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ORIGINAL PAPER

The fatty acid composition of the oil from *Lupinus albus* cv. *Luxe* as affected by environmental and agricultural factors

Giovanna Boschin · Alessandra D'Agostina · Paolo Annicchiarico · Anna Arnoldi

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Abstract There is a growing interest in white lupin (*Lupinus albus* L.) seed for food or feed, favoured by the availability of well-performing varieties with low content of alkaloids. The objective of this study was to assess the influence of the environmental and agricultural factors on the content and fatty acid composition of lupin oil. The investigation was performed on the sweet variety Luxe grown in three Italian locations (one continental and two Mediterranean) and 13 environments in total. Statistical analyses (analysis of variance and principal component analysis) indicated that oil content and composition of fatty acids were affected largely by the growing location. Mediterranean sites tended to lower crop yield, but to increase oil content and absolute α-linolenic acid content compared to the continental location; large variation occurred also between the Mediterranean sites. The α-linolenic acid content ranged from 1.41 to 3.24 mg/g flour, highlighting the possible value of white lupin in order to reach the recommended daily intake of this fatty acid. The observed ω-3/ω-6 ratio, ranging from 0.45 to 0.63, was much higher than that of most vegetable oils.

Keywords *Lupinus albus* · Growing environment · Oil composition · α-Linolenic acid · Linoleic acid · ω-3/ω-6 ratio

G. Boschin (✉) · A. D'Agostina · A. Arnoldi
Laboratory of Food Chemistry and Mass Spectrometry,
Department of Agri-Food Molecular Sciences (DISMA),
Faculty of Pharmacy, University of Milan,
Via Celoria 2,
20133 Milan, Italy
e-mail: giovanna.boschin@unimi.it

P. Annicchiarico
C.R.A. – Istituto Sperimentale per le Colture Foraggere,
Viale Piacenza 29,
26900 Lodi, Italy

Abbreviations FAME: Fatty acid methyl ester · GC-FID: Gas chromatography-flame ionisation detector · TSFA: Total saturated fatty acids · TUFA: Total unsaturated fatty acids · PUFA: Polyunsaturated fatty acids · MUFA: Monounsaturated fatty acid · PCA: Principal component analysis · RDI: Recommended daily intake

Introduction

Several experimental, clinical, and epidemiological studies support the hypothesis that ω-3 fatty acids are protective against cardiovascular diseases [1–3]. In addition, ω-3 fatty acids play a fundamental role during the fetal and infant development, in particular in the formation of the central nervous system and retina [4–6]. These considerations have prompted the Health Authorities of several countries to suggest specific recommended daily intakes (RDI) for ω-3 fatty acids. For example, the Italian Health Authority has proposed the following RDI values: 0.5 g/day for infants, 0.7–1 g/day for children depending on age, and 1–1.5 g/day for adults depending on gender [7].

Beside the absolute ω-3 fatty acid intake, another important dietary parameter is the ω-3/ω-6 fatty acid ratio [8–10]. This ratio used to be about 0.5 in the diet of our Paleolithic ancestors, based on gathering and hunting, while the current range is 0.1–0.08 because of the prevalence of cereals, having very low α-linolenic and high linoleic acid contents, and dairy products, whose fat contains less than 2.5% α-linolenic acid [11]. In addition, domestic livestock contains about five times less ω-3 polyunsaturated fatty acids than wild animals and birds [12, 13].

These facts suggest that it is important to promote the daily consumption of foods containing favourable ω-3/ω-6 fatty acid ratio, i.e. in the range 0.5–1 [8].

Lupinus albus L. (white lupin) belongs to the short set of vegetables whose lipid composition appears to be very favourable in this respect [14–17]. Its oil contains about 50–60% oleic acid, 16–23% linoleic acid, and 8–9% α-linolenic acid, and the oil percentage on the seed dry weight is rather high, falling in the range 9–14%. As a consequence of this fatty acid composition, the ω-3/ω-6 fatty acid ratio is about 0.4–0.6. These characteristics are typical of *L. albus*, whereas other domesticated lupin species, such as *L. angustifolius* and *L. luteus*, have lower ω-3/ω-6 fatty acid ratios due to much higher linoleic acid (34–48% and 45–48%, respectively) [14].

The increasing selection of new sweet varieties (containing very low amounts of bitter alkaloids) has widened the possibility to use white lupin seeds in human or livestock nutrition [18–20]. Considering the high protein content of this seed (> 35%), several companies have started to produce and commercialise food products in which lupin is used in replacement for animal ingredients [21–24]; in principle, these foods offer opportunities for improving the daily intake of α-linolenic acid. In addition, since the procedure for producing lupin protein isolates, starts with a defatting step [22], a great production of lupin ingredients would deliver also large amounts of oil to be used in human nutrition.

However, there is a lack of information on the influence of environmental and agricultural factors on the fatty acid composition of lupin oil. End users need to know whether the quality of different grain lots of the same variety can be consider homogeneous or, on the contrary, may vary depending on the growing environment. If the environment exerted a marked influence, preference could be given to grain lots that are produced under environmental conditions capable of maximizing their quality.

The objective of this study was to assess the influence on the content and the fatty acid composition of white lupin oil of Italian growing environments that were widely diversified in terms of climatic region (continental or Mediterranean), soil lime content (fairly high or very low), sowing time (autumn or late-winter) and cropping year. This assessment was performed on the variety Luxe, recently released and recommended for winter-sown cultivation in France [25].

Materials and methods

Sampling

The variety Luxe (obtained from Agri-Obtentions) was grown in 13 Italian environments that are listed in Table 1, in order to assess the adaptability and seed yield potential. Information on the crop management in each environment is provided elsewhere [26–28]. The three evaluation sites contrasted for climatic or soil characteristics. In particular, Lodi (Lombardy) has a continental climate with cold winters (Table 2) and soil characteristics (subacid pH; absence of $CaCO_3$) that are optimal for lupin growth [29]. Sanluri (Sardinia) and Foggia (Apulia) are characterized by a Mediterranean climate with mild winters and moderate spring rainfall (Table 2), with terminal drought mainly associated with higher evapotranspiration demand. The two Mediterranean sites differ in soil type; in Foggia, soil may be poorly apt to lupin cultivation because of fairly high $CaCO_3$ content, while it is suitable in Sanluri (Table 2). Two sowing times, either autumn and late-winter or early- and late-autumn, were assessed in each location for each of two cropping years (Table 1). An autumn-sowing environment was available for Sanluri in one additional year, leading to 13 the total number of evaluation environments (Table 1). Chemical analyses were carried out on three experiment replicates per growing

Table 1 Location, sowing time and date, harvest year, acronym, grain yield, and mean seed weight of 13 growing environments of *Lupinus albus*

Location	Environment						
	Sowing time	Sowing date (mm/dd/yy)	Harvest year	Acronym	Grain yield (t/ha, 13% humidity)	Seed weight (mg, 0% humidity)	Oil content (%)
Lodi	Early-autumn	10/23/02	2003	L1	3.99	236	8.45
Lodi	Late-autumn	11/07/02	2003	L2	3.88	232	8.80
Lodi	Late-autumn	11/15/04	2005	L3	3.85	225	7.97
Lodi	Late-winter	02/15/05	2005	L4	3.23	198	9.39
Foggia	Early-autumn	11/07/02	2003	F1	0.64	243	13.61
Foggia	Late-autumn	11/21/02	2003	F2	0.79	232	13.17
Foggia	Early-autumn	11/05/03	2004	F3	1.19	253	12.74
Foggia	Late-autumn	11/20/03	2004	F4	0.96	256	11.89
Sanluri	Early-autumn	11/05/02	2003	S1	2.15	187	9.45
Sanluri	Late-autumn	11/26/02	2003	S2	2.43	190	10.37
Sanluri	Mid-autumn	11/10/03	2004	S3	2.49	222	10.44
Sanluri	Late-winter	02/19/04	2004	S4	0.66	130	7.69
Sanluri	Early-autumn	10/29/04	2005	S5	1.13	202	6.98

Table 2 Soil and climatic characteristics of growing locations of *Lupinus albus* averaged across cropping years

Location	Soil pH (in H_2O)	Soil $CaCO_3$ (%)	Number of frost days	Absolute min. winter temp. (°C)	Spring rainfall (1 Mar–15 Jun) (mm)
Lodi	5.8	0.0	66	− 8.4	132
Foggia	7.6	3.2	7	− 2.7	133
Sanluri	7.4	1.6	5	− 1.1	161

environment represented by grain lots collected from three different field plots at crop maturity.

Materials

Hexane and methanol were HPLC grade and were purchased from Baker (Deventer, The Netherlands), water was produced with a MilliQ Water Purification System (Millipore, Billerica, MA, USA). Sodium methoxide in methanol (1%) was freshly prepared dissolving 0.34 g of metallic sodium in 100 ml of HPLC grade methanol. The following fatty acid methyl esters (FAME) standards were purchased from Fluka (Sigma–Aldrich, St. Louis, MO, USA): methyl pentadecanoate (99.5%), methyl palmitate (99.5%), methyl palmitoleate (99.0%), methyl heptadecanoate (99.7%), methyl stearate (99.5%), methyl oleate (99.0%), methyl linoleate (98.5%), methyl linolenate (99.0%), methyl eicosanoate (98.0%), methyl *cis*-11-eicosenoate (98.5%), methyl docosanoate (99.0%), methyl *cis*-13-docosenoate (99.0%).

Extraction of the crude oil

Lupin seeds were dehulled and ground in a house-hold mill (Braun, Germany); 12 g of flour were extracted with hexane (300 ml) for 6 h in a Soxhlet apparatus using cellulose extraction thimbles (123 mm × 45 mm e.d.; 43 mm i.d.; Whatman International, Brentford, UK). The solvent was then evaporated under reduced pressure.

Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared by trans-methylation using CH_3ONa in CH_3OH (1%) according to the official method published in the Official Journal of the European Union (Annex XB, 05/09/1991 num L248/44). The oil (1 g) was suspended in 20 ml of CH_3OH and 1 ml of a 30 mg/ml hexane solution of methyl heptadecanoate (C17:0) was added as internal standard, in order to quantify the fatty acids. CH_3ONa in CH_3OH (1.75 ml of 1% solution) was added under reflux, then the mixture was heated with continuous stirring for 3 h. The FAMEs were extracted with diethyl ether (3 × 20 ml) and methyl pentadecanoate (C15:0) (1 ml of a 30 mg/ml solution in hexane) was added as a second internal standard to evaluate the FAMEs recovery. Then the solvent was evaporated under reduced pressure and the residue was dissolved in hexane in order to obtain about 10 mg/ml total concentration.

GC-FID analysis

The FAMEs were analysed with a DANI 86.10HT gas-chromatograph equipped with a Flame Ionization Detector (FID) (gas pressure: H_2 at 1 bar; air at 1 bar), the detector temperature was set at 250 °C; a SP-2340 column (60 m × 0.25 mm i.d. × 0.2 μm film thickness) (Supelco, Sigma–Aldrich, St. Louis, MO, USA) was used. Analyses were performed in split-less mode using a PTV injector (operative conditions 45 °C for 30 s, then heating to 250 °C in 12 s). Operating conditions were carrier gas He (1.4 bar), auxiliary gas N_2 (0.8 bar). The temperature program was 16 min at 160 °C, from 160 to 210 °C at 1.5 °C/min, then 20 min at 210 °C. The analyses were processed with the Star GC Workstation software (Varian, version 5.52).

Peaks were identified by comparison of retention times with those of standard compounds. The percent fatty acid composition was calculated from the ratio of individual peak area to summation of all fatty acid areas after determination of the correction factors (Table 3). Compound concentrations were calculated using two internal standards (methyl heptadecanoate and methyl pentadecanoate) and were expressed as mg/g flour. Each analysis was performed at least in triplicate.

Statistical analysis

A one-way analysis of variance (ANOVA) was performed for oil content, absolute content of individual fatty acids, expressed as mg/g flour, total saturated fatty acids (TSFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), total unsaturated fatty acids (TUFA), also expressed as mg/g flour, and the ω-3/ω-6 ratio (α-linolenic acid / linoleic acid), partitioning the overall variation among environments into the following linear contrasts relative to the effects of climatic area, contrasting Mediterranean site or sowing time: (1) continental vs. Mediterranean locations under autumn sowing (equal to L1 + L2 + L3 vs. F1 + F2 + F3 + F4 + S1 + S2 + S3 + S5 according to acronym of environments reported in Table 1, weighting the contrast coefficients as appropriate), (2) between Mediterranean locations under autumn sowing (equal to F1 + F2 + F3 + F4 vs. S1 + S2 + S3 + S5), (3) autumn vs. late-winter sowing in same cropping years (equal to L3 + S3 vs. L4 + S4), and (4) early-autumn vs. late-autumn sowing in same cropping year (equal to L1 + F1 + F3 + S1

Table 3 Fatty acid composition (% ± SD) of *L. albus* sowed in different growing environments and sowing times (see Table 1 for acronyms)

Fatty acid (%)	L1	L2	L3	L4	F1	F2	F3	F4	S1	S2	S3	S4	S5
Palmitic acid (C16:0)	18.57 ± 0.10	18.47 ± 0.04	14.56 ± 1.97	16.48 ± 0.34	15.56 ± 0.12	16.01 ± 1.95	14.44 ± 0.11	15.13 ± 0.03	15.87 ± 0.20	14.93 ± 0.86	16.88 ± 0.02	21.57 ± 0.28	19.02 ± 1.32
Palmitoleic acid (C16:1)	0.77 ± 0.01	0.74 ± 0.01	0.91 ± 0.17	0.67 ± 0.11	0.36 ± 0.10	0.36 ± 0.03	0.41 ± 0.02	0.58 ± 0.01	0.47 ± 0.12	0.45 ± 0.01	0.51 ± 0.02	0.83 ± 0.03	1.03 ± 0.21
Stearic acid (C18:0)	2.83 ± 0.05	2.72 ± 0.07	3.15 ± 0.45	2.74 ± 0.07	3.91 ± 0.15	3.24 ± 0.35	3.81 ± 0.02	3.87 ± 0.05	2.76 ± 0.28	2.48 ± 0.07	3.56 ± 0.02	2.73 ± 0.01	1.37 ± 0.49
Oleic acid (C18:1)	48.08 ± 0.29	49.83 ± 0.42	52.87 ± 0.78	51.56 ± 0.10	52.09 ± 0.22	52.56 ± 0.27	51.16 ± 0.01	50.25 ± 0.24	42.86 ± 0.40	45.80 ± 0.59	47.21 ± 0.17	42.78 ± 0.05	43.79 ± 0.79
Linoleic acid (C18:2, ω6)	10.74 ± 0.15	10.07 ± 0.05	9.20 ± 0.20	10.41 ± 0.14	9.85 ± 0.07	10.09 ± 0.36	10.93 ± 0.01	10.06 ± 0.01	17.23 ± 0.33	16.78 ± 0.33	12.80 ± 0.01	13.41 ± 0.05	14.16 ± 0.15
α-Linolenic acid (C18:3, ω3)	5.31 ± 0.01	5.34 ± 0.09	4.81 ± 0.28	5.58 ± 0.01	5.94 ± 0.21	5.58 ± 0.45	6.78 ± 0.34	6.33 ± 0.08	9.02 ± 0.42	8.57 ± 0.09	6.55 ± 0.32	5.97 ± 0.02	8.33 ± 0.17
Arachidic acid (C20:0)	1.74 ± 0.11	1.61 ± 0.04	1.94 ± 0.01	1.67 ± 0.01	2.03 ± 0.01	2.00 ± 0.37	2.30 ± 0.02	2.22 ± 0.08	1.73 ± 0.03	1.78 ± 0.08	1.99 ± 0.07	1.71 ± 0.08	1.67 ± 0.02
11-Eicosenoic acid (C20:1)	4.92 ± 0.02	4.86 ± 0.25	5.30 ± 0.58	4.48 ± 0.10	4.40 ± 0.31	4.50 ± 0.06	4.24 ± 0.37	4.78 ± 0.13	3.98 ± 0.45	3.86 ± 0.36	4.57 ± 0.19	4.43 ± 0.11	4.04 ± 0.10
Behenic acid (C22:0)	5.57 ± 0.28	4.99 ± 0.28	5.99 ± 0.03	5.19 ± 0.30	5.05 ± 0.02	4.75 ± 0.52	5.09 ± 0.03	5.65 ± 0.33	5.37 ± 0.01	5.11 ± 0.19	5.15 ± 0.01	5.66 ± 0.32	5.72 ± 0.01
Erucic acid (C22:1)	1.47 ± 0.03	1.36 ± 0.21	1.28 ± 0.05	1.23 ± 0.10	0.82 ± 0.01	0.91 ± 0.07	0.82 ± 0.03	1.12 ± 0.06	0.70 ± 0.09	0.51 ± 0.01	0.78 ± 0.01	0.89 ± 0.03	0.86 ± 0.20

vs. L2 + F2 + F4 + S2). The variation between individual locations was assessed likewise, comparing the site means relative to autumn-sown environments to eliminate any bias derived from inconsistency of sowing season between sites.

A principal components analysis (PCA) holding the environments as individuals and the seed content of individual fatty acids as original variables assessed the overall similarity between growing environments for fatty acid composition. The relationships among grain yield, oil content and ordination on principal component (PC) axes of the environments was assessed by simple correlation analysis.

Results and discussion

Grain yield and oil content

The effect of environment on grain yields (Table 1) confirmed the good adaptation of white lupin in the continental location (Lodi), as well as its limited adaptation to the site whose soil characteristics may affect negatively the growth of some lupin cultivars (Foggia). The yield response in Sanluri was low only for the late-winter sowing (S4) and the last autumn sowing (S5) (Table 1). Sowings have to be delayed until late-winter when climatic conditions prevent their execution in autumn, but the yield penalty may be severe for winter-type material such as Luxe in Mediterranean environments, because of the difficulty in satisfying its vernalization requirement [20]. The low yield in the last autumn sowing of Sanluri was mainly due to severe water logging, to which white lupin is very susceptible [29]. The yields in the remaining environments of Sanluri, although lower than those in Lodi, were quite acceptable under the severe terminal drought stress that is typical of Mediterranean-climate locations. The drought stress at this site tended to reduce the seed size, whereas the soil lime stress at Foggia had no negative effect on this trait (Table 1) while leading to severe mortality of plants.

The seed oil content was affected by the growing environment. In particular, it was significantly affected by the location while being unaffected by sowing time (Table 4). On average, the continental location showed lower oil content than the Mediterranean sites (8.40% vs. 11.08%), under autumn sowing. However, the widest difference in oil content occurred between the two Mediterranean sites (12.85% in Foggia vs. 9.31% in Sanluri). A modest inverse correlation emerged between grain yield and oil content of the 13 environments ($r = -0.49, P < 0.10$).

The oil content observed in this work was always higher than those reported in literature for other white lupin seeds, namely 5.95% for a cultivar grown in Turkey [30], 7.1% for an Egyptian white lupin [31] and 4.86% for an American cultivar in Virginia [32]. A study by Jimenez et al. [16] sug-

Table 4 Variation between 13 growing environments and their subsets as defined by locations, linear contrasts between and within climatic areas and sowing seasons, for seed oil content (%), fatty acid composition (mg/g flour) and ω-3/ω-6 ratio (α-linolenic/linoleic acid) of *Lupinus albus*

Variable	All environments		Locations[a]					Sowing season[b]		
	Range	*P*	Lodi	Sanluri	Foggia	Continental *vs.* Mediterranean	Between Mediterranean	Autumn	Late-winter	*P*
Oil content	6.98–13.61	***	8.40 ± 0.13 c	9.31 ± 0.43 b	12.85 ± 0.29 a	***	***	9.20 ± 0.46	8.54 ± 0.60	NS
Palmitic acid (C16:0)	4.72–8.22	***	5.29 ± 0.27 b	5.23 ± 0.03 b	7.14 ± 0.16 a	***	***	5.46 ± 0.26	5.76 ± 0.12	NS
Palmitoleic acid (C16:1)	0.15–0.33	**	0.25 ± 0.01 a	0.19 ± 0.02 b	0.20 ± 0.02 b	**	NS	0.25 ± 0.02	0.23 ± 0.02	NS
Stearic acid (C18:0)	0.35–1.99	***	0.91 ± 0.06 b	0.83 ± 0.05 b	1.72 ± 0.10 a	***	***	1.16 ± 0.06	0.86 ± 0.03	**
Oleic acid (C18:1)	10.11–27.14	***	15.74 ± 0.31 b	14.34 ± 0.52 c	24.06 ± 0.84 a	***	***	17.49 ± 0.13	15.09 ± 0.41	**
Linoleic acid (C18:2, ω-6)	2.95–6.34	***	3.10 ± 0.08 b	4.88 ± 0.17 a	4.76 ± 0.19 a	***	NS	3.81 ± 0.03	3.61 ± 0.11	NS
α-linolenic acid (C18:3, ω-3)	1.41–3.24	***	1.60 ± 0.06 c	2.58 ± 0.09 b	2.86 ± 0.19 a	***	*	1.97 ± 0.07	1.79 ± 0.04	NS
Arachidic acid (C20:0)	0.41–1.04	***	0.55 ± 0.01 b	0.57 ± 0.01 b	0.99 ± 0.06 a	***	***	0.68 ± 0.01	0.53 ± 0.02	*
11-eicosenoic acid (C20:1)	1.01–2.33	***	1.57 ± 0.07 b	1.31 ± 0.10 c	2.09 ± 0.11 a	NS	***	1.73 ± 0.10	1.39 ± 0.02	**
Behenic acid (C22:0)	1.42–2.58	***	1.73 ± 0.03 b	1.69 ± 0.04 b	2.39 ± 0.12 a	**	***	1.95 ± 0.01	1.68 ± 0.09	*
Erucic acid (C22:1)	0.19–0.48	***	0.43 ± 0.03 a	0.22 ± 0.02 b	0.42 ± 0.02 a	***	***	0.36 ± 0.01	0.34 ± 0.01	NS
TSFA	6.91–13.53	***	8.48 ± 0.28 b	8.33 ± 0.12 b	12.24 ± 0.37 a	***	***	9.26 ± 0.20	8.82 ± 0.23	NS
MUFA	11.56–30.13	***	17.99 ± 0.41 b	16.06 ± 0.62 c	26.77 ± 0.91 a	***	***	19.83 ± 0.26	17.06 ± 0.44	**
PUFA	4.41–9.58	***	4.69 ± 0.10 b	7.46 ± 0.26 a	7.62 ± 0.38 a	***	NS	5.77 ± 0.04	5.40 ± 0.16	NS
TUFA	16.14–38.25	***	22.68 ± 0.51 b	23.52 ± 0.80 b	34.39 ± 1.30 a	***	***	25.61 ± 0.22	22.46 ± 0.60	*
ω-3/ω-6 ratio	0.45–0.63	***	0.51 ± 0.01 b	0.53 ± 0.01 b	0.60 ± 0.02 a	***	***	0.51 ± 0.02	0.49 ± 0.01	NS

***, **, *, NS = significant at $P < 0.001$, $P < 0.01$, $P < 0.05$ and not significant, respectively Mean ± standard error for subsets of environments.

[a] Under autumn sowing; three environments for Lodi (continental), and four for Sanluri and for Foggia (Mediterranean). Row means with same letter do not differ at $P < 0.05$ according to Duncan's test.

[b] Across locations, in same cropping years; two environments per sowing season.

gested a direct relationship between soil pH and oil content which may have contributed, together with other factors, to the trend towards higher oil content of the higher soil pH sites of Foggia and Sanluri relative Lodi (Table 2).

Content of fatty acids

On average, the fatty acids ranked in the following order of abundance: oleic acid > palmitic acid > linoleic acid > behenic acid ≈ α-linolenic acid > 11-eicosenoic acid > arachidic acid ≈ stearic acid > erucic acid ≈ palmitoleic acid. Their absolute content was affected by the growing environment, as confirmed by the ANOVA results for the amount of individual fatty acids, which are reported in Table 4. The variation between climatic areas (continental vs. Mediterranean) was significant in all cases except the 11-eicosenoic acid, but the sizeable variation observed also between the two Mediterranean sites suggested that other factors beside the climate are important in determining the response of locations. Lodi, characterized by continental climate and higher crop yield potential, showed higher values of palmitoleic acid, and low or bottom levels of all other fatty acids (including the polyunsaturated ones) in comparison with the Mediterranean locations (Table 4). Foggia displayed higher values of all fatty acids, except the palmitoleic and linoleic acids, compared with Sanluri (Table 4). The effect of sowing season reached $P < 0.01$ significance in just a few cases, namely oleic acid, stearic acid and 11-eicosenoic acid, for which autumn sowing revealed higher values than late-winter sowing (Table 4). Finally, no significant difference ($P < 0.01$) emerged between early- and late-autumn sowing for absolute content of any fatty acid.

The absolute contents of individual fatty acids were then submitted to PCA, holding the environments as individuals. The first two principal components (PC 1 and PC 2) summarize 90.7% of the overall variation among environments for the content of individual fatty acids. On the basis of trait eigenvectors reported in Table 4, PC 1 is based mostly on saturated fatty acids and two ω-9 monounsaturated fatty acids, i.e. oleic acid and 11-eicosenoic acid, and explains 66.9% of the variation. PC 2, which explains 23.8% of the variation, is based on the polyunsaturated fatty acids and the other two ω-9 monounsaturated fatty acids, i.e. palmitoleic acid and erucic acid (Table 5).

The ordination of the environments in the space of these PC axes emphasized the impact of the location factor on the absolute content of fatty acids, revealing a distinct trend towards greater similarity among the environments belonging to the same location (Fig. 1). On average, the similarity between Foggia's and Sanluri's environments did not appear markedly greater than that between either site's and Lodi's environments, confirming that the climatic area is not the only major factor affecting the content of fatty acids.

Table 5 Eigenvectors of the first two axes (PC) of a principal component analysis summarizing the variation among 13 growing environments for fatty acid composition of *Lupinus albus*

Variable (mg/g flour)	PC 1	PC 2
Palmitic acid (C16:0)	0.35	0.03
Palmitoleic acid (C16:1)	− 0.05	0.55
Stearic acid (C18:0)	0.37	0.02
Oleic acid (C18:1)	0.38	0.07
Linoleic acid (C18:2)	0.22	− 0.49
α-Linolenic acid (C18:3)	0.27	− 0.41
Arachidic acid (C20:0)	0.38	− 0.01
11-Eicosenoic acid (C20:1)	0.37	0.18
Behenic acid (C22:0)	0.38	0.07
Erucic acid (C22:1)	0.22	0.49

PC 1 and PC 2 summarize 66.9 and 23.8% of total variation among environments for fatty acid content.

A trend emerged within each site towards greater similarity of the autumn-sown environments that had same harvest year (L1 and L2; F1 and F2; F3 and F4; S1 and S2) (Fig. 1).

The variation in absolute content of TSFA, MUFA, PUFA and TUFA in each set of growing environments reflected that for the individual fatty acids, showing higher values in Foggia and lower values in Lodi and Sanluri in all cases except PUFA, for which also Sanluri had high values (Table 4). Autumn sowing led to higher MUFA and TUFA values than late-winter sowing ($P < 0.05$; Table 4). TSFA, MUFA, PUFA and TUFA were positively correlated with oil content ($r \geq 0.68$, $P < 0.01$). Their amounts reported in

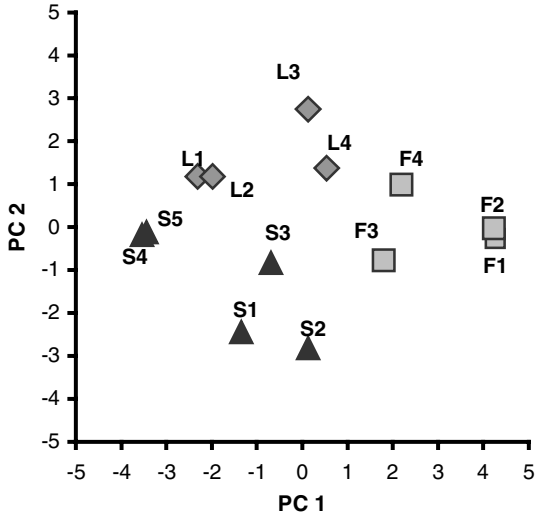

Fig. 1 Scores of growing environments of *L. albus* on the first two axes (PC) of a principal component analysis summarizing the variation for fatty acid composition (%) among 13 growing environments (see Table 1 for acronyms of environments); PC 1 and PC 2 summarize for 66.9 and 23.8%, respectively, of total variation among environments for fatty acid content (total variation explained 90.7%)

Table 6 Percentage content of oil, oleic, linoleic acid, α-linolenic acid, and ω-3/ω-6 ratio of some common vegetables

	Oil (%)	Oleic acid (%)	Linoleic acid (%)	α-Linolenic acid (%)	ω-3/ω-6
Peanut	42–52	41	35.5	0	/
Sesame	45–55	42	44.5	0	/
Cottonseed	22–24	16	55	0	/
Sunflower	25–30	23	63	< 0.5	> 0.01
Safflower	32–43	12	78	0.5	> 0.01
Corn germ	3.5–5	32.5	52	1	0.02
Olive	38–58	75.5	7.5	1	0.13
Soya	18–23	21	53	8	0.15
Wheat germ	8–11	20	52	10	0.19
Walnut	58–71	16	59	12	0.20
Canola	≈ 40	63	20	9	0.45
White lupin	8–13	48.5	11.9	6.5	0.54
Linseed	32–43	18	14	58	4.14

For white lupin: average value across 13 growing environments.

For all other species, see Ref. [11].

Table 4 are in good agreement with those of earlier studies [30–33].

Safety considerations

The average relative content of erucic acid was higher in Lodi (1.34 $\pm$ 0.10%) than in the Mediterranean locations of Sanluri (0.75 $\pm$ 0.15%) and Foggia (0.92 $\pm$ 0.14%). However, its content never exceeded 1.47% in the individual environments, indicating that the oil could directly be used for human and animal consumption. The Italian Health Authority has fixed at 5% the maximum level of erucic acid in oils and oil derivatives (margarine) for human nutrition and animal feeding (law 659/1980), whereas the US Food and Drug Administration (FDA) guidelines have suggested that every oil containing less than 2% erucic acid is safe for human consumption [34]. For rapeseed oil, FAO/WHO has developed the definition of "erucic acid free oil" when the content is lower than 1%, and of "oil with a low erucic acid content" when the content is lower than 2% [35]. Using the same definitions, almost all samples from Foggia and Sanluri may be defined as "erucic acid free", while those from Lodi may be defined "with a low erucic acid content".

The susceptibility to autoxidation is another crucial characteristic of oils, as it is related to a longer shelf life [15]. In this respect, lupin oil seems slightly more reactive than olive oil, because contains more α-linolenic acids, but is more stable than other very polyunsaturated vegetable oils, e.g. soybean oil [36].

Nutritional considerations

From a nutritional point of view, the most relevant parameters considered in this paper are the absolute α-linolenic acid and linoleic acid contents of the flour and the ω-3/ω-6 ratio (Table 4). The linoleic acid content ranged from 2.95 to 6.34 mg/g flour among environments, averaging 4.21 mg/g flour. The α-linolenic acid content ranged from 1.41 to 3.24 mg/g flour, averaging 2.31 mg/g flour. Since the average concentration of α-linolenic acid in the oil was about 23 mg/g, two spoonfuls of oil, equivalent to about 14 g and containing about 322 mg α-linolenic acid, would be sufficient to provide more than one third of the recommended daily intake of ω-3 for children, that is 0.7–1 g/day [7, 37].

The ω-3/ω-6 ratio of lupin oil ranged between 0.45 and 0.63, averaging 0.54, and showed higher values in Foggia (0.60) than in Sanluri (0.53) and in Lodi (0.51) (Table 4). This ratio tended to increase with increasing the oil content in the environments ($r = 0.58$, $P < 0.05$). The observed ω-3/ω-6 ratio values, although variable among locations, were always much higher than those reported for most vegetable oils. In particular, the average ratio value in white lupin oil was distinctly higher than that of olive oil (0.13), soybean oil (0.15), and walnut oil (0.20), comparable with that of canola oil, and definitely lower only in comparison with that of linseed oil (Table 6). The importance of a right balance between the intake of α-linolenic and linoleic acid appears evident considering that α-linolenic acid is the precursor of ω-3 long-chain polyunsaturated fatty acids (LC PUFA), in particular eicosapentenoic acid (EPA) and docosahexenoic acid (DHA), whereas linoleic acid is the precursor of arachidonic acid and its eicosanoids. In these processes, α-linolenic acid and linoleic acid are competing for the same enzymes, namely desaturases and elongases. Therefore, an adequate α-linolenic acid intake is important both to increase the production of ω-3 LC PUFA and to reduce the adverse effects of arachidonic acid and its eicosanoids.

Conclusion

Even if the oil content in lupinseed is lower than in soybeans, cottonseeds and sunflower seeds (Table 6), its fatty

acid composition confirms its potential role in increasing the daily intake of α-linolenic acid [38].

Our findings indicate an effect of the growing location on the fatty acid seed content and, although preliminary, suggest a slight positive effect on PUFA content of Mediterranean sites (regardless of their soil), as well as the better content and quality of oil produced in environments made unfavourable by high soil lime which, however, could hardly be exploited as a consequence of low crop yields in this condition. Further research is required also on the genetic variation for oil content and composition among elite *L. albus* varieties, as well as on the consistency of variety differences for these characteristics across contrasting growing environments.

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