



Analytical Methods

Relationship between cannabinoids content and composition of fatty acids in hempseed oils

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ABSTRACT

Hempseed oils acquired on the Croatian markets were characterised by cannabinoid content and fatty acid composition. The new method for determination of cannabinoid content was developed and validated in the range of 0.05–60 mg/kg, and the content of tetrahydrocannabinol varied between 3.23 and 69.5 mg/kg. Large differences among the samples were obtained for phenotype ratio suggesting that not all of analysed hempseed oils were produced from industrial hemp. Sample clustering based on cannabinoid content assigned samples to two groups closely related to the phenotype ratios obtained. The results of this study confirm that hempseed oil is a good source of polyunsaturated fatty acids, especially γ -linolenic and stearidonic acid, but the content varies a lot more than the omega-6/omega-3 ratio. The grouping of samples on fatty acid content assigned samples to two groups which were consistent with the groups obtained based on cannabinoid content clustering.

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1. Introduction

The *Cannabis* genera are well known for the psychoactive compound Δ^9 -tetrahydrocannabinol (THC). In contrast to *Cannabis sativa* subsp. *indica*, the dried leaves of which contain up to 20% THC, industrial hemp (*Cannabis sativa* subsp. *sativa*) is characterised by a low content of THC (United Nations Office for Drugs and Crime (UNODC), 2009). In most European countries, the current upper legal limit for cultivation of hemp for fibre and seeds production is 0.2% THC on dry basis (Official Journal of European Union, 2008). The ratio of THC and cannabidiol (CBD) (THC + CBN/CBD, phenotype ratio) is also used as a criterion; ratios less than 1 indicate fibre-type hemp while a ratio greater than 1 indicate a drug-type hemp (UNODC, 2009). Croatian regulations on hemp cultivation for the production of food and feed (Croatian Ministry of Agriculture, 2012) prescribe conditions for the cultivation of hemp and lists approved cultivars, which correspond to legally acceptable levels of THC (0.2%).

Hempseed oil, the most common food product using hemp, is produced from cold pressing of hempseeds. Its production has been growing in recent years due to its nutritional value. It contains up to 80% polyunsaturated fatty acids (PUFAs) (Da Porto, Decorti, & Tubaro, 2012; Matthaus & Bruhl, 2008; Oomah, Busson, Godfrey, & Drover, 2002; Teh & Birch, 2013) with an

omega-6/omega-3 ratio of 3:1, which is in good agreement with European Food Safety Agency recommendations (3–5:1, EFSA, 2009) for intake. Moreover, hempseed oil contains γ -linolenic (GLA, C18:3n-6) and stearidonic acid (SDA, C18:4n-3), which can only be found in a few plant families. These fatty acids are desaturation products of linoleic (LA) and α -linolenic acid (ALA) and are not contained in most of the oils used in human diet. Several studies reported putative health benefits associated with consumption of these fatty acids in cardiovascular diseases, rheumatoid arthritis and types of dermatitis (Callaway et al., 2005; Chow, 2008; Oomah et al., 2002). Hempseed oil contains tocopherols and high-quality proteins, which also have beneficial effects on human health (Kriese, Schumann, Weber, Beyer, Bruhl, & Matthaus, 2004; Callaway, 2004; Chen et al., 2010).

The highest concentration of THC and other cannabinoids in hemp is found in the seed coat. Because the majority of THC is located on the surface of the seed, the oil can absorb a certain amount during cold pressing (Ross, Mehmedic, Murphy, & ElSohly, 2000; Zoller, Rhy, & Zimmerli, 2000; UNODC, 2009). The absorbed amount depends on the variety of hemp, cultivation conditions and the extent of contamination with parts of the plant rich in cannabinoids during processing (Lachenmeier & Walch, 2005; Matthaus & Bruhl, 2008). However, administration via food has a lower bioavailability then, for example, smoking (Huestis, 2006). Limitation of the content in various foods produced from hempseeds is calculated based on the No-Observed Effect Level (NOEL) for THC and on the assumed daily intake of a specific food.

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Resulting differences are due to the application of different safety factors (Kraus, 2003).

Since the production and consumption of hempseed oil has rapidly increased, the oils on the Croatian market were characterised by cannabinoid content and fatty acid composition. The second goal of this study was to increase knowledge about the relationship between fatty acid composition and cannabinoid content, which has not been described in literature thus far.

2. Materials and methods

2.1. Materials

Eleven samples of commercial-grade hempseed oil were purchased at natural food stores or donated by manufacturers, between October 2012 and January 2013, and represented all

hempseed oils available on Croatian market, except via internet sales. Table 1 summarizes characteristics of the samples. Samples were kept at room temperature before analyses and sample codes were assigned to them in accordance with the order of acquisition.

2.2. Reagents and standards

Isooctane, methanol, ethanol and acetonitrile gradient grade were purchased from J.T. Backer (Devanter, The Netherlands). Tribenzylamine, p.a., was purchased from Merck (Damstadt, Germany) while potassium hydroxide, p.a., and sodium hydrogen sulphate monohydrate, p.a., were purchased from Kemika (Zagreb, Croatia). Analytical standards were: Δ^9 -tetrahydrocannabinol, cannabidiol and cannabinol (all 1 mg/mL, Lipomed AG, Arlesheim, Switzerland), Food Industry FAME, 37 components in methylene chloride (Restek, Bellefonte, PA, USA), cis-vaccenic acid methyl ester

Table 1

The origin of the analysed hempseed oil samples.

Sample label	Supplier	Manufacturer	Hempseeds origin
SMP1	Matičnjak d.o.o., Lug, Croatia	Matičnjak d.o.o., Lug, Croatia	Croatia
SMP2	Gorička ves d.o.o. Šalovci, Slovenia	Gorička ves d.o.o. Šalovci, Slovenia	Slovenia
SMP3	Harmonija narave Maribor, Slovenia	Hanf & Natur, Lindlar, Germany	Germany
SMP4	Ecorural Net, Osijek, Croatia	Hemp Oil Canada Inc., Ste. Agathe, Manitoba, Canada	Canada
SMP5	Biovega d.o.o., Zagreb, Croatia	Myristica d.o.o., Zagreb, Croatia	Romania
SMP6	Harmonija prirode d.o.o., Zagreb, Croatia	Hemp Oil Canada Inc., Ste. Agathe, Manitoba, Canada	Canada
SMP7	Uvita d.o.o., Debeljača, Serbia	Uvita d.o.o., Debeljača, Serbia	Serbia
SMP8	Herbio Plus d.o.o., Velika Gorica, Croatia	Herbio Plus d.o.o., Velika Gorica, Croatia	The Netherlands
SMP9	Herbio Plus d.o.o., Velika Gorica, Croatia	Herbio Plus d.o.o., Velika Gorica, Croatia	Croatia
SMP10	Herbarom d.o.o., Zagreb, Croatia	Suncokret d.o.o., Hajdukovo, Serbia	Unknown
SMP11	Vital.med. d.o.o., Rijeka, Croatia	Tara, Maribor, Slovenia	Slovenia

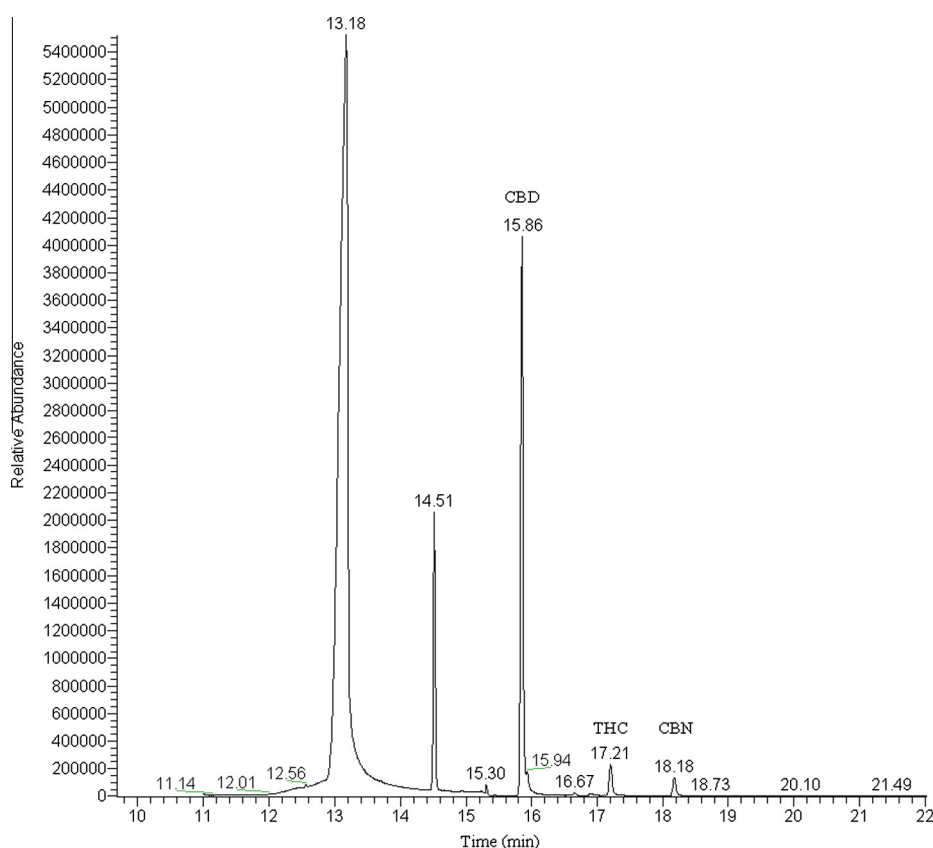


Fig. 1. Chromatogram obtained after injecting sample solution for determination of cannabinoids. CBD, cannabidiol; THC, Δ^9 -tetrahydrocannabinol; CBN, cannabinol.

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