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AUTHORS: Milica Pojić, Aleksandra Mišan, Marijana Sakač, Tamara Dapčević Hadnađev, Bojana Šarić, Ivan Milovanović, Miroslav Hadnađev

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Characterization of by-product originating from hemp oil processing

Milica **Pojić**[a], Aleksandra **Mišan**[a], Marijana **Sakač**[a], Tamara **Dapčević Hadnađev**[a], Bojana **Šarić**[a], Ivan **Milovanović**[a], Miroslav **Hadnađev**[a*]

[a]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000, Novi Sad, Serbia

[*] Corresponding author. Tel.: +381 214853811; fax: +381 21450725 (M. Hadnađev).

E-mail addresses: milica.pojic@fins.uns.ac.rs (M. Pojić), aleksandra.misan@fins.uns.ac.rs (A. Mišan), marijana.sakac@fins.uns.ac.rs (M. Sakač), tamara.dapcevic@fins.uns.ac.rs (T. Dapčević Hadnađev), bojana.beljkas@fins.uns.ac.rs (B. Šarić), ivan.milovanovic@fins.uns.ac.rs (I. Milovanović), miroslav.hadnadjev@fins.uns.ac.rs (M. Hadnađev)

Abstract

Valorization of hempseed meal, a by-product of hemp oil processing, was performed by measuring the distribution of nutritional and antinutritional compounds in different meal fractions. According to chemical composition, two different streams could be distinguished: cotyledon containing fraction (<250 μm) rich in protein, oil and sugar and hull containing fraction (>250 μm) rich in fiber. The radical scavenging capacity of fraction extracts increased with the increase in mean particle size. Cannabisin B and N trans-caffeoyltyramine were the most abundant phenolic compounds in hull containing fraction, while cotyledon fraction had higher content of catechin and p-hydroxybenzoic acid. Well balanced ω-6 to ω-3 fatty acid ratio (3:1) was determined in all fractions. Antinutrient (trypsin inhibitor, phytic acid, glucosinolates and condensed tannins) were mostly located in cotyledon fraction.

The obtained findings indicated that separation of hemp meal into different fractions could be used to concentrate target valuable compounds and consequently facilitate their recovery.

Keywords: hemp meal, fractionation, nutritients, antinutrients, phenolic compounds, fatty acids

Introduction

Food wastes, mostly considered as environmental problem, are now recognized as valuable sources of different nutraceuticals.[1] Oilseed processing, whether it is performed by solvent extraction or mechanical pressing, generates a significant amount of waste consisting of peels, seeds, defatted oilseed meals and oil sludge. Due to high amount of proteins, dietary fibers and other bioactive compounds which provide positive health benefits when consumed, oilseed meals have been identified as interesting by-product suitable for valorization either as human food or feed.[2] One of the promising, but not widely investigated oil seed meals is that which remains after processing of hemp seeds. Although primarily grown for hemp fiber used for production of durable fabrics and specialty papers, industrial hemp (*Cannabis sativa* L), has been attracting growing interest worldwide for oil production and been recognized as a new, "underdeveloped" industrial oilseed crops in EU in contrast to conventional oil crops such as rapeseed, sunflower, castor bean, and flax.[3] Due to the uniqueness of its composition, hemp oil has been positioned as highly valuable product utilizable in food, pharmaceutical, nutraceutical, and cosmetic industries, thus justifying their processing even with lower oil yields in comparison to conventional oil seeds.[4] The nutritional benefits of hemp seed are related to high content of polyunsaturated, essential fatty acids (over 80%), particularly linoleic (omega-6) and α-linolenic (omega-3) in the ratio 3:1 being perfectly balanced for human nutrition.[5] The hemp seed protein –edestin is of high biological value due to its structure similar to serum globulins and amino acid composition containing all essential amino acids.[4,6,7] The beneficial health effects of hemp (oil, seed) consumption in humans reported so far are related to its antihypertensive and hemostatic role and lowering a total-to-HDL cholesterol ratio.[8]

By-products of hemp processing have the potential to be used as ingredients in the formulation of specialty products for human consumption.[9,10] However, the unified research that indicates the content of different nutritional and antinutritional compounds in them is not available in the existing literature. 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 classification of hemp meal by sieving affected their distribution between the resulting fractions. The knowledge of their distribution may provide guidelines for the utilization of hemp meal either directly in production of value-added products, or as a starting material for isolation and production of bioactive compounds.

Materials and methods

Materials

Hemp meal, a by-product remained after cold mechanical pressing of hemp seeds was supplied by Svet konoplje, Kisač, Serbia. Hemp flour was obtained by grinding of hemp meal using 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 to four fractions of different particle size using a Universal Laboratory Sifter (Bühler AG, Uzwil, Switzerland) equipped with a stack of sieves of decreasing mesh size (>350 μm, >250 μm, >180 μm, <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$^{\cdot}$ free radical scavenging activity and phenolic compounds by HPLC method, as follows: hemp meal was extracted with metanol/water mixture (80:20, v/v), with the ratio of raw materials to methanol solution of 1:80. After the treatment in an ultrasonic bath at room temperature for 10 min, extracts were macerated for 2 h. Extracts were filtered through a filter paper (Whatman, Grade 4 Chr, UK) and dried by vacuum-evaporator at 40 °C. The dried extracts were redissolved in methanol (HPLC grade) and stored at -4 °C until the further use.

Color measurements

Color measurements were carried out in five replicates using 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 conducted under standard illuminant D65. The obtained results were reported in Commission Internationale d' Eclairage L^* (lightness, L^*=0, black; L^*=100, white), a^* (redness-greenness), b^* (yellowness-blueness) colorspace and were expressed as the total color differences (ΔE) between the whole hemp meal and separated hemp meal fractions.

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

where ΔL is the lightness difference (L^*=0, black; L^*=100, white), Δa is the redness difference (redness to greenness, positive to negative values, respectively) and Δb is the yellowness difference (yellowness to blueness, positive to negative values, respectively) values.

If $\Delta E<1$, color differences are not obvious to the human eye; $1<\Delta E<3$, color differences cannot be appreciated by the human eye; and $\Delta E>3$, color differences are obvious to the human eye.[11]

Chemical composition

The moisture content of hemp meal fractions was determined by oven-drying to a constant mass at 105 °C. The crude protein, crude lipid, crude fiber, ash and total sugars content were determined according to AOAC standard methods.[12]

DPPH˙ free radical scavenging activity

Radical-scavenging activity against the stable DPPH˙ (1,1-diphenyl-2-picryl-hydrazyl) radical was determined spectrophotometrically following the procedure of Espin et al.[13] The IC_{50} value (mg/mL), defined as the mass concentration of an antioxidant extract that was required to quench 50% of the initial DPPH˙ 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.[14]

Fatty acid determination

Fatty acid composition of the samples was expressed as fatty acid methyl esters (FAMEs). Lipids were extracted with chloroform:methanol (2:1, v/v) mixture following the Folch extraction procedure,[15] and the obtained extracts were dried by vacuum-evaporation at 40 °C. Methyl esters were prepared from the extracted lipids by transesterifitacion using 14% boron(III)-fluoride in methanol.[16] 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 um). 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, EU) and with data from internal data library, based on previous experiments. Results were expressed as mass of fatty acid or fatty acid group (g) in 100 g of fatty acids.

Antinutritional factors

Phitic acid was extracted from defatted flour with 0.2 M HCl and determined according to Haug and Lantzsch.[17] Tannins were extracted from defatted material using 70% acetone; the samples were evaporated to dryness and then resuspended in methanol. Condensed tannins were determined by the vanillin method (absorbance at 500 nm) using catehin as a standard.[18] 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.[19] using BAPA as a substrate for trypsin. One unit of trypsin inhibitor was defined as 0.01 decreases in absorbance at 410 nm under the assay conditions compared with the control (without inhibitor).The glucosinolate content was determined according to MSZ-08-1908.[20] The assay is based on measurement of absorbance of Pd-glucosinolate complex at 425 nm. A standard curve was constructed using synigrine as a standard.

Statistical analysis

The obtained results were analyzed by one-way analysis of variance with Fisher's LSD, which was performed using Statistica 12.0 (Statsoft, Tulsa, USA). The significance of differences among the mean values was indicated at the 95% confidence level.

Results and discussion

Physical characteristics of hemp meal fractions

Hemp seed oil is a product of a niche market, which is mainly produced in small-scale artisan-type plants by mechanical pressing using a screw press only. This type of oil production does not involve the application of refining procedures, but most often the oil purification is done by simple sedimentation to remove contaminants from oil, such as fine pulp, resins and water. Although this type of processing appears to be 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 flow processing diagram for mechanical hemp oil pressing. During the cold oil processing different by-products 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 by-product obtained after pressing the seeds to extract the oil.

The obtained meal was milled with simultaneous sample cooling to avoid thermolabile component decomposition. Subsequently, it was fractionated by sieving, which enabled the separation of particles on the basis of the differences in particle size. Generally, two groups of particles were identified: group of hull particles constituting coarser fractions (>350 and >250 μm) and group of cotyledon particles constituting finer fractions (>180 and <180 μm). The yield of obtained fractions was determined relative to the amount of starting material (Figure 1). The fraction with the highest yield was that with particle size in the range 180 – 250 μm, which, according to the appearance and structure, was mainly composed of ground cotyledon particles, while sieving yielded 33.0% of coarse fraction consisting of hulls. Different particle size distribution was due to different grinding behaviour of cotyledons and hulls, which were of higher elasticity due to higher content of structural carbohydrates which made them more resistant to the grinding.[21] The obtained fractions were clearly separated according to their colour, 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 to 53.46 for the finest fraction. The difference in lightness can be attributed to the difference in the scattering effect by particles of various sizes. Thus, the finest fraction due to the smallest particles and increased surface area, scattered more light and appeared to be lighter, unlike the fractions of the larger particle size.[22] The redness of the separated fractions increased with the increase in the particle size and varied between 1.15 for the finest fraction and 3.92 for the medium coarse fraction. Conversely, the yellowness of the fractions increased with the decrease in the particle size, being the highest for the finest fraction. The significant differences in redness (a^*) and yellowness (b^*) were observed between coarse and finer fractions. The increase in green component with decrease in particle size could be ascribed to presence of chlorophyll pigments which were found to be abundant in hemp oil.[23] Namely, fractions with smaller particle size (>180 µm and < 180 µm) had significantly higher oil content in comparison to other meal fractions which resulted in increase in chlorophyll content and thus led to pronounced green color of these fractions. Consequently, increase in yellowness of the fractions rich in oil (Table 2) could be related to high content of γ-tocopherol present in hemp oil.[23] 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 separated fractions and the whole hemp, 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 materials being dependent on variety, locality, applied agro-technical measures and weather conditions as well as processing conditions. 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 the significantly lowest ($p<0.05$) for seed coat fractions. Considering the fact that the cotyledons are the main reserve of proteins, carbohydrates and oils, cotyledon fractions (< 250 μm) appeared to be of the richest nutritive composition. More specifically, protein content of hemp meal fractions varied between 10.62% for seed coat fraction and 44.36% for finer cotyledon fraction. The same trend was observed for oil and total sugar content, ranging between 8.26% and 18.60%, and 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.[21] for pea flour and Sreerama et al.[24] 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.[25] in hemp meal and progressively increased in cotyledon containing fraction. The protein content in whole meal was also lower than that determined in defatted soy flour as reported by Sudha et al.[26] as well as in rapeseed and soybean meal.[27] The results of crude fibers content (which comprise only insoluble fibers) and ash content in hemp meal were in accordance with the results obtained for hempseed residue after oil extraction reported by Anwar et al.[28] Crude fiber content varied from 4.96 to 29.54% between the hemp meals fractions. Large quantities of crude fibers were found in coarse meal particles, i.e. in seed coat particles and consequently significantly lower content of crude fibers were determined 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. Obtained IC_{50} values significantly differed ($p<0.05$) between hemp meal fractions, being the lowest for the coarsest fraction indicating it's the strongest radical scavenging activity (5.29 mg/ml), unlike that of finest cotyledon fraction (< 180 μm) which appeared to be the weakest (17.18 mg/ml). Although reported for selected pulses, the same distribution of the radical scavenging capacity results was observed by Duenas et al.[29] and Sreerama et al.[30] Moreover, fractions originating from the peripheral parts of soybean seed manifested higher antioxidant capacity than that originating from cotyledons.[31]

The antioxidant potential of hemp oil has been relatively recently reported as it has been recognized as one of the non-traditional vegetable oils not so long ago introduced to the market.[32] Literature data on phenolic profile of hemp products other than oil are very limited, except that published by Chen et al.[33] 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 MeOH nm: 220, 294, 318) and cannabisin B (UV λ max MeOH nm: 220, 245, 283, 335) 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.[33]

Quantification 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 hemp meal fractions revealed that they were abundantly present in the hull containing fractions, being in accordance with the results of Chen et al.[33] Apart from them, important phenolic compounds detected in defatted hemp meal were catechin and *p*- hydroxybenzoic acid in cotyledon fractions and ferulic and sinapic acid in hull fractions. The content of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid and catehin increased from hull containing fraction to cotyledon containing fraction, thus disproving that hull is necessarily the main source of natural antioxidants (Table 3). Conversely, the content of ferulic and sinapic acids in hull fractions was significantly higher than that of cotyledon containing fractions. However, the content of ferulic and gallic acid was lower than these determined in defatted flaxseed.[34] Other phenolic compounds that were identified and quantified were gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, ferulic acid, sinapic acid and catehin. The content of *p*-hydroxybenzoic acid of all hemp meal fractions was higher than that of coconut, cottonseed, sesame, flax and peanut flours as reported by Dabrowski and Sosulski 1984.[35] Furthermore, whole hemp meal and cotyledon fractions contained higher content of *p*-hydroxybenzoic acid than rapeseed and sunflower flours, while the similar content 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.[32] The content of sinapic acid was lower than that determined in canola meal.[36]

Fatty acid composition of hemp meal fractions

Fatty acid compositions of different hemp meal fractions are provided in Table 4. According to obtained data it could be observed that fatty acid distribution was mainly uniform between different fractions. The main fatty acids were linoleic (54.09-55.42%), linolenic (17.31-18.42), oleic (12.96-13.93), followed by palmitic (6.48-7.90%), stearic (3.18 -3.86%) a γ-linolenic acid (2.61-2.76). Similar results were obtained for hemp seed oil according to Teh and Birch[23] and Da Porto et al.[5] Moreover, high content of polyunsaturated acids especially of linoleic (18:2 n6c) as well as ω-3 linolenic acid in the ratio which was approximately 3:1

indicate on their positive nutritional profile in all hemp meal fractions. Similar results were provided for fatty acid composition of hemp seed oil.[5]

Results presented in Table 5 showed relatively high content of polyunsaturated acids in all fractions. Hemp meal fraction with coarse particles (>250 μm) were characterized with higher content of polyunsaturated acids and consequently lower level of monounsaturated acids in comparison to other hemp meal fractions. Moreover, slightly higher content of saturated fatty acids was found in finer hemp meal particles (>180 μm and < 180 μm). Saturated fatty acids (SFA) have been labeled as a possible cause of cancers and coronary heart disease 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 suggested ratio of PUFA/SFA is above 0.4.[37,38] All of the investigated meal fractions showed a favourable PUFA/SFA ratio (from 6.02 to 7.14).

Antinutritive factors in hemp meal fractions

The concentrations of the antinutrients in different hemp meal fractions are shown in Table 6. Considerable variability in trypsin inhibitor activity between hemp meal fractions was evident, being the lowest in a fraction containing mainly husk and the highest for cotyledon containing fraction. The obtained results were higher than that obtained for watermelon, pumpkin and paprika seed flour by El-Adawy and Taha.[39] Considerable variability in phytic acid content between hemp meal fractions was observed being the highest in the cotyledon fractions (Table 6). Seed coat fraction appeared to be with the lowest phytic acid content. The obtained phytic acid content was lower than that of canola meal reported by Bell[40] and those determined in hempseed meal of Italian and French varieties.[41] Although the presence of certain antinutrients (condensed tannins, polyphenols, trypsin, chymotrypsin, α-amylase inhibitors, oligosaccharides, trypsin inhibitors, phytic acid, tannins, glucosinolates, 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 micro elements, in recent decades an increasing trend of application of oilseed proteins in food, cosmetic and pharmaceutical industries have been observed.[2,27] However, apart from known harmful effects antinutrients have, certain health-promoting and disease preventing properties have been attributed to them. A preventive impact of phytic acid, phenolics, saponins, protease inhibitors, phytoestrogens and lignanson diabetes, cardiovascular diseases and cancer have been demonstrated.[42,43]

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

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Figure captions:

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

Figure 2. The radical scavenging capacities (IC_{50} values) of fraction extracts

Table 1. Colour parameters of different hemp meal fractions

Hemp meal fractions	>350 µm	>250 µm	>180 µm	<180 µm	Whole meal
L^*	28.74±1.11[a]	39.95±1.02[b]	49.94±0.23[d]	53.46±0.23[e]	45.98±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]	12.60±0.46[b]	20.54±0.30[d]	21.35±0.30[d]	17.10±0.59[c]
ΔE	19.92±1.72[b]	8.00±1.94[a]	5.28±0.85[a]	8.61±0.93[a]	-

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

Table 2. Proximate analysis of hemp meal fractions (%)

Hemp meal fractions	>350 µm	>250 µm	>180 µm	<180 µm	Whole meal
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	10.62±0.10^{a}	20.29±0.25^{b}	41.25±0.04^{d}	44.36±0.02^{e}	27.86±0.12^{c}
Lipid content	8.26±0.02^{a}	10.04±0.05^{b}	15.10±0.02^{d}	18.60±0.04^{e}	11.83±0.01^{c}
Total sugar content	0.00±00^{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 fibre content	29.54±0.04^{e}	21.33±0.03^{d}	7.13±0.04^{b}	4.96±0.01^{a}	17.35±0.03^{c}

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

Table 3. Phenolic compounds of hemp meal fractions (mg/kg dry sample)

Hemp meal fractions	>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	14.55±1.67^{a}	22.19±1.88^{b}	31.67±2.28^{cd}	36.05±2.04^{d}	28.20±2.47^{c}
p-Hydroxybenzoic acid	33.27±3.20^{a}	29.83±1.64^{a}	128.78±8.56^{c}	123.66±6.47^{c}	78.56±8.00^{b}
Catechin	107.00±12.41^{a}	221.25±12.99^{b}	744.50±22.25^{e}	313.25±12.40^{c}	498.07±35.92^{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	88.40±6.05^{c}	81.99±6.26^{c}	9.67±0.74^{a}	4.72±0.55^{a}	47.43±5.37^{b}
Sinapic acid	66.80±5.48^{d}	58.33±2.39^{c}	26.43±2.21^{b}	17.34±1.45^{a}	22.25±1.89^{ab}
N-trans-caffeoyl tyramine*	286.68±23.11^{d}	267.38±15.88^{d}	54.61±3.17^{b}	41.70±3.42^{a}	152.50±11.22^{c}
Cannabisin B*	153.24±8.62^{e}	117.25±3.22^{d}	25.74±1.15^{b}	4.27±0.39^{a}	64.92±1.94^{c}

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

*Expressed in *trans*-cinnamic acid equivalents.

Table 4. Fatty acid composition in different hemp meal fractions (g/100g total fatty acids)

Hemp meal fractions	>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]
18:1n9c	13.93±0.08[c]	12.71±0.02[a]	13.56±0.51[bc]	13.86±0.01[c]	12.96±0.47[ab]
18:2n6c	54.61±0.32[ab]	55.42±0.01[c]	54.23±0.26[ab]	54.09±0.03[a]	54.82±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]
18:3n6	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]
18:3n3	17.64±0.09[b]	18.39±0.01[c]	17.31±0.13[a]	17.55±0.04[b]	18.42±0.10[c]

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

Table 5. Content and ratio of saturated and unsaturated fatty acids in different hemp meal fractions (g/100g total fatty acids)

Hemp meal fractions	>350 µm	>250 µm	>180 µm	<180 µm	Whole meal
SFA	10.78 ± 0.07^{a}	10.73 ± 0.03^{a}	12.32 ± 0.09^{d}	11.89 ± 0.01^{c}	11.04 ± 0.07^{b}
MUFA	14.33 ± 0.49^{c}	12.71 ± 0.02^{a}	13.56 ± 0.51^{abc}	13.86 ± 0.01^{bc}	12.96 ± 0.47^{ab}
PUFA	74.89 ± 0.42^{a}	76.56 ± 0.02^{b}	74.12 ± 0.42^{a}	74.24 ± 0.01^{a}	76.00 ± 0.40^{b}
UFA	89.22 ± 0.07^{d}	89.27 ± 0.03^{d}	87.68 ± 0.09^{a}	88.11 ± 0.01^{b}	88.96 ± 0.07^{c}
PUFA/SFA	6.94	7.14	6.02	6.24	6.88

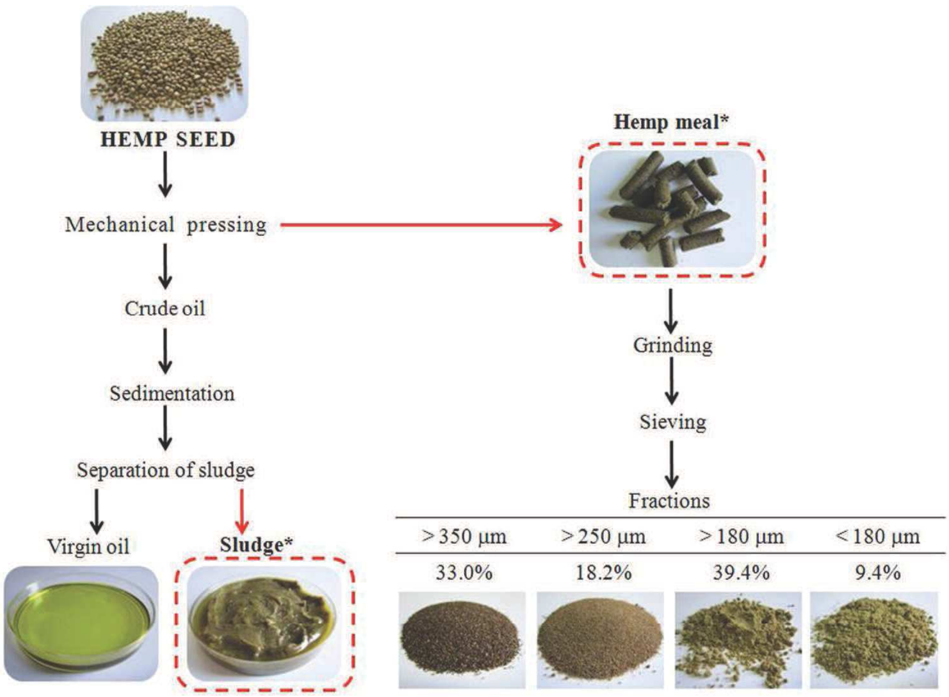
Values are means ± SD. Means in the same row followed by different superscript letters are significantly different ($P<0.05$)

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, UFA - unsaturated fatty acids

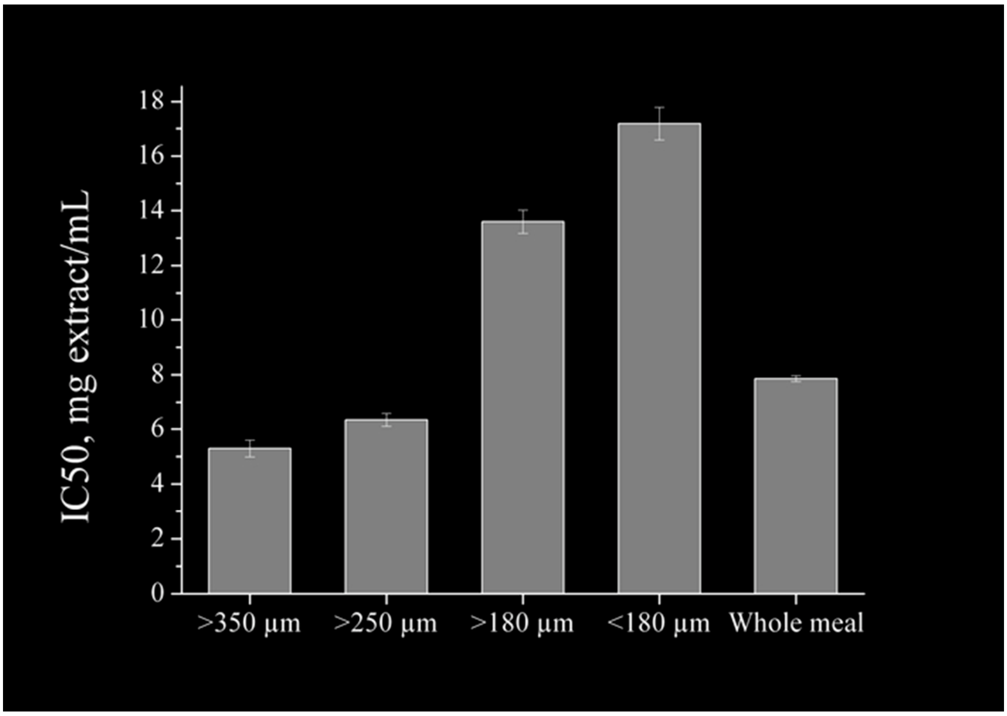
Table 6. Distribution of major antinutrients in different fractions of hemp meal

Hemp meal fractions	>350 µm	>250 µm	>180 µm	<180 µm	Whole meal
Trypsin inhibitor, TIU/mg 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]	21.22±0.11[c]	21.42±0.11[c]	22.50±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]

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



Simplified flow processing diagram for mechanical hemp oil pressing indicating by-products generated during processing and the yield of hemp meal fractions
127x92mm (300 x 300 DPI)



The radical scavenging capacities (IC50 values) of fraction extracts
59x42mm (300 x 300 DPI)