# Sterol and Fatty Acid Patterns in Wild and Cultivated Species of *Lupinus* (Leguminosae)

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*Lupinus*, Seed Lipids, Fatty Acids, Sterols, GLC

Seed oil components of wild and cultivated species of *Lupinus* were analyzed by gas liquid chromatography (GLC). The sterol and fatty acid patterns of *Lupinus albescens* and *L. gibertianus* that are considered important germoplasm resources of South America, are reported for the first time and compared with varieties of *Lupinus albus, L. angustifolius* and *L. mutabilis*. The taxonomic implication of seed oil composition was evaluated using a multivariate analysis system.

## Introduction

The genus *Lupinus* is a very large and one of the most complex groups of plants which occur predominately in North and South America. Only around 12 of large seeded species occur in the Mediterranean region and were cultivated by early civilizations for its edibles seeds (Planchuelo, 1978). The seeds composition have been primarly analyzed for its nutritional value as a main source of protein in many part of the world. The major components of the seeds by dry weight are protein (36–48%), oil (8–25%) and carbohydrates (11–14%) (Fuentes and Planchuelo, 1995).

Literature reviews related with seed lipid components show that the better known species are the cultivated lupins and a few wild species of the European and African Mediterranean region and North America (Bouthelier *et al.*, 1982; Huesa Lope *et al.*, 1985; Roemer and Jahn–Deesbach, 1988; Becker *et al.*, 1989; Souza *et al.*, 1989; Bertoni and Cattaneo, 1990; Mason *et al.*, 1990). The fatty acid and sterol composition of seeds have been frequently used in biochemical systematic and have proved to be a valuable tool in studies of *Quercus* (Rafii *et al.*, 1991), *Cupressus* (Rafii *et al.*, 1992) and *Solanum* (Zygadlo, 1994). For *Lupinus*, the relationships between species have been studied only from the morphological aspects and the chemotaxonomic implication of the alkaloid contents.

This paper compares seed oil content, fatty acid and sterol patterns of cultivated varieties of *Lupinus albus*, *L. angustifolius* and *L. mutabilis* with two wild South American lupins, *L. gibertianus* and *L. albescens* which are native to Northeastern Argentina, Paraguay and Uruguay. Actually, these two wild species are the object of intensive study in several areas of sciences, because, *L. gibertianus* has morphological characters very similar to *L. angustifolius* L. and has been considered as its possible ancestor (Planchuelo–Ravelo, 1991) and *L. albescens* is a pioneer species in sand dune fixation and a good nitrogen–fixing plant (Oliva and Planchuelo, 1996).

In this new approach, seed lipid analysis and numerical taxonomy are combined to establish possible relationships between wild and cultivated lupins.

## Results and Discussion

Seed weight, percentage of crude oil by dry matter and refractive index of each sample are presented in Table I. The percentage of oil content over dry matter found in seed of cultivated species agree with data published by other authors (Bertoni and Cattaneo, 1990; Mason *et al.*, 1990).

Seeds of cultivated varieties are much larger and heavier than those of the wild species; however, the percentage of crude oil is independent of the size and weight of the seeds. The highest percent

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Table I. Seed weight (mg), percent crude oil by dry matter and refractive index.

| Samples | | Seed Weight $\bar{X}$ | Seed Weight $\pm \delta$ | % Crude oil dry matter | Refractive Index |
|---|---|---|---|---|---|
| *L. albus* | L2034N | 0.3902 | 0.0532 | 15.60 | 1.4690 |
| | L2040N | 0.3452 | 0.0813 | 15.20 | 1.4699 |
| | L2053N | 0.3685 | 0.0427 | 12.70 | 1.4695 |
| | L2085N | 0.2854 | 0.0392 | 14.60 | 1.4702 |
| | Amiga | 0.4633 | 0.0696 | 11.60 | 1.4701 |
| | "Bitter" | 0.3711 | 0.0791 | 13.90 | 1.4719 |
| *L. angustifolius* | Danja | 0.1477 | 0.0240 | 9.69 | 1.4729 |
| | Emir | 0.1318 | 0.0271 | 7.27 | 1.4731 |
| | Gelen | 0.1508 | 0.0247 | 8.17 | 1.4728 |
| | Gungurru | 0.1258 | 0.0253 | 6.74 | 1.4711 |
| | Mirela | 0.1113 | 0.0183 | 6.98 | 1.4729 |
| | Wandoo | 0.1265 | 0.0270 | 9.37 | 1.4719 |
| | Yandee | 0.1683 | 0.0279 | 9.72 | 1.4720 |
| *L. mutabilis* | P33 | 0.2891 | 0.0578 | 19.40 | 1.4699 |
| | P106 | 0.2103 | 0.0306 | 15.80 | 1.4680 |
| | P154 | 0.2425 | 0.0398 | 15.50 | 1.4690 |
| | E001 | 0.2644 | 0.0462 | 17.40 | 1.4700 |
| | E042 | 0.3037 | 0.0489 | 18.00 | 1.4689 |
| *L. albecens* | | 0.0419 | 0.0067 | 10.70 | 1.4735 |
| *L. gibertianus* | | 0.0139 | 0.0138 | 6.71 | 1.4759 |

of oil content was observed in *L. mutabilis* (15.50–19.40%), followed by *L. albus* (11.60–15.60%), and *L. albescens* (10.70%). Among the cultivated, *L. angustifolius* is the one that has less oil, which values (6.74–9.72%) are similar to *L. gibertianus* (6.71%). The refractive indexes do not show any difference between cultivated and non cultivated species and agree with the values reported by Hilditch (1956).

*Fatty acid*

Fatty acid composition, oleic/linoleic acid ratio, nonsaturated/saturated acid ratio and iodine value are presented in Table II. The concentrations of major fatty acids in all cultivated species agree with the amounts cited by many authors, including Múzquiz *et al.* (1982), Huesa Lope *et al.* (1985), Mason *et al.* (1990).

Main fatty acids for all samples are oleic (C18:1) and linoleic acid (C18:2). The concentration of palmitic acid (C16:0) is higher than linolenic acid (C18:3) in *L. mutabilis*, *L. angustifolius* and *L. albescens*, the opposite occurs in *L. albus* and *L. gibertianus*. Stearic acid (C18:0) concentration ranges from traces to 8.8%, being highly variable within the varieties of the same species and between the different species analyzed. Other acids present in less quantities are myristic (C14:0), palmitoleic (C16:1) arachidic (C20:0), eicosenic (C20:1), eicosadienoic (C20:2), behenic (C22:0), erucic (C22:1) and docosadienoic acid (C22:2).

Erucic acid (22:1), fecuently referred as an indesirable fatty acid, (Múzquiz, *et al.*, 1982) is present as traces or in small quantities of the total fatty acid content in all varieties of cultivated species and in appreciably amount (2.1%) in *L. gibertianus*.

It is particularly notable that a large amount (3.7%) of a fatty acid can be ascribed to tetracosanoic acid (C24:0) present in *L. gibertianus* and only as traces in all other samples. This fatty acid was only cited (1.03%) for a wild european species (*L. hispanicus*) by Varejão *et al.* (1994).

The largest oleic/linoleic acid ratio (C18.1/C18.2) was observed in *L. albus* (2.34–3.42) followed by *L. mutabilis* (1.35–2.85) and *L. angustifolius*, (0.72–1.05). The two wild species show ratios below 0.5 which indicates a very poor oil quality. The nonsaturated/saturated acids ratio are

Table II. Fatty acid in percent of total amount.
Reference: tr: trace (< 0.1).

| | *Lupinus albus* | | | | | | *Lupinus mutabilis* | | | | | *Lupinus angustifolius* | | | | | | | *L. albescens* | *L. gibertianus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| F.A. | | | | | | | | | | | | | | | | | | | | |
| C14:0 | 0.1 | 0.1 | 0.6 | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | 0.2 | 0.2 | 0.2 | 0.2 | tr | tr | 1.5 |
| C16:0 | 7.1 | 8.5 | 7.0 | 8.5 | 7.3 | 7.1 | 9.6 | 11.0 | 12.0 | 9.7 | 12.2 | 11.4 | 12.3 | 10.0 | 11.7 | 11.9 | 10.7 | 10.0 | 7.7 | 9.5 |
| C16:1 | 0.4 | 0.5 | 0.6 | 0.5 | tr | 0.8 | tr | 0.3 | 0.2 | 0.2 | tr | tr | tr | tr | 0.2 | tr | tr | tr | tr | tr |
| C18:0 | tr | 0.5 | 1.7 | 2.0 | 2.2 | tr | 7.0 | tr | 7.8 | 6.1 | 7.8 | 4.7 | 5.3 | tr | 8.8 | 7.3 | tr | 6.7 | tr | 3.6 |
| C18:1 | 51.6 | 55.7 | 50.8 | 54.0 | 57.9 | 53.6 | 47.1 | 50.2 | 43.5 | 56.8 | 43.7 | 31.7 | 33.3 | 41.1 | 36.9 | 35.8 | 42.7 | 33.1 | 27.8 | 13.8 |
| C18:2 | 22.0 | 17.0 | 20.5 | 16.4 | 16.9 | 19.8 | 28.7 | 31.9 | 32.0 | 20.0 | 30.2 | 44.0 | 42.0 | 39.0 | 33.9 | 35.5 | 36.6 | 39.4 | 56.7 | 46.4 |
| C18:3 | 10.0 | 6.9 | 9.4 | 8.4 | 8.6 | 10.6 | 2.6 | 2.6 | 2.7 | 2.2 | 2.2 | 6.0 | 5.5 | 5.7 | 4.5 | 4.7 | 5.9 | 5.5 | 5.3 | 13.2 |
| C20:0 | 0.9 | 1.7 | 1.4 | 1.2 | tr | tr | tr | 1.1 | 0.6 | 2.1 | 0.6 | 0.8 | tr | 0.4 | 0.8 | 0.7 | 0.8 | 1.5 | 0.1 | 4.4 |
| C20:1 | 4.0 | 1.1 | 3.8 | 0.9 | tr | 0.6 | 0.7 | 0.7 | 0.7 | 0.6 | 0.7 | tr | 0.6 | 1.0 | 1.0 | 1.0 | 0.9 | 0.9 | 0.3 | 0.6 |
| C20:2 | 0.3 | 4.0 | 0.7 | 3.9 | 4.3 | 4.2 | 2.8 | 0.1 | tr | tr | tr | tr | tr | 0.3 | 0.3 | 0.4 | 0.6 | tr | tr | 0.7 |
| C22:0 | 2.3 | 2.5 | 2.2 | 2.4 | 2.7 | 1.6 | 0.7 | 0.8 | 0.7 | tr | 0.6 | 1.5 | 1.0 | 1.3 | 1.3 | 1.6 | 1.1 | 1.5 | 1.2 | 0.4 |
| C22:1 | 1.3 | 1.5 | 1.3 | 1.7 | tr | 1.3 | 0.6 | 0.9 | 0.5 | 0.6 | 0.4 | tr | tr | 0.2 | 0.3 | 0.2 | 0.3 | tr | tr | 2.1 |
| C22:2 | tr | 0.4 | tr | tr | tr | 0.2 | tr | 0.2 | 0.2 | 2.1 | 1.5 | tr | tr | 0.5 | 0.2 | 0.5 | 0.1 | 1.4 | tr | tr |
| C24:0 | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | 3.7 |
| 18:1/18:2 | 2.34 | 3.27 | 2.48 | 3.30 | 3.42 | 2.70 | 1.64 | 1.57 | 1.35 | 2.85 | 1.44 | 0.72 | 0.79 | 1.05 | 1.09 | 1.01 | 1.16 | 0.84 | 0.49 | 0.30 |
| I/S | 8.69 | 6.79 | 6.70 | 6.05 | 7.19 | 10.44 | 4.75 | 6.76 | 3.94 | 4.59 | 3.71 | 4.43 | 4.38 | 7.36 | 3.36 | 3.57 | 6.81 | 4.07 | 9.19 | 3.31 |
| $I_2$ | 114 | 109 | 118 | 110 | 113 | 122 | 124 | 112 | 105 | 97 | 103 | 107 | 121 | 125 | 108 | 112 | 123 | 119 | 142 | 135 |

quite large and variable and do not contribute to the typification of any oil pattern. The iodine value (Sallee, 1967) for all samples is in a range of semi-drying oil.

The present results suggest that *Lupinus albus* and *L. mutabilis* are the only two species whose seed oil content and fatty acid composition allow them to be considered as potential oil crops.

### *Sterols*

The amounts of sterol components are very similar between the varieties of the same species. Table III shows the mean value for each species. The dominant compound is β-sistoesterol, whose values range from 46.40% in *L. mutabilis* to 66.49% in *L. angustifolius*, followed by campesterol with 17.39% for *L. albescens* and 24.91% in *L. gibertianus*. Other sterols present with more than trace amounts are stigmasterol and cholesterol. The Δ5-avenasterol in cultivated and wild samples was only cited previously for *L. hispanicus* (Varejao *et al.*, 1994). The Δ7-stigmasterol and Δ7-avenasterol are cited for the first time for the genus *Lupinus*.

### *Cluster analysis*

The correlation in the two major cluster groups of the phenogram (Fig. 1) is highly negative. Thus, it may be attributed to the different genetic background (Wolff and Kwolek, 1971) of the species analyzed. The genetic improvement of grain for human nutrition, carried out for at least several

Table III. Free sterols in percent of total amount.
Reference: tr: trace (< 0.1).

| Compounds | *L. albus* | *L. angustifolius* | *L. mutabilis* | *L. albescens* | *L. gibertianus* |
|---|---|---|---|---|---|
| Cholesterol | 1.50 | 3.01 | 1.16 | 0.51 | 1.20 |
| Campesterol | 21.82 | 17.91 | 27.65 | 17.39 | 24.91 |
| Stigmasterol | 9.31 | 9.45 | 13.46 | 17.70 | 17.82 |
| 24-Methylcholest-/-enol | 7.29 | 1.54 | tr | 2.45 | tr. |
| β-Sitosterol | 52.14 | 66.49 | 46.40 | 59.75 | 56.07 |
| $\Delta^5$-Avenasterol | 5.25 | tr. | 11.33 | tr. | tr. |
| $\Delta^7$-Stigmastenol | 1.51 | 0.32 | tr. | 2.20 | tr. |
| $\Delta^7$-Avenasterol | 1.78 | 1.27 | tr. | tr. | tr. |

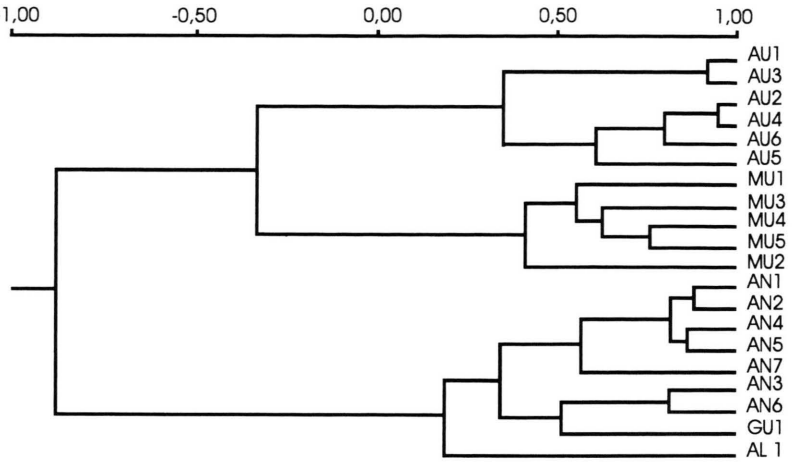


Fig. 1. Phenogram of similarities between 20 samples of *Lupinus* species.
Reference:
AU: *L. albus:* 1: Amiga; 2: L2085N; 3: "bitter"; 4: L2053N; 5: L2034N; 6: L2040 N.
MU: *L. mutabilis:* 1; E042; 2: E001; 3: P154; 4: P33; 5: P106.
AN: *L. angustifolius:* 1: Emir; 2: Mirela; 3: Danja; 4: Gungurru; 5: Wandoo; 6: Gelen; 7: Yandee.
AL 1: *L. albescens.*
GU 1: *L. gibertianus.*

hundred years in *L. albus* and *L. mutabilis* (Gladstones, 1974; Gross and von Baer, 1975; Antunez de Mayolo, 1982) differs completely from the crop management of *L. angustifolius* and the lack of amelioration in the two wild species, *L. albescens* and *L. gibertianus.*

The link between *L. angustifolius* and *L. gibertianus* agrees with results observed in morphological studies (Planchuelo–Ravelo, 1991) and alkaloid analysis (Planchuelo–Ravelo *et al.*, 1993), whereas *L. albescens* that is morphologically distinct and has a unique alkaloid profile (Planchuelo–Ravelo and Wink, 1993) shows for the first time some resemblance with *L. angustifolius.*

Variability between varieties and species could be considered as a result of environmental influences as has been previously reported for the Leguminosae (Souza *et al.*, 1989; Wolff and Kwolek, 1971).

As a conclusion it is possible to say that seed lipid components in *Lupinus* are good indicators of the quality of the grain for human comsumption and also contribute with valuable information in interpretating species relationship.

## Experimental

### *Plant material*

Seeds of improved lines and commercial varieties of *Lupinus albus, L. angustifolius* and *L. mutabilis* were obtained from breeders who sent samples for research programs carried out in Argentina.

The materials studied were:

*Lupinus albus*: the lines, L 2034 N, L 2040 N, L 2053 N, and L 2085 N, were obtained by Resource Seeds Inc. in California; the cultivar "Amiga" was developed by E. von Baer in Temuco, Chile, and the "Bitter" cultivar was from the lupin seeds collection of INTA–Pergamino, Argentina.

*L. angustifolius*: the cultivars, Danja, Emir, Gelen, Gungurru, Mirela, Wandoo and Yandee were all originated from the breeding program carried on by the Ministry of Agriculture of Western Australia, in Perth.

*L. mutabilis*: the lines, P 33, P 106, P 154, were originated from trials carried out in Cuzco by the University "La Molina," Lima, Peru. The lines E 001 and E 042 were from Santa Catalina Experimental Station in Izobamba, Ecuador.

The wild species were obtained from populations growing in sandy soil at each side of the Paraná River. Voucher specimens are: *L. albescens*: Catalogue N° 0050; *L. gibertianus*: Catalogue N° 0051.

Seed samples and voucher specimens are deposited in the *Lupinus*'s seed collection and herbarium at the Facultad de Ciencias Agropecuarias (ACOR) University of Córdoba, Argentina.

### *Oil extraction and GLC procedure*

Samples were ground to a fine powder and the oil was extracted by n–hexane in a Soxhlet during 12 hrs, and then it was evaporated under low pressure at 40° C. Saponification was accomplished by refluxing the oil for 45 minutes with a 1.0 N methanolic potassium hydroxide solution. The esterification of the methanolic extract was achieved by 1 N methanolic sulfuric acid solution.

Four decimicrolitres of the fatty acids' methyl esters were injected onto a GC–RIA Shimadzu with R PFR–G1 processor, FDI detector and capillary column AT–WAX (Superox II) 30 m x 0.25 mm ID. Column temperature was set from 180 to 240° C, with nitrogen as gas carrier. The analysis was run three times for each sample. Fatty acid identification was done by comparison, with a standard mixture of fatty acids methyl esters. Theoretical iodine absorption value was calculated based on the percentages of enoic fatty acids (Carreras *et al.*, 1989).

The unsaponifiable fractions were applied uniformly along a line 1.5 cm from one edge of the 20 x 20 cm plates with a 1 mm layer of silica gel 60 G (Merck). The solvent system chloroform:diethyl ether was 90:10 V/V (Kamal–Eldin *et al.*, 1991). Cholesterol, dehydrocholesterol, stigmasterol, β–sitosterol and campesterol were used as reference spots on both sides of the TLC. The sterols were analyzed with a CPB1 capillary column, SE–30. The column operates with an initial temperature of 200° C, increasing 2° C/min, to the final maximum temperature of 300° C. Nitrogen at 60 ml/min was the gas carrier. Identification of compounds was accomplished by comparison with standards (Sigma), other non standards sterols were identified by the relative retention time cited in Zygadlo (1994). All retention times were taken against cholesterol.

*Numerical analysis*

Characters considered for the 20 samples (OTUs) were the amount of fatty acids and sterols and the percentage of crude oil by dry matter. The data matrix was analyzed using correlation coefficient and weighted pair–group, Sperman's averages (WPGMS) method. The cluster analysis was accomplished using the Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1987).

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