Fatty Acid Spectrum of Mediterranean Wild Cruciferae

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ABSTRACT

Seed samples of 54 species of wild Cruciferae were newly collected from natural populations of the west Mediterranean and adjacent areas in a search for "new" oil crops. Oil contents and fatty acid compositions were determined simultaneously by gas liquid chromatography using methyl heptadecanoate as the internal standard. The study revealed large variations in oil content (6-48.8%), oleic acid (5-31.3%), linoleic acid (2-24.8%), linolenic acid (1.7-64.1%), and erucic acid (0-55.1%). Correlation coefficients between component fatty acids inter se and oil content were determined separately for all species, the tribe Brassiceae, and the genus *Brassica.* The promising species identified are being studied further.

INTRODUCTION

Several species of the family Cruciferae produce seed oils which differ in fatty acid composition from other vegetable oils. While the majority of cruciferous oilseeds are used in edible products such as edible oils, margarine, and shortening, some are utilized as raw materials for various technological purposes. Much interest has been shown in recent years in finding seed sources free from erucic and linolenic acids and high in linoleic acid (1-11), and rich in erucic acid (1,12-17) in wild and cultivated species of Cruciferae.

At this laboratory, we initiated a study of wild species of Cruciferae in 1975 in order to search for "new" oilseed crops having favorable lipid composition, viz., zero or low erucic and linolenic acids and high linoleic acid for the food industry, and high erucic or high linolenic acid for industrial raw material. In the present study, seeds of a wide array of wild species of Cruciferae which include 30 species, being reported for the first time and 24 species already reported earlier (12,14,15,17), were evaluated for their oil content and fatty acid composition. In addition, correlation coefficients between component fatty acids have been worked out so as to provide useful information to plant breeders.

MATERIALS AND METHODS

Seed Material

Seed samples analyzed were the original seeds of 54 species of wild Cruciferae collected from natural populations of the west Mediterranean and adjacent areas – Morocco, Algeria, Spain, Tenerife de Canarias, Portugal, and Madeira by the second author during the plant exploration of *Brassica* and allied genera in June-July 1975.

Analytical Methods

The procedures followed for the extraction and methylation of seed oil have been described in detail recently elsewhere by Kumar and Fujimoto (18). Oil content and fatty acid composition of seed samples were determined simultaneously in duplicate by gas liquid chromatography (GLC) using methyl heptadecanoate; (C17:0) as the internal standard (18). Dried seed samples (5 mg) were weighed and crushed in a test tube having a screw cap. Then, 1 mg of the benzene solution of methyl heptadecanoate and 2.5 ml of the mixture of methanol-acetyl chloride-benzene (20:1:4) were added and heated at 70 C for 1 hr. Contents were extracted with 5 ml of light petroleum ether, and the petroleum ether layer was washed with saturated NaCl solution. After dehydration, petroleum ether was evaporated under reduced pressure. Methyl esters of fatty acids were separated by GLC (Model JGC 20 KF) using a 1 m x 3 mm glass column packed with 10% LAC-2R-446 on 80-100 mesh, acid washed Chromosorb W. A column temperature of 190 C was used with nitrogen as the carrier gas. Detection was by flame ionization.

RESULTS AND DISCUSSION

Oil Content and Fatty Acid Composition

The results of our survey on oil content and fatty acid composition of 54 wild species of Cruciferae are presented in Table I. The data are classified into different tribes of Cruciferae on the basis of Schulz's system of classification (19), viz., Brassiceae, Arabideae, Sisymbrieae, Hesperideae, and Matthioleae. Further, species belonging to tribe Brassiceae are grouped into subtribes Brassicinae, Raphaninae, Cakilinae, Zillinae, Vellinae, Savignyinae, and Moricandiinae. Species of tribe Sisymbrieae are grouped into subtribes Sisymbrinae, Brayinae, and Descurainiinae.

The major fatty acids recorded are palmitic, stearic, oleic, linoleic, linolenic, eicosenoic, and erucic acids. Minor amounts of myristic, palmitoleic, and behenic were also detected in many seed samples.

As is evident from Table I, oil content of wild species show a wide range of variation from 6% in *Rapistrum rugosum* to 48% in *Cakile maritima.* The maximum frequency of species are observed between 30-35%. Miller et al. (14) evaluated the fatty acid composition of a large number of species of Cruciferae and reported similar oil content in *Cakile maritima,* but found higher oil content, 38% for *Rapistrum rugosum.*

The fatty acid composition of seed samples reveals a large variation for most of fatty acids examined. The variation, however, is found to be greater for erucic (0-55.1%) and linolenic (1.7-64.1%) acids than for oleic (5-31.3%) and linoleic (2-24.8%) acids (Table I). Since our objective was to identify genotypes having zero or low levels of erucic and linolenic acids and high linoleic acid; and high erucic or high linolenic acid, therefore, in the following text, reference will be made only to species exhibiting the aforesaid characteristics.

Most of the species of the tribe Brassiceae were found to produce oil rich in erucic acid, while those belonging to tribes Matthioleae, Hesperideae, and Sisymbrieae produced the lowest erucic acid (Table I). Among 54 species examined, 13 species, all belonging to tribe Brassiceae produced oil high in erucic acid concentration (45.5-55.1%). Of these, 7 belonged to subtribe Brassicinae, 4 to Raphaninae, and 2 to Vellinae. Mikolajczak et al. (12), Stefansson et al. (2), Downey (3), Miller et al. (14), Goering et al. (15), and Appelqvist (20) reported similar high concentrations of erucic acid in seed oil of cultivated and wild species of Cruciferae. *Crambe scaberrima* (55.1%), *Sinapis alba* (54.6%), and *Sinapidendron angustifolium* (52.7%) were found to produce the richest sources of erucic acid (Table I). Similar high concentration of erucic acid was reported in *Crambe hispanica, Sinapis alba,* and *Erucastrum strignosum,* respectively, by Miler et al. (14).

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TABLE I

Oil Content and Fatty Acid Composition of Seeds of Wild Species of Cruciferae

S. no.	Name of species	Place of collection	Oil content (% dry basis)	Fatty acid composition,[a] % 16:0	18:0	18:1	18:2	18:3	20:1	22:1	Other[b] acids
			Tribe Brassiceae (Subtribe Brassicinae)								
1.[c]	*Brassica amplexicaulis* (Desf.) Pomel	Morocco	29.7	6.0	2.1	13.0	11.7	23.0	10.8	33.4	---
2.	*Brassica adpressa* Boiss.	Algeria	22.6	7.5	3.0	10.2	11.8	26.4	5.9	33.5	1.6
3.	*Brassica barrelieri* (L.) Janka	Spain	43.5	6.0	3.9	15.6	12.9	20.1	7.4	34.1	---
4.[c]	*Brassica cossoneana* (Boiss. et Reuter) Maire	Spain	32.4	3.9	1.3	10.8	13.1	13.9	11.8	43.4	1.6
5.	*Brassica fruticulosa* Crrillo	Morocco	26.2	7.0	1.3	12.8	19.7	10.4	6.5	39.8	2.3
6.[c]	*Brassica gravinae* Ten. *Brachyloma* (Boiss. et Reuter) O.E. Schulz	Algeria	33.4	5.0	2.3	11.9	14.8	14.8	10.2	37.3	3.4
7.[c]	*Brassica maurorum* Dur.	Algeria	23.9	4.2	1.9	13.9	17.1	15.5	8.5	37.3	1.5
8.	*Brassica nigra* (L.) Koch.	Algeria	31.2	4.0	1.7	9.0	13.3	16.5	9.3	46.3	---
9.[c]	*Brassica oleracea* L. ssp. *robertiana* (Gay) Rouy et Fouq.	Spain	33.7	3.5	1.1	9.6	15.8	15.0	18.0	31.8	5.3
10.	*Brassica oxyrrhina* (Cosson) P.W. Ball et Heywood	Morocco	26.4	3.4	1.5	10.5	12.6	14.4	9.1	47.3	1.1
11.[c]	*Brassica repanda* (Willd.) DC.	Spain	17.2	6.8	1.4	15.9	9.1	18.9	20.9	24.0	3.0
12.[c]	*Brassica spinescens* Pomel	Algeria	35.0	4.0	2.5	11.2	14.9	12.7	9.2	44.6	1.0
13.	*Brassica tournefortii* Gouan.	Algeria	28.9	3.9	1.1	9.2	12.2	12.9	7.6	47.8	5.2
14.[c]	*Diplotaxis assurgens* (Del.) Gren.	Morocco	27.8	12.2	2.7	9.2	16.4	30.6	6.5	22.6	---
15.	*Diplotaxis catholica* (L.) DC.	Spain	30.8	9.01	4.3	11.4	15.8	32.2	2.6	24.4	---
16.	*Diplotaxis erucoides* (L.) DC.	Algeria	37.0	9.0	3.7	12.6	17.1	36.1	3.7	17.7	---
17.[c]	*Diplotaxis harra* (Forsk.) Boiss.	Morocco	36.3	10.2	1.8	13.4	16.3	25.5	6.96	25.0	0.8
18.[c]	*Diplotaxis muralis* (L.) DC.	Morocco	30.9	11.1	2.8	10.6	19.7	29.6	7.3	19.0	---
19.	*Diplotaxis silfolia* G. Kunze	Morocco	31.3	4.1	2.0	9.0	18.1	15.1	6.7	44.9	---
20.[c]	*Diplotaxis tenuisiliqua* Del.	Morocco	30.3	6.3	2.5	7.7	14.6	19.0	4.7	19.2	25.9[d]
21.	*Diplotaxis virgata* (Cav) DC	Spain	23.4	11.7	1.0	15.1	14.9	30.2	3.5	23.6	---
22.[c]	*Erucastrum cardaminoides* (Webb) O.E. Schulz.	Tenerife	29.4	4.1	2.1	9.9	12.0	13.7	4.7	51.7	1.7
23.[c]	*Erucastrum nasturtiifolium* (Poiret) O.E. Schulz	Spain	32.4	5.6	2.5	12.7	20.6	27.4	6.3	24.9	---
24.[c]	*Erucastrum varium* Durieu	Algeria	35.0	8.1	2.2	9.5	12.1	28.3	6.7	30.4	2.6
25.	*Eruca vesicaria* (L.) Cav.	Spain	22.7	3.5	2.0	8.8	11.4	11.4	10.7	49.5	2.7
26.[c]	*Hutera leptocarpa* Gonzalez-Albo	Spain	28.6	3.7	1.1	14.9	13.7	28.5	2.9	35.2	1.7
27.[c]	*Rhynchosinapis longirostra* (Boiss.) Heywood	Spain	23.2	6.1	1.5	11.7	17.9	23.9	6.1	31.2	1.7
28.[c]	*Sinapidendron augustifolium* (DC.) Lowe	Madeira	17.2	3.1	2.6	5.0	19.5	8.4	4.5	52.7	4.1
29.	*Sinapis alba* L.	Morocco	35.2	5.5	1.8	15.4	8.4	10.9	3.1	54.6	0.3
30.	*Sinapis arvensis* L.	Morocco	26.2*	4.6	2.1	13.0	14.9	15.1	15.4	33.0	1.7
			Tribe Brassiceae (Subtribe Raphaninae)								
31.[c]	*Crambe scaberrima* Webb.	Tenerife	11.0	3.2	1.0	14.1	12.2	13.0	1.5	55.1	---
32.[c]	*Crambe kralikii* Coss.	Morocco	19.0	4.0	1.2	22.2	8.4	7.5	11.2	45.5	---
33.[c]	*Crambe fruticosa* L. fil	Madeira	6.9	6.0	1.2	17.7	13.4	9.5	1.8	50.4	---
34.[c]	*Cordylocarpus muricatus* Desf.	Morocco	22.8	7.0	1.1	8.3	12.4	23.1	8.3	39.9	---
35.[c]	*Feziapterocarpa* Pitard	Morocco	14.1	15.9	3.9	13.6	2.0	4.9	13.0	46.7	---
36.[c]	*Guiraoa arvensis* Coss.	Spain	29.5	7.0	1.6	10.8	18.3	24.8	5.4	32.1	---
37.	*Muricaria prostata* (Desf.) Desv.	Algeria	33.6	9.7	3.0	23.6	15.6	18.6	10.4	19.1	---
38.[c]	*Raphanus maritimus* Sm. ssp. *landra* (Moretti) Rouy et Fouc.	Spain	39.7	6.2	1.7	14.3	12.8	15.1	10.1	37.8	2.0
39.	*Rapistrum rugosum* (L.) All.	Algeria	6.0	9.3	1.1	13.9	15.3	17.7	2.5	39.8	---
			Tribe Brassicea (Subtribe Cakilinae)								
40.	*Cakile maritima* Scop.	Morocco	48.8	5.4	2.1	13.1	20.7	20.6	7.9	27.6	2.5
			Tribe Brassiceae (Subtribe Zillinae)								
41.	*Zilla spinosa* (L.) Prantl.	Algeria	25.3	7.5	1.7	24.8	19.3	10.1	8.9	27.6	---
			Tribe Brassiceae (Subtribe Vellinae)								
42.	*Carrichtera annua* (L.) DC.	Spain	11.8	10.1	3.4	7.9	19.7	19.4	1.7	37.7	---
43.	*Vella annua* L.	Morocco	14.7	10.6	0.5	5.8	17.5	15.2	3.5	47.5	---
44.[c]	*Psychine stylosa* Desf.	Morocco	32.4	5.0	1.0	8.5	12.8	14.2	7.2	48.5	2.9
			Tribe Brassiceae (Subtribe Savignyinae)								
45.[c]	*Euzomodendrom bourgaenum* Cosson.	Spain	21.4	9.2	2.4	11.6	20.3	22.3	7.2	27.0	---
			Tribe Brassiceae (Subtribe Moricandiinae)								
46.	*Conringia orientalis* (L.) *Dumort.*	Algeria	15.1	4.4	0.3	9.2	24.8	3.7	28.8	23.3	5.7
47.	*Moricandia arvensis* (L.) DC.	Morocco	38.7	5.9	1.9	8.9	14.6	30.1	6.4	28.3	3.8
48.[c]	*Pseuderucaria teretifolia* (Desf.) O.E. Schulz	Algeria	27.2	9.8	2.5	17.9	12.0	28.9	9.4	16.1	2.6
			Tribe Arabideae								
49.	*Nasturtium officinale* R. Br.	Algeria	31.4	9.0	1.4	31.3	22.7	1.7	11.3	21.9	0.7
			Tribe Matthioleae								
50.[c]	*Matthiola parviflora* (Schouboe) R. Br.	Morocco	24.4	10.0	3.3	14.8	9.8	62.1	---	---	---
			Tribe Hesperideae								
51.[c]	*Malcolmia ramosissima*	Morocco	31.6	9.1	6.1	22.6	20.2	36.8	1.7	---	3.6
			Tribe Sisymbrieae (Subtribe Sisymbriinae)								
52.	*Sisymbrium erysimoides* (Desf.)	Morocco	26.7	14.3	0.6	13.4	16.3	30.5	4.0	19.7	1.2
			Tribe Sisymbrieae (Subtribe Brayinae)								
53.	*Torularia torulosa* (Desf.)	Algeria	23.7	12.3	1.8	12.5	9.4	64.1	---	---	---
			Tribe Sisymbrieae (Subtribe Descurainiinae)								
54.[c]	*Descurainia bourgaeana* Webb.	Tenerife	36.6	9.6	2.1	14.8	20.2	28.2	14.7	10.3	---

[a]Numbers refere to the length of fatty acid carbon chain and to the number of double bonds in the chain.

[b]Myristic and palmitoleic were the major components.

[c]New report.

[d]Contains 22.5% licosadienoic acid (C22:2).

TABLE II

Correlation Coefficients for Pair of Fatty Acids and Oil Content in Wild Species of Cruciferae

Fatty acids & oil content	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid	Oil content
				All Species				
Palmitic acid	---	0.268	0.192	-0.029	0.458[a]	-0.240	-0.549[a]	-0.139
Stearic acid		---	0.147	-0.037	0.352[b]	-0.269	-0.376[a]	0.256
Oleic acid			---	0.042	-0.057	0.052	-0.324[a]	0.058
Linoleic acid				---	-0.081	0.087	-0.282[b]	0.131
Linolenic acid					---	-0.461	-0.735[a]	0.184
Eicosenoic acid						---	0.014	0.059
Erucic acid							---	-0.254
Oil content								---
				Tribe Brassiceae				
Palmitic acid	---	0.371[b]	0.131	-0.048	0.389[a]	-0.177	-0.452[a]	-0.171
Stearic acid		---	0.053	-0.102	0.360[b]	-0.224	-0.299[b]	0.287
Oleic acid			---	-0.201	-0.079	0.073	-0.214	-0.003
Linoleic acid				---	0.170	-0.011	-0.393[a]	0.067
Linolenic acid					---	-0.362	-0.668[a]	0.361
Eicosenoic acid						---	-0.207	0.018
Erucic acid							---	-0.302[b]
Oil content								---
				Genus Brassica				
Palmitic acid	---	0.395	0.539	-0.132	0.555[b]	-0.081	-0.625[b]	-0.348
Stearic acid		---	0.380	-0.137	0.577[b]	-0.435	-0.221	0.446
Oleic acid			---	-0.086	0.219	0.219	-0.641[b]	-0.089
Linoleic acid				---	-0.590[b]	-0.345	0.242	0.193
Linolenic acid					---	0.005	-0.560[b]	-0.153
Eicosenoic acid						---	-0.582[b]	-0.245
Erucic acid							---	0.267
Oil content								---

[a]Significant at 1% level.
[b]Significant at 5% level.

Matthiola parviflora (Tribe Matthioleae), *Malcolmia ramosissima* (Tribe Hesperideae), and *Torularia torulosa* (Tribe Sisymbrieae) were found to produce oil free from erucic acid. Similar results were obtained by Miller et al. (14) in other species of *Matthiola* and *Malcolmia,* such as *Matthiola longipetala* and *Matthiola tritis; Malcolmia africana* and *Malcolmia cabulica;* and by Mikolajczak et al. (12) in *Matthiola bicornis.* However, Goering et al. (15) reported 2.4% erucic acid in *Matthiola bicornis,* and Joshi and Bhakuni (21) reported erucic acid as a major fatty acid component in *Matthiola incana.* Likewise, in *Malcolmia maritima* Mikolajczak et al. (12) reported higher concentration of erucic acid. Further, our results on erucic acid concentration in *Torularia torulosa* differ markedly from earlier findings of Miller et al. (14) where they reported higher concentration of erucic acid. Similar interspecific variations have been reported earlier within *Brassica campestris, B. napus, B. oleracea,* and *B. juncea* (13), and *B. napus* (2). Oils of *Matthiola parviflora* and *Torularia torulosa* found to be free from erucic acid were further characterized by high levels of linolenic acid. A comparison of the fatty acid composition of the three erucic acid free species shows the presence of nearly seven times as much linolenic, 64.1% and 62.1%, as linoleic acid, 9.4% and 9.8%, in *Torularia torulosa* and *Matthiola parviflora,* respectively (Table I). *Malcolmia ramosissima,* however, contains nearly twice as much linolenic (36.8%) as linoleic acid (20.2%). These results suggest that *Torularia torulosa* and *Matthiola parviflora* with the richest concentration of linolenic acid among 54 species studied may possibly be utilized in industry for preparing high quality paints and varnishes.

Taking into consideration the above-mentioned facts, we tried to find a possible relationship between the erucic acid and the phylogeny of cruciferous plants based on Schulz's system of classification (19). It was observed that tribes which include species having the zero level of erucic acid are positioned at the top of the Schulz's phylogenetic tree, while those with higher concentrations of erucic acid occupy a lower position.

With regard to polyenoic fatty acids, none of the 54 species studied produced oil with zero linolenic acid. However, two species, e.g., *Conringia orientalis* belonging to tribe Brassiceae (Subtribe Moricandiinae) and *Nasturtium officinale* of the tribe Arabideae produced oil with very low concentration, 3.7% and 1.7% of linolenic acid, respectively (Table I). Surprisingly *Conringia orientalis* also showed the highest concentration of linoleic acid (24.8%) among species examined. Further, the eicosenoic and erucic acid concentrations in the seed oil of this species were found to be 28.8% and 23.3%, respectively. Such a favorable composition of fatty acids of *Conringia orientalis* suggests that this species might have potential as a "new" oilseed crop for the food industry if the growth and the yield behavior can be improved. Miller et al. (14) and Appelqvist (16) reported similar values of linoleic and linolenic acids in *Conringia orientalis.* For *Nasturtium officinale,* we found that this species, besides producing oil with the lowest (1.7%) linolenic acid content, produced the highest concentration of oleic acid (Table I). Furthermore, the linoleic concentration was also relatively high (22.7%) in this species. These findings are in agreement with the earlier report of Mikolajczak et al. (12).

The promising species identified have been grown, and evaluation of their potential as a possible "new" oilseed crop is in progress.

Relationship between Fatty Acids and Oil Content

Correlation coefficients between various fatty acids inter se and oil content were determined separately for all species, the tribe Brassiceae and the genus *Brassica* (Table II).

The long chain erucic acid showed strong negative correlations with C16 and C18 fatty acids significant either at

5% or 1% probability level. The decrease in the concentration of erucic acid will result in an increase of C16 and C18 fatty acids. Similar inverse relationships between oleic and erucic acid, and linolenic and erucic acid have been reported earlier (1,7,12,22). The correlation between oleic and linoleic acid was found to be not significant in each of the groups. Gross and Stefansson (23) carried out similar correlation studies in rapeseed and reported a negative correlation. Loof and Appelqvist (24) and Shiga et al. (25), however, found positive correlation between oleic and linoleic acid. Shiga et al. (25) explained such discrepancies as being caused by the variation of the materials used. The correlation coefficients between linoleic and linolenic acid for all species and the Brassiceae tribe were also found to be low (0.08 and 0.17, respectively) and nonsignificant. However, a strong negative correlation (-0.590) was observed within the genus *Brassica.* A similar high negative correlation between linoleic and linolenic acid was reported earlier (26). However, a number of workers (7,23-25) have reported positive correlation between linoleic and linolenic acid in their studies with rapeseed. Another interesting feature was the negative correlation between oil content and erucic acid concentration among the species of tribe Brassiceae (Table II).

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