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Food Chemistry 74 (2001) 449–453

Food Chemistry

www.elsevier.com/locate/foodchem

Total lipid content and fatty acid composition of the seeds of some *Vicia* L. species

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Received 17 September 2000; received in revised form 23 February 2001; accepted 23 February 2001

Abstract

The seed oils of eight *Vicia* species (Leguminosae) were investigated for their total lipid contents and fatty acid compositions. The seed lipid contents were found to be between 2.30 and 3.91%. The fatty acid compositions of these eight different species were determined by gas chromatography of the methyl esters of their fatty acids. The seed oils of *Vicia* species contain palmitic and stearic acids as the major component fatty acids, among the saturated acids, with a small amounts of myristic, pentadecanoic, arachidic and behenic acids. The major unsaturated fatty acids found in the seed oils were oleic, linoleic and linolenic acids.

Keywords: *Vicia*; Seed; Fatty acids

1. Introduction

Some species of family Fabaceae (Leguminosae) are a source of cheap protein for both humans and animals (Tewatia & Virk, 1996). Some of these leguminous species are groundnut (*Arachis hypogea*), cowpea (*Vigna anguiculata*), soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), lentil (*Lens culinaris*) and broad bean (*Vicia faba*). Groundnut and soybean have received considerable attention because of their high oil as well as high protein contents. Therefore, their fat characteristics and fatty acid components have been extensively investigated (Grela & Gunter, 1995; Howells, Brim, & Rinne, 1972; Onochie, 1972; Smouse & Chang, 1967). However, with the exception of soybean and groundnut, the leguminous seeds were reported to be usually low in lipid (Jones & Earle, 1966). According to Aykroyd and Doughty (1964) the percentage of lipids in leguminous seeds is between 1 and 2.

The seed lipid content, of 704 legume samples studied averages 5.5% while many leguminous seeds used for food contain only 1–2% lipids (Harborne, Boulter, & Turner, 1971), who also showed that the percentages of the unsaponifiable fraction of leguminous seed fixed oils range from 0.5 to 4.0% and revealed the presence of myristic, palmitic, stearic, oleic, linoleic and linolenic acids in the seed oils of certain *Vicia* species.

Studies on the oil contents and fatty acid compositions of *Vicia* species are very scant in Turkey. Therefore, it was found to be a matter of interest to carry out a study of the lipid contents and fatty acid compositions of some *Vicia* species.

2. Material and methods

2.1. Seed material

The *Vicia* species used in this study were *V.cassubica* L., *V. cracca* L. subsp. *tenuifolia* (Roth) Gaudin, *V. hyrcanica* Fisch. et Mey., *V. peregrina* L., *V. hybrida* L., *V. sativa* L. subsp. *nigra* (L.) Ehrh., *V. galilaea* Plitm. et Zoh. and *V. faba* L. Matured seeds of these species were collected from various locations in Sivas (Turkey) between June and August 1998.

2.2. Total lipid and fatty acid analysis

The air-dried seed material was ground. From of each of the milled samples, 1 g was taken and stored in chloroform/methanol (2/1, v/v) for 48 h at 4°C. Thereafter,

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0308-8146/01/$ - see front matter
PII: S0308-8146(01)00162-5

Table 1
Total lipid amounts of the seeds of *Vicia* species[a]

Species	Total lipid amounts (mg g^{-1}) (Mean±S.E.)	% of Total lipid (Mean±S.E.)
V. cassubica L.	35.40±0.26 ac	3.54±0.04
V. cracca L. ssp. *tenuifolia* (Roth) Gaudin	36.40±0.87 ac	3.64±0.08
V. hyrcanica Fisch. et Mey.	39.13±0.43 c	3.91±0.04
V. peregrina L.	28.46±3.26 ab	2.84±0.32
V. hybrida L.	35.03±0.20 ac	3.50±0.02
V. sativa L. ssp *nigra* (L.) Ehrh.	31.26±0.96 abc	3.12±0.09
V. galilaea Plitm. et Zoh.	25.20±0.90 b	2.52±0.09
V. faba L.	38.70±3.25 c	3.87±0.32

[a] Each value represents the mean of three experiments. Means with the same letter in the column do not significantly differ at 0.05 level.

they were extracted and purified according to the procedure described by Folch, Lees, and Stanley (1957). Extracts of each part of the seeds were saponified by refluxing with methanol (50%) containing 5% sodium hydroxide for 1 h. The unsaponifiable fraction was removed with petroleum ether.

The saponifiable lipids were converted to their methyl esters using the standard Boron trifluoride-methanol (BF_3) method (Moss, Lambert, & Mervin, 1974). The resultant mixture of fatty acid methyl esters (FAME) in hexane/chloroform (4/1, v/v) was injected onto a UNICAM-610 gas chromatograph equipped with a flame ionization detector (FID), capillary column (15 m×0.32 mm) packed with 70% Biscyanopropyl Polysilphenylene Silaxane (BPX-70), at a column temperature of 185°C. The injection port and detector temperatures were maintained at 200°C. Separations were carried out with N_2 carrier gas at 2.5 ml/min. A small quantity of methyl ester solution (1 μl) was introduced onto the column. FAME were identified by comparison of retention times with authentic standards from Sigma Chemical Co. The peak area of FAME was obtained with a Unicam-4815 integrator. All determinations were performed in triplicate and the mean values were reported.

The statistical analysis of the total lipid contents and percentages of fatty acid were tested by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were reported as significant if $P<0.05$.

3. Results and discussion

The amounts and percentages of total lipids in *Vicia* seeds are presented in Table 1. The lipid contents of seeds were found to be between 2.52 and 3.91%. The percentages of lipid content was highest in *V. hyrcanica* (3.91%) and lowest in *V. galilaea* (2.52%; Table 1). The statistical analysis showed that there were no differences between percentages and total lipid contents of *V. hyrcanica* (3.91%), *V. faba* (3.87%), *V. cracca* subsp. *tenuifolia* (3.64%), *V. cassubica* (3.54%), *V. hybrida* (3.50%) and *V. sativa* subsp. *nigra* (3.12%). However, percentages and total lipid contents of *V. galilaea* (2.52%) and *V. peregrina* (2.84%) showed significant differences from all the other species. Similar interspecific variations have been reported earlier within *V. faba* L. var. *minor*, *V. sativa* L. and *V. calcarata* Desf. However, the oil content was higher in *V. calcarata* than in the other species (Darwish Sayed, Afifi, & Hassan, 1980).

The fatty acid compositions of eight species of *Vicia* are presented in Table 2. Fatty acid levels greater than docosatetraenoic acid (C 22: 4) were not detected in the seed oils of any member of the *Vicia* investigated. Saturated acid components of the seed oils revealed that low molecular weight acids (lauric, myristic and pentadecanoic acids) commonly occur in all the investigated species. *V. cracca* subsp. *tenuifolia* had the highest level of lauric (C 12:0) and myristic (C 14:0) acids: 2.73 and 5.07%, respectively. Pentadecanoic acid (C 15:0) was detected in all species but found at the highest level in *V. cassubica* (6.99%). Palmitic (C 16:0) and stearic (C 18:0) acids were higher in *V. hyrcanica* (23.0, 19.4%, respectively) than in other species. Margaric acid (C 17:0) content was higher in *V. faba* and *V. peregrina*. Nonadecanoic acid (C 19:0) was not detected in the seed oils of *V. cracca* subsp. *tenuifolia*, *V. hybrida*, *V. galilaea* and *V. faba*. Small amounts of nonadecanoic acid were detected in *V. cassubica*, *V. hyrcanica* and *V. sativa* subsp. *nigra*, except *V. peregrina* (2.12%). Arachidic acid (C 20:0) occurred in all species but found at the lowest level in *V. sativa* subsp. *nigra* (0.32%), *V. hyrcanica* (0.57%) and *V. faba* (0.94%). Behenic acid (C 22:0) was also detected in all seed samples but the seed oils of *V. faba*, *V. sativa* subsp. *nigra*, *V. galilaea* and *V. peregrina* contained very low levels of this.

It is evident from our results that the seed oils of all species of *Vicia* studied contain, on average, palmitic and stearic acids as the only major saturated acids. The results of the present study, with respect to saturated acids, confirm the earlier reports of Harborne et al. (1971). Darwish Sayed et al. (1980) reported that *V. sativa* subsp. *nigra*, *V. calcarata* and *V. faba* var. *minor* contain considerable amounts of higher molecular

Table 2
Percentages of fatty acid in seed oils of some *Vicia* species[a]

Fatty acids	*V.cassubica* (Mean±S.E.)	*V.cracca* (Mean±S.E.)	*V.hyrcanica* (Mean±S.E.)	*V. peregrina* (Mean±S.E.)	*V. hybrida* (Mean±S.E.)	*V. sativa* (Mean±S.E.)	*V. galilaea* (Mean±S.E.)	*V. faba* (Mean±S.E.)
C 12:0	0.97±0.08 a	2.73±0.09 b	0.23±0.06 c	1.81±0.07 d	0.67±0.02 ac	1.10±0.16 a	1.11±0.13 a	0.82±0.19 a
C 14:0	0.74±0.04 a	5.07±0.36 b	1.24±0.09 a	1.61±0.30 a	2.85±0.39 c	3.79±0.21 cd	4.09±0.09 bd	3.97±0.06 bc
C 14:1	1.75±0.04 a	3.41±0.17 b	0.67±0.02 c	0.58±0.08 c	3.20±0.04 b	4.81±0.03 d	0.88±0.08 c	0.76±0.05 c
C 15:0	6.99±0.69 a	1.20±0.05 b	0.75±0.04 b	0.43±0.07 b	0.48±0.12 b	2.91±0.22 c	0.89±0.11 b	0.63±0.02 b
C 15:1	–	–	0.29±0.09	2.40±0.07	–	–	–	–
C 16:0	10.8±0.22 a	18.7±0.30 b	23.0±0.46 c	6.99±0.12 d	9.20±0.26 af	8.05±0.06 df	16.7±0.65 e	17.4±0.65 be
C 16:1	4.83±0.37 a	3.02±0.48 b	5.81±0.20 a	1.91±0.09 bc	5.94±0.06 a	1.24±0.39 c	5.56±0.34 a	9.65±0.46 d
C 16:2	–	–	0.23±0.06 a	1.83±0.07 b	–	4.50±0.08 c	–	–
C 17:0	–	1.14±0.21 a	0.37±0.06 a	4.20±0.35 b	0.32±0.16 a	0.76±0.05 a	2.94±0.13 c	6.24±0.27 d
C 18:0	3.85±0.04 a	13.2±0.19 b	19.4±0.16 c	7.26±0.57 d	9.13±0.07 d	7.31±0.28 d	15.94±0.34 e	9.03±0.88 d
C 18:1	35.0±0.70 a	22.4±1.34 be	29. 5±0.44 c	20.0±0.26 b	25.1±0.17 e	23.7±0.45 ef	22.0±0.13 bf	14.6±0.55 d
C 18:2	5.66±0.01 a	4.82±0.29 a	6.29±0.19 a	9.42±0.10 b	5.76±0.35 a	4.33±0.38 a	9.04±0.12 b	9.40±1.37 b
C 18:3	3.11±0.43 ac	3.90±0.35 ac	4.93±0.28 ab	9.20±0.23 d	8.99±0.02 d	1.95±0.28 c	5.20±0.18 ae	6.68±1.03 be
C 19:0	1.30±0.22 a	–	0.26±0.03 b	2.12±0.10 c	–	0.25±0.03 b	–	–
C 20:0	2.29±0.29 a	3.11±0.09 ac	0.57±0.05 b	3.87±0.14 c	2.65±0.02 ad	0.32±0.03 b	3.47±0.36 cd	0.94±0.03 b
C 20:1	6.55±0.52 a	2.09±0.10 bd	0.55±0.04 c	6.86±0.15 a	6.53±0.24 a	0.34±0.01 c	1.32±0.13 cd	2.57±0.47 d
C 20:2	0.38±0.04 a	3.72±0.29 b	0.39±0.01 a	10.9±0.81 c	–	9.25±0.33 c	2.84±0.17 b	6.47±0.32 d
C 20:3	–	–	0.69±0.01 a	–	–	1.48±0.03 b	0.71±0.02 a	0.97±0.03 c
C 20:4	1.37±0.39 a	4.94±0.20 d	2.02±0.14 ac	1.23±0.06 a	6.83±0.23 b	4.80±0.34 d	3.33±0.27 cd	4.34±0.73 d
C 22:0	1.49±0.01 a	3.04±0.07 b	1.53±0.08 a	0.49±0.04 c	2.99±0.06 b	0.29±0.06 cd	0.47±0.01 c	0.15±0.02 d
C 22:1	0.38±0.02 a	–	0.23±0.05 a	1.24±0.06 b	3.01±0.10 c	0.93±0.34 ab	1.67±0.15 bd	2.04±0.23 d
C 22:2	6.99±0.01 ac	1.09±0.19 b	0.48±0.02 b	2.45±0.26 b	5.89±0.61 a	8.97±0.81 c	1.88±0.54 b	2.34±0.33 b
C 22:4	5.48±0.16 a	2.40±0.10 b	0.68±0.01 c	3.22±0.15 b	0.42±0.09 c	8.90 ±0.84 d	–	–
TSFA	28.5±1.25 a	48.2±0.67 b	47.3±0.59 b	28.8±0.64 a	28.31±0.40 ac	24.8±0.41 c	45.6±0.58 b	40.2±0.87 d
TUFA	71.6±1.26 a	51.8±0.67 b	52.7±0.60 b	71.2±0.64 a	71.69±0.40 ac	75.2±0.41 c	54.4±0.60 b	59.8±0.86 d

[a] Each value represents the mean of three experiments. Means with the same letter in each row do not significantly differ at 0.05 level. TSFA, Total Saturated Fatty Acid; TUFA, Total Unsaturated Fatty Acid.

weight saturated acids in addition to arachidic acid. However, according to our results, saturated acids greater than C 22:0 were not detected. This may be related to differences in the ecological and geographical zones where the seeds were collected, as suggested by Aaes-Jorgensen (1961).

V. faba, *V. sativa* subsp. *nigra*, *V. galilaea* and *V. peregrina* showed the lowest concentrations of behenic acid (Table 2). This result is important because some researchers have indicated that oils with high levels of behenic acid may be difficult for digestive enzymes in humans and animals (Balogun & Fetuga, 1985; Hilditch, Meare, & Patel, 1951).

Myristoleic acid (C 14:1), which is an unsaturated fatty acid of the seed oil, was found in all the investigated species. This fatty acid was present at highest level in seeds of *V. sativa* subsp. *nigra*, *V. cracca* subsp. *teuifolia* and *V. hybrida* (Table 2). Pentadecenoic acid (C 15:1) was present in only two species; *V. hyrcanica* and *V. peregrina*. Palmitoleic acid (C 16:1) was detected in all the species. Palmitoleic acid content was higher in *V. faba* (9.65%) than in all other species. Hexadecadienoic acid (C 16:2) was present in the seed oils of *V. hyrcanica* (0.23%), *V. peregrina* (1.83%) and *V. sativa* subsp. *nigra* (4.50%).

The major unsaturated acids in the seed oils of all species were oleic (C 18:1), linoleic (C 18:2) and linolenic (C 18:3) acids. Oleic acid was found in the greatest proportion in the seed oils. Oleic acid content was at the highest level in *V. cassubica* (35.0%), but found to be at the lowest level in *V. faba* (14.6%). The seed oils of all the species were richer in linoleic than linolenic acid contents, with the exception of *V. hybrida*. Linoleic acid content was highest in *V. peregrina* (9.42%), *V. faba* (9.40%) and *V. galilaea* (9.04%). Linolenic acid was also detected at low level in *V. sativa* subsp. *nigra* (1.95%). Eicosenoic acid (C 20:1) was detected in all species, but it was at the lowest level in *V. hyrcanica* (0.55%), *V. sativa* subsp. *nigra* (0.34%) and *V. galilaea* (1.32%). Surprisingly, *V. peregrina* and *V. sativa* subsp. *nigra* also possessed a higher concentration of eicosadienoic acid (C 20:2) than of linoleic and linolenic acids. Trace amounts of eicosatrienoic acid (C 20:3) were detected in *V. hyrcanica*, *V. galilaea*, *V. faba* and *V. sativa* subsp. *nigra*.

Furthermore, significant quantities of arachidonic acid (C 20:4) in the seed oils of all the examined species were found and these were between 1.23 and 6.83%. This acid was detected at a high level in *V. hybrida* (6.83%), *V. cracca* subsp. *tenuifolia* (4.94%), *V. sativa* subsp. *nigra* (4.80%) and *V. faba* (4.34%). Erucic acid (C 22:1) was not detected in *V. cracca* subsp. *tenuifolia*. Erucic acid content was higher in *V. hybrida* (3.01%) and *V. faba* (2.04%) than in all the other species. In addition, docosadienoic (C 22:2) and docosatetraenoic acid (C 22:4) concentrations were also relatively higher than the linoleic and linolenic acids in the seed oil of *V. sativa* subsp. *nigra*. Docosatetraenoic acid was not detected in *V. galilaea* and *V. faba*.

The seed oils of all *Vicia* members had lower levels of monoenoic fatty acids, than polyenoic fatty acids, with the exception of oleic acid (Table 2). From the average figures presented, it appears that there are no major differences in the linoleic acid contents of the members of the *Vicia*, with the exception of *V. galilaea*, *V. faba* and *V. peregrina*. Oils rich in oleic and linoleic acids, according to Bailey (1951) and Hemavathy and Prabhakar (1989), are the most adaptable of all oils and are excellent edible oils.

Thus, as far as unsaturated fatty acid content is concerned, the present study is supported by previous studies (Daulatabad, Hosamani, Desai, & Alagawadi, 1987; Hamberg & Fahlstadius, 1992; Kwiecinska & Matyka, 1986; Hilditch & Williams, 1964; Sengupta & Basu, 1978; Rao et al., 1984; Tharib & Veitch, 1983). All these studies suggest that the unsaturated fatty acid contents of Leguminosae seed oils closely resemble each other and that the chief components are oleic and linoleic acids. Linolenic acid occurred in all species but was found at the lowest levels in *V. sativa* subsp. *nigra*, *V. cassubica* and *V. cracca* subsp. *tenuifolia*. According to Hilditch and Williams (1964) and Tharib and Veitch (1983), linolenic acid is either entirely absent from, or present in only very small amounts, in most Leguminosae seed oils. Our study revealed large variations in arachidonic acid content (1.23–6.83%) among species examined. Although the results found in our study were surprising, similar high concentrations of arachidonic acid were reported in leaves of *V. calcarata* and *V. faba*, by Darwish Sayed et al. (1980).

Such a favourable composition of unsaturated fatty acids of *V. cassubica*, *V. peregrina*, *V. hybrida* and *V. sativa* subsp. *nigra* suggests that these species might have potential as a new oilseed crop for the food industry if growth and yield behaviour can be improved. We found that these species, besides producing oil with the lowest saturated fatty acid contents, produce the highest concentration of unsaturated fatty acids.

Acknowledgements

We are grateful to Dr. V. Konar (Fýrat University, Faculty of Science and Arts, Department of Biology, Elazýd-Turkey) for analysis of fatty acid methyl esters by gas chromatography.

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