Fatty Acid and Tocochromanol Patterns of Some *Salvia* L. species

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In the course of our investigations of new sources of higher plant lipids, seed fatty acid compositions and the tocochromanol contents of *Salvia bracteata*, *S. euphratica* var. *euphratica*, *S. aucherii* var. *canascens*, *S. cryptantha*, *S. staminea*, *S. limbata*, *S. virgata*, *S. hypargeia*, *S. halophylla*, *S. syriaca* and *S. cilicica* were investigated using GLC and HPLC systems. Some of the species are endemic to Turkey. All the *Salvia* sp. showed the same pattern of fatty acids. Linoleic, linolenic and oleic acid were found as the abundant components. Tocochromanol derivatives of the seed oil showed differences between *Salvia* species. γ-Tocopherol was the abundant component in most of the seed oils except of *S. cilicica*. The total tocopherol contents of the seed oils were determined to be more than the total of tocotrienols.

Key words: Salvia, Chemotaxonomy, Fatty Acids and Tocochromanols

Introduction

The *Salvia* L. genus comprises 900 species all over the world (Standley and Williams, 1973) and it is represented with 88 species in the flora of Turkey. Anatolia is a major centre for the genus in Asia (Davis, 1970, 1988; Güner *et al.*, 2000). Some of the studied *Salvia* species from East Anatolia and the Mediterranean area studied in here are endemic to Turkey. The genus has economic *i.e.* medicinal importance and has rich potential as far as the species number and natural widespreading in Turkey is concerned. Studies on the distribution of fatty acids (FAs) of seed oils have been driven by economic and taxonomic interests. Previous knowledge about lipids in the Lamiaceae relates mainly to the discovery of new, economically important oil resources in which a number of species from different genera were analysed (Earle *et al.*, 1959). Chia (*Salvia hispanica*), *Perilla*, *Lallementia*, *Elsholtzia*, *Dracocephalum* (Labiatae) and some others (Aitzetmüller, 1995) were also investigated and evaluated for use as alternative oilseed crops or renewable resources (Aitzetmüller and Tsevegsüren, 1998). More recently, Velasco and Goffman (1999) and Bağci *et al.* (2003, 2004) demonstrated the taxonomic potential and significant distribution of an evaluation of seed fatty acids and tocochromanols for some taxa.

Chia (*Salvia hispanica* L.), a source of industrial oil for the cosmetics industry and of ω-3 α-linolenic acid for the food industry, is one new crop that could help diversify the local economy (Coates and Ayerza, 1998). The present work describes results of analyses of fatty acid composition and content of nutlet lipids of a number of species from the genus *Salvia*. The aim was to characterize their fatty acids and tocochromanols to establish the taxonomic value and contribution as the renewable resources of FA patterns of these plant taxa.

Experimental

Plant materials

Following plant seeds were collected from natural habitats from different regions of Turkey: *Salvia bracteata* Banks & Sol., Afyon-Kütahya, 1000 m, Dirmenci-1387; *S. euphratica* Montbret & Aucher ex Benth. var. *euphratica*, Sivas-Gürün, Vural-6275; *S. aucherii* Benth. var. *canascens* Boiss. & Heldr., Konya-Ermenek, 1100 m, Vural-6189; *S. virgata* Jacq., Afyon-Kütahya, 1000 m, Dirmenci-1388; *S. cryptantha* Montbret & Aucher ex Benth., Afyon-Kütahya, 1000 m, Dirmenci-1384; *S. halophylla* Hedge, Aksaray-Eskil-Gülyazý, 950 m, Vural-7075; *S. syriaca* L. Elazığ-Oymaağaç

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Table I. Fatty acid composition of some *Salvia* sp. from Turkey (mean ± SD).

Salvia sp.	14:0	16:0	16:1 Δ7	16:1 Δ9	17:0	18:0	18:1 Δ9	18:1 Δ11
S. bracteata	0.60 ± 0.21	3.80 ± 1.12	0.04 ± 0.00	0.08 ± 0.01	0.04 ± 0.00	2.05 ± 0.03	20.43 ± 1.22	0.74 ± 0.00
S. euphratica var. *euphratica*	0.03 ± 2.10	5.40 ± 0.25	0.00 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	2.17 ± 0.75	18.98 ± 1.15	0.97 ± 0.15
S. aucherii var. *canascens*	0.08 ± 1.36	7.77 ± 0.98	0.02 ± 0.00	0.10 ± 0.00	0.05 ± 0.02	2.05 ± 0.75	16.76 ± 2.32	1.21 ± 0.27
S. cryptantha	0.05 ± 0.00	4.73 ± 0.07	0.07 ± 0.00	0.07 ± 0.02	0.04 ± 0.10	2.33 ± 1.00	23.14 ± 1.40	0.84 ± 0.05
S. limbata	0.04 ± 0.92	4.95 ± 0.05	0.03 ± 0.10	0.06 ± 0.02	0.05 ± 0.00	2.90 ± 0.15	21.20 ± 0.18	0.86 ± 0.09
S. hypargeia	0.75 ± 0.29	7.80 ± 1.12	0.10 ± 0.02	0.04 ± 0.00	0.07 ± 0.10	2.01 ± 0.75	21.84 ± 1.87	0.91 ± 0.10
S. halophila	0.23 ± 3.00	5.03 ± 0.85	0.08 ± 0.03	0.00 ± 0.00	0.36 ± 0.05	1.22 ± 0.15	20.10 ± 1.00	0.62 ± 0.02
S. virgata	0.03 ± 7.00	5.41 ± 0.22	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.00	2.63 ± 0.98	10.09 ± 0.21	0.77 ± 0.01
S. syriaca	0.05 ± 2.01	5.70 ± 1.02	0.03 ± 0.00	0.10 ± 0.02	0.06 ± 0.02	2.11 ± 1.02	19.70 ± 0.03	1.22 ± 0.03
S. staminea	0.11 ± 0.24	8.24 ± 0.25	0.06 ± 0.01	0.30 ± 0.06	0.11 ± 0.03	1.83 ± 0.05	20.29 ± 1.01	1.65 ± 0.06
S. cilicica	0.57 ± 0.02	8.66 ± 0.09	0.08 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.83 ± 0.89	19.90 ± 0.82	0.88 ± 0.84

TSFA: Total saturated fatty acids; TUFA: total unsaturated fatty acids.

village, 850 m, Bagci-1975; *S. cilicica* Boiss. & Kotschy, Niğde-Ulukışla-Çiftehan, 1050 m, Vural-6882; *S. hypargeia* Fisch. & Mey., Adana-Kamışlı-Hamidiye, 1380 m, Vural-6904. *S. limbata* C. A. Mey. (Tr-59072), Ağrı-Tutak, 1700 m and *S. staminea* Montbret & Aucher ex Benth. (Tr-59107), Van-Haşap, 2800 m, seed samples were obtained from seed bank in Aegean Agricultural Research Institute, Izmir.

Oil extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground to powder using a ball mill. Lipids were extracted with heptane in a straight through extractor. The triglycerides were transesterified to methyl esters with potassium hydroxide in methanol according to ISO method 5509 (1989).

Capillary GLC

The fatty acid methyl ester composition was determined by using different gas chromatographs: Hewlett-Packard HP5890 (A), HP6890 (B), each equipped with a fused silica WCOT capillary and an Unicam-610 (C) FID detector. The results are confirmed with a HP-5973 N GC-MS in Plant Products and Biotechnology Research Laboratory of Firat University, Biology Department.

Conditions:

For (A): Silar 5 CP (50 m × 0.25 mm in diameter, 0.24 μm film thickness); carrier gas: nitrogen; split ratio: 1:50, pressure: 160 kPa; oven temperature: 5 min isothermal at 163 °C, then 163 to 205 °C at 1 °C/min; injection temperature: 230 °C; detector temperature: 260 °C.

For (B): DB-23 (60 m × 0.32 mm in diameter, 0.25 mm film thickness); carrier gas: hydrogen; split ratio: 1:50; pressure: 69 kPa; oven temperature: 1 min isothermal at 80 °C, then 80 to 150 °C at 25 °C/min, then 150 to 240 °C at 3 °C/min, 5 min isothermal; PTV-injection temperature: 80 °C, 12 °C/min to 250 °C, 5 min isothermal; detector temperature: 250 °C.

For (C): BPX-70 (15 m x 0.32 mm); carrier gas: nitrogen; split ratio: 1:40; oven temperature: 3 min isothermal at 80 °C, then 80 to 185 °C at 5 °C/min, then 185 to 220 °C at 3 °C/ min.

Data analysis was done with a chromato-integrator D 2500 (Merck-Hitachi) and a Chemstation integration software, respectively. Peak identification was achieved by comparison of relative retention times with those obtained from test mixtures of a known composition on two different columns. All determinations were performed in duplicate and the mean values were obtained.

Tocochromanol analysis

Tocochromanols were determined by high-performance liquid chromatography (HPLC) according to the procedure of Balz *et al.* (1992). An aliquot of a solution of 50 mg oil in 1 ml heptane was injected in an HPLC system via a Rheodyne valve with a sample loop volume of 20 μl. Tocopherols

Table I (cont.).

18:2 Δ9,12	18:3 Δ9,12,15	20:0	22:0	22:1	24:0	24:1 Δ15	TSFA	TUFA	Oil content (%)
68.50 ± 2.25	2.70 ± 0.11	0.09 ± 0.00	0.16 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.79 ± 0.72	92.50 ± 1.98	23.10
69.2 ± 2.24	0.83 ± 0.09	0.09 ± 0.00	0.38 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.10	8.14 ± 0.12	90.20 ± 2.20	19.90
68.13 ± 1.45	1.10 ± 0.25	0.10 ± 0.00	0.20 ± 0.25	0.07 ± 0.02	0.05 ± 0.02	0.12 ± 0.09	10.30 ± 0.89	87.50 ± 1.11	22.40
65.43 ± 0.76	0.98 ± 0.01	0.09 ± 0.01	0.08 ± 0.02	0.09 ± 0.00	0.04 ± 0.01	0.17 ± 0.25	7.36 ± 0.25	90.80 ± 0.99	17.70
28.10 ± 0.18	40.80 ± 2.25	0.08 ± 0.02	0.07 ± 0.22	0.09 ± 0.02	0.02 ± 0.00	0.11 ± 0.00	8.12 ± 0.65	91.30 ± 0.87	24.30
44.19 ± 0.29	20.81 ± 1.13	0.34 ± 0.02	0.00 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.87	87.90 ± 1.65	20.10
34.45 ± 1.17	36.68 ± 0.98	0.00 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.32 ± 0.09	0.00 ± 0.00	7.20 ± 0.45	91.90 ± 0.42	19.20
22.90 ± 0.09	55.53 ± 0.95	0.10 ± 0.00	0.09 ± 0.00	0.01 ± 0.00	0.08 ± 0.00	0.08 ± 0.20	8.41 ± 1.11	89.50 ± 0.84	25.20
37.76 ± 2.34	31.15 ± 0.06	0.09 ± 0.01	0.15 ± 0.03	0.11 ± 0.01	0.04 ± 0.01	0.13 ± 0.02	8.20 ± 0.78	90.20 ± 1.14	22.70
58.50 ± 3.21	2.68 ± 0.01	0.18 ± 0.02	0.53 ± 0.01	0.11 ± 0.00	0.09 ± 0.00	0.06 ± 0.03	10.50 ± 0.21	83.60 ± 0.62	22.90
36.40 ± 0.89	30.00 ± 1.00	0.00 ± 0.00	0.23 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	0.09 ± 0.00	12.4 ± 0.75	87.40 ± 0.34	23.00

were separated on a LiChrospher 100 Diol phase, 5 mm particle size (Merck, Darmstadt, Germany). HPLC column (25 cm × 4.6 mm in diameter) with an additional guard column (4 mm × 4 mm in diameter), filled with LiChrospher Si 60, 5 mm particle size. The system was operated with the eluent heptane/*tert.*-butyl methyl ether (96:4 v/v) and detection was made using a fluorescence detector F-1000 (Merck) at 295 nm excitation wavelength and 330 nm emission wavelength. A D-2500 Chromato Integrator (Merck, Darmstadt) was used for data acquisition and processing. Calibration was done by external standards with α-, β-, γ- and δ-tocopherol (Calbiochem, Bad Soden, Germany). Tocotrienols were calculated with the same response factors as the corresponding tocopherols and plastochromanol-8 was calculated with the response factor as γ-tocopherol (Balz *et al.*, 1992).

Results and Discussion

The fatty acid (FA) composition of total nutlet lipids and tocochromanol contents of some *Salvia* species naturally growing in Turkey were determined. The results of the fatty acid analyses and the oil yield of the *Salvia* sp. are shown in Table I; the tocopherol and tocotrienol contents are shown in Table II. The analyses showed no significant qualitative difference in fatty acid composition of the analysed *Salvia* species (Table I). *Salvia virgata* was showing the highest and *S. cryptantha* the lowest oil content (Table I). We found usual fatty acids, from C_{14} to C_{24} with their unsaturated forms in the studied taxa. It is possible to say that *Salvia* species studied in here showed qualitatively uniform FA data. Palmitic acid (16:0) was determined in very small amounts. It was ranged between 3.85 and 8.66%. It not found more variable in these and in the other *Salvia* sp. reported by Aitzetmüller *et al.* (2003).

The seed oils of five of the studied *Salvia* sp. (*Salvia bracteata*, *S. euphratica* var. *euphratica*, *S. aucherii* var. *canascens*, *S. cryptantha*, *S. staminea*) amounted 69.2 to 58.5% for linoleic acid (18:2 Δ 9,12). The other studied *Salvia* sp. had ca 22.9 to 44.19% content of this component (Table I). The linolenic acid (18:3 Δ9,12,15) contents of these genera showed very different compositional patterns between species. Whereas some species had lower linolenic acid content than 10%, others ranged from ca 20.8 to 55.5%. The large differences between groups in *Salvia* sp. are very interesting. Oleic acid had similar concentrations between studied *Salvia* species (23.1 to 16.8%) except *S. virgata* (10.1%). Oleic acid was the third abundant and more constant component in the studied taxa.

GLC analysis of the studied *Salvia* sp. showed that there were three different groups. The first group has high linoleic (> 50%) and very low linolenic acid contents (< 10%), respectively (*Salvia bracteata*, *S. euphratica* var. *euphratica*, *S. aucherii* var. *canascens*, *S. cryptantha*, *S. staminea*). The second group is the high in linolenic and low in linoleic acid (*S. limbata* and *S. virgata*). The third group (*S. hypargeia*, *S. halophylla*, *S. syriaca* and *S. cilicica*) has medium linolenic and linoleic acid contents (Table I). These results give some clues on the infrageneric relationships in the genus *Salvia.* Poly-saturated (PSFA) and unsaturated fatty acids (PUFAs) were detected in low levels and ei-

Table II. Tocochromanol (tocopherol and tocotrienol) composition of some *Salvia* sp. from Turkey (mean ± SD).

Salvia sp.	α-T	β-T	γ-T	δ-T	α–T3	β-T3	γ-T3	δ-T3	P-8	TToc	TT3
S. bracteata	4.94 ± 0.07	3.69 ± 0.12	30.39 ± 2.01	11.45 ± 1.02	49.53 ± 1.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	50.47 ± 1.15	49.53 ± 1.21
S. euphratica var. *euphratica*	2.72 ± 0.11	4.20 ± 0.34	27.60 ± 1.12	14.21 ± 0.12	46.30 ± 1.72	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	48.71 ± 1.36	46.30 ± 1.70
S. cryptantha	15.25 ± 0.25	2.94 ± 0.09	73.13 ± 2.34	2.72 ± 0.03	3.72 ± 0.06	0.00 ± 0.00	0.21 ± 0.03	0.05 ± 0.00	1.98 ± 0.34	94.00 ± 0.98	3.98 ± 0.05
S. limbata	7.42 ± 1.12	0.00 ± 0.00	77.70 ± 0.75	2.85 ± 0.12	5.45 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.01	2.23 ± 0.14	88.00 ± 1.23	5.52 ± 0.08
S. virgata	8.55 ± 0.56	0.00 ± 0.00	74.00 ± 1.13	1.04 ± 0.05	14.39 ± 0.09	0.55 ± 0.01	1.36 ± 0.24	0.12 ± 0.20	0.00 ± 0.00	83.60 ± 0.75	16.40 ± 0.12
S. syriaca	9.61 ± 0.23	0.00 ± 0.00	88.71 ± 0.95	0.87 ± 0.03	0.74 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.01	99.19 ± 0.48	0.74 ± 0.01
S. staminea	71.61 ± 0.00	2.89 ± 0.23	14.90 ± 0.05	2.87 ± 0.12	3.70 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.97 ± 0.09	92.30 ± 0.87	3.70 ± 0.08
S. cilicica	46.21 ± 0.16	43.7 ± 0.72	0.00 ± 0.00	0.01 ± 0.00	0.57 ± 0.01	0.00 ± 0.00	9.20 ± 0.42	0.22 ± 0.03	0.00 ± 0.00	90.00 ± 0.25	9.77 ± 0.25

T: Tocopherol; T3: tocotrienol; P-8; plastochromanol-8, TToc: total tocopherol; TT3: total tocotrienols.

cosanoic acid homologues in general were lower than 1% (20:0, 22:0, 24:0 with unsaturated forms) (Table I). Total saturated fatty acid (TSFA) composition of the studied *Salvia* sp. ranged between 6.79 and 12.4%. Total unsaturated FA (TUSFA) content of the *Salvia* sp. were found very high, 87.5% (*S. aucherii* var. *canascens*) to 92.9% (*S. halophylla*) except for *S. staminea.* All studies (Ayerza, 1995; Ferlay *et al.*, 1993; Coates and Ayerza, 1998) suggested that the unsaturated fatty acid (USFA) contents of *Salvia* oils closely resemble each other and chief components are linoleic, oleic and linolenic acid.

The chemotaxonomic significance of the presence or absence of some unusual FAs like phlomic (*Phlomis tuberosa*) and labellenic (*Leonurus sibiricus*, *Marrubium vulgare*) acid in the Lamioideae is not yet known. It is reported that labellenic (18:2 Δ 5,6), phlomic (20:2 Δ 7,8), lamen-allenic (18:3 Δ 5,6, Δ 16 *trans*), *cis*-11-eicosenoic, *cis*-9-eicosenoic acid are the unusual fatty acids found in different genera of the Labiatae like *Lamium* and *Phlomis* (Bagby *et al.*, 1965; Mikolajczak *et al.*, 1967; Aitzetmüller and Tsevegsüren, 1998).

The tocochromanol (tocopherol and tocotrienol) derivatives, α-, β-, γ-, δ-tocopherols and tocotrienols, and 8-plastochromanol were determined in *Salvia* sp. oils. α-Tocopherol was detected in all of the studied taxa. This result was reported by Demo *et al.* (1998) for *S. fruticosa* and *S. pomifera.* On the other hand, β-tocopherol was not determined or present in very low amount except for *S. cilicica* (43.7%). γ-Tocopherol was the most abundant tocochromanol derivative in most of the seed oils. It had maximum concentration in *S. cryptantha*, *S. syriaca*, *S. virgata* and *S. limbata.* It was not found in *S. cilicica* (Table II). δ-Tocopherol was also found in all of the seed oils at contents lower than 10% except for *S. bracteata* and *S. euphratica* var. *euphratica.* Total tocopherol contents were very high (in general > 90%) compared to total tocotrienol contents (< 50%) in the whole seed oils of *Salvia* species. β-, γ- and δ-tocotrienols were not found or present lower than one percent except γ-tocotrienol in *S. cilicica* (Table II). Plastochromanol-8 was lower than 4% in all the seed oils. The tocopherol profiles of *Salvia* species showed varying contents of α-, γ- and δ-tocopherols (Table II). The fatty acid and tocopherol composition of plant seed oils can provide characteristic information in order to confirm phylogenetic and taxonomical relations in the plant kingdom (Aitzetmüller, 1993). The Labiatae has shown 18:3 type FAs as dominant, except *Scutelleria* L. (Marin *et al.*, 1991) and several plants of the family Labiatae are known to produce highly unsaturated seed oils which contain a range of unusual fatty acids (Bohannon and Kleiman, 1975). Enlarged studies on the genera patterns in this family have been continued with the use of different locations patterns as far as chemotaxonomic relationships are concerned.

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