JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf102636b

Evaluating the Quality of Protein from Hemp Seed (*Cannabis sativa L.*) Products Through the use of the Protein Digestibility-Corrected Amino Acid Score Method

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The macronutrient composition and the quality of protein of hemp seed and products derived from hemp seed grown in Western Canada were determined. Thirty samples of hemp products (minimum 500 g), including whole hemp seed, hemp seed meal from cold-press expelling, dehulled, or shelled, hemp seed and hemp seed hulls, were obtained from commercial sources. Proximate analysis, including crude protein (% CP), crude fat (% fat) and fiber, as well as full amino acid profiles, were determined for all samples. Protein digestibility-corrected amino acid score (PDCAAS) measurements, using a rat bioassay for protein digestibility and the FAO/WHO amino acid requirement of children (2−5 years of age) as reference, were conducted on subsets of hemp products. Mean (±SD) percentage CP and fat were 24.0(2.1) and 30.4(2.7) for whole hemp seed, 40.7(8.8) and 10.2(2.1) for hemp seed meal, and 35.9(3.6) and 46.7(5.0) for dehulled hemp seed. The percentage protein digestibility and PDCAAS values were 84.1−86.2 and 49−53% for whole hemp seed, 90.8−97.5 and 46−51% for hemp seed meal, and 83.5−92.1 and 63−66% for dehulled hemp seed. Lysine was the first limiting amino acid in all products. Removal of the hull fraction improved protein digestibility and the resultant PDCAAS value. The current results provide reference data in support of protein claims for hemp seed products and provide evidence that hemp proteins have a PDCAAS equal to or greater than certain grains, nuts, and some pulses.

KEYWORDS: Hemp seed; hemp seed meal; *Cannabis sativa L.*; protein quality; proximate analysis; amino acid composition; arginine

INTRODUCTION

The commercial production of hemp (*Cannabis sativa L.*) in Canada was permitted in 1998 following a long period of discontinuation. Traditionally, hemp was cultivated as a multiuse crop, serving as a source of fiber, food, and medicinal products. Despite the utility of this crop, hemp farming in Canada and many other countries was banned starting in the 1930s, due to the presence of the psychoactive compound delta-9-tetrahydrocannabinol (THC) in the plant flowers and leaves. The breeding in Europe of industrial hemp varieties with a low-THC content, typically less than 0.3%, allowed reintroduction of this crop into the Canadian production systems in 1998. Farmers must exclusively use permitted low-THC varieties and obtain a license to grow the crop from Health Canada. In 2008/9, in excess of 20 000 ha were licensed for industrial hemp production in Canada with 90% of the production based in the eastern prairie region (*1*). In the U.S., the largest market for hemp seed grown in Canada, commercial hemp production remains illegal under federal law, although some states, such as North Dakota, have passed legislation approving its production. The captive U.S. market offers some farmers in Western Canada an alternative, profitable rotation crop.

Current commercial hemp cultivars have been bred for either seed oil production, fiber production, or as dual-purpose crops. Because the market demand is primarily for seed products, Canadian farmers generally grow early flowering seed varieties. Hemp seeds contain approximately 33−35% oil (*2*), most of which is expelled through “cold-pressing”. Hemp oil is marketed as a dietary oil with a unique spectrum of fatty acids. The remaining seed cake or meal, containing approximately 10% residual oil, has a high protein content, typically 30−50% (*2*−*4*), and milled hemp seed cake is now offered commercially as a source of vegetable protein and dietary fiber in the form of hemp protein powder, hemp flour, and in shake drinks. Food products made from whole hemp seeds and shelled (hulled) seeds are also commercially available, including energy bars and hemp milk. While data exists as to the protein content of hemp seed and hemp seed meal, the scientific literature offers little information on the quality of hemp protein. Numerous factors are known to influence the nutritional quality of plant proteins, as measured by their amino acid composition and the digestibility of the protein (*5*). The amino acid composition may be affected by variety/genetics, agronomic conditions such as soil fertility, and postharvest

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Table 1. Characteristics of the Analyzed Hempseed Products

sample key	product	variety	cropping year	location
HS1	hempseed	USO 31	2004	Manitoba
HS2	hempseed	USO 31	2003	Manitoba
HS3	hempseed	USO 14	2004	n.a.[a]
HS4	hempseed	USO 14	2003	n.a.
HS5a	hempseed	Finola	2004	n.a.
HS5b	hempseed	Finola	2004	n.a.
HS6	hempseed	Finola	2003	Manitoba
HS7a	hempseed	Crag	2004	Manitoba
HS7b	hempseed	Crag	2004	Saskatchewan
HS8	hempseed	Crag	2003	n.a.
HS9	hempseed	Crag	2003	n.a.
DHS1a	dehulled hemp seed	USO 31	2004	n.a.
DHS1b	dehulled hemp seed	USO 31	2004	Saskatchewan
DHS2	dehulled hemp seed	USO 31	2003	Manitoba
DHS4	dehulled hemp seed	USO 14	2003	n.a.
DHS5	dehulled hemp seed	Crag	2004	Manitoba
DHS6[b]	dehulled hemp seed	USO-31	2004	Manitoba
HPF1	hemp seed meal	USO 31	2004	n.a.
HPF2	hemp seed meal	USO 31	2003	n.a.
HPF4	hemp seed meal	USO 14	2003	n.a.
HPF4a	hemp seed meal	Finola	2004	Manitoba
HPF4b	hemp seed meal	Finola	2004	Saskatchewan
HPF5a	hemp seed meal	Crag	2004	Manitoba
HPF7	hemp seed meal	unknown	2004	n.a.
HPF8	hemp seed meal	Crag	2004	Saskatchewan
HPF9	hemp seed meal	Finola	2004	n.a.
HPF10	hemp seed meal	Finola	2005	n.a.
HH1	hemp hulls	USO-31	2004	Manitoba
HH1F	hemp hulls	unknown	n.a.	n.a.
HH2	hemp hulls	unknown	n.a.	n.a.

[a] n.a. = not available [b] Includes fines.

processing that alters the ratio of seed components, such as shelling. The digestibility of proteins may be affected by protein structure, the presence of antinutritional compounds and high-temperature processing (*6*). Yet, to date no comprehensive evaluation of the composition and in vivo digestibility of hemp protein has been published. Therefore, the current study was undertaken to (a) determine the representative macronutrient content of hemp seed products derived from hemp cultivars grown commercially in Western Canada and (b) determine the nutritional quality of the protein found in select hemp products, through the use of the protein digestibility-corrected amino acid score (PDCAAS) method.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents used were purchased from Sigma Chemical Co (Oakville, ON, Canada).

Sample Procurement. Thirty samples of hemp seed products (minimum sample size: 500 g) were obtained from two commercial hemp receiving and crushing plants (Hemp Oil Canada, Ste. Agathe, MB, Canada; Manitoba Harvest, Winnipeg, MB, Canada). The details of these products are presented in **Table 1**. They included 11 samples of whole hemp seed, 10 samples of hemp seed meal (cold-press expelling), 6 samples of shelled hemp seeds (hemp nuts), and 3 samples of hemp seed hulls. Hemp products were derived from one of four hemp cultivars: Finola, USO-14, USO-31, and Crag. The USO-14 and USO-31 cultivars are early maturing with significant stalk yield and seed yield potentials of approximately 400 kg per acre (*7*). Therefore, these two varieties are dual-purpose (grain and fiber) crops. Crag and Finola, a Finnish variety that is shorter in stature, very early maturing, and with a high-yield potential (*7*), are predominantly grown for seed. In 2007, all four varieties were approved for cultivation, and USO 14, 31, and Crag were exempt from otherwise mandatory field testing of hemp field for THC. Finola is under observation by Health Canada and still requires THC testing to ensure levels are below 0.3% THC (*8*).

Analytical Procedures. Prior to analysis, all samples were ground in a hand-held electric coffee mill. For all samples, percent crude protein (CP; $N \times 6.25$) was determined through the use of a LECO CNS-2000 Nitrogen Analyzer (LECO Corporation, St Joseph MI., U.S.A., Model No. 602-00-500), and percent dry matter (DM) and ash were determined according to standard procedures (*9*). The use of 6.25 for the correction factor for converting nitrogen to crude protein was chosen based on the official methodology employed for the determination protein digestibility (see below). The gross energy content (MJ/kg) was determined with an adiabatic bomb calorimeter (Parr Instrument Company Inc. Moline, Illinois, U.S.A.). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the Ankom nylon-bag procedure. The percent crude fat was determined by extracting crude fat into hexane and by gravimetrics (*9*). The amino acid contents of the samples were determined by acid hydrolysis using the AOAC Official Method 982.30 (*9*). Methionine and cysteine were determined by the performic acid oxidized hydrolysis procedure, and tryptophan was determined using alkaline hydrolysis.

Protein Digestibility-Corrected Amino Acid Score Determination. A rat bioassay, as described previously (*5*), was used to determine the PDCAAS for two samples of whole hemp seeds, three samples of shelled seeds, and three samples of hemp meal. Amino acid ratios for the eight test samples and the reference protein casein were derived by dividing for each essential amino acid its relative abundance in a hemp or casein test protein, expressed in milligrams of amino acid per gram of test protein, by the relative abundance of the same amino acid in the protein reference pattern adopted by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO)

$$\text{Amino Acid Score (AAS)} = \frac{\text{mg of amino acid per gram of protein (test protein)}}{\text{mg of amino acid per gram of protein (reference pattern)}}$$

The reference pattern used was the 1985 FAO/WHO/UNU (*10*) pattern of requirements for children 2 to 5 years of age (amino acid, mg/g protein): Histidine, 19; Isoleucine, 28; Leucine, 66; Lysine, 58; Methionine + Cysteine, 25; Phenylalanine + Tyrosine, 63; Threonine, 34; Tryptophan, 11; Valine, 35. Amino acid scores were determined by selecting the value of the amino acid with the lowest ratio (first limiting amino acid).

True protein digestibility was determined using the AOAC Official Method 991.29 rat bioassay (*9*), using casein as a reference standard, and correcting for endogenous protein losses using a protein-free diet. Hemp seed and dehulled hemp seed samples were defatted prior to analysis. All test articles were ground to pass through a 2 mm screen prior to preparation of the test diets. Diets were formulated to contain 10% protein, supplied by the test hemp article, 10% total fat (total of residual hemp oil and supplemental corn oil), and 5% cellulose with the remaining energy derived from corn starch. Vitamins and minerals (AIN-93 formulations; Harlan Teklad, Madison, WI) were added to diets to meet the micronutrient requirements of laboratory rats (*11*). Male weanling laboratory rats ($n = 6$ per treatment; initial weight 70 g) were individually housed in suspended wire-bottomed cages, with absorbent paper placed underneath. Water was available for ad libitum consumption. Feed was restricted to a maximum of 15 g/day over a four day acclimation period followed by a 5 day balance period, during which daily feed intake was calculated. Total fecal output was collected during the balance period, air-dried, and analyzed for its dry matter and nitrogen content. True protein digestibility (TPD%) was calculated as follows

$$\text{TPD\%} = \left(\frac{\text{nitrogen intake} - (\text{fecal nitrogen loss} - \text{metabolic nitrogen loss})}{\text{nitrogen intake}}\right) \times 100$$

where nitrogen intake and fecal nitrogen loss represent the product of food intake or fecal weights and their respective nitrogen values. The value for metabolic nitrogen loss was determined as the amount of fecal nitrogen produced per gram of diet consumed by rats consuming a protein-free diet. The PDCAAS was calculated as

$$\text{PDCAAS (\%)} = \text{TPD\%} \times \text{AAS}$$

As an additional marker of protein quality, rat weights were recorded throughout the acclimation and balance periods, and feed conversion efficiency, measured as the amount of weight gain per unit of feed, was calculated and expressed as a percentage of that afforded by the rats consuming the casein reference diet. This value is consistent with the protein efficiency ratio (PER).

Statistical Treatment of Data. Means, standard deviations, and percent coefficients of variation (% CV) were calculated for chemical constituents within a hemp product group. Because of the small sample size re: year, cultivar, and growing condition, no attempt was made to analyze the variance associated with these parameters. Regression analysis was performed for proximate variables and digestibility data, using SigmaPlot 2000 (SPSS Inc.).

RESULTS AND DISCUSSION

Proximate Analyses of Hemp Seed and Hemp Seed Products. The contents of proximate components, or macronutrients, for the four kinds of hemp products tested are given in **Table 2**. On average, intact, whole, hemp seed contains (on a fresh weight basis) approximately 24% CP, 30% CF, 32% NDF, and 5% ash with water and nitrogen-free extractives accounting for the remainder. These values are in general agreement with data published previously (*2–4*). Silversides and Lefrançois (*4*) reported CP, crude lipid, and gross energy values of 24.9%, 33.2%, and 24.9 MJ/kg, respectively in a sample of Uniko-B hemp, a Hungarian variety grown in Eastern Canada for fiber. Callaway (*2*) reported a CP and CF content of 24.8 and 35.5%, respectively, for Finola hemp seed. In the current study, the mean protein and oil content of seeds collected from Finola varieties (samples HS5a, 5b, and 6) were somewhat lower, 23.0 and 30.4%, respectively, indicating that geography, climatic conditions, and local agronomic factors may impact hemp seed composition. The lack of controls does not permit the drawing of extensive conclusions on the impact of these factors on proximate composition of hemp seed. In general, the % CVs for protein, fat, and fiber in the hemp seed samples tested in the current study were less than 10%.

Because much of the fiber (NDF) fraction of whole hemp seeds resides in the seed hull (**Table 2**), shelling of whole seeds is expected to yield a product that is enriched in fat and protein. In fact, the shelled hemp seed was found to contain typically 1.5 times (by weight) as much fat and protein as the respective whole seed (**Table 2**). The higher oil content of shelled seeds is also visible in their gross energy content (27.7 MJ/kg) versus the parent seed (24.2 MJ/kg). In general, the percent CVs for the dehulled hemp seed products are below 12% with the exception of the ADF and NDF fractions. The latter is explained by one sample (DHS6) with a substantially higher NDF and ADF value and a correspondingly lower lipid and protein content, relative to the mean values. The variability in fiber content is a result of processing conditions and serves to highlight the need to control processing conditions to minimize variation in the nutrient profile of dehulled hemp seed.

Hemp seed meal is produced from whole hemp seed by expelling its oil fraction. One would expect the removal of hemp oil to cause (a) an increase in the content of other proximate components and (b) a decrease in the gross energy content. The mean crude protein value for the hemp seed meals was 40.7%, 1.7 times the corresponding mean value for hemp seed (**Table 2**). The crude fat content was reduced from 30.4% in the whole seed to a significant mean residual crude fat content of 10.2% in the meal. The data reflect the fact that the removal of oil from hemp seeds involves a single-stage mechanical expelling, which leaves a higher proportion of oil in the meal when compared to a solvent extraction process. Furthermore, the efficiency of oil extraction may vary depending on expeller equipment and processing conditions. Silversides and Lefrançois (*4*) reported crude protein, crude lipid, and gross energy values of 30.7%, 16.4%, and 21.2 MJ/kg in a sample of hemp seed meal, suggesting that they tested a sample of hemp seed meal with a higher residual crude fat content. Post-expeller processing of hemp seed meal further affects the final composition of the hemp protein flours. These processes include milling of the meal, followed by sifting or air-classification. Some commercial products now claim a protein content of 50%. This and the comparatively high % CVs for fat, protein, and ADF in the tested meal, generally greater than 20%, demonstrate the

Table 2. Proximate Analysis and Gross Energy Content of Hemp Seed Products (As Is Basis)

	DM[a] (%)	CF[b] (%)	CP[c] (%)	ADF[d] (%)	NDF[e] (%)	ash (%)	GE[f] (MJ/kg)
			Hemp Seeds				
HS1	90.8	25.6	21.9	24.3	33.0	5.7	23.6
HS2	94.3	30.0	25.5	23.4	34.2	3.7	23.6
HS3	95.6	31.4	24.0	21.9	31.9	5.2	24.0
HS4	91.2	25.4	21.3	26.1	36.2	4.5	23.5
HS5a	93.7	29.5	21.9	25.0	33.2	5.2	24.5
HS5b	91.8	30.3	23.8	21.8	29.3	4.3	24.7
HS6	95.6	31.3	23.2	25.2	34.5	3.7	24.8
HS7a	95.1	31.7	27.5	21.9	27.8	5.1	24.3
HS7b	95.3	32.9	23.3	22.4	32.3	4.7	24.3
HS8	95.6	33.0	27.2	23.9	31.4	4.9	24.2
HS9	96.0	33.0	24.1	22.5	29.6	5.9	24.5
mean	94.1	30.4	24.0	23.5	32.1	4.8	24.2
S.D.	2.0	2.7	2.1	1.5	2.5	0.7	4.5
% CV	2.1	8.9	8.6	6.4	7.8	15.0	18.6
			Dehulled Hemp Seed				
DHS1a	93.7	45.9	38.5	2.6	6.7	6.9	27.7
DHS1b	93.7	46.5	38.5	1.4	5.6	7.1	27.9
DHS2	94.3	49.3	36.5	0.9	6.1	7.0	28.5
DHS4	96.6	48.9	38.7	2.1	6.0	5.6	29.0
DHS5	97.0	52.3	32.7	0.6	4.6	6.2	28.0
DHS6	95.4	37.6	30.3	12.0	18.1	5.4	25.3
mean	95.1	46.7	35.9	3.3	7.8	6.4	27.7
S.D.	1.4	5.0	3.6	4.3	5.1	0.8	1.3
% CV	1.5	10.8	9.9	133.1	64.8	11.8	4.6
			Hemp Seed Meal				
HSM1	98.8	8.9	31.5	23.1	38.1	6.8	20.0
HSM2	91.9	10.6	44.3	20.4	23.1	7.1	20.3
HSM4	93.9	15.5	44.7	14.1	21.8	7.1	21.7
HSM4a	92.3	8.8	53.3	12.4	20.9	6.8	20.2
HSM4b	94.3	9.5	47.7	16.9	26.9	6.3	20.7
HSM5a	94.9	10.5	33.1	32.0	41.5	6.8	19.6
HSM7	94.2	8.4	35.1	27.9	37.2	4.6	20.6
HSM8	95.4	11.9	33.7	23.5	38.6	6.8	21.1
HSM9	98.6	8.6	31.0	27.9	37.4	6.1	20.4
HSM10	96.6	9.2	52.5	16.7	19.0	8.7	19.6
mean	95.1	10.2	40.7	21.5	30.5	6.7	20.4
S.D.	2.3	2.2	8.8	6.5	8.8	1.0	0.6
% CV	2.5	21.3	21.6	30.3	29.0	15.0	3.1
			Hemp Hulls				
HH1	97.0	15.8	16.3	44.9	55.7	4.1	21.4
HHIF	93.4	10.9	12.8	48.8	64.7	3.1	20.2
HH2	94.5	4.3	8.8	56.9	74.2	4.4	18.8
mean	94.9	10.3	12.7	50.2	64.9	3.9	20.2
S.D.	1.8	5.8	3.7	6.1	9.3	0.6	1.3
% CV	1.9	55.7	29.6	12.2	14.3	16.6	6.6

[a] [1]DM = dry matter content. [b] CF = crude fat content determined by hexane extraction. [c] CP = crude protein = nitrogen content × 6.25 (determine by LECO analysis). [d] ADF = acid detergent fiber. [e] NDF = neutral detergent fiber. [f] GE = gross energy, determined by adiabatic bomb calorimetry.

Table 3. Amino Acid Composition of Hempseed Products (% As Is Basis)[a]

	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
Whole Seeds																		
HS1	2.25	0.89	1.08	3.55	1.00	1.00	0.97	0.35	1.07	0.45	0.85	1.41	0.72	0.97	0.50	0.83	1.96	0.21
HS2	2.22	1.11	1.22	3.52	0.81	0.95	0.81	0.37	1.00	0.52	0.68	1.39	0.51	0.98	0.49	0.77	2.07	0.23
HS3	2.51	0.79	1.01	3.79	1.12	1.23	1.02	0.39	1.49	0.57	0.54	1.19	0.47	0.68	0.60	0.90	2.12	0.15
HS4	2.20	0.82	1.02	3.28	0.79	0.97	0.95	0.42	0.99	0.56	0.79	1.40	0.74	0.95	0.48	0.76	2.05	0.19
HS5a	2.14	0.77	0.95	3.36	0.81	0.99	0.93	0.37	1.08	0.52	0.87	1.36	0.81	1.01	0.48	0.76	2.01	0.21
HS5b	2.34	0.93	1.23	3.90	0.93	1.11	1.04	0.39	1.08	0.47	0.88	1.60	0.73	1.05	0.56	0.84	2.38	0.22
HS6	2.40	0.80	1.12	4.00	0.82	1.10	1.00	0.40	1.18	0.56	0.93	1.56	0.76	1.11	0.67	0.84	2.53	0.28
HS7a	2.72	1.34	1.44	4.21	0.87	1.21	1.12	0.46	1.18	0.71	0.81	1.73	0.68	1.11	0.61	1.02	2.76	0.20
HS7b	2.48	1.11	1.28	3.61	0.88	0.98	0.82	0.45	1.09	0.65	0.78	1.41	0.55	0.97	0.52	0.83	2.27	0.26
HS8	2.43	1.21	1.28	3.87	0.90	1.01	0.87	0.57	1.15	0.66	0.81	1.65	0.72	1.13	0.57	0.91	2.49	0.19
HS9	2.63	1.31	1.43	4.08	0.96	1.14	0.99	0.35	1.18	0.53	0.84	1.66	0.75	1.34	0.56	0.96	2.41	0.37
mean	2.39	1.01	1.19	3.74	0.90	1.06	0.96	0.41	1.14	0.56	0.80	1.49	0.68	1.03	0.55	0.86	2.28	0.23
S.D.	0.18	0.22	0.17	0.30	0.10	0.10	0.09	0.06	0.14	0.08	0.11	0.16	0.11	0.16	0.06	0.09	0.26	0.06
% CV	7.68	21.64	13.97	8.03	10.88	9.44	9.85	15.36	12.17	14.16	13.58	11.04	16.59	15.67	11.30	9.92	11.39	25.59
Dehulled Hemp Seed																		
DHS1a	3.86	1.37	1.83	6.68	2.04	1.78	1.71	0.68	1.94	0.98	1.53	2.39	1.64	1.63	1.14	1.29	4.51	0.39
DHS1b	3.79	1.30	1.76	6.54	1.43	1.60	1.55	0.73	1.74	0.99	1.46	2.20	1.16	1.62	0.97	1.31	4.48	0.42
DHS2	4.06	1.41	1.90	7.21	2.10	1.73	1.64	0.66	1.95	1.10	1.52	2.33	1.16	1.64	1.02	1.22	4.74	0.45
DHS4	3.84	1.26	1.69	6.27	1.35	1.60	1.42	0.67	1.94	0.97	1.56	2.32	1.44	1.60	0.97	1.21	4.21	0.42
DHS5	3.32	1.15	1.49	5.49	1.08	1.36	1.32	0.57	1.52	0.85	0.87	1.84	1.03	0.94	0.88	1.31	5.31	0.33
DHS6	3.10	1.13	1.51	5.17	1.73	1.57	1.48	0.57	1.59	0.75	0.83	1.74	1.25	1.17	0.83	1.21	4.04	0.27
Mean	3.66	1.27	1.70	6.23	1.62	1.61	1.52	0.65	1.78	0.94	1.29	2.14	1.28	1.43	0.97	1.26	4.55	0.38
S.D.	0.37	0.11	0.17	0.77	0.41	0.15	0.14	0.07	0.19	0.12	0.35	0.28	0.22	0.30	0.11	0.05	0.45	0.07
%CV	10.09	8.93	9.84	12.30	24.98	9.11	9.45	10.13	10.79	12.89	26.74	13.05	17.38	21.15	11.11	3.88	9.82	17.91
Hemp Seed Meal																		
HSM1	3.04	1.09	1.35	4.76	1.33	1.27	1.05	0.62	1.52	0.53	1.31	2.21	1.21	1.67	0.78	1.03	3.40	0.26
HSM2	4.13	1.34	1.90	7.08	1.63	1.87	1.80	0.83	2.10	1.08	1.60	2.48	1.10	1.76	1.13	1.52	4.68	0.47
HSM4	3.81	1.47	1.86	6.44	1.79	1.77	1.59	0.78	1.83	0.97	1.39	2.24	0.98	1.58	0.96	1.30	3.93	0.44
HSM4a	4.80	1.74	2.25	7.98	1.95	2.14	2.05	0.93	2.38	1.32	1.75	3.16	1.46	2.08	1.23	1.76	5.37	0.55
HSM4b	4.45	1.56	2.06	7.44	1.98	2.12	2.04	0.83	2.29	1.09	1.77	2.99	1.63	2.03	1.14	1.65	4.98	0.46
HSM5a	3.08	1.08	1.34	4.69	1.41	1.17	1.59	0.55	2.00	0.75	1.39	1.82	1.11	1.20	0.89	1.03	2.93	0.27
HSM7	3.08	1.11	1.48	5.25	1.32	1.57	1.47	0.60	1.69	0.73	1.33	2.13	1.12	1.49	0.79	1.21	3.45	0.33
HSM8	3.42	1.29	1.61	5.67	1.84	1.71	1.59	0.61	1.80	0.74	1.42	2.09	1.11	1.43	0.80	1.19	3.32	0.36
HSMF9	3.12	1.45	1.71	4.96	1.06	1.35	1.33	0.53	1.57	0.73	1.05	1.98	0.66	1.37	0.70	1.15	3.11	0.41
mean	3.66	1.35	1.73	6.03	1.59	1.66	1.61	0.70	1.91	0.88	1.45	2.35	1.15	1.62	0.93	1.32	3.91	0.39
S.D.	0.67	0.23	0.32	1.24	0.32	0.35	0.32	0.15	0.30	0.25	0.23	0.45	0.28	0.30	0.19	0.27	0.89	0.10
% CV	18.22	17.01	18.28	20.61	20.39	21.16	19.98	20.74	15.96	28.10	15.89	19.33	23.91	18.17	20.24	20.18	22.69	24.35
Hemp Hulls																		
HH1	1.23	0.47	0.56	1.76	1.23	0.52	0.51	0.18	0.91	0.18	0.44	0.96	0.46	0.58	0.40	0.47	1.82	0.09
HHIF	0.93	0.39	0.45	1.27	0.54	0.49	0.50	0.23	0.58	0.30	0.49	0.75	0.42	0.58	0.24	0.35	0.71	0.06
HH2	0.54	0.22	0.24	0.53	0.31	0.22	0.21	0.11	0.30	0.05	0.24	0.43	0.33	0.44	0.11	0.16	0.28	0.02
mean	0.90	0.36	0.42	1.19	0.69	0.41	0.40	0.18	0.60	0.18	0.39	0.71	0.40	0.53	0.25	0.33	0.94	0.06
S.D.	0.35	0.13	0.16	0.62	0.48	0.16	0.17	0.06	0.31	0.12	0.14	0.27	0.07	0.09	0.15	0.16	0.80	0.04
% CV	38.82	35.84	38.93	52.53	69.32	39.90	41.24	34.45	51.16	69.45	34.76	37.41	17.43	15.90	59.65	47.75	85.38	64.53

[a] Key: ASP = asparagine; THR = threonine; SER = serine; GLU = glutamate/glutamine; PRO = proline; GLY = glycine; ALA = alanine; CYS = cysteine; VAL = valine; MET = methionine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; HIS = histidine; LYS = lysine; ARG = arginine; TRP = tryptophan.

significant impact of processing conditions on proximate composition and nutritional profile of hemp protein flours.

Protein Quality of Hemp Seed and Hemp Seed Products. In human nutrition, the quality of a protein is defined by (1) the relative contribution that the amino acids contained in the protein make to an individual's amino acid requirement and (2) the digestibility of the protein. The amino acid profiles of hemp seed and hemp seed products are given in **Table 3**. Hemp seed and its derived products contain all essential amino acids required by humans. The respective amino acid scores are presented in **Table 4**. The amino acid score of a protein reflects the extent to which a dietary protein meets the needs of an individual for a particular amino acid. Scores of 1.0 or greater for an amino acid indicate that this amino acid is not limiting relative to requirements. When scores are less than 1.0, the provision of the dietary protein source will yield an intake for a specific amino acid below its requirement level. The lowest score over the range of all essential amino acids is taken as the amino acid score for the entire protein source, irrespective of the relative contributions of other amino acids. On the basis of the amino acid composition of hemp seed and hemp protein products and the use of the FAO/WHO reference protein for children 2−5 years of age, lysine is the first limiting amino acid in all hemp protein sources tested (**Table 4**), and the amino acid scores for hemp seed, dehulled hemp seed, hemp seed meal and hemp hulls are 0.62, 0.61, 0.58, and 0.50, respectively. Depending on the product, leucine or

Table 4. Amino Acid Scores of Hemp Protein Products[a]

	HIS[b]	ILE[b]	LEU[b]	LYS[b,c]	M + C[b]	P + T[b]	THR[b]	TRP[b]	VAL[b]
				Hemp Seed					
HS1	1.19	1.39	0.97	0.66	1.47	1.22	1.20	0.85	1.40
HS2	1.00	0.96	0.83	0.52	1.40	0.93	1.28	0.84	1.12
HS3	1.31	0.80	0.75	0.65	1.60	0.76	0.96	0.57	1.78
HS4	1.20	1.32	1.00	0.61	1.85	1.26	1.13	0.82	1.32
HS5a	1.14	1.41	0.94	0.60	1.61	1.32	1.03	0.88	1.40
HS5b	1.23	1.31	1.02	0.61	1.44	1.18	1.15	0.82	1.30
HS6	1.52	1.44	1.02	0.62	1.65	1.28	1.01	1.08	1.46
HS7a	1.16	1.04	0.95	0.64	1.70	1.03	1.44	0.65	1.23
HS7b	1.18	1.19	0.92	0.61	1.89	1.04	1.40	1.00	1.33
HS8	1.10	1.07	0.92	0.57	1.81	1.07	1.31	0.62	1.21
HS9	1.21	1.24	1.05	0.69	1.47	1.38	1.60	1.39	1.41
mean	1.20	1.20	0.94	***0.62***	1.63	1.13	1.23	0.87	1.36
S.D.	0.13	0.21	0.09	***0.05***	0.17	0.19	0.20	0.23	0.17
				Dehulled Hemp Seed					
DHS1A	1.56	1.42	0.94	0.58	1.72	1.34	1.04	0.92	1.44
DHS1B	1.33	1.36	0.87	0.59	1.79	1.15	1.00	1.00	1.29
DHS2	1.47	1.49	0.97	0.58	1.92	1.22	1.14	1.11	1.52
DHS4	1.31	1.44	0.91	0.54	1.69	1.25	0.95	1.00	1.44
DHS5	1.42	0.95	0.85	0.69	1.73	0.96	1.04	0.91	1.33
DHS6	1.44	0.98	0.87	0.69	1.74	1.26	1.10	0.81	1.50
mean	1.42	1.27	0.90	***0.61***	1.77	1.20	1.04	0.96	1.42
S.D.	0.09	0.24	0.05	***0.06***	0.08	0.13	0.07	0.10	0.09
				Hemp Seed Meal					
HSM1	1.31	1.48	1.07	0.57	1.46	1.45	1.02	0.74	1.37
HSM2	1.34	1.29	0.85	0.59	1.72	1.03	0.89	0.96	1.36
HSM4	1.12	1.11	0.76	0.50	1.57	0.91	0.96	0.89	1.17
HSM4a	1.21	1.17	0.90	0.57	1.69	1.05	0.96	0.93	1.27
HSM4b	1.26	1.33	0.95	0.60	1.61	1.22	0.96	0.87	1.37
HSM5a	1.41	1.50	0.83	0.54	1.57	1.11	0.96	0.75	1.72
HSM7	1.18	1.35	0.92	0.59	1.52	1.18	0.93	0.86	1.38
HSM8	1.25	1.51	0.94	0.61	1.60	1.20	1.13	0.98	1.53
HSM9	1.19	1.21	0.97	0.64	1.61	1.04	1.38	1.19	1.45
mean	1.25	1.33	0.91	***0.58***	1.60	1.13	1.02	0.91	1.40
S.D.	0.09	0.15	0.09	***0.04***	0.08	0.16	0.15	0.13	0.16
				Hemp Hulls					
HH1	1.30	0.96	0.89	***0.50***	0.90	1.02	0.85	0.52	1.59
reference casein	1.65	2.54	1.65	1.51	1.50	1.46	1.40	1.19	2.43

[a] Reference protein = FAO/WHO amino acid requirement pattern for school children. [b] HIS = histidine; ILE = isoleucine; LEU = leucine; LYS = lysine; M + C = methionine plus cysteine; P + T = phenylalanine plus tyrosine; THR = threonine; TRP = tryptophan; VAL = valine. [c] Values that are in bold/italic reflect limiting amino acid score = lysine.

tryptophan will be the second or third limiting amino acid. All other amino acids yielded scores greater than 1.0. The lower amino acid scores for hemp protein flour apparently reflect a relatively higher proportion of proteins with lower lysine content in the hull fraction.

Relative to other dietary protein sources, the limitation in the lysine content of hemp protein positions it in the same range as the main cereal grains (whole wheat = 0.44; corn = 0.54.) (*12*) Oil seed meals, due to their higher proportion of lysine, yield higher relative amino acid scores (soybean meal = 1.05; canola meal = 1.01.) (*12*) As the amino acid score provides only one measure of protein quality, to better quantify the quality of a dietary protein, allowances should be made for how well the protein is digested and utilized by the body. This is the concept behind the protein digestibility-corrected amino acid score.

The data for the digestibility of select hemp protein sources are provided in **Table 5**, along with the reference protein casein. The use of a reference protein, such as the highly digestible protein casein, provides a "benchmark" for comparisons against other studies assessing protein quality in foods. In the current study, the digestibility of casein was determined to be 97.6%. The digestibility of the protein in the two intact hemp seed samples studied averaged 85.2%. A similar protein digestibility was observed for the hemp protein flour samples (86.7%). These results suggest that the process commonly used in Canada to extract oil from hemp seed does not lower the digestibility of the seed protein. Heat-damaged proteins have been shown to have a lower protein digestibility (*6*). Large-scale oil expellers operate at high pressure and temperatures, which may reduce the digestibility of protein. In contrast, the expellers used in Canada to process hemp seeds usually "cold-press" the seeds, resulting in lower overall temperature increases. Additionally, the estimate of net protein efficiency ratio (PER) presented in **Table 5** is similar for hemp seeds and the hemp seed meal. The PER calculated in the current study provides a different measure of the protein quality and reflects the ability of the test subject (i,e., a growing rat) to deposit body protein. The values obtained for protein digestibility and PER are consistent for both the hemp seed and the hemp seed meal.

Table 5. Protein Digestibility-Corrected Amino Acid Score Estimates for Selected Hemp Protein Products

	protein digestibility %	amino acid score[a]	PDCAAS[b] %	net protein efficiency ratio[c]
casein	97.6	1.19	100	1.00
		Hemp Seed		
HS3	86.2	0.57	49	0.62
HS7a	84.1	0.64	54	0.69
mean	85.2	0.60	51	0.65
S.D.				
% CV				
		Dehulled Hemp Seed		
DHS4	97.5	0.54	53	0.76
DHS5	96.2	0.69	66	0.73
DHS6	90.8	0.69	63	0.76
mean	94.9	0.64	61	0.75
S.D.	3.5	0.09	7	0.02
% CV	3.72	13.44	11.66	2.10
		Hemp Seed Meal		
HSM1	84.4	0.57	48	0.64
HSM4	92.1	0.50	46	0.71
HSM8	83.5	0.61	51	0.64
mean	86.7	0.56	48	0.66
S.D.	4.8	0.05	2	0.04
% CV	5.49	9.73	4.93	6.09

[a] Lysine, the first limiting amino acid, using the FAO/WHO amino acid requirement pattern for school children. [b] Protein digestibility-corrected amino acid score, calculated as the product of protein digestibility and the amino acid score. [c] Surrogate measure, determined as the ratio of feed conversion efficiency (FCE) in rats consuming test article divided by FCE of rats consuming casein.

Wang et al. (*13*) have previously characterized the amino acid composition and in vitro digestibility of isolated hemp proteins. In general, their data is in good agreement with the current results with the exception that the current data provide measures of tryptophan, as measured by alkaline hydrolysis, and methionine and cysteine, as measured following performic acid oxidation. The inclusion of tryptophan analysis is important as this amino acid is identified as the second limiting amino acid in hemp proteins, using the FAO reference protein pattern. The data of Wang et al. (*13*) report total sulfur amino acid concentrations of approximately 1.6 g per 100 g protein for hemp protein isolate. Recalculation of the data in the present study yields a total sulfur

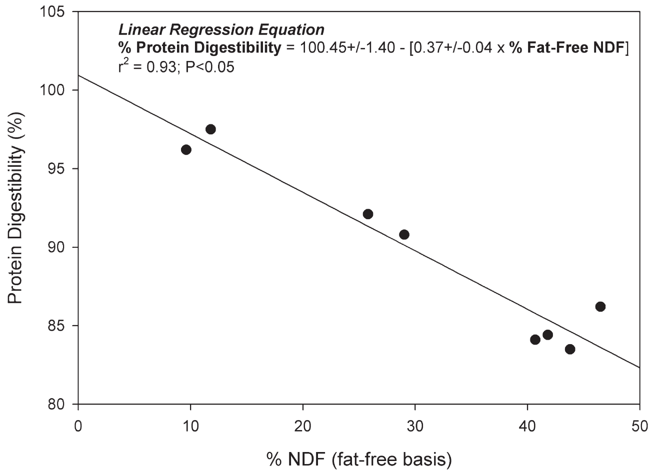

Figure 1. Relationship between protein digestibility and the neutral detergent fiber (NDF) content (corrected to a fat-free basis) of hemp protein samples.

amino acid content (methionine plus cysteine) of 4.04 g per 100 g protein for hemp seed. The discrepancy between the two studies is likely because the previous authors did not employ a performic acid oxidation step in advance of the acid hydrolysis, thus leading to an underestimation of the total sulfur amino acid, in particular cysteine, content due to the presence of unaccounted mixed oxidation products (*14*). The in vitro digestibility (as measured by nitrogen release) of hemp protein isolates ranged between 88 and 91% (*13*), closely matching the observed in vivo results from the current study. The good agreement between the two approaches is encouraging; however in vitro analytical approaches are limited by the fact that the biological response of the animal to the ingredient is not assessed.

While a detailed description of the factors affecting within sample variability in protein digestibility is not possible, due to the low numbers of samples tested, the one hemp protein flour that had the highest protein digestibility value (HPF4 = 92.1%) also had approximately half of the total NDF (21.8 vs 38.1 and 38.6%) as the other two samples (HPF1 and HPF8). Further evidence that NDF content influences the digestibility of the protein fraction is provided by the data for the dehulled hemp seed protein. Removal of the hull fraction from the hemp seed leads to an average increase in protein digestibility from 85.2 to 94.9%. Within the hemp nut samples, the one sample with the lowest protein digestibility (DHS6 = 90.8%) had the highest content of NDF (18.12%). Regression of the protein digestibility values against the percent NDF of the samples (corrected to a fat-free basis to account for the fact that samples were defatted prior to feeding as per the PDCAAS protocol) provided strong evidence of the digestibility depressing effect of the hemp hull (**Figure 1**). The exact nature of the depressing effect of the NDF fraction on protein digestibility, however, remains unknown. In general, the range of true protein digestibility values observed falls within the values observed for other high quality food proteins (*5*, *6*). Thus, while the commercially available hemp protein flours offer both vegetable protein and dietary fiber, the presence of the fiber will also have a depressant effect on protein digestibility. Protein digestibility values below 80% are often related to heat-damaged proteins or other processing effects (*6*). It is important to note that for the hemp samples studied in the current study no additional processing steps beyond oil extraction and milling were involved prior to the determination of the protein digestibility values. Caution must be used in extending protein digestibility values to hemp-containing foods that have

Table 6. Protein Digestibility-Corrected Amino Acid Scores of Hemp Protein Sources in Comparison to Other Food Proteins[a]

protein source	PDCAAS (%)
casein	100
egg white	100
beef	92
soy protein isolate	92
chickpeas (canned)	71
pea flour	69
kidney beans (canned)	68
dehulled hemp seed	61
pinto beans (canned)	57
rolled oats	57
lentils (canned)	52
hemp seed	51
hemp seed meal	48
whole wheat	40
almond	23

[a] Data for all non-hemp protein sources derived from the Joint FAP/WHO expert consultation on protein quality evaluation,(*5*) with the exception of the data for almonds.(*15*)

been subjected to high heat or oxidizing conditions during processing.

The product of the true protein digestibility values and the amino acid score is the PDCAAS (**Table 5**). In comparison to other protein foods, the PDCAAS value for hemp protein sources is positioned in the same range as the major pulse protein sources (lentils, pinto beans), and above cereal grain products, such as whole wheat (**Table 6**). This is especially true for the protein in dehulled hemp seed. In general, the amino acid score has the largest impact on the PDCAAS value, due to the high values observed for protein digestibility. Therefore, unless protein digestibility is substantially depressed due to dramatic increases in hull fraction (i.e., added hulls or breeding efforts) or further processing (high heat or oxidizing conditions), the PDCAAS value of hemp protein products will continue to remain in the 0.5 to 0.6 range due to the limitation in lysine content. Future efforts to breed for enhanced lysine content may be warranted if the value of the hemp protein component for human consumption dictates future market development for this crop.

A limitation of the PDCAAS method remains the fact that it is primarily focused on the relative ability of a given protein to meet the amino acid needs of the host and that it uses as reference the protein needs of children who require a larger proportion of lysine. It does not, however, provide an indication of other potential attributes of the protein. For example, like other nut proteins, hemp proteins contain a high amount of arginine (94−128 mg/g protein), relative to other food proteins, including whole wheat (48 mg/g protein (*12*);). Arginine serves as a dietary precursor for the formation of nitric oxide (*16*), a potent mediator of vascular tone and, therefore, may have implications for the health of the cardiovascular system. Additionally, arginine, or nitric oxide specifically, has been linked to optimal immune function (*17*) and to muscle repair (*18*). Therefore, the potential exists to position hemp proteins as a source of digestible arginine.

In summary, the protein from hemp seed is highly digestible in either its native form or as a hemp seed meal. Removal of the hull fraction from the hull improves the digestibility of the protein and the corresponding PDCAAS, likely due to the removal of significant NDF components that may limit protein digestion. While the lysine content of hemp proteins limits the PDCAAS valuation, using current accepted methods for the estimation of protein quality, the overall pattern of amino acid supply, including the relative abundance of arginine, position this protein source as a viable, vegetable-based protein for the human diet.

ACKNOWLEDGMENT

The authors would like to thank Mr. Prakash Sharma for assistance with the amino acid analysis, and the staff of the Animal Holding Facility for the provision of excellent animal care.

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Received for review July 7, 2010. Revised manuscript received October 18, 2010. Accepted October 18, 2010. In-kind contributions of hemp products from Manitoba Harvest, Hemp Oil Canada, and Canadian Hemp Trade Alliance, as well as funding from the Agricultural Research and Development Initiative (ARDI), Province of Manitoba, are gratefully acknowledged.