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Nutritional quality of hemp seeds (*Cannabis sativa* L.) in different environments

Nutričná kvalita semena konopy siatej (*Cannabis sativa* L.) pestovanej v rôznom prostredí

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ABSTRACT

Hemp seed (*Cannabis sativa* L.) is rich in many substances beneficial in human nutrition, especially proteins, lipids, and total dietary fibre. Contents of these primary metabolites were analysed in seeds of hemp cultivar Finola grown at three locations in Slovakia (Borovce, Vígľaš-Pstruša, Milhostov) within two years (2013, 2014). The average content of total dietary fibre was 36.10 ± 1.92%, lipids 32.05 ± 0.42%, and proteins 24.66 ± 0.55%. The main fatty acids in oil were linoleic, α-linolenic, and oleic acids. About 75% of all fatty acids were polyunsaturated ones. The content of lipids and fatty acids such as γ-linolenic, stearidonic, linoleic, α-linolenic, oleic, *cis*-vaccenic, stearic, and gadoleic acids, as well as total dietary fibre were significantly affected (P≤0.05) by the year of cultivation. Content of proteins, gadoleic and arachidonic acids were significantly (P≤0.05) influenced by maturity of seeds. The growing location significantly influenced the content of lipids, total dietary fibre, proteins, fatty acids, and the ratio saturated:polyunsaturated fatty acids.

Keywords: hemp, seed quality, environment, proteins, dietary fibre, lipids, fatty acids

ABSTRAKT

Semeno konopy siatej (*Cannabis sativa* L.) je bohaté na mnohé látky prospešné pre ľudskú výživu, zvlášť bielkoviny, lipidy a celkovú potravinovú vlákninu. Obsah týchto primárnych metabolitov bol analyzovaný v semenách odrody Finola, ktorá bola pestovaná v troch lokalitách Slovenska (Borovce, Vígľaš-Pstruša, Milhostov) počas dvoch rokov (2013, 2014). Priemerný obsah celkovej potravinovej vlákniny bol 36,10 ± 1,92 %, lipidov 32,05 ± 0,42 % a bielkovín 24,66 ± 0,55 %. Hlavnými mastnými kyselinami v oleji boli kyseliny linolová, α-linolénová a olejová. Približne 75 % všetkých mastných kyselín boli polynenasýtené mastné kyseliny. Obsah lipidov a mastných kyselín, ako γ-linolová, stearidónová, linolová, α-linolénová, olejová, *cis*-vakcénová, stearová a gadolejová, ale aj potravinovej vlákniny bol štatisticky významne (P≤0,05) ovplyvnený stupňom zrelosti semien. Obsahy bielkovín, kyseliny gadoleovej a kyseliny arachidonovej boli významne (P≤0.05) ovplyvnené stupňom zrelosti semien. Pestovateľská lokalita bola významným zdrojom variability v obsahu

lipidov, celkovej potravinovej vlákniny, bielkovín, mastných kyselín a pomeru nasýtené:polynenasýtené mastné kyseliny.

Kľúčové slová: konopa siata, kvalita semena, prostredie, bielkoviny, potravinová vláknina, lipidy, mastné kyseliny

INTRODUCTION

Hemp (*Cannabis sativa* L.) is one of the oldest plants grown for fibres and edible seeds (Callaway, 2004a; 2004b; Schluttenhofer and Yuan, 2017; Tererycz et al., 2021). It is used also in pharmacy due to content of psychoactive substances (Pollastro et al., 2018; Pavlovic et al., 2019). More than eighty biologically active substances (Pollastro et al., 2018) have a great potential (Rupasinghe et al., 2020) and their applications are increasing worldwide (Schluttenhofer and Yuan, 2017). Hemp is used also for innovative industrial applications including production of new biomaterials and biofuels (Amaducci et al., 2015; Bonini et al., 2018; Petit et al., 2020). Most often, hemp is a dioecious plant, but in some areas the monoecious individuals may occur. However, monoecious plants are more agriculturally valuable (Faux et al., 2013).

Hemp seeds are rich in many substances advantageous for human nutrition (Rupasinghe et al., 2020). Hemp flour can be used for production of functional foods (Teterycz et al., 2021). Due to relatively high content of lipids (approximately 25-35%, w/w) (Callaway, 2004a) the hemp is considered also as the oil crop (Deferne and Pate, 1996; Pate, 1999). Water content in mature seeds is approximately 8% (Oomah et. al., 2002), 20-30% of the dry matter represent saccharides, and 10-15% insoluble fibre (Deferne and Pate, 1996; Pate, 1999). Moreover, hemp seeds contain 20-25% of proteins (Tang et al., 2006) including all essential amino acids (Pate, 1999). Seeds are rich also in iron, calcium, magnesium, phosphorus, potassium, sodium, zinc, copper, selenium, and manganese (Callaway, 2004b). They are also a source of carotene (Deferne and Pate, 1996) with a content of 2-5.3 mg/100 g (Oomah et al., 2002).

The content of beneficial fatty acids in hemp oil is relatively stable (Prociuk et al., 2008; Kaul et al., 2008). Up to 80% of the total fatty acids are polyunsaturated fatty acids (PUFAs), the linoleic acid and α-linolenic acid, both with preventive health effects in reducing cancer and coronary heart diseases (Oomah et al., 2002), anti-inflammatory and antithrombotic properties, enhancing overall metabolism, and promoting fat burning (Russo and Reggiani, 2013). Other important fatty acids in hemp oil are oleic (11%), palmitic (5%), γ-linolenic (3%), and stearic acids (1-2%), respectively. The ratio of omega-3 to omega-6 fatty acids in hemp oil is 1:3 what is optimal in terms of nutritional value (Oomah et al., 2002; Matthäus and Brühl, 2008; Da Porto et al., 2012). High ratio of polyunsaturated to saturated fatty acids lowers cholesterol level in the blood serum and prevents atherosclerosis and heart diseases (Oomah et al., 2002). Tocopherols are also present in hemp seeds, mostly (about 85%) the γ-tocopherol (Leizer et al., 2000; Matthäus and Brühl, 2008; Teh and Birch, 2013), followed by α-tocopherol (Teh and Birch, 2013; Vonapartis et al., 2015).

Mature seeds contain high variations in the content of primary and secondary metabolites (lipids, proteins, fatty acids, tocopherols, phytosterols) compared to immature seeds (Mölleken et al., 2000). Climatic conditions and associated harvest time may affect the ripeness of the seeds. Agro-climatic, geographical (Mölleken et al., 2000), and agro-ecological conditions as well as the type of agricultural practices (conventional or unconventional) also influence the quality of hemp seeds. The origin of seeds affects lipids content, fatty acids composition (Ross et al., 1996; Mölleken and Theimer, 1997), as well as the quality of lipids (Leizer et al., 2000; Bağci et al., 2003; Kriese et al., 2004; Anwar et al., 2006).

Cultivar Finola is suitable for seed production used in the food industry. It is an oilseed hemp cultivar developed in Finland. Finola is the shortest and fastest auto-flowering cultivar, it is dioecious (Schluttenhofer and Yuan, 2017) with distinct male and female plants. Typically begins to flower at 25-30 days after sowing and matures in less than 100 days in most locations by producing huge buds containing low level of tetrahydrocannabinol (THC)

(<0.2%). According to Pavlovic et al. (2019) Finola is a good source of cannabidiol (2614 µg/g) and cannabidivarin (4888 µg/g), but results vary according to the growing conditions and time of harvest. The European Union (EU) Plant Variety Rights were granted for the Finola cultivar in 1999. After additional delays in Europe, Finola was eventually admitted to the EU list of hemp cultivars after a special category for oilseed hemp was created in November 2003. Before that, only fibre hemp varieties were recognized as hemp in the EU. Finola was the first industrial hemp cultivar registered as an oilseed crop (Fadel et al., 2020).

The quality parameters of hemp seeds are influenced by genetic and environmental factors, respectively. The cultivar Finola, three growing locations, field experiment with four randomly repeating blocks, and two growing seasons were the basic factors of our experiment. The aim was to determine their impact on the content of proteins, total dietary fibre, and lipids in hemp seeds.

MATERIAL AND METHODS

Plant material

Hemp (*Cannabis sativa* L.) cultivar Finola was cultivated at three locations for two years (2013 and 2014). Basic characteristics of locations: Borovce (48°58' N, 17°72' E), 179 meters above sea level (MASL), mean annual temperature 9.2 °C, mean annual precipitation 593 mm, black earth Chernozem, degraded on loess; Milhostov (48°40' N, 21°43' E), MASL 101 m, mean annual temperature 8.9 °C, mean annual precipitation 559 mm, heavy, loamy soil; Vígľaš-Pstruša (48°32' N, 19°19' E), MASL 375 m, mean annual temperature 8.0 °C, mean annual precipitation 666 mm, fluvial soil on loess clays with a high content of clay and clay minerals.

Field experiments were performed in four replicates. Size of plots was 12.5 m^2, inter-row stand distance 0.25 m, seed rate 1 million of germinating seeds per hectare. No pesticides or fertilizers were applied. The sowing date was second half of May (2013) and the first half of April (2014). Seed harvest at 75% maturity was at the end of September (2013) and in the beginning of August (2014). The full maturity of seeds in all locations was approximately 5-8 days later. Temperature and precipitations at all three locations present Table 1 (2013) and Table 2 (2014).

Samples at all locations were hand collected gradually in two terms, at 75% maturity (collection 1) and at the full (100%) maturity (collection 2). Seeds were stored in paper bags at 18 °C, humidity 68%, in dark. Seeds were milled to pass a 0.5 mm sieve and stored in plastic closed doses just before the analysis of nutritional parameters (total proteins, total dietary fibre, lipids, fatty acids composition).

Total dietary fibre determination

The total dietary fibre (TDF) content was determined using the Total dietary fibre assay procedure (Megazyme, Ireland). The procedure is based on the method of Prosky et al. (1988) and McCleary et al. (2013) and is also included in the standard AOAC 991.43. TDF was determined on duplicate samples of dried and defatted material.

Table 1. Temperature and precipitation in the year 2013

Month	Borovce		Milhostov		Vígľaš-Pstruša	
	Average air temperature [C°]	Sum of precipitation [mm]	Average ai temperature [C°]	Sum of precipitation [mm]	Average air temperature [C°]	Sum of precipitation [mm]
April	9.1	35.4	11.1	10.2	10.0	22.6
May	13.5	52.5	16.1	21.3	14.1	166.9
June	17.5	75.4	20.1	78.2	17.3	116.9
July	20.7	3.8	21.1	33.8	19.3	48.3
August	20.1	115.8	21.1	13.1	19.4	38.9
September	11.6	78.5	14.1	49.3	12.3	47.4

Table 2. Temperature and precipitation in the year 2014

Month	Borovce		Milhostov		Vígľaš-Pstruša	
	Average air temperature [C°]	Sum of precipitation [mm]	Average ai temperature [C°]	Sum of precipitation [mm]	Average air temperature [C°]	Sum of precipitation [mm]
April	9.8	64.4	12.5	48.5	9.8	44.3
May	13.1	116.2	15.0	78.2	13.0	64.3
June	18.2	38.2	19.2	18.7	16.7	58.1
July	19.8	120.1	21.5	155.0	18.8	138.7
August	16.8	51.4	19.7	96.4	15.8	123.5
September	13.5	54.6	15.9	30.0	13.0	97.7

Samples were incubated at ~100 °C with thermo-stable α-amylase, then at 60 °C with protease, amyloglucosidase, and finally treated with ethanol to precipitate fibre. The residue was filtered, washed gradually with 78% ethanol, 95% ethanol, and acetone, subsequently dried and weighed. One duplicate was analysed for proteins and the other was burned at 525 °C to determine ash. The TDF was the weight of the filtered and dried residue less the weight of the proteins and ash. The TDF was calculated on a dry-weight basis using Sartorius MA 45 (Sartorius AG, Göttingen/Germany).

Total proteins determination

Total proteins determination was performed using analyser operating by the Dumas principle. The Dumas method (AACC Method 46-30.01, AOAC 992.23, ICC Standard No. 167) based on the combustion of the nitrogen components in sample at high temperature (1100 °C), reduction of the resulting nitrogen oxides, and thermal conductometry. The nitrogen level is translated using the transformation factor as the desired protein content (McAuley and McLean, 1998). Homogenized samples in amount of about 200 mg were analysed with the TruMac CNS 2000 working unit (LECO Corporation, St. Joseph, MI). The proteins content was calculated on the dry weigh by a conversion factor of 6.25.

Lipids content and fatty acids determination

Lipid content was determinated in two replications using the Soxhlet extractor according to the technical norm STN 461011-28. Samples of milled seeds were extracted in n-hexane. The content of lipids was measured gravimetrically.

Methyl esters of fatty acids prepared according to Christopherson and Glass (1969) were analysed by gas chromatography with mass spectrometry (GC-MS) (7890B GC system, 5977A MSD, Agilent Technologies). Column temperature program was: initial temperature 150 °C (4 min.), then increased by 3 °C/min to 230 °C, maintain for 5 min., final increasing by 15 °C/min. to 280 °C, maintain for 19 min. Inlet parameters (capillary column inlet): temperature 250 °C, pressure 10.8 psi, total helium pressure 169.32 ml/min, split ratio 200:1. HP-5ms ultra inert column: dimensions 30 m x 250 µm x 0.25 µm, initial temperature 150 °C, pressure 10.8 psi, flow rate 0.82746 ml/min, and average value 34.613 cm/s. Injection volume of the sample was 1 µl.

MS parameters: MSD Transfer-line temperature 280 °C, ion source temperature 230 °C, quadrupole temperature 150 °C, electron energy 70 eV, record full mass spectra (SCAN type), gain factor 1, scanning range 50-550 m/z, and scan speed 1.562 (N=2). Fatty acid identification was performed by comparing the mass spectra of the samples with the spectra in the NIST2007 library databases. Sums of saturated (SFAs), mono- (MUFAs), and poly-(PUFAs) unsaturated fatty acids were calculated from the fatty acid values.

Statistical evaluation

Results obtained from chemical analyses were statistically analysed using the JMP 11.0 software. Following statistical methods were used: Correlation Analysis (CA), Analysis of Variance (ANOVA) with Post Hoc test (LSD) and Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

The average content of total dietary fibre was 36.10 ± 1.92%, lipids 32.05 ± 0.42%, and proteins 24.66 ± 0.55% (Table 3). These amounts correlate with results of Deferne and Pate (1996) and Pate (1999). They determined that 20-30 % of the dry matter represented

Table 3. Contents of lipids, TDFs, and proteins in hemp seeds harvested in two years and at three locations

Year		2013			2014		
Location	Collection	Lipids (%)	TDF (%)	Proteins (%)	Lipids (%)	TDF (%)	Proteins (%)
Borovce	1	34.52	39.80	25.17	31.96	38.21	24.24
Borovce	1	34.17	42.20	25.14	31.54	33.44	23.62
Borovce	1	34.73	40.43	25.16	31.31	38.39	23.43
Borovce	1	34.50	41.00	25.11	32.73	33.93	25.65
Borovce	2	34.00	42.19	25.05	31.97	35.12	27.02
Borovce	2	34.02	36.74	25.87	31.04	35.16	25.16
Borovce	2	33.95	39.70	25.46	31.45	35.77	25.72
Borovce	2	33.89	39.99	25.50	31.22	35.14	25.02
Vígľaš-Pstruša	1	33.23	38.97	24.23	28.79	24.23	22.67
Vígľaš-Pstruša	1	33.67	37.54	24.00	29.53	26.34	24.82
Vígľaš.Pstruša	1	33.12	36.87	24.13	29.60	34.57	24.24
Vígľaš.Pstruša	1	34.56	38.00	24.20	27.96	33.13	22.70
Vígľaš.Pstruša	2	33.08	36.78	25.23	31.79	30.92	27.53
Vígľaš.Pstruša	2	33.00	36.45	25.43	29.56	33.85	28.24
Vígľaš.Pstruša	2	33.18	36.98	25.12	31.73	37.29	25.36
Vígľaš.Pstruša	2	33.00	36.00	25.73	32.15	27.45	25.10
Milhostov	1	32.43	40.06	22.59	30.43	36.56	23.54
Milhostov	1	32.12	36.12	24.14	30.98	34.12	24.12
Milhostov	1	32.33	36.59	23.37	31.00	34.76	24.22
Milhostov	1	32.00	36.72	23.42	30.54	38.65	23.87
Milhostov	2	31.34	38.10	23.58	31.00	32.34	24.55
Milhostov	2	31.55	39.51	24.21	31.21	30.54	24.34
Milhostov	2	32.11	38.81	23.90	31.43	33.76	24.54
Milhostov	2	31.78	38.90	24.00	31.00	34.67	24.31

Abbreviations: TDF – total dietary fibre

saccharides and 10-15% insoluble fibre. Hemp seeds contain usually 20-25% of proteins (Tang et al., 2006), where all essential amino acids are present (Pate, 1999) and the content of lipids is generally 25-35% (Deferne and Pate, 1996; Pate, 1999; Callaway, 2004a). All these parameters are influenced by external factors during the seed development and by determined by genotype.

The major fatty acids in hemp oil were linoleic, α-linolenic, and oleic acids (Table 4). Palmitic, γ-linolenic, stearidonic, *cis*-vaccenic, stearic, gadoleic, and arachidonic acids were detected also, but in much lover amounts. The largest proportion (up to 80% of the total fatty acids) represents polyunsaturated fatty acids (Oomah et al., 2002; Russo and Reggiani, 2013). Other important fatty acids of hemp oil are oleic (11%), palmitic (5%), γ-linolenic (3%), and stearic acids (1-2%), respectively (Oomah et al., 2002; Matthäus and Brühl, 2008; Da Porto et al., 2012). The presence of fatty acids in the hemp oil is relatively stable (Prociuk et al., 2008; Kaul et al., 2008), but environmental factors influence the quality of hemp seed (Mölleken and Theimer, 1997; Mölleken et al., 2000; Anwar et al., 2006).

Very interesting is a profile of unsaturated fatty acids in the hemp oil. The linoleic and α-linolenic acids (PUFA) and oleic acid (MUFA) were identified as dominant fatty acids in all analysed samples of hemp seeds. The palmitic, stearic, and arachidonic acids were minor fatty acids (Figure 1). The gadoleic acid was present in amount less than 1%. The hemp lipids consist 50-60% of linoleic acid (C18:2, omega-6) and 20-25% of α-linolenic acid (C18:3, omega-3) (Wright and Burton, 1982; Anstey et al., 1990; Fiocchi et al., 1994; Pate, 1999; Pavlovic et al., 2019).

Effects of growing season

The year of cultivation (growing season) influenced statistically significantly (P≤0.05) contents of lipids, total dietary fibre, and fatty acids (γ-linolenic, stearidonic, linoleic, α-linolenic, oleic, *cis*-vaccenic, stearic, gadoleic) (Table 5). Proteins content was not affected what indicates relative stability of this parameter. Irakli et al. (2019) confirmed that proteins, dietary fibre, lipids, and fatty acids profile strongly varied between growing year and genotype, even if this observation was not described by Vogl et al. (2004).

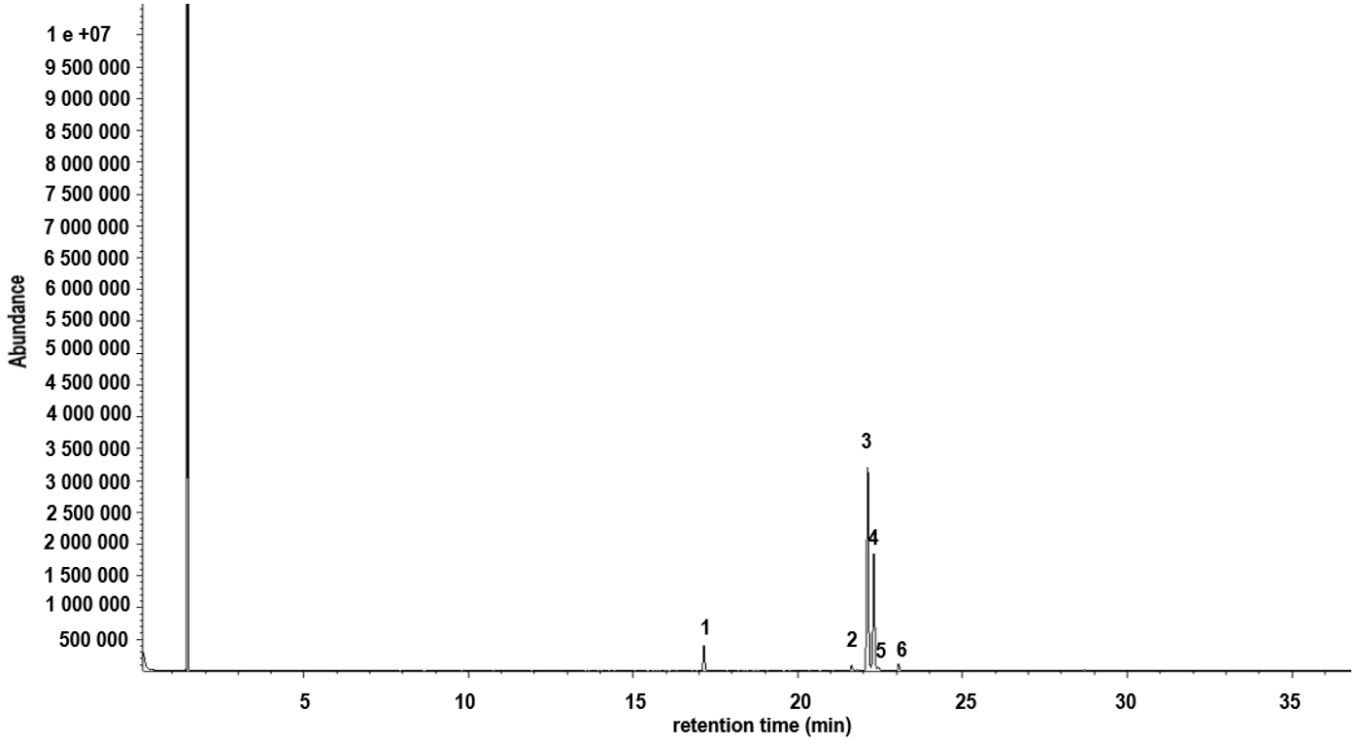

Figure 1. Chromatogram of fatty acids in hemp oil (1 - C 16:0, palmitic acid; 2 - C 18:3 n6, γ-linolenic acid; 3 - C 18:2, linoleic acid; 4 - C 18:1-9C, oleic acid; 5 - C 18:1-11C, *cis*-vaccenic acid; 6 - C 18:0, stearic acid)

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Table 4. The profile of fatty acids (in %) in hemp seeds grown in 2014 on two locations according to two collecting dates

Location	Collection	Palmitic	γ-linolenic	Stearidonic	Linoleic	α-linolenic	Oleic	*cis*-vaccenic	Stearic	Gadoleic	Arachidonic	SFA	MUFA	PUFA
Borovce	1	7.14	4.40	1.57	5228	17.36	11.42	1.58	2.92	0.42	0.91	10.97	13.42	75.62
Borovce	1	7.08	4.37	1.53	51.79	16.10	13.08	1.79	2.95	0.41	0.90	10.92	15.29	73.79
Borovce	1	7.17	4.29	1.47	51.67	16.35	13.04	1.68	2.93	0.41	1.00	11.09	15.13	73.78
Borovce	1	7.16	4.95	1.67	51.87	15.84	12.25	1.47	3.15	0.53	1.10	11.42	14.25	74.34
Borovce	2	6.91	4.89	1.66	52.66	16.65	11.45	1.66	2.51	0.52	1.09	10.51	13.63	75.86
Borovce	2	6.51	4.48	1.49	53.54	15.25	13.33	1.50	2.38	0.49	1.03	9.92	15.32	74.76
Borovce	2	7.23	5.65	1.86	51.46	16.54	10.63	1.67	3.19	0.56	1.22	11.64	12.86	75.50
Borovce	2	7.30	5.86	1.96	51.15	16.26	10.79	1.65	3.22	0.59	1.23	11.75	13.03	75.22
Vígľaš.Pstruša	1	6.16	5.33	1.83	52.42	16.28	12.19	1.43	2.85	0.47	1.05	10.06	14.09	75.85
Vígľaš.Pstruša	1	6.36	5.77	1.94	53.84	14.93	11.88	1.21	2.55	0.46	1.08	9.99	13.54	76.47
Vígľaš.Pstruša	1	6.06	5.35	1.83	52.56	15.51	13.01	1.28	2.91	0.45	1.05	10.02	14.74	75.24
Vígľaš.Pstruša	1	6.24	5.70	1.96	53.02	16.51	10.89	1.44	2.64	0.50	1.09	9.97	12.83	77.20
Vígľaš.Pstruša	2	6.56	5.97	2.03	52.22	16.40	10.93	1.46	2.80	0.50	1.13	10.49	12.89	76.63
Vígľaš.Pstruša	2	6.24	5.70	1.94	52.95	15.59	12.14	1.30	2.59	0.47	1.08	9.91	13.91	76.19
Vígľaš.Pstruša	2	6.18	5.57	1.93	52.18	16.83	11.59	1.35	2.82	0.49	1.05	10.05	13.44	76.51
Vígľaš.Pstruša	2	6.48	5.39	1.89	53.24	15.78	11.80	1.28	2.52	0.51	1.10	10.11	13.58	76.31

Table 5. Analysis of variance in contents of analysed parameters of seed samples grown in two years (2013, 2014). The table contains only parameters significantly influenced by year (P-value≤0.05)

		Sum of Squares	d.f.	Mean Square	F	P-value
Lipids	**Between Groups**	**61.623**	**1**	**61.623**	**53.128**	**0.000**
	Within Groups	53.355	46	1.160		
	Total	114.979	47			
TDF	**Between Groups**	**280.983**	**1**	**280.983**	**33.462**	**0.000**
	Within Groups	386.270	46	8.397		
	Total	667.253	47			
γ-linolenic	**Between Groups**	**6.747**	**1**	**6.747**	**29.211**	**0.000**
	Within Groups	5.082	22	0.231		
	Total	11.829	23			
Stearidonic	**Between Groups**	**1.308**	**1**	**1.308**	**48.131**	**0.000**
	Within Groups	0.598	22	0.027		
	Total	1.907	23			
Linoleic	**Between Groups**	**21.554**	**1**	**21.554**	**51.561**	**0.000**
	Within Groups	9.197	22	0.418		
	Total	30.751	23			
α-linolenic	**Between Groups**	**2.016**	**1**	**2.016**	**7.173**	**0.014**
	Within Groups	6.183	22	0.281		
	Total	8.199	23			
Oleic	**Between Groups**	**8.032**	**1**	**8.032**	**15.253**	**0.001**
	Within Groups	11.585	22	0.527		
	Total	19.617	23			
***Cis*-vaccenic**	**Between Groups**	**6.379**	**1**	**6.379**	**300.716**	**0.000**
	Within Groups	0.467	22	0.021		
	Total	6.845	23			
Stearic	**Between Groups**	**0.258**	**1**	**0.258**	**5.634**	**0.027**
	Within Groups	1.006	22	0.046		
	Total	1.264	23			
Gadoleic	**Between Groups**	**0.021**	**1**	**0.021**	**11.550**	**0.003**
	Within Groups	0.041	22	0.002		
	Total	0.062	23			

d.f. – degrees of freedom, F – Fisher statistics, P – value represents statistical

Climatic conditions are associated with the harvest time therefore affect also quality of hemp seed (Leizer et al., 2000; Bağci et al., 2003; Kriese et al., 2004; Anwar et al., 2006). Differences between the monitored years expressed as average air temperature and sum of precipitation were recorded also in years 2013 and 2014 at our testing locations (Table 1, Table 2).

The average content of total dietary fibre was higher in the year 2013 compared to 2014. TDF content decreased in location Borovce from 40.26 ± 1.63% to 35.64 ± 1.49%, in Milhostov from 38.10 ± 1.19% to 34.43 ± 1.92%, and in Vígľaš-Pstruša from 37.20 ± 0.66% to 30.97 ± 4.62%, respectively.

The average lipids content in hemp seeds harvested in the year 2013 at individual locations were 34.22 ± 0.14% (Borovce), 33.36 ± 0.37% (Milhostov), 31.96 ± 0.26% (Vígľaš-Pstruša). In the year 2014, the average content of lipids decreased at all locations to 31.65 ± 0.51% (Borovce), 30.14 ± 0.97% (Milhostov), and 30.95 ± 0.25% (Vígľaš-Pstruša). Relationships between oil content and climatic conditions presented Kostić et al. (2013) and Rezvankhah et al. (2018). Kriese et al. (2004) indicated significant effect of year and year x genotype interaction on oil content in hemp seeds. The average oil content in hemp seeds was lower during season with frequent precipitation than during drought (Iványi, 2006).

Different studies presented that hemp plants grown in mild or warm climate contain lower amounts of γ-linolenic acid compared to plants grown in cooler regions (Deferne and Pate, 1996; Ross et al., 1996; Mölleken and Theimer, 1997). Unsaturated fatty acids, especially oleic acid, accumulate preferably at low temperatures (Hilditch and Williams, 1964). During the two years of our study the linoleic and oleic acids levels were higher in 2013 (54.44% for linoleic acid and 13.13% for oleic acid) compared to 2014 (52.43% and 11.90%, respectively). On the opposite, the content of α-linolenic acid was higher in seeds cultivated in 2014 (16.13%) compared to 15.52% in 2013.

The quality of lipids was evaluated by the saturation of fatty acids that indicates the presence or absence of double bonds in molecule. Excessive intake of saturated fatty acids (SFAs, palmitic and stearic acids) is associated with increased level of LDL-cholesterol, related obesity or other health problems. Non-significant differences in content of SFAs, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in seeds harvested in two years were detected. The mean contents of SFAs were 10.56% (2013) and 10.55% (2014), MUFAs 14.21% (2013) and 13.87% (2014), and PUFAs 75.24% (2013) and 75.58% (2014), respectively.

Hemp seeds contain well-balanced ratio (optimal ratio is 3:1) of two essential fatty acids, the linoleic acid (omega-6) and α-linolenic acid (omega-3) (Oomah et al., 2002; Callaway, 2004a). This ratio was in our study different in both years, 3.8 : 1 in 2013 and 2.5 : 1 in 2014. However, Pavlovic et al. (2019) detected that this ratio strongly deviated (6.22: 1) from optimal in seeds of Finola cultivar grown at mountain environment of Italian Alps. Generally, the lipid content and its composition in hemp seeds is strongly influenced by environmental factors (Ross et al., 1996; Kriese et al., 2004) and this confirmed also our results. Hemp oil contains about 75% of PUFAs (Callaway, 2004a; Pavlovic et al., 2019) making it an exceptional nutritional source of lipophilic substances including essential fatty acids for human consumption.

Effect of seed maturity

Hemp seeds were harvested in two dates according to their maturity. The first harvest was done when 75% of seeds were mature, the second at full (100%) maturity. The optimal harvest time is in hemp very important to obtain the highest yield and quality of seeds (Burczyk et al., 2009). However, the harvest time statistically significantly ($P \leq 0.05$) influenced only the content of proteins and two fatty acids, the gadoleic acid and arachidonic acid (Table 6).

Proteins content is usually a stable feature. Their metabolism is essential for health, development, and adaptation to environmental conditions (Young and Pellett, 1994; Shewry et al., 1995). Nevertheless, statistically significant influence of seed maturity to proteins content was observed and increased from partial

Table 6. Analysis of variance in content of proteins and two fatty acids in hemp seeds harvested in two different harvest time. Table contains only parameters influenced significantly (P-value≤0.05)

		Sum of Squares	d.f.	Mean Square	F	P-value
Proteins	**Between Groups**	**16.563**	**1**	**16.563**	**17.291**	**0.000**
	Within Groups	44.061	46	0.958		
	Total	60.624	47			
Gadoleic	**Between Groups**	**0.014**	**1**	**0.014**	**6.316**	**0.020**
	Within Groups	0.048	22	0.002		
	Total	0.062	23			
Arachidonic	**Between Groups**	**0.030**	**1**	**0.030**	**7.066**	**0.014**
	Within Groups	0.092	22	0.004		
	Total	0.122	23			

d.f. – degrees of freedom, F – Fisher statistics, P – value represents statistical significance

(75%) to full (100%) seed maturity. Process of ripening deposit proteins into seeds, decreases the fresh weight of embryo and whole seed, decreases water content therefore, seed maturation impacts on composition of some compounds in seed (Mbofung, 2012).

Analysis of total dietary fibre content revealed differences in seeds harvested later (collection 2) in comparison with earlier harvest in both years and in all three locations. Nevertheless, these differences were not statistically significant. The same was true for lipid content. Differences between harvest time in both years and in all locations were not statistically significant.

Mature hemp seeds showed visible differences in content of different substances (including proteins) in comparison with juvenile, immature ones (Mölleken et al., 2000). At the beginning of embryonal development, the endosperm contains mainly free glycids and amino acids (for energy supply and protein synthesis), and also plant-growth regulators, enzymes, and vitamins. Storage substances such as polysaccharides, lipids, proteins, accumulate during last phases of maturations in higher amounts (Mbofung, 2012). This process relates to climatic and geographical conditions, soil management practices, plant fertilization and protection as well as to optimal harvest time.

Effect of location

Growing location was source of variation in content of lipids, total dietary fibre, proteins, selected individual fatty acids, and proportion of saturated fatty acids and polyunsaturated fatty acids (Table 7). Locality Borovce was characterized by the highest level of lipids in both analysed years and seeds contained also the highest level of total dietary fibre and proteins (Table 3). Differences in protein content in hemp seeds have been detected in different agro-ecological zones (Anwar et al., 2006). Another factor influencing qualitative parameters of the hemp seed are also cultivation practices used at the growing area (Ross et al., 1996; Mölleken et al., 2000; Kriese et al., 2004; Anwar et al., 2006, Kiralan et al., 2010). The hemp cultivar Finola is sensitive to precipitation from the beginning of vegetation period to the flowering. Another important period is grain filling. The fertile soil in locality Borovce was responsible for the highest content of proteins, lipids and total dietary fibre in comparison with other two locations.

Principal Component Analysis of analysed seeds quality parameters

The Principal Component Analyses (PCA) (Table 2) separated samples according to the year of cultivation.

Table 7. Analysis of variance in contents of analysed parameters of hemp seeds grown at three locations. The table presents only parameters influenced significantly (P-value≤0.05)

		pro	d.f.	Mean Square	F	P-value
Lipids	**Between Groups**	**19.717**	**2**	**9.858**	**4.657**	**0.015**
	Within Groups	95.262	45	2.117		
	Total	114.979	47			
TDF	**Between Groups**	**120.172**	**2**	**60.086**	**4.942**	**0.011**
	Within Groups	547.080	45	12.157		
	Total	667.253	47			
Proteins	**Between Groups**	**13.645**	**2**	**6.823**	**6.535**	**0.003**
	Within Groups	46.978	45	1.044		
	Total	60.624	47			
Palmitic	**Between Groups**	**0.926**	**1**	**0.926**	**7.476**	**0.012**
	Within Groups	2.724	22	0.124		
	Total	3.650	23			
γ-linolenic	**Between Groups**	**6.610**	**1**	**6.610**	**27.864**	**0.000**
	Within Groups	5.219	22	0.237		
	Total	11.829	23			
Stearidonic	**Between Groups**	**1.077**	**1**	**1.077**	**28.545**	**0.000**
	Within Groups	0.830	22	0.038		
	Total	1.907	23			
SFAs	**Between Groups**	**2.780**	**1**	**2.780**	**16.678**	**0.000**
	Within Groups	3.667	22	0.167		
	Total	6.447	23			
PUFAs	**Between Groups**	**8.330**	**1**	**8.330**	**24.013**	**0.000**
	Within Groups	7.632	22	0.347		
	Total	15.962	23			

d.f. – degrees of freedom, F – Fisher statistics, P – value represents statistical significance

Samples from location Borovce and from the year 2013 were very similar, grouped very close to each other, disregarding the seed maturity. On the opposite, samples from year 2014, from the same location, were significantly scattered that indicated very high variation in all analysed seed quality parameters. This indicates a high influence of climatic conditions in this locality on the content parameters of cannabis seeds. All samples from the location Vígľaš-Pstruša were located in only one quadrant and were quite similar in measured quality parameters, disregarding the maturity. This in turn suggests a smaller impact of the year and relatively uniform climatic conditions in this locality.

Figure 2 and Table 8 present correlations between seed compounds. The most interesting statistically significant (P≤0.05) positive correlations were observed between contents of total dietary fibre and lipids and between contents of lipids and α-linolenic acid.

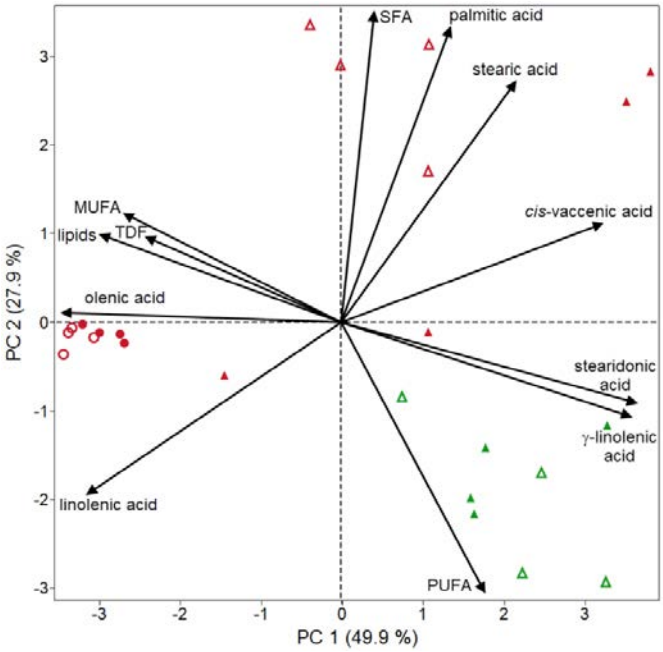

Figure 2. Principal Component Analysis of analysed hemp seeds quality parameters

Red colour – samples from location Borovce, green colours – Vígľaš-Pstruša, circles – year 2013, trigons – 2014, empty points – 75% maturity (collecting 1), filled points – full (100%) maturity (collecting 2)

CONCLUSIONS

The content of total dietary fibre, proteins, lipids, and fatty acid profiles were analysed in the hemp cultivar Finola cultivated at three locations during two growing seasons. Seeds were harvested in two maturity stages. The average content of total dietary fibre was 36.10%, lipids 32.05%, and proteins 24.66%. Major fatty acids in hemp oil were linoleic, α-linolenic, and oleic acids. Hemp oil contained about 75% of polyunsaturated fatty acids. The year of cultivation (growing season) statistically significantly (P≤0.05) influenced the content of lipids, total dietary fibre, and some of fatty acids. Also, the growing location statistically significantly (P≤0.05) influenced content of all monitored primary metabolites, selected fatty acids, and the ratio of saturated fatty acids and polyunsaturated fatty acids. The degree of seed maturity was the source of variation only in content of proteins, gadoleic acid and arachidonic acids. Statistically significant (P≤0.05) positive correlations were observed between contents of total dietary fibre and lipids and between contents of lipids and α-linolenic acid.

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Table 8. Correlations between analysed quality parameters of hemp seeds

	TDF	Proteins	PUFAs	Linoleic FA	α-linolenic FA	Oleic FA
Lipids	0.6625*	0.3305		0.6104*		0.4998
TDF				0.5061		0.5359
Proteins						
SFAs			-0.5850*			
MUFAs			-0.8314*		-0.4747	0.8268*
PUFAs						-0.6404*
Linoleic FA					-7.7070*	0.6390*

Abbreviations: TDF – total dietary fibre, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids, FA – fatty acid. * - significant correlations (P<0.001)

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