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THE ALIMENTARY IMPACT OF THE HEMP SEED

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Hemp seed and hemp seed oil can supply us with many important substances. Their essential fatty acid compositions are favorable, but they may contain non-psychotropic cannabinoids. Emerging data show that these components can influence the civilization health status beneficially. Some data also showed trace amount of tetrahydrocannabinol in seed oils, the main psychotropic cannabinoid that is controversial.

Our aim was to examine cannabinoids and fatty acid composition as well as metal and non-metal element compositions in hemp seed oil and chopped hemp seed capsula..

The cannabinoids were separated by thin layer chromatography. Fatty acid composition was determined by gas-chromatographic and elements (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sn, Sr, V, Zn) were measured by inductively coupled plasma optical emission spectrometric method. Selenium was determined with polarograpic analyzer.

We could not detect cannabinoids by thin layer chromatography, so hemp seed oil as well as the capsula have no psychotropic adverse effect. Our data showed that hemp seed contains essential fatty acids close to the recommended ratio. The B and Se concentration of oils and the P concentration of capsula are also relevant.

Keywords: hemp seed, cannabinoids, fatty acid, metal and non-metal elements

Hemp seed oil has been used for ages, but in the last few years it became popular in Hungary. It is a rational notion because Hungary has good fiber varieties, and that may give a chance to grow proper oil varieties. These varieties have low Δ9-tetrahydro-cannabinol (Δ9-THC) levels, under 0.2%, and the oil practically doesn't contain this psychotropic material, despite the fact, that the provisional maximum tolerable daily

intake of Δ9-THC is 0.0004 mg/kg body weight (0.024 mg/day for an adult) as it was proposed by EFSA (EFSA, 2011). In the study of LEIZER et al. (2000) cannabidiol (CBD) was also present just in a trace amount, nevertheless the oil, as well as the fiber varieties contain much higher concentrations from this non-psychotropic cannabinoid, than Δ9-THC.

Hemp seed oil contains many beneficial nutrient compounds, especially polyunsaturated fatty acids. LEIZER et al. (2000) measured its fatty acid composition and found that it had 52-62% linoleic acid (ω-6 fatty acid) and 12-23% α-linolenic acid (ω-3 fatty acid). DEFERNE & PATE (1996) found 50-70% linoleic acid, and 15-25% α-linolenic acid in the hemp seed oil. These oil components are essential fatty acids, and they play a really important role in signal transduction. ω-6 fatty acids are the precursors of series 1 and 2 prostaglandins and series 4 leukotrienes. These eicosanoids are responsible for inflammatory processes and platelet aggregation (GRANSTRÖM et al. 1983, GRANSTRÖM 1984, VARGA 2008, INSEL et al. 2011).

The hemp seed oil's α-linolenic acid, and its further elongated and desaturated productions (e. g. docosahexaenoic acid and eicosapentaenoic acid), as produced in mammalian cells are compounds that are in competition for the same enzymes but they are the precursors of series 3 prostaglandins and series 5 leukotrienes. These ω-3 fatty acid derived mediators all in all have anti-inflammatory, antiaggregatory, and antithrombotic effects (INSEL et al. 2011). The ω-3 fatty acid derivates also influence on intracellular receptors, for example the peroxisome proliferator-activated receptor (PPAR) (KARÁDI 2008, VARGA 2008).

It is necessary to study the metal element compositions and concentrations in foodstuff, because some transition metal ions (e.g. Cu, Fe, Mn, Zn) and non-metal elements (e.g.

S, Se, P) have a great influence on the redox balance and signal transduction processes (SCHRAMM et al. 1986, POWIS et al. 1997, KUDRIN 2000, MILOSEVIC et al. 2000, BLÁZOVICS 2011). It is beneficial obtaining the optimal tissue concentrations by supplementation with essential trace elements from natural sources. A study showed that hemp seed may be a potential material (SZENTMIHÁLYI et al. 2002).

Objectives

The aims of our study are to show that the hemp seed oil as well as the whole ground hemp seed could be an important part of our meals. To test our hypothesis, we tested the Δ9-THC levels in three products, measured their metal ion, and some other non-metal element concentrations and determined the fatty acid composition.

1. Materials and methods

1.1. Detection of the cannabinoids

The presence of cannabinoids has been examined in the Vita Crystal chopped hemp seed capsula, Herbarium and Solio hemp seed oils from hemp (*Cannabis sativa* L.) by thin layer chromatography from one-one sample. From the chopped seed, cannabinoids were extracted by petroleum aether in ultrasonic extractor. The extract was filtered and evaporated. The cannabinoids from the hemp seed oils were extracted 3 times with chloroform-methanol (1:3) solution. Merck Silicagel 60 thin layer and hexan-dioxan (4:1) eluent-mixture was used to analyze the cannabinoid compounds. After the separation 2% Echtblausalz B reagent was applied in methanol to develop the chromatogram (PARIS & DEMESY 1971).

1.2. Measurement of the elements

Samples were digested for elemental analysis with nitric acid (5 mL 65%) and H_2O_2 solution (2 mL 30%) from the chopped seed, with sulfuric acid (10 mL 98%), nitric acid (5 mL 65%) and H_2O_2 solution (2 mL 30%) from the hemp seed oils, then the elements were measured by ICP-OES (inductively coupled plasma optical emission spectrometric) method (SZENTMIHÁLYI & THEN 2007) with Spectro Genesis ICP equipment (Kleve, Germany). For the standardization of equipment and measurements of elements (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sn, Sr, V, Zn), Spectro multielement and Spectrum 3D standards were used. Computer guided TraceLab 50 type polarographic analyzer was also used for the determination of Se from the digested samples, because OES method is not enough precise for this element (MAY et al. 2005). Three parallel measurements were made.

1.3. Analysis of the fatty acids

The fatty acid composition was determined by gas chromatographic method, in methyl ester form (FAME). We used one-one sample pro each product, that were in warranty period. Lipids were extracted with chloroform-methanol (2:1) mixture and were saponified with sodium hydroxide solution. Fatty acids were methylated by methanolic boron-trifluoride solution. FAMEs were extracted to hexane, and this extract was used for determination of fatty acid composition (AOAC 1990, FOLSCH et al. 1957).

The composition of FAME was analyzed using a Shimadzu 2010 gas chromatograph, equipped with a flame ionization detector (FID). A ZB-WAX (Zebron, Phenomenex) capillary column (30 m x 0.25 mm x 0.25 μm) was used. The carrier gas was helium at 30 mL/min flow rate; 0.2 mL hexane extract was injected. Split injection was made with 1:40 split ratio. The temperatures of the injector and detector were 220 °C and 250 °C,

respectively. The oven temperature was initially maintained at 60 °C for 2 min then programmed at 13 °C min^{-1} to 150 °C, then increased at 2 °C min^{-1} to 240 °C which was held for 20 min. The identification of peaks were made by the comparison of retention times of standard FAME mix (Supelco 37 component FAME mix) (VÁLI et al. 2007).

2. Results and discussion

Figure 1 shows that cannabidiol or cannabinoid compounds could not be detected in the samples undergoing analysis. The reagent can also show THC and other cannabinoids (PARIS & DEMESY 1971), but there weren't any sign of these compounds. It means that these products haven't got psychotropic side effects, but the beneficial effect of the cannabidiol is also not present.

Table 1 shows that the seed contains high proportion of essential fatty acids. The chopped seed capsula contained about three times more linoleic acid (ω-6 fatty acid) than α-linolenic acid (ω-3 fatty acid), but its proportion was still really high. Other fatty acids, like palmitic acid, oleic acid and stearic acid were in a lower, but relevant proportion.

In the hemp seed oils the same composition was found. The Solio hemp seed oil contained 17.12% and the Herbarium hemp seed oil contained 19.59% of α-linolenic acid. ω-6 fatty acids were mainly linoleic acid. In the Solio hemp seed oil linoleic acid was 61.23% and Herbarium hemp seed oil contained 59.81% of linoleic acid. The proportions of nonessential fatty acid components were close to the chopped hemp seed capsule. For example the proportion of oleic acid was 9.67% and palmitic acid was

6.90% in the Solio oil. Their proportion in the Herbarium hemp seed oil was 8.36% and 6.99%, respectively. The other fatty acid components were also present in traces.

It should be also notified, that the ω-6/ω-3 ratio is under 3.61. Emerging data show that our ω-6 fatty acid consumption is too high and ω-3 fatty acid consumption is too low because of using popular sunflower oil. Nowadays the recommended ω-6/ω-3 ratio is under 5, so the supplement of our diet with hemp seed oil can accomplish this requirement (Gigaud & Combes 2008, VARGA 2008). Other studies showed 70% decrease in the cardiovascular mortality under the 4/1 ω-6/ω-3 ratio (SIMOPOULOS 2002).

The composition of elements in the oils as well as in the chopped hemp seed capsule can be seen in Table 2. The concentration of Sn was under the detection limit in all cases, therefore it was omitted from the table. In the oils, the concentrations of Cd, Co, Li, Pb and V in the oils were also under the detection limits (under the LOD).

In the chopped hemp seed capsule the element contents were similar as in the general plant samples expect P, the concentration of which was higher (9624 μg/g) than an average concentration in plants (3000-4000 μg/g). Toxic Cd and Pb were detectable, but under the limited concentration, because a capsula is 0,5 g and the maximum dose is 2 capsules (COMMISSION REGULATION 629/2008).

The concentrations of Al and Sr were detectable. B concentration was high in the oils, especially in Herbarium hemp seed oil. Solio hemp seed oil contains 53.49 μg/g, while Herbarium oil contains 157.4 μg/g B, which is almost 3 times higher concentration than in the other oil measured. It should mention the about 2 μg/g Se concentration in Solio hemp seed oil as it is relevant. It seems that B as well as Se are accumulated in the oils compared to the hemp seed.

Sulfur could not be measured neither in Solio, nor in Herbarium hemp seed oil since the digestion was made by sulfuric acid. In the oils, P was present in a lower concentration, than in the ground hemp seed, as well as Si.

The hemp seed, as every crop, accumulates beneficial and toxic elements. Its oil also could be an important source of essential compounds.

From the metal ion assay it was visible, that the concentrations of B and Se reach high level in the hemp seed oils. The daily Dietary Reference Intake of Se is more than 55 μg for a man (DRI 2002, VENTURA et al. 2009) and the daily Dietary Reference Intake of B is about 1-3 mg, and they are enough for a healthy life. . For the easier way to calculate, 2 mg B was considered for the daily intake (DRI 2002, MEACHAM et al. 2010). A spoonful of oil, that advised mostly for a portion of salad is about 6 g. It means that only a dish of salad with Solio hemp seed oil can fulfill about the 20% of the daily Se intake and 16% of daily B intake. This salad, with Herbarium hemp seed oil can fulfill approximately 23% of the daily Se intake and 47% of daily B intake.

The P concentration in the ground hemp seed is remarkable, and the capsule may help to fulfill the daily intake of this element. The chopped seed usually contained higher concentration of elements, expect from B, Mo, Se, but the toxic metal cations, like Cd and Pb were also in a higher levels.

3. Conclusion

According to the results it can be concluded that consuming hemp seed oil, or ground hemp seed is healthy and harmless, because of futile heavy metal element concentration and not detectable Δ9-THC levels. Although the CBD was also not detectable, its high

essential fatty acid, especially ω-3 fatty acid proportion can slow down the progression of civilization diseases. The oils contain significant amounts of B and Se as well as the chopped hemp seed has relevant P concentration, which are favorable in nutritional aspect and can play a role in healthy life in various ways.

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REFERENCES

AOAC (1990): Official methods for analysis. 15th. ed. Arlington, pp. 963-965.

BLÁZOVICS, A. (2011): Small molecules in cancer therapy: cytotoxics and molecularly targeted agents. *Curr. Signal. Transduct. Ther.*, *6*, 2-19.

DEFERNE, J.L., PATE, D.W. (1996): Hemp seed oil: A source of essentially fatty acids. *Journal of the International Hemp Association.*, *3,* 4-7.

EFSA (2011): Scientific opinion on the safety of hemp (Cannabis genus) for use as animal feed. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), *EFSA Journal, 9,* 1-41

FOLSCH, J., LEES, M., SLOANE-STANEY, G.H. (1957): A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem., 226,* 497-509.

COMMISSION REGULATION (EC) 629/2008. *Off. J. Eur. Comm.* L 173/6-9.

DRI (2002): Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutritional Board, Institute of Medicine. National Academies Press, Boston, pp. 772-773.

GIGAUD, V., COMBES, S. (2008): The effect of decreasing the omega 6 / omega 3 ratio in feed on fatty acid content of rabbit meat to meet human dieatry recommendations. *9th World Rabbit Congress,* Verona, 1353-1358.

GRANSTRÖM, E., DICZFALUSY, U., HAMBERG, M. (1983): The thromboxanes. -in: Pace-Asciak, C., Granström, E. (Eds) *Prostaglandins and Related Substrates.* Elsevier. Amsterdam. 45-94.

GRANSTRÖM, E. (1984): The arachidonic acid cascade. The prostaglandins, thromboxanes and leukotrienes. *Inflammation., 8,* S15-25.

INSEL, P., ROSS, D., MCMAHON, K., BERNSTEIN, M. (2011): *Nutrition.* Jones & Bartlett Learning. Sudbury. pp. 181-224.

KARÁDI, I. (2008): Metabolikus szindróma. (Metabolic syndrome.) –in: Kerpel-Fronius, S., Gyires, K., Fürst, Zs. (Eds), *Farmakoterápia, Válogatott betegségek bizonyítékon alapuló kezelésének klinikai farmakológiai elvei. (Pharmacotherapy, Clinical pharmacologic principes of evidence based treatment of assorted illnesses.)* Medicina Könyvkiadó Zrt., Budapest, pp. 182-187.

KUDRIN, A.V. (2000): Trace elements in regulation of NF-κB activity. *J. Trace Elem. Med. Biol.*, *14*, 129-42.

LEIZER, C., RIBNICKY, D., POULEV, A., DUSHENKOV, S., RASKIN, I. (2000): The composition of hemp seed oil and its potential as an important source of nutrition. *Journal of Nutraceuticals*. *Functional & Medical Foods.*, *2*, 35-53.

MAY, Z., TABA, G., BLÁZOVICS, A., SZÉKELY, E., BÍRÓ, E., FÉBEL, H., SZENTMIHÁLYI, K. (2005): The application of stripping technique for the determination of selenium content in various samples with voltammetric method. –in: Majdik K. (Ed) *XI. International Chemical Conference, Proceedings.* Erdélyi Magyar Tudományos Társaság, INCITATO Nyomda, pp. 354-356.

MEACHAM, S., KARAKAS, S., WALLACE, A., ALTUM, F. (2010): Boron in human: Evidence for dietary recommendation and public policies. *Open Miner. Process. J.*, *3*, 36-53.

MILOSEVIC, N., MAIER, P. (2000): Lead stimulates intercellular signaling between hepatocytes and Kupffer cells. *Eur. J. Pharmacol.*, *401*, 317-328.

PARIS, M., DEMESY, D. (1971): Nouvelles méthod d'identification et d'évaluation de l'activité du Cannabis. (New methods for the evaluation and identification of Cannabis activity.) *Plant. Med. Phytother., 5,* 28-38.

POWIS, G., GASDANSKA, J.R., BAKER, A. (1997) Redox signaling and the control of cell growth and death. *Adv. Pharmacol.*, *38*, 329-58.

SCHRAMM, V.L., WEDLER, F.C. (1986): *Manganese in metabolism and enzyme function.* Academic Press, New York, pp. 437.

SIMOPOULOS, A.P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.*, *56,* 365-379.

SZENTMIHÁLYI, K., BENKŐ, A., THEN, M. (2002): Magyarországi vadkender (Cannabis sativa var. spontanea) és mikrohullámú kivonatainak esszenciális és toxikus fémelem-tartalma. (Essential and toxic metal element content of the Hungarian feral hemp (Cannabis sativa var. spontanea) and its microwave extracts.) *Olaj, Szappan, Kozmetika*, *51*, 143-147.

SZENTMIHÁLYI K., THEN M. (2007): Examination of microelements in medicinal plants of the Carpathian Basin. *Acta Alimentaria*, *36*, 231-236

VÁLI, L., STEFANOVITS-BÁNYAI, E., SZENTMIHÁLYI, K., FÉBEL, H., SÁRDI, É., LUGASI, A., KOCSIS, I., BLÁZOVICS, A. (2007): Liver-protecting effect of table beet (Beta vulgaris var. rubra) during ischaemia-reperfusion. *Nutrition, 23,* 172-181.

VARGA, ZS. (2008): Az omega-3 többszörösen telítetlen zsírsavak az atherosclerosis megelőzésében. (Cardioprotective role of omega-3 polyunsaturated fatty acids.) *Orv. Hetil. 14*, 627-637.

VENTURA, M.G., FREITAS, M.C., PACHECO, M.G. (2009): Determination of selenium daily intakes in two small groups of the Portuguese population by replicate sample neutron activation analysis. *J. Radioanal. Nucl. Chem., 281,* 193-196.

Figure 1.: The thin layer chromatogram of the cannabinoid compounds (DITRÓI, K)

1. Hemp seed capsule
2. Solio oil
3. Herbarium oil
4. CBD standard
5. CBN standard

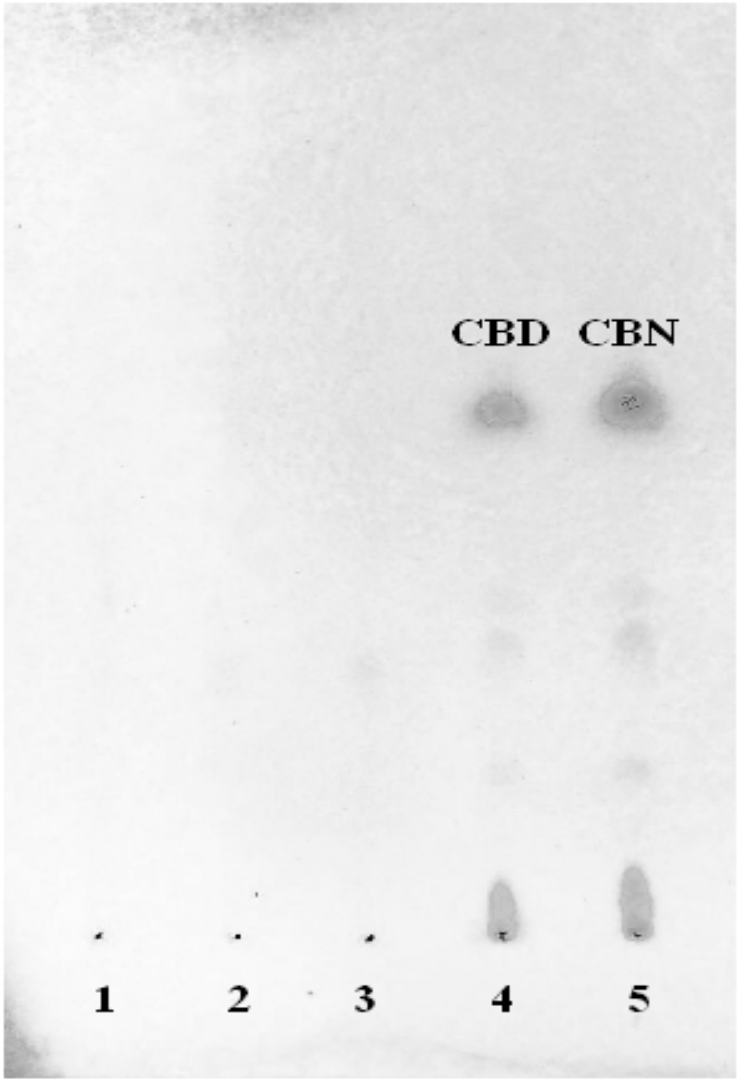

Table 1.: Fatty acid composition of the chopped hemp seed and the hemp seed oils

(weight% of total fatty acids) (< dl: under detection limit)

	Chopped seed capsula	Solio hemp seed oil	Herbarium hemp seed oil
C14:0 (myristic acid)	0.11	0.03	0.04
C15:0 (pentadecanoic acid)	0.08	0.025	0.02
C16:0 (palmitic acid)	7.49	6.90	6.95
C16:1 (palmitoleic acid	0.17	0.11	0.12
C17:0 (heptanoic acid)	0.17	0.07	0.08
C18:0 (stearic acid)	2.96	2.98	3.01
C18:1ω-9c (oleic acid)	7.62	9.67	8.36
C18:2ω-6c (linoleic acid)	59.03	61.23	59.81
C18:3ω-6 (γ-linolenic acid)	1.22	0.49	0.56
C18:3ω-3 (α-linolenic acid)	19.45	17.12	19.59
C20:0 (arachidic acid)	0.80	0.68	0.74
C20:1ω-9 (eicosenoic acid)	0.42	0.36	0.36
C20:2ω-6 (eicosadienoic acid)	0.14	0.07	0.08
C20:3ω-3 (eicosatrienoic acid)	< dl	0.01	0.01
C22:0 (behenic acid)	0.35	0.27	0.28
Saturated fatty acids	11.95	10.94	11.12
Unsaturated fatty acids	88.05	89.06	88.88
ω-6 fatty acids	60.39	61.79	60.45
ω-3 fatty acids	19.45	17.13	19.60
ω-6/ω-3 ratio	3.11	3.61	3.08

Table 2.: The element concentration (mean ± standard deviation) in the hemp seed capsula and oils. (< dl: under detection limit)

Elements	Vita Crystal hemp seed capsula (µg/g)	Solio hemp seed oil (µg/g)	Herbarium hemp seed oil (µg/g)
Al	208.8 ± 7.0	11.44 ± 1.15	11.17 ± 0.36
B	40.42 ± 5.63	53.49 ± 1.73	157.4 ± 4.8
Ba	8.05 ± 0.42	2.30 ± 0.07	2.40 ± 0.09
Ca	1364 ± 121	23.65 ± 0.54	18.84 ± 0.98
Cd	0.045 ± 0.013	< dl	< dl
Co	0.224 ± 0.012	< dl	< dl
Cr	0.875 ± 0.169	0.311 ± 0.010	0.312 ± 0.058
Cu	16.32 ± 0.35	0.283 ± 0.101	1.215 ± 0.158
Fe	284.5 ± 9.3	6.00 ± 0.10	8.36 ± 0.20
K	2298 ± 121	28.03 ± 1.46	20.15 ± 2.54
Li	1.47 ± 0.06	< dl	< dl
Mg	4792 ± 221	4.45 ± 0.55	5.46 ± 0.48
Mn	109.0 ± 5.1	0.485 ± 0.098	0.513 ± 0.060
Mo	0.260 ± 0.021	0.174 ± 0.006	0.359 ± 0.148
Na	114.9 ± 4.0	38.25 ± 5.70	33.66 ± 5.97
Ni	1.42 ± 0.16	0.382 ± 0.005	0.502 ± 0.016
P	9628 ± 89	43.18 ± 4.81	83.02 ± 0.87
Pb	1.55 ± 1.17	< dl	< dl
S	2530 ± 177	digestion: with sulphuric acid	
Se	0.463 ± 0.034	1.84 ± 0.31	2.08 ± 0.17
Si	120.2 ± 0.2	13.22 ± 0.13	21.29 ± 0.17
Sr	29.40 ± 6.30	0.200 ± 0.014	0.107 ± 0,001
V	0.152 ± 0.024	< dl	< dl
Zn	74.87 ± 4.63	1.84 ± 0.07	2.20 ± 0.66