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Characterization of Byproducts Originating from Hemp Oil Processing

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ABSTRACT: Valorization of hemp seed meal, a byproduct of hemp oil processing, was performed by measuring the distribution of nutritional and antinutritional compounds in different hemp seed meal fractions. According to chemical composition, two cotyledon-containing fractions (>180 and <180 μm) were significantly richer in protein ($p < 0.05$) (41.2% ± 0.04% and 44.4% ± 0.02%, respectively), lipid (15.1% ± 0.02% and 18.6% ± 0.04%, respectively), and sugar content (4.96% ± 0.11% and 3.46% ± 0.08%, respectively) in comparison to the hull-containing fractions (>350 and >250 μm), which were significantly richer in crude fiber content (29.5% ± 0.04% and 21.3% ± 0.03%, respectively). The free radical scavenging capacity (IC_{50}) of fraction extracts increased ($p < 0.05$) with increasing mean particle size (from 17.18 ± 0.59 to 5.29 ± 0.30 mg/mL). Cannabisin B and *N-trans*-caffeoyltyramine were the most abundant phenolic compounds in the hull fractions (from 267 ± 15.9 to 287 ± 23.1 mg/kg), while cotyledon fractions had higher content of catechin (from 313 ± 12.4 to 744 ± 22.2 mg/kg) and *p*-hydroxybenzoic acid (from 124 ± 6.47 to 129 ± 8.56 mg/kg ($P < 0.05$). Well-balanced ω-6 to ω-3 fatty acid ratio (3:1) was determined in all fractions. Antinutrients (trypsin inhibitors, phytic acid, glucosinolates, and condensed tannins) were mostly located in the cotyledon fractions. These findings indicate that the separation of hemp seed meal into different fractions could be used to concentrate valuable target compounds and consequently facilitate their recovery.

KEYWORDS: *hemp meal, fractionation, nutritive and antinutritive value, antioxidant potential*

INTRODUCTION

Food waste, mostly considered an environmental problem, has been recognized lately as an abundant source of different nutraceuticals and valuable compounds.[1] Oilseed processing, whether performed by solvent extraction or mechanical pressing, generates a significant amount of waste consisting of peels, seeds, defatted oil seed meals, and oil sludge. Due to high amounts of proteins, dietary fibers, and other bioactive compounds that provide positive health benefits when consumed, oil seed meals have been identified as an interesting byproduct suitable for valorization as either human food or feed.[2] One of the promising, but not widely investigated, oil seed meals is the remainder of hemp seed (*Cannabis sativa* L.) processing. Although primarily grown for hemp fiber used for production of durable fabrics and specialty papers, industrial hemp has been attracting growing interest worldwide for oil production. It has been recognized as a new, underdeveloped industrial oilseed crop in the European Union, in contrast to conventional oil crops such as rapeseed, sunflower, castor bean, and flax.[3] It must be noted that industrial hemp is characterized by trace amounts of δ-9-tetrahydrocannabinol (THC) (<0.3%), which cannot cause any intoxication, and therefore it is suitable for agricultural production. Moreover, THC and other cannabinoids have been recognized as potent lipophilic antioxidants, which have been related to a certain therapeutic potential.[4] Due to the uniqueness of its composition, hemp oil has been positioned as a highly valuable product usable in food, pharmaceutical, nutraceutical, and cosmetic industries, thus justifying hemp processing, even if its oil yields are lower than the conventional oilseeds.[4,5] The beneficial effects of hemp seed and its phytochemical character are mainly attributed to the to high content of polyunsaturated, essential fatty acids (over 80% in hemp seed oil), particularly linoleic (ω-6) and α-linolenic (ω-3), with the 3:1 ratio being perfectly balanced for human nutrition Its unsaponifiable fraction also is an important source of interesting minor compounds such as β-sitosterol, campesterol, phytol, cycloartenol, and γ-tocopherol.[4−7]

The hemp seed protein edestin is of high biological value due to its structural similarity to serum globulins and its amino acid composition representing all essential amino acids.[4,8,9] The beneficial health effects of hemp (oil, seed) consumption in humans reported so far are related to its antihypertensive and hemostatic roles and to lowering the total-to-HDL cholesterol ratio.[10]

Byproducts of hemp processing have the potential to be used as ingredients in the formulation of specialty products for human consumption.[11,12] However, the content of different nutritional and antinutritional compounds in hemp meal has not yet been reported. Therefore, the main objective of this study was to characterize the types and content of nutritional and antinutritional compounds in hemp meal and to determine to what extent the separation of hemp meal by sieving affected their distribution between the resulting fractions. Our results may provide guidelines for the utilization of hemp meal either directly in the production of value-added products or as a source of bioactive compounds for their isolation and production.

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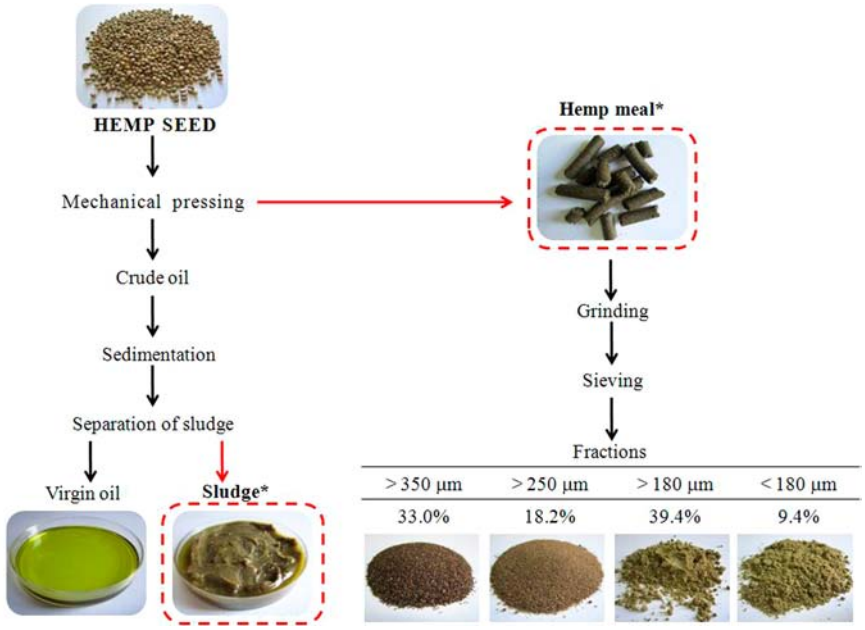

Figure 1. Simplified processing flow diagram for mechanical hemp oil pressing indicating byproducts generated during processing and the yield of hemp meal fractions.

MATERIALS AND METHODS

Chemicals. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, gallic acid, protocatechuic acid, caffeic acid, vanillic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, myricetin, rosmarinic acid, *trans*-cinnamic acid, naringenin, luteolin, kaempferol, apigenin, phytic acid dodecasodium salt, (+)-catechin, vanillin, BAPA (*N*-benzoyl-DL-arginine *p*-nitroanilide hydrochloride), trypsin, sinigrin hydrate, ecteola cellulose, Supelco 37 component fatty acid methyl ester mix, 2,2′-bipyridine, palladium(II) chloride, ammonium iron(III) sulfate dodecahydrate, and tris(hydroxymethyl) aminomethane were purchased from Sigma−Aldrich GmbH (Sternheim, Germany). Methanol (HPLC, gradient-grade), quercetin, formic acid (HPLC-grade), *n*-heptane (GC-grade), and 50% boron(III) fluoride in methanol were purchased from Merck (Darmstadt, Germany). Anhydrous methanol and sodium chloride were obtained from Avantor Materials (Gliwice, Poland). Thioglycolic acid was purchased from Kemika (Zagreb, Croatia). Calcium chloride dehydrate, dimethyl sulfoxide, methanol, and other chemicals used for proximate composition were of p.a. (pro analysis) quality and obtained from Lach-Ner (Neratovice, Czech Republic). Water used throughout the experiments was purified on a Millipore Elix UV and simplicity water purification system (Milford, MA).

Materials. Hemp meal, a byproduct remaining after cold mechanical pressing of hemp seeds, was supplied by the company Svet Konoplje (Kisač, Serbia). Cannabinoid-free hemp variety Helena, bred by the Institute of Field and Vegetable Crops, Novi Sad, Serbia, was grown in 2013 in the South Bačka district in the northern part of Serbia. Hemp flour was obtained by grinding hemp meal in a laboratory mill, Foss Knifetec 1095 (Foss, Hillerød, Denmark), fitted with tubing to allow circulation of water to cool the sample during milling. Ground hemp meal was separated into four fractions of different particle size by use of a universal laboratory sifter (Bühler AG, Uzwil, Switzerland) equipped with a stack of sieves of decreasing mesh size (>350 μm, >250 μm, >180 μm, and <180 μm). Along with the individual fractions, the whole ground hemp meal was analyzed.

The hemp meal extracts were prepared for subsequent determination of DPPH$^{\bullet}$ free radical scavenging activity and phenolic compounds by an HPLC method as follows: hemp meal was extracted with methanol/water mixture (80:20 v/v), with the ratio of raw materials to methanol solution 1:80. After treatment in an ultrasonic bath at room temperature for 10 min, the extracts were macerated for 2 h, filtered through a filter paper (Whatman, grade 4 Chr), and dried by vacuum evaporation at 40 °C. The dried extracts were redissolved in methanol (HPLC-grade) and stored at −4 °C until further use.

Color Measurements. Color measurements were carried out in five replicates on a Minolta Chroma Meter CR 400 colorimeter equipped with accessories for granular materials attachment CR-A50 (glass light-protection tube with plate 40 mm CR-A33b; Konica Minolta Sensing Inc., Japan). The instrument was calibrated against a standard light white reference tile, and the measurements were conducted under standard illuminant D65. The results were recorded according to the CIELab color system, where the determined parameters were expressed as L^* describing lightness ($L^* = 0$ for black, $L^* = 100$ for white), a^* describing intensity in greenness/redness ($a^* < 0$ for green, $a^* > 0$ for red), and b^* describing intensity in blueness/yellowness ($b^* < 0$ for blue, $b^* > 0$ for yellow). Moreover, the total color difference (ΔE) between whole hemp meal and separated hemp meal fractions was expressed as

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where ΔL is the lightness difference, Δa is the redness/greenness difference, and Δb is the yellowness/blueness difference. For $\Delta E < 1$, color differences were not considered obvious to the human eye; for $1 < \Delta E < 3$, color differences were not considered appreciated by the human eye; and for $\Delta E > 3$, color differences were considered obvious to the human eye.[13]

Chemical Composition. The moisture content of hemp meal fractions was determined by oven-drying to a constant mass at 105 °C. Crude protein, crude lipid, crude fiber, ash, and total sugars content were determined according to AOAC standard methods.[14]

DPPH$^{\bullet}$ Free Radical Scavenging Activity. Free radical scavenging activity against the stable DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical was determined spectrophotometrically following the procedure of Espin et al.[15] The IC_{50} value (milligrams per milliliter), defined as the mass concentration of an antioxidant extract that was required to quench 50% of the initial DPPH$^{\bullet}$ under the given experimental conditions, was obtained by interpolation from linear regression analysis.

Separation and Determination of Phenolic Compounds. Determination of phenolic compounds was performed by a single rapid resolution reverse phase HPLC method as previously described by Mišan et al.[16]

Fatty Acid Determination. Fatty acid composition of the samples was expressed as fatty acid methyl esters (FAMEs). Lipids were

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Table 1. Color Parameters of Different Hemp Meal Fractions[a]

color parameter[b]	hemp meal fractions				whole meal
	>350 μm	>250 μm	>180 μm	<180 μm	
L^*	28.7 ± 1.11 a	39.9 ± 1.02 b	49.9 ± 0.23 d	53.5 ± 0.23 e	46.0 ± 0.67 c
a^*	3.68 ± 0.19 c	3.92 ± 0.14 c	1.59 ± 0.14 b	1.15 ± 0.01 a	1.22 ± 0.09 a
b^*	7.44 ± 0.29 a	12.6 ± 0.46 b	20.5 ± 0.30 d	21.3 ± 0.30 d	17.1 ± 0.59 c
ΔE	19.9 ± 1.72 b	8.00 ± 1.94 a	5.28 ± 0.85 a	8.61 ± 0.93 a	

[a]Values are means ± SD. Means in the same row followed by different letters are significantly different ($P < 0.05$). [b]L^*, lightness; a^*, greenness/redness; b^*, blueness/yellowness, ΔE, total color difference between whole meal and single fraction.

Table 2. Proximate Analysis of Hemp Meal Fractions[a]

	hemp meal fractions				whole meal
	>350 μm	>250 μm	>180 μm	<180 μm	
moisture content (%)	6.98 ± 0.01 b	6.63 ± 0.04 a	7.39 ± 0.04 c	7.34 ± 0.02 c	7.88 ± 0.06 d
protein content (%)	10.6 ± 0.10 a	20.3 ± 0.25 b	41.2 ± 0.04 d	44.4 ± 0.02 e	27.9 ± 0.12 c
lipid content (%)	8.26 ± 0.02 a	10.0 ± 0.05 b	15.1 ± 0.02 d	18.6 ± 0.04 e	11.8 ± 0.01 c
total sugar content (%)	<0.05 a	0.56 ± 0.08 a	4.96 ± 0.11 d	3.46 ± 0.08 c	1.49 ± 0.08 b
ash content (%)	3.46 ± 0.02 a	5.51 ± 0.06 b	9.60 ± 0.01 d	9.83 ± 0.01 e	6.74 ± 0.02 c
crude fiber content (%)	29.5 ± 0.04 e	21.3 ± 0.03 d	7.13 ± 0.04 b	4.96 ± 0.01 a	17.3 ± 0.03 c

[a]Values are means ± SD. Means in the same row followed by different letters are significantly different ($P < 0.05$).

extracted with chloroform/methanol (2:1 v/v) mixture following the Folch extraction procedure,[17] and the obtained extracts were dried by vacuum evaporation at 40 °C. Methyl esters were prepared from the extracted lipids by transesterification with 14% boron(III) fluoride in methanol.[18] The obtained samples were analyzed by a GC Agilent 7890A system with flame-ionization detector (FID), autoinjection module for liquid samples, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 μm). Helium was used as a carrier gas (purity >99.9997 vol %, flow rate = 1.26 mL/min). The fatty acids peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl ester mix (Sigma–Aldrich) and with data from an internal data library based on previous experiments. Results were expressed as the mass of fatty acid or fatty acid group (grams) in 100 g of fatty acids.

Antinutritional Factors. Phytic acid was extracted from defatted flour with 0.2 M HCl and determined according to Haug and Lantzsch.[19] Tannins were extracted from defatted material with 70% acetone, evaporated to dryness, and then resuspended in methanol. Condensed tannins were determined by the vanillin method (absorbance at 500 nm) with catechin as a standard.[20] Trypsin inhibitors were extracted from defatted flour with 0.01 M NaOH (pH adjusted to 8.4–10.0). Trypsin inhibitor activity was measured according to Hamerstrand et al.[21] with *N*-benzoyl-DL-arginine *p*-nitroanilide hydrochloride (BAPA) as a substrate for trypsin. One unit of trypsin inhibitor was defined as 0.01 decrease in absorbance at 410 nm under the assay conditions compared with the control (without inhibitor).The glucosinolates content was determined according to MSZ-08-1908.[22] The assay is based on measurement of absorbance of Pd–glucosinolate complex at 425 nm. A standard curve was constructed with synigrine as a standard.

Statistical Analysis. The results were analyzed by one-way analysis of variance with Fisher's least significant difference (LSD), which was performed by Statistica 12.0 (Statsoft, Tulsa, OK). The significance of differences among the mean values is indicated at the 95% confidence level.

RESULTS AND DISCUSSION

Physical Characteristics of Hemp Meal Fractions. Hemp seed oil is a niche market product, mainly produced in small-scale artisan-type plants by mechanical pressing with a screw press. Although this type of oil production does not involve any refining procedures, the oil is purified by simple sedimentation to remove contaminants, such as fine pulp, resins, and water. Although this type of processing appears to be rather simple, the disposal of waste generated throughout the processing can pose a number of problems to the processors having not enough resources to cope with it.

Figure 1 shows the simplified processing flow diagram of mechanical hemp oil pressing. During the cold oil processing, different byproducts could be distinguished: sludge or sediment and press cake or meal. The sludge represents a complex mixture comprising small pieces of pressed seed and hull as well as phospholipids, oil, waxes, and minor amounts of other constituents like phytosterols, tocopherols, pigments, and fatty acids. Hemp seed press cake or meal is a byproduct obtained after the seeds are pressed to extract the oil.

The obtained hemp meal was milled with simultaneous sample cooling to avoid the decomposition of thermolabile components. Subsequently, it was fractionated by sieving, which enabled the separation on the basis of the differences in particle size. Generally, two groups of fractions were identified: coarse fractions of particle sizes >350 and >250 μm, containing hull particles, and fine fractions of particle sizes >180 and <180 μm, containing predominantly cotyledon particles. The yield of these fractions was determined relative to the amount of starting material (Figure 1). The fraction with the highest yield (39.4%) was that with particle size in the range 180–250 μm, which, according to the appearance and structure was predominantly composed of ground cotyledon particles, while sieving yielded 33.0% coarse fraction consisting of hulls. Different particle size distribution was due to different grinding behavior of cotyledons and hulls, which were of higher elasticity due to higher content of structural carbohydrates. which made them more resistant to the grinding.[23] The obtained fractions were clearly separated according to their color, which was quantified by total color difference (Table 1).

The obtained results indicated the existence of a significant difference in lightness (L^*), ranging from 28.74 for the coarsest fraction (>350 μm) to 53.46 for the finest fraction (<180 μm). The difference in lightness can be attributed to the difference in scattering effect by the particles of various sizes. Thus the finest fraction, with the smallest particles and increased surface area, scattered more light and appeared to be lighter, unlike the

fractions with larger particle size.[24] The redness of the separated fractions increased with increasing the particle size and varied between 1.15 for the finest fraction (<180 μm) and 3.92 for the medium coarse fraction (>250 μm). Conversely, the yellowness of the fractions increased with decreasing particle size, being highest for the finest fraction (<180 μm). Significant differences in redness (a^*) and yellowness (b^*) were observed between coarse and fine fractions. The increase in greenness with decreasing particle size could be associated with the presence of chlorophyll pigments, which were found to be abundant in hemp oil.[25] Namely, fractions with smaller particle size (>180 μm and <180 μm) had significantly higher oil content in comparison to other meal fractions (Table 2), which resulted in increased chlorophyll content and thus led to pronounced green color of these fractions. Consequently, the increase in yellowness of the fractions rich in oil (lipid; Table 2) could be related to high content of γ-tocopherol present in hemp oil.[25] In order to quantify the difference in the appearance between separated fractions, the total color difference was calculated relative to the whole hemp meal. Since the total color difference was greater than 3 between all the fractions and the whole hemp meal, an indication of the perception of a color difference by human eye was confirmed.

Proximate Composition of Hemp Meal Fractions. The proximate composition of hemp meal is primarily determined by the quality of starting raw material, itself dependent on variety, locality, applied agrotechnical measures, weather conditions, and processing parameters. The proximate composition of hemp meal fractions is shown in Table 2. The moisture content of hemp meal fractions varied between 6.98% and 7.88%, being significantly the lowest ($p < 0.05$) for hull-containing fractions. When it is considered that the cotyledons are the main reserve of proteins, carbohydrates, and oils, cotyledon fractions (of particle size <250 μm) appeared to be of the richest nutritive composition. More specifically, protein content of hemp meal fractions varied between 10.62% for the coarsest fraction and 44.36% for the finest cotyledon fraction. The same trend was observed for oil and total sugar content, ranging between 8.26% and 18.60% and between 0.00% and 4.96%, respectively. Differentiation of hemp meal fractions in relation to protein, fat, total sugar, ash, and crude fiber content was achieved by sieving as previously indicated by Maaroufi et al.[23] for pea flour and Sreerama et al.[26] for chickpea and horse gram flours.

The protein content in the whole meal was lower than that reported by Callaway[4] and Tang et al.[27] in hemp meal, but it progressively increased in cotyledon-containing fractions. The protein content in whole meal was also lower than that determined in defatted soy flour, as reported by Sudha et al.[28] as well as in rapeseed and soybean meal.[29] The results of crude fiber content (which comprise only insoluble fibers) and ash content in hemp meal were in accordance with the results obtained for hemp seed residue after oil extraction reported by Anwar et al.[30] Crude fiber content varied from 4.96% to 29.54% between the hemp meal fractions. Large quantities of crude fibers were found in coarse meal fractions and consequently were significantly lower in cotyledon fractions. Oil content in the whole meal was consistent with the results reported by Callaway.[4]

Antioxidant Compounds and Activities of Hemp Meal Fractions. The radical-scavenging capacities (IC_{50} values) of fraction extracts are presented in Figure 2. IC_{50} values significantly differed ($p < 0.05$) between hemp meal fractions,

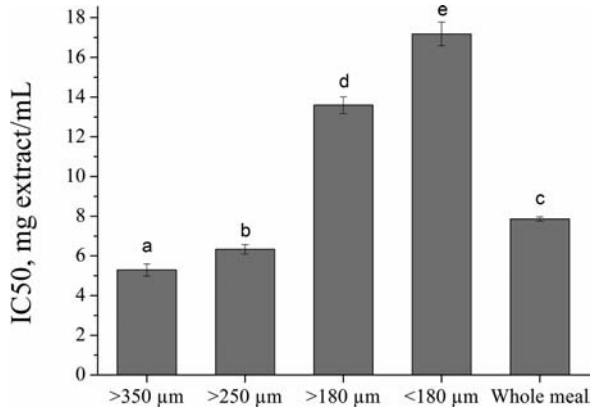

Figure 2. Radical scavenging capacities (IC_{50} values) of fraction extract.

the lowest being the coarsest fraction (>350 μm), indicating the strongest radical-scavenging activity (5.29 mg/mL), unlike that of the finest cotyledon fraction (<180 μm), which appeared to have the weakest radical-scavenging activity (17.18 mg/mL). Although reported for selected pulses, the same distribution of the radical-scavenging capacity was observed by Duenas et al.[31] and Sreerama et al.[32] Moreover, fractions originating from the peripheral parts of soybean seed manifested higher antioxidant capacity than those originating from cotyledons.[33]

The antioxidant potential of hemp oil has been recently reported, as it has been recognized as one of the nontraditional vegetable oils not so long ago introduced to the market.[34] Literature data on phenolic profile of hemp products other than hemp oil are very limited, except that published by Chen et al.,[35] who reported only phenolic compounds with major significance. Phenolic compounds of hemp meal fractions are presented in Table 3. Apart from *N-trans*-caffeoyltyramine and cannabisin B, identification of phenolic compounds in crude extracts was performed by comparing the retention times and spectra of phenolic compounds of extracts with those of the corresponding external standards. *N-trans*-caffeoyltyramine (UV λ_{max} in MeOH 220, 294, 318 nm) and cannabisin B (UV λ_{max} in MeOH 220, 245, 283, 335 nm) were identified on the basis of their spectral characteristics, as they were previously isolated and identified as phenolic compounds with predominant radical-scavenging activity in hemp seed.[35] The identification of *N-trans*-caffeoyltyramine and cannabisin B within the present research, solely based on UV spectral characteristics, has been only tentative. However, the presence of previously known and new lignanamides and cannabisins was recently reported by Lesma et al.,[6] who performed a thorough investigation of the metabolic content of hemp seeds in various cultivars, making an important contribution to their NMR spectroscopic characterization. Quantification of phenolic compounds was based on external standards calibration except for *N-trans*-caffeoyltyramine and cannabisin B, which were expressed in *trans*-cinnamic acid equivalents due to the lack of corresponding external standards. The determination of distribution of *N-trans*-caffeoyltyramine and cannabisin B between our hemp meal fractions revealed that they were abundantly present in the hull-containing fractions, in accordance with the results of Chen et al.[35] Apart from those, important phenolic compounds detected in defatted hemp meal were catechin and *p*-hydroxybenzoic acid in the cotyledon fractions, and ferulic and sinapic acid in the hull

Table 3. Phenolic Compounds of Hemp Meal Fractions[a]

	hemp meal fractions				
phenolic compd	>350 μm	>250 μm	>180 μm	<180 μm	whole meal
gallic acid	0.43 ± 0.06 a	0.63 ± 0.05 b	0.79 ± 0.04 c	1.06 ± 0.05 d	0.82 ± 0.08 c
protocatechuic acid	14.5 ± 1.67 a	22.2 ± 1.88 b	31.7 ± 2.28 cd	36.0 ± 2.04 d	28.2 ± 2.47 c
p-hydroxybenzoic acid	33.3 ± 3.20 a	29.8 ± 1.64 a	129 ± 8.56 c	124 ± 6.47 c	78.6 ± 8.00 b
catechin	107 ± 12.4 a	221 ± 13.0 b	744 ± 22.2 e	313 ± 12.4 c	498 ± 35.9 d
vanillic acid	0.41 ± 0.04 ab	0.43 ± 0.02 b	0.55 ± 0.03 c	0.54 ± 0.03 c	0.35 ± 0.04 a
ferulic acid	88.4 ± 6.05 c	82.0 ± 6.26 c	9.67 ± 0.74 a	4.72 ± 0.55 a	47.4 ± 5.37 b
sinapic acid	66.8 ± 5.48 d	58.3 ± 2.39 c	26.4 ± 2.21 b	17.3 ± 1.45 a	22.2 ± 1.89 ab
N-trans-caffeoyltyramine[b]	287 ± 23.1 d	267 ± 15.9 d	54.6 ± 3.17 b	41.7 ± 3.42 a	152 ± 11.2 c
cannabisin B[b]	153 ± 8.62 e	117 ± 3.22 d	25.7 ± 1.15 b	4.27 ± 0.39 a	64.9 ± 1.94 c

[a]Values are means ± SD and are given in milligrams per kilogram dry sample. Means in the same row followed by different letters are significantly different ($P < 0.05$). [b]Expressed in *trans*-cinnamic acid equivalents.

Table 4. Fatty Acid Composition in Different Hemp Meal Fractions[a]

	hemp meal fractions				
fatty acid	>350 μm	>250 μm	>180 μm	<180 μm	whole meal
16:0	6.48 ± 0.01 a	6.54 ± 0.03 ab	7.05 ± 0.08 d	6.90 ± 0.01 c	6.63 ± 0.03 b
18:0	3.26 ± 0.04 b	3.18 ± 0.01 a	3.86 ± 0.02 d	3.69 ± 0.01 d	3.30 ± 0.03 b
18:1 n9c	13.9 ± 0.08 c	12.7 ± 0.02 a	13.6 ± 0.51 bc	13.9 ± 0.01 c	13.0 ± 0.47 ab
18:2 n6c	54.6 ± 0.32 ab	55.4 ± 0.01 c	54.2 ± 0.26 ab	54.1 ± 0.03 a	54.8 ± 0.30 b
20:0	1.04 ± 0.01 b	1.00 ± 0.01 a	1.40 ± 0.01 e	1.31 ± 0.01 d	1.10 ± 0.01 c
18:3 n6	2.64 ± 0.02 b	2.75 ± 0.01 c	2.57 ± 0.02 a	2.61 ± 0.01 a	2.76 ± 0.01 c
18:3 n3	17.6 ± 0.09 b	18.4 ± 0.01 c	17.3 ± 0.13 a	17.5 ± 0.04 b	18.4 ± 0.10 c

[a]Values are means ± SD and are given in grams per 100 g total fatty acids. Means in the same row followed by different letters are significantly different ($P < 0.05$).

Table 5. Content and Ratio of Saturated and Unsaturated Fatty Acids in Different Hemp Meal Fractions[a]

	hemp meal fractions				
fatty acid fraction[b]	>350 μm	>250 μm	>180 μm	<180 μm	whole meal
SFA	10.8 ± 0.07 a	10.7 ± 0.03 a	12.3 ± 0.09 d	11.9 ± 0.01 c	11.0 ± 0.07 b
MUFA	14.3 ± 0.49 c	12.7 ± 0.02 a	13.6 ± 0.51 abc	13.9 ± 0.01 bc	13.0 ± 0.47 ab
PUFA	74.9 ± 0.42 a	76.6 ± 0.02 b	74.1 ± 0.42 a	74.2 ± 0.01 a	76.0 ± 0.40 b
UFA	89.2 ± 0.07 d	89.3 ± 0.03 d	87.7 ± 0.09 a	88.1 ± 0.01 b	89.0 ± 0.07 c
PUFA/SFA ratio	6.94	7.14	6.02	6.24	6.88

[a]Values are means ± SD and are given as grams per 100 g total fatty acids. Means in the same row followed by different letters are significantly different ($P < 0.05$). [b]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

fractions. The content of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and catehin increased from hull fraction to cotyledon fraction, thus disproving the fact that hull is necessarily the main source of natural antioxidants (Table 3). Conversely, the content of ferulic and sinapic acids in the hull fractions was significantly higher than that of cotyledon fractions. However, the content of ferulic and gallic acid was lower than those determined in defatted flaxseed.[36] The content of *p*-hydroxybenzoic acid in all hemp meal fractions was higher than that of coconut, cottonseed, sesame, flax, and peanut flours as reported by Dabrowski and Sosulski.[37] Furthermore, whole hemp meal and cotyledon fractions contained higher content of *p*-hydroxybenzoic acid than that of rapeseed and sunflower flours and a similar level as soybean flour. The content of all determined phenolic compounds was higher than that determined for cold-pressed hemp oil, wherein protocatechuic acid was not detected in hemp oil.[34] The content of sinapic acid was lower than that determined in canola meal.[38]

Fatty Acid Composition of Hemp Meal Fractions. Fatty acid compositions of different hemp meal fractions are provided in Table 4. According to obtained data, fatty acid distribution was mainly uniform across different fractions. The main fatty acids were linoleic (54.09−55.42%), linolenic (17.31−18.42%), and oleic (12.96−13.93%) acids, followed by palmitic (6.48−7.90%), stearic (3.18−3.86%), and γ-linolenic (2.61−2.76%) acids. Similar results were obtained for hemp seed oil according to Teh and Birch[25] and Da Porto et al.[7] Moreover, high content of polyunsaturated acids, especially of linoleic (18:2 n6c) as well as ω-3 linolenic acid in the ratio of approximately 3:1, indicated a positive nutritional profile of all hemp meal fractions. Similar results were reported for fatty acid composition of hemp seed oil.[7]

Results presented in Table 5 show relatively high content of polyunsaturated fatty acids (PUFA) in all fractions. Hemp meal fraction with coarse particles (>250 μm) was characterized by higher content of polyunsaturated fatty acids and consequently lower level of monounsaturated fatty acids (MUFA) in comparison to other hemp meal fractions. Moreover, slightly

Table 6. Distribution of Major Antinutrients in Different Fractions of Hemp Meal[a]

	hemp meal fractions				
antinutrient	>350 μm	>250 μm	>180 μm	<180 μm	whole meal
trypsin inhibitor (TIU/mg of protein)	1.39 ± 0.00 a	1.96 ± 0.04 b	3.70 ± 0.02 d	3.90 ± 0.07 e	2.88 ± 0.09 c
phytic acid (mg/g)	4.36 ± 0.05 a	18.35 ± 0.57 b	21.2 ± 0.11 c	21.4 ± 0.11 c	22.5 ± 0.07 d
glucosinolates (μmol/g)	3.14 ± 0.20 a	3.66 ± 0.08 ab	5.33 ± 0.17 c	5.64 ± 0.30 c	3.80 ± 0.27 b
condensed tannins (mg/g)	0.19 ± 0.04 a	0.33 ± 0.05 b	0.25 ± 0.00 a	0.26 ± 0.00 ab	0.23 ± 0.01 a

[a]Values are means ± SD. Means in the same row followed by different letters are significantly different ($P < 0.05$).

higher content of saturated fatty acids was found in fine hemp meal fractions (>180 μm and <180 μm). Saturated fatty acids (SFA) have been labeled as a possible cause of cancer and coronary heart diseases when present in excessive amounts in human diet. The mean ratio of PUFA/SFA recommended by the British Department of Health is more than 0.45, and WHO/FAO experts have reported guidelines for a "balanced diet" in which the suggested ratio of PUFA/SFA is above 0.40.[39,40] In this respect, all investigated meal fractions exhibited a favorable PUFA/SFA ratio (from 6.02 to 7.14).

Antinutritive Factors in Hemp Meal Fractions. The concentrations of antinutrients in different hemp meal fractions are shown in Table 6. Considerable variability in trypsin inhibitor activity between hemp meal fractions was evident, the lowest being in the fraction containing mainly husk (>350 μm) and the highest for cotyledon fraction. These results were higher than those obtained for watermelon, pumpkin, and paprika seed flour reported by El-Adawy and Taha.[41] Considerable variability in phytic acid content between hemp meal fractions was observed, being the highest in the fine fractions (Table 6). The coarsest fraction (>350 μm) appeared to have the lowest phytic acid content. The phytic acid content was lower than that of canola meal reported by Bell[42] and those determined in hemp seed meal of Italian and French varieties.[43] The presence of certain antinutrients (condensed tannins, polyphenols, trypsin, chymotrypsin, α-amylase inhibitors, oligosaccharides, trypsin inhibitors, phytic acid, tannins, glucosinolates, and saponins) may limit their conversion into edible-grade products and utilization in human nutrition as they influence protein digestibility, organoleptic properties, and bioavailability of macro- and microelements. However, in recent decades an increasing trend of reutilization of plant byproducts in food, cosmetic, and pharmaceutical industries has been observed.[2,29] Apart from the well-known harmful effects of antinutrients, certain health-promoting and disease-preventing properties also have been attributed to them. A preventive impact of phytic acid, phenolics, saponins, protease inhibitors, phytoestrogens, and lignans on diabetes, cardiovascular diseases, and cancer has been demonstrated.[44,45]

Finally, it can be concluded that fractionation by sieving can be used as a processing operation in order to preserve and concentrate target valuable compounds from hemp meal, a byproduct of hemp oil processing. While certain fractions might be used as food functional ingredients, the other fractions, due to increased antinutritive factors, could be used as a substrate for recovery of valuable compounds or in cosmetic and pharmaceutical industries.

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