Physico-chemical characteristics of cannabis seed oil (*Cannabis sativa L.)* from different varieties grown in the conditions of the Syrdarya region

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Abstract. The article considers the physico-chemical composition of cold pressed hemp oil from different varieties to ensure food safety and choose the most promising variety for cultivation in the conditions of Syrdarya region. The content of cold pressing oil in technical cannabis seeds (Cannabis sativa L) from three different varieties of Felina 32, Santica 27 and Rodnik, grown in the Syrdarya region, ranged from 29.1% to 31.70%. The protein, ash and moisture content was 23.44-26.95, 4.95-6.10 and 7.66-8.10 per cent, respectively. Some other physico-chemical results were: moisture 0.10-0.13, iodine number 148-160; density (25°C) 0.9150-0.9250 mg/ml ; acid value 1.22-1.76 mg KOH/g; peroxide value 5.5-7.8 meq O2/kg. It has been found that the investigated fatty acids hemp oils contain Linoleic acid(C18:2((cis-9,12) 54.15-59.48%, palmitic acid(C16:0), Palmitoleic acid ((C16:1(cis-10), stearic acid (C18:0), Acid (Behc8:0), Acid (C20:20:0), Archicic acid (C1:20:0:0:0), Acid (C8:0:0:0:0:0:0:0:0:0:0:0:8:8:8:0) (C22:1(cis-22:1(cis-13)) и Lignoceric acid (C24:0): 5.85-7.82, 0.10-0.18, 2.78-3.25, 11.80-12.65, 0.55-0.86, 0.20-0.32, 0.02-0.09, и 0.085-0.15 %, respectively. Flavonoids, rutins, quercetin and gallic acid were found to be 0.1-0.8, 0.5-0.12 and 1.2-2.4 mg/l. The following elements of calcium(Cd), iron(Fe), magnesium(Mg), sodium(Na), zinc(Zn), cadmium(Cd), and mercury(Hg) have been identified as 1.1-1.8, 0.4-0.9, 0.3-0.7, 0.08-0.15, 0.06-0.11 mg/100g. The results of this analytical study compared to the results found in the literature on hemp oils showed that technical cannabis (Cannabis sativa L.) for Uzbekistan is a potentially valuable non-traditional oil crop.

1 Introduction

Industrial hemp, like hops, is part of the Cannabis family (Cannabaceae). Industrial cannabis, like marijuana, originates from the same family, Cannabis sativa L. (NPGS, 2018). However, there are many different varieties of the same species that contain approximately delta-9-tetrahydrocannabinol (THC), which is a psychotropic component of

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this species. According to the Minister of Justice of Canada (2017), industrial cannabis is defined as: plants or plant parts of the genus Cannabis derived from certified seeds of varieties included in the approved list of plant varieties, in accordance with the Cannabis Industrial Regulations, leaves and heads of flowers, like their derivatives, do not contain more than 0.3% TNS. Seeds are mainly use to supply the food market. It can be sold row or cleaned. Also it can be use to produce oil. The initial quality of the grain and the extraction method used determine whether the oil will be edible or industrial. From the extracted seed oil leaves a solid by-product, oil cake. It can be dried and then milled to produce a plant-protein-rich, gluten-free flour for human consumption. The above-mentioned guide was published in Canada partly still prohibited to use industrial cannabis or its derivatives as a food additive for livestock [1-2]. This prohibition does not apply to pets (cat, dog, parrot, etc.) and wild animals (deer, goose, turkey, etc.). Cannabis has economic and pharmaceutical significance. In Uzbekistan, not more than (0.2%) d-9-tetrahydrocannabinol. Low-THC industrial hemp is legal for cultivation in several states, however, the global low-THC hemp market is estimated to be worth $100–$2,000 million annually.

Cannabis seeds are rich in vitamins A, C and E; minerals; and b-carotene and are said to have exceptional nutritional value [3-5]. It contains 20-25% protein, 20-30% carbohydrates, 25-35% oil, 10-15% insoluble fiber and a rich set of minerals, especially phosphorus, potassium, magnesium, sulfur and calcium, and a moderate amount of iron and zinc, The latter is an important enzyme co-factor of human fatty acid metabolism [6-7]. Cannabis seeds have long been used as a food ingredient or are ground to produce oil and flour. Like soy flour, cannabis flour contains enough protein for a vegetarian diet. In addition to its nutritional value, cannabis seeds have shown positive health benefits, including reduced cholesterol and high blood pressure. It is consumed in food and traditional medicines, and is also used as fodder for birds and fish [3, 7]. Hemp oil has a pleasant taste and has a number of advantages over other vegetable oils. It is considered to be perfectly balanced in terms of the ratio (3:1) of two indispensable for human nutrition PHP - polyunsaturated linolenic acids. In addition, due to the large amount of PHP and the presence of g-linolenic acid, hemp oil is ideal as an ingredient of light body oils and creams enriched with lipids known for their skin penetration ability [3]. Hemp oil is currently used in personal care products such as lotions, moisturizers, shampoos and lip balms. Highly unsaturated cannabis oil is used in the same way as linseed oil (for example, as a light fuel, in printer ink, as a wood preservative) but is also used as a raw material for detergents and soap [7-8]. The quality of hemp oil is currently being studied to improve the economic and environmental performance of this non-traditional crop through the innovative use of its components or by-products[9-10].

2 Materials and methods

Hemp oil extraction and moisture content. After removing impurities from industrial hemp seeds, the oil was extracted by cold pressing. Before pressing, seed samples pass through a huller, then rolling, after which the seeds are sent to the roaster after 40 minutes, the finished product is sent for pressing at this time the moisture of the product is 10-12%, and the temperature is 38-42 0C [11-12]. The official FOSFA method is used to determine the percentage of protein in the residue of the oilcake. The samples were decomposed for 10 minutes by a mixture of sulfuric acid, hydrogen peroxide, potassium sulfate and selenium dioxide as a catalyst. The next step was to weigh the finely ground sample (2.4 g) and remove the fat with a 15 ml n hexane extraction. The mount was boiled with sulfuric acid (0.25 mol/l) with the subsequent separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.312 mol/l cNaOH=1.001), then

separated, washed and dried. The dried residue was weighed and calcified in a muffle furnace (ELF 1100/14V; Corbolite) at a temperature of 600°C, determined the mass loss (Table 1). The ash content was determined by ISO [23].

Analysis obtained of cold pressing oil. Physical chemistry of hemp oil. ISO [20] method was used to determine mass fraction and volatiles, ISO [19] acid number, ISO [21] peroxide number, ISO [21] iodine number by ISO [22] different standard methods density was determined on the plate (Table 2).

Fatty acid composition of industrial hemp oil (Cannabis sativa L.). used the IUPAC method 2.301 to produce Fatty Acid Methyl Esters and analyzed on gas chromatograph the Chromotogrof Crystal 9000 model, which has a polar capillary column CR-WAXms, coated with methylgnose (30*0.32*0.5, #6.914.) and flame ionization detectors (PID). used nitrogen as a carrier gas with a flow rate of 4.0 ml/min. We have created an analysis environment: initial furnace temperature 170°C; linear change rate, 5°C min; final temperature 225°C; nozzle temperature 230°C; detector temperature, 250°C; and temperature maintenance 2 minutes before and 10 minutes after jogging. The 1.5 μL sample was then introduced and the results were identified by comparing their relative and absolute retention time with the authentic standards purchased from Sigma Aldrich (Germany). Chromotec Analytics Version 3.0.0.2 was used for quantitative determination. the composition of the fatty acids indicated (Table 3) as a relative percentage of the total peak area [13-14].

Identification of flavonoids in technical cannabis oil (Cannabis sativa L.). The study was conducted with the help of equipping High-efficient liquid chromatography (HPLC). For the analysis we needed exactly measured one gram of oil, after the oil was reduced to an ethanol volume in a 10 ml measuring flask wrapped in foil to inhibit oxidation. We used HPLC Shimadzu 2030. The 20 μm sample was injected into the Shim-pack GIST-HP C18 150 x 4.6, 3 μm (Shimadzu, Japan.). The moving phase consisted of 0.5% of the acetic acid-acetonitrile (35:65 o.) mixture. Testing was carried out at a excitation wavelength of 280 nm and a radiation wavelength of 354 nm. The flavonoids were recorded by comparing the retention time with the pure rutin, quercetin, gallic acid standards and quantifying the percentage of the peak area of unknown substances with that of the pure standards (Sigma Aldrich Chemical Co.). The quantitative definition was taken from the external standard[15].

Statistical analysis. Three specimens of technical cannabis oil have been analyzed individually in three specimens; from each variety (Felina 32, Santica 27 and Rodnik) the data are presented as average values of standard deviation (n = 3 3) [16].

The elemental composition of technical cannabis oil (Cannabis sativa L.) In order to dry the moisture contained in the sample for analysis, it was first dried in a drying cabinet (VWR DRY-line, Germany) until its mass did not change. 200 mg of the fully dried sample is taken out on an analytical balance (FA220 4N) for mineralization, i.e. to make it a clear solution. A mineralization device (MILESTONE Ethos Easy, Italy) was used to convert the sample into a mineral state. For this purpose, Sample (200 mg), 6 ml of nitric acid (HNO_3) purified on the basis of distillation, i.e., acid distilled on an infrared acid purification device (Distillacid BSB-939-IR) and 2 ml of hydrogen peroxide (H_2O_2) as an oxidizer are placed in the test tube of the device. . 20 min. during 1800C, all the mixture is mineralized. After the mineralization process is completed, the mixture in the test tube is diluted with distilled water (BIOSAN, Latvia) to 25 ml in a separate conical volumetric flask. The solution in the flask is placed in special test tubes in the AutoSampling Department for analysis. The prepared sample was analyzed in an Avio200 ICP-OES Inductively Coupled Plasma Optical Emission Spectrometer (Perkin Elmer, USA). The accuracy level of the device is high [17,18], it allows measuring the elements in the solution to an accuracy of 10-9 g (Table 5).

3 Results and Discussion

The analysis of the data of oilseeds of cannabis (C. sativa) and extracted oils from three different varieties of Felina 32, Santica 27 and Rodnik grown in the conditions of the Syrdarya region are summarized in tables 1-5. The values for the analysis are given a standard deviation as the average for three samples of cannabis seeds from each three varieties, all 3 specimens were analyzed individually. The cold pressing oil content ranged from 29.1% to 31.70% (Table 1). The oil concentration was highest (31.70%) in Santica 27 seed samples compared to other seed varieties derived from cannabis plants grown in the Syrdarya region. the lowest oil content (29.1%) in Rodnik seed samples [17-18]. The decrease in the oil content of the seeds could be influenced by seasonal variations in air temperature as well as by the physical and chemical composition of the soil, which is characteristic of the Syrdarya region. Average oil content (30.44%) in hemp seeds from different varieties grown on the terms of the Syrdarya region does not correspond to the indicator reported by the manufacturer. However, the oil content of cannabis seeds grown in the Syrdarya region was significantly lower compared to other different regions of Russia (30-34%). This is explained by the fact that the physical and chemical composition of the soil, climatic conditions of cultivation of technical cannabis, much better suited to the country of Russia. Analysis shows that Pancake seed residues contain many proteins. Protein, ash and moisture content ranged from 23.44-26.95%, 4.95-6.10% and 7.66-8.10% respectively [24-25].

Table 1. Analysis of industrial hemp seeds (*Cannabis sativa L.*)*.

Constituent, %	Sample 1	Sample 2	Sample 3
Oil	30.52 ± 0.48	31.70 ± 0.32	29.1 ± 0.51
Protein	24.12 ± 0.33	23.44 ± 0.45	26.95 ± 0.32
Ash content	4.95 ± 0.62	6.10 ± 0.51	5.82 ± 0.72
Moisture	7.88 ± 0.27	7.66 ± 0.42	8.10 ± 0.62

*The values represent the average standard deviation, the calculation of the percentage of dry weight of seeds for 3 samples of cannabis seed (Cannabis sativa L.). Specimen 1 (Felina 32), specimen 2 (Santica 27), specimen 3 (Spring). All varieties grown in Syr Daryn region.

Edestin and albumin are mainly found in cannabis protein which is easily assimilated by the components of human plasma [26]. which allows for protein absorption is the thermal treatment of whole cannabis seeds that denatures protein. by-product Crushed hemp seed is suitable for animal feed additive and as the main food product for humans due to the range of amino acids (which is necessary for human life) as well as carbohydrate [27]. Analyses prove that the flour by-product is a good source of protein that can be consumed in human and animal diets as a source of nutrients and calories. It can also be used as fertilizer and potential animal feed.

Table 2. Physicochemical properties of industrial hemp oil (*Cannabis sativa L.*)*.

Constituent	Sample 1	Sample 2	Sample 3
Moisture (%)	0.10 ± 0.002	0.10 ± 0.002	0.13 ± 0.003
Acid value (mg KOH/g)	1.22 ± 0.004	1.32 ± 0.003	1.76 ± 0.003
Peroxide value (meq O_2/kg)	5.50 ± 0.004	6.20 ± 0.003	7.80 ± 0.004
Iodine value (g of I/100g of oil)	148 ± 1.32	155 ± 1.10	160 ± 1.42
Density (25 ^{0}C, mg ml $^{-1}$)	0.9150 ± 0.002	0.9250 ± 0.002	0.9210 ± 0.002

*The table represents average values of standard deviation for three grades of oils, technical hemp (Cannabis sativa L.), analysed individually in three samples. For abbreviations, see Table. 1.

The various physico-chemical values of hemp oil are given in Table 2. The values of moisture (0.1-0.13%), acid value (1.22-1.76 mg KOH/g), peroxide value (5.5-7.80 meq

O_2/kg), iodine of value (148-160 g I/ of 100 g), density value with 25 °C. (0.9150-0.9250, mg ml^{-1}), hemp oil, from different varieties grown on the conditions of Syrdarya region, it is not possible to compare with the theoretical data, because these parameters have not been previously analyzed cannabis. However, the iodine content of cannabis oil was higher than that of cotton (99-119 g iodine/100 g of oil), soy (120-143 g iodine/100 g of oil) and sunflower oil (110 g iodine/100 g of oil). - 143 g of iodine/100 g of oil), but lower than flaxseed oil (155-205 g of iodine/100 g of oil) [28].

Table 3. Fatty acid composition (g/100 g fatty acids) of industrial hemp seed oil (*Cannabis sativa L.*)*.

Constituent %	Sample 1	Sample 2	Sample 3
Linoleic acid(C18:2((cis-9,12)	59.48 ± 1.00	54.15 ± 1.10	57.66 ± 1.12
palmitic acid(C16:0)	5.85 ± 0.05	7.82 ± 0.04	6.88 ± 0.05
Palmitoleic acid ((C16:1(Cis-10))	0.10 ± 0.01	0.18 ± 0.02	0.14 ± 0.02
stearic acid (C18:0)	2.78 ± 0.02	2.44 ± 0.03	3.25 ± 0.07
Oleic acid (C18:1(cis-9))	11.80 ± 0.03	12.65 ± 0.03	12.20 ± 0.04
Archidic acid (C20:0)	0.55 ± 0.02	0.67 ± 0.03	0.86 ± 0.02
Behanic acid (C22:0)	0.20 ± 0.02	0.32 ± 0.02	0.28 ± 0.01
Erucic acid (C22:1(cis-22:1(cis-13))	0.02 ± 0.001	0.05 ± 0.002	0.09 ± 0.001
Lignoceric acid (C24:0)	0.085 ± 0.001	0.10 ± 0.002	0.15 ± 0.002

*The table represents average values of standard deviation for three grades of oils, technical hemp (Cannabis sativa L.), analysed individually in three samples. For abbreviations, see Table. 1.

Previously there were no studies on unrefined cannabis oils with which to compare the results of the induction period in our present work grown in the conditions of Uzbekistan. Hemp oil, due to its high PHP content, is rather unstable and quickly burns out if not stored under optimal conditions. Table 3 shows the composition of fatty acids of cannabis oils grown in the Syrdarya region. The content of stearic (C18:0) and palmitic (C16:0) acids in hemp oil ranged from 2.44 to 3.25 and 5.85 to 7.82%, respectively. The oils were found to contain a high unsaturated level (85.12-90.47%). Linoleic acid(C18:2(cis-9,12) ranged from 54.15 to 59.48%, followed by Palmitoleic acid ((C16:1(Cis-10)), Oleic acid (C18:1(cis-9)), Archidic acid (C20:0), Behanic acid (C22:0)), Erucic acid (C22:1(cis-22:1(cis-13)) and Lignoceric acid (C24:0) with ranges 0.10-0.18, 11.80-12.65, 0.55-0.86, 0.20-0.32, 0.02-0.09 and 0.085-0.15%, respectively. Based on the literature , that cannabis oil contains many fatty acids, among which linoleic (18:2) and linolenic (18:3) acids predominate [3, 7, 27, 28]. Rumyantseva and Lemeshev [29] reported that 18:2 and content 18:3 are usually about 50-70% and 15-25% of total fatty acids in hemp oil, respectively.

With the exception of one class from Jamaica, all cannabis varieties contain different amounts of γ-linolenic acid. The occurrence and distribution of γ-linolenic acid in the plant kingdom may have chemotaxy-sonic significance in some families. γ-linolenic acid is highly valued and is of considerable interest due to its dietary and therapeutic properties. γ-linolenic acid is one of the important fatty acids used both as a health nutrient and as a therapeutic agent, and only recently its potential physiological benefits have been extensively researched [4, 29]. The study showed that the composition of fatty acids of cannabis oil grown in the conditions of the Syrdarya region falls into the category of high linoleic and α-linolenic acid and contains a ratio of 18:2n-6 to 18:3n-3 3 3:15:1,0, which is very close to the recommended food ratio of 3:1; thus, it is a potato crop that could be an acceptable substitute for oils with high content of linoleic acid, such as soy, sunflower, maize and cotton oil, as edible fats. Our current research has shown that cannabis oil from the Syrdarya region can be successfully used as a valuable source of nutraceutical NLC (18:2n-6, 18:3n-3). It can also be used in the preparation of various foods due to its

nutritional and therapeutic properties, and can also be used with other high oleinic vegetable oils for the preparation of nutritionally balanced mixtures of oils [15, 17].

Table 4. Comparison of flavonoids in industrial hemp (*Cannabis sativa L.*) seed oil*.

Constituent (mg/l)	Sample 1	Sample 2	Sample 3
Rutin	0.10 ± 0.01	0.80 ± 0.02	0.56 ± 0.02
quercetin	0.5 ± 0.01	0.10 ± 0.02	0.12 ± 0.03
gallic acid	1.20 ± 0.02	2.40 ± 0.03	1.88 ± 0.03

*The table represents average values of standard deviation for three grades of oils, technical hemp (Cannabis sativa L.), analysed individually in three samples. For abbreviations, see Table 1.

Table 4 shows the standard deviation of flavonoids and refined hemp oils determined by HPLC. Mean levels of rutin, quercetin and gallic acid in unrefined oils from different regions ranged from 0.10-0.80, 0.1-0.5 and 1.20-2.40 mg/l, respectively.

Table 5. Elemental composition in industrial hemp seed oil (Cannabis sativa L.)*.

Constituent (mg//100g)	Sample 1	Sample 2	Sample 3
Calsuim(Ca)	1.10 ± 0.02	1.25 ± 0.03	1.80 ± 0.02
Iron (Fe)	0.40 ± 0.001	0.90 ± 0.002	0.60 ± 0.001
Magnesium(Mg)	0.30 ± 0.001	0.57 ± 0.002	0.70 ± 0.002
Cadmium(Cd)	-	-	-
Sodium(Na)	0.08 ± 0.001	0.15 ± 0.004	0.10 ± 0.003
Zinc(Zn)	0.06 ± 0.001	0.11 ± 0.002	0.09 ± 0.001
Mercury(Hg)	-	-	-

*The table represents average values of standard deviation for three grades of oils, technical hemp (Cannabis sativa L.), analysed individually in three samples. For abbreviations, see Table. 1.

Table 5 shows the value of different macro-trace elements in unrefined cannabis oils as determined by IPP-OES. Average levels of Calsuim(Ca), Iron(Fe), Magnesium(Mg), Sodium(Na) and Zinc(Zn) in the unfinished oils of different grades ranged from 1.10-1.80, 0.4-0.9, 0.30-0.70, 0.08-0.15 and 0.06-0/11. No Cadmium(Cd) or Mercury(Hg) were found in these oils.

4 Conclusion

After a detailed analysis of the physico-chemical composition of cold pressing oil 3 strains of hemp seeds grown in the conditions of the Syrdarya region, found many useful fatty acids and other components as microelements and flavonoids. Cannabis oil is almost entirely fatty acids, about 70% of the major fatty acids as shown in Table 3. These results are described in the literature and confirmed in this study. The value of cold pressed hemp oil is just beginning to be recognized in the world market. It's ideal composition of omega 3 fatty acids, omega 6 is just one of several potential beneficial qualities listed above. Cold Pressed Hemp Oil is a full complete food product that also demonstrates many active pharmacological properties it will undoubtedly be attractive for many potential global markets and consumers.

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