Journal of Applied Biology & Biotechnology Vol. 6(05), pp. 37-41, September-October, 2018
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2018.60506
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Hemp seed oil: Chemical characterization of three non-drug varieties cultivated in Morocco

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ARTICLE INFO

Article history:
Received on: December 09, 2017
Accepted on: April 03, 2018
Available online: August 01, 2018

Key words:
Cannabis sativa L. oil,
Fatty acids,
Omega-6,
Omega-3.

ABSTRACT

Cannabis sativa L. (hemp) seed oil is known for their food use, is the amount of polyunsaturated fatty acids from hemp seeds that would make these interesting products. The yield and the chemical characterization of three varieties of non-drugs hemp oil cultivated in Morocco were determined. Three varieties were used, namely, Santhica 27, Futura 75, and Epsilon 68 listed in the French and European catalogs. The chemical composition of all varieties from hemp oils was determined using gas chromatography–mass spectrometry method. The oil content of the hemp seeds ranged between 30.45% and 34.93%. Among the 09 detected fatty acids, a high level of linoleic acid (omega-6) was recorded between 56.71 ± 0.1% and 57.70 ± 0.1%, α-linolenic acid (omega-3) ranged from 13.66 ± 0.04% to 14.80 ± 0.06%, and the oleic acid ranged from 12.80 ± 0.04% to 13.30 ± 0.03%. Moreover, palmitic, stearic, and arachidic acids contents varied between 7.32 ± 0.02% (Futura 75) to 7.70 ± 0.03% (Santhica 27), 2.40 ± 0.02% (Futura 75) to 2.60 ± 0.04% (Epsilon 68), and 0.9% for all varieties, respectively. These results show that the hemp seeds cultivated in Morocco could be used as a source of food, characterized by high nutritional value.

1. INTRODUCTION

In Morocco, the cultivation of *Cannabis sativa* L. concerns the drug variety of this plant, covers an area of about 120,000 ha which covers 20,000 km^2 in the northern region of the country with an average production of 750 kg per hectare [1-3]. The report of the United Nations Office on Drugs and Crime (UNODC), published in 2016, suggests that the world's largest producer of cannabis resin continues to be Morocco, and a large amount of cannabis production has essentially exported to Europe through traffickers [4].

Despite the reduction recorded in the province of Chefchaouen, Rif region, where cannabis cultivation increased from 79,195 ha in 2004 to 40,529 ha in 2005 (−46%), the northern region of Morocco, especially the center of the Rif, remains the preferred region by cannabis resin producers [5]. Since 2005, the authorities have conducted a campaign to eradicate the cultivation of cannabis, which has reduced production in the provinces of Larache, Al Hoceima, and Taounate [5,6]. A study realized by Stambouli *et al.* [5] about 30 samples of hemp seeds oils collected in north region from Morocco confirms that the delta-9-tetrahydrocannabinol (Δ-9-THC) content of approximately 6%. With the same objective, the composition of *C. sativa* L. seed oil grown in Northern of Morocco was also carried [7]. Moreover, a recent study realized by Bouayoun *et al.* [8] about three non-drug varieties of hemp (Epsilon 68, Santhica 27, and Futura 75) revealed that the average contents of delta-9-tetrahydrocannabinol were ranged from 0.013% to 0.035%. The production of cannabis in Morocco remains illegal, which leads to judicial proceeding against farmers and transporters. In fact, the production and cultivation of the industrial varieties of cannabis in Morocco will have a great socioeconomic impact.

Industrial hemp is an annual plant selected for its low delta-9-tetrahydrocannabinol content, developed from *C. sativa* L. It has undergone a significant evolution since the 1990s, more than 51 species were developed in 2013 compared to 12 species cultivated in 1995 [9]. This is the result of legislative authorization by the majority of European countries [10].

Hemp seed oil is rich in essential fatty acids, characterized by a height quantity of unsaturated fatty acids, linoleic and α-linolenic acids (omega-6 and omega-3) are the unsaturated fatty acids most represented in vegetable oils of cannabis, with values between 50–70% and 15–25% of total oil, respectively [11]. Several researchers have studied the effects of hemp oils in cosmetic [12], antioxidant [13,14], lipid metabolism [15], cardiovascular health [16], immunomodulatory

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effects [17], and dermatological diseases [18]. In addition to the uses of the cannabis by ancient civilizations as sources of food and medication [19], and actually several products were developed by industries in food, cosmetic, and pharmaceutics areas.

In this sense, our study consists to analysis the chemical compositions of hemp seed oils from three non-drug varieties cultivated in Morocco.

2. MATERIALS AND METHODS

2.1. Plant Material

The hemp seeds used in this work come from the experimental plots of the cultivation of varieties of industrial hemp [8]. During this experiment, three varieties were used, namely, Santhica 27, Futura 75, and Epsilon 68 (S27, F75, and E68) listed in the French and European catalogs were received from the company "The National Federation of Hemp Producers," France. Agronomic and industrial characteristics of the industrial hemp are presented in Table 1. The major criterion adopted in breeding is to achieve very low Δ-9-THC contents (see the trace). This criterion cancels the drug effect of the plant. The seeds used are from the Moroccan experimental plots.

2.2. Morphological Characteristics of Seeds

Microscopic examination of cannabis seeds was conducted at the Royal Gendarmerie Criminal Institute (Rabat) using an FEI Quanta 650 environmental microscope. The selected specimens are fixed on a carbon double-sided adhesive backing and to avoid any accumulation of parasitic charges which decrease the quality of the image, they are metalized in carbon before being introduced into the microscope enclosure. The observations are carried out under vacuum (7.4 10^{-4} Pascal) at a voltage of 1–2 KV.

2.3. Soxhlet Extraction of Oils

About 30 g of hemp seeds (Santhica 27, Futura 75, and Epsilon 68) were slightly crushed and extracted by hexane using Soxhlet apparatus. Hexane was removed by a rotary evaporator and the oily portion was recovered. The oils obtained were weighed and the yields were calculated.

2.3.1. GC Analysis of Fatty Acids

Preparations of each sample of the fatty acid methyl esters (FAME) by transesterification of with 2 N KOH in methanol and hexane. The gas chromatographic conditions were as follows: Capillary column CARBOWAX 20 M (25 m × 0.32 mm with 0.25 µm); The Agilent autosystem brand chromatograph is equipped with a divider injector 240°C; detector 260°C; nitrogen as the carrier gas; and flow rate 2.5 ml/min, and the analyzes are carried out in isotherm (200°C). 1 mL of each sample was injected in the splitless mode, and the determination of the fatty acids was carried out by comparing the retention times with the corresponding methyl esters of fatty acid standards. Palmitic acid (W283207), palmitoleic acid (P9417), stearic acid (W303518), oleic acid (W281506), linoleic acid (W338020), linolenic acid (L2376), arachidic acid (10930), and arachidonic acid (10931) have been used as standards, and FAME was obtained from Sigma Chemical Co. (Sigma-Aldrich, Germany).

FAME samples were analyzed and the values are presented as the mean ± standard error of mean of triplicate analysis using one-way analysis of variance.

3. RESULTS

3.1. Morphological Characteristics of Female Fruits: Seeds

Figure 1 represents the microscopy of three varieties (Santhica 27, Futura 75, and Epsilon 68) of female hemp seeds characterized by an achene, contains a single seed with a hard shell tightly covered by the thin wall of the ovary, ellipsoid, lightly compressed, smooth, about 2-5 mm long, with a brown color.

3.2. Yields of Seeds and Oils of the Three Hemp Varieties

Oils contents of the seeds determined by Soxhlet extraction were 34.93, 33.86, and 30.45% (w/w) of Epsilon 68, Santhica 27, and Futura 75 varieties, respectively [Table 2].

3.3. Fatty Acid Quality of Three Varieties of Seeds Oil

Table 3 summarizes the chemical composition of all the varieties oils. The results show that the fatty acids composition of the all varieties studied has a very close composition. The saturated fatty acids contents (% of total oils) such as palmitic, stearic, and arachidic acids ranged from 7.32 ± 0.02% (Futura 75) to 7.70 ± 0.03% (Santhica 27), 2.40 ± 0.02% (Futura 75 and Santhica 27) to 2.60 ± 0.04% (Epsilon 68), and 0.9% (Futura 75, Santhica 27, and Epsilon 68), respectively. The saturated fatty acid fraction represents between 10.6% and 11% of the total fatty acids present in hemp seeds.

4. DISCUSSION

The values of yields obtained in this study were within the expected range reported previously for hemp seed [20,21], a study was conducted by Anwar *et al.* [22] proved that the yield of hemp seeds grown in Pakistan (28.87%) was less than that found in our study. Regarding the yield of hemp seed oil, values between 35.27% and 36.88% were recorded in the northwest of Turkey [23]. The quantitative difference in the content of hemp oils could be explained by the effects of environmental factors and the storage methods [20,24,25].

Among the chemical composition, our results were similar to that of the previous studies [11,19,20,22]. Parker *et al.* [26] and Kriese *et al.* [20] were reported that stearic and palmitic acid concentrations are in accordance with our results. Several studies have shown that stearic acid does not increase the concentration of cholesterol, and oil with high concentration of stearic acid is more preferred for the food industries and in hypoglycemic diets than oils rich in palmitic acid [27-29].

Table 1: Some agronomic characteristics of varieties of industrial hemp.

Varieties	Flowering	Registration year	Breeder	THC (%)	PMG	Fiber production (%)
Santhica 27	Average	2002	NFHP	<0.001	16.8	35.81
Epsilon 68	tardy	1996	NFHP	0.04	19.3	30.45
Futura 75	tardy	1998	NFHP	0.06	18.9	30.77

NFHP: National Federation of Hemp Producers, PMG: Weight of thousand seeds; THC: Δ-9-trans-tetrahydrocannabinol.

In this study, the most abundant fatty acids were linoleic (Ω-6), *α*-linolenic (Ω-3), and oleic acids, which together ranged between 83.6% (Santhica 27) and 87.3% (Futura 75) of the total fatty acid composition. A study realized by Da Porto *et al.* [30] have been determined a total of linoleic, α-linolenic, and oleic acids from non-drug varieties (Felina 32, Chameleon, Uso 31, and Finola), with values between 84.93 and 88.61% of the total fatty acid composition**.**

Moreover, compared with the fatty acid compositions of olive and soya vegetable oils, established under the same operating conditions [Table 4 and Figure 2], proved that there is a qualitative resemblance between the three types of oils. The chromatographic profiles of the fatty acids of cannabis seed oils and soybean oil seem to have the most similarities, particularly in their oleic (between 13% and 22%) and linoleic (between 53% and 57%) acid contents. However, cannabis seed oil is also characterized by a relatively high level (between 13.6% and 14.8%) of linolenic acid (omega-3) compared with soybean oil (7.34%), which could have favorable nutritional and physiological effects for the prevention of cardiovascular diseases and cancer [31].

Table 2: Yields of seeds and oils of the three hemp varieties.

Variety	Yield of seeds (kg/ha)	Yield of oil (%)
Santhica 27 (S27)	1035	33.86
Epsilon 68 (E68)	1336	34.93
Futura 75 (F75)	996	30.45

The ratio of polyunsaturated acids to saturated acids for the oils studied was evaluated at 6/1. This relatively high ratio would be considered favorable for reducing blood cholesterol and preventing cardiovascular disease [32]. Moreover, the results of our study indicate that the ratio of omega-6 to omega-3 acid in hemp seeds is 3/1, this results were in accordance with this reported in the literature [21,30], which is widely regarded as a ratio highly beneficial to human health [14,22].

The lipid composition obtained in the present study is also quite similar to those reported by Stambouli *et al.* [7] of the seeds drug-type cultivated in the north of Morocco. The weight ratios of polyunsaturated to saturated acids (P/S) and omega-6 to omega-3 fatty acids were 6:1 and 3:1, respectively.

5. CONCLUSION

In our study, the oil contents of the seeds were 34.93, 33.86, and 30.45% of Epsilon 68, Santhica 27, and Futura 75 varieties, respectively. The content values of linoleic, linolenic, and oleic acids were ranged between 83.6% and 87.3% of the total fatty acid composition. On the

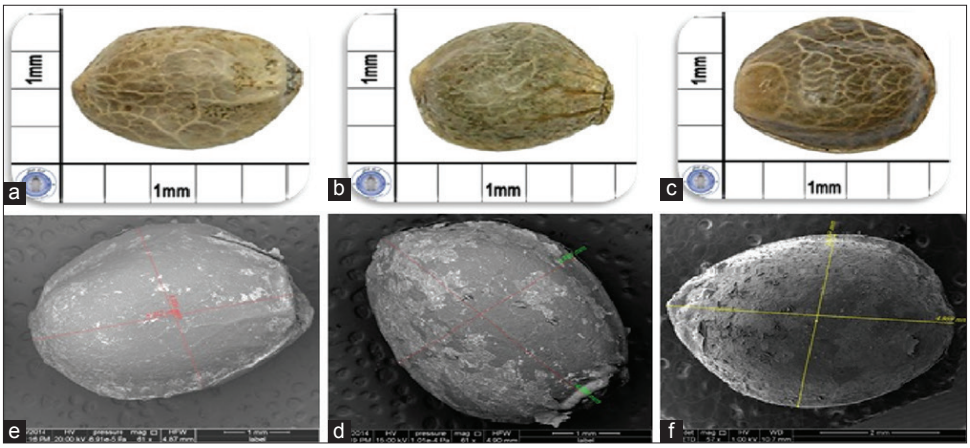

Figure 1: Microscopy of three varieties (Santhica 27, Futura 75, and Epsilon 68) of female hemp seeds cultivated in Morocco. (a) Palmitic acid (C16.0). (b) Palmitoleic acid (C16.1). (c) Stearic acid (C18.0). (d) Oleic acid (C18.1). (e) Linoleic acid (C18.2). (f) Linolenic acid (C18.3). (g) Arachidic acid (C20.0). (h) Arachidonic acid (C20.1).

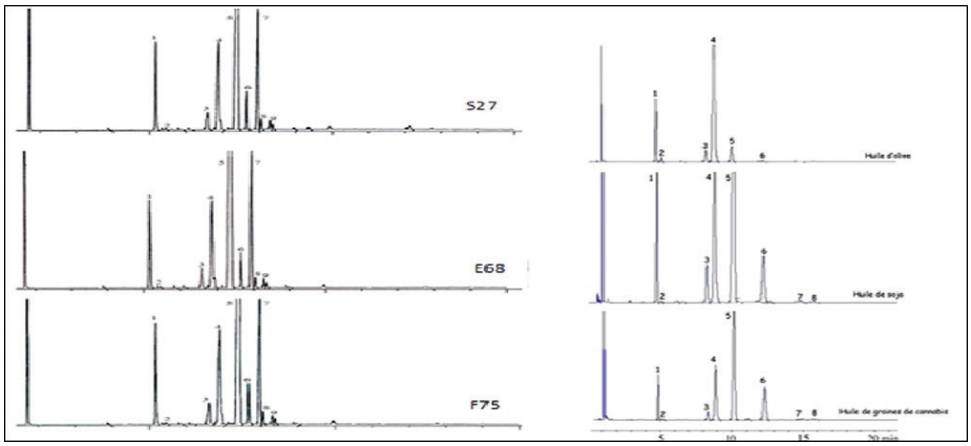

Figure 2: Gas chromatography/flame ionization detector chromatographic profile of methyl esters of vegetable oils extracted from olive, soya, and seeds of *Cannabis sativa.*

Table 3: Content of fatty acid composition (% of total oils) of hemp seed oils from three varieties of hemp (Santhica27, Epsilon 68, and Futura75) cultivated in Morocco.

Hemp variety	*Fatty acid composition (%)								
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 (trans)	C18:3	C20:0	C20:1
Santhica 27	7.70±0.03	0.20±0.01	2.44±0.04	13.30±0.03	56.71±0.1	3.10±0.05	13.66±0.04	0.92±0.05	0.82±0.02
Epsilon 68	7.40±0.04	0.20±0.01	2.60±0.04	13.13±0.03	57.12±0.1	2.74±0.05	14.80±0.06	0.90±0.05	0.70±0.02
Futura 75	7.32±0.02	0.20±0.01	2.40±0.02	12.80±0.04	57.70±0.1	2.80±0.04	14.72±0.03	0.92±0.05	0.84±0.02

*Palmitic acid (C16.0); Palmitoleic acid (C16.1); Stearic acid (C18.0); Oleic acid (C18.1); Linoleic acid (C18.2); Linolenic acid (C18.3); Arachidic acid (C20.0); Arachidonic acid (C20.1).

Table 4: Content (% of total oils) of fatty acid composition of hemp seed oils of hemp (Santhica 27, Epsilon 68, and Futura75) in comparison with olive and soybean oils cultivated in Morocco.

Fatty acid	Hemp seeds oil (%)			Olive oil (%)		Soya oil (%)	
	S27	E68	F75	Experiment	Standard (Fournier, 2003)	Experiment	Standard (Fournier, 2003)
Palmitic acid (C16:0)	7.7	7.4	7.3	10.11	(7.5–20.0)	10.93	(8.0–13.5)
Palmitoleic acid (C16:1)	0.2	0.2	0.2	0.83	(0.3–3.5)	0.09	(ND–0.2)
Stearic acid (C18:0)	2.4	2.6	2.4	3.73	(0.5–5.0)	4.64	(2.0–5.4)
Oleic acid (C18:1)	13.3	13.1	12.8	78.66	(55.0–83.0)	22.21	(17.0–30.0)
Linoleic acid (C18:2) (Oméga-6)	56.7	57.1	57.7	5.44	(3.5–21.0)	53.42	(48.0–59.0)
Linolenic acid (C18:3) (Oméga-3)	13.6	14.8	14.7	0.67	(ND–1.0)	7.34	(4.5–11.0)
Arachidic acid (C20:0)	0.9	0.9	0.9	0.40	(ND–0.6)	0.36	(0.1–0.6)
Arachidonic acid (C20:1)	0.8	0.7	0.8	ND	(ND–0.4)	0.18	(ND–0.5)

ND: Not determined.

other hand, saturated fatty acids such as palmitic, stearic, and arachidic acids ranged from 7.32% ± 0.02% to 7.70% ± 0.03%, 2.40% ± 0.02% to 2.60% ± 0.04%, and 0.9%, respectively. Moreover, the results of our study indicate that the ratio of omega-6 to omega-3 acid in hemp seeds is 3:1. The results of this study showed that hemp seeds cultivated in Morocco could be used as a source of food characterized by high nutritional value.

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How to cite this article:
Bouayoun T, Stambouli H, Ez zoubi Y, El Bouri A, Farah A, Tabyaoui M. Hemp seed oil: Chemical characterization of three non-drug varieties cultivated in Morocco. J App Biol Biotech. 2018;6(05):37-41. DOI: 10.7324/JABB.2018.60506