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CANNABINOIDS CONTENT AND FATTY ACIDS COMPOSITION IN TWELVE EUROPEAN FIBER HEMP VARIETIES

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ABSTRACT

The objective of this study was to determine cannabinoids content and fatty acids composition in twelve European fiber hemp varieties from Serbia, Romania, Hungary, Italy, Ukraine, France, Nederland and Poland.
The CBD content in analysed varieties ranged from 0.00 to 1.96% and THC content was lower than the legal limit in EU.
Total oil content in seeds of investigated varieties ranged from 25.09 to 34.09%. Fatty acid analysis showed that seed oil of all analyzed varieties is high in PUFAs (polyunsaturated fatty acids) ranging from 69.2 to 78.2% of the total fatty acids. Gamma-linolenic acid (C18:3 n-6) ranged from 0.62 to 2.97% and omega-6/omega-3 ratio varied from 2.93 to 4.47.
Obtained results indicate that all analysed hemp varieties are an excellent source of polyunsaturated fatty acids, especially gamma-linolenic acid. In addition, according to these results there is low risk of oil contamination with cannabinoids. Most of the analyzed varieties can be used as a source for CBD extraction.
***Keywords**: fiber hemp, hemp seed, fatty acids, cannabinoids*

INTRODUCTION

Cannabis sativa L. is an important herbaceous species originating from Central Asia, which has been used in folk medicine and as a source of textile fiber since the dawn of times.
Although primarily grown for fiber, hemp plant has been attracting growing interest worldwide for oil production. It has been recognized as a new, underdeveloped industrial oilseed crop in the European Union, in contrast to conventional oil crops such as rapeseed and sunflower (Zanetti *et al.,* 2013).
In the varieties bred mainly for the production of fibre, the seed component is often overlooked simply as a by-product (Oomah et al., 2002; Kriese et al., 2004). Nonetheless, hemp seeds have proven to be of great nutritional value, generally composed of 250–350 g/kg lipids, 20–25 g/kg protein, and 20–30 g/kg carbohydrates (Oliver and Joynt, 1999; Leizer et al., 2000; House et al., 2010). In particular, the lipid portion of hemp seeds is very rich in essential fatty acids, consisting of large amounts of alpha-linolenic (omega-3) and linoleic acid acid (omega-6), often in a favourable 3:1 ratio (Carvalho et al., 2006; Matthaus and Bruhl, 2008; Chen et al., 2010; Da Porto et al., 2012; Teh and Birch, 2013). Notably, hemp seeds are also a good source of highly digestible protein, well-suited for human and animal consumption (Deferne and Pate, 1996; Mustafa et al., 1999; Callaway, 2004; Tang et al., 2006).
While fiber and seed are the main products, there is a growing interest over the valorization of hemp secondary metabolites. Hemp vegetative and reproductive organs are rich in various unique bioactive secondary metabolites, particularly cannabinoids (Hazekamp *et al.,* 2010).
Cannabinoids are terpenophenolic compounds, typical of the genus *Cannabis*. Commonly, there are cannabidiol (CBD), delta 9-tetrahydrocannabinol (THC), cannabichromene (CBC) and cannabigerol (CBG). Cannabinol (CBN) is a degradation product of THC and it does not occur naturally in plants (Peschel and Politi, 2015). CBD, as a non-psychoactive cannabinoid, is currently the cannabinoid of considerable interest. CBD, along with THC, has been demonstrated to have a wide range of pharmacological activity, with the potential to be

developed for a number of therapeutic areas (Pertwee, 2004). It is likely, that other cannabinoids, present in small amounts in *Cannabis sativa* L., may also have interesting pharmacological properties, for example THCV, CBC, CBG (McPartland & Russo, 2001; Russo & McPartland, 2003).
The objective of this study was to determine cannabinoids content and fatty acids composition in twelve European fiber hemp varieties in order to determine their potential for multipurpose usage (fiber, oil and CBD production).

MATERIAL AND METHODS

Twelve varieties of fiber hemp used in this research belong to collection of Institute of Field and Vegetable Crops, Department for Alternative Crops, Novi Sad, Serbia).
Varieties were grown in 2017 at the Department for Alternative Crops of the Institute of Field and Vegetable Crops.

Table 1. Name, country of origin and sex type of the 12 Cannabis sativa varieties used in this study

	Variety	Origin (country)	Sex type
1	Helena	Serbia	monoecious
2	Marina	Serbia	dioecious
3	Novosadska	Serbia	dioecious
4	Lovrin 110	Romania	dioecious
5	Monoica	Hungary	monoecious
6	Tiborszallasi	Hungary	dioecious
7	Carmagnola	Italy	dioecious
8	USO 31	Ukraine	monoecious
9	Santhica	France	monoecious
10	Fedora 17	France	monoecious
11	Chameleon	Netherlands	monoecious
12	Silesia	Poland	monoecious

Plants used for analysis of cannabinoids were harvested in flowering phase and only flowering tops were used. Hemp seeds (approx. moisture content 6.6%) were used for the analyses of oil composition.
Hemp flowering tops were dried and then homogenised using laboratory mill. Absolute ethanol (20 ml) was added to 200 mg of dry and homogenised sample weighed into an erlenmayer with a stopper, after which the solution was sonicated for 15 min. Decarboxylation step was performed prior to GC analysis by transferring 1 ml of solution to a 2 ml GC vial and heating it in a heating unit (150 °C) for 12 min. Solvent was evaporated and cannabionids were decarboxylated. The residue was dissolved in 1.5 ml of ethanol and shaken well prior to analysis.
Analysis of cannabinoids was performed on Agilent 7890 gas chromatograph equipped with flame ionisation detector (FID). The separation was performed on a fused silica capillary column (HP-5, 30 m × 0.25 mm i.d., and 0.25 μm film thickness). Helium was used as carrier gas at a constant flow of 1 ml/min. The temperature program was as follows: initial temperature of 200 °C was held for 2 min, then increased to 240 °C at a rate of 10 °C/min, and hold for 10 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The injected sample volume was 1 μl and split ratio was 1:10. Individual analytical standards for cannabidiol (CBD), cannabigerol (CBG) and cannabinol (CBN) were used for calibration. Quantitation of THC was performed with CBN analytical standard in accordance to method given by Poortnman-van der Meer *et al.* (1999) and expressed as % in dry weight.
To determine hempseed fatty acid composition and content 4 g of seeds were pressed in a hydraulic press to yield approximately 0.5 ml of oil available for GC analysis. Then, oils were converted into fatty acid methyl esters by transesterification using TMSH (transesterification

agents). To the reaction vial with 270 µl of TMSH, exactly 30 µl of oil was added, well shaken in the vortex, and kept at room temperature for an hour.
Analysis of fatty acid methyl esters was performed on Agilent 7890 gas chromatograph equipped with flame ionisation detector (FID) and split/splitless injector (split ratio of 1:50). The separation was performed on a fused silica capillary column (HP-INNOWAX, 30 m × 0.25 mm i.d., and 0.25 µm film thickness). Helium was used as carrier gas at a constant pressure of 53 kPa at 50 °C min. The temperature program was as follows: initial temperature of 50 °C was held for 1 min, increased to 200 °C at a rate of 25 °C/min, then increased to 230 °C at a rate of 3 °C/min, and hold for 18 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. Injected sample volume was 1 µl. The results were processed using ChemStation software and expressed as the percentage of individual fatty acids in the oil sample.

RESULTS AND DISCUSSION

Cannabinoids content in twelve analyzed varieties of fiber hemp flowering tops is presented in Figures 1 and 2. Fiber hemp (*Cannabis sativa* subsp. *sativa)* contains primarily CBD and little or no THC. CBD is credited with analgesic, anticonvulsant, antiemetic, antiepileptic, anti-inflammatory, muscle relaxant, anxiolytic (anxiety-reducing), neuroprotective, antioxidant, and antipsychotic activity (Russo and Guy 2006; Mechoulam et al. 2007). Hemp varieties relatively high in CBD and low in THC can be used as a natural source for CBD extraction.
The CBD content in analyzed varieties ranged from 0% (Santhica) to 1.97% (Carmagnola) (Figure 1.). Obtained results was similar to previously reported data that USO 31 and Santhica are varieties with very low cannabinoid content (Small, 2017), as well as other varieties grown in France (Thouminot, 2015).
Presented results have slightly lower than average CBD content than these reported from the study (Sikora *et al.*, 2011) on the effects of agroclimatic conditions on CBD synthesis and accumulation in fiber hemp varieties experimentally grown in Serbia from 1999 to 2005.

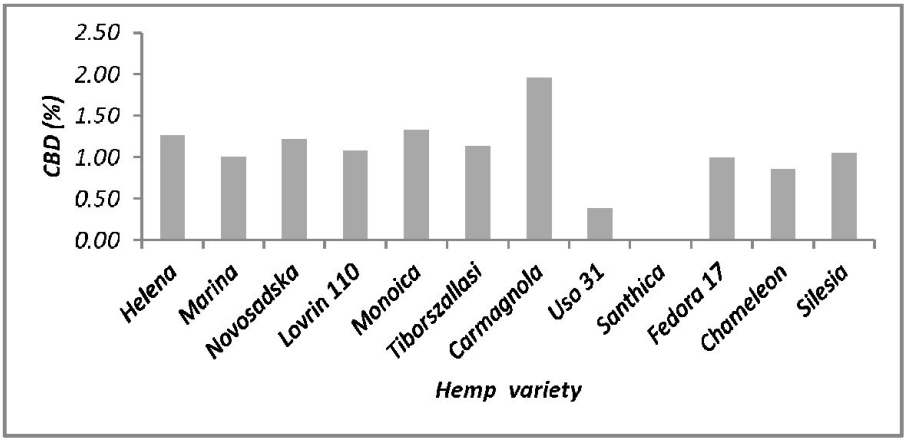

Figure 1. Cannabidiol content (%) in fiber hemp flowering tops

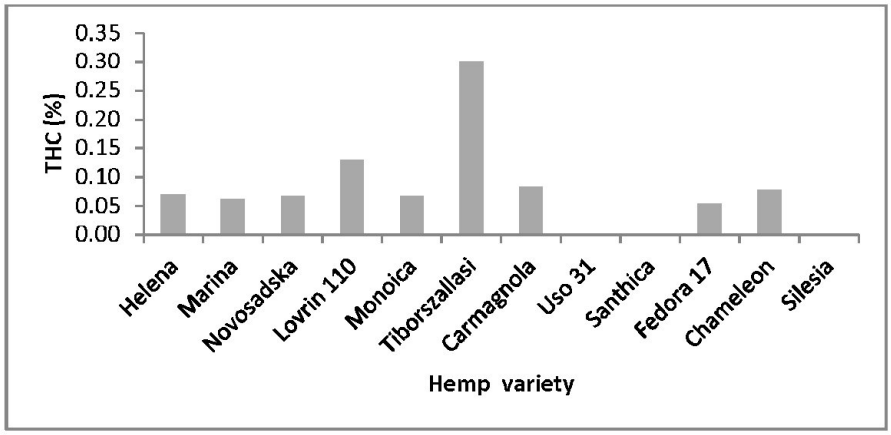

Figure 2. Delta 9-tetrahydrocannabinol (THC) content (%) in fiber hemp flowering tops

The THC content in analyzed varieties ranged from 0% (USO 31, Santhica and Silesia) to 0.29% (Tiborszallasi) (Figure 2.) In most EU countries the current upper legal limit for cultivation is 0.2% THC (Sarmento *et al.*, 2015) and in Serbia the limit is 0.3% THC. The THC content in eleven of twelve analyzed hemp varieties was significantly lower than legal limit in EU. Flowering tops of hemp variety Tiborszallasi had significantly higher TCH content then current upper legal limit in EU, but lower than legal limit in Serbia. Relatively high THC content in variety Tiborszallasy can pose a problem if seed is used for hemp oil production.

The highest concentration of THC and other cannabinoids in hemp is found in female flowers. Because the majority of cannabinoids is located on the surface of the seed during and after seed formation, the oil can absorb a certain amount during cold pressing. The absorbed amount mostly depends on the variety of hemp and the extent of contamination with parts of the plant rich in cannabinoids during processing. Mandatory limits for THC in edible oils in EU range from 5 mg/kg in Germany to 20 mg/kg in Switzerland.

In agreement with previous reports, the hemp seed oil of the twelve varieties evaluated in this study contained on average 83% unsaturated fatty acids and 73.9% polyunsaturated fatty acids (Deferne and Pate, 1996; Matthaus and Bruhl, 2008; Chen et al., 2010; Da Porto et al., 2012). Similar to the results of Carvalho et al. (2006), Chen et al. (2010) and Da Porto et al. (2012), the major monounsaturated fatty acid in the hemp seed oil analyzed in the present study was oleic acid (an average of 13.26% for all varieties). Of all varieties, Monoica contained the most oleic acid (16.80%) (Table 2).

No significant differences among the twelve varieties analyzed were observed for linoleic acid, the fatty acid present in hemp seed in the greatest proportion (52.7% in average). However, noticeable differences in α-linolenic and γ-linolenic acids concentration were observed. The greatest concentration of α-linolenic acid was measured in Carmagnola (18.16%) and the lowest concentration in Monoica (11.61%), while the greatest concentration of γ-linolenic acid was measured in Santhica and Fedora 17 (2.97%) and the lowest concentration in Marina (0.62%). These fatty acids, known as omega-3 and omega-6, are essential fatty acids because humans cannot produce them themselves, and must obtain them in their diet.

Hempseed oil has been suggested to be perfectly balanced in regards to the ratio (3:1) of these two essential polyunsaturated fatty acids (linoleic and linolenic acids) for human nutrition (Leizer et al., 2000;Matthaus and Bruhl, 2008; Da Porto et al., 2012; Teh and Birch, 2013). The average ratio of 3.6:1 linoleic to α-linolenic acid for the varieties presented in this study indicates that these varieties potentially represent a highly nutritional food source. In addition, the oil is mostly used in cosmetic industry, because of the presence of α-linolenic acid, which is an ideal ingredient for light body oils and lipid-enriched creams, known for high penetration into the skin (Rausch, 1995).

Table 2. Fatty acid composition (%) of hempseed oils

	Hemp variety											
Fatty acid	**Helena**	**Marina**	**Novosadska**	**Lovrin 110**	**Monoica**	**Tiborszallasi**	**Cargmanola**	**USO 31**	**Santhica**	**Fedora 17**	**Chameleon**	**Silesia**
Palmitic C16:0	7,18	6,11	6,22	6,77	6,94	8,13	6,50	6,20	6,26	7,48	6,70	6,49
Stearic C18:0	2,69	3,03	2,80	2,98	2,67	2,33	2,73	2,70	2,52	2,69	2,72	2,68
Oleic C18:1	11,0	12,5	12,3	16,1	16,8	13,1	12,3	15,1	9,74	14,3	12,8	13,6
Linoleic C18:2ω6	53,5	53,2	54,1	51,1	50,9	52,3	51,8	52,6	54,1	53,2	53,5	51,9
α-Linolenic C18:3ω3	15,5	16,5	17,2	13,3	11,6	15,1	18,2	13,6	15,7	13,4	13,9	13,3
γ-Linolenic C18:3ω6	1,72	0,62	0,78	2,21	0,94	0,89	1,40	2,01	2,97	2,97	2,28	1,50
Arachidic C20:0	0,84	0,83	0,76	1,02	0,83	0,88	0,87	0,96	0,77	0,87	1,03	0,62
Eicosenic 20:1	0,38	0,40	0,37	0,45	0,39	0,49	0,42	0,46	0,39	0,46	0,49	0,42
Behenic 22:0	0,56	0,35	0,32	0,42	0,38	0,42	0,33	0,41	0,30	0,36	0,49	0,29
% PUFA*	**75,5**	**74,9**	**75,7**	**70,4**	**69,2**	**72,7**	**75,3**	**72,3**	**78,2**	**72,5**	**74,0**	**73,4**
n6/n3 ratio	**3,55**	**3,27**	**3,20**	**3,99**	**4,47**	**3,52**	**2,93**	**4,01**	**3,62**	**4,19**	**3,99**	**4,01**

* Polyunsaturated fatty acids

CONCLUSIONS

Obtained results indicate that all analysed hemp varieties are an excellent source of polyunsaturated fatty acids, especially gamma-linolenic acid with the exception of Tiborszallasi variety, which bears a low risk of contamination with cannabinoids if used for oil production. Most of the analysed varieties are rich in CBD, which makes them promising source of cannabinoids for pharmaceutical industry.

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