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Oil yield and fatty acid profile of seeds of three *Salvia* species. A comparative study

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Summary

A comparative study of the oil yield and fatty acid composition of three *Salvia* species seeds collected in different locations has been conducted. Seed oil extraction was made using a Soxhlet-extractor and fatty acid analysis was undertaken using a GC-FID. The effect of the collecting site on oil yield, as well as the content of individual fatty acid and total fatty acid and fatty acid content was significant. Seed oil yield varied from 14.94 to 22.83% and the total fatty acids ranged from 67.36 to 82.49 mg/g DW. α-Linolenic (24.02–49.19%), linoleic (20.13–42.88%), oleic (12.97–17.81%) and palmitic (8.37–16.63%) acids were the most abundant fatty acids in all analyzed samples. α-Linolenic acid was found to be the major fatty acid in *S. verbenaca* and *S. officinalis* species, however, *S. aegyptiaca* was characterized by the prevalence of linoleic acid. Among the unsaturated fatty acids, which were represented in all samples in high amounts (78.16–89.34%), the polyunsaturated fatty acids (α-linolenic and linoleic acids) showed important levels ranging from 63.09 to 74.71%. Seeds of *S. verbenaca* were the richest in polyunsaturated fatty acids.

Key words: *fatty acids, principal component analysis, polyunsaturated fatty acids, Salvia, seed oil*

INTRODUCTION

The genus *Salvia* is one of the largest and most widely distributed genera of the *Lamiaceae* family. In Tunisia, the genus is represented by ten species [1]. *Salvia* species are mainly used for their terpenoids and phenolics, which have a broad range

of applications in culinary, cosmetic, pharmaceutical and industrial fields [2]. Since ancient times, many species of *Salvia* have been used in folk medicine for treatment of stomach ailments and common cold [3]. Recently, it has been demonstrated that sage can be used to improve memory, has shown promising outcomes in the treatment of Alzheimer's disease [4] and has a potential for treating cancer as it shows strong antitumorigenic activities [5].

Several *Salvia* species have been characterized by palmitic, stearic, oleic, linoleic and α-linolenic acids in their seed oils and marked by high levels of unsaturated fatty acids [3, 6-9]. The traditional use of *Salvia* seeds for nutrition and medicinal purposes had been reported [10]. Also, oils of *Salvia* seeds have shown a considerable antioxidant activity [7]. Among *Salvia* species, *S. hispanica* is very important in cosmetic and food industries because of its oil content and richness in ω-3 α-linolenic acid [8]. The fatty acid profile is a main determinant of the oil quality of seeds [11]. It is noticeable that oil concentration and fatty acid composition vary greatly, mainly as a response to variations in environmental parameters [12-14] during seed development and genetic differences [15-17].

The polyunsaturated fatty acids (PUFA) have a natural preventive role in cardiovascular diseases and in alleviation of some other health problems, because they promote the reduction of both total and HDL cholesterol [18]. It is worth to mention that oils with high proportions of oleic acid are more stable than others and contribute to the reduction of cardiovascular diseases [19]. In addition, linoleic and α-linolenic acids are essential for normal growth, health promotion, and disease resistance [20]. Furthermore, a balanced source of *n*-3 and *n*-6 essential fatty acids in human diets is necessary in prevention of asthma, coronary heart disease, many forms of cancer, autoimmunity and neurodegenerative diseases [21]. In fact, these disorders are caused by an excess of *n*-6 fatty acids in the diet and are suppressed by *n*-3 fatty acids uptake [21].

To the best of our knowledge, few studies have been carried out on fatty acids contents in the genus *Salvia* and only one report was dedicated to evaluate the variation of fatty acid composition of tunisian *S. verbenaca* [6]. The aim of current study was to determine seed oil yields and to establish the fatty acid profile of three tunisian *Salvia* species, namely *S. verbenaca, S. officinalis* and *S. aegyptiaca*, growing in different habitats. The present investigation will highlight the potential utility of some *Salvia* seeds in human diets and as a raw material source of useful industrial oils components.

MATERIALS AND METHODS

Plant material

Salvia seeds were randomly collected from different regions in north and center of Tunisia at full ripeness (March, April, May and June 2007–2008). *S. officinalis* seeds were obtained from cultivated plants and those of *S. verbenaca* and *S. aegyptiaca* were provided by wild plants. A voucher specimen was deposited at the Herbarium of the Laboratory of Biochemistry and Molecular Biology at the Faculty of Sciences, Bizerte. Details of collection sites and voucher specimens are provided in table 1.

Table 1.

Voucher specimen and eco-geographical characteristics of *S. verbenaca*, *S. officinalis* and *S. aegyptiaca* seeds collection sites

N°	Code	Collection sites	Species	Bioclimatic stage	Soil pH	Geographical location			Voucher specimen
						Longitude (N)	Latitude (E)	Altitude (m)	
1	VT	Tunis	*S. verbenaca*	Higher semi-arid	7.94	36°49'	10°08'	67	SV 2008-125
2	VB	Bir Mroua		Sub-humid	7.95	36°47'	10°37'	86	SV 2008-126
3	VN	Enfida		Lower semi-arid	7.96	36°02'	10°24'	10	SV 2008-127
4	VC	Chott Meriem		Lower semi-arid	8.05	35°53'	10°35'	8	SV 2008-128
5	VA	Bou Arada		Semi-arid moderate	8.15	36°20'	09°39'	252	SV 2008-129
6	VR	Rass Zebib		Sub-humid	7.50	37°16'	10°04'	14	SV 2008-130
7	VE	Beja		Sub-humid	7.50	36°41'	09°10'	284	SV 2008-131
8	VO	Touiref		Higher semi-arid	7.60	36°15'	08°33'	447	SV 2008-132
9	VS	Sers		Semi-arid moderate	7.32	36°00'	09°07'	487	SV 2008-133
10	VH	Hancha		Higher arid	7.50	35°07'	10°44'	52	SV 2008-134
12	OS	Soliman	*S. officinalis*	Higher semi-arid	8.02	36°41'	10°29'	16	SO 2008-122
13	OB	Bou Arada		Semi-arid moderate	8.15	36°21'	9°37'	252	SO 2008-123
15	AE	Enfida	*S. aegyptiaca*	Lower semi-arid	7.96	36°02'	10°24'	10	SA 2008-135
16	AC	Chott Meriem		Lower semi-arid	8.05	35°53'	10°35'	8	SA 2008-136
17	AG	Ghraba		Higher arid	7.50	34°59'	10°44'	98	SA 2008-137

Reagents and standards

All solvents used in the experiments (chloroform, ethanol, hexane, methanol and toluene) were purchased from Merck (Darmstadt, Germany). Sodium methylate (CH_3ONa), sodium chloride (NaCl), sulphuric acid (H_2SO_4) and anhydrous sodium sulphate (Na_2SO_4) were obtained from Sigma-Aldrich (Steinheim, Germany). Fatty acid standards were supplied by Fluka (Ridel de Haën, Switzerland) and Sigma-Aldrich (Steinheim, Germany). All reagents and chemicals used in this study were of analytical grade.

Oil extraction

Triplicate samples of seeds of each collection site were finely ground and 5 g of plant material was extracted in a Soxhlet-extractor with 50 ml of hexane for 4 h. The extraction was protected from light. The extracts were taken to dryness in vacuum conditions in an evaporator system and the oil content was determined (tab. 2). Oil extractions were performed in triplicate for each collection site.

Table 2.

Oil content % (w/w) of *S. verbenaca*, *S. officinalis* and *S. aegyptiaca* seeds collected in different locations

Species	Samples	Oil content (%, w/w)
S. verbenaca	Tunis	14.94±0.40g
	Bir Mroua	22.09±0.74a
	Enfida	18.31±0.31d
	Chott Meriem	22.83±0.29a
	Bou Arada	19.47±0.80c
	Rass Zebib	22.65±0.30a
	Beja	20.63±0.36b
	Touiref	18.59±0.16d
	Sers	18.36±0.83d
	Hancha	16.67±0.20f
S. officinalis	Soliman	17.01±0.24a
	Bou Arada	16.48±0.32a
S. aegyptiaca	Enfida	21.06±0.20a
	Chott Meriem	19.82±0.40b
	Ghraba	18.60±0.14c

Values followed by the same letter within the same species did not share significant differences at 5% (Duncan test)

Total lipid extraction and fatty acid methylation

Triplicate sub-samples of 1 g were extracted using the modified method of Bligh and Dyer [22]. Thus, seed samples were kept in a boiling water for 5 min to inactivate lipases [23] and then ground manually in a mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v). After washing with water and decantation during 24 h at 4°C, the organic layer containing total lipids was recovered and dried under a nitrogen stream. The residue was then dissolved in a known volume of toluene-ethanol (4:1, v/v) and stored at 20°C for further analyses.

Total fatty acids (TFA) of total lipids were transformed into their corresponding methyl esters as described by Cecchi et al. [24]. Transmethylation was conducted by the addition of 2 ml of hexane, 0.5 ml of 3% sodium methylate, a known amount of heptadecanoic acid methyl ester (C17:0) as the internal standard, 0.2 ml of 1 N H_2SO_4 and 1.5 ml of 10% sodium chloride. The organic layer containing fatty acid methyl esters (FAMEs) was recovered and its volume reduced in a stream of nitrogen, prior to analysis.

Gas chromatography (GC-FID)

FAMEs were analyzed by gas chromatography (GC) using a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A HP-Innowax capillary column (polyethylene glycol: 30 m x 0.25 mm i.d, 0.25 mm film thickness; Agilent Technologies, Hewlett–Packard, CA, USA) was used, the flow of the carrier gas (N2, U) was 1.5 ml/min and the split ratio 60:1. Analyses were performed using the following temperature program: the initial oven temperature was held at 150°C for 1 min, increased at a rate of 15°C/min to 200°C, and then held for 3 min and finally ramped at 2°C/min to 242°C. The detector and injector temperatures were set at 275°C and 250°C, respectively. FAMEs were identified by comparison of their retention times with those of co-injected authentic standards. Gas chromatograph was connected to HP Chemstation (Rev. A.0401) software for peak area and fatty acid percentage calculation.

Statistical analysis

All data were reported as means ± standard deviation of three experiments. The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test were employed and the differences between individual means were deemed to be significant at ($p<0.05$). A principal component analysis (PCA) was performed in order to discriminate between different collection sites on the basis of their

fatty acids composition. Correlation coefficients were also calculated based on fatty acids contents. Results were processed by computer programs Excel and STATISTICA software version 5.1.

RESULTS AND DISCUSSION

Oil content

Table 2 shows the seed oil yields obtained for the studied *Salvia* species. *S. verbenaca* oil content varied from the highest levels attributed to Chott Meriem (22.83%), Rass Zebib (22.65%) and Bir Mroua (22.09%) collection sites to the lowest in Tunis (14.94%). *S. officinalis* oil content was 17.01% and 16.48% respectively in Soliman and Bou Arada. The *S. aegyptiaca* oil yield rose from 18.60% in Ghraba to 21.06% in Enfida. The results showed a significant ($p<0.05$) variation in the oil yield between collection sites except for *S. officinalis*. Variations could be attributed to some environmental factors such as drought and high temperature, which reduces amounts of seed oil [25]. In agreement with our findings, Ayerza [26] confirmed the location effect on *S. hispanica* oil content. Our oil yields fall within the range 1.1–27.1% reported for several *Salvia* species [3, 9, 27]. However, much higher oil yields (29.86–50.42%) were recorded for *Salvia* species collected in Iran [28]. Also *S. hispanica*, an oilseed crop, produced 33.0–35.7% of oil [26].

Fatty acid composition

The total fatty acid content and fatty acid profile of *S. verbenaca, S. officinalis* and *S. aegyptiaca* are shown in table 3. Total fatty acid (TFA) levels were found to be the highest in *S. officinalis* seeds (80.65, 82.49 mg/g DW) *vs* the lowest contents detected in *S. aegyptiaca* samples (57.36–61.76 mg/g DW). A total of nine fatty acids were identified in *Salvia* seed oils. Although the plants were collected from varying geographical and ecological conditions and belonged to different species, the major fatty acid components remained the same, namely α-linolenic acid (C18:3), linoleic acid (C18:2), oleic acid (C18:1) and palmitic acid (C16:0). Similar results were obtained for samples of *S. verbenaca* harvested at three different localities in Tunisia [6]. The effect of the collection site of *S. verbenaca* on the percentages of the predominant fatty acids (α-linolenic acid, linoleic acid, oleic acid and palmitic acid) was significant ($p<0.05$). The contents of α-linolenic acid, linoleic acid, oleic acid and palmitic acid ranges were 43.58–49.19%, 20.13–26.67%, 13.58–17.81% and 8.37–10.89%, respectively. As previously reported, oleic, linoleic and α-linolenic acids concentrations in *S. hispanica* were significantly affected by geographical location [26]. For *S. officinalis*, no significant variation ($p>0.05$) was detected between the two collection sites for the main fatty acids. The two

Table 3.

Fatty acid composition (expressed as a percentage of TFA) of *Salvia* species seeds collected in different habitats

Fatty acids	*S. verbenaca*									
	Tunis	Bir Mroua	Enfida	Chott Meriem	Bou Arada	Rass Zebib	Beja	Touiref	Sers	Hancha
C14:0	0.12±0.00cd	0.13±0.01c	0.16±0.01b	0.14±0.00c	0.06±0.00g	0.04±0.00h	0.25±0.03a	0.12±0.00cd	0.11±0.00df	0.10±0.00f
C15:0	0.03±0.00f	0.04±0.01f	0.10±0.00a	0.06±0.00cd	0.02±0.00g	0.02±0.00g	0.09±0.00b	0.05±0.00d	0.06±0.00c	0.05±0.00d
C16:0	9.36±0.15b	10.53±0.35a	10.58±0.02a	9.68±0.03b	9.40±0.32b	8.37±0.13c	10.55±0.11a	10.50±0.15a	10.58±0.19a	10.89±0.69a
C16:1	2.76±0.09a	0.04±0.01h	1.00±0.00d	0.13±0.01g	0.10±0.01g	0.15±0.00g	0.26±0.02f	1.96±0.01c	2.59±0.03b	0.99±0.01d
C18:0	2.62±0.05c	2.61±0.03c	3.13±0.07b	1.31±0.11f	2.49±0.26c	1.78±0.03d	3.01±0.41b	2.46±0.01c	3.53±0.26a	3.68±0.23a
C18:1n-9	16.45±0.30c	13.58±0.19g	16.14±0.24cd	13.66±0.25g	16.42±0.22c	14.48±0.02f	17.08±0.03b	16.01±0.27d	16.27±0.12cd	17.81±0.26a
C18:2n-6	23.36±1.63cd	24.01±0.72bc	20.13±0.63g	25.51±0.79ab	26.15±1.96a	26.67±0.87a	21.94± 0.09df	22.50±0.39cdf	21.21± 0.66fg	22.65±0.85cdf
C18:3n-3	45.02±1.63cd	48.72±0.89a	48.38±1.26ab	49.19±0.95a	45.00±1.50acd	48.04±0.74b	46.64±0.20bc	46.15±0.82c	45.41±0.68cd	43.58±0.63d
C20:0	0.28±0.01f	0.35±0.00cd	0.37±0.02b	0.34±0.01d	0.36±0.01bc	0.46±0.01a	0.17±0.00j	0.24±0.01h	0.25±0.01gh	0.26±0.00g
SFA	12.41±0.20f	13.65±0.27cd	14.35±0.06ab	11.51±0.10g	12.32±0.55f	10.66±0.09h	14.09±0.22bc	13.37± 0.11d	14.53±0.06ab	14.98±0.93a
UFA	87.59±0.21abcd	86.35±0.37cdf	85.65±2.13df	88.49±0.13ab	87.68±0.57abc	89.34±1.59a	85.91±0.28cdf	86.63±0.15bcdf	85.47±0.07f	85.02±1.74f
UFA/SFA	7.06±0.14c	6.33±0.20df	5.97±0.17gh	7.69±0.10b	7.13±0.39c	8.38±0.22a	6.10±0.14fg	6.48±0.09d	5.88±0.03gh	5.69±0.24h
PUFA	68.38±0.38d	72.73±0.19b	68.51±1.89d	74.70±0.33a	71.15±0.74c	74.71±1.61a	68.58±0.29d	68.65±0.42d	66.62±0.02f	66.23±1.48f
C18:3/C18:2	1.94±0.21cd	2.03±0.10bc	2.40±0.01a	1.93±0.10cd	1.73±0.19f	1.80±0.03df	2.13±0.00bc	2.05±0.07bc	2.14±0.10b	1.92±0.04cd
Total (mg/g DW)	71.99±2.33 c	75.56±1.78 ab	73.01±0.99bc	72.33±1.09 c	69.54±2.09 cd	75.98±1.01 a	76.98±1.33 a	67.46±1.19 cd	66.63±0.88 c	62.78±1.54 e

Fatty acids	*S. officinalis*		*S. aegyptiaca*		
	Soliman	Bou Arada	Enfida	Chott Meriem	Ghraba
C14:0	1.05 ±0.01a	0.94±0.01b	0.04±0.00a	0.04±0.00a	0.03±0.00a
C15:0	0.06±0.00b	0.08±0.00a	0.18±0.00a	0.17±0.00a	0.14±0.00b
C16:0	13.06±0.08a	12.88±0.33a	16.63± 0.19a	16.12±0.73ab	15.61±0.28b
C16:1	1.18±0.01a	0.91±0.01b	0.05±0.00a	0.06±0.00a	0.05±0.00a
C18:0	1.64±0.02a	1.49±0.01b	4.87±0.01a	3.60±0.20b	3.11±0.01c
C18:1n-9	15.68±0.48a	16.10±0.10a	13.23±0.20a	12.97± 0.73a	13.57±0.10a
C18:2n-6	20.63±0.53a	20.97±0.95a	39.45±0.39c	42.88± 0.97a	40.91±0.56b
C18:3n-3	42.46±0.46a	42.69±0.72a	25.42±0.18b	24.02± 0.71c	26.45±0.08a
C20:0	4.25±0.08a	3.94±0.02b	0.13±0.00a	0.13±0.00a	0.12±0.00b
SFA	20.06±0.20a	19.33±0.37b	21.77±0.25a	20.07± 0.93b	18.99±0.30b
UFA	79.94±1.47a	80.68±1.79a	78.16±0.74b	79.93±0.93a	80.98±0.54a
UFA/SFA	3.98±0.03b	4.17±0.01a	3.59±0.07b	3.99±0.24a	4.27±0.10a
PUFA	63.09±0.99a	63.67±1.68a	64.88±0.56b	66.91±1.67ab	67.36±0.49a
C18:3/C18:2	2.06±0.03a	2.04±0.06a	0.64±0.00a	0.56±0.01b	0.65±0.01a
Total (mg/g DW)	82.49±1.71a	80.65±0.97a	60.55±0.76a	61.76±1.84a	57.36±2.07b

Values followed by the same letter did not share significant differences at 5% within the same species.

mentioned species were characterized by α-linolenic acid, a compound of increasing pharmaceutical interest [29, 30], as the major fatty acid. Similar prevalence of α-linolenic acid, followed by linoleic acid, oleic acid and palmitic acid in several *Salvia* species, namely *S. candidissima* ssp. *occidentalis, S. virgata, S. ceratophylla*, *S. euphratica* var. *euphratica* and *S. verbenaca* have been reported [3, 6- 8].

Linoleic acid was the most abundant fatty acid in *S. aegyptiaca* oil seeds, followed by α-linolenic, palmitic and oleic acids. Seeds collected in Chott Meriem were characterized by the highest level of linoleic acid (42.88%) and lowest of α-linolenic acid (24.02%). Oils of Enfida contained the lowest level of linoleic acid (39.45%) and the highest of α-linoleic acid (26.45%) were shown in the Ghraba collection site. The levels of linoleic and α-linolenic acids showed significant differences ($p<0.05$) between the studied collection sites. However, oleic acid (12.97–13.57%) did not show a significant variation ($p>0.05$). Different results were reported by Malik et al. [27]. These authors revealed the presence of capric acid (1.34%) in *S. aegyptiaca* seeds and high level of linoleic acid (84.53%) but oleic and linoleic acids were not represented. The prevalence of linoleic acid in several *Salvia* species such as *S. syriaca, S. potentillifolia, S. macrochlamys, S. halophyla, S. hypargeia, S. cilicica, S. bracteata* and *S. aethiopis* had been previously reported [3, 7-9].

Literature indicates that α-linolenic and linoleic acids are among the most important oil components since they are dietary essential fatty acids [31]. Ohlrogge and Browse [32] suggested that in oilseed plants the activity of the Δ^{12}-desaturase responsible for desaturation of oleic to linoleic acid is leading to the accumulation of the latter fatty acid, which is the case of *S. aegyptiaca* in our study. However, the accumulation of α-linolenic acid over the linoleic in our *S. officinalis* and *S. verbenaca* samples could be explained by the fact that the Δ^{15}-desaturase involved in desaturation of linoleic acid into α-linolenic is more active than the Δ^{12}-desaturase [6].

Good levels of stearic acid (C18:0) were found in *S. verbenaca* (1.31-3.68%) and *S. aegyptiaca* (3.11-4.87%) and reasonable amounts of arachidic acid (C20:0) in *S. officinalis* (3.94, 4.25%). Much lower contents of myristic acid (C14:0), pentadecanoic acid (C15:0) and palmitoleic acid (C16:1) were detected in the three studied species. It is worth noting that the fatty acids identified in our samples were previously reported in several *Salvia* species analyzed by Azcan et al. [7] and Gören et al. [3].

S. verbenaca, S. officinalis and *S. aegyptiaca* were characterized by high proportions of unsaturated fatty acids (UFA) versus much lower amounts of satured fatty acids (SFA). The range of UFA was the lowest in *S. officinalis* and *S. aegyptiaca* samples (78.16–80.98%) and the highest in *S. verbenaca* (85.02–89.34%) seeds. In the same way, Ben Taarit et al. [6] reported high contents of UFA (81.65–88.07%) in *S. verbenaca* samples. UFA showed significant differences ($p<0.05$) between *S. verbenaca* collection sites and between *S. aegyptiaca* collection sites. The UFA proportions of our samples were within the range reported for several *Salvia*

species: from 50.3% in *S. potentillifolia* to 92.5% in *S. bracteata* [3, 7, 8]. Significant differences ($p<0.05$) were recorded in SFA ranging from 10.66 to 14.98% in *S. verbenaca*, from 19.33 to 20.06% for *S. officinalis* and from 18.99 to 21.77% in *S. aegyptiaca*.

The PUFA, ranging in our samples from 63.09% in *S. officinalis* collected in Soliman to 74.71% in *S. verbenaca* sampled in Rass Zebib, have been reported to have be beneficial in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases [33, 34]. Moreover, the potential damages, namely oxidative DNA damage, DNA strand breakage, necrosis and apoptosis in human cells *in vitro* caused by palmitic acid, the most abundant fatty acid in human diet, could be suppressed when consumed with other fatty acids such as PUFA [20].

The ratio of unsaturated fatty acids to saturated fatty acids (UFA/SFA) changed significantly ($p<0.05$) between collection sites in all analyzed species. The values ranged from 3.59 in *S. aegyptiaca* collected in Enfida to 8.38 in *S. verbenaca* sampled in Rass Zebib. Similar findings illustrated high variation in the ratio UFA/SFA values for several *Salvia* species ranging from 2.4 in *S. candidissima* to 13.6 in *S. bracteata* [3, 7, 8].

The effect of the collection site of *S. verbenaca* and *S. aegyptiaca* on the ratio C18:3/C18:2 was significant ($p<0.05$) and the values varied from 0.56 to 2.40. Compared to our results, several *Salvia* species showed much lower ratios for C18:3/C18:2 such as *S. euphratica* var. *euphratica* (0.01), *S. fruticosa* (0.01) and *S. aucheri* var. *canescens* (0.02) [7, 8], while other species showed values in the same range as those obtained for our samples, namely *S. macrochlamys* (0.55), *S. sclarea* (1.57), *S. chianantha* (1.71) and *S. staminea* (2.64) [9, 28]. It should be noted that as a consequence of the prevalence of C18:3*n*-3 in *S. verbenaca* and *S. officinalis* seeds, the ratio C18:3*n*-3/C18:2*n*-6 is higher. A balanced source of *n*-3 and *n*-6 essential fatty acids is required to prevent several diseases caused by an excess of *n*-6 fatty acids in the human diet [21].

Principal component analysis (PCA) was carried out to examine the relative distribution of different species and collection sites according to their fatty acid composition. The correlations between fatty acids and two principal components are shown in table 4 and the distribution of fatty acids and collection sites of the three *Salvia* species are illustrated in figure 1. Two principal components (PC1 and PC2) accounted for 76.48% of total variance. As shown in figure 1, the PC1 explained 52.11% of total variance and was positively correlated with α-linolenic acid and negatively correlated with pentadecanoic, palmitic and linoleic acids and the PC2 absorbed 24.37% of the whole variation and was positively correlated with myristic and arachidic acids (tab. 4). The plot established according to the first two PC axes showed a wide range of compositions and permitted the differentiation of the three principal groups. Collection sites of *S. officinalis* constituted the first group, *S. verbenaca* populations formed the second group and *S. aegyptiaca* populations represented the third group.

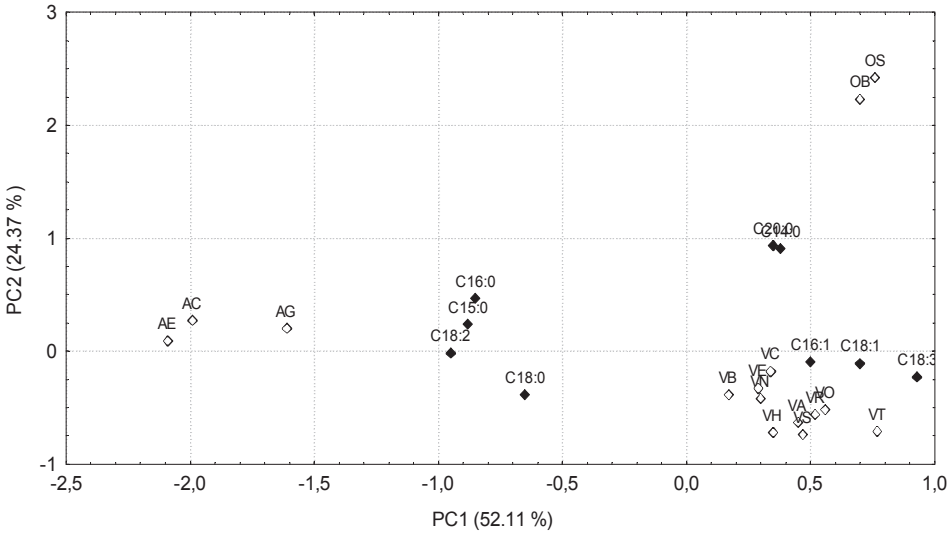

Figure 1.

Principal component analysis performed on fatty acids of three *Salvia* species seeds collected in different habitats (variance of each principal component is listed in parentheses).

S. officinalis (OS: Soliman, OB: Bou Arada), *S. verbenaca* (VT: Tunis, VB: Bir Mroua, VN: Enfida, VC: Chott Meriem, VA: Bou Arada, VR: Rass Zebib, VE: Beja, VO: Touiref, VS: Sers, VH: Hancha), *S. aegyptiaca* (AC: Chott Meriem, AE: Enfida, AG: Ghraba)

Table 4.

Correlations of individual fatty acids with the two main axes of PCA

	Principal component 1	Principal component 2
Variables	*r*	
C14:0	0.38	0.91*
C15:0	– 0.88*	0.24
C16:0	– 0.85*	0.47
C16:1	0.50	– 0.10
C18:0	– 0.65	– 0.39
C18:1	0.70	– 0.11
C18:2	– 0.95*	– 0.02
C18:3	0.93*	– 0.23
C20:0	0.35	0.93*

* Significant correlation at $p<0.05$

S. officinalis species was positively correlated with the first two principal components (PC1 and PC2) and was distinguished by high contents of

α-linolenic, myristic and arachidic acids. However, *S. aegyptiaca* was negatively correlated with the same axis, and was characterized by the highest levels of pentadecanoic, palmitic and linoleic acids. It was observed that within the species *S. aegyptiaca*, samples collected in lower semi-arid Chott Meriem and Enfida showed a tight cluster, indicating similar fatty acids profiles. The populations of the *S. verbenaca* species could be divided into three sub-clusters. The first sub-cluster was formed by samples collected in lower semi-arid and sub-humid namely Chott Meriem, Beja, Enfida and Bir Mroua. The second regrouped populations of Rass Zebib (sub-humid), Bou Arada (moderate semi-arid), Touiref (higher semi-arid), Sers (moderate semi-arid) and Hancha (higher arid). The third sub-cluster comprised the samples collected in higher semi-arid, Tunis. The PCA did not show a clear classification of populations of *S. verbenaca* according to climate, altitude or geographical location on the basis of fatty acid composition. As it was reported previously, quantitative variation in seed oil fatty acid composition can be related to habitat, environment [12, 13] and genetic differences [15-17]. Ghebretinsae et al. [12] affirmed that among environmental components, temperature was the most influential in previous studies of angiosperm seed oils. Also Linder [16] and Canvin [35] suggested that the proportion of saturated to unsaturated acids produced by seeds increases at high temperatures. Conversely, more unsaturated acids are produced by the same species at lower temperatures. According to Harris et al. [36], this result could be attributed to the reduction in desaturase at high temperatures.

Correlations between individual fatty acids, SFA, UFA, PUFA and the UFA/SFA and C18:3/C18:2 ratios

Correlation analysis among the three *Salvia* species with twelve collection sites, was performed to explore the trend of association between individual fatty acids, SFA, UFA, PUFA and the UFA/SFA and C18:3/C18:2 ratios (tab. 5). Significant positive correlations ranging from 0.39 to 0.98 ($p<0.01$) were detected among fatty acids of *Salvia* species. Strong positive correlation was observed between C20:0 and C14:0 ($r=0.98$), SFA and C16:0 ($r=0.94$), C16:0 and C15:0 ($r=0.90$) as well as PUFA and UFA ($r=0.82$). The latter relationship suggested that the increase of PUFA could be a result of increasing UFA. A similar finding was reported for *Artemisia* spp. [20]. Furthermore, the UFA/SFA ratio showed a significant positive correlation with C18:3n-3 ($r=0.74$) content and a significant negative correlation with C14:0, C15:0, C16:0, C18:0, C18:2*n*-6, C20:0 and SFA contents, at $p<0.01$. In addition, a significant positive correlation ($p<0.01$) characterized the relationship between the C18:3/C18:2 ratio and C16:1, C18:1*n*-9, UFA and UFA/SFA ratio.

Table 5.

Linear correlation coefficients between individual fatty acids, SFA, UFA, PUFA and the UFA/SFA and C18:3/C18:2 ratios

	C14:0	C15:0	C16:0	C16:1	C18:0	C18:1*n*–9	C18:2*n*–6	C18:3*n*–3	C20:0	SFA	UFA	UFA/SFA	PUFA	C18:3/ C18:2
C14:0	1.00													
C15:0	–0.08	1.00												
C16:0	0.13	0.90**	1.00											
C16:1	0.14	–0.33*	–0.32*	1.00										
C18:0	–0.52**	0.60**	0.50**	–0.01	1.00									
C18:1n–9	0.23	–0.48**	–0.51**	0.55**	–0.07	1.00								
C18:2n–6	–0.41**	0.73**	0.76**	–0.51**	0.48**	–0.73**	1.00							
C18:3n–3	0.14	–0.85**	–0.92**	0.32*	–0.57**	0.55**	–0.91**	1.00						
C20:0	0.98**	–0.10	0.15	0.10	–0.54**	0.16	–0.34*	0.09	1.00					
SFA	0.41**	0.79**	0.94**	–0.19	0.39**	–0.31*	0.52**	–0.79**	0.42**	1.00				
UFA	0.40**	–0.76**	–0.91**	0.18	–0.38**	0.32*	–0.50**	0.78**	–0.41**	–0.96**	1.00			
UFA/SFA	–0.41**	–0.77**	–0.91**	0.11	–0.43**	0.22	–0.45**	0.74**	–0.41**	–0.98**	0.96**	1.00		
PUFA	–0.53**	–0.47**	–0.61**	–0.30*	–0.34*	–0.24	–0.06	0.47**	–0.51**	–0.78**	0.82**	0.84**	1.00	
C18:3/C18:2	0.33*	–0.73**	–0.80**	0.46**	–0.49**	0.67**	–0.99**	0.94**	0.26	–0.60**	0.57**	0.52**	0.17	1.00

Significant correlation: ** at 0.01 level and * at 0.05 level

CONCLUSIONS

The *Salvia* species included in the current study showed a rich content of PUFA, particularly α-linolenic and linoleic acids, that could contribute to the potential use of the species in numerous applications such as food and pharmaceutical industries. Intra- and interspecies differences in oil yields, TFA and fatty acid composition were demonstrated. *S. verbenaca* collected in Chott Meriem, Rass Zebib and Bir Mroua were the richest in oil yield contents and *S. officinalis* species was characterized by the highest levels of TFA. The fatty acid profile of *Salvia* seeds could present the species as alternate sources of PUFA and optimizing the availability of a balanced source of *n*-3 and *n*-6 fatty acids required to prevent numerous diseases and participate to health promotion.

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WYDAJNOŚĆ OTRZYMYWANIA OLEJU I PROFIL KWASÓW TŁUSZCZOWYCH NASION TRZECH GATUNKÓW *SALVIA*. BADANIA PORÓWNAWCZE

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S t r e s z c z e n i e

W niniejszej pracy przeprowadzono badanie porównawcze oleju i składu kwasów tłuszczowych otrzymywanych z nasion trzech gatunków szałwii pochodzących z różnych stanowisk. Ekstrakcję oleju z nasion przeprowadzono przy użyciu aparatu Soxhleta. Analizę kwasów tłuszczowych przeprowadzono za pomocą GC-FID. Wpływ miejsca zbioru na wydajnosć otrzymywania oleju, całkowitą zawartość kwasów tłuszczowych i zawartość poszczególnych kwasów tłuszczowych był istotny. Zawartość oleju w nasionach wahała się od 14,94 do 22,83%, a suma kwasów tłuszczowych od 67,36 do 82,49 mg/g suchej masy. W analizowanych próbach dominował kwas α-linolenowy (24,02–49,19%), linolowy (20,13–42,88%), oleinowy (12,97–17,81%) i palmitynowy (8,37–16,63%). Głównym kwasem tłuszczowym w *S. verbenaca* i *S. officinalis* okazał się kwas α-linolenowy, natomiast w *S. aegyptiaca* zanotowano przewagę kwasu linolowego. Spośród nienasyconych kwasów tłuszczowych, które były w dużej ilości reprezentowane we wszystkich próbach (78,16–89,34%), wielonienasycone kwasy tłuszczowe (kwas α-linolenowy i kwas linolowy) stanowiły od 63,09 do 74,71%. Ich największą zawartość stwierdzono w nasionach *S. verbenaca*.

Słowa kluczowe: *kwasy tłuszczowe, analiza składowych głównych, wielonienasycone kwasy tłuszczowe, Salvia, olej z nasion*