Comparison of Chemical Composition in Lupin (Lupinus spp.) Species

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The aim of this study was to investigate the chemical characteristics of seeds in two cultivated bitter landraces of Lupinus albus and two wild Lupinus angustifolius types in Mediterranean environment (Hatay, southern Turkey). Fat quantity and fatty acid quality were considerably different depending on the genotype. L. albus showed the higher oil content (74.0-85.1 g/kg), saturated fatty acids (16.88-19.3 g/100 g), monounsaturated fatty acids (55.80-57.62 g/100 g) and polyunsaturated fatty acids (24.8-25.5 g/100 g) than did L. angustifolius with oil content of 38.8-46.69 g/kg; saturated fatty acids of 23.40-25.12 g/100 g, monounsaturated fatty acids of 51.48-52.98 g/100 g and polyunsaturated fatty acid of 23.4-23.6 g/100 g. The data obtained suggest L. albus as the most interesting lupin species for aiding the crop-livestock food chain and wild L. angustifolius type as a promising crop due to its high nutritive traits for the Mediterranean environment.

Keywords: Fat, Fatty acid content, Lupin species

Lupin is one of the prospective multipurpose crops to be utilized as a homegrown inexpensive protein source in the developing countries due to its low agronomic requirements. Lupin is a legume used as human and animal consumption since ancient Roman times. Over 200 lupin species are cultivated worldwide. Lupin can tolerate extreme conditions such as frost, drought, and poor soils [1]. White or Mediterranean Lupin (Lupinus albus ssp. albus) is adapted to Mediterranean climate and widely cultivated or naturally grown in such regions [2, 3]. However, since its domestication, utilization of lupin has remained inadequate, and this may be partially due to its alkaloid content and low yield. Breeding programs have produced “sweet” varieties with as low as 0.002% alkaloid content, which makes them safe for human consumption [1].

Lupinus seeds are rich in protein content. Therefore, attention is particularly given to the quantity and quality of protein (the amino acid profile) due to their potential significance in human and animal diets. However, there is less attention consideration on the content and quality of Lupin oil. Although lupin belongs to the legumes and is not described as an oilseed crop, it has a considerable amount of oil in its seeds [4]. Unlike protein, whose level varies in a wide range (30-52%) depending on a certain variety, the oil content is substantially lower (5-20%) [1, 5-11]. Lupin seeds shows variation in lipid content; yellow lupin (Lupinus luteus L.) seeds contain about 6%, white lupin (Lupinus albus L.) 7–14%, and Andean lupin (Lupinus mutabilis Sweet) about 20% of lipids by dry mass [1, 11].

In general, lupin oil is characterized by a balanced fatty acid composition with total saturated fatty acids of 10% and total unsaturated fatty acids of 90%, of which 32% to 50% is oleic (18:1) acid, 17% to 47% is linoleic (18:2) acid, and 9% to 11% is linolenic (18:3) acid [11-13]. The variation in composition is due to genetic and environmental differences [14]. Vegetable oil compositions were successfully assessed using GC-MS and H-NMR methods [15, 16]. A better understanding of the chemical composition of different lupin species and land races will aid to improve new varieties for potential use in human and animal diets. The objective of this study is to evaluate and compare the chemical composition of white lupin (Lupinus albus) landraces with that of wild lupin species such as Lupinus angustifolius from East Mediterranean region of Turkey. Therefore protein, ash, fat and fatty acid content of three lupin species were compared.

Experimental part
Materials and methods

The experimental materials of the present study were; two white lupin landraces ((Lupinus albus L. (c.v.Hüyük and c.v.Seydişehir) and two wild narrow-leaved blue lupin Lupinus angustifolius) types (black and white seed color) collected from wild environment of Hatay. All genotypes were grown in botanical garden of Mustafa Kemal University, Hatay, Turkey located at 36° 15' N and 36° 30' and Lupin seeds were obtained from plants grown during growth seasons of 2012-2013 in Mustafa Kemal University. In order to minimize environmental variations, the seeds of collected plant materials were planted to the experimental area. The weather temperature mean was 13.98 °C and total precipitation was 1132 mm during the plant growth season. The experimental area showed typical Mediterranean climate. The experiment was arranged as randomized complete block design with three replications. Seeds of land races and other lupins collected from wild were planted on 4 rows with 5 m row length and 70 cm row space. Samples were harvested from middle two rows of 4.8 m² area. Chemical analyses were performed to harvested seeds.

Crude Protein, Lipid, and Ash Determinations

Levels of crude lipid, and ash of anchovy were determined by modified Bligh & Dyer Method [17], EEC recommended oven drying method ISOR 1442 [18], and [19] method no 938.08, respectively. Determinations were done in triplicates.
Preparation of Fatty Acid Methyl Esters

Fatty acids were analyzed with GC-MS (Gas Chromatography-Mass Spectrometry) using a Hewlett Packard GC (model 6890) and coupled with Hewlett Packard (model 5972A, HP 6890 system) MS detector. Separations of fatty acids were achieved with HP-INNOWAX Polyethylene Glycol Capillary Column (Model number HP 19091N-133, 0.25 mm * 30m * 0.25μm) and HP 6890 automatic injection system was used. Injection and detector temperatures were set at 250°C and 270°C, respectively. Split ratio was 1:20 with a total injection volume of 1 μL. Injector was washed three times with iso-octane and with the FAME containing iso-octane prior to injection. Post injection, injector program was also set to triple wash of injector for next injection. Fatty acid class content determination was performed according to [20].

Chromatographic Conditions

Oven temperature was programmed initially at 20°C and hold for 3 minute. Then, the temperature was increased to 250°C with a 10°C per minute ramp rate and hold at this temperature four minutes. Total separation was achieved in 20 minutes. Identification of individual fatty acids was made by comparing those retention time of FAME standard (Supelco 47085U PUSA No: 3) and Supelco 37 component Fame mix (Supelco 47885-U). Confirmation of fatty acid methyl esters was also performed by using MS data base library (PAMEDBWAX).

All data were subject to analysis of variance (ANOVA) procedures using the SAS statistical software package SAS statistical software version 9.1 (SAS Institute, 2003). The post hoc comparisons were performed using Tukey’s Honestly Significant Difference (HSD) P < 0.05 test.

Results and discussions

Lupin species varied significantly for their fat content in their seeds. The highest fat content was obtained from white lupin landraces and the lowest was from white *L. angustifolius*. The fat content was about twice higher in the white lupin landraces than in other two lupin species. Black seeded *L. angustifolius* had similar fat content to white seeded one (table 1). Considering fat content, there was also significant difference between two white lupin land races and Seydişehir landrace had lower fat content than did Hüyük [1, 21, 22].

Fat contents also differed with respect to lupin genotypes used in this study. Although white lupin landraces had the highest fat rate, they had the lowest rate of saturated fatty acids (SFA). The highest rate of SFA was obtained from *L. angustifolius* seeds. SFA content for cultivar-environment combination ranged from about 10% to 27% [5 - 7, 20]. Of the most lupin species, *L. angustifolius* generally had the highest the SFA content compared to *L. albus* and *L. luteus* [5, 20]. In our study, Palmitic acid was the major saturated fatty acid followed by stearic acid, behenic acid and arachidic acid. The lowest rate of palmitic acid was found in *L. albus* landraces while it was similar in the other two species. Similar to our results, Chiofalo et al. [20] also found that palmitic acid content of *L. angustifolius* was about 30% more than that of *L. albus*. Stearic acid was the highest in black seeded *L. angustifolius* and it was the lowest in *L. albus*. Stearic acid of Seydişehir landrace was significantly higher than that of Hüyük. There was also significant variation among and within species in terms of stearic acid. For example, stearic acid content in some *L. albus* landraces and cultivars ranged from 1.34% to 3.56% [4-6, 20] suggesting that stearic acid content is amenable for selection processes of breeding lupin cultivars. Similarly, *L. angustifolius* cultivars significantly differed in their stearic acid contents [20]. In our study, there was also significant variation within species. Behenic acid was significantly the highest in Hüyük landrace while it was the lowest in *L. angustifolius* (table 1). Contrary to our findings, behenic acid contents were similar within *L. albus* and *L. angustifolius* species [20]. However, results of Boschin et al. [5] was similar to ours in that there was significant variation within *L. albus* species. Arachidic acid was also the highest in Hüyük landrace and the lowest was obtained in black *L. angustifolius*. We found significant variation in arachidic acid content among and within species, conversely Chiofalo et al. [20] found no significant differences within *L. albus* while the difference was significant within *L. angustifolius* species. This reason may be attributed to different genetic background and environments.

<table>
<thead>
<tr>
<th>L.albus</th>
<th>L. albus</th>
<th>L. angustifolius</th>
<th>L. angustifolius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hüyük</td>
<td>Seydişehir</td>
<td>white</td>
<td>black</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>31.45 a</td>
<td>29.8 ab</td>
<td>26.30 bc</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.79 c</td>
<td>3.65 c</td>
<td>4.48 a</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>8.51 a</td>
<td>7.40 a</td>
<td>3.88 bc</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>9.75b</td>
<td>9.00 b</td>
<td>13.80 a</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>0.60 a</td>
<td>0.39 ab</td>
<td>0.23 c</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>4.09 c</td>
<td>3.14 d</td>
<td>5.98 b</td>
</tr>
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<td>Oleic acid (C18:1)</td>
<td>50.02 b</td>
<td>52.73 a</td>
<td>48.65 bc</td>
</tr>
<tr>
<td>Linoleic acid (C18:2, ω-6)</td>
<td>18.20 a</td>
<td>18.40 a</td>
<td>17.40 b</td>
</tr>
<tr>
<td>α-linolenic acid (C18:3, ω-3)</td>
<td>6.60 b</td>
<td>7.10 a</td>
<td>6.20 c</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>1.56 a</td>
<td>1.17 c</td>
<td>1.31 c</td>
</tr>
<tr>
<td>11-eicosenoic acid (C20:1)</td>
<td>3.50 a</td>
<td>3.05 ab</td>
<td>2.70 b</td>
</tr>
<tr>
<td>Behenic acid (C22:0)</td>
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<td>3.57 b</td>
<td>2.33 c</td>
</tr>
<tr>
<td>Erucic acid (C22:1)</td>
<td>1.78 a</td>
<td>1.45 b</td>
<td>1.40 b</td>
</tr>
</tbody>
</table>

Table 1 CRUDE PROTEIN, CRUDE ASH, FAT AND FATTY ACID COMPOSITION OF LUPINUS SPECIES FROM TURKEY

Mean values with different letters (a-d) within the same line differ significantly (P<0.05).
There was also significant variation in the rate of monounsaturated fatty acid (MUFA) among species and within species. The highest MUFA was obtained in landraces of white lupin (fig. 1). The reason for that is *L. albus* contained relatively higher level of oleic acid. Black seeded *L. angustifolius* had higher MUFA content than did white seeded one. Similarly, *L. albus* landraces and *L. angustifolius* types showed variation in terms of MUFA [5, 20]. Oleic acid (C18:1) content was the highest in all lupin species although it significantly varied depending on the genotype (table). In general, content of oleic acid is around 20-55% in most Lupin species [4 - 6]. Seydişehir landrace genotypes (table). In general, content of oleic acid is around 20-55% in most Lupin species [4 - 6]. Seydişehir landrace of white lupin had the highest oleic acid content compared to Hüyük landrace and other species. Chlofalo et al. [20] and [5] also found significant differences in oleic acid contents among cultivars within Lupin species. White seeded *L. angustifolius* had higher oleic acid than black seeded one. In terms of oleic acid, different seed color did not yield significant difference within *L. angustifolius*.

The second highest MUFA was 11-eicosenoic acid which was highest in Hüyük Landrace followed by white seeded *L. albus*. Black and white seed colored *L. angustifolius* had higher MUFA content than did white seeded one. Similarly, *L. albus* landraces and *L. angustifolius* types showed variation in terms of MUFA [5, 20]. Oleic acid (C18:1) content was the highest in all lupin species although it significantly varied depending on the genotype (table). In general, content of oleic acid is around 20-55% in most Lupin species [4 - 6]. Seydişehir landrace of white lupin had the highest oleic acid content compared to Hüyük landrace and other species. Chlofalo et al. [20] and [5] also found significant differences in oleic acid contents among cultivars within Lupin species. White seeded *L. angustifolius* had higher oleic acid than black seeded one. In terms of oleic acid, different seed color did not yield significant difference within *L. angustifolius*.

The rate of polyunsaturated fatty acid (PUFA) was the highest in white lupin landraces and the lowest in *L. angustifolius*. Majority portion of PUFA was composed of linoleic acid which was significantly lower in *L. angustifolius* than in *L. albus* (p<0.05). Although there was variation between species, the variation existed only within *L. angustifolius*. Significant linoleic acid variation both within *L. angustifolius* and *L. albus* was reported earlier [5, 20, 23]. Similar values within *L. albus* species may be due to utilization of different plant materials and environment suggesting that linoleic acid may be affected by the genetic background and/or environmental conditions. Black seeded *L. angustifolius* had the lowest α-linolenic acid amount of all the genotypes. The highest portion of α-linolenic acid was obtained in Seydişehir landrace of white lupin. Both species showed significant within species variation in previous studies [5, 20, 23].

**Conclusions**  
Our results showed that lupin species in Mediterranean condition yielded substantial amount of protein and oil profile. Considering the fact that lupins are good for amelioration of soil properties, they can be also potential source of protein and energy for human and animal diets. Composition of fatty acids especially linoleic and α-linolenic acid is comparable to most of the oil crops. In our study, we showed among and within species variation in terms of protein, ash, fat and fatty acids. We also showed that lupin landraces and wild species have large gene pool which can be used to improve lupin cultivars with desirable characteristics. We analyzed black and white seed colored *L. angustifolius*, however we could not find any correlation especially with fatty acid content (data not shown). More detailed studies should be performed whether seed colour is correlated with other seed characteristics. Although lupin seeds are known to have bitter taste due to alkaloids and erucic acid content, there is enough variation within species to improve lupins with more desirable characteristics for human and animal consumption.

**References**

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