γ-Linolenic Acid from Caryophyllaceae Seed Oil

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ABSTRACT: Seeds from 50 species belonging to the family Caryophyllaceae, subfamilies Alsinoideae, Silenoideae, and Spergulaceae, were surveyed in a search for new sources of γ-linolenic acid (GLA) having low levels of other interfering PUFA and therefore appropriate for GLA purification. It was detected mainly in Alsinoideae species, with a maximum of 15.6% of total FA in *Minuartia laricifolia* subsp. *ophiolitica*. Different amounts of stearidonic acid (18:4n-3) were present in the Alsinoideae species, ranging from undetectable levels in seven species to 3.30% of total FA in *Stellaria media*. An ANOVA showed a taxonomical correlation between GLA percentages and the genus/subfamily within this family.

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KEY WORDS: Alsinoideae, Caryophyllaceae, Caryophylloideae, α-linolenic acid (ALA), γ-linolenic acid (GLA), *Minuartia laricifolia*, Spergulaceae.

The beneficial effect of γ-linolenic acid (GLA) on a variety of human diseases, as well as its importance as a dietary (1–5) and cosmetics (1,2) component, have been widely reported. The GLA appears in plant species from several families in addition to the usual α-linolenic acid (ALA). Although it occurs infrequently in several species, only two plant families have species in which GLA reaches an appreciable concentration in their seed oil (>15% of total FA), specifically, in Boraginaceae and Grossulariaceae, although other minor plant sources such as Onagraceae species are commercially exploited (1). In addition, GLA is present in a variety of fungi and microorganisms (1,2).

Oils rich in GLA have traditional uses in nutrition and cosmetics. To concentrate and/or purify GLA, natural oils devoid of other C_{18} PUFA would be desirable so as to supply higher levels of GLA. During purification by urea concentration, HPLC, or argentated silica gel column chromatography, other PUFA could interfere (6) owing to co-purification of GLA with these other PUFA. The discovery of new sources of seeds containing GLA but lacking these PUFA and development of methods to cultivate them are important in order to design any industrial process for recovering GLA in quantity.

In general, the relative percentages of C_{18} PUFA found in green tissues and seed lipids are substantially different. For example, concentrations of ALA are usually higher in green tissues, whereas linoleic acid (LA) is the most abundant FA in seeds. Through the same enzymatic step, Δ6 desaturation, GLA is generated from LA and stearidonic acid (SDA) from ALA (1). In the botanical families mentioned above, different plant tissues exhibit different percentages of GLA and SDA. The work of Jamieson and Reid (8) indicates the leaf lipids of some members of the Caryophyllaceae contain only moderate amounts of GLA (maximum of 2.3% on total FA in *Stellaria media*); thus, taking into account the high oil content in seeds, we decided to study the seed FA of several species from this family.

The family Caryophyllaceae comprises annual or biennial cosmopolitan species, mainly herbs; a few of them are either small trees or shrubs. They produce abundant quantities of seeds of small size in amounts large enough to be harvested.

This paper reports on the FA composition of the seed oil of 50 species from the Caryophyllaceae family whose seed FA profiles have not been previously reported.

EXPERIMENTAL PROCEDURES

Materials. Some seeds were collected at maturity from their natural habitats. Samples of *Cerastium ramosissimum*, *Dianthus brachyanthus*, *D. lusitanus*, *Holosteum umbellatum,* and *Petrorhagia nanteuilii* were collected in April 2001 in Sierra de los Filabres (Almeria province, Spain). Seeds of *Silene tamaranae* were gathered from Grand Canary island (Spain) in March 2001. The remaining seeds were purchased from B&T World Seeds® (Olonzac, France). Just before analysis, seeds were freeze-dried and ground to powder with a mortar at room temperature. They were then analyzed immediately.

Oil extraction and transesterification. Rapid simultaneous oil extraction and transesterification were made according to the method of Lepage and Roy (9), as described elsewhere (7). Triplicates were accomplished for each sample, and mean values are reported in Table 1 (variation among the triplicates was routinely less than 5%).

Mixed FAME were analyzed by GLC and GLC–MS as previously described (7). FA contents in samples were determined using methyl heptadecanoate (17:0) as internal standard. Unidentified peaks were taken into account for further calculations (7).

Statistical analyses. Means, SD, and variances were calculated using the software package Statgraphics for Windows v. 4.1 (Manugistics, Rockville, MD). The significance level was $P < 0.01$.

RESULTS AND DISCUSSION

Seed oil content and FA composition from 50 Caryophyllaceae species are shown in Table 1. Total FA content in the seeds ranged from 1.42% in *Cucubalus baccifer* to 19.1% in

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TABLE 1
FA Composition of Seed Oils from 50 Species of the Family Caryophyllaceae Juss.

		FA percentages[a]											
	% Oil[b,c]	16:0	18:0	18:1n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:1n-9	22:0	22:1n-9	24:0
Subfam. Alsinoideae Burnett													
Arenaria stricta Michx.	1.79	10.68	3.26	28.54	50.04	3.92	0	0	0	0	0	0	0
Cerastium arvense Linn.	4.87	12.39	2.15	14.36	39.39	15.53	0.83	0.43	0.51	0	0	0	0
Cerastium ramosissimum Boiss.	4.63	7.03	1.61	13.69	38.53	7.81	1.81	1.29	0	0	0	0	0
Cerastium tomentosum Linn.	4.79	11	1.36	27.47	37.76	9.81	0.47	0	0.4	0.46	0.36	0	0.61
Holosteum umbellatum Linn.	2.27	13.24	6.19	32.74	31.65	8.57	2.47	2.41	0	0	0	0	0
Minuartia laricifolia subsp. *ophiolitica* Pignatti	2.69	16.21	1.76	18.72	41.52	15.59	0.65	0	0	0.67	0.55	0	1.24
Sagina subulata Presl.	4.51	13.19	1.46	20.95	49.02	3.8	0.96	0	0.36	0.42	0	0	1.04
Scleranthus biflorus Hook. f.	1.94	20.94	3.17	23.96	29.53	11.2	0	0	4.1	0	2.91	0	4.14
Stellaria graminea Linn.	3.29	14.13	2.39	21.22	42.65	10.1	1.44	2.5	0	0	0	0	0
Stellaria holostea Linn.	2.29	19.85	2.78	18.81	42.55	9.58	2.63	0	0	0	0	0	0
Stellaria media Cyrill.	1.84	21.56	6.28	20.78	36.23	6.85	3.87	3.29	0	0	0	0	0
Subfam. Paronychioideae A. St.-Hil. ex Endl													
Spergula arvensis Linn.	12.43	15.17	2.95	25.94	46.21	0.2	6.93	0	0.36	0.4	0	0	0
Subfam. Caryophylloideae Arn.													
Agrostemma githago Linn.	5.89	14.84	1.46	16.57	36.2	Trace	25.68	0	0.42	0.69	0.64	0	0
Cucubalus baccifer Linn.	1.42	15.22	3.08	25.76	46.14	1.48	2.89		0	0	0	0	0
Dianthus arenarius Linn.	5.58	9.9	2.18	28.94	50.89	2.37	2.67	0	0.46	0.51	0	0	0.4
Dianthus barbatus Linn.	5.3	10.25	1.64	28.51	51.62	2.66	1.93	0	0.51	0.63	0.35	0	0.53
Dianthus basuticus Burtt Davy	19.1	13.62	2.55	26.61	48.31	2.62	3.33	0	0	0	0	0	0
Dianthus brachyanthus Boiss.	15.49	19.41	7.2	49.59	9.13	0.73	2.97	0	0.64	1.94	0.86	0	0.73
Dianthus caespitosus ssp. *pectinatus* Eckl. & Zeyh.	5.37	15.38	2.25	22.52	50.25	3.13	2.99	0	0.71	0.39	0.33	0	0.45
Dianthus carthusianorum Linn.	5.88	8.82	2.28	29.17	52.85	2.01	1.85	0	0.48	0.6	0.24	0	0.42
Dianthus caryophyllus Linn.	6.75	10.27	1.96	31.17	49.66	1.72	2.1	0	0.44	0.57	0.25	0	0.46
Dianthus deltoides Linn.	4.34	10.72	1.5	28.39	53.26	Trace	2.62	0	0.56	0.7	0.46	0	0.65
Dianthus gratianopolitanus Vill.	4.62	9.46	1.34	32.29	49.42	2.63	2.29	0	0.37	0.64	0	0	0.5
Dianthus lusitanus Brot.	7.22	10.03	2.85	38.76	42.38	Trace	3.34	0	0.55	0.54	0.24	0	0.43
Dianthus plumarius Linn.	6.67	8.19	1.62	32.28	51.15	1.87	2.41	0	0.4	0.58	0.25	0	0.38
Dianthus superbus Linn. var. *Rose*	4.9	8.34	2.21	28.62	51.11	3.88	2.65	0	0.59	0.53	0.39	0	0.46
Subfam. Silenoideae													
Gypsophila elegans Bieb.	5.06	9.43	1.83	32.17	47.64	Trace	1.52	0	0	0.84	0	0	0.45
Gypsophila paniculata Linn.	4.28	8.66	1.5	26.83	57.95	Trace	1.62	0	0	1.01	0	0	0.57
Gypsophila repens Linn.	4.68	8.44	1.43	26.9	55.04	2.07	1.35	0.43	0.42	1.22	0.42	0	0.85
Lychnis arkwrighti Hort. ex Heydt	4.69	8.41	1.1	19.89	62.6	0.77	5.04	0	0	0	0	0	0
Lychnis chalcedonica Linn.	7.57	9.01	1.73	22.87	59.56	0.92	1.49	0.54	0.45	0.4	0.27	0	0.39
Lychnis coronaria Desr.	1.64	12.2	2.86	21.81	58.6	1.24	1.99	0	0	0	0	0	0
Lychnis flos-cuculi Linn.	2.15	14	2.04	14.36	65.75	Trace	2.68	0	0	0	0	0	0
Lychnis haageana Hort. ex Vilm.	6.47	8.22	1.12	16.96	61.77	1.59	6.02	0.75	0.3	0.33	0.2	0	0.3
Lychnis viscaria Linn.	3.69	11.98	1.54	17.94	65.29	0.97	1.03	0	0	0	0	0	0
Petrorhagia nanteuilii (Burnat) P.W. Ball & Heywood	5.28	19.67	2.09	29.11	40.08	1.83	2.04	0	0.56	0.42	0.29	0	0.44
Petrorhagia prolifera (L.) P.W. Ball & Heywood	4.73	15.35	1.77	36.38	38.83	1.14	1.78	0	0.56	0.42	0.29	0	0.44
Saponaria ocymoides Linn.	1.62	29.3	11.72	31.43	22.75	4.77	0	0	0		0	0	0
Silene alba Burnat	3.23	10.14	1.68	17.15	61.54	1.16	4.57	0.61	0	0.55	0	0	0.84
Silene alpestris Jacq.	2.46	13.09	1.24	19.12	55.32	4.45	1.59	0.33	0	0	0	0	0
Silene armeria Linn.	3.38	12.38	2.05	16.47	64.46	Trace	1.07	0.72	0.56	0	0	0	0.55
Silene bellidioides Sond.	6.9	13.97	0	0	8.82	Trace	0	0	0	0	0	0	0
Silene dioica (L.) Clairv.	3.87	11.67	1.28	13.5	62.51	3.18	1.94	0.39	0.38	0	0.31	0	0.42
Silene latifolia Britten & Rendle	3.65	11.16	1.57	15.44	60.33	1.13	4.2	0.46	0.43	0.5	0.19	0	0.4
Silene nutans Linn.	3.58	9.24	1.71	23.65	49.65	1.83	1.36	0.4	0.39	0.4	0	0	0.39
Silene noctiflora Linn.	2.48	10.85	1.36	17.54	62.32	Trace	2.04	0	0	0	0	1.43	0
Silene schafta Gmel. ex Hohen.	3.62	14.52	1.89	13.08	65.86	0.38	1.37	0	0.55	0	0.25	0	0.48
Silene tamaranae D. Bramwell	1.9	14.79	1.48	20.68	55.1	1.85	1.85	0.58	0	0	0	0	0
Silene vulgaris Garcke	3.49	10.6	1.49	16.52	59.62	1.3	4.23	0.55	0.38	0	0.25	0.26	0.38
Vaccaria hispanica (Mill.) Rauschert		3.35	10.51	1.73	41.55	41.97	Trace	1.53	0	0	0.79	0	0

[a]The difference between the sum of all FA for each species and 100% represents FA of undetermined structure.
[b]Values reported on a dry weight basis.
[c]g/100 g of seeds.

D. basuticus. The oils from all 50 species had GLA, although 10 species from the Silenoideae had GLA only at a trace level. *Minuartia laricifolia* subsp. *ophiolitica* was at the top of the range, with 15.6% GLA of total FA, followed closely by *Cerastium arvense* (15.5%).

Among the three subfamilies surveyed, Alsinoideae seems to be the better source of GLA (9.3 ± 3.9% for 11 species). From Paronychioideae, only one species *(Spergula arvensis*) was analyzed, yielding 0.2%. Finally, Caryophylloideae species exhibited low levels of GLA (1.5 ± 1.3% for 38 species). The ALA percentages were different in the subfamilies: The Alsinoideae had very low amounts of ALA (1.4 ± 1.2%); the only Paronychioideae species analyzed had relatively high percentages of this acid (6.9%), whereas Caryophylloideae seeds reached moderate percentages (2.9 ± 4.0%). SDA was present in some species in low amounts. This can be due to a low level of ALA in the seeds or to a low activity of the Δ6 desaturase that leads to a reduced synthesis of SDA (7).

In all species, the absence of 16:1n-7, 18:1n-7, and 24:1n-9 was noted; these FA usually appear in other plant species that possess GLA.

LA was the major PUFA in all species investigated, being higher in Caryophylloideae (50.7 ± 13.6%) than Alsinoideae seeds (38.1 ± 9.0%). The biosynthesis of GLA from LA effected by the Δ6 desaturase could be responsible for this fact. On the other hand, the only Paronychioideae species considered here had an LA percentage comparable to the other Caryophyllaceae species analyzed (46.2%). The content of oleic acid was fairly homogeneous among the three subfamilies: Alsinoideae (21.9 ± 5.9%), Caryophylloideae (25.1 ± 8.4%), and Paronychioideae (25.9%).

ANOVA of the GLA data indicated that the GLA percentage has taxonomical significance both at the genus level (*F*-ratio 4.5; $P < 0.0001$) and at the subfamily level (*F*-ratio 28.8; $P < 0.0000$).

The FA profile of the seeds from this family indicates they are particularly suitable for GLA purification, since the GLA content is substantial and both ALA and SDA are present in low amounts, thus potentially simplifying any GLA concentration and/or purification bioprocess (6) by minimizing the co-isolation of other PUFA along with the desired GLA.

GLA in this work was found in considerable percentages in some Caryophyllaceae species. *Minuartia laricifolia* subsp. *ophiolitica* and *Cerastium arvense* can be considered as potential new sources of GLA. Our data indicate that the Alsinoideae subfamily should be considered as the main target when looking for new species rich in GLA within the Caryophyllaceae.

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