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## Chemical composition and nutritional value of seeds of *Lupinus luteus* L., *L. angustifolius* L. and new hybrid lines of *L. angustifolius* L.

Elena BARTKIENE<sup>1</sup>, Vadims BARTKEVICS<sup>2,3</sup>, Vytaute STARKUTE<sup>1</sup>,  
Vita KRUNGLEVICIUTE<sup>1</sup>, Dalia CIZEIKIENE<sup>4</sup>, Daiva ZADEIKE<sup>4</sup>,  
Grazina JUODEIKIENE<sup>4</sup>, Zita MAKNIKIENE<sup>5</sup>

<sup>1</sup>Lithuanian University of Health Sciences  
Tilžės 18, Kaunas, Lithuania

<sup>2</sup>University of Latvia  
Kr. Valdemara 48, Riga, Latvia

<sup>3</sup>Institute of Food Safety, Animal Health and Environment  
Lejupes 3, Riga, Latvia

<sup>4</sup>Kaunas University of Technology  
Radvilėnų 19, Kaunas, Lithuania  
E-mail: dalia.cizeikiene@ktu.lt

<sup>5</sup>Vokė Branch, Lithuanian Research Centre for Agriculture and Forestry  
Žalioji 2, Trakų Vokė, Vilnius, Lithuania

### Abstract

The aim of the current study was to evaluate the chemical composition, nutritional value, fatty acid composition, macro- (Na, Mg, K, Ca) and microelements, including essential (Cr, Mn, Co, Ni, Fe, Zn, Se and Cu) and nonessential (Al, As, Sr, Cd, Pb and Ag) elements, in the seeds of the yellow lupine (*Lupinus luteus* L.) variety 'Vilčiai', the narrow-leaved lupine (*L. angustifolius* L.) variety 'Vilniai' and the six new hybrid lines (Nos. 1700, 1701, 1703, 1072, 1734 and 1800) bred from the narrow-leaved lupine (*L. angustifolius*) in Lithuania. Gas chromatography with a flame ionization detector showed that oleic and linoleic unsaturated fatty acids were the dominant fatty acids in lupine seeds, which accounted for on average 33.2% and 38.4% of the total fatty acids, respectively.

The highest protein content, measured by the Kjeldahl method, was detected in the 'Vilčiai' lupine seeds (40.8%) and for the tested hybrid lines it was by 20.1–25.7% lower. The content of alkaloids in the seeds of lupine varieties 'Vilčiai' and 'Vilniai' was 0.021% and 0.030%, respectively; while in the new hybrid lines of *L. angustifolius* the content of alkaloids was less and varied from 0.011% to 0.012%. Analysis of macro- and microelements of lupine seeds performed using inductively coupled plasma mass spectrometry revealed that the new hybrid lines had the highest content of Na (from 1.07 to 1.19 mg g<sup>-1</sup> dry mass (d.m.), respectively), whereas the highest content of Mg and K was found in 'Vilčiai' seeds (3.44 and 13.9 mg g<sup>-1</sup> d.m., respectively). Among the *L. angustifolius* hybrid lines tested, the highest content of Mg and K in the seeds was determined for the four hybrid lines Nos. 1701, 1703, 1072 and 1734.

The highest contents of Mn, Fe, Zn and Se were observed in 'Vilčiai' seeds (147.65, 73.52, 59.84 and 0.13 µg g<sup>-1</sup> d.m., respectively). The concentration of nonessential elements in the tested lupine seeds was far below the levels that cause a health risk.

Key words: fatty acid, inductively coupled plasma mass spectrometry, lupine, macro- and microelements, nutrition value.

### Introduction

The demand of the ever-growing world population for protein foods is no longer sustainable through animal products alone. To compensate for this deficiency, soya bean has become the prevalent source of plant proteins for food and feed. Europe has become

heavily dependent on soya bean imports, entailing trade agreements and quality standards that do not fully satisfy the European citizens' expectations. During the recent years human consumption of lupine seeds has increased worldwide as lupine seeds are a good source of nutrients,

not only proteins but also lipids, dietary fibre, minerals, and vitamins. There are variations in the protein content between species and cultivars, the growing conditions and soil types (Martínez-Villaluenga et al., 2006). Although lupine belongs to the legumes and is not described as an oilseed crop, it has a considerable amount of oil in its seeds (Uzun et al., 2007).

Lupine seeds are a good source of macro- and microelements. Essential elements are classified as macronutrients (N, P, K, Ca, Mg and S) and micronutrients (Fe, Cu, Mn, Zn, B, Mo, Ni and Cl), and the classification is based on the relative abundance in plants. Heavy metals and metalloids represent a series of environmental hazards worldwide (Ehsan et al., 2015). The accumulation of heavy metals in plants is related to the concentration and chemical fractions of the metals in soils (Ehsan et al., 2007). *Lupinus* species have shown a relatively high tolerance to various environmental stresses, nitrate excess, low root temperature, lime excess and salinity, and therefore could be cultivated worldwide. Some *Lupinus* species are able to accumulate Zn, Cd, Mg and Al, Hg (Esteban et al., 2008), Pb and Cr (Ximenez-Embun et al., 2001), therefore new species of lupine should be examined before application for food. White lupine (*Lupinus albus* L.) tolerance to Cd and As with few toxic visual symptoms has been reported, as well as its ability for phosphate assisted phytoextraction of As and for phytostabilization of acidified multi-contaminated soils (Vazquez et al., 2006). Ximenez-Embun et al. (2001) reported that *L. albus*, *L. luteus*, *L. angustifolius* and *L. hispanicus* were able to grow under extreme conditions (wastewater, pH lower than 2) and to remove 98% of the initial amount of toxic metals present in the sample. The presence of quinolizidine alkaloids and some anti-nutritional factors hinders lupine consumption in the form of raw seeds. To make the lupine suitable for human consumption, different modern and traditional processing methods have been developed. Among the methods, soaking after roasting, boiling, germination, fermentation and alkaline treatments can be mentioned (Erbaş, 2010).

In Lithuania, the narrow-leaved lupine breeding program was started in 1995 at Vokė Branch of Lithuanian Institute of Agriculture (currently – Lithuanian Research Centre for Agriculture and Forestry). The breeding work is done in three directions: 1) low-alkaloids narrow-leaved lupine lines bred for food industry, 2) low-alkaloids narrow-leaved lines bred for animal feed and 3) narrow lupines lines bred for green manure. The seeds of hybrid lines Nos. 1675, 1700, 1702 and 1703 have low content of alkaloids (0.039–0.064%) (Maknickienė, Ražukas, 2007). Hybrid lines Nos. 1672, 1719 and 1700 were tested as an ingredient for wheat bread, and it was found that addition of up to 10% of these seeds can improve wheat bread quality (Bartkiene et al., 2013). The chemical composition of lupine seeds is one of the decisive factors for deciding on further use in food or feed industry, therefore the chemical composition of seeds of new hybrid lines should be examined before application in food industry.

The aim of the study was to evaluate the chemical composition, nutritional value, fatty acids composition, macro- and micronutrients in the seeds of the yellow lupine (*Lupinus luteus* L.) variety ‘Vilčiai’, the narrow-leaved lupine (*L. angustifolius* L.) variety ‘Vilniai’ and the six new hybrid lines (Nos. 1700, 1701, 1703, 1072, 1734 and 1800) bred from the narrow-leaved lupine *L. angustifolius* in Lithuania.

## Materials and methods

**Materials.** The seeds of lupine (*Lupinus* L.) varieties ‘Vilčiai’ and ‘Vilniai’ and hybrid lines Nos. 1700, 1701, 1703, 1072, 1734 and 1800 bred at the Vokė Branch of Lithuanian Research Centre for Agriculture and Forestry (Trakų Vokė, Lithuania) were analysed in this study. The research was conducted in 2014–2015.

**Evaluation of proximate composition of lupine seeds.** Chemical composition of lupine seeds was investigated according to the ICC standard methods. Moisture content was determined by drying the samples at  $105 \pm 2^\circ\text{C}$  to constant weight (ICC 109/01:1976. Determination of the moisture content of cereals and cereal products). Ash content was determined by calcinations at  $900^\circ\text{C}$  (ICC 104/1:1990. Determination of ash in cereals and cereal products). Nitrogen content was determined using Kjeldahl method with a factor of 5.7 to determine protein content (ICC 105/2:2001. Determination of crude protein in cereals and cereal products for food and for feed). The total lipid content was determined by extraction in the Soxhlet apparatus (“Boeco”, Germany) with hexane technical grade (Fisher Scientific, USA) (ICC 136:1984. Cereals and cereal products – Determination of total fat content). Carbohydrates content in lupine seeds was calculated by the following formula:  $100 - (\text{weight in grams} [\text{protein} + \text{fat} + \text{water} + \text{ash}] \text{ in } 100 \text{ g of seeds})$ . Energy value was calculated by multiplying the content of protein, fat and carbohydrates by the appropriate factor – 4, 4 and 9 for protein, carbohydrates and fat, respectively. All the measurements of analysed samples were made in triplicate.

**Determination of alkaloids.** Determination of alkaloids concentration was carried out as described by Maknickiene et al. (2013).

**Analysis of fatty acid composition.** Fatty acid composition of lupine seed oil was determined using a gas chromatography-flame ionization detector GC-FID, gas chromatograph Agilent 6890N (Agilent Technologies, USA). Methyl esters of fatty acids were dissolved in anhydrous 99.5% (Sigma-Aldrich, Germany) cyclohexane (100 mg in 4 mL) and were prepared by transmethylation using 8 mL 1.5% sulphuric acid ( $\geq 95\%$ , Sigma-Aldrich, Germany) in the pure (99.9%) methanol (Sigma-Aldrich, Germany), and kept at  $60^\circ\text{C}$  for 12 h in the dark. Samples were cooled, shaken for 30 s and centrifuged for 10 min, at 3000 relative centrifugal force at  $17^\circ\text{C}$  and injected (100  $\mu\text{L}$  of the upper part of supernatant, diluted before in cyclohexane 1:9, respectively) into a capillary BPX90 column (60 m  $\times$  0.32 mm, ID  $\times$  0.25  $\mu\text{m}$  film thickness) (SGE, USA). The following conditions were used: flame

ionization detector – 280°C, H<sub>2</sub> flow – 40 ml min<sup>-1</sup>, air flow – 450 ml min<sup>-1</sup>, helium (carrier gas) flow – 1 ml min<sup>-1</sup>, injector – 250°C (split 1:10), oven temperature – 50°C (2 min), 4°C min<sup>-1</sup> to 245°C and 245°C for 15 min. The identification of fatty acid was carried out by retention times and expressed as percentage of the total peak area of all the fatty acids in the oil sample.

*Analysis of macro- and microelements of lupine seeds using inductively coupled plasma mass spectrometry (ICP-MS).* The seeds were milled and homogenised (final particle size ≤150 µm). For the analysis the following chemicals were used: nitric acid (concentration ≥69.0%), for-trace element analysis (Sigma-Aldrich, France), hydrogen peroxide, 30% w/w (weight/weight), extra pure (Scharlau, Spain), multielement standard solution V for ICP-MS calibration (Sigma-Aldrich, France). Agilent 7700x ICP-MS (Agilent Technologies, Japan), software *Mass Hunter Work Station* for ICP-MS, version B.01.01 (Agilent Technologies, Japan) were used for analysis. For sample preparation for ICP-MS analysis, 0.3 g of milled lupine seeds was accurately weighed in a microwave vessel. 2 mL of de-ionized water, 8 mL of concentrated nitric acid and 2 mL of concentrated hydrogen peroxide were added and waited for 2–8 h for reaction stabilization until the formation of bubbles had finished. The vessel was sealed and heated in the microwave system. The following thermal conditions were applied: 150°C temperature was reached in approx. 20 min and remained such for 30 min, and then 200°C was reached in approx. 20 min and remained such for 30 min for the completion of specific reactions. After cooling (approx. 40 min) the prepared solution was filtered through the filter with a pore size of 8–10 µm. The solution was transferred to a 50 mL volumetric flask and filled with water to 50 mL volume. The following operating conditions of Agilent 7700x ICP-MS were used for the analysis of

the samples: plasma mode – normal, robust; RF forward power 1300 W; sampling depth 8.00 mm; carrier gas flow 0.6 L min<sup>-1</sup>; dilution gas flow 0.4 L min<sup>-1</sup>; spray chamber temperature 2°C; extraction lens 1 V; kinetic energy discrimination 3V.

*Statistical analysis.* In order to evaluate the influence of variety and hybrid line on the chemical composition of lupine seeds, the data were subjected to one-way analysis of variance (ANOVA), statistical program SPSS, version 11.0 (SPSS Inc., USA). Differences between groups were considered significant at  $p < 0.05$ .

## Results and discussion

*Proximate composition and energy value of analysed lupine seeds.* Proximate composition of lupine seeds is presented in Table 1. The highest protein content was found in the *Lupinus luteus*, variety ‘Vilčiai’ (40.8%), while in *L. angustifolius* seeds protein content was lower (from 20.1% to 25.7%). Similar results of protein content were reported by Porres et al. (2007). The least content of proteins was observed for hybrid lines Nos. 1700 and 1800 (30.3 ± 0.11% and 30.3 ± 0.13%, respectively). According to literature, there is considerable variation of protein content among lupine species (from 24% to 61%), for example yellow lupine seeds contain 39–47% and blue lupine 31–38% of proteins. Moreover, there are variations in the protein content between species and cultivars, growing conditions and soil types (Martínez-Villaluenga et al., 2006). According to literature, lupine seed protein has a relatively good amino acid profile with high content of arginine (4.1–11.2%), leucine (7.5–9.4%), lysine (4.3–5.2%) and phenylalanine (3.0–6.8%). Among pulses, lupine ranked third in protein quality after soybean and chickpea (Nwokolo, Smartt, 1996.).

**Table 1.** Composition (%) of lupine seeds (dry mass)

Lupine varieties and hybrid lines	Proteins	Fat	Carbohydrates	Moisture	Ash	Alkaloids
<i>L. luteus</i> ‘Vilčiai’	40.8 ± 0.1 c	4.4 ± 0.1 a	41.6 ± 0.1 a	9.0 ± 0.1 a	4.3 ± 0.2 c	0.021 ± 0.005 c
<i>L. angustifolius</i> ‘Vilniai’	32.2 ± 0.1 b	5.8 ± 0.1 c	49.3 ± 0.1 b	9.7 ± 0.1 b	3.0 ± 0.2 a	0.030 ± 0.003 d
<i>L. angustifolius</i> hybrid lines						
No. 1701	31.0 ± 0.1 a	5.2 ± 0.1 b	50.2 ± 0.2 bc	10.4 ± 0.1 bc	3.2 ± 0.2 b	0.012 ± 0.002 b
No. 1072	31.9 ± 0.7 ab	5.8 ± 0.1 c	47.4 ± 0.2 b	11.8 ± 0.1 e	3.2 ± 0.1 ab	0.012 ± 0.002 b
No. 1700	30.3 ± 0.1 a	6.4 ± 0.1 d	49.2 ± 0.1 b	11.1 ± 0.2 d	3.0 ± 0.2 a	0.011 ± 0.002 a
No. 1703	32.0 ± 0.1 ab	7.0 ± 0.1 de	46.9 ± 0.2 b	11.1 ± 0.2 d	2.9 ± 0.2 a	0.012 ± 0.002 b
No. 1734	32.6 ± 0.1 b	6.7 ± 0.1 d	47.7 ± 0.2 b	9.9 ± 0.2 b	3.1 ± 0.1 ab	0.011 ± 0.002 a
No. 1800	30.3 ± 0.1 a	5.6 ± 0.2 c	50.8 ± 0.3 bc	10.4 ± 0.1 bc	2.9 ± 0.1 a	0.012 ± 0.002 b

*Notes.* Data expressed as means (n = 5) ± SD (standard deviation). Values within each group in the same superscript letter are not different at  $P \leq 0.05$ .

Alkaloid content in the seeds of *L. angustifolius* hybrid lines was in range 0.011–0.012%, whereas in the seeds of *L. luteus* variety ‘Vilčiai’ and *L. angustifolius* variety ‘Vilniai’ the contents of alkaloids were 0.021% and 0.030%, respectively. Higher contents of alkaloids were reported by Maknickiene et al. (2013) in *L. luteus* variety ‘Trakiai’ (0.022%) and *L. angustifolius* line No. 1702 (0.085%).

Higher carbohydrate content was observed in *L. luteus* variety ‘Vilčiai’ (41.6%) compared with *L. angustifolius* variety ‘Vilniai’ (49.3%), whereas in two hybrid lines (Nos. 1701 and 1800) bred from *L. angustifolius* the content of carbohydrates was higher (50.2% and 50.8%, respectively).

The highest content of mineral elements was found in ‘Vilčiai’ seeds (4.25%), whereas the lowest

content was observed in the seed of line No. 1800 (2.93%). The lowest fat content was found in 'Vilčiai' seeds (4.4%), while the highest fat content was in the hybrid lines Nos. 1700, 1703 and 1734 (6.4, 7.0 and 6.7 %, respectively). According to Hudson (1979), *L. mutabilis* Sweet seeds are one of the richest in fats (13–23%), whereas the content of fat in other species such as *L. albus* (5–14%), *L. luteus* (5–7%) and *L. angustifolius* (4–8.5%) was found to be lower. In general, the content of fat in lupine is relatively high and only a few pulses like soybean exceed lupine in this respect. According to Uzun et al. (2007), the content of fat in lupine is ranked third after ground nut (*Arachis hypogaea* L.) and soybean (*Glycin max* L. Merrill) among the legumes. The high fat content confers a high energy value on lupine meal as food and feed. As dietary oil, lupine compares favourably with soybean and rape seed oils.

Energy value of *L. luteus* variety 'Vilčiai' and *L. angustifolius* variety 'Vilniai' was 369 and 378 kcal 100 g<sup>-1</sup> seeds, respectively, whereas in *L. angustifolius* hybrid lines energy value ranged in 369–382 kcal 100 g<sup>-1</sup> seeds. The highest energy value of seeds was observed in *L. angustifolius* hybrid line (No. 1734) – 382 kcal 100 g<sup>-1</sup> seeds.

Results of ANOVA test indicated that the contents of proteins (F(483.139) = 244.136,  $p = 0.0001$ ), fat (F(138.794) = 14.816,  $p < 0.0001$ ), carbohydrates

(F(686.995) = 174.986,  $p < 0.0001$ ), minerals (F(23.574) = 3.956,  $p < 0.0001$ ) and alkaloids (F(148.527) = 0.001,  $p < 0.0001$ ) in lupine seeds significantly depended on the hybrid line.

**Fatty acid composition of lupine seeds.** Fatty acid composition of lupine seeds is presented in Table 2. We found that the main fatty acids in hybrid lines bred in Lithuania are unsaturated fatty acids – oleic acid (C18:1) and linoleic acid (C18:2), which accounted for respectively 33.2 ± 3.9% and 38.4 ± 4.5% of the total content of fatty acids, whereas the concentrations of saturated fatty acids were lower. Monounsaturated fatty acids – palmitic acid (C16:0) and 10-pentadecenoic acid methyl ester (C20:1) were found only in the *L. luteus* variety 'Vilčiai', 1.3 ± 0.09% and 0.4 ± 0.05%, respectively. Palmitic acid (C16:0) content in lupine seeds was on average 10.8 ± 2.7% of the total fatty acid content, whereas the content of other saturated fatty acids was found significantly less. Similar results were reported by Uzun et al. (2007) as they found that among the unsaturated fatty acids from *L. albus* seed oleic and linolenic acids were dominant. High content of unsaturated fatty acids indicates that lupine can be a potential source of considerable amount of useful fats. Moreover, the high content of linoleic and oleic fatty acids make lupine seed a good source of essential fatty acids.

**Table 2.** Fatty acid composition (% of total fatty acids) of lupine seeds

Lupine varieties and hybrid lines	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	C22:0	C24:0+C20:5
<i>L. luteus</i> 'Vilčiai'	4.2 ± 0.1 a	2.4 ± 0.3 a	25.2 ± 0.2 a	48.0 ± 0.3 d	2.9 ± 0.2 f	7.9 ± 0.2 e	6.9 ± 0.3 e	0.8 ± 0.1 c
<i>L. angustifolius</i> 'Vilniai'	11.4 ± 0.1 c	6.5 ± 0.1 b	30.8 ± 0.2 b	41.7 ± 0.3 c	0.9 ± 0.1 a	6.1 ± 0.1 c	2.1 ± 0.2 b	0.5 ± 0.1 b
<i>L. angustifolius</i> hybrid lines								
No. 1701	11.6 ± 0.1 c	8.0 ± 0.2 d	32.6 ± 0.1 c	37.5 ± 0.2 b	1.2 ± 0.1 d	6.4 ± 0.2 cd	2.3 ± 0.1 c	0.4 ± 0.0 a
No. 1072	12.0 ± 0.1 cd	8.2 ± 0.2 d	33.4 ± 0.2 c	37.3 ± 0.2 b	1.0 ± 0.1 b	5.5 ± 0.2 b	2.1 ± 0.1 b	0.5 ± 0.0 b
No. 1700	11.7 ± 0.2 c	9.1 ± 0.2 e	36.0 ± 0.1 d	34.4 ± 0.3 a	1.1 ± 0.03 c	5.1 ± 0.1 a	2.1 ± 0.1 b	0.5 ± 0.0 b
No. 1703	12.0 ± 0.2 cd	7.5 ± 0.2 c	34.0 ± 0.1 c	38.3 ± 0.2 b	1.0 ± 0.0 b	4.9 ± 0.1 a	1.8 ± 0.2 a	0.5 ± 0.0 b
No. 1734	11.5 ± 0.2 c	8.2 ± 0.2 d	36.2 ± 0.2 d	35.2 ± 0.2 a	1.1 ± 0.0 c	5.3 ± 0.1 b	2.0 ± 0.3 b	0.5 ± 0.0 b
No. 1800	11.6 ± 0.3 c	7.4 ± 0.3 c	37.2 ± 0.2 d	34.8 ± 0.2 a	1.0 ± 0.0 b	5.4 ± 0.1 b	2.1 ± 0.3 b	0.5 ± 0.0 b
Average	10.8 ± 2.7 b	7.2 ± 2.1 c	33.2 ± 3.9 c	38.4 ± 4.5 b	1.3 ± 0.7 e	5.8 ± 1.0 c	2.7 ± 1.7 d	0.5 ± 0.1 b

Notes. Data expressed as means (n = 5) ± SD (standard deviation). C16:0 – palmitic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2 – linoleic acid, C20:0 – arachidic acid, C18:3 –  $\alpha$ -linolenic acid, C22:0 – behenic acid, C24:0+C20:5 – lignoceric acid + eicosapentaenoic acid, C15:1 – ginkgolic acid, C20:1 – eicosenoic acid. Values within each group in the same superscript letter are not different at  $P \leq 0.05$ .

Results of ANOVA test indicated a significant influence of variety and hybrid line on palmitic acid (C16:0) (F(854.266) = 148.201,  $p = 0.0001$ ), stearic acid (C18:0) (F(260.178) = 89.696,  $p < 0.0001$ ), oleic acid (C18:1) (F(1450.286) = 310.905,  $p < 0.0001$ ), linoleic acid (C18:2) (F(1064.961) = 432.840,  $p < 0.0001$ ), arachidic acid (C20:0) (F(157.827) = 9.225,  $p < 0.0001$ ),  $\alpha$ -linolenic acid (C18:3) (F(128.186) = 19.785,  $p < 0.0001$ ), behenic acid (C22:0) (F(203.897) = 61.605,  $p < 0.0001$ ), and lignoceric acid + eicosapentaenoic acid (C24:0+C20:5) (F(17.512) = 0.285,  $p < 0.0001$ ) % of total fatty acid in lupine seeds.

**Macro- and microelements of lupine seeds.** Macroelements such as Na, Mg, K and Ca are essential for

a wide variety of metabolic and physiological processes in the human body; therefore should be included in daily diets to prevent chronic diseases (Williams, 2006; Alsafwah et al., 2007). The content of macroelements in lupine seeds depended on lupine variety and hybrid line (Table 3). The highest content of Na was observed in hybrid lines Nos. 1700, 1701, 1703, 1072, 1734 and 1800 (from 1.07 ± 0.04 to 1.19 ± 0.04 mg g<sup>-1</sup>). While Na is an essential nutrient, dietary intakes in Europe today far exceed nutritional requirements. Mean daily Na intakes of populations in Europe range from about 3–5 g and are well in excess of dietary needs (about 1.5 g per day in adults, according to Dietary reference intakes... (DRI, 2005). The content of Na in lupine seeds did not

exceed the recommended level; therefore the tested lupines are not harmful for human health. The highest content of Mg and K was observed in 'Vilčiai' seeds,  $3.44 \pm 0.09$  and  $13.9 \pm 0.12$  mg g<sup>-1</sup> d.m., respectively. The content of Mg ranged from 2.00 (in the seeds of 'Vilniai') to  $3.44$  mg g<sup>-1</sup> d.m. (in the seeds of 'Vilčiai'). The lowest content of K and Ca was found in the seeds of 'Vilniai' –  $12.6$  and  $1.46$  mg g<sup>-1</sup> d.m., respectively. The content of Ca and Na was the highest in the hybrid lines Nos. 1700, 1701, 1703, 1072, 1734 and 1800 – varied in

range  $1.65$ – $2.41$  and  $1.01$ – $1.19$  mg g<sup>-1</sup> d.m., respectively. Among the lines of *L. angustifolius*, the highest content of Mg and K was determined in the seeds of hybrid line No. 1700 ( $2.48$  and  $13.61$  g kg<sup>-1</sup> d.m., respectively). Porres et al. (2007) reported similar Mg and K values in *L. angustifolius* seeds. Dietary reference intakes... (DRI, 2005) recommendations for adults (31–50 years old) are 420, 4700 and 100 mg per day of Mg, K and Ca, respectively. The lowest coefficient of variation in lupine seeds was found for K (3.61%).

**Table 3.** Macroelements (mg g<sup>-1</sup> dry mass) in lupine seeds

Lupine varieties and hybrid lines	Na	Mg	K	Ca
<i>L. luteus</i> 'Vilčiai'	$0.93 \pm 0.06$ a	$3.44 \pm 0.09$ e	$13.91 \pm 0.12$ b	$1.93 \pm 0.25$ c
<i>L. angustifolius</i> 'Vilniai'	$1.01 \pm 0.09$ b	$2.00 \pm 0.11$ a	$12.63 \pm 0.09$ a	$1.46 \pm 0.14$ a
<i>L. angustifolius</i> hybrid lines				
No. 1701	$1.07 \pm 0.04$ c	$2.45 \pm 0.14$ d	$12.72 \pm 0.21$ a	$2.23 \pm 0.09$ d
No. 1072	$1.11 \pm 0.07$ c	$2.33 \pm 0.15$ c	$12.91 \pm 0.15$ a	$2.53 \pm 0.15$ f
No. 1700	$1.12 \pm 0.04$ c	$2.48 \pm 0.12$ d	$13.61 \pm 0.17$ b	$1.92 \pm 0.12$ c
No. 1703	$1.18 \pm 0.02$ d	$2.14 \pm 0.21$ b	$13.40 \pm 0.23$ b	$1.65 \pm 0.20$ b
No. 1734	$1.18 \pm 0.09$ d	$2.29 \pm 0.23$ c	$13.49 \pm 0.20$ b	$2.41 \pm 0.08$ e
No. 1800	$1.19 \pm 0.04$ d	$2.44 \pm 0.17$ cd	$13.30 \pm 0.19$ ab	$2.04 \pm 0.06$ c
Statistical analysis				
Mean	1.10	2.45	13.23	2.02
Standard deviation (SD)	0.09	0.43	0.48	0.36
P value (two tailed)			<0.0001	
Coefficient of variation %	8.38	17.75	3.61	17.99

Note. Data expressed as means (n = 5) ± SD; significant, when  $P \leq 0.05$ . Values within each group in the same superscript letter are not different at  $P \leq 0.05$ .

Krejpcio et al. (2006) analysed *L. angustifolius* variety 'Baron' and observed similar content of Ca ( $2.71$  mg g<sup>-1</sup> d.m.), whereas the content of Mg ( $1.74$  mg g<sup>-1</sup> d.m.) was determined significantly lower in this variety compared with Lithuanian varieties of *L. angustifolius*. Moreover, Ca content was observed higher in the seeds of *L. angustifolius* variety 'Vilniai' and hybrid lines in comparison with *L. angustifolius* var. *troll* and *L. angustifolius* var. *emir*. Results of ANOVA test indicated that there is a significant influence of variety and hybrid line on Na ( $F(6.798) = 0.178$ ,  $p = 0.001$ ), Mg ( $F(22.331) = 3.959$ ,  $p < 0.0001$ ), K ( $F(20.526) = 4.436$ ,  $p < 0.0001$ ) and Ca ( $F(17.913) = 2.776$ ,  $p < 0.0001$ ) content in seeds. The contents of Zn and Fe reported in *L. angustifolius* variety 'Baron' were similar to those in the hybrid lines.

The content of Zn and Fe in the seeds of the *L. luteus* L. variety 'Vilčiai' was observed significantly higher ( $59.84$  and  $73.52$  µg g<sup>-1</sup> d.m., respectively), but did not exceed the dietary recommendations (DRI, 2005). As a result, the tested lupine seeds could be recommended in human diet as a source of essential elements such as Zn and Fe. Essential and non-essential microelements in lupine seeds are presented in Table 4.

In humans and animals, Cr is an essential nutrient that plays a significant role in a metabolism of glucose, fat and protein through potentiation of insulin action. There is limited data on which to base tolerable daily intakes for chromium. The highest concentration of chromium was observed in the seeds of hybrid line No. 1703 ( $1.81$  µg g<sup>-1</sup> d.m.), whereas the lowest concentration was in the seeds of 'Vilčiai' variety ( $0.73$  µg g<sup>-1</sup> d.m.).

The content of Mn in lupine seeds strongly depended on the field conditions and the species. Hung

et al. (1987) examined 33 samples of seeds from two species of lupin and found that *L. angustifolius* had a much lower Mn content ( $61$  µg g<sup>-1</sup> d.m.) than *L. albus* ( $1316$  µg g<sup>-1</sup> d.m.). The content of Mn in the tested Lithuanian lupine seeds varied from  $28.1$  (hybrid line No. 1701) to  $147.7$  (variety 'Vilčiai') µg g<sup>-1</sup> d.m.

The highest Fe and Zn concentrations ( $73.52$  and  $59.84$  µg g<sup>-1</sup> d.m., respectively) were found in the seeds of lupine variety 'Vilčiai'. The concentration of Ni in the investigated lupine seeds ranged from  $1.25$  (hybrid line No. 1701) to  $2.31$  (hybrid line No. 1703) µg g<sup>-1</sup> d.m. Shah et al. (2011) found that deficiency of Ni can lead to anaemia as Ni is considered synergistic to Fe by promoting its intestinal absorption. The highest concentration of Ni was found in the two lines Nos. 1703 and 1734. Deficiencies of only four elements – cobalt as vitamin B12, I, Fe and Zn – occur with known sufficient frequency in humans so as to be of concern to health professionals (Trace elements..., 1996). The concentration of Co in lupine seeds ranged from  $0.06$  (hybrid line No. 1800) to  $0.18$  (hybrid line No. 1703) µg g<sup>-1</sup> d.m.

Se is an essential trace element and integral part of many antioxidant enzymes such as glutathioneperoxidase and selenoprotein P in humans and animals, therefore the deficiency of Se leads to various clinical consequences including cancer, cardiovascular diseases, type 2 diabetes and lung disorders (Kumar, Priyadarsini, 2014). The highest content of Se was found in the seeds of hybrid line No. 1703 and 'Vilčiai' ( $0.16$  and  $0.13$  µg g<sup>-1</sup> d.m., respectively). Certain elements are essential for human health but if present in excessive concentrations they become toxic (Zelalem, Chandravanshi, 2014). The

**Table 4.** Microelements ( $\mu\text{g g}^{-1}$  dry mass) in lupine seeds

Lupine varieties and hybrid lines	Essential microelements								Non-essential microelements					
	Mn	Fe	Zn	Cu	Ni	Cr	Se	Co	Sr	Al	Pb	Cd	As	Ag
<i>L. luteus</i> L. 'Vilčiai'	147.65 ± 0.30 e	73.52 ± 0.24 d	59.84 ± 0.08 e	8.07 ± 0.11 e	1.80 ± 0.11 e	0.73 ± 0.09 a	0.13 ± 0.02 c	0.14 ± 0.02 e	16.60 ± 0.21 e	1.19 ± 0.07 b	0.07 ± 0.01 b	0.04 ± 0.01 b	0.03 ± 0.01 b	<0.005
<i>L. angustifolius</i> L. 'Vilniai'	88.16 ± 0.15 c	53.06 ± 0.15 a	36.90 ± 0.23 a	5.53 ± 0.18 b	1.61 ± 0.13 d	1.19 ± 0.07 c	0.09 ± 0.01 a	0.12 ± 0.03 d	11.26 ± 0.15 b	1.73 ± 0.12 c	0.06 ± 0.02 a	0.04 ± 0.01 b	0.03 ± 0.03 b	<0.005
<i>L. angustifolius</i> L. No. 1701	28.13 ± 0.19 a	52.54 ± 0.21 a	35.15 ± 0.17 a	5.10 ± 0.17 a	1.25 ± 0.09 a	1.38 ± 0.11 d	0.10 ± 0.01 b	0.03 ± 0.01 a	9.09 ± 0.13 a	0.94 ± 0.06 a	0.06 ± 0.02 a	0.03 ± 0.01 a	0.02 ± 0.01 a	<0.005
<i>L. angustifolius</i> L. No. 1072	122.82 ± 0.36 d	63.97 ± 0.19 c	53.66 ± 0.11 d	6.15 ± 0.09 c	1.51 ± 0.10 bc	1.40 ± 0.10 d	0.10 ± 0.02 b	0.09 ± 0.02 c	26.10 ± 0.09 g	6.00 ± 0.23 f	0.11 ± 0.02 d	0.03 ± 0.01 a	0.02 ± 0.02 a	<0.005
<i>L. angustifolius</i> L. No. 1700	90.19 ± 0.13 c	64.27 ± 0.17 c	40.92 ± 0.20 b	6.46 ± 0.15 cd	1.44 ± 0.13 b	1.32 ± 0.13 d	0.09 ± 0.01 a	0.10 ± 0.03 c	14.21 ± 0.14 d	4.00 ± 0.24 e	0.07 ± 0.01 b	0.04 ± 0.01 b	0.02 ± 0.02 a	<0.005
<i>L. angustifolius</i> L. No. 1703	123.23 ± 0.27 d	55.62 ± 0.13 ab	39.79 ± 0.11 b	6.44 ± 0.17cd	2.31 ± 0.12 g	1.81 ± 0.14 f	0.16 ± 0.01 d	0.18 ± 0.04 f	20.89 ± 0.20 f	2.99 ± 0.31 d	0.08 ± 0.01 c	0.04 ± 0.01 b	0.02 ± 0.04 a	<0.005
<i>L. angustifolius</i> L. No. 1734	118.84 ± 0.23 d	63.59 ± 0.19 c	45.17 ± 0.15 c	6.40 ± 0.13 c	1.95 ± 0.15 f	1.51 ± 0.21 e	0.09 ± 0.02 a	0.12 ± 0.02 d	28.49 ± 0.17 h	6.27 ± 0.19 f	0.08 ± 0.01 c	0.05 ± 0.01 c	0.03 ± 0.04 b	<0.005
<i>L. angustifolius</i> L. No. 1800	48.68 ± 0.14 b	54.05 ± 0.23 a	39.95 ± 0.23 b	6.03 ± 0.06 c	1.40 ± 0.09 b	0.91 ± 0.09 b	0.09 ± 0.01 a	0.06 ± 0.02 a	12.95 ± 0.14 c	1.79 ± 0.17 c	0.08 ± 0.01 c	0.04 ± 0.01 b	0.02 ± 0.01 a	<0.005
Statistical analysis														
Mean	95.96	60.08	43.92	6.273	1.659	1.281	0.106	0.105	17.45	3.114	0.076	0.038	0.023	–
Standard deviation (SD)	40.69	7.44	8.60	0.87	0.34	0.34	0.02	0.05	7.05	2.11	0.02	0.01	0.01	–
P value (two tailed)	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0002	0.0042	<0.0001	<0.0001	<0.0001	–
Coefficient of variation %	42.40	12.39	19.58	13.89	20.83	26.51	24.09	44.38	40.42	67.79	20.96	16.54	21.79	–

Note. Data expressed as means ( $n = 5$ )  $\pm$  SD; significant, when  $P \leq 0.05$ . Values within each group in the same superscript letter are not different at  $P \leq 0.05$ .

concentration of Ni in the tested lupine seeds did not exceed the concentrations that the European Food Safety Authority derived as an upper level for the intake of nickel (EFSA, 2006).

Among non-essential elements Al, As, Sr, Cd, Pb and Ag were evaluated.

The EFSA has established for the lifelong intake of Al a tolerable weekly intake of 1 mg per kg body weight (b.w.) (EFSA, 2008). The least content of Al was observed in the lupine seeds of variety 'Vilčiai' and hybrid line No. 1701 (1.19 and 0.94  $\mu\text{g g}^{-1}$  d.m., respectively) while hybrid lines contained higher content of Al, especially Nos. 1072 and 1734 (6.00 and 6.27  $\mu\text{g g}^{-1}$  d.m., respectively).

The concentration of Sr in seeds ranged from 9.09 (hybrid line No. 1701) to 26.10 (hybrid line No. 1072)  $\mu\text{g g}^{-1}$  d.m. The concentration of Ag was found below the detection limit in all lupine seed samples.

Cd is toxic to a wide range of organs and tissues, and a variety of toxicological endpoints (reproductive toxicity, neurotoxicity, carcinogenicity) have been observed in experimental animals and subsequently investigated in human populations (ATSDR, 2008). However, at a meeting in June 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) withdrew the provisional tolerable weekly intake and established

a provisional tolerable monthly intake of 25  $\mu\text{g kg}^{-1}$  b.w. (Joint FAO/WHO Expert Committee..., 2010). A monthly intake was established due to consideration of the long half-life of Cd, and consequently the small to negligible influence of daily ingestion on overall exposure. Concentration of Cd ranged from 0.026 (hybrid line No. 1701) to 0.049 (hybrid line No. 1734)  $\mu\text{g g}^{-1}$  d.m. in lupine seeds.

The concentration of Pb ranged from 0.06 (hybrid line No. 1701) to 0.11 (hybrid line No. 1072 seeds)  $\mu\text{g g}^{-1}$  d.m. in lupine seeds. In average adult consumers, lead dietary exposure ranges from 0.36 to 1.24, up to 2.43  $\mu\text{g kg}^{-1}$  body weight (b.w.) per day in high consumers in Europe (EFSA, 2010).

The risk-specific dose of 0.0086  $\mu\text{g kg}^{-1}$  b.w. per day, derived from the As concentration in drinking water determined to represent "negligible risk" (0.3  $\mu\text{g L}^{-1}$ ) by Canadian agencies (FPTCDW, 2006), is recommended. In the investigated lupine seeds arsenic concentration ranged from 0.015 (No. 1701 seeds) to 0.033 ('Vilčiai' seeds)  $\mu\text{g g}^{-1}$  d.m.

The concentration of nonessential elements in the tested lupine seeds was far below the levels that cause a health risk (EFSA, 2006; 2008; FPTCDW, 2006; Joint FAO/WHO Expert Committee..., 2010).

Results of *ANOVA* test indicated that variety and hybrid line has a significant effect on Al ( $F(363.070) = 93.559, p = 0.0001$ ), Cr ( $F(22.357) = 2.422, p < 0.0001$ ), Mn ( $F(90216.207) = 34772.709, p < 0.0001$ ), Fe ( $F(4508.221) = 1164.079, p = 0.0001$ ), Co ( $F(10.218) = 0.046, p = 0.0001$ ), Ni ( $F(26.274) = 2.506, p = 0.0001$ ), Cu ( $F(118.689) = 15.931, p = 0.0001$ ), Zn ( $F(7794.620) = 1553.663, p = 0.001$ ), Se ( $F(10.759) = 0.014, p = 0.001$ ) and Sr ( $F(6341.740) = 1044.511, p < 0.0001$ ) content in lupine seeds. Therefore, significant effect of variety and hybrid line on As, Cd and Pb content in lupine seeds was not found.

## Conclusions

1. The content of alkaloids in the seeds of lupine varieties 'Vilčiai' and 'Vilniai' was 0.021% and 0.030%, respectively; while in the new hybrid lines of *Lupinus angustifolius* the content of alkaloids was lower and varied from 0.011% to 0.012%.

2. The concentration of Mg and K in the seeds of *L. luteus* L. variety 'Vilčiai' was higher (3.44 and 13.91 g kg<sup>-1</sup> d.m., respectively) in comparison with *L. angustifolius* variety 'Vilniai' (2.00 and 12.63 g kg<sup>-1</sup> d.m., respectively). Higher contents of Ca and Na were detected in the seeds of hybrid lines Nos. 1700, 1701, 1703, 1072, 1734 and 1800 (varied in range 1.65–2.41 and 1.01–1.19 g kg<sup>-1</sup> d.m., respectively) in comparison with *L. luteus* variety 'Vilčiai' and *L. angustifolius* variety 'Vilniai'. Among the *L. angustifolius* hybrid lines the highest content of Mg and K was determined in the seeds of line No. 1700 (2.48 and 13.61 g kg<sup>-1</sup> d.m., respectively).

3. The highest energy value was established in the seeds of hybrid line No. 1734 (382 kcal 100 g<sup>-1</sup> seeds).

4. The dominant fatty acids in the seeds of *L. luteus* variety 'Vilčiai', *L. angustifolius* variety 'Vilniai' and hybrid lines are unsaturated fatty acids – oleic (C18:1) and linoleic (C18:2) (an average content, respectively, 33.2% and 38.4% of the total content of fatty acid). The seeds of the six hybrid lines of *L. angustifolius* Nos. 1700, 1701, 1703, 1072, 1734 and 1800 had higher content of oleic and linoleic acids compared with the *L. angustifolius* variety 'Vilniai'.

5. Higher carbohydrate content was observed in the seeds of the *L. luteus* variety 'Vilčiai' (41.6%) compared with the *L. angustifolius* variety 'Vilniai' (49.3%), whereas in the seeds of the two hybrid lines Nos. 1701 and 1800 the content of carbohydrates was higher (50.2% and 50.8%, respectively).

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## *Lupinus luteus* L., *L. angustifolius* L. bei *L. angustifolius* L. hibridų sėklų cheminė sudėtis ir mitybinė vertė

E. Bartkienė<sup>1</sup>, V. Bartkevics<sup>2,3</sup>, V. Starkutė<sup>1</sup>, V. Krunglevičiūtė<sup>1</sup>, D. Čižeikienė<sup>4</sup>, D. Žadeikė<sup>4</sup>,  
G. Juodeikienė<sup>4</sup>, Z. Maknickienė<sup>5</sup>

<sup>1</sup>Lietuvos sveikatos mokslų universitetas

<sup>2</sup>Latvijos universitetas

<sup>3</sup>Latvijos maisto saugos, gyvūnų sveikatos ir aplinkos institutas

<sup>4</sup>Kauno technologijos universitetas, Lietuva

<sup>5</sup>Lietuvos agrarinių ir miškų mokslų centro Vokės filialas

### Santrauka

Tyrimo tikslas – ištirti geltonžiedžio lubino (*Lupinus luteus* L.) veislės ‘Vilčiai’ bei siauralapio lubino (*L. angustifolius* L.) veislės ‘Vilniai’ ir siauralapio lubino (*L. angustifolius* L.) šešių naujų hibridų Nr. 1700, 1701, 1703, 1072, 1734 bei 1800, išvestų Lietuvoje, sėklų cheminę sudėtį, mitybinę vertę, riebalų rūgščių sudėtį, makro- ir mikroelementų kiekį. Taikant dujų chromatografijos su liepsnos jonizacijos detektoriumi metodą lubinų sėklose daugiausia buvo nustatyta nesočiųjų oleino ir linolo riebalų rūgščių, vidutiniškai 33,2 ir 38,4 % bendro riebalų rūgščių kiekio, o sočiųjų riebalų rūgščių buvo nustatyta gerokai mažiau. Didžiausias baltymų kiekis, įvertintas Kjeldalio metodu, buvo nustatytas veislės ‘Vilčiai’ lubinų sėklose (40,8 %), o lubinų veislės ‘Vilčiai’ sėklose ir hibriduose baltymų kiekis buvo nuo 20,1 iki 25,7 % mažesnis. Ekstrahuojant su heksanu mažiausias riebalų kiekis buvo veislės ‘Vilčiai’ lubinų sėklose – 4,4 %, o lubinų veislės ‘Vilčiai’ sėklose ir hibriduose riebalų kiekis buvo nuo 18,2 iki 59,1 % didesnis. Angliavandenių didžiausias kiekis buvo Nr. 1800 hibridų sėklose (50,8 %), mažiausias – veislės ‘Vilčiai’ sėklose (41,6 %). Alkaloidų kiekis siauralapio lubino (*L. angustifolius* L.) naujai išvestų hibridų sėklose buvo 0,011–0,012 %, o veislių ‘Vilniai’ ir ‘Vilčiai’ lubinų sėklose jis sudarė 0,030 ir 0,021 %. Lubinų sėklų mikro- ir makromineralinių medžiagų analizė, atlikta taikant induktyviai susietos plazmos masių spektrometrija, parodė, kad didžiausias kiekis natrio (Na) buvo nustatytas naujai išvestų lubinų hibridų (nuo 1,07 iki 1,19 mg g<sup>-1</sup> sausųjų medžiagų), didžiausi kiekiai magnio (Mg) ir kalio (K) – veislės ‘Vilčiai’ (atitinkamai 3,44 ir 13,9 mg g<sup>-1</sup> sausųjų medžiagų) sėklose. Didžiausi kiekiai Mg nustatyti *L. angustifolius* naujai išvestų hibridų sėklose. Be to, didžiausias kiekis biologiškai svarbių mikromineralinių medžiagų Mn, Fe, Zn ir Se buvo nustatytas veislės ‘Vilčiai’ sėklose, atitinkamai 147,65, 73,52, 59,84 ir 0,13 μg g<sup>-1</sup> sausųjų medžiagų. Lubinų sėklose biologiškai nesvarbių (neesminių) mineralinių elementų koncentracija nustatyta gerokai mažesnė už pavojų sveikatai sukelti kiekį.

Reikšminiai žodžiai: induktyviai susietos plazmos masių spektrometrija, lubinai, mitybinė vertė, makro- ir mikroelementai, riebalų rūgštys.

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