupine proteins is good *in vitro* and compares favorably with soy protein (Cerletti and Duranti, 1979).

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The different *Lupinus* species (domesticated and the recently studied wild) possesses useful adaptation, plant and seed quality attributes that makes the genus a valuable resource for farming practice, production and use in established feed and emerging food and health industries. Although *Lupinus* have played a major role as an animal feed, subsistence food and soil improvers, current and future use has entered the arena of health food and functional ingredient, offering a viable option for world agriculture in the future (Wolko *et al*., 2011).

The objective of this study was to compare the chemical composition (moisture, total protein, lipids, fiber, carbohydrates and ashes) and protein digestibility, besides carotenoids, tannins and alkaloids presents, of two wild *Lupinus* species (*barkeri* and *montanus*) and one domesticated (*L. albus*).

MATERIALS AND METHODS

Raw material and flours elaboration: Wild *Lupinus barkeri* and *Lupinus montanus* seeds were collected at Mineral del Chico, Hidalgo, in central-eastern Mexico at an altitude of 2,700 m.s.l., in the coordinates latitude 20°10'05" N longitude and 98°41'47" W and latitude 20°13'25" N longitude 98°45'31" W. *Lupinus albus* seeds were kindle provided by the Universidad Autonoma de Guadalajara (Mexico). Species were phenotypically identified at the Forestry Research Center of the Universidad Autónoma Estado Hidalgo at Tulancingo City (Mexico).

Flours of the different *Lupinus* seed were elaborated first dehulling the seeds with 40°C water soak overnight. Dry seeds were milled using a laboratory disc mill and sieved on an 8XX sieve. Flours were kept in hermetic flasks until further analysis.

Chemical composition, *in vitro* digestibility and carotenoids content: Standard AOAC methods for grain analysis (Official Method 945.38) were employed to determine: Moisture (Official Method 925.09B), Ash (Official Method 923.03), Crude fiber (Official Method 962.09) and Ether extract (Official Method 920.39C). Total protein content was determined according to the Official Method 955.04 (referred in the Official Method 978.04, conversion factor = 6.25) (AOAC, 1995).

Protein digestibility was determined according to Hsu *et al*. (1977). A portion of sample was taken that provided 5.25 mg protein per 1 mL enzymatic solution. This was suspended in 50 mL water adjusting pH to 8 with 0.1 N HCl or 0.1 N NaOH and agitated in a water bath at 37°C. Simultaneously, a multienzymatic solution was prepared containing 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per mL of solution and pH adjusted to 8. Then, 5 mL multienzymatic solution was added to the sample suspension, agitation continued at 37°C for 10 min and pH measured 10 min after incubation. Apparent *in vitro* protein digestibility was calculated using the formula:

$$\text{Apparent digestibility} = 210.46 - 18.10\ \text{pH}$$

Carotenoids content in seed flours was determined by first placing one g flour in a glass, adding 50 mL acetone and agitating for 12 h to extract the pigments. The glass was covered during extraction to prevent contact with light and consequent pigment degradation. The extract was filtered and its absorbance read at 472 and 508 nm in a spectrophotometer (Spectronic, Genesys 5, La Joya, USA). Carotenoids concentration was reported for the red and yellow isochromic fractions and the concentration of each calculated using the absorbance values and the following formulas (Hornero-Mendez and Minguez-Mosquera, 2001):

$$C^R = \frac{(A_{508})\,(2144.0) - (A_{472})\,(403.3)}{270.9}\ \mu g/ml$$

$$C^Y = \frac{(A_{472})\,(1724.3) - (A_{508})\,(2450.1)}{270.9}\ \mu g/ml$$

Where:

C^R = Red isochromic fraction
C^Y = Yellow isochromic fraction
A_{508} = Absorbance at 508 nm
A_{472} = Absorbance at 472 nm

Tanins and alkaloid extraction and analysis: Tannins content was determined following the methodology proposed by Burns (1971). Briefly, a chromogenic agent was prepared by mixing equal parts of an 8% HCl/methanol solution with a 4% vanillin/methanol solution. Immediately thereafter, a standard curve was run by preparing a catechin/methanol solution containing 100 mg catechin in 5 mL methanol. Four solution dilutions were prepared in duplicate from the catechin solution by diluting at a 1:10 ratio each time. Then, 5 mL chromogenic solution were added to each tube, the tubes agitated in a vortex and transmittance measured at 500 nm in a spectrophotometer. The chromogenic agent was used as a blank.

Alkaloid extraction was done by first homogenizing *Lupinus* seed flour with 0.5 M HCl, allowing this mixture to settle for 30 min at room temperature and centrifuging at 10 000 g for 10 min. The supernatant was removed pH adjusted to 12 by adding 20 mL 6 M NaOH and placed in an Extrelut (Merck) column. The alkaloids were eluded with methyline chloride and the solvent almost completely evaporated at 45°C in a rotavapor. Quinolizine alkaloids analysis was done using a gas chromatographer (GC, model 6890, Hewlett Packard, MS MOD-5972) with an HP-1 column (non-polar; 30m x 0.25

mm. i.d., 0.25 μm thin film) under the following conditions: 180-250°C programmed temperature, one mL/min helium gas vehicle, 250°C injection temperature, 300°C auxiliary temperature and 2 μL QA extract. Identification and quantification were done with a Mass Selective Detector (MSD) under the conditions: EI mode at 70 eV; acquisition mode scan, 50-500 mass range, 150 threshold and 1.53 scans/s. Data were analyzed in the Chemstation using the Wiley 275 library (Bermudez-Torres *et al*., 1999).

Statistical analyses: Results were analyzed employing PROC ANOVA procedure and significantly difference was determined by the Duncan's mean tests in the SAS software v. 8.0 (SAS Institute, Cary, USA). Correlation among the obtained results was determined with the PROC CORR procedure in same statistical software.

RESULTS AND DISCUSSION

Chemical composition *in vitro* protein digestibility and carotenoids: Moisture content was significantly ($p<0.05$) higher for *L. albus* than for *L. barker* and *L. montanus*. *L. barkeri* presented significantly ($p<0.05$) higher ash and crude fiber contents. Ether extract was significantly ($p<0.05$) higher for *L. montanus*. Protein content was significantly ($p<0.05$) higher for both *L. barkeri* and *L. montanus* flours. Nonetheless, protein digestibility was not significantly ($p>0.05$) different between the three *Lupinus* samples (Table 1).

Chemical composition differences are related to implicit differences due location and season or climatic conditions, with slightly variation among varieties (Wolko *et al*., 2011). But in general, chemical composition related to ash, fiber and ether extract contents are close to the reported range for other *Lupinus* species (Hill, 1977; Yanez *et al*., 1983; Zdunczyk *et al*., 1994; Ruiz and Sotelo, 2001).

Ash content is related to high levels of macronutrients, like phosphorus and potassium and micronutrients like iron, besides lower levels of essential minerals like calcium or magnesium reported in *Lupinus* seeds (Ortega-David *et al*., 2010). Regarding to fiber, it has been reported that *Lupinus* fiber reduce transit time in human digestion and beneficial effects on stool bulking (Johnson *et al*., 2006), reduce blood glucose in non-insulin diabetics (Hall *et al*., 2005) and blood pressure (Lee *et al*., 2009). Enzymatic, acid and alkaline hydrolysis or acetylation modifications enhanced the *in vitro* bile acid binding capacity of lupin dietary fibre (Cornfine *et al*., 2010). Concerning to the extract, related to oil content, a high proportion of unsaturated fatty acids has been reported for *Lupinus* varieties (Uzun *et al*., 2007). Lupin seed is a potentially useful source of high quality oil with a ω-3/ω-6 fatty acids ratio of 1:3.7 for whole seed and 1:3.8 for kernel (Suchy *et al*., 2008). Protein content resulted high for the wild varieties, but with no difference in the in vitro protein digestibility, although protein digestibility values obtained were higher than the reported previously for other *Lupinus* varieties (Egana *et al*., 1992; Lqari *et al*., 2002). *In vitro* protein digestibility of *Lupinus* ranged from 82-89% (Cerletti and Duranti, 1979; El-Adawy *et al*., 2001; Pastor-Cavada *et al*., 2009).

A proportional high significantly ($p<0.01$) correlation was detected between total protein with both crude fiber and ash, as well for crude fiber with ash content. An inverse highly significantly correlation ($p<0.01$) was observed for crude fiber and protein digestibility. This is that the relatively higher crude fiber contents resulted in lower protein digestibility. This is consistent with the reported by Baer *et al*. (1997), who reported that as the diet fiber content increased protein digestibility decreased. Nonetheless, the results obtained in the present study were on raw flour with no further treatment as the applied in food processing. Processing methods (soaking, cooking, dehulling) of lupin seeds improved protein digestibility and mineral availability, reducing both tannins and phytic acid contents with some treatments (Hassan *et al*., 2005; Embaby, 2010). In same manner, an inverse significantly ($p<0.05$) correlation was found between moisture with protein and fiber content (Table 2).

Table 1: Chemical composition of commercial *L. albus* and wild *L. barkeri* and *L. montanus* samples

Lupinus	Moisture (%)	Ash (%)	Crude fiber (%)	Ether extract (%)	Total protein (%)	Protein digestibility (%)
L. albus	7.03±0.10^{a}	2.79±0.28^{b}	10.71±0.81^{c}	7.80±0.45^{b}	29.33±2.07^{b}	80.72±1.48^{a}
L. barkeri	6.55±0.10^{b}	5.00±0.75^{a}	15.84±0.29^{a}	8.18±0.84^{b}	37.07±1.68^{a}	78.58±1.47^{a}
L. montanus	6.57±0.15^{b}	3.28±0.19^{b}	14.07±1.08^{b}	9.97±0.23^{a}	35.27±0.75^{a}	81.43±1.48^{a}

^{a,b,c}Means with same letter in same column are not significantly different ($p>0.05$)

Table 2: Correlation coefficients for the chemical composition and protein digestibility of the analyzed *Lupinus* samples

	Moisture	Total protein	Ether extract	Crude fiber	Ash	Protein digestibility
Moisture	1.0000	-0.7108*	-0.3565•	-0.7221*	-0.4742•	0.4818•
Total protein		1.0000	0.5235•	0.9661**	0.8262**	-0.0758•
Ether extract			1.0000	0.3689•	0.0431•	0.6362•
Crude fiber				1.0000	0.8451**	-0.8308**
Ash					1.0000	-0.3605•
Protein digestibility						1.0000

**Highly significantly ($p<0.01$); *Significantly ($p<0.05$); •No significantly ($p>0.05$)

Table 3: Carotenoids and tannins content commercial of *L. albus* and wild *L. barkeri* and *L. montanus* samples

Lupinus	Carotenoids isochromic red fraction (mg/g)	Carotenoids isochromic yellow fraction (mg/g)	Tanins (mg/g)
L. albus	0.284±0.07[b]	0.394±0.07[ab]	0.74280[a]
L. barkeri	0.726±0.15[a]	0.511±0.15[a]	0.84280[a]
L. montanus	0.174±0.07[b]	0.272±0.07[b]	0.92850[a]

[a,b]Means with same letter in same column are not significantly different (p>0.05)

Table 4: Quinolizidine alkaloids content of commercial of *L. albus* and wild *L. barkeri* and *L. montanus* samples

Lupinus	Alkaloid	Concentration (mg/kg)	Total (%)
*L. albus**	Amondendrine	270.0	1.26
	Albine	4590.0	21.41
	Angustifoline	250.0	1.16
	Isolupanine	70.0	0.33
	5-6 Dehidrolupanine	160.0	0.75
	Lupanine	13600.0	63.47
	11,12-seco-12,13-Didehydromultiflorine	480.0	2.24
	Multiflorine	1830.0	8.54
	13-hydroxy-lupanine	160.0	0.75
	13-Tigloyloxylupanine	20.0	0.09
	Total	21430.0	100.00
L. barkeri	NI	71.2	6.23
	Lupanine	106.3	9.31
	Oxoesparteine	1.7	0.15
	NI	13.8	1.21
	Nuftalline	24.2	2.12
	Multiflorine	26.0	2.27
	Oxylupanine	595.2	52.13
	Oxylupanine 2	119.2	10.44
	Sparteine	1.4	0.12
	Virgiline	48.4	4.24
	11,12-dehydrolupanine	134.4	11.78
	Total	1141.8	100.00
L. montanus	Lupanine	11.6	4.46
	NI	57.9	22.35
	Nuftalline	28.2	10.86
	Multiflorite	49.9	19.22
	Virgiline	111.9	43.11
	Total	259.6	100.00

The content of carotenoids (both isochromic red and yellow fractions) resulted significantly ($p<0.05$) higher for *L. barkeri* as compared to *L. montanus* and *L. albus* (Table 3). The difference in carotenoids contents observed among *Lupinus* samples agrees with interspecies variation, where characteristics pigments in this kind of legumes include lutein, zeaxanthin and β-carotene (Cerletti *et al*., 1978; Entisar and Hudson, 1979; Paiva and Russell, 1999; Wang *et al*., 2008). Antioxidant activity was found in lupin flour (Tsaliki *et al*., 1999; Lampart-Szczapa *et al*., 2003; Siger *et al*., 2011). Recent epidemiology studies showed that carotenoids can prevent the development of some chronic diseases in humans, including cancers and cardiovascular diseases, in addition to other biological activities, including antioxidant activity, influences on the immune system, control of cell growth and differentiation and stimulant effects on gap junction communication (Wang *et al*., 2008). Lupinus seeds are a source of functional compounds, such as antioxidant with health-promoting properties (Pastor-Cavada *et al*., 2010).

Tanins and quinolizidine alkaloids: Tanins content was not significantly ($p>0.05$) different for *Lupinus* samples (Table 3). Total and condensed tannin (responsible for negative effect in protein binding) levels in *Lupinus* were reported to be approximately 0.29% and 0.01%, respectively (Petterson *et al*., 1997). Tannins content in the present study resulted higher than the reported for *L. mutabilis* (Guemes-Vera *et al*., 2008), but lower than for *L. rotundiflorus*, *L. simulans* or *L. madrensis* (Ruiz and Sotelo, 2001).

Different quinolizidine alkaloids determined in the different *Lupinus* samples analyzed are listed in Table 4. Two alkaloids were found the three samples: lupanine and multiflorine. For *L. albus*, lupanine was the higher alkaloid detected (63.47%), in contrast to wild lupin samples with a lower content of this compound (9.31% and 4.46% for *L. barkeri* and *L. montanus*, respectively). The second one, multiflorine, was detected in higher concentration in *L. montanus* samples (19.22%), in comparison with *L. albus* (8.54%) or *L. barkeri* (2.27%). *Lupinus* contain quinolizidine alkaloids

and different species have unique alkaloid profiles of usually 4-5 major and several minor alkaloid types. These alkaloids are toxic to herbivores such as bees, caterpillars, beetles, aphids, locusts, snails, nematodes, rabbits and cows and have antiviral, antibacterial and antifungal properties (Wolko *et al*., 2011). The major alkaloids found, depending on crop lupin species are, 13-a-hydroxylupanine, angustifoline, cytosine, lupanine, lupinine and sparteine (Wink *et al*., 1995; Petterson, 1998; Torres *et al*., 2002; Wink, 2006), but Lupanine is the most common quinolizidine alkaloids found in most *Lupinus* species (Ruiz and Sotelo, 2001). However, alkaloids levels higher than the 0.2 $mg \cdot g^{-1}$ are not allowed for human consumption (Chango *et al*., 1993). The most employed detoxifying *Lupinus* seed reported method is the use of an alkaline medium, reducing alkaloid content above 98% (Torres *et al*., 1980; Ortiz and Muckherjee, 1982; Ruiz and Sotelo, 2001; Jimenez *et al*., 2001; 2003). Nonetheless, processing of lupin flours will reduce the contents of alkaloids, since the presence of these compounds is particularly low in lupin protein isolates and in foods containing these ingredients (Resta *et al*., 2008).

Conclusion: Lupin has been a poor exploited crop for human consumption although the high protein content of this legume. Wild varieties of *Lupinus* could represent a viable alternative looking for new protein resources, from the techno-functional and nutritional point of view. Fiber from lupin could be also a good source of dietary fiber or even as prebiotic ingredient. Lupin flour is as well a good source of minerals and functional compounds, like carotenoids as antioxidant, with health-promoting properties. Antinutritional factors of the wild lupin analyzed, related to tannins and quinolizidine alkaloids, besides resulted lower than the domesticated *L. albus* or to the reported for other varieties, can be minimized or eliminated by process (soaking, dehulling and cooking). The elaboration functional protein isolates to fortify other food products is another viable alternative. These characteristics of wild *Lupinus* varieties result in a revalorization of these crops as a protein and other healthy promoting compounds for human or animal consumption.

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