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FIBER HEMP AS A VALUABLE SOURCE OF NUTRIENTS AND NUTRACEUTICALS

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ABSTRACT

Hemp (*Cannabis sativa* L.) is considered one of the oldest crops known to man. It was used in the Old World for thousands of years as an important source of food, fiber, and medicine. Although primarily grown for hemp fiber used for production of durable plastics and specialty papers, fiber hemp plant has been attracting growing interest worldwide as a valuable source of nutrients and nutraceuticals. There is also a growing interest over the valorization of hemp secondary metabolites especially cannabinoids, which are believed to be beneficial in a number of physiopathological processes. There are 54 fiber hemp varieties registered in EU and 4 hemp varieties registered in Serbia.

The aim of this study was to evaluate medical cannabinoids and nutrients (oil and protein) contents of all fiber hemp varieties registered in Serbia.

Total oil content in seeds of investigated varieties ranged from 21.09 to 32.09%. Fatty acid analysis showed that hempseed oil is high in PUFA (polyunsaturated fatty acids) content and polyunsaturated/saturated (P/S) ratio with linoleic (C18:2 ω -6) and linolenic (C18:3 ω -3) acids as the major polyunsaturated fatty acids.

The cannabidiol (CBD) content in four analyzed varieties ranged from 1.00 to 1.27% and delta 9-tetrahydrocannabinol (THC) content was approximately 3 times lower than the legal limit in EU.

The obtained results indicate that all analyzed hemp varieties are an excellent source of nutrition and a valuable resource for the functional foods, nutraceutical and pharmaceutical industry.

Keywords: fiber hemp, hemp seed, oil, cannabinoids

INTRODUCTION

Hemp (*Cannabis sativa* L.) is considered one of the oldest crops known to man. 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 hemp fiber used for production of durable plastics and specialty papers, 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 rapeseed and sunflower (Zanetti *et al.*, 2013).

Hemp seeds are traditionally used in food and folk medicinal preparations (Jones, 1995) or employed as a feed for birds and fishes (Deferne and Pate, 1996). Recent characterization of oil (Latif and Anwar, 2009) and protein isolates (Tang *et al.*, 2006, 2009) from hemp seeds have highlighted that not only the fiber, but also the seeds hold very interesting commercial potential in food (Callaway, 2004; Matthäus and Brühl, 2008), feed (Hessle *et al.*, 2008) and cosmetic applications (Sapino *et al.*, 2005). Hempseed, in addition to its nutritional value, has demonstrated positive health benefits, including the lowering of cholesterol and high blood pressure (Jones, 1995).

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, namely cannabinoids, terpenoids and flavonoids (Hazekamp *et al.*, 2010).

Cannabinoids are terpenophenolics, a group of 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 plant (Peschel and Politi, 2015). Cannabinoids have modulating effects on the human endocannabinoid system and are believed to be beneficial in a number of physiopathological processes (Izzo *et al.*, 2009). There are 54 fiber hemp varieties registered in EU (EC, 2016) and 4 hemp varieties registered in Serbia.

The aim of this study was to evaluate all fiber hemp varieties registered in Serbia as a source of nutrients and medical cannabinoids.

MATERIAL AND METHODS

Four registered varieties of fiber hemp plant: Helena, Marina, Novosadska and Fedora 17 were grown in 2015, at the Department for Alternative Crops of the Institute of Field and Vegetable Crops in Novi Sad, Serbia.

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 content and composition, as well as total protein content.

Hemp flowering tops were dried and then homogenized using laboratory mill. Absolute ethanol (20 ml) was added to 200 mg of dry and homogenized 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, than putting it into a heating unit (150 °C) for 12 min where the 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 μ m 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 μ l 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).

Total oil content were determined by the extraction of oil from ground hempseed (8h, 70 °C), performed in a Soxhlet extractor using 5 g of seed and 200 ml petroleum-ether followed by solvent removal under vacuum at 60 °C.

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 agent). To the reaction vial with 270 μ l of TMSH, exactly 30 μ l 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 ionization 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 μ m film thickness). Helium was used as carrier gas at a constant pressure of 53 kPa at 50 °C. 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 $^{\circ}$ C, respectively. Injected sample volume was 1 μ l. The results were processed using ChemStation software and expressed as the percentage of individual fatty acids in the oil sample.

Determination of protein content was performed according to AOAC Official Method 972.43.

RESULTS AND DISCUSSION

Cannabionids content in four analyzed varieties of fiber hemp flowering tops extracts is presented in Table 1 and typical chromatogram of fiber hemp flowering tops extracts is given in Figure 1. Fiber hemp flowering tops extract is characterized by low THC content and high cannabidiol (CBD) content.

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 all four analyzed hemp varieties was approximately 3 times lower than legal limit in EU.

The CBD content in analyzed varieties ranged from 1.00 to 1.27% which is in good correlation with CBD content reported in fiber hemp varieties grown in France (Thouminot, 2015) and slightly lower than average CBD content in eight varieties experimentally grown in Serbia from 1999 to 2005 in order to evaluate the effects of agroclimatic conditions on CBD synthesis and accumulation (Sikora *et al.*, 2011). A strong influences of soil temperature at 5 cm and growing degree days (GDD), which are the most significant agroclimatic parameters influencing cannabinoids synthesis (Mandolino *et al.*, 2003; Sikora *et al.*, 2011), could have contributed to lower CBD content in varieties grown in 2015 (Table 1).

Table 1. Phytocannabinoids content (%) in fiber hemp plant

Hemp variety	CBD	THC	CBG
Helena	1.27	0.07	nd*
Marina	1.00	0.06	nd
Novosadska	1.21	0.07	nd
Fedora 17	1.00	0.05	nd

^{*}nd - not detected

CBD is the most prevalent cannabinoid in the fiber-type hemp and presents a large array of pharmacological properties, as recently reviewed by Burstein (2015). CBD itself has been shown in animal and *in vitro* studies to possess, among others, anti-anxiety, anti-nausea, anti-arthritic, anti-psychotic, anti-inflammatory, and immuno modulatory properties (Burstein, 2015). It has also shown potential as therapeutic agent in preclinical models of central nervous system diseases such as epilepsy, neurodegenerative diseases, schizophrenia, multiplesclerosis, affective disorders and the central modulation of feeding behavior (Hill *et al.*, 2012).

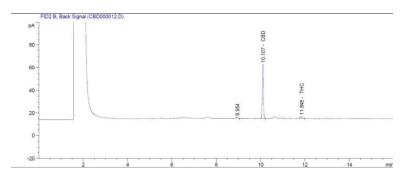


Figure 1. GC-FID chromatogram of flowering tops extracts of fiber hemp variety Helena

Total oil content in seeds of investigated varieties ranged from 21.09 to 32.09%, and it was similar to findings of other authors (Callaway, 2004; Peschel and Politi, 2015). Obtained results showed that hemp seed oil contained linoleic (C18:2 ω 6) and linolenic (C18:3 ω 3) acids as the major polyunsaturated fatty acids (Table 2), as it was confirmed in some previous studies (Callaway, 2004; Da Porto *et al.*, 2012).

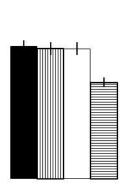
'Novosadska' had the highest linoleic and α -linolenic acids and 'Fedora' γ -linolenic acid contents (Table 2). These fatty acids are known as essential fatty acids because humans cannot produce them themselves, and must obtain them in their diet. Hempseed oil has been suggested to be perfectly balanced in regards to the ratio (3:1) of these two essential polyunsaturated fatty acids (linoleic and linolenic acids) for human nutrition. Also, 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).

Hempseed oil was characterized by high content of PUFA (polyunsaturated fatty acids) and polyunsaturated/saturated (P/S) ratio (Table 2). When compared to other vegetable oils, hempseeds has much higher PUFA content (approximately 10% higher than corn, soy, sunflower seeds and 60% than olive fruits). A high ratio of P/S is regarded favorably for the reduction of serum cholesterol and arteriosclerosis and prevention of heart diseases (Rudel et al., 1998). Similarly, the ratio of ω -6 to ω -3 fatty acids was 3.3-4.2, which is lower than in sunflower and olive seeds, but higher than in rape seeds or flax seeds (Callaway, 2004).

Table 2. Oil content and fatty acid composition (%) of hempseed oils.

	Hemp variety				
Fatty acid	Helena	Marina	Novosadska	Fedora 17	
Palmitic C16:0	7.18	6.11	6.22	7.48	
Stearic C18:0	2.69	3.03	2.80	2.69	
Oleic C18:1	10.99	12.48	12.36	14.29	
Linoleic C18:2ω6	53.55	53.21	54.09	53.22	
α-Linolenic C18:3ω3	15.58	16.48	17.16	13.41	
γ-Linolenic C18:3ω6	1.72	0.62	0.78	2.97	
Arachidic C20:0	0.84	0.83	0.76	0.87	
Eicosenic 20:1	0.38	0.39	0.37	0.46	
Behenic 22:0	0.56	0.35	0.31	0.36	
% PUFA*	75.78	75.20	75.94	72.69	
n6/n3 ratio	3.55	3.27	3.20	4.19	
total oil content - Soxhlet	32.09	31.88	29.38	21.09	

^{*} Polyunsaturated fatty acids



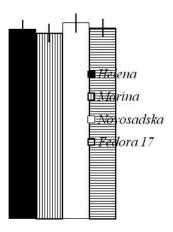


Figure 2. Protein content (%) in whole and dehulled hemp seeds

From a nutritional standpoint, numerous factors are known to influence the quality of dietary proteins, most notably the amino acid composition and the digestibility of the protein (FAO/WHO, 1990). The amino acid composition of a plant protein can be influenced by factors such as variety/genetics, agronomic conditions such as soil fertility, and post-harvest processing effects that alter the ratio of seed components (i.e., dehulling) (House *et al.*, 2010). Due to the latter, we analyzed protein content in both, whole and dehulled seeds. As it is presented in Figure 2, protein content was similar in the investigated hempseeds. The highest protein content was determined in seeds of Novosadska variety (35.38%).

The remaining seed cake or meal after the cold pressing/extrusion-based processing of hempseed has significant oil content (approx. 10%) and high (>30%) protein content (Callaway, 2004; Silversides *et al.*, 2005), and has been marketed as a source of vegetable protein. In addition to the hemp seed meal, whole hemp seed and dehulled hemp seed (hemp nuts) are found in the human food marketplace.

CONCLUSIONS

Due to high levels of PUFAs in the oil and high protein content in seeds, analyzed fiber hemp varieties are excellent source of nutrition and valuable resource for the functional food and nutraceutical industries. Analyzed varieties are rich in CBD which makes them promising source of cannabinoids for pharmaceutical industry.

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