Fatty Acids and Oil Content in White Lupin Seed as Affected by Production Practices

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ABSTRACT: Characteristics of the seed oil of white lupin (*Lupinus albus* L.), a potential alternative winter crop in the mid-Atlantic region of the United States, are not well established. Replicated experiments were conducted during the 1998–1999 and 1999–2000 growing seasons with a determinate and an indeterminate cultivar to characterize oil and FA in lupin seed in relation to production practices. The experiments were planted in early October, late October, and mid-November using row spacings of 0.3, 0.6, and 0.9 m at each planting time. Seeds from the planting date of early October had significantly ($P < 0.05$) higher oil content than the later plantings (late October and mid-November). A closer row spacing (0.3 m) also had significantly ($P < 0.05$) higher oil content than the wider row spacing (0.9 m). Planting date effects on FA content were significant for some FA, but row spacing did not affect FA contents. Oil content in the seed varied from 7.2 to 8.2% (w/w). The oil from white lupin seed contained FA in the order of 18:1 > 18:2 > 18:3 > 16:0 > 20:1 > 22:1 > 22:0 > 18:0 > 24:0 > 20:0. The saturated FA/unsaturated FA ratio in lupin oil was 0.14. White lupin seed contained higher contents of oil and FA than literature values for seed of navy, kidney, and pinto beans.

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KEY WORDS: Cultivars, MUFA, planting dates, PUFA, row spacing, saturated fatty acids.

World agriculture is faced with two challenges: production of adequate food, including feed and food grains, for an ever-increasing human population and protection of natural resources from pollution. Use of N that is biologically fixed by legumes to support production of nonlegume crops can improve soil fertility with fewer detrimental effects on the environment as compared with use of inorganic fertilizers. White lupin, a winter-annual legume, has been used as a green-manure N source in the past. Estimates suggest that if lupin replaced a quarter of the wheat acreage in the southeastern United States, it could save 95,000 tons of N fertilizer, worth $50 to $60 million per year (1).

Production of lupin as a grain crop could provide alternative grains for use as food and feed. A preliminary study with white lupin (2) indicated that lupin seed may contain 4.7 to 10.6% oil, depending upon growing location. This study also showed variation among lupin genotypes for fatty acids in lupin seed oil. However, characteristics of oil are not well established for seed produced in the mid-Atlantic region of the United States. Objectives of our studies therefore were to characterize the effects of cultivar, planting date, and row spacing on oil traits in lupin seed. In addition, we were interested in comparing the oil in lupin seed to that of other legumes.

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MATERIALS AND METHODS

Seeds for oil extraction were obtained from two lupin cultivars: 'Lucyanne' (determinate) and 'Lunoble' (indeterminate), and grown during the 1998–1999 and 1999–2000 seasons at Randolph Farm of Virginia State University, which is located in Ettrick, Virginia (37°15′ N and 77°30.8′ W). The experiments were planted on three dates during each season (5 October, 28 October, and 16 November 1998; and 8 October, 28 October, and 19 November 1999) and in three row spacings (0.3, 0.6, and 0.9 m between the rows). The experiment during each year was designed as a split plot with planting dates as main-plots, cultivars as sub plots, and row spacings as sub sub plots. Each plot consisted of three rows with 0.9 m distance between plots. Each experiment consisted of three replications per planting date. The rows were 4 m long. Approximately 100 seeds were planted in each row with a cone-type manual planter at a depth of approximately 0.04 m. These plots received no fertilizer applications because the seed were inoculated with a commercial bradyrhizobial inoculum for N fixation and the field sites were known to have high levels of P and K. The soil type was an Abel sandy loam (fine loamy mixed thermic Aquatic Hapludult). Plots were manually kept free of weeds. These plots were not irrigated. At maturity, in June, the plants in the middle row of each plot were harvested. The seeds were analyzed for oil and FA.

Oil extraction. The oil was extracted from 2 g of ground seed at room temperature by homogenization (3) for 2 min in 10 mL hexane/isopropanol (3:2, vol/vol) with a Biospec Model 985-370 Tissue Homogenizer (Biospec Products, Inc., Racine, WI) and centrifuged at 4000 × *g* for 5 min, as previously described (4,5). The oil extraction was repeated three times for each sample to ensure full oil recovery. The hexane-lipid layer was washed and separated from the combined extract by shaking and centrifugation with 10 mL of 1% $CaCl_2$ and 1% NaCl in 50% methanol. The washing procedure was repeated, and the purified lipid layer was removed by aspiration and dried over anhydrous Na_2SO_4. The oil percentage (g/100 g dry basis) was determined gravimetrically after drying in a vacuum oven at 40°C and stored under N_2 at −10°C until analyzed.

FA. FAME were prepared by acid-catalyzed transestrification method as previously described (5,6). The oil samples (5 mg) were vortexed with 2 mL sulfuric acid/methanol (1:99, vol/vol) in 10-mL glass vials containing a Teflon boiling chip. The open vials were placed in a heating block at 90°C until the sample volume was reduced to 0.5 mL. After cooling to room temperature, 1 mL of hexane, followed by 1 mL of distilled water was added. The mixture was vortexed, and the upper hexane layer containing the FAME was then dried over anhydrous Na_2SO_4. The hexane phase containing FAME was transferred to a suitable vial and kept under N_2 at 0°C for GC analysis.

Analyses of FAME were carried out as described by Bhardwaj and Hamama (4). A 1-μL aliquot of FAME in hexane was injected into a SupelcoWax 10 capillary column (25 m × 0.25 mm i.d. and 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA) in a Varian model Vista 6000 gas chromatograph equipped with an FID (Varian, Sugar Land, TX). Helium was used as a carrier gas at 25 cm/s, with a split ratio of 1:100. The column temperature was isothermal at 210°C. The injector and detector temperatures were 250 and 260°C, respectively. A Spectra Physics Model 4290 Integrator (San Jose, CA) was used to determine relative concentrations of the detected FA. Peaks were identified by reference to the retention of FAME standards and quantitated by the aid of heptadecanoic acid (17:0) as an internal standard. The concentration of each FA was calculated as the percentage (w/w) of the total FA.

Data were analyzed *via* procedures in version 6.11 of SAS (7) using a split plot design. Fisher's protected least squares difference was used for mean separation with a significance level of 5%. The oil traits of Virginia-grown lupin seed were compared with the literature values for navy, kidney, and pinto beans (8).

RESULTS AND DISCUSSION

The ANOVA indicated that year × cultivar interactions were nonsignificant for all traits under consideration except for 18:3/18:2 ratio. The year × planting date interaction was nonsignificant for all traits under consideration except for the contents of 16:0, 18:0, 18:1, 18:2, monounsaturated FA (MUFA), PUFA, and PUFA/MUFA ratio. The year × row spacing interactions were nonsignificant for all traits under consideration except for 16:0, saturated FA (SFA), unsaturated FA (UFA), and UFA/SFA ratio. In the case where interactions with year were significant, the interaction mean squares instead of the residual error were used for mean separation (2). The means reported in Tables 1, 2, and 3 are averages over both seasons.

The oil from white lupin seed contained FA in the order of 18:1 > 18:2 > 18:3 > 16:0 > 20:1 > 22:1 > 22:0 > 18:0 > 24:0 > 20:0. The oil was quite high in UFA (87.6% w/w), with a SFA/UFA ratio of 0.14, which makes lupin oil similar to that from corn, sesame, and quinoa, which have SFA/UFA values of 0.16, 0.19, and 0.15, respectively (9).

The two white lupin cultivars differed significantly ($P < 0.05$) in total oil content and in contents of 16:0, 18:1, 18:2, total SFA, total UFA, MUFA, and PUFA in the oil from white lupin seed (Tables 1 to 3). The determinate cultivar (Lucyanne) had significantly ($P < 0.05$) higher oil content, and contents of 16:0, 18:2, SFA, and PUFA whereas the indeterminate cultivar (Lunoble) had higher contents of 18:1, UFA, and MUFA. Based on the higher contents of oleic acid, MUFA, and a ratio approximately between 1:4 and 1:10 of 18:3 (ω3):18:2 (ω6), which is recommended for human health, Lunoble cultivar has a nutritional advantage over Lucyanne. The present data indicate that lupin oil is characterized by a balanced FA composition with <12% SFA (of which about 9% is palmitic, 16:0) and ≥88% UFA, of which approximately 46% is oleic (18:1), 25% linoleic (18:2), and 12% linolenic acid (18:3). These findings

TABLE 1
Cultivar, Planting Date, and Row Spacing Effects[a] on Contents of Oil (g/kg) and Saturated FA (weights as % of total FA) in White Lupin Seed Grown in Ettrick, Virginia, During the 1998–1999 and 1999–2000 Growing Seasons

		16:0	18:0	20:0	22:0	24:0
	Oil (g/kg)	% of total FA				
Cultivar						
Lunoble	72 b	8.7 b	0.85 a	0.54 a	1.32 a	0.53 a
Lucyanne	79 a	9.4 a	0.78 a	0.56 a	1.46 a	0.59 a
Planting date						
Early Oct.	82 a	8.8 b	0.92 a	0.52 b	1.44 a	0.52 a
Late Oct.	73 b	9.1 ab	0.82 b	0.61 a	1.25 a	0.61 a
Mid-Nov.	72 b	9.3 a	0.71 c	0.52 b	1.48 a	0.56 a
Row spacing						
0.3 m	77 a	9.1 a	0.81 a	0.57 a	1.45 a	0.57 a
0.6 m	76 a	9.0 a	0.84 a	0.53 a	1.38 a	0.61 a
0.9 m	73 b	9.1 a	0.80 a	0.57 a	1.34 a	0.51 a

[a]Means followed by the same letters within a column and main effect were not different according to least squares difference at the 5% probability level. The cultivar means are from 54 observations (2 yr, 3 planting dates per yr, 3 row spacings per yr, and 3 replications per yr). The planting date means are from 36 observations (2 yr, 2 cultivars per yr, 3 row spacings per yr, and 3 replications per yr). The row spacing means are from 36 observations (2 yr, 2 cultivars per yr, 3 planting dates per yr, and 3 replications per yr).

TABLE 2
Cultivar, Planting Date, and Row Spacing Effects[a] on Contents of Unsaturated FA (weights as % of total FA) in White Lupin Seed Grown in Ettrick, Virginia, During the 1998–1999 and 1999–2000 Growing Seasons

	16:1	18:1	18:2	18:3	20:1	22:1
	% of total FA					
Cultivar						
Lunoble	0.29 a	45.82 a	24.15 b	11.30 a	4.09 a	2.36 a
Lucyanne	0.26 b	43.87 b	24.78 a	11.26 a	4.35 a	2.73 a
Planting date						
Early Oct.	0.30 a	46.03 a	23.86 b	10.86 b	4.18 a	2.57 a
Late Oct.	0.28 a	45.02 a	24.74 a	10.90 b	4.29 a	2.44 a
Mid-Nov.	0.25 a	43.49 b	24.80 a	12.08 a	4.19 a	2.63 a
Row spacing						
0.3 m	0.28 a	44.67 a	24.43 a	11.04 a	4.50 a	2.62 a
0.6 m	0.26 a	45.06 a	24.29 a	11.41 a	4.09 b	2.50 a
0.9 m	0.29 a	44.81 a	24.68 a	11.39 a	4.06 b	2.52 a

[a]Means followed by the same letters within a column and main effect were not different according to least squares difference at the 5% probability level. The cultivar means are from 54 observations (2 yr, 3 planting dates per yr, 3 row spacings per yr, and 3 replications per yr). The planting date means are from 36 observations (2 yr, 2 cultivars per yr, 3 row spacings per yr, and 3 replications per yr). The row spacing means are from 36 observations (2 yr, 2 cultivars per yr, 3 planting dates per yr, and 3 replications per yr).

TABLE 3
Cultivar, Planting Date, and Row Spacing Effects[a] on Contents of Total SFA, Total UFA, Total MUFA, and Total PUFA (weights as % of total FA) in White Lupin Seed Grown in Ettrick, Virginia, During the 1998–1999 and 1999–2000 Growing Seasons.

	SFA	UFA	MUFA	PUFA			
	% of total FA				R1[b]	R2[b]	R3[b]
Cultivar							
Lunoble	11.99 b	88.01 a	52.56 a	35.45 b	7.34 a	0.67 b	0.47 a
Lucyanne	12.75 a	87.25 b	51.22 b	36.04 a	6.84 b	0.70 a	0.45 b
Planting dates							
Early Oct.	12.21 a	87.79 a	53.07 a	34.72 c	7.19 a	0.65 c	0.46 b
Late Oct.	12.33 a	87.67 a	52.03 b	35.64 b	7.11 a	0.69 b	0.44 b
Mid Nov.	12.57 a	87.43 a	50.56 c	36.87 a	6.96 a	0.73 a	0.49 a
Row spacings							
0.3 m	12.47 a	87.53 a	52.07 a	35.47 a	7.02 a	0.68 a	0.45 a
0.6 m	12.40 a	87.60 a	51.90 a	35.70 a	7.07 a	0.69 a	0.47 a
0.9 m	12.24 a	87.76 a	51.69 a	36.07 a	7.17 a	0.70 a	0.46 a

[a]Means followed by the same letters within a column and main effect were not different according to least significant difference at the 5% probability level. The cultivar means are from 54 observations (2 yr, 3 planting dates per yr, 3 row spacings per yr, and 3 replications per yr). The planting date means are from 36 observations (2 yr, 2 cultivars per yr, 3 row spacings per yr, and 3 replications per yr). The row spacing means are from 36 observations (2 yr, 2 cultivars per yr, 3 planting dates per yr, and 3 replications per yr).
[b]R1, unsaturated FA (UFA)/saturated FA (SFA) ratio; R2, PUFA/monounsaturated FA (MUFA) ratio; R3, 18:3/18:2 ratio.

are in close agreement with those previously reported (10,11). Furthermore, lupin oil was found to be more digestible than soybean oil (12). The PUFA/MUFA ratio of 0.7 in lupin oil (Table 3) was similar to that of canola (5,13) and was lower by about 75–80% than corn, soybean, and cottonseed oils (13,14). These results suggest that lupin oil is much less susceptible to auto-oxidation as compared to most common vegetable oils. Lupin oil was found to be stable for 90 days at 51°C (15).

Oil from Lunoble seed contained approximately 2% less PUFA and a 4.3% lower PUFA/MUFA ratio than that from Lucyanne, thus, indicating its higher oxidative stability (Table 3). On the other hand, Lucyanne, with approximately 7% higher oil content, was desirable, as the seed grains with higher oil content are expected to provide higher energy. Our results indicate that for human consumption, seed from Lunoble would be preferable. However, for use as feed grains, it may be desirable to use the seed from Lucyanne. Whether this observation would hold true for determinate vs. indeterminable cultivars in general would need to be verified from a larger sample of cultivars in each group.

Planting date affected the oil content and contents of 16:0, 18:0, 20:0, 18:1, 18:2, 18:3, MUFA, and PUFA (Tables 1 to 3). In general, earlier planting dates resulted in higher ($P < 0.05$) contents of oil, 18:0, 18:1, and MUFA, whereas later plantings resulted in higher ($P < 0.05$) contents of 16:0, 18:2, 18:3, and PUFA. Seeds from lupin planted in early or late October contained 5.8 and 3.5% higher ($P < 0.05$) contents of oleic acid, respectively, as compared to the seed of lupin planted in mid-November. Delay in planting from early or late October to mid-November resulted in an 11% increase in the content of 18:3. Delayed planting also resulted in an increased content of PUFA by 2 to 3%. These results indicate that later plantings of lupin result in better quality seed oil, but later plantings are also associated with lower seed yield and lower oil contents (16). We are currently breeding white lupin for development of winter-hardy, high-yielding cultivars to facilitate later planting dates without adversely affecting seed yields. Availability of these newer cultivars may lead to a situation where later planting dates would produce not only a higher quantity of oil but also oils with a more desirable FA profile.

Row spacing generally did not affect the quality of white lupin seed (Tables 1 to 3). The only significant effect of row spacing was that 0.3 m row spacing produced approximately 10% higher content of 20:1 FA than the 0.9 m row spacing (Table 2). Row spacings of 0.3 and 0.6 m were also observed to be superior to the 0.9 m row spacing in total oil content.

The content of erucic acid varied from 2.4 to 2.7% of the total FA. We had previously observed that content of erucic acid among 12 lupin genotypes, grown in Virginia during 1994, varied from 0.6 to 1.5% with a mean of 1.1%. This amount of erucic acid, which is considered an anti-quality factor, is of little significance for use of white lupin seed as food or feed grains. As is known from research with canola (*Brassica napus* L.), a content of erucic acid up to 3% is not considered detrimental for human health (13,14). Our results indicate that it may be advisable to be cognizant of the content of erucic acid in the oil from lupin seed.

White lupin, with an oil content of approximately 8%, is not a true oilseed like canola and soybean. Based on oil content, its use would need to be discussed in the context of consumption as a whole grain similar to that of other edible legumes such as navy, kidney, and pinto beans or as secondary products of defatted lupin meal. A comparison of oil from white lupin with other edible legume seeds (Table 4) indicates that lupin seeds have higher contents of all oil traits than navy, kidney, and pinto beans. Lupin seeds are used as food in many forms: fermented foods such as tempe and miso, bread and pasta, milk products, and sprouts (17). Hung *et al.* (18) utilized

milk from Australian lupin (*L. angustifolius* L.) for manufacture of tofu and observed that up to 30% of lupin "milk" could be incorporated in soymilk for manufacture of acceptable tofu. Australian lupin contains 45 to 72 g/kg of oil in its seed with a mean of 58 g/kg (19). The white lupin in our studies contained 72 to 82 g/kg oil with a mean of 75 g/kg, indicating that it may be suitable for many food uses including manufacture of tofu.

Our results have established a benchmark for quantity and quality of oil in white lupin seed produced in the mid-Atlantic region of the United States. We observed that oil from the indeterminate cultivar possessed a better FA composition for human health than that from the determinate cultivar. These studies were conducted with only two cultivars; therefore, results comparing determinate vs. indeterminate cultivars need to be acquired with larger samples of cultivars from each group. Our results also indicate that lupin planted in mid-November produces seed with better oil quality as compared with lupin planted in early or late October. White lupin seed had higher contents of oil and FA than seeds of navy, kidney, and pinto beans. We concluded that lupin seed, based on their oil content and FA characteristics, may be a desirable dietary element and may be dietetically recommended.

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TABLE 4
Comparison of Mean Oil Traits of White Lupin Seed Grown in Ettrick, Virginia, During 1998–1999 and 1999–2000 to the Literature Values for Navy, Kidney, and Pinto Beans.

Variable	White lupin[a]	Navy bean[b]	Kidney bean[b]	Pinto bean[b]
Oil (%)	7.537	1.280	1.060	1.350
FA (g/100 g seed)				
16:0	0.686	0.310	0.136	0.229
18:0	0.603	0.020	0.018	0.005
20:0	0.415	NA	NA	NA
22:0	0.105	NA	NA	NA
24:0	0.042	NA	NA	NA
16:1	0.021	0.000	0.000	0.000
18:1	3.381	0.111	0.082	0.229
18:2	1.845	0.301	0.228	0.170
18:3	0.851	0.252	0.358	0.237
20:1	0.318	0.000	0.000	0.000
22:1	0.192	0.000	0.000	0.000
SFA	0.933	0.331	0.154	0.235
UFA	6.607	0.663	0.668	0.636
MUFA	3.913	0.111	0.082	0.229
PUFA	2.696	0.552	0.586	0.407
UFA/SFA	7.1	2.0	4.3	2.7
PUFA/MUFA	0.7	5.0	7.2	1.8
18:3/18:2	0.5	0.8	1.6	1.4

[a]Grown in Virginia during 1998–1999 and 1999–2000 seasons; means over 54 observations: 2 yr, 3 replications per yr, 2 cultivars, 3 planting dates per yr, and 3 row spacings per yr. NA, not available; for other abbreviations see Table 3.
[b]Information from USDA Nutrient Database for Standard Reference, Release 14. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp/ (checked May 14, 2004).

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