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## POSSIBILITY OF FIBER HEMP ESSENTIAL OIL UTILIZATION AS AN AROMA AND FRAGRANCE ADDITIVE

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### ABSTRACT

Essential oils of two fiber hemp cultivars (Fedora 17 and Helena) were investigated in order to determine their composition and their suitability for utilization as fragrance and aroma additive. The fresh fiber hemp inflorescences were hydrodistilled and obtained essential oils were characterized by GC-MS. In both cultivars, there were 45 compounds detected, of which monoterpene hydrocarbons were most abundant (36.7% in Fedora 17 and 37.44% in Helena). In significant percentage there were  $\alpha$ -pinene (5.67% in Fedora 17 and 10.12% in Helena) which has woody fragrance, myrcene which has pleasant herbaceous aroma (9.18% in Fedora 17 and 10.14% in Helena) and terpinolen with pine fragrance (8.26% in Fedora 17 and 4.63% in Helena). Limonene, with its fruity aroma, was present with 1.36% in Fedora 17 and 1.19% in Helena. Among sesquiterpenes most abundant was bitter E- $\beta$ -caryophyllene. Non-psychoactive cannabidiol (CBD) was present with 9.30% and 7.22% in Fedora 17 and Helena, respectively, while psychoactive tetrahydrocannabinol (THC) was not detected. Based on investigated essential oils composition, Fedora17 and Helena possess terpenes with desirable scent, which makes them favorable for application as flavor and fragrance additive.

**Keywords:** *cannabis sativa*, fiber hemp, essential oils, terpenes

### INTRODUCTION

Fiber hemp (*Cannabis sativa* L.) is an annual herbaceous plant belonging to the family *Cannabaceae* originating from Eastern and Central Asia. It is considered one of the oldest crops known to man and it was used as an important source of food, fiber and medicine for thousands of years in the Old World (Zias *et al.*, 1993). Although primarily grown for fibers (Li, 1973; Synowiec *et al.*, 2016), nowadays fiber hemp is recognized as multi-use crop because of its great potential for application in oil, pharmaceutical, cosmetic and other industries (Ranalli and Venturi, 2004). While hempseed oil already has application in human nutrition due to its high polyunsaturated fatty acids content, there is a growing interest over the valorization of hemp secondary metabolites, especially cannabinoids, terpenoids and flavanoids (Hazekamp *et al.*, 2010).

The hemp essential oil has generally been considered a niche high value product with promising potential marketing (Mediavilla and Steinemann, 1997; Meijer, 1998; Thomas *et al.*, 2000). Due to characteristic aroma it is used in some products such as cosmetics, soaps, shampoo, cream, cosmetic oil, perfume or in candles and a flavor to foodstuffs (particularly candy and beverages) or medicines. Alcoholic beverages made with hemp utilize hemp essential oil as a flavorant food. Additionally, hemp essential oil can also be used in natural medicine (aromatherapy) and as a plant protection preparation as two compounds: limonene and  $\alpha$ -pinene terpenes show insect-repellent properties. Another important aspect of essential oil is that the monoterpenes and sesquiterpenes contained in it have bacteriostatic effect comparable to the effect of thyme essential oil. The effect is apparent especially towards Gram-positive bacteria (*Staphylococcus* and *Streptococcus*).

Hemp inflorescences, which pose a waste in fiber industry can be used for production of essential oil which gives opportunity for fiber hemp to become multi-use crop.

The objectives of this study were to determine essential oils profile of two monocious fiber hemp cultivars Fedora 17 and Helena, which are widely cultivated in Serbia in order to



determine their suitability for possible multi-usage as fiber crop and natural fragrance or flavor additive.

## MATERIAL AND METHODS

Two registered cultivars of fiber hemp, Helena (RS) and Fedora 17 (France) were grown in 2016, at the Department for Alternative Crops of the Institute of Field and Vegetable Crops in Novi Sad, Serbia.

The fresh hemp inflorescences (150–200 g) were hydrodistilled (2 h, 2 l water distilled, flow 2.0 ml min<sup>-1</sup>) by a Clevenger apparatus. The essential oils were dissolved in absolute ethanol (Carlo Erba, HPLC-PLUS Gradient), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and injected in GC-MS. GC-MS analysis was performed on an Agilent 6890N series gas chromatograph equipped with a CombiPal CTC Analytics autosampler, a HP5-MS column (30 m length, 250 µm internal diameter, film thickness 0.25 µm, Agilent J&W GC Columns, Agilent Technologies), and a single quadrupole mass spectrometer Agilent 5975B. The injector temperature was 230 °C with an injection volume of 1 µl, a split ratio of 1:50, and a carrier gas (He) flow rate of 1.0 ml min<sup>-1</sup>. The oven temperature program: 60 °C, ramp rate of 3 °C min<sup>-1</sup>, final temperature 240 °C held for 2 min. The MS source temperature was set to 230 °C, the single quad temperature was 150 °C, and the transfer line temperature was set to 280 °C. The mass range analyzed by the mass spectrometer was 35.00 – 500.00 amu. The identification of essential oil constituents was performed by a computer matching of mass spectra with two commercial data base (ADAMS 2007; NIST 2005) and by comparison of their Linear Retention Indices (LRI) relative to a series of n-hydrocarbons (C9–C40). Quantitative data were obtained from normalized area values.

## RESULTS AND DISCUSSION

Hemp essential oil is a mixture of volatile compounds, including monoterpenes, sesquiterpenes, and other terpenoidlike compounds. About 140 terpenoids are known in *C. sativa* (Giese *et al.*, 2015), although none is unique to just this species. Depending on biotype, monoterpenes represent 48%–92% of the volatile terpenes and sesquiterpenes represent 5%–49% (Mediavilla and Steinemann, 1997).

Essential oils of two different cultivars of *C. sativa* were analyzed. In total, 45 compounds were detected and identified, accounting for 89.5 and 89.4% of the whole GC profile for Fedora 17 and Helena, respectively. The essential oils composition is reported in Table 1 and typical chromatogram of fiber hemp essential oil is given in Figure 1.

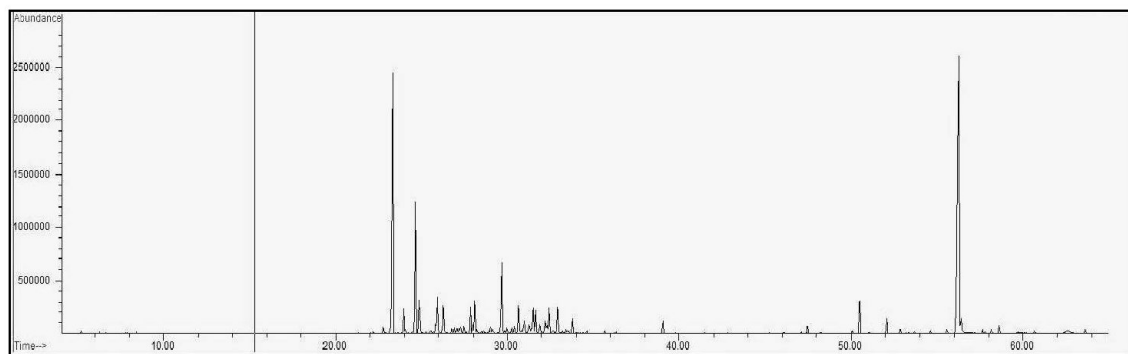


Figure 1. A typical GC-MS chromatogram of fiber hemp essential oil

The most abundant class in both cultivars are monoterpene hydrocarbons with 36.87% and 38.09% in Fedora 17 and Helena, respectively. In essential oils of both cultivars there were 24 monoterpenes identified, among which  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene, E-

ocimene and terpinolene were present in the highest percentage. The main sesquiterpenes in analyzed cultivars were E- $\beta$ -caryophyllene,  $\alpha$ -humulene and caryophyllene oxide. It has been documented by other authors that the same terpenes are main constituents of fiber hemp essential oil (Novak *et al.*, 2001; Bertoli *et al.*, 2010; Nissen *et al.*, 2010; Da Porto *et al.*, 2014; Synowiec *et al.*, 2016). Aroma of *C. sativa* originate from the volatile monoterpenes and sesquiterpenes. Many terpenes are extremely odiferous, detectable by smell at very low concentrations (Small, 2017). Monoterpenes pinene and limonene are particularly responsible for the pleasant smell and aroma of *C. sativa* (Hood *et al.*, 1973). Limonene has fruity and citrusy odor (Small, 2017) and besides that, its presence is connected with uplifting effect on mood. Limonene was present with 1.36% of the total essential oil in Fedora 17 and with 1.19% in Helena.  $\alpha$ -pinene has woody and resinous smell (Schreiner *et al.*, 2018) and can reduce the short-term memory (Small, 2017). In our study,  $\alpha$ -pinene content was 5.67% and 10.12% of the total essential oil of Fedora 17 and Helena cultivars, respectively. Myrcene was present with 9.18% in Fedora 17 and 10.14% in Helena and is most abundant monoterpene in investigated essential oils. It has sedative effect (Piomelli and Russo, 2016) and is used in perfume industry due to its pleasant herbaceous odor.

Terpinolene is a monoterpene with a pine fragrance and the notable difference between its content in Fedora 17 (8.26%) and Helena (4.63%) has been observed. Novak *et al.* (2001) suggested that cannabis cultivars can be separated in two groups based on their terpinolene content: high terpinolene cultivars originating from France and low terpinolene cultivars originating from Eastern Europe. According to Novak *et al.* (2001) terpinolene content could be genetic marker for distinguishing two gene pools for breeding different varieties.

Monoterpenes are principally responsible for differences in fragrance among hemp cultivars. In our study, general monoterpene content was lower than in the cultivars analyzed by Bertoli *et al.* (2010). It may be due to monoterpene characteristic to evaporate relatively faster than other components and thus their content can be reduced during the drying of fresh inflorescences (Ross and ElSohly, 1996). Because of that, composition of essential oil in extracts obtained from the harvested plant may differ from the volatiles released around the fresh plant and therefore the odor of the living plant is usually different from the odor of the dried plant. When fresh inflorescences are dried, a greater loss of monoterpenes and also sesquiterpenes is observed, but none of the major components of the oil completely disappears (Ross and ElSohly, 1996).

Table 1. Essential oil composition of two monoecious varieties of *Cannabis sativa* L.

	Compound	LRI <sub>lit</sub> <sup>a</sup>	LRI <sub>exp</sub> <sup>b</sup>	Fedora 17 (%)	Helena (%)
1.	$\alpha$ -Thujene	927.8	925.9	0.11	0.00
2.	$\alpha$ -Pinene	936.1	933.8	5.67	10.12
3.	Camphene	950.3	947.4	0.12	0.28
4.	Sabinene	973.0	972.7	0.11	0.19
5.	$\beta$ -Pinene	977.7	977.0	2.41	3.23
6.	Myrcene	989.2	990.9	9.18	10.14
7.	$\alpha$ -Phellandrene	1004.1	1005.2	0.40	0.20
8.	$\delta$ -3-Carene	1011.3	1011.1	0.92	0.18
9.	$\alpha$ -Terpinene	1017.1	1016.5	0.31	0.16
10.	p-Cymene	1024.3	1023.8	0.04	0.07
11.	Limonene	1029.5	1028.2	1.36	1.19
12.	Eucalyptol	1031.8	1030.1	0.00	0.12
13.	Z-Ocimene	1037.8	1036.3	0.55	0.67
14.	E-Ocimene	1047.7	1048.6	7.02	6.20
15.	$\gamma$ -Terpinene	1059.7	1057.4	0.24	0.18



16.	Sabinene hydrate	1066.5	1065.1	0.03	0.03
17.	<b>Terpinolene</b>	<b>1086.9</b>	<b>1087.8</b>	<b>8.26</b>	<b>4.63</b>
18.	Linalool	1099.0	1099.6	0.00	0.05
19.	Nonanal	1103.3	1104.0	0.10	0.10
20.	Pinene hydrate	1121.2	1120.4	0.02	0.04
21.	Pinocarveol	1140.0	1137.1	0.02	0.08
22.	Pinocarvone	1160.6	1160.8	0.02	0.05
23.	Borneol	1166.2	1163.7	0.00	0.06
24.	Terpinen-4-ol	1177.1	1175.7	0.08	0.19
25.	$\alpha$ -Terpineol	1189.7	1189.3	0.00	0.03
26.	<b>Z-<math>\beta</math>-Caryophyllene</b>	<b>1406.5</b>	<b>1406.0</b>	<b>0.26</b>	<b>0.42</b>
27.	<b>E-<math>\beta</math>-Caryophyllene</b>	<b>1420.1</b>	<b>1425.0</b>	<b>20.43</b>	<b>18.73</b>
28.	$\alpha$ -Bergamotene	1434.5	1436.9	1.27	1.13
29.	<b><math>\alpha</math>-Humulene</b>	<b>1453.1</b>	<b>1455.9</b>	<b>7.51</b>	<b>6.63</b>
30.	$\beta$ -Farnesene	1455.9	1459.6	2.25	2.23
31.	$\gamma$ -Muurolene	1476.2	1476.3	0.07	0.00
32.	$\gamma$ -Curcumene	1480.3	1480.2	0.09	0.08
33.	$\beta$ -Selinene	1486.1	1485.9	1.08	1.53
34.	$\alpha$ -Selinene	1493.4	1494.2	1.09	1.35
35.	$\beta$ -Bisabolene	1508.4	1508.6	0.66	0.26
36.	$\alpha$ -7-epi-Selinene	1517.2	1516.4	0.22	0.00
37.	$\beta$ -Sesquiphellandrene	1523.5	1523.5	0.31	0.25
38.	Selina-3,7-diene	1540.5	1540.7	0.68	0.19
39.	$\alpha$ -Bisabolene	1540.3	1542.7	0.00	0.06
40.	Germacrene B	1550.9	1555.2	0.69	0.00
41.	Nerolidol	1560.9	1564.1	0.00	0.51
42.	<b>Caryophyllene oxide</b>	<b>1580.6</b>	<b>1581.7</b>	<b>4.39</b>	<b>6.40</b>
43.	Humulene epoxide II	1604.7	1606.9	1.43	2.57
44.	Caryophylla-4-dien-5-beta-ol	1640.3	1634.1	0.80	1.65
45.	CBD			<b>9.30</b>	<b>7.22</b>
	<b>Total monoterpenes</b>			<b>36.87</b>	<b>38.09</b>
	Monoterpene hydrocarbons			36.7	37.44
	Monoterpenes oxygenated			0.17	0.65
	<b>Total sesquiterpenes</b>			<b>43.23</b>	<b>43.99</b>
	Sesquiterpene hydrocarbons			36.61	32.86
	Sesquiterpenes oxygenated			6.62	11.13
	<b>Others</b>			<b>9.4</b>	<b>7.32</b>
	<b>Total</b>			<b>89.5</b>	<b>89.4</b>

<sup>a</sup> RI<sub>lit</sub> – literature retention indices

<sup>b</sup> RI<sub>exp</sub> experimental retention indices on DB-5ms column

Sesquiterpene E- $\beta$ -caryophyllene was the most abundant compound in analyzed essential oil with 20.43% and 18.73% of total compounds in Fedora 17 and Helena, respectively. The content of E- $\beta$ -caryophyllene in Fedora 17 and Helena was in accordance with the literature

(Novak *et al.*, 2001; Bertoli *et al.*, 2010; Da Porto *et al.*, 2014), where is stated that  $\beta$ -caryophyllene is usually dominant sesquiterpene in *C. sativa* (Mediavilla and Steinemann, 1997) and because of its bitterness it protects plant from insect herbivores.

Terpenes are biosynthesized in the epidermal glands or glandular hairs of *C. sativa*, where the cannabinoids are also produced (Kim and Mahlberg, 1981; Malingré *et al.*, 1975). Cannabinoids, secondary metabolites characteristic for *C. sativa*, were also found in fiber hemp essential oil and their content was determined. The content of non-psychoactive cannabidiol (CBD) was 9.30% and 7.22% in Fedora 17 and Helena, respectively, while hallucinogen tetrahydrocannabinol (THC) was not detected in analyzed cultivars. Unlike the terpenes, the cannabinoids are odorless (Clarke and Watson, 2002).

## CONCLUSIONS

Essential oils consist of secondary metabolites of plants, mostly terpenes, which are responsible for scent of plants. Both analyzed fiber hemp cultivars (Fedora 17 and Helena) showed similar qualitative composition of essential oils, dominating volatile monoterpenes with a pleasant fragrance. The aroma of *C. sativa* could be of a considerable commercial value in flavor industry, especially when the amount of odoriferous terpenes is significant. Since Fedora 17 and Helena have high monoterpene content, such as limonene,  $\alpha$ -pinene, myrcene and terpinolene, they have a potential to be used as food and beverages additives. This study confirm the hypothesis to grow fiber hemp as a multi-use crop through a complete utilization of the plant material using stems for fiber industry and inflorescences to produce essential oils as natural flavor and fragrance additives.

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