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Effect of genotype on the physicochemical, nutritional, and antioxidant properties of hempseed



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ABSTRACT

Hempseed products has been used as nutraceutical supplements and pharmaceutical products. However, hempseed has been underutilized as a food crop for human consumption. To fill the gap of limited knowledge of the variation of hempseed for food consumption, thirteen hemp varieties were selected to evaluate the effect of genotype on the physicochemical, nutritional, and antioxidant properties of hempseed. The tested hempseed contains 26.48–32.03% crude protein with average of 28.48%, 28.03–33.23% crude oil with average of 29.54%, 28.78–36.55% crude fiber with average of 33.49%, and 5.43%–6.32% ash with average of 5.89. Average test weight of 36.85 lbs/bu was relatively low compared to the standard test weight of 44 lbs/bu. Hempseed oil contained high portions of about 80% unsaturated fatty acids such as linoleic and α -linolenic acid. The DPPH scavenging activities varied greatly (0.37–28.78%) for the hydrolysates from different hempseed varieties. This study provides comprehensive understanding of the nutritional value of hempseed for human food and potential of a new crop in agricultural food system.

1. Introduction

Industrial hemp (Cannabis sativa L.) is an ancient annual plant that originated in central Asia, and has been traditionally cultivated in many regions of Europe as a fiber source or fiber-seed dual purpose crop, with only limited application as an oilseed crop [1,2]. Hemp was cultivated in temperate Eurasia for millennia, and was first brought to North America in 1606. In 1994, Canada issued licenses to allow research on industrial hemp. Two years later, new regulations permitted commercial cultivation of hemp under the licensing and control of Health Canada. Section 7606 of the US Agricultural Act of 2014 and the 2018 Farm Bill authorized state departments of agriculture to permit pilot programs for industrial hemp research. Recent interest in this multipurpose crop for fibers, shives, and seeds production has grown rapidly worldwide not only for natural fibers but also for high quality protein and oil [3]. Hempseed has been an important crop for protein, oil, and dietary for human food and animal feed. Currently, commercial hempseed products are receiving a growing interest with the rapid growth in the US market [4,5].

Nutritionally, hempseed consists of 20–25% protein, 20–30% carbohydrates, 25–35% oil, 10–15% fiber, and minerals, such as phosphorus, potassium, magnesium, sulfur, calcium, iron, and zinc [6]. Hempseed oil is an important product due to its nutritional and health benefits which could lower cholesterol and blood pressure, and even prevent cardiovascular diseases and cancers [7]. The main nutritional and health benefits of hempseed oil are attributed not only to its high amount of polyunsaturated fatty acids (PUFA), but also to the significant amount of other bioactive minor components, such as tocopherols, polyphenols, and phytosterols. These minor components have strong antioxidant properties that may protect oil from oxidation, and also provide health benefits to humans [8].

Hempseed oil consists of more than 80% PUFA, and is rich in both *omega*-6 and *omega*-3 essential fatty acids (EFAs), with the optimal ratio between 2:1 and 3:1 good for human metabolism. EFAs such as linoleic acid (LA) and alpha-linolenic acid (LNA) cannot be generated naturally by the human body and must be ingested from the diet [8].

The essential amino acid amount of hempseed proteins is superior to that of soybean, and lysine and tryptophan are the main limiting amino

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2666-1543/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/bynend/4.0/). acids in hempseed protein. The meal after oil extraction shows great potential as a high-protein flour for human food. In addition, a considerable amount of the amino acid arginine (a metabolic precursor for nitric oxide) in the hempseed protein is an essential chemical for the normal regulation of blood pressure. The sulfur-rich protein in hempseed protein can serve as a rich thiol resource to formulate highly nutritious foods, and as a nutritional tool to boost the antioxidant capacity in human body [9–16].

However, hempseed appears to have been underutilized as a grain crop for human consumption. This study provides comprehensive understanding of the nutritional value of hempseed for human food and potential of a new crop in agricultural food system.

2. Materials and methods

2.1. Materials

Seeds of thirteen varieties of industrial hemp including five grain varieties, and eight grain and fiber dual-purpose varieties were planted at Haysville, KS, detailed information referred to Table 1. Seeds were planted into a freshly prepared seed bed at an approximate depth of 2.0 cm in late June 2019 and immediately irrigated to hasten germination. Seed was planted at a rate of 33.6 kg/ha into each of four plots measuring $1.2 \text{ m} \times 6.7 \text{ m}$. Plots were irrigated to prevent plant stress if natural precipitation was lacking. Thirteen varieties of hempseed were harvested when 75% of the seed head had matured and brown seed is visible in early September 2020, and air dried to low moisture content for long-term storage. The moisture content of industrial hempseed was determined using the conventional air oven drying method at 103 °C for 24 h. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Physical properties

Two hundred seeds were counted out and weighed, then multiplied by 5 to obtain 1000 kernel weight (n = 2 replicates). Standard ASABE method was used to determine test weight, which was measured in terms of pounds of grains per volumetric bushel. Toluene test, toluene displacement method, was used to obtain the true density of the grains. Kernel hardness, kernel weight, and kernel size were analyzed using the single kernel characteristics system 4100.

2.3. Chemical composition

Starch content was analyzed following the Megazyme assay procedure with Total Starch assay kit (K-TSTA, Megazyme, Bray, Ireland) according to AACC Approved Method 76–13.01 [29]. Protein content was measured using nitrogen combustion via a LECO FP-2000 nitrogen

| Table 1 | | | | |
|----------|------------|-------------|----------|-----------|
| Physical | properties | of thirteen | hempseed | varieties |

determinator (St. Joseph, MI) according to AOAC method 990.03. Nitrogen value was converted to protein using a conversion factor of 6.25. Oil content was determined by using the Soxhlet petroleum ether extraction method according to AOAC method 920.39C for oil and expressed as a weight percentage on a dry basis [30]. Cruder fiber was determined according to AOCS approved procedure. Ash content was measured using muffle furnace at 575 ± 25 °C and dried to a constant weight.

2.4. Oil extraction and fatty acid composition

Mechanical cold press method was used for oil extraction. Fatty acid analysis was performed at the Kansas Lipidomics Research Center. GC-FID using an Agilent 6890 N GC coupled to the FID (flame ionization detector). The GC was fitted with a DB-23 capillary column (column length - 60 m, internal diameter - 250 μ m, film thickness - 0.25 μ m). Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The back inlet was operated at a pressure of 36.01 psi and 250 °C. Agilent 7683 autosampler was used to inject 3 μ L of the sample in the splitless mode. The GC oven temperature ramp was operated as follows: initial temperature of 150 °C hold 1 min, increase at 25 °C/min to 175 °C, then increase at 4 °C/min to 230 °C, hold 8 min. Total rum time was 23.75 min. The FID detector was operated at 260 °C. The hydrogen flow to the detector was 30 mL/min and air flow was 400 mL/min. The sampling rate of the FID was 20 Hz. The data were processed with Agilent Chemstation software.

2.5. Protein isolation

Isoelectric-precipitation method was used to isolate hemp protein from defatted hemp meal. The meal was suspended in water at 1:10 ratio (w/v), and pH was adjusted to 10.0 by adding 1.0 M NaOH under constant stirring for 2 h at 35 °C, which was then centrifuged for 1 h at 7000×g. The supernatant was collected and adjusted to pH 5.0 with 1 M HCL under constant stirring for 1 h at 35 °C, and then centrifuged for 1 h at 7000×g to collect the precipitated protein.

2.6. Antioxidant properties of hempseed protein hydrolysates

2.6.1. Preparation of hempseed protein hydrolysates

Hempseed protein was first decolorized by mixing with acetone (1:10, w/v) for 3 h in a fume hood [12]. The resulting protein was air-dried overnight and then stored at 4 °C before uses. Enzymatic hydrolysis was conducted following the method of [17]. Briefly, 5% hempseed protein aqueous suspension was heated to 37 °C and adjusted to pH 2.0. Pepsin (4%, protein basis) was then added into the suspension and allowed to react for 2 h at 37 °C in a water bath shaker. After peptic hydrolysis, the suspension was adjusted to pH 7.5, followed by adding

| Variety | Purpose | 1000 kernel weight (g) | Kernel hardness index | Single kernel weight (mg) | Kernel diameter (mm) | Moisture (%) | Test weight (lb∕ bu) | True density (kg/ m ³) |
|-------------|---------|---------------------------|--------------------------|------------------------------|-------------------------|-----------------|-------------------------|---------------------------------------|
| Fedora 17 | Grain | 14.06 | 16.77 | 16.53 | 2.02 | 8.57 | 36.85 | 1.09 |
| Helena | Dual | 17.01 | 16.67 | 17.06 | 2.04 | 8.20 | 38.24 | 1.09 |
| Joey | Dual | 16.93 | 7.07 | 18.90 | 2.24 | 8.14 | 38.11 | 1.10 |
| Hlukhivs'ki | Dual | 14.30 | 19.95 | 16.09 | 2.06 | 8.77 | 33.25 | 1.08 |
| 51 | | | | | | | | |
| Katani | Grain | 13.01 | 25.20 | 14.81 | 1.99 | 7.92 | 36.37 | 1.07 |
| Felina 32 | Dual | 14.88 | 16.76 | 16.59 | 1.97 | 8.14 | 38.29 | 1.09 |
| Futura 75 | Dual | 15.84 | 20.46 | 17.50 | 2.02 | 9.38 | 38.01 | 1.08 |
| Tygra | Dual | 14.93 | 27.60 | 15.21 | 1.90 | 8.13 | 37.73 | 1.05 |
| Hlesia | Dual | 14.52 | 22.87 | 16.25 | 2.07 | 8.26 | 34.69 | 1.08 |
| CRS1 | Grain | 16.01 | 17.08 | 16.46 | 2.04 | 7.88 | 38.05 | 1.10 |
| Canda | Dual | 18.54 | 11.53 | 19.33 | 2.28 | 8.66 | 36.69 | 1.10 |
| USO31 | Grain | 15.38 | 15.97 | 16.48 | 2.08 | 8.15 | 35.75 | 1.09 |
| CFX1 | Dual | 14.68 | 14.41 | 16.64 | 2.03 | 8.27 | 37.03 | 1.09 |

pancreatin (4%, protein basis), which was then allowed to react for another 4 h. Enzymatic hydrolysis was terminated by adjusting pH to 4.0 and heating at 95 °C for 15 min. The mixture was then centrifuged (7000 g, 4 °C) for 30 min. The supernatant was lyophilized and stored at -18 °C until further analysis. Hydrolysate yield was calculated as: yield = (W₁/W₀) × 100%, where W₁ was the weight of hydrolysate, and W₀ was the weight of the sample (protein basis) used for hydrolysis.

2.6.2. Characterization of hempseed protein hydrolysates

Degree of hydrolysis (DH) was determined according to a previously method [18]. Total phenolic content (TPC) of hempseed protein hydrolysates (HPH) was measured using Folin-Ciocalteu assay [19]. Gallic acid was used as a standard to establish the calibration curve. TPC of the hydrolysates was expressed as mg gallic acid equivalent per gram of sample (mg GAE/g). DPPH radical (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity of HPH was analyzed following the method of Shen et al. (2018) [33]. Briefly, HPH solution (5 mg/mL, 500 µL) was added into 4 mL of DPPH (0.2 mmol/L in 95% ethanol). Aliquot of 1 mL of the mixture was diluted with 2 mL 95% ethanol and allowed to react for 5 min in dark. Absorbance was measured at 517 nm using a double beam spectrophotometer (VMR UV-6300PC). DPPH radical scavenging activity was calculated using the equation as follows: DPPH% = $((A_0-A_1)/A_0) \times 100$, where A₀ was absorbance of blank, and A₁ was absorbance of the sample [31,32]. Metal (Fe²⁺) chelating activity was determined according to our previous method [18]. Briefly, 25 µL of HPH solution (1 mg/mL) was loaded into microplate followed by adding 150 µL DI water and 25 µL FeCl₂ solution (0.2 mM). After reacting for 30 s, 50 µL ferrozine solution (1 mM) was added into the mixture. The absorbance was determined at 562 nm immediately using a Biotek Synergy H1 Hybrid microplate reader (Winooski, VT, USA). The chelating ability was calculated as follows: Fe^{2+} chelating ability (%) = $((A_0-A_1)/A_0) \times 100$, where A_0 was absorbance of blank, and A_1 was the absorbance of sample. All the measurements were conducted in at least triplicate.

2.7. Statistics

Means values and standard deviations from the duplicated experiments are reported.

3. Results and discussion

3.1. Effect of genotype on the physical properties of hempseed

Nine hemp varieties typically bred for dual fiber and grain purpose, and four hemp varieties for grain purpose only were selected in this study. Table 1 shows the physical properties of 13 hempseed varieties. Thousand seed weight varied considerably from 13.01 g (Katani) to 18.54 g (Canda) with the mean value of 15.39 g. In North Dakota, the lower thousand seed weight were reported in the range of 14.1 g-16.7 g (Hanson et al., 2018). However, the five Romanian hemp varieties reported a relatively higher thousand seed weight range of 17-23 g [20]. Single kernel weight of all tested industrial hemp varieties averaged 16.76 mg with a range of 14.81 mg (Katani) to 19.33 mg (Canda). Kernel hardness index varied significantly from very soft 7.07 (Joey) to relatively hard 27.60 (Tygra) with the mean value of 17.87. The size of hempseed kernel was relatively small, averaged 2.06 mm with a range of 1.90 mm (Tygra) to 2.28 mm (Canda). Canda had significantly higher thousand kernel weight, single kernel weight, and kernel size compared to other varieties. Katani had considerably lighter thousand kernel weight, and single kernel weight, and smaller kernel size. Average test weight of all thirteen varieties were 36.85 lbs/bu with a range of 33.25 lbs/bu (Hlukhivs'ki 51) to 38.29 lbs/bu (Felina 32), relatively low compared to the standard test weight for hemp of 44 lbs/bu. All hemp varieties had a relatively similar true density in the range of 1.05 kg/m³ (Tygra) to 1.10 kg/m³ (Joey, Canda, and CRS1) with the average value of 1.08 kg/m^3 .

3.2. Effect of genotype on the chemical composition of hempseed

Whole ground seed samples were analyzed for chemical composition, including crude protein, crude oil, crude fiber, starch, and ash contents (Table 2). Hempseed has been reported as a valuable source of protein. The crude protein content for the tested 13 varieties varied from 26.48% (Fedora 17) to 32.03% (CRS1) with a mean value of 28.48%. The oil content ranged between 28.03% (Joey) and 33.23% (Katani) with 30.31 g/100 g as mean amount. The starch content for all thirteen hemp varieties was typically small, less than 2% of whole kernel, averaged 1.84% with a range of 1.69% (Canda) to 1.97% (Helena). The crude fiber content for all tested thirteen varieties varied from 28.78% (CRS1) to 36.55% (Canda) with the mean value of 5.89%. The ash content for all tested thirteen varieties varied from 5.43% (Joey) to 6.32% (Fedora 17) with the mean value of 5.89% [21]. reported the crude protein content of industrial hemp flour from 10 varieties ranged from 32.7% to 35.9%, crude oil content ranged from 24.3% to 28.1%. Hempseed dehulling showed no significant impact on protein composition, but greatly enhanced the protein extraction yield [22].

The oil contents of hempseed ranged from 29.61% to 36.47% with the average of 32.13% were reported in Northwestern Turkey [23]. The oil contents of hempseed in Kansas obtained in this study were within the expected range reported previously for hempseed.

3.3. Effect of genotype on the fatty acid profiles of hempseed

Hemp oil is one of the major compositions that accounts for about 35% weight of the whole seed [7]. Hemp oil is highly valuable for human health because of its high PUFA and minor bioactive components; however, the properties of hemp oil are still little known for those grown in Kansas. It has been reported the genotype had a significant effect on oil content, particularly fatty acid profiles [23]. A huge difference in the fatty acid profiles between oil hempseed and fiber hempseed was reported [8]. The oil contains high amounts of unsaturated fatty acids like linoleic and α -linolenic acid, which comprised about 80% of the total fatty acids. Hempseed oil also consisted of remarkable amounts of γ -linolenic and stearidonic acid. The concentrations varied from 2.43 to 4.50% and 0.63–1.12%, respectively, dependent on the genotype.

Rapid growth of interest in hempseed as a food source has considerably focused on its oil content and fatty acid profile [23]. The fatty acid composition (15 fatty acids) of thirteen different hemp varieties were evaluated. The fifteen fatty acids such as myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, gama-linolenic, alpha-linolenic, stearidonic, arachidic, eicosadienoic, eciosadienoic, behenic, and lignoceric acid were identified in the oil of the hempseeds analyzed. The fatty acid composition of the hempseed oils was shown in Table 3. The principal saturated fatty acid in hempseed was palmitic acid (C16:0), ranged from 8.11% (Hlesia) to 8.45% (Fedora 17), and it is also one of the most common saturated fatty acids in animal or plant oil. It was followed by stearic acid (C18:0), varying 2.12% (CFX1) to 2.49% (Hlesia). The remaining saturated fatty acids, including myristic, heptadecanoic, arachidic, behenic, lignoceric acids, were typically below 1%. The saturated fatty fraction represented 11.70% (Tygra) to 12.07% (Helena) of the total fatty acid in hempseeds. This relatively low level of saturated fatty acids was comparable to those previous published data.

Out of the 15 measured fatty acids, the omega-6 linoleic acid (C18:2n-6) was a predominant unsaturated acid, and fluctuated from 55.97% (CRS1) to 57.34% (Hlesia), while the omega-3 α -linolenic acid (C18:3n-3) ranged from 13.28% (Tygra) to 15.47% (CRS1), and the omega-9 oleic acid (C18:1n-9) ranged from 10.77% (CFX1) to 14.00% (Tygra). Of the minor fatty acids, the highest concentrations were found for γ -linolenic acid (C18:3n-6), range 2.43% (Helena) to 4.50% (CFX1), followed by stearidonic acid (C18:4n-3), ranged 0.63% (Helena) to 1.12% (CFX1). These results show that hempseed grown in Kansas provides a well-

Table 2

Chemical composition of thirteen hempseed varieties.

| Variety | Crude protein (%, db) | Crude oil (%, db) | Starch (%, db) | Crude fiber ¹ (%, db) | Ash (%, db) |
|----------------|------------------------------------|------------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| Fedora 17 | $\textbf{26.48} \pm \textbf{0.05}$ | 30.24 ± 0.55 | 1.95 ± 0.07 | 35.02 | 6.32 ± 0.21 |
| Helena | $\textbf{27.49} \pm \textbf{0.02}$ | 29.17 ± 0.17 | 1.97 ± 0.07 | 35.64 | 5.72 ± 0.01 |
| Joey | 30.20 ± 0.03 | $\textbf{28.03} \pm \textbf{1.24}$ | 1.78 ± 0.27 | 34.54 | $\textbf{5.43} \pm \textbf{0.06}$ |
| Hlukhivs'ki 51 | 29.31 ± 0.02 | $\textbf{29.93} \pm \textbf{1.04}$ | 1.82 ± 0.07 | 32.87 | $\textbf{6.08} \pm \textbf{0.08}$ |
| Katani | 30.07 ± 0.08 | 33.23 ± 1.13 | 1.84 ± 0.13 | 28.85 | 6.00 ± 0.07 |
| Felina 32 | $\textbf{27.00} \pm \textbf{0.03}$ | $\textbf{32.92} \pm \textbf{2.33}$ | $\textbf{1.88} \pm \textbf{0.20}$ | 32.36 | 5.84 ± 0.15 |
| Futura 75 | $\textbf{27.27} \pm \textbf{0.06}$ | $\textbf{20.98} \pm \textbf{0.18}$ | 1.79 ± 0.07 | 34.25 | 5.70 ± 0.18 |
| Tygra | $\textbf{28.96} \pm \textbf{0.03}$ | $29.82 \pm 1.1.$ | 1.82 ± 0.07 | 33.19 | 6.21 ± 0.10 |
| Hlesia | 27.06 ± 0.05 | $\textbf{30.72} \pm \textbf{1.48}$ | 1.88 ± 0.07 | 34.55 | 5.80 ± 0.08 |
| CRS1 | $\textbf{32.03} \pm \textbf{0.13}$ | 31.530.67 | 1.71 ± 0.07 | 28.78 | 5.95 ± 0.27 |
| Canda | $\textbf{26.81} \pm \textbf{0.10}$ | $\textbf{28.92} \pm \textbf{1.18}$ | 1.69 ± 0.13 | 36.55 | 6.03 ± 0.02 |
| USO31 | 28.05 ± 0.12 | 29.52 ± 0.24 | 1.84 ± 0.07 | 34.79 | 5.81 ± 0.07 |
| CFX1 | 29.50 ± 0.07 | $\textbf{28.98} \pm \textbf{0.47}$ | 1.890.13 | 33.93 | 5.71 ± 0.01 |
| Average | 28.48 | 29.54 | 1.84 | 33.49 | 5.89 |

1 Crude fiber = 100 - all other components.

Table 3

Fatty acid profiles of thirteen hempseed varieties.

| Fatty Acid Composition (| %) | Fedora 17 | Helena | Hlukhivs'ki 51 | Felina 32 | Tygra | Hlesia | CRS1 | USO31 | CFX1 |
|--------------------------|----------|-----------|--------|----------------|-----------|-------|--------|-------|-------|-------|
| Myristic Acid | C14:0 | 0.07 | 0.06 | 0.09 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.08 |
| Palmitic Acid | C16:0 | 8.45 | 8.40 | 8.36 | 8.32 | 8.17 | 8.11 | 8.33 | 8.15 | 8.39 |
| Palmitoleic Acid | C16:1 | 0.14 | 0.13 | 0.15 | 0.13 | 0.13 | 0.12 | 0.13 | 0.13 | 0.13 |
| Heptadecanoic Acid | C17:0 | 0.06 | 0.07 | 0.06 | 0.06 | 0.06 | 0.07 | 0.07 | 0.06 | 0.06 |
| Stearic Acid | C18:0 | 2.18 | 2.46 | 2.28 | 2.36 | 2.39 | 2.49 | 2.14 | 2.25 | 2.12 |
| Oleic Acid | C18:1 | 12.09 | 13.83 | 12.58 | 11.42 | 14.00 | 11.96 | 12.01 | 13.12 | 10.77 |
| Linoleic Acid | C18:2ω-6 | 56.80 | 56.02 | 56.27 | 56.78 | 56.21 | 57.34 | 55.97 | 56.22 | 56.24 |
| γ-Linolenic Acid | C18:3 | 4.11 | 2.43 | 3.31 | 3.61 | 3.44 | 3.60 | 3.38 | 3.41 | 4.50 |
| | ω-6 | | | | | | | | | |
| α-Linolenic Acid | C18:3 | 13.57 | 14.44 | 14.53 | 14.70 | 13.28 | 13.85 | 15.47 | 14.05 | 14.91 |
| | ω-3 | | | | | | | | | |
| Stearidonic Acid | C18:4 | 0.94 | 0.63 | 0.84 | 0.96 | 0.82 | 0.90 | 0.91 | 0.83 | 1.12 |
| | ω-3 | | | | | | | | | |
| Arachidic Acid | C20:0 | 0.72 | 0.71 | 0.69 | 0.71 | 0.64 | 0.67 | 0.67 | 0.77 | 0.74 |
| Eicosenoic Acid | C20:1 | 0.37 | 0.36 | 0.33 | 0.36 | 0.34 | 0.36 | 0.33 | 0.38 | 0.36 |
| Eciosadienoic Acid | C20:2 | 0.08 | 0.07 | 0.10 | 0.10 | 0.08 | 0.09 | 0.09 | 0.08 | 0.11 |
| Behenic Acid | C22:0 | 0.29 | 0.28 | 0.29 | 0.29 | 0.27 | 0.24 | 0.29 | 0.33 | 0.30 |
| Lignoceric Acid | C24:0 | 0.13 | 0.10 | 0.12 | 0.13 | 0.12 | 0.12 | 0.14 | 0.15 | 0.15 |
| EFAs | | 70.37 | 70.46 | 70.80 | 71.47 | 69.49 | 71.20 | 71.44 | 70.27 | 71.15 |
| SFAs | | 11.90 | 12.07 | 11.88 | 11.93 | 11.70 | 11.76 | 11.71 | 11.78 | 11.84 |
| UFAs | | 88.10 | 87.93 | 88.12 | 88.07 | 88.30 | 88.24 | 88.29 | 88.22 | 88.16 |
| MUFAs | | 12.60 | 14.33 | 13.06 | 11.92 | 14.47 | 12.45 | 12.47 | 13.62 | 11.27 |
| PUFAs | | 75.51 | 73.60 | 75.05 | 76.15 | 73.82 | 75.79 | 75.82 | 74.60 | 76.89 |
| w6/w3 ratio | | 4.20 | 3.88 | 3.88 | 3.86 | 4.23 | 4.13 | 3.62 | 4.01 | 3.79 |

balanced and rich source of dietary omega-6 and -3 essential fatty acids, and appears to be a potentially nutritious food source. The ω -6/ ω -3 ratio of 3.5 in oil was reported from hemp variety planted in Seville, Spain [24]. The results of this study revealed the oil in hempseed from Kansas were high in PUFA (73.60–76.89%).

 γ -Linolenic acid (GLA, C18:3n-6) is generally derived from linoleic acid and serves as an intermediary for the formation of long-chain polyunsaturated fatty acids and eicosanoids. Eicosanoids are shortlived, hormone-like substances with an important role in the control of vital processes, such as inflammation control, and initiation of contractions during delivery. Relatively high GLA ranged from 2.43 to 4.50% were observed in this study, compared to reported approximately reported 2% GLA in other published data (Matthäus et al., 2005). In the tested hempseeds, stearidonic acid (SDA, C18:4n-3) was not in such high percentages as GLA and varied from 0.63 to 1.12%. Although stearidonic acid seems to present a very limited amount in domesticated plants, it can function as an important human dietary component of hempseed oil, especially in combination of GLA. SDA is a polyunsaturated fatty acid consists of the first metabolite of α -linolenic acid in the metabolic pathway leading to longer chain omega-3 fatty acids, eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3).

3.4. Effect of genotype on the antioxidant properties of hempseed protein hydrolysates

Hydrolysate yield, degree of hydrolysis, and antioxidant properties (TPC, DPPH, and metal chelating) of the nine HPHs from different hempseed varieties are summarized in Table 4. The sequential hydrolysis of hempseed proteins with pepsin and pancreatin resulted in high yield of hydrolysates, between 68.7 and 96.9% depending on the hempseed variety. The highest hydrolysate yield was observed for Hlukhivs'ki 51, while Fedora 17 had the lowest hydrolysate yield. The DH values of the nine HPHs were in a similar range of 20-23%, and TPC values were also very close, ranged from 0.42 to 0.48 mg GAE/g. However, the DPPH scavenging activities varied greatly (0.37-28.78%) for the hydrolysates from different hempseed varieties. Tygra exhibited the highest DPPH value of 28.78%, followed by CFX1 (26.85%) and Hlukhivs'ki 51 (22.33%). USO31 showed the lowest DPPH scavenging activity. For the metal chelating activity, CFX1 hydrolysate exhibited the highest value of 42.65%, followed by Helena, Fedora 17, and Hlukhivs'ki 51. Previous studies reported that antioxidant properties of protein hydrolysates are largely dependent on the type and specificity of protease, hydrolysis conditions, degree of hydrolysis, and molecular features of the peptide

Table 4

Antioxidant activities of nine hempseed protein hydrolysates.

| Sample | Yield % | DH (%) | DPPH% | TPC (mg GAE equiv./g) | Metal chelating capacity % |
|----------------|---------|------------------------------------|------------------------------------|-----------------------------------|------------------------------------|
| Fedora 17 | 68.7 | 22.47 ± 0.67 | 10.71 ± 0.64 | 0.46 ± 0.04 | 39.02 ± 0.55 |
| Helena | 77.1 | 21.58 ± 0.22 | 13.34 ± 0.50 | $\textbf{0.48} \pm \textbf{0.01}$ | $\textbf{39.58} \pm \textbf{1.49}$ |
| Hlukhivs'ki 51 | 96.9 | 21.26 ± 0.80 | 22.33 ± 0.31 | 0.47 ± 0.01 | $\textbf{38.40} \pm \textbf{1.40}$ |
| Felina 32 | 92.3 | $\textbf{20.94} \pm \textbf{0.19}$ | 21.43 ± 0.56 | $\textbf{0.47} \pm \textbf{0.01}$ | $\textbf{36.45} \pm \textbf{2.24}$ |
| Tygra | 70.1 | 19.43 ± 0.14 | $\textbf{28.78} \pm \textbf{0.31}$ | $\textbf{0.48} \pm \textbf{0.01}$ | $\textbf{28.85} \pm \textbf{1.19}$ |
| Hlesia | 82.4 | 21.48 ± 0.72 | $\textbf{7.93} \pm \textbf{0.68}$ | $\textbf{0.48} \pm \textbf{0.01}$ | $\textbf{28.64} \pm \textbf{1.51}$ |
| CRS1 | 86.1 | 20.77 ± 0.27 | 13.34 ± 0.70 | $\textbf{0.47} \pm \textbf{0.01}$ | 38.19 ± 2.72 |
| USO31 | 79.7 | 20.68 ± 0.64 | $\textbf{0.37}\pm\textbf{0.00}$ | $\textbf{0.47} \pm \textbf{0.01}$ | $\textbf{28.64} \pm \textbf{1.87}$ |
| CFX1 | 91.0 | 20.81 ± 0.60 | 26.85 ± 7.04 | 0.42 ± 0.01 | $\textbf{42.65} \pm \textbf{1.12}$ |

domains (peptide sequence, amino acid composition, molecular size, etc.) released during hydrolysis (Aluko et al., 2008; Kim et al., 2007; Udenigwe et al., 2009). Hydrophobic, branched chain, or aromatic amino acid residues, such as Phe, Ile, Leu, Pro may have a greater contribution to the antioxidant capacity of peptides [18,25,26]. [17] reported that the identified peptide sequences from hempseed protein hydrolysate with higher antioxidant capacity contained about 80% hydrophobic amino acid residues, 15% aromatic amino acid residues, and 28% branched chain amino acid residues. Besides, peptides with lower molecular weight usually exhibited better antioxidant capacity due to better accessibility to be adsorbed to the oxidative agents [27,28]. [17] found the peptides of <1 kDa and 3–10 kDa fractions from hempseed protein hydrolysates had the highest DPPH radical scavenging activities.

4. Conclusions

Hempseed oil contains high portions of unsaturated fatty acids such as linoleic and α -linolenic acid, which comprised about 80% of total fatty acids. Hempseed show a well-balanced and rich source of dietary omega-6 and -3 essential fatty acids, and appears to be a potentially nutritious food source, in addition to a great source of essential amino acids found in easily digestible protein. Antioxidant properties of protein hydroly-sates are found to largely depend on hempseed varieties with the DPPH scavenging activities varied greatly from 0.37 to 28.78%. The results provided comprehensive information which could be used for the development of new and improved hemp hybrids for human food application.

Declaration of competing interest

No Conflict of interesting.

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