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Evening Primrose (*Oenothera* Spp.) Oil and Seed

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ABSTRACT

Evening primrose (*Oenothera* spp.) seed contains ca. 15% protein, 24% oil, and 43% cellulose plus lignin. The protein is unusually rich in sulphur-containing amino acids and in tryptophan. The component fatty acids of the oil are 65–80% linoleic and 7–14% of γ-linolenic, but no α-linolenic acid.

The 1.5–2% unsaponifiable matter has a composition very similar to that of cottonseed oil. The sterol fraction contains 90% β-sitosterol and the 4-methyl sterol fraction contains 48% citrostadienol; γ-tocopherol dominates its class, with some α- but no other tocopherols.

INTRODUCTION

Evening primrose (*Oenothera* spp., particularly *Oenothera biennis*) is of special interest because its seed contains an oil characterized by its content of γ-linolenic acid (all *cis*-6:9:12-octadecatrienoic acid) (1), sometimes also designated 18:3-ω6. Although comparatively scarce in seed oils, this acid also occurs in a number of other oilseed bearing plants such as the *boraginaceae* (2) and *aceraceae* (3) families. In these oilseeds, however, it is normally accompanied by the common α-linolenic acid. γ-Linolenic acid also occurs in unicellular organisms such as the blue-green alga *Spirulina* (4), fungi such as the phycomycetes (5) and protozoa, e.g., *tetrahymena pyriformis* (6). Particular interest attaches to the recent observation that γ-linolenic acid is present in human milk fat (7). Indeed, this acid is probably more widely distributed than is at present realized since simple methods have only recently become generally available for identifying and differentiating it from α-linolenic acid.

At present, evening primrose oil is the most important source of γ-linolenic acid, which is in growing demand for its clinical and pharmaceutical applications as a very active essential fatty acid, and the precursor of prostaglandin E_1 and its derivatives (8). Studies in this field are very active, e.g., at the laboratories of Efamol Research Inc., Kentville, Nova Scotia, Canada. Although the evening primrose plant does not produce a high yield of seeds compared with the well-known commercial oilseeds, it is preferred to other sources of γ-linolenic acid because it is easy to produce and because it does not contain any α-linolenic acid. Very little information on this seed or its oil composition has been published. The object of this communication is to report some compositional characteristics and properties of evening primrose oil and seed in order to assist in setting quality standards and to provide means for authentication.

EXPERIMENTAL PROCEDURES

Materials

Evening primrose seed and oil samples were supplied by Efamol Ltd., Efamol House, Woodbridge Meadows, Guildford, Surrey GU1 1BA, England. The extensive seed production and plant breeding programs in progress under the auspices of this company have made a wide range of seed types available. Cottonseed oil (unrefined) was from Tropical Products Institute, 52–62 Grays Inn Road, London WCIX 8LU, England.

Seed analyses were by standard methods (9), except for fiber analysis, which was carried out by the acid-detergent method of the USDA (10). Component fatty acids were determined by gas-liquid chromatography (GLC) of methyl esters prepared by transmethylation of the oil with sodium methoxide in methanol. A 10% EGSSY (ethylene glycol succinate-methyl silicone copolymer, U.S.P. 3,253,401) column operating at 180 C was used. Injection point and flame ionization detector were at 200 C in the Pye 104 Gas Chromatograph with a nitrogen flow rate of 30 mL/min.

Saponification of the oil was carred out by reflux for 1 hr of 10 g of oil, 10 mL 60% KOH solution, 60 mL ethanol and 10 mL light petroleum (b.p. 60–80 C). After cooling, 100 mL of distilled H_2O was added and unsaponifiable matter (USM) extracted from the mixed soaps by three successive extractions with diethyl ether (100 mL each). After washing with water and drying over anhydrous Na_2SO_4, the combined extracts were evaporated to dryness to recover USM.

The USM sample (30 mg) was applied (in benzene) to the baseline of a silica gel G60 plate for preparative TLC. The chromatogram was developed with hexane/diethyl ether (4:1), and the plate dried and sprayed with a 0.01% of Rhodamine 6G in ethanol. Observed under UV, the separated fractions, in decreasing order of polarity, showed orange, faint orange-yellow, orange-yellow and two or three dull purple fluorescent bands corresponding to sterols, 4-methyl sterols, triterpene alcohols and two or three tocopherols, respectively. The bands were scraped from the plate and organic material quantitatively extracted with diethyl ether and weighed.

Sterols and 4-methyl sterols were analyzed directly by GLC in a Pye 104 Chromatograph, using a 3% SE-30 column operating at 250 C with a nitrogen flow rate of 50 mL/min. Triterpene alcohols could be handled under the same conditions but slightly better resolution was obtained by derivatizing them as acetates (11), which were run on a 1% SE-30 column operating at 230 C and flow rate of 50 mL/min.

Tocopherols were separated from USM by the same method used for the other classes of components, α-, γ- and δ-tocopherols appearing as separate bands, which were individually identified by comparison with standards. For quantitation, samples were spiked with known levels of stigmasterol and run on a 3% SE-30 column operating at 240 C with a flow rate of 50 mL/min.

RESULTS AND DISCUSSION

Seed Composition

The mature, dry seeds of *Oenothera biennis* are very small (about 2,800 per g), dark brown and somewhat irregular and angular in shape. Three seed samples, taken from different growing locations, were analyzed by standard procedures (9, 10). The figures quoted in Table I are mean results since very little sample-to-sample variation was found. The total figure is a little high because fiber, and sometimes protein, tend to be overestimated. The outstanding features of evening primrose are its high fiber content (implying that a high proportion of the seed, perhaps 50%, is seed coat) and its high ash content. The fiber comprises cellulose (27%) and lignin (16%).

Protein Quality

The protein was estimated from the content of individual amino acids. Those of interest from a biological point of view are listed in Table II, from which it will be seen that the essential amino acid profile of evening primrose protein is curiously unbalanced but nevertheless protentially useful. Though deficient in lysine and four other amino acids, it is very rich in tryptophan and the sulphur-containing amino acids. This suggests that defatted meal may be useful as a supplement for protein concentrates (e.g., soybean protein) deficient in these. However, in view of the presence in the seed of trypsin inhibitors (12), the defatted meal would have to be cooked before use.

Ash Composition

The elements contributing to ash are listed in Table III, which shows an unusually high contribution from calcium, iron and possibly zinc.

Expressing the quantities of these elements as a total of the forms in which they occur in ash gives an ash figure of 6.3% of seed as against 7.8% found. The difference is probably due mainly to silica, which was not determined.

Oil Extraction

Evening primrose oil is readily recovered from seed by conventional solvent extraction. Great care must be taken, however, to avoid conditions promoting oxidative deterioration or other chemical transformations. Like soybean and rapeseed oils, evening primrose oil contains a substantial quantity of linolenic acid, which is very sensitive to autoxidation. At the same time, since the applications of the oil depend crucially on the retention, intact, of the all-*cis* 6:9:12 double-bond system, processes that could in any way promote isomerization, polymerization or other subtle chemical changes must be rigidly avoided. This includes not only hydrogenation but also excessive heat, exposure to catalysts and probably even bleaching earths.

In practice the seed is roller milled and extracted cold by percolation with pure hexane in stainless steel or glass-lined tanks. The extract is washed with water and solvent removed under reduced pressure without allowing the temperature to exceed 50 C. This "partial degumming" operation is the only permissible refining step.

Evening primrose oil is obtained as a clear, pale yellow oil with a slight, but not unpleasant, "musty" flavor. The flavor as could be expected from the chemistry of the autoxidation process, is distinct from the "painty" or "beany" flavor of soybean oil, being based on products of higher average molecular weight. The oil is purged with nitrogen and stored in sealed, filled, polyethylene-lined drums under cool, dry conditions.

The oil as marketed is made up of ca. 97–98% triglycerides, 1.5 to 2% unsaponifiable matter and 0.5 to 1% polar lipids consisting of phospholipids and glycolipids, which have not so far been examined in detail. The component fatty acids and the unsaponifiable matter have been studied, with the following results.

TABLE I

Overall Seed Composition (%)

Water	8.3
Protein	15.2
Oil	24.3
Fiber	42.9
Starch, dextrins and sugars	5.6
Ash	7.8
Total	104.1

TABLE II

Essential Amino Acid Profile

	Evening primrose		FAO ideal
	a	b	b
Tryptophan	1.60	10.5	1.0
Lysine	0.31	2.0	5.5
Threonine	0.35	2.3	4.0
Cystine/methionine	1.68	11.0	3.5
Valine	0.52	3.4	5.0
Isoleucine	0.41	2.7	4.0
Leucine	0.87	5.7	7.0
Tyrosine/phenylanine	1.05	6.9	6.0

[a]Expressed as % of whole seed.
[b]Expressed as % of protein.

TABLE III

Elements Contributing to Ash (mg per 100 g)

Ca	1,800	Fe	39
Mg	530	Na	18
K	460	Zn	7
P	410	Cu	1.1
Mn	0.5		

TABLE IV

Component Fatty Acids (%)

Acid	RRT*	Normal range	Extreme range
16:0	0.58	7 – 10	5 – 13
18:0	1.00	1.5 – 3.5	0.5 – 5
18:1-ω-9	1.14	6 – 11	3 – 15
18:2-ω-6	1.40	65 – 80	50 – 85
18:3-ω-6	1.66	8 – 14	2 – 20

*Relative retention time. 18:3-ω-3 has RRT 1.84

Component Fatty Acids

A very large number of analyses have been undertaken, the results of which are summarized in Table IV. A better picture of the variability in composition that can be expected can be obtained from Figure 1 which analyzes data obtained from *Oenothera* spp. during the period 1979–1980 about the two essential fatty acids. Analyses were of harvested crops, usually from different locations, and plant breeding material available at the time. Linolenic acid content had a median value of 73%, as high as that of any known vegetable oil, and γ-linolenic acid a median of 10.4%. However, the latter showed a wide spread of

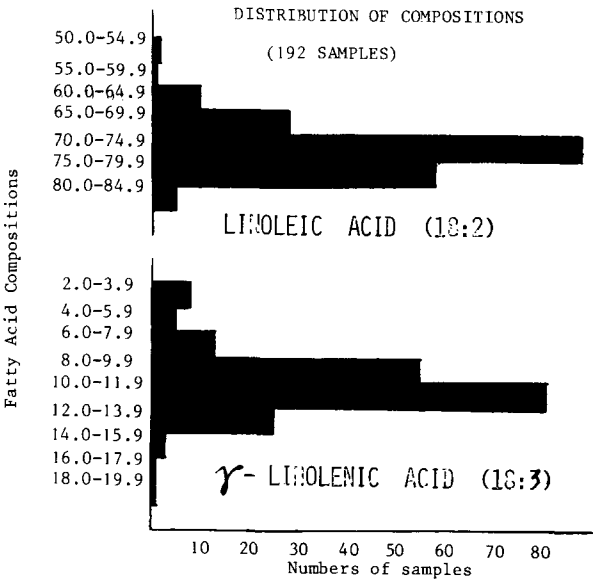

FIG. 1. Distribution of fatty acid composition.

levels, a fact which emphasizes the need for accurate quality control to meet customers' specifications.

Unsaponifiable Matter (USM)

Evening primrose oil contains USM that can be isolated and charomatographed (TLC) in the usual way. The relative proportions of the different classes of components and their individual compositions are markedly characteristic of the oil under study and provide means for its authentication. Of the common oils, the USM of evening primrose oil most closely resembles that of cottonseed oil. In Table V the USM compositions of both oils are listed. The sterols (4-demethyl sterols) are listed in Table VI. The remarkable similarity between evening primrose oil USM and cottonseed oil USM data will be noted. Analyses of sterol, 4-methyl sterol and triterpene alcohol fractions of cottonseed oil USM have been previously reported by Itoh et al. (13) and Kornfeldt and Croon (14). On the whole, the results reported here agree with theirs.

Two unusual features, common to both oils, will be noted, the absence of stigmasterol and the very high proportion (12:1) of sitosterol to campesterol.

4-Methyl sterols are listed in Table VII. Though the similarities between the two oils persist, evening primrose shows significantly lower obtusifoliol and gramisterol contents. Triterpene alcohols (4,4-dimethyl sterols) are listed in Table VIII. This is usually the most difficult group to characterize, but allowing for some as yet unidentified material, evening primrose oil triterpene alcohols are notable for an unusually low proportion of methylene cycloartanol, normally a major triterpene alcohol component. A major proportion of the triterpene alcohols of evening primrose oil are eluted between β-amyrin and cycloartenol, comprising α-amyrin and others. Tocopherols are listed in Table IX. Evening primrose oil USM contains only two identifiable tocopherols, α- and γ-, and there is no evidence of tocatrienols. The tocopherol profile of evening primrose oil is not distinctive.

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TABLE V

Unsaponifiable Matter (USM) of Crude Oils

Oil	% USM	% Sterols	% Methyl-sterols	% Triterpene alcohols	% Other[a]
Evening primrose	1.5–2	44	8	13	35
Cottonseed	1.0–1.5	56	7	8	29

[a]Includes hydrocarbons, primary alcohols and tocopherols.

TABLE VI

Sterols (%)

Oil	Cholesterol	Campesterol	β-Sitosterol	Other[a]
Evening primrose	tr	7.8	89.8	2.4
Cottonseed	tr (tr[b])	7.4 (7[b])	90.0 (86[b])	2.6 (tr[b])
RRT	0.61	0.80	1.00	1.12

[a]Probably Δ^7 stigmastenol, also present in sunflower and safflower seed oils.
[b]Data from Kornfeldt & Croon (14).

TABLE VII

4-Methyl Sterols (%)

Oil	Obtusifoliol	Gramisterol	Citrostadienol	Other
Evening primrose	11	15	48	26
Cottonseed	18	20 (11[a])	48.5 (42[a])	13.5
RRT	0.71	0.78	1.00	

[a]Data from Kornfeldt & Croon (14).

TABLE VIII

Triterpene Alcohols (%)

Oil	β-Amyrin	α-Amyrin+ unknown	c-Artenol	Methylene c-artanol
Evening Primrose	12	64	16	5
Cottonseed	6.5 (7[a])	22 (60[a])	27 (12[a])	40 (21[a])
RRT (acetates)	0.88	0.92–0.98	1.00	1.14

[a]Data from Kornfeldt & Croon (14).

TABLE IX

Tocopherols (ug/g of Oil)

Oil	α-Tocopherol	γ-Tocopherol	δ-Tocopherol
Evening primrose	76.0	187.0	–
Cottonseed	102.0	216.9	2.2
RRT[a]	0.78	0.64	0.49

[a]Stigmasterol, used as an internal standard, had RRT 1.00.

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Oil and Water Analysis of Sunflower Seed by Near-Infrared Reflectance Spectroscopy

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ABSTRACT

The applicability of NIR for oil and moisture analyses of sunflower seed was determined using a NIR spectrocomputer system. The method was compared with the wide-line NMR method for oil analysis and with the A.O.C.S. oven method for moisture analysis. The NIR was calibrated with 120 samples for oil (96 for calibration, 24 for prediction) and 63 samples for moisture (55 for calibration, 8 for prediction). Twenty-two sunflower seed samples were analyzed for oil and moisture by NIR and by methods used by industry. The oil contents of the samples by NMR and NIR were not significantly different. The overall mean oil contents and mean of the standard deviations for the samples were: NMR, 44.2% ± 0.35% and NIR, 44.34% ± 0.74%. A significant difference was found between the moisture values obtained by the oven-drying method and NIR. The average standard deviation for moisture by NIR was 0.57% compared with 0.07% for the oven-drying method. The variability of the oil content in one of the commercial seed samples was 1.52% oil as determined by NMR and 2.52% as determined by NIR. The advantages and disadvantages of both methods are discussed.

INTRODUCTION

The standard method for the determination of oil content of oilseeds since about the 1880's has been the direct solvent extraction method. This is a time-consuming process involving the use of flammable solvents. Moreover, the sample is destroyed, which is an inconvenience, particularly for plant breeders who often have only a few seed available for planting and analysis. These serious drawbacks resulted in the development of wide-line nuclear magnetic resonance (NMR) and near-infrared reflectance (NIR) spectroscopy techniques.

In 1960, Conway (1) first used NMR to analyze whole seed for oil content. Since the process is nondestructive and feasible even with single seeds, plant breeders have used the technique extensively (2–4). NMR provides a rapid, accurate means of measuring the oil content of oilseeds (5–6) and has been found to be more reproducible and statistically more reliable than A.O.C.S. and other extraction methods (5, 7–9).

Robertson and Morrison (6) reported that NMR gave accurate estimates of the oil content of sunflower seed, but they found that the NMR response varied depending on the linoleic acid content. In addition, NMR analysis required a predrying step to remove moisture interference before the oil content was determined.