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Lupinus angustifolius L. cultivar "Boregine" from South of Bulgaria: a source of nutrients and natural biologically active components☆

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Abstract – Nowadays, the requirements of new sources of natural food components are constantly expanding worldwide. On one hand, the constituents derived from the common agriculture plants satisfy the needs of the body to function properly. On the other hand, the price of producing ordinary foods is gradually increasing. For that reason, it is necessary to find a cheaper alternative industrial crops, such as a specific variety of lupin (*Lupinus angustifolius* L. cultivar "Boregine"). The chemical and lipid composition of lupin seeds as well as the physicochemical characteristics of the oil were examined. The seeds are rich in proteins and carbohydrates, mainly starch, but have low oil content. Sucrose was the main soluble sugar and the major amino acids were phenylalanine, arginine, tyrosine and serine. Linoleic and oleic acids were predominate in the oil; β-sitosterol and γ-tocopherol were the main components in the sterol and tocopherol fractions, respectively. Phosphatidylinositol and phosphatidylcholine represented more than 50% of all phospholipids and oleic acid was in the highest amount in all phospholipid classes. All physicochemical characteristics of lupin seed oil were in agreement with the requirements for edible oils and its oxidative stability at 100 °C and an air flow rate of 20 L/h was extremely high (more than 100 h). Lupin seeds have high nutritional value and their oil depicts to be stable, which makes them a possible source of high quality lipids with long shelf life.

Keywords: *Lupinus angustifolius* L. cultivar "Boregine" / chemical composition / biologically active compounds / physicochemical characteristics / South of Bulgaria

Résumé – Le cultivar « Boregine » *Lupinus angustifolius* L. du Sud de la Bulgarie : une source de nutriments et de composants naturels biologiquement actifs. De nos jours, les besoins en nouvelles sources de composants alimentaires naturels sont en constante augmentation dans le monde entier. D'une part, les composants dérivés de plantes agricoles communes répondent aux besoins nutritionnels de l'homme. D'autre part, le prix de production des aliments de base augmente progressivement. Pour cette raison, il est nécessaire de trouver des cultures industrielles alternatives moins chères, comme une variété spécifique de lupin (*Lupinus angustifolius* L., cultivar « Boregine »). La composition chimique et lipidique des graines de lupin ainsi que les caractéristiques physicochimiques de l'huile ont été examinées. Les graines sont riches en protéines et en hydrates de carbone, principalement en amidon, mais présentent une faible teneur en huile. Le saccharose est le principal sucre soluble et les principaux acides aminés sont la phénylalanine, l'arginine, la tyrosine et la sérine. Les acides linoléique et oléique prédominent dans l'huile ; le β-sitostérol et le γ-tocophérol sont respectivement les principaux composants des fractions stérol et tocophérol. Le phosphatidylinositol et la phosphatidylcholine représentaient plus de 50 % de tous les phospholipides et l'acide oléique prédominait dans toutes les classes de phospholipides. L'ensemble des caractéristiques physico-chimiques de l'huile de graines de lupin répondait aux exigences des huiles alimentaires et sa stabilité oxydative à 100 °C et à un débit d'air de 20 L/h était extrêmement élevée (plus de

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100 h). Les graines de lupin possèdent une valeur nutritionnelle élevée et leur huile est stable, ce qui en fait une source possible de lipides de haute qualité avec conservation de longue durée.

Mots clés : *Lupinus angustifolius* L. cultivar « Boregine » / composition chimique / composés biologiquement actifs / caractéristiques physico-chimiques / sud de la Bulgarie

Highlights

The chemical and lipid composition of seeds from *Lupinus angustifolius* L. cultivar "Boregine" as well as physicochemical characteristics of the oil were examined in detail for the first time. The seeds have high nutritional value. The oil is rich in fat-soluble bioactive components and has long shelf life.

1 Introduction

Legumes are considered to be important plants, because they are a main source of proteins for the human body. They are widespread in Asia and America and are used as animal feed and human consumption. In the recent years, it has been observed a lack of protein consumption in Europe, which encourages the cultivation of legumes that are characterized with high protein content (25–31.33%) (Bakoglu *et al.*, 2009; Kökten *et al.*, 2010). The bean crops, in Bulgaria, occupy less area than wheat, and the most common are chickpeas, peas, lentils, beans, and soybeans, which are the richest in protein of all legumes. The proteins from the legumes are biologically complete because they contain all necessary amino acids as well as all of the essential ones. Protein, derived from beans, is the cheapest and can be used as an additive to increase the protein content of foods, feeds, etc. They are also unique foods because of their rich content of other nutrients such as starch, fibres, oligosaccharides and minerals.

Over the past few years, the cost of growing and producing conventional foods (corn, soybean, and wheat) are constantly increasing, so it is necessary to find a cheaper alternative way. There is an ongoing search for new raw materials that contain more essential fatty and amino acids; tocopherols, carotenoids, phospholipids and sterols that are not synthesized in the human body but are provided only by the food, and their balanced intake is important for protecting the health.

Therefore, there is an increasing interest in some non-traditional crops that could be the source of new functional food ingredients rich in valuable nutrients and biologically active substances. Such representatives are the plants from the Fabaceae family. Lupin seeds (*Lupinus angustifolius* L.) can be offered as an alternative to the main legumes. Lupin belongs to the Fabaceae family and is believed to contain all essential amino acids, making its protein preferable for consumption. Recently, lupin has become a source of protein in the food industry, and may even replace soybeans in some of the markets worldwide. According to some studies, these seeds are also useful in the prevention of cardiovascular disease because they have the potential to lower the cholesterol level (Starkute *et al.*, 2016).

Besides that, the climatic conditions in Bulgaria are favorable for the cultivation of lupin, especially in areas with acidic soils and where other legumes do not normally develop.

The studies on lupin seeds worldwide are focused mainly on their chemical composition as follow: oil content (3.88–12.58%), proteins (28.4–45.9%), carbohydrates (41.60–51.68%), starch (3.00–10.26%), fibres (8.05–22.20%), ash (2.9–4.8%), non-nitrogenous extracts (285.9–436.5 g/kg) (Mohamed and Rayas-Duarte, 1995; Bartkiene *et al.*, 2016; Sedláková *et al.*, 2016; Lara-Rivera *et al.*, 2017; Tarasenko *et al.*, 2017) and the lipid composition comprises the fatty acid composition, the total and individual tocopherol composition, and the total content of sterols and phospholipids. The composition of the seeds varies within certain limits and depends on the type of lupin, the geographical location, and the climatic conditions in which the respective plant species are grown.

Unsaturated fatty acids predominate in the fatty acid composition of triacylglycerols, such as oleic (31.9–66.2%), linoleic (13.7–48.3%), and linolenic (5.4–12.8%) (Hansen and Czochanska, 1974; Alamri, 2012; Rybiński *et al.*, 2018). The fatty acid composition also varies widely depending on the geographical area where the plant is grown. In the fatty acid composition of lipids from seeds of Russian lupin varieties, oleic acid prevails (57.0%) (Tarasenko *et al.*, 2017), while in those from the Baltic republics linoleic acid predominates (41.7–48.0%) (Bartkiene *et al.*, 2016). In the lipids of Egyptian lupin seeds, oleic acid is predominant (41.9%) and the content of linoleic is much lower (23.4%) (Hassanein *et al.*, 2011).

Major classes of tocopherols are present in the tocopherol fraction (α-, γ- and δ-tocopherols) and γ-tocopherol prevails. Total content of phospholipids and monoglycerides in the lipids is 1.18–2.24% and sterols are about 4.0% (Alamri, 2012). It is found that total lipids (8.6%) derived from lupin seeds of the species *L. angustifolius* var. Uniwhite consists of triglycerides (71.1%), phospholipids (14.9%), free sterols (5.2%), glycolipids (3.5%), sterol esters (0.5%), free alcohols (0.4%), hydrocarbons (0.4%), and waxes (4.0%) (Hansen and Czochanska, 1974).

In the past few years, specific cultivars of lupin with low alkaloid content have been developed, which makes them really suitable for human consumption (Mihailović *et al.*, 2008). One of this species is *Lupinus angustifolius* cultivar "Boregine" which have been under examination about their possible introduction in Serbia (Mihailović *et al.*, 2008). On the other hand, the information of the chemical and lipid composition of this specific cultivar of lupin is rather limited. In order to evaluate the possible application of *L. angustifolius* cultivar "Boregine" seeds and depict them as a potential alternative food, the main purpose of the current study is to determine in detail the proximate composition of the seeds from the above mentioned lupin cultivar grown in Bulgaria.

2 Materials and methods

2.1 Materials

The investigations were carried out with lupin seeds (*L. angustifolius* German cultivar "Boregine") which were provided from a manufacturer in Bulgaria. The seeds were harvested at the full maturity and the weight of 1000 seeds was 128 g. The seeds were grown in the southern part of Bulgaria (elev. 200 m; 42°29'8.12" N; 24°78'1.53" E) and harvested in 2018 (average temperature: 17 °C; average humidity: 67%; average pressure: 1011 mbar; precipitation: 603 mm). The seeds were ground into a flour before the analysis, using laboratory mill in order to pass through 30 mesh sieve. Then, the samples were kept in a closed container in the refrigerator (at 5 °C) prior the analysis.

2.2 Chemical composition

Crude fiber, moisture, ash content, and total protein were determined according to AOAC (2016). Carbohydrate content was calculated as follows: 100 – (weight in grams [protein + lipids + water + ash] in 100 g of dry seeds) (FAO, 2003). The soluble carbohydrates and the starch content were identified by using standard methods (BS 7169, 1989; BS 13488, 1976). The oil was extracted from the seeds in a Soxhlet using *n*-hexane (ISO 659, 2014).

The energy value of the seeds was determined according to FAO (2003)'s procedure using the formula below:

$$EV = C \times 4 + L \times 9 + P \times 4(\text{kcal}/100\text{g}),$$

where EV is the energy value, C is the total carbohydrates (%), L is the total lipids (%), and P is the total proteins (%).

2.3 Soluble sugars

Soluble sugars are determined by high performance liquid chromatography (HPLC) on an Agilent® LC 1220 instrument (USA) equipped with Zorbax Carbohydrate column (150 × 4.6 mm; pore size: 70 Å; particle size: 5.0 µm, Agilent) and Zorbax Reliance Cartridge guard-column (Agilent) and refractive index detector (RID 1260) (Georgiev *et al.*, 2012). The mobile phase was acetonitrile/water (AcN/H_2O) (80/20) at 1.0 mL/min. All individual pure monosaccharides (purity 98%) were purchased from Merck (Darmstadt, Germany).

2.4 Amino acids

The seeds (300 mg) were hydrolyzed with 6 N HCl solution at 105 °C for 24 h and after that, dried in a vacuum chamber at 50 °C. The residue was diluted with 20 mM HCl and filtered. The derivatization was performed with AccQ-Fluor kit (WATO52880, Waters Corporation, USA) and 20 µL of the filtrate were used. The solution was heated to 55 °C and 20 µL were injected. An ELITE LaChrome HPLC chromatograph (Hitachi) equipped with a diode array detector (DAD) and a reverse phase C 18 AccQ-Tag column (3.9 × 150 mm; particle size: 4 µm; packing material: Silica base bonded with C_{18}) was used. The mobile phases in the gradient elution were WATO52890 buffer (Waters Corporation, USA) and 60% acetonitrile in a double distilled water. The detection wavelength was 254 nm and the column temperature was 37 °C (Popova *et al.*, 2021).

2.5 Physicochemical characteristics of glyceride oil

The physicochemical properties (iodine, acid, peroxide, saponification values, refractive index, and relative density) of the oil were analyzed following the standard procedures (AOCS, 1999; EN ISO 6320, 2000; ISO/FDIS 3657, 2001; ISO 3960, 2007; ISO 660, 2009; ISO 6883, 2017). Oxidative stability is measured at 100 °C and an air flow rate of 20 L/h by Rancimat 679 equipment (Metrohm Switzerland) (ISO 6886, 2006).

2.6 Fatty acid composition

Fatty acid composition of the glyceride oil was determined by gas chromatography (GC) (ISO 12966-1, 2014). Fatty acid methyl esters (FAMEs) were prepared by transesterification of the oil with sulfuric acid in methanol (ISO 12966-2, 2011). Determination was performed on HP 5890 gas chromatograph equipped with a capillary column Supelco of 30 m × 0.25 mm × 0.2 µm (film thickness) and a flame ionization detector (FID). The column temperature was programmed from 70 °C (1 min), at 6 °C/min to 190 °C (3 min), and at 10 °C/min to 240 °C; the injector and detector temperatures were 250 °C; the carrier gas was hydrogen. Identification was carried out by comparison of the retention times with those of a standard mixture of FAME.

2.7 Phospholipids

Ground seeds were subjected to extraction with a mixture of chloroform and methanol (2:1, v/v) (Folch *et al.*, 1957). Individual phospholipids were isolated by two-dimensional thin-layer chromatography (TLC) (Schneiter and Daum, 2006). Identification was performed by comparing the R_f values with authentic standards. The spots of phospholipids were scrapped and mineralized with a mixture of perchloric and sulphuric acid, 1:1 (v/v), and the quantification was performed spectrophotometrically at 700 nm (ISO 10540-1, 2014). The total phospholipids were determined in the seeds and then their quantity in the lipids was calculated on the basis of the oil content of the seeds.

Fatty acids of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidic acids (PA) were isolated from the phospholipid fraction by preparative TLC. They were subjected to saponification with 2 N KOH in ethanol and extracted with hexane. After that, the preparation of the FAME and their GC determination were the same as for the glyceride oil described in Section 2.6. (Supplementary Material 1).

2.8 Sterols

Glyceride oil was subjected to saponification and the unsaponifiables were extracted with n-hexane (ISO 18609,

2000). Total sterols were measured spectrophotometrically (at 597 nm) after the isolation of sterols from the unsaponifiable matter by TLC (Ivanov *et al.*, 1972).

Sterol composition was determined on HP 5890 gas chromatograph equipped with DB 5 capillary column (25 m × 0.25 mm × 0.25 μm (film thickness)) and FID. Temperature gradient was from 90 °C (3 min) up to 290 °C at a rate of change of 15 °C/min and then up to 310 °C at a rate of 4 °C/min (10 min); detector temperature: 320 °C; injector temperature: 300 °C and carrier gas was hydrogen. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols (ISO 12228-1, 2014) (Supplementary Material 2).

2.9 Tocopherols

Tocopherols were determined by high performance liquid chromatography with Nucleosil Si 50-5 column (250 × 4 mm, particle size: 5 μm), fluorescent detection at 295 nm excitement and 330 nm emission. The operating conditions were the mobile phase of hexane:dioxane, 96:4 (v/v) and flow rate of 1 mL/min (ISO 9936, 2016). The total tocopherols were determined in the oil and then their quantity in the seeds was calculated on the basis of their oil content.

2.10 Carotenoids

Total carotenoid content was determined according to method described by Borello and Domenici (2019). The measurement was done spectrophotometrically at 470 nm.

2.11 Statistics

All measurements were performed in triplicate (n = 3) and the results were presented as mean value ± standard deviation (SD).

3 Results and discussions

3.1 Chemical composition

Main chemical compositions (proteins, carbohydrates, oil, ash, fibers and moisture) of the lupin seeds (*L. angustifolius* cultivar "Boregine") are presented in Table 1.

The protein content of the examined lupin seeds was 23.9%, which confirmed that these seeds belonged to the legumes with high total protein content (23.0–31.33%) (Iqbal *et al.*, 2006; Bakoglu *et al.*, 2009; Kökten *et al.*, 2010) and possessed a high nutritional value. The obtained results corresponded to the findings from previous authors who reported that the total proteins in lupin seeds ranged from 28.4 to 45.8% (Sedláková *et al.*, 2016; Tarasenko *et al.*, 2017; Bartkiene *et al.*, 2016; Mohamed and Rayas-Duarte, 1995; Lara-Rivera *et al.*, 2017). On the other hand, these seeds had rather low oil content (7.4%) but it was higher than those in other seeds from Fabaceae family (1.0–3.0%). Their oil content was similar to those of corn germ and oats (5.0%) but it was significantly lower than those of soybeans (17.0–20.0%). Similar results about the oil content were observed also in the seeds from different lupin varieties (6.9–14.1%) (Mohamed and Rayas-Duarte, 1995; Sedláková *et al.*, 2016; Tarasenko *et al.*, 2017; Rybiński *et al.*, 2018), but Borek *et al.* (2009) and Mohamed and Rayas-Duarte (1995) observed that the oil content of the seeds from *Lupinus mutabilis* и *Lupinus angustifolius* was higher (19.0–20.0%). Contrariwise, Lara-Rivera *et al.* (2017) and Bartkiene *et al.* (2016) reported that the oil content of the seeds from six accessions of *Lupinus angustifolius* ranged from 3.88 to 5.80%.

Table 1. Chemical composition of lupin seeds.

Chemical composition	Lupin seeds
Proteins, %	23.9 ± 0.4
Oil content, %	7.4 ± 0.4
Carbohydrates	57.1 ± 0.9
» starch, %	24.1 ± 0.2
» water-soluble sugars, %	4.1 ± 0.2
» fibres, %	10.1 ± 0.6
Ash, %	3.9 ± 0.2
Moisture, %	7.7 ± 0.2
Energy value, kJ/100 g (kcal/100 g)	1658 (391)

The examined lupin seeds possessed a higher carbohydrate content (57.1%) than the results obtained by other researchers (42.24–52.28%) (Mohamed and Rayas-Duarte, 1995; Bartkiene *et al.*, 2016; Lara-Rivera *et al.*, 2017). The studied seeds had a significantly higher starch content (24.1%) than the results obtained by previous authors who examined the seeds of different lupin species *Lupinus albus* and *Lupinus angustfolius* (2.81–4.53%) (Mohamed and Rayas-Duarte, 1995; Kohajdová *et al.*, 2011). It was established that the starch content of all examined lupin seeds was lower than those of other legumes (30–50%). The content of available sugars in the examined lupin seeds (4.1%) was higher than the results by Kohajdová *et al.* (2011) (1.21–2.58%).

Recently, there has been a growing emphasis on fiber content, which is important for the normal digestion. Leguminous plants contain a great amount of these substances and, given their high protein and carbohydrate content, these crops are suitable foods for human consumption. The results showed that the fiber content of lupin seeds was 10.1%, which corresponded well with the results from previous studies on the fiber content of the seeds of different lupin species (8.05–15.4%) (Sedláková *et al.*, 2016; Lara-Rivera *et al.*, 2017). Higher values for the fibers (22.2%) were obtained by Tarasenko *et al.* (2017) who examined the chemical composition of the meal from lupin seeds cultivated in Russia.

Legumes are rich in minerals (potassium, calcium, phosphorus, iron, zinc, etc.). For that reason, was examined the total mineral content (ash) of lupin seeds. The ash content was 3.9%, which was in agreement with the ash content of different legume seeds (3.0–4.9%) examined in previous studies. They also corresponded to the finding about lupin seeds of Lara-Rivera *et al.* (2017) (3.13–3.51%), Bartkiene *et al.* (2016) (3.0–4.3%) and Mohamed and Rayas-Duarte (1995) (4.0–4.4%).

The moisture content of the lupin seeds (7.7%) was close to the previous findings for different lupin species (8.3–9.7%) (Tarasenko *et al.*, 2017; Bartkiene *et al.*, 2016), but it was

Table 2. Composition of soluble sugars in lupin seeds.

Content, mg/100 g	Lupin seeds
Fructose	244.9 ± 2.1
Glucose	221.6 ± 10.8
Galactose	186.5 ± 14.6
Xylose	109.5 ± 6.1
Rhamnose	109.4 ± 3.3
Sucrose	2341.4 ± 141.3
Cellobiose	883.1 ± 9.5

higher than the results reported by Lara-Rivera *et al.* (2017) who examined six varieties of *Lupinus angustifolius* (3.59–4.38%).

The tested lupin seeds had a high content of their main components, which determined their high energy value (1658 kJ/100 g or 391 kcal/100 g).

Table 2 presents the content of soluble carbohydrates in lupin seeds.

Five monosaccharides and two disaccharides were detected in the seeds. Total content of water soluble carbohydrates was found to be 4096.2 mg/100 g. The fructose (244.9 mg/100 g) was found in the highest amount among all monosaccharides, followed by glucose (221.6 mg/100 g) and galactose (186.5 mg/100 g). The content of xylose and rhamnose were detected in similar quantities (109.5 and 109.4 mg/100 g, respectively). The main disaccharide was found to be sucrose (2341.4 mg/100 g) and the amount of cellobiose was much lower (883.1 mg/100 g). Similar results about the content of fructose were obtained by Piotrowicz-Cieslak (2005) (from 1.33 to 2.80 mg/g) who examined the composition of soluble carbohydrates of lupin seeds from Mediterranean Sea. According to the same author, only the seeds from variety *L. pilosus* possessed higher content of fructose (6.26 mg/g). On the other hand, the content of glucose and galactose of the lupin seeds in the present study was higher than that observed by Piotrowicz-Cieslak (2005) (from 0.07 to 0.89 mg/g for glucose and from 0 to 0.85 mg/g for galactose). The content of sucrose, reported by the mentioned author (from 18.86 to 27.9 mg/g) was similar to our results, apart from varieties *L. pilosus* where the amount of sucrose was higher (32.65 mg/g), and *L. palaestinus* and *L. hispanicus* subsp. *hispanicus* where the quantity was lower (15.6 and 15.54 mg/g, respectively).

Table 3 shows the results about the content of amino acids (mg amino acid (AA)/g sample) in the examined lupin seeds.

Seventeen amino acids were identified in the seeds. The major amino acid was phenylalanine (24.8 mg/g), followed by arginine (13.6 mg/g), tyrosine (12.8 mg/g), and serine (12.6 mg/g). On the other hand, leucine (1.4 mg/g), lysine (2.8 mg/g), proline (2.8 mg/g), and valine (2.9 mg/g) were identified in the lowest content. The amount of the other amino acids was observed to range between 3.2 and 11.5 mg/g. These results differed significantly from others where leucine was found to be in the highest content (7.05–9.34% from total amino acids) while methionine (0.15–1.38% from total amino acids) was with the lowest content in all examined seeds from variety *L. angustifolius* (Starkute *et al.*, 2016). Overall, lupin seeds as all leguminous plants were deficient in methionine, cystine and cysteine (Iqbal *et al.*, 2006).

Table 3. Amino acid composition of lupin seeds.

Amino acid, mg AA/g	Lupin seeds
Aspartic acid	7.1 ± 0.2
Serine	12.6 ± 0.1
Glutamic acid	7.1 ± 0.1
Glycine	3.2 ± 0.2
Histidine	11.5 ± 0.2
Arginine	13.6 ± 0.3
Threonine	5.8 ± 0.2
Alanine	3.8 ± 0.1
Proline	2.8 ± 0.1
Cysteine	7.8 ± 0.3
Tyrosine	12.8 ± 0.1
Valine	2.9 ± 0.2
Methionine	5.0 ± 0.1
Lysine	2.8 ± 0.2
Isoleucine	9.7 ± 0.3
Leucine	1.4 ± 0.1
Phenylalanine	24.8 ± 0.2

3.2 Physicochemical characteristics of lupin seed oil

Physicochemical characteristics are important indicators for the quality of the glyceride oils and play a role in the establishing of their shelf life. The main physicochemical characteristics of lupin seed oil have been determined and the results are shown in Table 4.

The peroxide value of the crude glyceride oil of lupin seeds met the requirements for peroxide value of vegetable oils (up to 15 meqO_2/kg) and was close to that of oil isolated from sweet and bitter types of lupin seeds (1.85 and 1.97 meqO_2/kg; 1.80 and 1.89 meqO_2/kg) (Alamri, 2012; Khalid and Elhardallou, 2019).

The acid value of the examined oil was higher than the requirements for edible oils (4.0 mgKOH/g) and much higher than the results reported by Alamri (2012) (0.935 and 0.853 mgKOH/g). This difference in the acid values is probably caused by the higher amount of linoleic acid in the examined oil while the same author indicates that in the bitter and sweet lupin seed oil the predominant fatty acid is oleic (52.22 and 44.93%, respectively). According to Negash *et al.* (2019) the higher the fatty acid unsaturation, the higher the acid value of the oils.

The results for relative density and refractive index were within the limits of the requirements for these indicators for vegetable oils (Codex-Stan 210, 1999).

Saponification value of the lupin seed oil was higher than the results reported by Alamri (2012) (192.92 and 187.90 mgKOH/g) and Khalid and Elhardallou (2019) (193.54 and 190.0 mgKOH/g).

The iodine value, which was an indicator of the degree of unsaturation of fatty acids in oils, was relatively high (116 gI_2/100 g) and this was due to the higher content of

Table 4. Physicochemical characteristics of lupin seed oil.

Physicochemical characteristics	Lupin seed oil
Peroxide value, meqO_2/kg	2.5 ± 0.3
Acid value, mgKOH/g	6.0 ± 0.2
Iodine value, gI_2/100 g	116 ± 2.0
Saponification value, mgKOH/g	231 ± 2.0
Relative density, 20 °C	0.9022 ± 0.0002
Refractive index, 20 °C	1.4770 ± 0.0008
Oxidative stability, h	105

unsaturated fatty acids in the lupin seed oil. These values were close to that reported by Alamri (2012): 118.4 and 108.7 gI_2/100 g. This makes it possible to classify lupin seed oil as semi-dry oil, characterized by an iodine value between 100–150 gI_2/100 g. The iodine value of lupin seeds oil was close to that of rapeseed oil (94–120 gI_2/100 g) and sesame oil (104–120 gI_2/100 g) (Codex-Stan 210, 1999).

Despite the higher iodine value, the oil had a very high oxidative stability (the induction period was 105 h, which was probably due to the high amount of tocopherols and carotenoids.

3.3 Lipid composition of the oil

Lipids are biological components which include more than 1000 different compounds and the major lipid groups are fatty acids, triacylglycerols, phospholipids, glycolipids, sterols, isoprenoids, etc. They are an important nutrient, a major source of energy, and a major carrier of fat-soluble vitamins. Essential fatty acids, linoleic and linolenic, are extremely important in the formation of cell membranes, especially neural tissue.

The lipid composition of glyceride oil isolated from lupin seeds (*Lupinus angustifolius* L. cultivar "Boregine") was also examined, including fatty acid composition the composition of some representatives of unsaponifiables (sterols, tocopherols and carotenoids), the content and composition of phospholipids, as well as the fatty acid composition of the major phospholipid classes.

The content of biologically active substances (sterols, tocopherols, carotenoids and phospholipids) in glyceride oil and in lupin seeds are present in Table 5.

Unsaponifiable matters in the examined oil was 3.0%, which was higher than the results about the content of unsaponifiables in other seed oils (0.9–2.0%) (Codex-Stan 210, 1999). These results were similar to the data about bitter and sweet types of lupin seed oils (3.51–3.66%) (Alamri, 2012). The sterol content of the oil was 1.0%, which was significantly lower than the results obtained by Alamri (2012) (4.32–4.11%), but it was much higher than these by Hassanein *et al.* (2011) (0.19%). On the other hand, total sterol content of lupin seed oil was similar to those of rapeseed oil (1.1%) (Codex-Stan 210, 1999).

It was observed a high content of tocopherols (vitamin E) in the lipids and seeds: 1585 mg/kg and 117.3 mg/kg, respectively. The total content of tocopherols in lupin seed oil was close to that of palm, sunflower and soybean oils (1500, 1520 and 1680 mg/kg) (Codex-Stan 210, 1999; O'Brien *et al.*, 2000), but it was twice higher than the reported amounts in the oils isolated from bitter and sweet lupin seed oils (795.7 and 635.8 mg/kg) (Alamri, 2012), and the data obtained by Hassanein *et al.* (2011) (939 mg/kg).

Table 5. Content of biologically active components in the glyceride oil and seeds from lupin.

Biologically active components	Lupin seed oil
Unsaponifiable matters, %	
in the oil	3.0 ± 0.2
in the seeds	0.2 ± 0.0
Sterols, %	
in the unsaponifiable matters	34.4 ± 3.4
in the oil	1.0 ± 0.1
in the seeds	0.07 ± 0.0
Tocopherols, mg/kg	
in the oil	1585 ± 14
in the seeds	117.3 ± 1.0
Carotenoids, mg/kg	
in the oil	2068 ± 50
in the seeds	153 ± 4
Phospholipids, %	
in the oil	5.5 ± 0.5
in the seeds	0.4 ± 0.1

Lupin seed oil contained 2068 mg/kg of carotenoids which amount was similar to the carotenoid content of other crude vegetable oils (500–2500 mg/kg) (Codex-Stan 210, 1999). Carotenoids, such as α- and β-carotene and lycopene, have antioxidant properties and provitamin A activity.

The content of phospholipids in the isolated oil was 5.5%, and in the seeds it was 0.4%, respectively. It was established that a higher amount of phospholipids was found in the lipids of the examined lupin seeds compared to the results reported by Alamri (2012) (2.24% and 1.18% for lupin seed oils from bitter type and sweet type, respectively). The total phospholipid content was higher than that in other vegetable oils (1.0–1.5%) (Codex-Stan 210, 1999; O'Brien *et al.*, 2000).

Lupin seeds possessed relatively low oil content but were rich in biologically active substances, such as tocopherols, carotenoids, phospholipids and sterols.

3.3.1 Fatty acid composition

Fatty acid composition of the oils may vary in some limits, depending on the climatic conditions where the plants are grown. It also serves to evaluate the quality and nutritional properties of the oil. The fatty acid composition was determined by gas chromatography of the corresponding methyl esters.

Fatty acid composition of the lupin seed oil is presented in Table 6.

Nineteen fatty acids were identified in lupin seed oil. Linoleic acid (41.0%) was the major component in the triacylglycerols, followed by oleic (32.9%), palmitic (11.9%) and stearic (7.4%) acids. It was established that the amount of the essential linolenic acid was rather higher (4.4%) than in the sunflower oil, but the content of the same fatty acid in lupin

Table 6. Fatty acid composition of lupin seed oil.

Fatty acids, %	Lupin seed oil
Caprylic ($C_{8:0}$)	0.1 ± 0.02
Capric ($C_{10:0}$)	0.1 ± 0.0
Lauric ($C_{12:0}$)	0.1 ± 0.05
Myristic ($C_{14:0}$)	0.3 ± 0.1
Myristoleic ($C_{14:1}$)	0.2 ± 0.1
Pentadecanoic ($C_{15:0}$)	0.1 ± 0.05
Palmitic ($C_{16:0}$)	11.9 ± 0.9
Palmitoleic ($C_{16:1}$)	0.2 ± 0.1
Margaric ($C_{17:0}$)	0.1 ± 0.05
Stearic ($C_{18:0}$)	7.4 ± 0.9
Oleic ($C_{18:1}$)	32.9 ± 2.8
Linoleic ($C_{18:2}$), n-6	41.0 ± 2.5
Linolenic ($C_{18:3}$), n-3	4.4 ± 1.7
Arachidic ($C_{20:0}$)	0.2 ± 0.05
Eicosadienoic ($C_{20:2}$), n-6	0.1 ± 0.02
Eicosatrienoic ($C_{20:3}$), n-3	0.2 ± 0.05
Behenic ($C_{22:0}$)	0.1 ± 0.02
Erucic ($C_{22:1}$)	0.2 ± 0.05
Docosahexaenoic ($C_{22:6}$), n-3	0.4 ± 0.1
Σ n-6	**41.1**
Σ n-3	**5.0**
Ratio n-3/n-6	**0.12**

seed oil was closed to that of soybean oil (5–11%) (Codex-Stan 210, 1999).

Fatty acid composition of the examined oil was also similar to those of sesame, soybean and sunflower oils (Codex-Stan 210, 1999). Other than that, it was close to the fatty acid composition of oils from different types of lupin seeds (*Lupinus albus* and *L. angustifolius*), where the main components were also linoleic (41.7–48.3%), oleic (25.2–31.2%), palmitic (4.2–11.4%) and linolenic (0.1–7.9%) acids (Hansen and Czochanska, 1974; Bartkiene *et al.*, 2016).

It was established that the ratio n-3/n-6 in the tested oil was lower than those reported by Rybiński *et al.* (2018) (0.51%) who examined seed oil from variety *L. albus*. This indicated that the examined seed oil had lower content of n-3 fatty acids.

The content of saturated and unsaturated (mono- and polyunsaturated) fatty acids in the lupin seed oil is shown in Figure 1.

The amount of unsaturated fatty acids (UFA) predominated in the oil (79.6%) and the content of polyunsaturated fatty acids (PUFA) was higher (46.1%) than those of monounsaturated fatty acids (MUFA) (33.5%). The main representatives of PUFA were linoleic and linolenic acids, while in the MUFA was oleic acid.

The obtained results correlated well with these from previous studies for seed oils from *L. angustifolius*, where the content of UFA were 78.6% (Bartkiene *et al.*, 2016) and from sp81.1–87.0% (Bartkiene *et al.*, 2016; Alamri, 2012). On the other hand, according to Rybiński *et al.* (2018) the relative percent of the UFA in the oils from different accessions of *L. albus* varied from 41.2 to 66.2%, which was twice less than the present data.

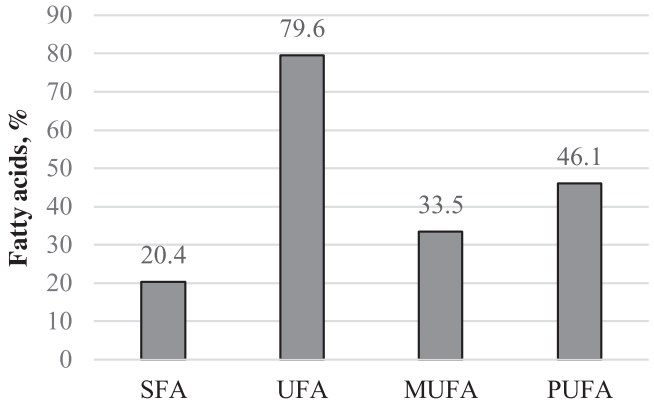

Fig. 1. Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in lupin seed oil.

Table 7. Individual sterol, tocopherol and phospholipid composition of lupin seed oil.

Components	Lupin seed oil
Sterols, %	
Cholesterol	1.0 ± 0.2
Campesterol	24.3 ± 0.5
Stigmasterol	3.3 ± 0.3
β-sitosterol	71.3 ± 1.1
Δ^7-stigmasterol	0.1 ± 0.0
Tocopherols, %	
α-tocopherol	5.2 ± 0.2
γ-tocopherol	92.2 ± 0.6
γ-tocotrienol	2.6 ± 0.5
Phospholipid, %	
Phosphatidylcholine	21.2 ± 3.2
Phosphatidylinositol	33.8 ± 1.5
Phosphatidylethanolamine	6.6 ± 1.1
Sphingomyelin	5.8 ± 1.1
Phosphatidylserine	3.5 ± 1.0
Phosphatidic acids	7.1 ± 1.7
Lysophosphatidylcholine	6.1 ± 1.4
Lysophosphatidylethanolamine	8.9 ± 1.6
Monophosphatidylglycerol	7.0 ± 1.2

The content of SFA was relatively lower (20.4%) and the main representatives were palmitic (11.9%) and stearic (7.4%) acids.

3.3.2 Sterol, tocopherol and phospholipid composition

Sterol, tocopherol and phospholipid composition of the lupin seed oil are presented in Table 7.

The main component of the sterol fraction was β-sitosterol (71.3%), followed by campesterol (24.3%). These results were close to the sterol composition of oils from lupin seeds, cultivated in Egypt where β-sitosterol also predominated (54.6%), followed by campesterol (27.9%) and stigmasterol (11.2%) (Hassanein *et al.*, 2011). The content of campesterol

Table 8. Fatty acid composition of the main phospholipid classes.

Fatty acids, %	Phospholipid classes			
	Phosphatidylinositol	Phosphatidylcholine	Phosphatidylethanolamine	Phosphatidic acids
Caprylic ($C_{8:0}$)	0.2 ± 0.01	–[†]	0.1 ± 0.01	–
Capric ($C_{10:0}$)	0.1 ± 0.01	–	–	0.1 ± 0.01
Lauric ($C_{12:0}$)	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.3 ± 0.01
Myristic ($C_{14:0}$)	1.0 ± 0.01	0.3 ± 0.04	0.4 ± 0.05	0.7 ± 0.02
Myristoleic ($C_{14:1}$)	–	0.1 ± 0.01	–	0.1 ± 0.01
Pentadecanoic ($C_{15:0}$)	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01
Palmitic ($C_{16:0}$)	20.1 ± 0.3	17.5 ± 0.1	16.4 ± 0.2	13.6 ± 0.2
Palmitoleic ($C_{16:1}$)	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.01	0.3 ± 0.02
Margaric ($C_{17:0}$)	–	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.01
Heptadecenoic ($C_{17:1}$)	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	–
Stearic ($C_{18:0}$)	9.1 ± 0.1	7.1 ± 0.2	5.1 ± 0.2	4.3 ± 0.1
Oleic ($C_{18:1}$)	44.3 ± 0.3	43.1 ± 0.2	44.6 ± 0.3	48.3 ± 0.4
Linoleic ($C_{18:2}$)	23.8 ± 0.2	28.7 ± 0.1	30.6 ± 0.3	28.1 ± 0.2
Linolenic ($C_{18:3}$)	1.0 ± 0.1	2.3 ± 0.3	1.7 ± 0.2	3.8 ± 0.3
Arachidic ($C_{20:0}$)	–	–	0.2 ± 0.01	–
Eicosadienoic ($C_{20:2}$)	–	0.3 ± 0.02	0.3 ± 0.01	0.1 ± 0.01

[†] Not detected.

in lupin seed oil was in the given limits for crude oils in Codex-Stan 210 (1999): from 6.4 to 38.6%. Cholesterol and stigmasterol contents in the lipids of lupin seeds were found to be relatively low (1.0 and 3.3%, respectively).

Two main tocopherols were identified in the examined lupin seed oil (α- and γ-tocopherols). The main component was γ-tocopherol (92.2%), while α-tocopherol was found in small quantities (5.2%). The only representative from the unsaturated tocopherols was γ-tocotrienol which was also found in low amounts (2.6%).

Present results were similar to the tocopherol composition of oils from lupin seeds, cultivated in Egypt where γ-tocopherol (93.6%) predominated, followed by α- and δ-tocopherols (3.4 and 3.0%, respectively) (Hassanein *et al.*, 2011).

Tocopherol composition of the examined oil was similar to those of soybean, corn and sesame oils (Codex-Stan 210, 1999). On the other hand, the individual tocopherol composition of lupin seed oil was completely different from these of other seed oils (such as sunflower, safflower, etc.) where the main component was α-tocopherol (Codex-Stan 210, 1999).

Nine phospholipid classes were identified in lupin seed oil. Phosphatidylinositol (33.8%) and phosphatidylcholine (21.2%) predominated in the fraction. Low amount of phosphatidylserine (3.5%) was established, while the content of the other phospholipids were from 5.8 to 8.9%.

Phospholipid composition of lupin seeds differed from other legumes (soybean, lentil, peas, vigna, etc.), that contained bigger amount of phosphatidylcholine (35.0–46.0% of total phospholipids) (Codex-Stan 210, 1999).

Fatty acid composition of the main phospholipid classes (phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and phosphatidic acids) has been also examined and the results are shown in Table 8.

Oleic acid (43.1–48.3%) predominated in all phospholipid classes, followed by linoleic (23.8–30.6%) and palmitic (13.6–20.1%) acids. The other main saturated fatty acid was stearic with amount between 4.3 and 9.1%. The quantity of the essential linolenic acid varied from 1.0 to 3.8% and the content of the other fatty acids was insignificant (0.1–1.0%).

A tendency of increasing the amount of oleic acid was observed in the direction: phosphatidylcholine < phosphatidylinositol < phosphatidylethanolamine < phosphatidic acids, while for linoleic acid was: phosphatidylinositol < phosphatidic acids < phosphatidylcholine < phosphatidylethanolamine.
The contents of palmitic and stearic acids decreased in the direction: phosphatidylinositol > phosphatidylcholine > phosphatidylethanolamine > phosphatidic acids.

The content of total saturated and unsaturated fatty acids of the main phospholipid classes in lupin seed oil is presented in Figure 2.

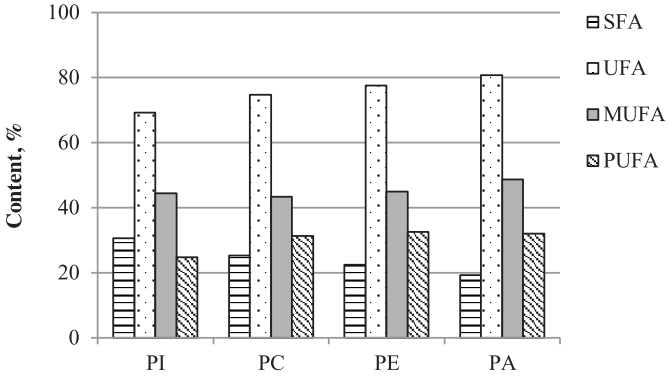

Fig. 2. Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of the main phospholipid classes. [†] PI: Phosphatidylinositol; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PA: Phosphatidic acids.

The content of SFA increased in the following direction: phosphatidic acids (19.3%) < phosphatidylethanolamine (22.5%) < phosphatidylcholine (25.3%) < phosphatidylinositol (30.7%). A decrease of the UFA was observed in the same direction. The amount of the MUFA remained almost unchanged in all examined phospholipid classes (43.4–48.7%), while those of PUFA increased in the following direction: phosphatidylinositol < phosphatidylcholine < phosphatidic acids < phosphatidylethanolamine.

Fatty acid composition of the main phospholipids did not differ significantly from those of glyceride oil. Linoleic acid (41.0%) predominated in the latters, while the amount of the same fatty acid in the phospholipid fraction was 23.8–36.0% which was at the expense of the higher quantity of oleic acid (43.1–48.3%). It was established that there were not significant differences in the content of SFA, but differences were observed in the amount of MUFA and PUFA (33.5:46.1% in the glyceride oil, and 43.4–48.7%:24.8–32.6% in the phospholipids).

The differences in the fatty acid composition could be explained by the different stages of biosynthesis of the separated phospholipids on the one hand and triacylglycerols on the other. The saturated fatty acids were synthesized in the beginning of this process alongside with the phospholipids as follows: first was synthesized phosphatidylinositol, followed by phosphatidylethanolamine, phosphatidic acids and eventually – triacylglycerols. For that reason, more SFA were included in phospholipid molecules – first in phosphatidylinositol and after that in phosphatidylethanolamine, phosphatidic acids and in triacylglycerols when the biosynthesis of polyunsaturated fatty acids also began to accelerate (Munshi *et al.*, 1983).

4 Conclusion

Detailed examinations on the chemical and lipid composition of *L. angustifolius* cultivar "Boregine" seeds were carried out for the first time. According to the results lupin seeds are a promising industrial crop which have a nutritional value, because of their high content of proteins, carbohydrates, dietary fibers and several lipid-soluble biologically active components such as essential fatty acids, tocopherols, sterols, carotenoids and phospholipids. What is more, lupin seed oil depicts to be an alternative source of high quality lipids with a nutritional value and a long shelf life which is due to its extremely high oxidative stability.

Supplementary Material

Supplementary Material 1. Chromatogram of the fatty acid composition of phosphatidylethanolamine isolated from lupin seeds.
Supplementary Material 2. Chromatogram of sterol composition of lupin seed oil.

The Supplementary Material is available at http://www.ocl-journal.org/10.1051/ocl/2022003/olm.

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