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Characterization, amino acid composition and *in vitro* digestibility of hemp (*Cannabis sativa* L.) proteins

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Abstract

The protein constituents and thermal properties of hemp (*Cannabis sativa* L.) protein isolate (HPI) as well as 11S- and 7S-rich HPIs (HPI-11S and HPI-7S) were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and different scanning calorimetry (DSC), and their amino acid composition and *in vitro* digestibility were also evaluated, as compared to soy protein isolate (SPI). SDS-PAGE analysis showed that the edestin (consisting of acidic and basic subunits, AS and BS) was the main protein component for HPI and HPI-11S, while HPI-7S was composed of the BS of edestin and a subunit of about 4.8 kDa. DSC analysis characterized thermal transition of the edestin component and the possible present form of different subunits. Except lysine and sulfur-containing amino acids, the essential amino acids of various HPIs met the suggested requirements of FAO/WHO for 2–5 year old infants. The proportion of essential amino acids to the total amino acids (E/T) for HPI (as well as HPI-11S) was significantly higher than that of SPI. In an *in vitro* digestion model, various protein constituents of various HPIs were much easily digested by pepsin plus trypsin, to release oligo-peptides with molecular weight less than 10.0 kDa (under reduced condition). Only after pepsin digestion, *in vitro* digestibility of HPIs was comparable to that of SPI, however after pepsin plus trypsin digestion, the digestibility (88–91%) was significantly higher than that (71%) of SPI ($P < 0.05$). These results suggest that the protein isolates from hempseed are much more nutritional in amino acid nutrition and easily digestible than SPI, and can be utilized as a good source of protein nutrition for human consumption.

Keywords: Hemp protein isolate (HPI); *Cannabis sativa* L.; Thermal property; Amino acid composition; *In vitro* digestibility

1. Introduction

Cannabis sativa L., commonly referred to as hemp, is a widely cultivated plant of industrial importance, as an important source of food, fiber and medicine. The industrial hemp with a low level of δ-9-tetrahydrocannabinol (THC) has been developed in several countries (e.g., Canada and China), as a good source for valuable hemp fiber. The hemp fiber is widely used in the modern production of durable fabrics and specialty papers in some countries. In the commercial utilization of hemp fiber, the seed becomes an interesting byproduct. In addition to considerable amounts of dietary fiber, the seed typically contains over 30% oil and about 25% protein (Callaway, 2004). The hempseed oil, over 80% in polyunsaturated fatty acids (PUFAs), is an exceptionally nutritional oil source for human consumption. The proteins (mainly edestin and albumin) in hempseed are also very nutritional in essential amino acids, and easily digested (Callaway, 2004). From the seed, even a methionine-and cystine-rich seed protein (a 10-kDa protein) has been isolated and identified (Odani & Odani, 1998). Thus, the proteins from hempseed have good potential to be applied as a valuable source of protein nutrition.

The investigation of the proteins from hempseed originated from the early 20 century. Osborne (1902a, 1902b)

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reported some properties of the globulin protein (edestin and edestan) from hempseed. Svedberg and Stamm (1929) reported the molecular weight of edestin by ultracentrifuge method. Hall (1949) investigated the electron micrographs of crystalline edestin. In 1964, Stockwell and others using the DEAE-cellulose chromatography found that the edestin from the seed consists of several protein and non-protein components, among which there is a high degree of interaction (Stockwell, Dechary, & Altschul, 1964). Recently, we reported the physicochemical and functional properties of hemp protein isolate (HPI), and pointed out that although it is very nutritional in amino acid composition, its functional properties (especially protein solubility) are poor as compared to soy protein isolate (SPI) (Tang, Ten, Wang, & Yang, 2006). In a further work, we also tried to apply the technique of limited enzymatic hydrolysis with trypsin to improve the functional properties of HPI, and confirmed that this technique could be effective to improve its protein solubility, but led to further declines in other properties (Yin et al., 2007). However, information about the nutritional properties of different fractions of HPIs is still very limited.

The main objective of the study was to characterize the protein constituents of various HPIs (including 11S- and 7S-rich HPIs). The amino acid composition and *in vitro* digestibility of these HPIs were also investigated, as compared to SPI.

2. Materials and methods

2.1. Materials

Defatted hempseed flour, a byproduct during the utilization of the valuable hempseed oil, was kindly supplied by YUNNAN Industrial Hemp Co., Ltd. (Yuannan Province, China). This meal had been obtained from hemp (*C. sativa* L.) seeds on a large scale through dehulling, disintegrating and defatting with supercritical liquid (CO_2) at a low temperatures of less than 40 °C. The denaturation extent of the protein components in this meal can be considered to be low, since all the steps were carried out at a temperature of less than 35 °C (except the disintegrating process). Defatted soybean seed flour was provided by XIANGCHI Cereal and Oil Co. Ltd. (Shandong Province, China). Pepsin (catalog no. P7000, 600–1000 units/mg) and trypsin powder (from porcine pancreas; catalog no. T4799, 1000–5000 BAEE units/mg solid) were purchased from Sigma Chemical Co (St. Louis, MO). Low molecular weight protein markers were purchased from Shanghai DINGUO Biotech. Co., Ltd. (China). All the chemical agents used in the present study were of analytical or better grade.

2.2. Preparation of HPI and SPI

The HPI and SPI were prepared at room temperature as follows. One hundred grams of defatted hempseed or soybean flour was mixed with 1.5 L deionized water, and the mixture was adjusted to pH 8.5 (soybean) or 10.0 (hemp) with 1.0 N NaOH. After continuously stirred for 1 h, the suspensions were centrifuged at 8000*g* for 30 min and the residue discarded. Then, the pH of the supernatants was adjusted to pH 4.5 (soybean) or 5.0 (hemp) at 4 °C with 1 N HCl, and the precipitates were collected by centrifugation (6500*g*, 25 min). The obtained precipitates were washed with pre-cooled deionized water, and dispersed in the deionized water. The dispersions were adjusted to pH 7.0 with 1 N HCl, and then dialyzed at 4 °C before freeze drying.

2.3. Preparation of 11S- and 7S-rich HPIs (HPI-11S and HPI-7S)

HPI-11S and HPI-7S were prepared at room temperature as follows. One hundred grams of defatted hempseed flour was dispersed in distilled water (1:20, w/v), and adjusted to pH 10.0 with 1 N NaOH. The dispersion was then stirred at room temperature for 1 h, and centrifuged at 10,000*g* for 30 min (at 20 °C) to obtain the supernatant. Then, $NaHSO_3$ was added to the supernatant (the concentration is 0.98 g $NaHSO_3$/L), and the supernatant was adjusted to pH 6.4 with 1 N HCl (to precipitate the 11S fraction) and kept overnight at 4 °C. The resultant dispersion was centrifuged at 6500*g* for 25 min at 4 °C. The obtained precipitate (HPI-11S) was suspended in deinonized water, and the suspension was adjusted to pH 7.0 with 1 N NaOH, and then dialyzed and freeze dried. The obtained supernatant was further adjusted to pH 4.6 with 1 N HCl (to precipitate the 7S fraction). The corresponding suspension was also centrifuged at 6500*g* for 20 min at 4 °C. The obtained precipitate (HPI-7S) was suspended in deinonized water, and the suspension was adjusted to pH 7.0 with 1 N NaOH, and then dialyzed before freeze-drying.

2.4. Chemical analysis

Protein ($N \times 6.25$, %), ash and moisture contents of the protein isolates were analyzed using AOAC methods (1985).

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the discontinuous buffer system of Laemmli (1970) at 5% stacking gel and 12.5% separating gel using gel electrophoresis apparatus DYCZ-30 (Beijing LIUYI Instrument Factory, China). The protein samples were directly dissolved in the sample buffer, namely 0.125 M Tris–HCl buffer (pH 8.0) containing 1.0% (w/v) SDS, 0.05% (w/v) bromophenol blue, 30% (v/v) glycerol and 5% (v/v) β-mercaptoethanol (2-ME). The electrophoresis was run at 20 mA in stacking gel and at 40 mA in separating gel until the tracking dye reached the bottom of the gel. The gel was dyed and destained

according to the method of Kwanyuen and Wilson (2000). Before electrophoresis, the samples were heated at 100 °C for 5 min, and centrifuged (10,000*g*, 10 min).

The SDS-PAGE under reducing conditions was carried out as mentioned above, just using the samples dissolved in the sample buffer without the addition of 2-ME. The protein electrophoretic patterns were analyzed by Image Master VDS (Pharmacia Biotech. Co.) in combination with Image Master 1D Edit soft (quantity one V46). The relative protein quantity of each subunit (protein band) of protein was calculated from their respective percent area on the densitograms against the total subunits area of protein (Kwanyuen & Wilson, 2000).

2.6. Differential scanning calorimetry (DSC)

The thermal properties of protein samples were examined using a TA Q100-DSC thermal analyzer (TA Instruments, New Castle, Delaware 19720 USA), according to the procedure of Meng and Ma (2001) with some modifications. Approximately 2.0 mg of protein samples were weighed into the aluminum pans (Dupont), and 10 μL of 0.05 M phosphate buffer (pH 7.0) was added. The pans were hermetically sealed and heated from 20 to 110 °C at a rate of 5 °C/min. A sealed empty pan was used as a reference. Onset temperature (T_m), peak transition or denaturation temperature (T_d), enthalpy of denaturation (ΔH) and cooperativity, represented by the width at half-peak height ($\Delta T_{1/2}$), were computed from the thermograms by the Universal Analysis 2000, Version 4.1D (TA Instruments-Waters LLC). All experiments were conducted in triplicate. The sealed pans containing protein isolate samples and buffers were equilibrated at 25 °C for more than 6 h.

2.7. Amino acid analysis

The amino acid composition of the protein samples was determined by an automatic amino acid analyzer (Waters M510, USA), using PICO.TAG column. The determination was carried out at 38 °C, and the detection wavelength 254 nm and flow rate 1.0 mL per minute. The samples were hydrolyzed with 6 N HCl for 24 h at 110 °C in a sealed tube. The amino acid composition was reported as g/100 g protein. The amino acid tryptophan was not determined.

2.8. Evaluation of nutritional parameters

The amino acid composition of sample was used for calculation of the nutritional value of hemp and soybean proteins as summarized below (Chavan, McKenzie, & Shahidi, 2001). The proportion of essential amino acids (E) to total amino acids (T) of the protein:

$$\frac{E}{T}\% = \frac{\text{Ile} + \text{Leu} + \text{Lys} + \text{Met} + \text{Cys} + \text{Phe} + \text{Tyr} + \text{Thr} + \text{Val} + \text{His}}{\text{Ala} + \text{Asp} + \text{Arg} + \text{Gly} + \text{Glu} + \text{Ile} + \text{Leu} + \text{Lys} + \text{Met} + \text{Cys} + \text{Phe} + \text{Tyr} + \text{Pro} + \text{Ser} + \text{Thr} + \text{Val} + \text{His}} \times 100$$

2.9. Sequential in vitro protein digestion procedure

The *in vitro* digestibility of protein isolates was evaluated using sequential pepsin and trypsin digestion model according to the method of Chavan et al. (2001) and Nunes et al. (2004), with minor modifications. For pepsin digestion, in a 50 mL centrifuge tube, 0.5 g of protein material was suspended in 9.5 mL of 0.1 N HCl, and mixed with 5 mg pepsin power in 0.5 mL of 0.1 N HCl. The mixture of protein and pepsin was incubated at 37 °C for up to 120 min, under gently shaking condition. After that, the pepsin-digested hydrolysate was neutralized with 1.0 M phosphate buffer (pH 8.0), followed by the addition of appropriate trypsin (the enzyme to initial protein ratio was 1:100, w/w). This mixture (of pepsin-digested hydrolysate and trypsin) was incubated at 37 °C for another 120 min.

For SDS-PAGE analysis of digestion process, aliquots (200 μL) of the protein and enzyme mixtures were taken at specific periods of incubation time (0–120 min), during pepsin and subsequent trypsin digestion. These mixtures were directly mixed with the same volume of the sample buffer (in order to inactivate the enzyme and at the same time prepare the samples suitable for SDS-PAGE analysis).

2.10. Determination of nitrogen release during digestion

For the nitrogen release analysis of digestion process, the trichloroacetic acid (TCA)-precipitation method was used. At the specific periods of digestion time (during pepsin and trypsin digestion process), the TCA-soluble nitrogen fraction of the pepsin (or plus trypsin) digest was obtained by directly mixing the digest with the same volume of 10% (w/w) TCA and centrifugation at 8000*g* for 15 min. The nitrogen content of the corresponding precipitates or other protein samples was measured by the micro-Kjeldahl method ($N \times 6.25$). The % nitrogen release was defined as follows:

$$\frac{N_0 - N_t}{N_{tot}} \times 100,$$

where N_t represents the TCA-precipitated nitrogen content after pepsin (and trypsin) digestion for t min (mg), N_0 the TCA-precipitated nitrogen content in protein samples before the digestion (mg), and N_{tot} the total nitrogen content in the protein samples (mg).

2.11. Statistical analysis

An analysis of variance (ANOVA) was performed on the data, and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

3.1. Proximate analysis

The protein, moisture, ash and fat contents of HPI (as well as HPI-7S and HPI-11S) and SPI, used in the present study, are shown in Table 1. The obtained HPI was mainly composed of protein (90.5%), moisture (2.8%), ash (2.4%) and other components (e.g., carbohydrate). The contents of these constituents for HPI are similar to that of SPI. By comparison, HPI-11S had significantly higher protein content, while HPI-7S had significantly lower protein content ($P < 0.05$; Table 1). The difference in protein content for different HPIs may be attributed to the differences in carbohydrate content, since most globulins from plants are usually a kind of glycoprotein. All the protein samples had similar low lipid contents of about 0.32–0.42% and moisture contents of 2.6–2.9%.

3.2. SDS-PAGE analysis

The SDS-PAGE profiles of protein constituents of HPI (including HPI-7S and HPI-11S) and SPI in the presence and absence of 2-ME are presented in Fig. 1, and the estimated molecular weight (MW) and relative contents of different subunits are summarized in Table 2. Under reduced condition, there were two major kinds of protein constituents (marked as the numbers 2, 3 and 4) for HPI, corresponding to acidic and basic subunits (AS and BS) of edestin, respectively (Fig. 1). Patel, Cudney, and McPherson (1994) using crystallographic technique, had showed that like the hexamer of soy glycinin, the edestin molecule is also composed of six identical subunits, and each subunit consists of AS and BS linked by one disulfide bond. In the present study, the AS of about 34.0 kDa was relatively homogenous, while the BS mainly consisted of two subunits of about 20.0 and 18.0 kDa, respectively. From these MW and relative content data, the MW of edestin (hexamer form) can be estimated to be about 300 kDa, which is close to that (290 kDa) reported by Hall (1949), and much higher than that (212 kDa) reported by Svedberg and Stamm (1929).

Unlike the soy protein case, HPI only contained a minor component (marked as the number 1) of about 48.0 kDa, which corresponds to the β-subunit of soy β-conglycinin. In the HPI-11S, the relative contents of the subunits (2 and 3) were significantly increased, while the subunit *1* sig-

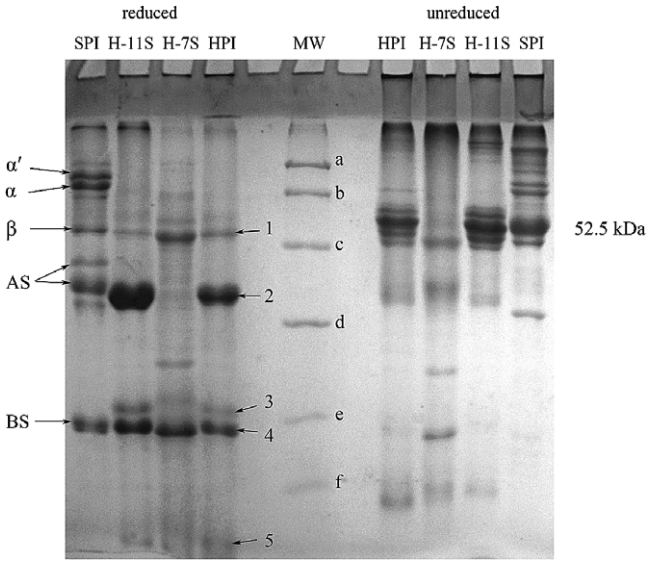

Fig. 1. SDS-PAGE profiles of HPI and SPI in the presence (reduced) and absence (unreduced) of 2-ME. MW: Molecular weight markers (a, b, c, d, e and f correspond to 97.4, 66.2, 43, 31, 20.1 and 14.4 kDa, respectively). AS and BS represent the acidic and basic subunits of soy glycinin, respectively, while α′, α and β represent the corresponding major subunits of soy β-conglycinin.

Table 1
Proximate compositions of HPI, HPI-7S, HPI-11S and SPI

Constituents (wet basis, %)	HPI	HPI-7S	HPI-11S	SPI
Protein	90.53 ± 0.71^{b}	87.67 ± 0.28^{c}	93.02 ± 0.38^{a}	92.72 ± 0.19^{a}
Moisture	2.79 ± 0.12^{a}	2.85 ± 0.05^{a}	2.61 ± 0.01^{b}	2.89 ± 0.04^{a}
Ash	2.38 ± 0.01^{b}	3.91 ± 0.01^{a}	2.54 ± 0.03^{b}	2.63 ± 0.01^{b}
Fat	0.36 ± 0.17^{a}	0.32 ± 0.07^{a}	0.41 ± 0.03^{a}	0.42 ± 0.42^{a}

Each value was the mean and standard deviation of duplicate measurements.
Different superscript characters (a–c) represent the significant difference at $P \leqslant 0.05$ within a same row.

Table 2
Molecular weight (MW) and relative content of the major subunits of HPI, HPI-7S and HPI-11S

Proteins/MW and content		Different subunits			
		1	2	3	4
HPI	MW (kDa)	47.99 ± 0.70	34.39 ± 0.94	20.26 ± 0.27	18.61 ± 0.28
	Relative content (%)	6.60 ± 0.22^{b}	43.34 ± 0.08^{b}	11.95 ± 0.20^{b}	35.21 ± 0.22^{b}
HPI-7S	MW (kDa)	47.25 ± 0.45	34.73 ± 0.24	21.15 ± 0.20	17.86 ± 0.22
	Relative content (%)	31.61 ± 0.41^{a}	11.17 ± 0.17^{c}	7.04 ± 0.25^{c}	47.56 ± 0.08^{a}
HPI-11S	MW (kDa)	48.58 ± 0.25	33.93 ± 0.28	19.82 ± 0.10	18.19 ± 0.11
	Relative content (%)	3.65 ± 0.06^{c}	50.42 ± 1.47^{a}	13.73 ± 0.77^{a}	30.38 ± 0.35^{c}

Each value is the mean and standard deviation of duplicate determinations.
Different superscript characters (a, b and c) indicate the significant difference at $P < 0.05$ level within a same column.

nificantly decreased, relative to HPI ($P < 0.05$; Table 2). By comparison, the subunits (2 and 3) were almost absent in HPI-7S, while the relative content of the subunit 1 increased by almost 5-fold relative to that of HPI (Fig. 1 and Table 2).

In the absence of 2-ME, the disulphide bonds between AS and BS of edestin (or glycinin) will not be disrupted, and as the result, the subunits of edestin would be in the form of different AB units. As expected, the subunits (2, 3 and 4) were absent in the SDS-PAGE profiles (unreduced), and correspondingly, there were miscellaneous bands of about 52.5 kDa, clearly attributed to the AB units (Fig. 1). Under this non-reduced condition, HPI-11S showed similar SDS-PAGE profiles to that of HPI. However, in the case of HPI-7S, the corresponding AB units were almost absent, and most of the protein constituents were in the form of aggregates with MW of above 97.4 kDa (Fig. 1). Furthermore, the relative content of the subunit 1 also remarkably decreased as compared to that in the presence of 2-ME. The data suggest that there be strong hydrophobic interactions between the subunit 1 and the BS of about 18 kDa, and the disulphide bond be clearly involved. Under non-reduced condition, all HPI samples contained a relatively high content of aggregates stacking on the top of separating gel, or even not entering the stacking gel. This phenomenon confirms our previous assumption that the poor solubility of hemp protein isolate is largely due to the disassociation and/or re-association of disulphide bonds between AS and BS of edestin (Tang et al., 2006).

3.3. Thermal property

The thermal transition of the edestin component for various HPIs (including HPI, HPI-11S and HPI-7S) was investigated by conventional DSC, as shown in Fig. 2, and some DSC characteristics are shown in Table 3. In the DSC profiles, the endothermic events are usually associated with the rupture of hydrogen bonds, or the unfolding of globular proteins during thermal denaturation (Arntfield & Murray, 1981; Fitzsimons, Mulvihill, & Morris, 2006; Meng & Ma, 2001; Privalov, 1982). HPI and HPI-11S exhibited similar DSC profiles. Both showed a major endothermic peak with on-set and denaturation temperatures of 85.7 and 92.0 °C, respectively (Fig. 2 and Table 3), clearly assigned to thermal denaturation of the edestin component. This is consistent with the result of SDS-PAGE analysis that HPI and HPI-11S had similar protein constituent patterns (Fig. 1).

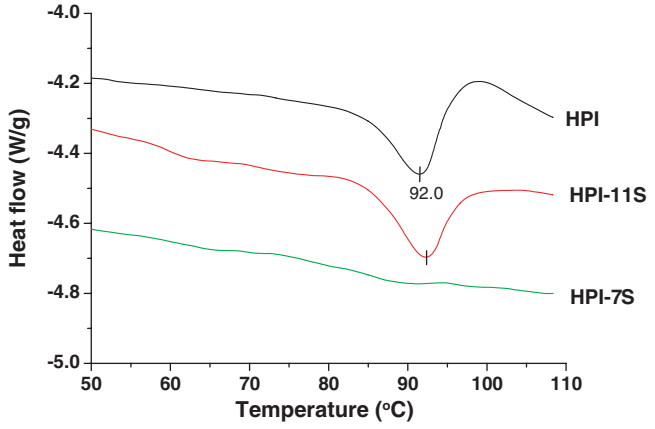

Fig. 2. Typical DSC thermograms of HPI, HPI-7S and HPI-11S in 0.05 M phosphate buffer (pH 7.0).

Table 3
The onset temperatures (T_m), peak temperatures (T_d), enthalpy change (ΔH) and width at half-peak height of major endothermic peak ($\Delta T_{1/2}$) for various HPIs[a]

Samples	T_m (°C)	T_d (°C)	ΔH (J/g protein)	$\Delta T_{1/2}$ (°C)
HPI	85.7 ± 0.51	92.0 ± 0.42	9.4 ± 0.27	6.2 ± 0.32
HPI-7S	No transition signals			
HPI-11S	85.4 ± 0.40	91.9 ± 0.45	7.3 ± 0.77	6.3 ± 0.30

[a] Means ± standard deviations of triplicate analyses. The protein sample (with 1.5–2.0 mg protein content) was dispersed in 10 μL of 0.05 M phosphate buffer (pH 7.0).

The enthalpy change (ΔH) of the endothermic peak of HPI-11S was significantly ($P < 0.05$) lower than that of HPI (Table 3). The ΔH reflects the proportion of undenatured protein in a sample, or extent of ordered structure (Arntfield & Murray, 1981). Thus, this data suggests that the extent of ordered structure in HPI was higher than that in HPI-11S. The width at half-peak height of major endothermic peak ($\Delta T_{1/2}$), which is an index of the cooperativity of the transition from native to denatured state (Privalov, 1982), was not significantly different for both HPIs.

Interestingly, in the case of HPI-7S, there were no detectable thermal transition signals (Fig. 2). This phenomenon confirmed that the thermal denaturation of edestin was due to the disruption of intact structure (hexamer form). The absence of acidic subunits (Fig. 1) may facilitate the formation of aggregate of basic subunits, or its complex with the subunit 1, due to strong hydrophobic interactions between these subunits. These results confirmed that the thermal property of HPI was affected by the difference of its protein constituent.

3.4. Amino acid composition and evaluation

The amino acid compositions (g/100 g of protein) of HPI (including HPI-11S and HPI-7S) and SPI are shown in Table 4. In the table, the FAO/WHO suggested requirements of the essential amino acids for 2–5 year old are also included (FAO/WHO, 1990). Like SPI, various HPIs are rich in glutamic acid, aspartic acid, serine, arginine and leucine, which are consistent with the previous data of these amino acids (Callaway, 2004; Tang et al., 2006; Yang & Bai, 2001). Although the sulfur-containing amino acids (Met and Cys) might be to some extent destroyed by the HCl-hydrolysis method used in this study, their contents in HPI were remarkably higher than that of SPI (Table 4). This may be related to the presence of a methionine-

Table 4
Amino acid compositions of HPI (including HPI-11S and HPI-7S) and SPI, and the FAO/WHO suggested requirements (2–5 year old) for the essential amino acids

Amino acids	HPI	HPI-7S	HPI-11S	SPI	FAO/WHO suggested requirements (2–5 year old)
Ile[a]	3.99 ± 0.08	3.09 ± 0.21	3.30 ± 0.14	4.35 ± 0.11	2.8
Leu[a]	6.63 ± 0.23	6.17 ± 0.46	6.49 ± 0.27	6.79 ± 0.83	6.6
Lys[a]	4.16 ± 0.87	3.32 ± 0.25	3.36 ± 0.15	5.23 ± 0.01	5.8
Met[a]	1.39 ± 0.06	1.50 ± 0.13	2.36 ± 0.12	0.92 ± 0.07	
Cys	0.17 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.05 ± 0.00	
Tyr	3.67 ± 0.23	2.95 ± 0.22	3.34 ± 0.15	3.61 ± 0.18	
Phe[a]	4.57 ± 0.11	4.03 ± 0.31	4.03 ± 0.14	5.14 ± 0.58	
Thr[a]	4.57 ± 0.35	3.99 ± 0.32	4.01 ± 0.16	3.98 ± 0.13	3.4
Val[a]	4.98 ± 0.13	4.30 ± 0.32	4.26 ± 0.18	4.28 ± 0.32	3.5
His[a]	2.81 ± 0.47	3.13 ± 0.22	3.31 ± 0.20	2.81 ± 0.24	1.9
Trp	Not determined				1.1
Asp	9.41 ± 0.39	8.76 ± 0.59	8.82 ± 0.27	11.47 ± 0.71	
Glu	16.14 ± 0.26	15.88 ± 1.19	16.66 ± 0.43	20.67 ± 0.83	
Ser	5.18 ± 0.02	5.53 ± 0.41	5.34 ± 0.41	5.32 ± 0.09	
Gly	3.99 ± 0.06	3.83 ± 0.23	3.88 ± 0.13	3.74 ± 0.27	
Arg	9.91 ± 0.91	7.60 ± 0.59	9.35 ± 0.15	7.35 ± 0.35	
Ala	4.50 ± 0.36	4.27 ± 0.35	4.24 ± 0.06	3.72 ± 0.06	
Pro	4.53 ± 0.39	4.42 ± 0.37	4.31 ± 0.04	5.13 ± 0.33	
Total sulfur-containing amino acids (Met and Cys)	1.55	1.67	2.58	0.96	2.5
Total aromatic amino acids (Phe and Tyr)	8.43	6.97	7.37	8.75	6.3
Total essential amino acids[b]	37.12	32.64	34.69	37.15	32.8
Total non-essential amino acids	53.67	50.29	52.6	57.40	
E/*T* (%) [c]	45.16	42.38	44.90	42.72	

Each value is the mean and standard deviation of duplicate measurements, and all values are expressed in g of amino acid per 100 g of protein.
[a] Essential amino acids.
[b] Total essential amino acids exclude the amino acid Trp.
[c] The proportion of essential amino acids to the total amino acids.

and cystine-rich seed protein in hemp seeds (Odani & Odani, 1998). In comparison, the essential amino acids Ile, Lys and Phe of HPI were to a various extent lower than that of SPI, however, the others were similar or higher. The proportion of essential amino acids to the total amino acids (*E*/*T*) for HPI was significantly higher than that of SPI, suggesting that the HPI have more nutritional amino acid pattern than that of SPI. The *E*/*T* of HPI-11S was close to that of HPI, while that of HPI-7S is much less (Table 4).

Infants have very critical nutritional requirements due to rapid growth and immaturity of gastrointestinal function, and nine amino acids have been identified to be essential for infants: Thr, Val, Leu, Ile, Lys, Trp, Phe, Met and His. Arg and Cys are also essential for low birth weight infants (Chau, Cheung, & Wong, 1998). According to the FAO/WHO suggested requirements for 2–5 year old infants, only Lys and sulfur-containing amino acids (Met and Cys) in HPI are limiting amino acids, which is consistent with our previous data (Tang et al., 2006). Except these amino acids, other essential amino acids are sufficient for the FAO/WHO suggested requirements for 2–5 year old infants. Interestingly, the sulfur-containing amino acids of HPI-11S met the requirement of FAO/WHO for 2–5 year old infants (Table 4). The data suggests that the protein rich in sulfur-containing amino acids is enriched in HPI-11S, obtained by precipitation at pH 6.4 in the presence of a low concentration of $NaHSO_3$.

3.5. In vitro digestibility

The *in vitro* digestibility of HPI and SPI was evaluated using the sequential pepsin and trypsin digestion model, by reducing SDS-PAGE and nitrogen release analyses, as shown in Figs. 3 and 4. The *in vitro* digestibility of HPI, HPI-11S and HPI-7S was also compared. During the pepsin digestion, the protein constituents of edestin (including the AS and BS) were rapidly digested by pepsin within about 1 min, to release oligo-peptides with molecular weight (MW) less than 10.0 kDa in reducing SDS-PAGE profile (Fig. 3, Lane 14). In a similar way, the AS and BS of soy glycinin were digested by pepsin. Upon further incubation with pepsin, it could be distinctly observed that the MW distribution of the oligo-peptides decreased with increasing the incubation time from 1 to 120 min (Fig. 3, Lanes 14–19). After the pepsin-digested hydrolysate were adjusted to pH 8.0, the addition of trypsin led to further decline in the MW distribution of the oligo-peptides (Fig. 3, Lanes 20–24).

The pepsin and trypsin digestion pattern of HPI-11S and HPI-7S are similar to that of HPI (data not shown).

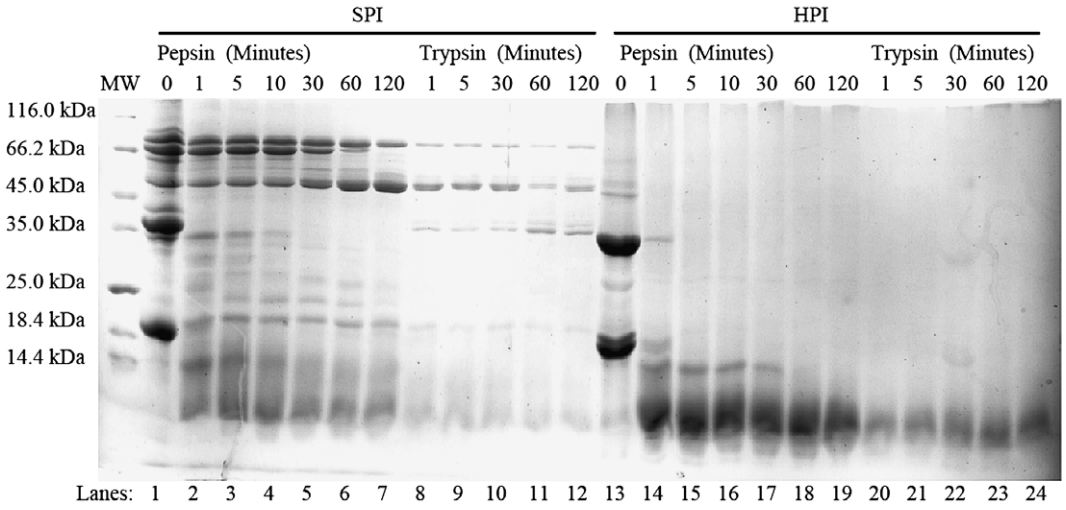

Fig. 3. Reducing SDS-PAGE profiles for SPI and HPI digested with sequential pepsin and trypsin, in the presence of 5% 2-ME. Lanes 1–7, SPI digested by pepsin for 0, 1, 5, 10, 30, 60 and 120 min, respectively; Lanes 8–12, the SPI pepsin-hydrolysate further digested by trypsin for 1, 5, 30, 60 and 120 min, respectively; Lanes 13–19, HPI digested by pepsin for 0, 1, 5, 10, 30, 60 and 120 min, respectively; Lanes 20–24, the HPI pepsin-hydrolysate further digested by trypsin for 1, 5, 30, 60 and 120 min, respectively. M, protein molecular weight markers.

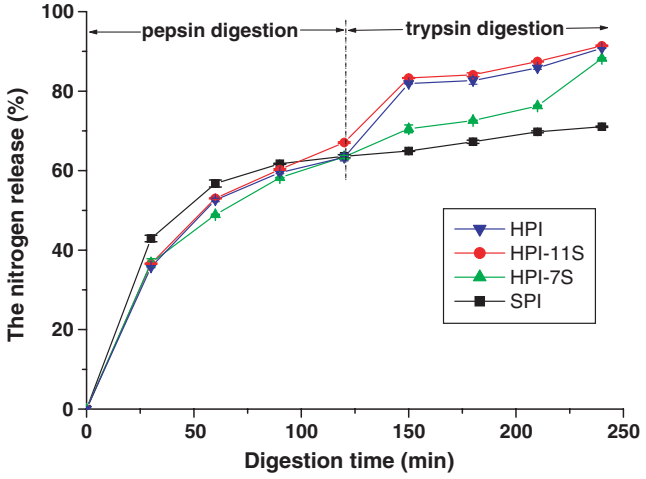

Fig. 4. The % nitrogen release of HPI, HPI-11S, HPI-7S and SPI during pepsin and subsequent trypsin digestion. The error bars indicate the standard deviations of duplicate measurements.

Interestingly, the subunit 1 of 4.8 kDa in HPI-7S, accounting for 31.6% total dyed proteins, was also easily digested by pepsin, like the subunits of edestin (data not shown). In contrast, the subunits of soy β-conglycinin (including α′-, α- and β-subunits) were much less prone to pepsin digestion (Fig. 3, Lanes 2–8). This difference may be attributed to the difference in protein stability of these subunits in acid medium (at about pH 2.0). With respect to the subunit 1 (which may be in the form of complexes with the basic subunits) of HPI-7S, the more intact structure of β-conglycinin at about pH 2.0 may greatly decrease its susceptibility to the pepsin digestion.

The TCA-soluble nitrogen release during pepsin and trypsin digestion can reflect the changes in the digestibility, and the differences in digestion pattern (especially the trypsin digestion). During the pepsin digestion, different HPIs showed a similar change trend of the % nitrogen release: increased fast and linearly at the initial stage (0–25 min), and then slowly and gradually reached the maximum upon further digestion (Fig. 4). In the same process, the TCA-soluble nitrogen of SPI was more easily released during initial pepsin digestion (0–60 min) with respect to HPI, and the % nitrogen release values at 30 and 60 min were significantly higher ($P < 0.05$). However, the % nitrogen release after the whole pepsin digestion was similar for SPI and HPI. The difference in the pepsin digestion pattern may be attributed to the difference in the availability of catalytic sites of protein substrates to pepsin. The poor solubility or the presence of insoluble aggregates of HPI (Tang et al., 2006) might result in direct decline in the availability of these sites, during the initial pepsin digestion.

In the further trypsin digestion of SPI, the % nitrogen release increased gradually and slowly from the initial value (63.6%) to a maximum value (71%). The presence of high activity of trypsin inhibitors in beans (Khokar & Chanhan, 1986; Marquez, Fernandez, & Alonso, 1998) may account for this phenomenon. In the same process, the % nitrogen release for HPI and HPI-11S increased fast during the initial incubation with trypsin (0–30 min), and then increased gradually up to the maximum (Fig. 4). In the case of HPI-7S, the % nitrogen release increased much slower than that of HPI (and HPI-11S), during the initial 30 min digestion. However, it increased much faster during last (90–120 min) trypsin digestion. The pepsin-released peptides with high hydrophobicity are usually unstable at pH 8.0, and might interact to form some kinds of aggregates. Thus, the difference of trypsin digestion pattern for various HPIs may be attributed to the differences in the form and size of aggregates formed from the pepsin-digested hydrolysates. It is obvious that the formation of

the aggregates in the HPI-7S largely comes from the contribution of the digestion of the subunit 1. At the end of trypsin digestion, the % nitrogen release was insignificant at $P < 0.05$ level for various HPIs (Fig. 4). On the other hand, the % nitrogen release (88–91%) of various HPIs after pepsin and trypsin digestion was much significantly higher than that (71%) of SPI ($P < 0.05$; Fig. 4). The results suggest that HPI is a good source of much more digestible protein as compared to SPI, which is highly suitable for the human consumption.

4. Conclusions

The HPI was composed of a major component, edestin (consisting of acidic and basic subunits), and a minor component with MW of about 48.0 kDa. The minor component was enriched in 7S-rich HPI, which may affect the present form of protein constituents in HPI. Except the lysine and sulfur-containing amino acids, the essential amino acids of HPI (including 11S- and 7S-rich HPIs) met up with the suggested requirements of FAO/WHO for 2–5 year old infants. The proportion of essential amino acids to the total amino acids (E/T) for HPI (as well as HPI-11S), and their *in vitro* pepsin plus trypsin digestibility were significantly higher, as compared to that of SPI. Thus, hemp proteins can be suitable for human consumption as a more superior source of protein nutrition, relative to widely recognized soy proteins.

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