# ΦΥΤΟΝ

REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY

FUNDACION ROMULO RAGGIO Gaspar Campos 861, 1638 Vicente López (BA), Argentina www.revistaphyton.fund-romuloraggio.org.ar

# Grain yield, and chemical and protein composition of *Lupinus angustifolius* varieties grown in Mexico

Rendimiento de grano, y composición química y proteica de variedades de *Lupinus angustifolius* en México

# Lara-Rivera AH<sup>1</sup>, MA Ruiz-Lopez<sup>1</sup>, R Rodriguez-Macias<sup>1</sup>, C Soto-Velasco<sup>1</sup>, PM Garcia-López<sup>1</sup>, L Barrientos-Ramirez<sup>2</sup>, JF Zamora-Natera<sup>1</sup>

Abstract. Given that the agronomic potential of Lupinus angustifolius is not yet known in Mexico, we evaluated the yield and chemical composition of six L. angustifolius varieties (Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta) in Zapopan, Jalisco, Mexico. Studies were conducted during the Autumn-Winter seasons of 2012-2013. We identified the varieties with the highest and lowest protein concentrations and analyzed their respective amino acid profiles. The major protein constituents were determined by electrophoresis (SDS-PAGE). This experiment was conducted in an agricultural facility at the University of Guadalajara using a completely randomized block design and four replicates. The highest grain yield (kg/ha) was recorded on the Probor variety (4950 kg/ha) followed by Borlu (4940 kg/ha) and Sonate (4930 kg/ha). The Haags Blaue and Boruta varieties showed the lowest yields. There were considerable differences in seed protein content (P<0.05), with values ranging from 28.4 to 36.6% on Boruta and Probor, respectively. Analyses of amino acid composition showed that the Probor seeds had a higher concentration of lysine and methionine than Boruta (2.45 and 1.93 versus 1.09 and 1.63 g/100 g protein, respectively). Electrophoresis revealed prominent protein bands from 6 to 64 kDa for both varieties. The grain yield, chemical composition, and amino acid and protein concentrations of the tested varieties indicate that they could be successfully cultivated in the agricultural region of Zapopan, Jalisco, Mexico.

Keywords: Lupins; Chemical Composition; Yield; Amino Acid Analysis; Electrophoresis.

Resumen. Se evaluó el rendimiento y la composición química de seis variedades de Lupinus angustifolius (Haags Blaue, Boregine, Borlu, Probor, Sonate, y Boruta) en Zapopan, Jalisco, México dado que el potencial agronómico de dicha especie todavía no se conoce en dicho país. Los estudios se condujeron durante las estaciones de Otoño-Invierno de 2012-2013. Se identificaron las variedades con las mayores y menores concentraciones de proteína y analizaron sus respectivos perfiles de amino-ácidos. Los principales constituyentes proteicos se identificaron por electroforesis (SDS-PAGE). Este estudio se condujo en una unidad agrícola de la Universidad de Guadalajara, usando un diseño en bloques completamente al azar y cuatro réplicas. El mayor rendimiento de grano se registró en la variedad Probor (4950 kg/ha) seguida por Borlu (4940 kg/ha) y Sonate (4930 kg/ha). Las variedades Haags Blaue v Boruta mostraron los rendimientos más bajos. Hubo diferencias considerables en las concentraciones de proteína de la semilla (P<0,05), con valores que variaron entre 28,4 y 36,6% en Boruta y Probor, respectivamente. La composición de amino ácidos mostró que las semillas de Probor tuvieron una mayor concentración de lisina y metionina que Boruta (2,45 y 1,93 versus 1,09 y 1,63 g/100g de proteína, respectivamente). La electroforesis reveló bandas de proteína prominentes de 6 a 64 KDa en ambas variedades. El rendimiento de grano, composición química, y concentraciones de aminoácidos y proteínas de las variedades estudiadas indican que pueden ser exitosamente cultivadas en la región agrícola de Zapopan, Jalisco, México.

**Palabras clave:** Lupino; Composición Química; Rendimiento; Análisis de aminoácidos; Electroforesis.

<sup>1</sup>Departamento de Botánica y Zoología. Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara. CP. 45110, Zapopan, Jal., Mexico. <sup>2</sup>Departamento de Madera Celulosa y Papel. Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, C.P. 45220, Zapopan, Jal. Mexico. Address correspondence to: Juan F. Zamora-Natera, *e-mail:* jfzamora@cucba.udg.mx, Tel. 013337771192. Received 14.III.2016. Accepted 20.V.2017.

## INTRODUCTION

The genus *Lupinus* is one of the most diverse in the legume family; about 280 species are known (Eastwood et al., 2008) of which only *L. angustifolius*, *L. albus*, *L. luteus* and *L. mutabilis* are of agronomic interest (Reinhard et al., 2006). In general, lupins are characterized by their substantial adaptability to poor soils and cool climates (López-Bellido & Fuentes, 1986). Their seeds serve as a valuable source of protein, lipid, and fiber, for both animal and human nutrition in various parts of the world (Sujak et al., 2006). Typically, lupin seeds also contain a significant content of oligossacharides (Gulewicz et al., 2000). Since the recent development of varieties with low levels of alkaloids (sweet varieties), lupin consumption has increased and is now considered, at least in some parts of the world, to be a food ingredient with nutraceutical properties (Leterme, 2002; Martinez-Villalluenga et al., 2006a).

The grain yield and protein content of lupins vary with the species and variety, weather conditions, crop management, and soil types (Capraroa et al., 2008). Proteins isolated from lupin seeds largely comprise globulins and albumins (85% and 15% respectively). The protein constituent of seeds demonstrate a good balance of essential amino acids, and are considered a source of lysine, although they are generally poor in methionine, cysteine, and threonine (Martinez-Villaluenga et al., 2006b; Drakos et al., 2007; Gulewicz at al., 2008; Pisarikova et al., 2008).

Lupinus angustifolius is one of the most important grain legumes in Australia, which is considered to be the world's leading producer and exporter (DAFWA, 2010). Recently, its cultivation has spread to European countries, as well as Africa and South America, where lupins are now used as an alternative crop to soybean.

In Mexico, approximately 100 wild lupin species have been reported, although only 12 are native to Jalisco (Ruiz & Sotelo, 2001), but none of them is useful for consumption because of their high alkaloid content. As a way to increase the production of vegetable protein, especially in regions with cold climates and acidic soils, we have studied the adaptation of domesticated species of lupin in Mexico. The aim of this experiment was to evaluate the grain yield and chemical composition of six varieties of *L. angustifolius* using agricultural soil common to Zapopan, Jalisco. We identified the varieties with the highest and lowest protein concentrations, then analyzed their respective amino acid profiles, and the major proteins obtained by electrophoresis (SDS-PAGE).

## MATERIALS AND METHODS

**Plant material.** Seeds of the varieties under study (Sonate, Haags Blaue, Borlu, Probor, Boregine and Boruta) were provided by Saatzucht Steinach GmbH, which is a medium-size private breeding company in Germany. **Description of the experimental agricultural site.** Our agronomic evaluation was conducted during the autumnwinter cycle of 2012-13, using an experimental agricultural field at the University Center for Biological and Agricultural Sciences (with the Spanish acronym CUCBA), of the University of Guadalajara in Zapopan, Jalisco, Mexico.

This site is located at the geographic coordinates 20° 44' 47" N and 103° 30' 43" W, at an altitude of 1523 m.a.s.l. It is characterized by a temperate, humid climate, with summer rains. Rainfall varies from 700 to 1400 mm per year, with an average annual temperature of 12.0 to 18.0 °C, with frosts (García, 1988). The soil type is Regosol, with a high sand concentration (51.8%), a low concentration of organic matter (1.72%), and acidic pH (5.04). Table 1 shows some of the chemical and physical properties of the soil used in this experiment.

Table 1. Soil analyses during the experimental culture period.Tabla 1. Análisis de suelo durante el período de cultivo experimental.

Characteristics	Value
pH	5.04
Electrical conductivity dSm-1%	0.47
Organic matter %	1.72
Estimated nitrogen %	0.09
* Phosphorus mg/kg	68.53
Sodium (Na+) cmol(+)/kg	0.36
Potassium (K+) cmol(+)/kg	0.84
Calcium (Ca++) cmol(+)/kg	1.37
Magnesium (Mg++) cmol(+)/kg	0.18
Texture	Sand 51.8%
	Clay 20.9%
	Loam 27.3%
Method used	NOM-021-RECNAT-2000
	*Bray

**Experimental cultivation conditions.** After conventional land preparation with a moldboard plow and disk harrow, seeding was performed manually under an experimental randomized block design, with four replicates. We established plots 3.20 m wide x 8 m long (25.6 m<sup>2</sup> total area). Each plot comprised four rows, with 80 cm between rows, and 10 cm between plants (giving an approximate density of 12-13 plants/m<sup>2</sup>). After seeding, drip irrigation was performed at field capacity; this was repeated every 15 days, and suspended 10 days before harvest. Due to the limited presence of weeds, these were removed manually during cultivation. At the time of sowing, seeds were not inoculated and no fertilizers were used. This was because early studies reported that lupins are known for their ability to thrive on soils of low fertility (Dracup et al., 1998). Pesticide application was not necessary for

growth and development of the crop because of the low incidence of pests and diseases. Table 2 shows the minimum, maximum, and average temperatures during crop growth.

 Table 2. Precipitation, maximum and minimum temperatures, and

 radiation measurements during the experimental culture period.

 Tabla 2. Precipitación, temperaturas máxima y mínima, y mediciones

 de radiación durante el período de cultivo experimental.

Month	T max (°C)	T min (°C)	Rainfall (mm)	Radiation (cal/cm <sup>2</sup> )
November	24.9	9.3	0.4	157.4
December	16.8	5.4	2.0	179.6
January	14.6	5.7	0.5	309.3
February	22.6	8.8	0.0	421.8
March	29.2	10.3	0.0	610.6
April	32.3	12.9	0.0	535.2

Source: Weather station of experimental agricultural field (Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, México).

**Agronomic variables.** At maturity, all plants found in a linear meter of the central rows of each plot were harvested. Grain yield, number of pods per plant, number of grains per pod, and the 1000-grain weight were recorded.

**Proximate composition.** Dry seeds of each lupin variety were separately ground using a cutter mill to obtain flour of 0.5 mm in diameter. The following flour parameters were then assessed according to the methodologies detailed in AOAC (2000): moisture content (925.09), crude fat (920.30), crude protein (979.09), ash (923.03), crude fiber (962.09), and carbohydrate content.

Amino acid analyses. Amino acid composition was analyzed by HPLC according to Khayat et al., (1982). In these analyses we utilized Probor for their highest protein concentration (36.6%), and Boruta with the lowest value (28.4%). A sample equivalent of 2 mg of protein was hydrolyzed with 4 mL of 6N HCl for 21 hours at 110 °C under vacuum. To remove HCl, hydrolysates were concentrated using a rotary evaporator under vacuum, then rinsed twice with water and filtered with Millipore filters (Merck Millipore Mexico). Amino acids were dansylated using dansyl chloride [5-(DimethylAmino) Naphthalene-1-Sulfonyl chloride]. Aliquots of 20 µL were subsequently injected into an HPLC (Waters, Milford, MA, USA) using a WISP Autosampler 712 with a UV-VIS detector. A C-18 column was used as the stationary phase (Lichrosorb, 4.6 mm internal diameter x 250 mm in length, with 5 µm particles) with a mixture of acetonitrile (5%) with 4 mL of H<sub>3</sub>PO<sub>4</sub> and 31mM Na<sub>2</sub>HPO<sub>4</sub> at a pH of 7.6, as the mobile phase. The flow rate was 1 mL/min with an

acetonitrile gradient of 30 to 80%. Amino acids were identified by comparison of their retention times versus external standards (Sigma A-9656).

Protein isolates. Likewise were utilized Probor and Boruta for their highest and lowest protein content, respectively. Flour was defatted using n-hexane (1:20 w/v) for 4 hours in a Soxhlet apparatus, filtered, and then dried at room temperature. Flour (100g) was then mixed in 1L of distilled water containing 0.025% Na<sub>2</sub>SO<sub>2</sub> (with pH adjusted to 9.0 using 1N NaOH). The suspension was stirred for 1 hour at room temperature and then centrifuged at 10000 rpm for 10 min. The supernatant was then collected and the pH adjusted to 4.5 using 1N HCl. After a further 1 hour stirring, the solution was centrifuged at 10000 rpm for 10 min at 4 °C. Proteins were recovered, neutralized with 1N NaOH to pH 7, and immediately washed several times with distilled water, according to the method described by Martinez-Villaluenga et al. (2007). The protein precipitate was freeze dried (Labconco) and the content of soluble protein determined by the technique described by Lowry et al., (1985), using bovine serum albumin as a standard (BSA) (Sigma, USA).

**Protein electrophoresis.** Protein isolates (2 mg per variety) were analyzed by 12% SDS-PAGE under non-denaturing conditions (Laemmli, 1970), using a Protean II apparatus (Bio-Rad). Samples were denatured at 95 °C for 5 minutes (Bio-Rad, California, USA) with loading buffer containing 2-Mercaptoethanol (2%). Proteins were subsequently revealed by staining with Coomassie Brilliant Blue R250 solution, with molecular weight estimates made by inclusion of a reference mix of pre stained molecular weight protein standards (Standards, Kaleidoscope, Bio-Rad, Hercules, CA, USA).

Statistical analysis. Agronomic and chemical composition data were subjected to analyses of variance (ANOVA) using the Statgraphics Plus software (v.4.1). Tukey's test was used to determine the significance of differences between treatments at P<0.05.

#### RESULTS

**Yield components and grain yield.** Analyses of variance for the lupin varieties revealed significant differences (P<0.05) for the following variables: number of pods per plant, weight of 1000 seeds, and grain yield. The lowest number of pods per plant was found for the Haags Blaue variety (61.1 ± 13.2), with the highest value recorded for the Boregine variety (95.08 ± 4.47). The number of seeds per pod ranged from 3.2 ± 0.1 in Boregine to 4.4 ± 0.03 in Probor. The variety with the highest weight per 1000 seeds was Boregine (142.0 ± 6.5 g). Equivalent weights for the other varieties ranged from 119.0 ± 10.1g for Probor to 137.0 ± 14.4 g for Borlu. The highest grain yields (kg/ha) were achieved using the Borlu variety, with the lowest yield showed on Boruta (Table 3).

**Chemical analyses.** Significant differences in protein content for the different lupin varieties (P<0.05) were also identified. The highest protein content (dry basis) corresponded to Probor (36.6%), with the lowest value found for Boruta (28.4%). The Borlu, Sonate and Haags Blaue varieties were of intermediate protein values (33.4%, 32.5% and 32.3%, respectively). Ash content ranged from 3.1 to 3.5%, although no statistically significant differences were identified.

Values for oil concentrations (dry basis), which were statistically different (P<0.05) between varieties, varied from 3.9 to 5.3%, with Boruta showing the highest oil concentration, and Boregine the lowest. Crude fiber levels ranged from 8.1% in Haags blaue to 15.1% in Boruta (Table 4). The Boregine variety contained the highest carbohydrate concentration (51.7%), followed by Haags Blaue at 48.1%; the varieties with the lowest carbohydrate concentration were Probor and Sonate.

Amino acid concentration. While amino acid patterns were similar for both the Boruta and Probor varieties, their absolute values differed (Table 5). Phenylalanine, tyrosine, lysine, methionine, and alanine were found at greater quantities in Boruta than in Probor (with values of 4.27, 8.07, 2.45, 1.93, and 3.76 g/100 g protein, respectively). In contrast, the concentrations of isoleucine, leucine, valine, histidine, aspartic acid, and glutamic acid were higher in Probor than Boruta (with values of 3.12, 8.04, 2.36, 7.98, 4.94, and 17.48 g/100 g protein, respectively). The content of threonine, arginine, glycine, and serine were similar for both varieties.

**Protein pattern.** Figure 1 shows the electrophoretic profiles (SDS-PAGE) of protein isolates from Probor and Boruta under non-denaturing (lanes 1, 2). Under these conditions, the protein bands determined for the Boruta and Probor varieties were distributed between 6 and 64 kDa.

## DISCUSSION

**Yield components and grain yield.** The varieties tested in this study (Probor, Haags Blaue, Boruta, Borlu, Sonate and Boregine) have also been evaluated in other countries in terms of their yield and chemical composition. Yeheyis et al. (2012) in Kosober, Ethiopia, reported values of 17 to 38 pods per plant for the following varieties: Probor, Haags Blaue, Boruta,

Table 3. Yield components (average) for the indicated varieties of Lupinus angustifolius grown in the state of Jalisco, Mexico.Tabla 3. Componentes del rendimiento (promedio) para las variables indicadas de Lupinus angustifolius que crecieron en el estado de Jalisco,México.

Variety	Number of pods/plant	Number of seeds/pod	1000 seed weight (g)	Seed yield (kg/ha)
Haags Blaue	61.1 ± 13.2 b	3.9 ± 0.1 a	129.7 ± 4.0 ab	3650 ± 790 b
Boregine	95.1 ± 4.5 a	3.2 ± 0.1 a	142.0 ± 6.5 a	4710 ± 680 a
Borlu	72.0 ± 5.5 b	4.3 ± 0.3 a	137.0 ± 14.4 ab	4940 ± 440 a
Probor	81.8 ± 3.7 b	4.4 ± 0.03 a	119.0 ± 10.1 b	4950 ± 1820 a
Sonate	83.3 ± 18.7 b	4.0 ± 0.2 a	133.3 ± 16.0 ab	4930 ± 640 a
Boruta	67.8 ± 18.3 b	4.2 ± 0.1 a	116.7 ± 5.8 b	3630 ± 1380 b

Different letters in the same column indicate significant differences (P<0.05).

± Standard deviation

Table 4. Proximal chemical-composition (% dry basis) of varieties of *Lupinus angustifolius* grown in the state of Jalisco, Mexico. Tabla 4. Composición química (% sobre una base seca) de variedades de *Lupinus angustifolius* que crecieron en el estado de Jalisco, México.

Variety	Protein	Ash	Moisture	Oil	Crude fiber	*Carbohydrates
Haags blaue	32.3 b	3.5 a	3.6 b	4.4 b	8.1 c	48.1 a
Boregine	29.2 с	3.3 a	3.7 b	3.9 b	8.2 c	51.7 a
Borlu	33.4 b	3.1 a	4.4 a	4.2 b	10.1 b	44.5 b
Probor	36.6 a	3.3 a	3.8 b	4.2 b	9.9 ab	42.2 b
Sonate	32.5 b	3.5 a	3.8 b	5.3 a	12.5 a	42.4 b
Boruta	28.4 c	3.4 a	3.7 b	5.3 a	15.1 a	44.1 b

\*Carbohydrates obtained by difference (% dry basis); different letters in the same column indicate statistically significant differences (P<0.05).

Table 5. Amino acid composition of two *Lupinus angustifolius* varieties (g/100 g protein) grown in the state of Jalisco, Mexico. Tabla 5. Composición de amino ácidos de dos variedades de *Lupinus angustifolius* (g/100 g de proteína) que crecieron en el estado de Jalisco, México.

	Varieties			
Amino acid	Probor	Boruta		
Phe	2.657	4.269		
Tyr	3.458	8.077		
Ile	3.121	2.889		
Leu	8.045	6.484		
Lys	1.094	2.451		
Met	1.637	1.931		
Thr	14.355	14.046		
Val	2.361	1.506		
His	7.984	4.522		
Asp	4.946	3.352		
Glu	17.483	13.528		
Ala	3.293	3.763		
Arg	2.764	2.448		
Gly	6.339	6.147		
Ser	3.027	3.625		

Borlu and Boregine. In our study, the number of pods per plant for the same varieties ranged from 61 to 95. Other studies in Poland and Serbia recorded an average of 13 pods per plant, which is also lower than the average reported in this study (Mihailovic et al., 2008; Podlesny & Podlesna, 2011). Plausible explanations for the higher pod number reported here include differences in the agronomic and climate conditions during the growing season. In other countries, *L. angustifolius* varieties are cultivated under conditions of low to medium rainfall and at high plant density. In this experiment, a low plant density was used, with unlimited irrigation.

No significant differences between varieties were found in terms of seed number per pod; the values we report are consistent with those previously published by Clements et al. (2005), who noted that a pod might contain 3-7 seeds.

Weights per 1000 seeds (116-142 g) were comparable to those reported in different agro-ecological zones including Ethiopia, where values from 95.8 to 213.6 g were recorded for the Probor, Haags Blaue, Boruta, Borlu and Boregine varieties (Yeheyis et al., 2012). On the other hand, Stoddard (2011) reported a mean value of 174.6 g for the Boregine, Haags Blaue, and Boruta varieties, which is higher than that found in this study.

Previous investigations of the yield of *L. angustifolius* have revealed significant effects incurred by the genotype, year, and cultivation system. The average yield for all the varieties tested in this study was 4460 kg/ha, which is higher than that re-



Fig. 1. SDS-PAGE of *L. angustifolius* (Boruta variety) protein isolates, with major protein species revealed by staining with Coomassie Brilliant Blue. Lanes 1 and 2 indicate protein lysates resolved under non-denaturing conditions. Protein standards are shown in lane 3.

Fig. 1. SDS-PAGE de aislamientos de proteína de *L. angustifolius* (variedad Boruta) con las principales clases de proteínas reveladas utilizando el colorante Azul Coomassie Brilliant. Las columnas 1 y 2 indican roturas de proteínas determinadas bajo condiciones de no desnaturalización. Los estándares proteicos se muestran en la columna 3.

ported for the same varieties in Germany (3800 kg/ha) over a three year period (Jansen, 2015). On the other hand, our results are similar to those reported in Kosober-2, Ethiopia, of 4540 kg/ha using Probor, Haags Blaue, Boruta, Borlu and Boregine (Yeheyis et al., 2012).

Our data showed a high number of pods per plant, in comparison to values obtained in other countries, although grain yields were similar. The lower number of pods per plant reported in other studies is compensated for by a greater number of pods and seeds/m<sup>2</sup>, because of the use of higher plant densities. On the other hand, the high yields obtained in this experiment, indicate that climate and irrigation conditions were favorable for lupin cultivation.

**Chemical analyses.** The mean crude protein concentrations found in this study are comparable with those reported in other countries, including 28.0-35.6% for Poland, Finland, and Ethiopia (Sujak et al., 2006; Yeheyis et al., 2012; Saastamoinen et al., 2013). In particular, the Probor variety had the highest seed crude protein concentrations of the six varieties (36%), which was comparable with protein values reported for Probor in Merawi and Finoteselam, Ethiopia (35.5 and 34.9 %, respectively; Yeheyis et al., 2012). However, in Ethiopia, the Haags Blaue variety recorded the lowest protein concentrations (27%), whereas our data revealed a much higher protein concentrations for this variety (32%) (Yeheyis et al., 2012).

The average concentration of oil recorded in this study (4.5%) was much lower than that reported previously by Sujak et al. (2006) (7%) for eight lupin varieties grown in Poland. Beyer et al. (2015) also reported a mean oil concentration of 6.3% in 50 genotypes, including the varieties assayed in this study. In this work, the protein concentration and yield did not show a relationship with the oil concentration. Therefore, these differences could be attributed to other factors, including temperatures, soil type, and rainfall during lupin growth. In this regard, Williams (1979) reported that oil percentage is subject to environmental effects. Although the lupin varieties cultivated in this study had a lower oil concentration, their high mean seed protein concentration make *Lupinus angustifolius* an alternative species for production, and a viable alternative for animal nutrition.

The crude fiber values were lower than those reported in Finland for the Haags blaue and Sonate varieties (23.9 and 15.7%, respectively: Saastamoinen et al., 2013). However, Sujak et al. (2006) reported comparable values to those found in this research (11.6-14.1%), when evaluating eight distinct varieties of lupin, in Poland. The results for carbohydrate concentration reported in this study are similar to those reported for other varieties of *L. angustifolius* (41.0-51.0%) (Sujak et al., 2006), and lower than those found in other legumes such as peas, lentils and beans (55%) (de Almeida et al., 2006; Moon, 2007).

Amino acid concentration. In general, the seeds of Lupinus species contain a good balance of essential amino acids (Drakos et al., 2007). They are considered to be a good source of lysine, although they are generally low in sulfur-containing amino acids (methionine and cysteine) (Gulewicz et al., 2008), and threonine (Pisariková et al., 2008). The concentrations of amino acids in Probor and Boruta are similar to those reported by Martinez-Villaluenga et al. (2006b), except for leucine. In general, there is similarity with the amino acid concentration reported by Lqari et al. (2004). However, the levels of some amino acids were found to be higher than those reported by Lqari et al., (2004), who measured 2.7 and 4.9 g/100g of protein for histidine and threonine, respectively. The amount of arginine found in the Probor and Boruta varieties were comparatively low at 2.76 and 2.45 g/100 g, respectively, which disagrees with previous reports where values as high as 11 g/100 g were shown (Sujak et al., 2006; Yeheyies et al., 2012; Lgari et al., 2004). In addition, Sujak et al. (2006) reported that sweet lupine seeds cultivated in Poland were deficient in sulfur amino acids, as found for other legumes. Methionine levels in Boruta and Probor (1.64 and 1.93 g/100 g of protein, respectively) are comparable to data previously reported for other species of lupin (El-Adawy et al., 2001).

**Protein pattern.** The electrophoretic profiles (SDS-PAGE) of the protein isolates that we report are comparable to those identified by Vargas-Guerrero et al. (2014), and Garzon-de la Mora et al. (2008), using protein isolates of *L. albus.* The results are also consistent with data reported for *L. mutabilis* (Acuña et al., 1996).

Globulins are the main soluble proteins in leguminous seeds (Lgari et al., 2004). Lupinus albus, L. luteus, and L. angustifolius have all been shown to contain three main globulins:  $\gamma$ -conglutin,  $\beta$ -conglutin (known as vicilin), and  $\alpha$ -conglutin (known as legumin) (Santos et al., 1997). Studies of the protein content of *L. albus* have revealed the presence of  $\beta$ -conglutin, which is composed of monomers of a molecular weight ranging from 17 to 20 kDa, 25 to 46 kDa, and 53 to 64 kDa (Duranti et al., 2008). Furthermore, the 40-kDa bands represent  $\gamma$ -conglutin, while the bands at 53, 60, 66 and 70 kDa represent  $\alpha$ -conglutins (Melo et al., 1994). The presence of globulins in the seeds of the Boruta and Probor L. angustifolius varieties (Fig. 1) is suggested by their SDS PAGE profile, with banding patterns at 10-15 kDa, 20-30 kDa, and 35-50 kDa, which probably correspond to the three monomers of  $\beta$ -conglutin. The 40 kDa bands likely indicate y-conglutin, while the higher molecular weigh bands (>50 kDa) could represent the  $\alpha$ -conglutins. Further protein analyses are needed to corroborate these findings. In studies with seeds of L. angustifolius, Lqari et al. (2004) reported that the globulins were the most abundant species, with the 60 kDa  $\alpha$ -conglutin protein being the most abundant species, comprising 76.6% of the seed's protein reserve.

In conclusion, the results obtained in this study, in terms of grain yield and seed composition for the varieties, suggest that climate and soil conditions in Zapopan, Jalisco, Mexico, are compatible for cultivation of *L. angustifolius* under irrigation. The essential amino acid concentrations (leucine, histidine, isoleucine, threonine, and tyrosine) recorded for these varieties meet the requirements suggested by FAO. The protein profiles of the varieties under study are similar to other species of sweet lupin that have been commonly used for food. These characteristics are indicative of an important source of vegetable protein that could be used to obtain protein isolates for human consumption.

#### ACKNOWLEDGEMENTS

The authors thank the National Council for Science and Technology of Mexico for financial support and collaboration that made this work possible.

#### REFERENCES

Acuña, O., P. Castillo, M. Orbea, M. Cherrez & M. Guerrero (1996). Fraccionamiento de proteína de lupino por solubilidad y determinación de pesos moleculares (*Lupinus mutabilis* sweet). Simposio Iberoamericano sobre proteínas para alimentos (CYTED). Buenos Aires, Argentina, pp. 239-249.

- A.O.A.C. (2000). Official Methods of Analysis 17<sup>th</sup> Ed. Association of Official Analytical Chemists. Gaithersburg, Maryland, EUA.
- Beyer, H., H.U. Jurgens, G. Jansen, R. Uptmoor & F. Ordon (2015). Composition, environmental stability and potential of Genetic improvement of fatty acids on *Lupinus angustifolius*. *Journal of Applied Botany and Food Quality* 88: 192-196.
- Capraroa, J., C.H. Magni, M. Fontanesi, A. Budelli & M. Duranti (2008). Application of two-dimensional electrophoresis to industrial process analysis of proteins in lupin-based pasta. *LWT- Food Science and Technology* 41: 1011-1017.
- Clements, J.C., M. Dracup, B.J. Buirchell & C.G. Smith (2005). Variation for seed coat and pod wall percentage and other traits in a germplasm collection and historical cultivars of lupins. *Australian Journal of Agricultural Research* 56: 75-83.
- DAFWA (2010). Lupin in Western Australian farming. Department of Agriculture and Food, Government of Western Australian. Available in http://www.agric.wa.gov.au/PC\_93318.html
- De Luna-Jiménez, A. (2007). Composición y procesamiento de la soya para consumo humano. *Investigación y Ciencia de la Universidad Autónoma de Aguascalientes* 37: 35-44.
- De Almeida Costa, G.E., K.D. Queiroz-Monici, S.M. Machado Reis & A. Costa de Oliveira (2006). Chemical composition, dietary fibre and resistant starch contents of raw cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry* 94: 327-330.
- Dracup, M., N.C.Turner, C. Tang, M. Reader & J. Palta (1998). Responses to Abiotic Stresses. In: Gladstones J., Atkins CA. & Hamblin, J. (eds), pp. 227-262. Lupins as Crop Plant: Biology, Production and Utilization. CAB International, UK.
- Drakos A., G. Doxastakis & V. Kiosseoglou (2007). Functional effects of lupin proteins in comminuted meat and emulsion gels. *Food Chemistry* 100: 650-655.
- Duranti, M., A. Consonni, C. Magni, F. Sessa & A. Scarafoni (2008). The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science and Technology* 19: 624-633.
- Eastwood, R.J., C.S. Drummond, M.T. Schifino-Wittmann & C.E. Hughes (2008). Diversity and evolutionary history of lupins insights from new phylogenies. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, Fremantle, Western Australia, Sept 14-18. pp. 346-354.
- El-Adawy, T.A., E.H. Rahma, A.A. El-Bedawey & A.F. Gafar (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry* 74: 455-462.
- FAO/WHO (2007). Codex Alimentarius Commission. ftp://ftp.fao. org/codex/ Circular\_letters /CXCL2007/cl07\_31e.pdf.
- García, E. (1988). Modificaciones al sistema de clasificación climática de Köppen. Ind, México, DF.
- Garzón-de la Mora, P., G. Avalos-Alcantara, J.R. Villafán-Bernal, E.A. Maciel Hernández, C. Gurrola-Díaz, J. López, D.C. Brune, P.M. García-López & M. Ruiz-López (2008). Chemical physical Properties of Globulins and Γ-Conglutins Isolated at Different pH Values from *Lupinus albus*. Proceedings of the 12th International Lupin Conference, Fremantle, Western Australia, Sept 14-18. pp: 157-161.
- Gulewicz, P., D. Ciesiolka, J. Frias, C. Vidal-Valverde, S. Frejnagel, K. Trojanowska & K. Gulewicz (2000). Simple method of isolation and purification of α-galactosides from legumes. *Journal of Agriculture and Food Chemistry* 48: 3120-3123.

- Gulewicz, P., C. Martínez-Villaluenga, J. Frias, D. Ciesiołka, K. Gulewicz & C. Vidal-Valverde (2008). Effect of germination on the protein fraction composition of different lupin seeds. *Food Chemistry* 107: 830-844.
- Khayat, A., P.K. Redenz & L.A. Gorman (1982). Quantitative determination of amino acids in food by high-pressure liquid chromatography. *Food Technology* 36: 46-66.
- Kurlovich, B.S., F.L. Stoddard & P. Earnshaw (2008). Potential and problems of *Lupinus polyphyllus* Lindl. domestication. Proceedings of the 12th International Lupin Conference, Fremantle, Western Australia, Sept 14-18. pp. 304-307.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Leterme, P. (2002). Recommendations by health organizations for pulse consumption. *British Journal of Nutrition* 88: 239-242.
- López-Bellido, L. & M. Fuentes (1986). Lupin crop as an alternative source of protein. Advances in Agronomy 40: 239-289.
- Lowry, O.H., B. German & J.E. Kinsella (1985). A collaborative study to develop a standarized food protein solubility procedure. *Journal of Food Science* 50: 1715-1718.
- Lqari, H., J. Pedroche, J. Girón-Calle, J. Vioque & F. Millán (2004). Purification and partial characterization of storage proteins in *Lupinus angustifolius* seeds. *Grasas y Aceites* 4: 364-369.
- Martínez-Villaluenga, C., J. Frías & C. Vidal-Valverde (2006a). Functional lupin seeds (*Lupinus albus* L. and *Lupinus luteus* L.) after extraction of α-galactosides. *Food Chemistry* 98: 291-299.
- Martinez-Villaluenga, C., E. Sironi, C. Vidal-Valverde & M. Duranti (2006b). Effects of oligosaccharide removing procedure on the protein profiles of lupins seeds. *European Food Research and Technology* 223: 691-696.
- Martínez-Villaluenga, C., G. Urbano, J.M. Porres, J. Frías & C. Vidal-Valverde (2007). Improvement of food intake and nutritive utilization of protein from *Lupinus albus* var *multolupa* protein isolates supplemented with ascorbic acid. *Food Chemistry* 103: 944-951.
- Melo, T.S., R.B. Ferreira & A.N. Teixeira (1994). The seed storage proteins from *Lupinus albus*. *Phytochemistry* 37: 641-648.
- Mihailovic, V., A. Mikic, S. Katic, S. Vasiljevic, I. Pataki, D. Milic & D. Karagic (2008). Future challenges in breeding annual forage legumes in the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Proceedings International Conference Conventional and Molecular Breeding of Field and Vegetable Crops, Novi Sad, Serbia, 24-27. pp.438-442.
- Pisariková, B., Z. Zraly., F. Bunka & M. Trckova (2008). Nutritional value of white lupine cultivar Butan in diets for fattening pigs. *Veterinarni Medicina* 53: 124-134.
- Podlesny, J. & A. Podlesna (2011). Effect of rainfall amount and distribution on growth, development and yields of determinate and indeterminate cultivars of blue lupine. *Polish Journal of Agronomy* 4: 16-22.
- Reinhard, H., H. Rupp, F. Sager, M. Streule & O. Zoller (2006). Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food. *Journal of Chromatography A* 1112: 353-360.
- Ruíz-López, M.A. & A. Sotelo (2001). Chemical composition, nutritive value, and toxicology evaluation of Mexican wild lupins. *Journal of Agricultural and Food Chemistry* 49: 5336-5339.
- Santos, C.N., R.B. Ferreira & A.N. Teixeira (1997). Seed proteins of Lupinus mutabilis. Journal of Agricultural and Food Chemistry 45: 3821-3825.

- Saastamoinen, M., M. Eurola & V. Hietaniemi (2013). The Chemical Quality of Some Legumes, Peas, Fava Beans, Blue and White Lupins and Soybeans Cultivated in Finland. *Journal of Agricultural Science and Technology* 3: 92-100.
- Schumacher, H., H.M. Paulsen, A.E. Gau, W. Link, H.U. Jürgens, O. Sass & R. Dieterich (2011). Seed protein amino acid composition of important local grain legumes *Lupinus angustifolius* L., *Lupinus luteus* L., *Pisum sativum* L. and *Vicia faba* L. *Plant Breeding* 130: 156-164.
- Stoddard, F.L. & C.I. Lizarazo (2011). Introducing narrow-leafed lupin (*Lupinus angustifolius* L.) into Finnish cropping systems. *Proceedings of the 13th International Lupin Conference*. Lupin crops: an opportunity for today, a promise for the future, Poznan (Poland), June 6-10. pp: 141-143.
- Sujak, A., A. Kotlarz & W. Strobel (2006). Composition and nutritional evaluation of several lupin seeds. *Food Chemistry* 98: 711-719.
- Williams, W. (1979). Studies on the development of lupins for oil and protein. *Euphytica*, 28: 481-488.
- Yeheyis, L., C. Kijora, E. Van Santen, M. Wink, J. Danier & K.J. Peters (2012). Crude protein, amino acid and alkaloid contents of annual sweet lupin (*Lupinus* spp. L.) forages and seeds grown in Ethiopia. *Australian Journal of Experimental Agriculture* 48: 414-427.
- Zamora-Natera, J.F., P. García-López, M.A. Ruíz-López, E. Salcedo-Pérez & R. Rodríguez-Macías (2009). Composición y concentración de alcaloides en *Lupinus exaltatus Zucc*. durante su crecimiento y desarrollo. *Interciencia* 34: 672-676.