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Physicochemical and Functional Properties of Hemp (*Cannabis sativa* L.) Protein Isolate

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The amino acid composition and physicochemical and functional properties of hemp (*Cannabis sativa* L.) protein isolate (HPI) were evaluated and compared with those of soy protein isolate (SPI). Edestin, a kind of hexameric legumin, was the major protein component. HPI had similar or higher levels of essential amino acids (except lysine), in comparison to those amino acids of SPI. The essential amino acids in HPI (except lysine and sulfur-containing amino acids) are sufficient for the FAO/WHO suggested requirements for 2−5 year old children. The protein solubility (PS) of HPI was lower than that of SPI at pH less than 8.0 but similar at above pH 8.0. HPI contained much higher free sulfhydryl (SH) content than SPI. Differential scanning calorimetry analysis showed that HPI had only one endothermic peak with denaturation temperature (T_d) of about 95.0 °C, attributed to the edestin component. The T_d of the endotherm was nearly unaffected by 20−40 mM sodium dodecyl sulfate but significantly decreased by 20 mM dithiothreitol ($P < 0.05$). The emulsifying activity index, emulsion stability index, and water-holding capacity of HPI were much lower than those of SPI, and the fat adsorption capacity was similar. The data suggest that HPI can be used as a valuable source of nutrition for infants and children but has poor functional properties when compared with SPI. The poor functional properties of HPI have been largely attributed to the formation of covalent disulfide bonds between individual proteins and subsequent aggregation at neutral or acidic pH, due to its high free sulfhydryl content from sulfur-containing amino acids.

KEYWORDS: Hemp protein isolate (HPI); *Cannabis sativa* L.; amino acid composition; physiochemical property; functional property

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. Its cultivation in most of countries has been prohibited due to the presence of the phytochemical drug component δ-9-tetrahydrocannabinol (THC). A low THC form of industrial hemp is now legal to grow in Canada and China, and the global market for low THC hemp is increasing rapidly (*1*, *2*). The industrial hemp is a good source for producing hemp fiber, while the hemp fiber is widely used in the modern production of durable fabrics and specialty papers in some countries. In the processing of hemp fiber, the seed becomes an interesting byproduct.

Hempseed has been consumed as a source of food throughout recorded history (raw, cooked, or roasted) or employed as a feed (*1*). Within the last 10 years, it has also been legally used as food for humans in both Canada and the United States. In addition to considerable amounts of dietary fiber, the seed typically contains over 30% oil and about 25% protein (*3*). Hempseed oil, over 80% in polyunsaturated fatty acids (PUFAs), is an exceptionally rich source of linoleic (omega-6) and α-linolenic (omega-3) acids and thus is suggested to be perfectly balanced in regards to the ratio (3:1) of the two essential fatty acids for human nutrition (*1*, *3*). Besides the nutritional value, the hempseed oil also has some potential health benefits, such as lowering of cholesterol and high blood pressure, since there is a well-characterized relationship between these benefits and the high polyunsaturated fatty acid content in vegetable oils. Recently, a clinical report on dietary hempseed oil has shown that this oil can be used to treat atopic dermatitis in humans (*4*).

In addition, the hempseed contains high-quality storage proteins (edestin and albumin), which are easily digested and rich in all essential amino acids (*3*). From hempseed, even a methionine- and cystine-rich seed protein (a 10-kDa protein) has been isolated and identified (*5*). Thus, the proteins from hempseed have good potential to be applied as a source of protein nutrition. However, little information is available concerning the physiochemical and functional properties of this protein.

The objective of this work was to investigate the physiochemical and functional properties of the protein isolate from hempseed (HPI). The amino acid composition was also deter-

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mined and compared with the FAO/WHO suggested requirements for infants and children. In addition, the properties of HPI were compared with those of soy protein isolate (SPI).

MATERIALS AND METHODS

Materials. Defatted hempseed meal, a byproduct during the utilization of the valuable hempseed oil, was kindly supplied by YUNNAN Industrial Hemp Co. Ltd. (China). This meal had been obtained from hemp (*Cannabis sativa* L.) seeds on a large scale through dehulling, disintegrating and defatting with supercritical liquid (CO_2) at low temperatures (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). All the agents used in the present study are of analytical grade or better grade.

Preparation of Protein Isolates. Hemp protein isolate (HPI) was prepared from defatted hemp meal according to the well-known process used for soybean protein isolate (SPI), with a few modifications (*6*). Defatted hemp meal was mixed with 20-fold (w/v) deionized water at 35 °C, and the mixture was adjusted to pH 10.0 with 2 N NaOH. After more than 1 h of extraction by stirring, samples were centrifuged at 8000*g* for 30 min at 20 °C. The pellet was discarded, the supernatant was adjusted to pH 5.0 with 2 N HCl, and the precipitate or curd was collected by centrifugation (8000*g*, 10 min). The isoelectric precipitate was resuspended in deionized water, and after homogenization, the suspension was adjusted to about pH 6.8 with 1 N NaOH. Then, the suspension was freeze-dried to produce HPI products.

SPI was prepared from defatted soybean meal (XIANGCHI Cereal and Oil Co. Ltd., Shandong Province, China), according to the method described by Tang et al. (*6*). The protein content of HPI and SPI was determined by Kjeldahl method ($N \times 6.25$). The chemical compositions of hemp meal and protein isolate were determined according to AOAC procedures (*7*).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was performed on a discontinuous buffered system according to the method of Laemmli (*8*) using 12% separating gel and 4% stacking gel. The protein samples were heated for 5 min in boiling water before electrophoresis. For each sample, 10 μL was applied to each lane. Before the sample entered the separating gel, electrophoresis was performed at 10 mA, and afterwards it was performed at 20 mA. The gel was stained with 0.25% Coomassie brilliant blue (R-250) in 50% trichloroacetic acid and destained in 7% acetic acid [methanol/acetic/water, 227:37:236 (v/v/v)].

For protein quantification by densitometric scanning, the individual lanes of the stained gels were scanned by a white/ultraviolet transilluminator (UVP Inc., Upland, CA) and analyzed by the software of Labworks (version 4.0). The relative content of proteins was calculated as the sum of the area density of their subunit or polypeptide bands with respect to total area density of the densitogram.

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

Protein Solubility (PS). PS was determined according to the method of Tomotake et al. (*9*), with a few modifications. Protein dispersions (1.0%, w/v) were prepared in 0.01 M phosphate buffer adjusted to pH 2−10. To achieve desirable pH above 10, the dispersion was adjusted directly using 1 N NaOH. For total soluble protein content (control), the samples were dispersed in 0.1 N NaOH. The protein dispersions were stirred at ambient temperatures for 1 h, centrifuged (8000*g* for 30 min), and filtered through filter paper. Protein contents of the filtrate were determined according to the Bradford method using bovine serum albumin as a standard. Percent protein solubility was calculated as PS (%) = (protein content of sample/protein content of control) × 100. Each measurement was carried out in triplicate.

Free Sulfhydryl (SH) Content. The SH content was determined according to the method of Beveridge et al. (*10*). About 8 mg of each protein sample was solubilized in 8 mL of 0.086 M Tris buffer (pH 8.0), containing 0.09 M glycine, 0.004 M EDTA, and 8 M urea. One milliliter of protein solutions was then mixed with 40 mL of Ellman's reagent (4 mg/mL in methanol). The absorbance of the mixture was measured at 412 nm in a UV−vis spectrophotometer **(**Shanghai JINMI Science Instrument Co. Ltd., China**)**, and the buffer with same volume was used as the blank. The values of SH content were obtained by dividing the absorbance value by the molar extinction coefficient of 13 600. Each sample was determined in triplicate.

Emulsifying Activities. Emulsifying activity index (EAI) and emulsion stability index (ESI) of the samples were determined according to the method of Pearce and Kinsella (*11*), with minor modifications made by Tang et al. (*12*). For the emulsion formation, 6 mL of 0.2% HPI or SPI dispersion in 0.05 M Tris-HCl buffer (pH 7.5) and 2 mL of soybean oil were homogenized in an FJ-200 high-speed homogenizer (Shanghai Specimen Model Co., China) for 1 min at the maximum velocity. Fifty microliters of emulsion was taken from the bottom of the homogenized emulsion immediately (0 min) or 10 min after homogenization and diluted (1:100, v/v) in 0.1% (w/v) SDS solution. After shaking in a vortex mixer for a moment (about 5 s), the absorbance of diluted emulsions was read at 500 nm in the spectrophotometer. EAI and ESI values were calculated by the following equations:

$$\mathrm{EAI}\ (\mathrm{m^2/g}) = \frac{2 \times 2.303 \times A_0 \times \mathrm{DF}}{c \times \phi \times 10\,000} \quad (1)$$

$$\mathrm{ESI}\ (\mathrm{min}) = \frac{A_0}{A_0 - A_{10}} \times 10 \quad (2)$$

where DF is the dilution factor (100), c is the initial concentration of protein (g/mL), ϕ is the optical path (0.01 m), θ is the fraction of oil used to form the emulsion (0.25), and A_0 and A_{10} are the absorbance of the diluted emulsions at 0 and 10 min. Measurements were performed in triplicate.

Water Holding and Fat Absorption Capacities (WHC and FAC). WHC and FAC were determined according to the method of Tomotake et al. (*9*), with minor modifications. Two grams of sample was weighed into 25 mL preweighed centrifuge tubes. For each sample, deionized water was added in small increments to a series of tubes under continuous stirring with a glass rod. After the mixture was thoroughly wetted, samples were centrifuged (5000*g* for 30 min). After the centrifugation, the amount of added desalted water in the supernatant liquid in the test tube was recorded. WHC (grams of water per gram of protein) was calculated as WHC = $(W_2 - W_1)/W_0$, where W_0 is the weight of the dry sample (g), W_1 is the weight of the tube plus the dry sample (g), and W_2 is the weight of the tube plus the sediment (g). Each sample was determined in triplicate.

For FAC, 0.5 g of sample was weighed into 25 mL preweighed centrifuge tubes, and thoroughly mixed with 5 mL of sesame oil. The protein−oil mixture was centrifuged at 5000*g* for 30 min. Immediately after centrifugation, the supernatant was carefully removed, and the tubes were weighed. FAC (grams of oil per gram of protein) was calculated as FAC = $(F_2 - F_1)/F_0$, where F_0 is the weight of the dry sample (g), F_1 is the weight of the tube plus the dry sample (g), and F_2 is the weight of the tube plus the sediment (g). Each sample was determined in triplicate.

Differential Scanning Calorimetry (DSC). The thermal denaturation of HPI was examined using a TA Q100-DSC thermal analyzer (TA Instruments, New Castle, DE), according to the procedure of Meng and Ma (*13*), with some modifications. Approximately 2.0−3.0 mg protein samples were accurately weighed into aluminum liquid pans, 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. Peak or denaturation temperature (T_d) of different protein components and enthalpy of denaturation (ΔH), were computed from the thermograms by the Universal Analysis 2000, version 4.1D (TA Instruments-Waters LLC, USA).

For experiments involving additives (e.g., protein structure perturbants), the phosphate buffers containing the additives were added to the pans. All experiments were conducted in triplicate. In all cases,

Table 1. Proximate Analyses of Defatted Hemp and Soy Meals, HPI and SPI[a]

samples	protein (%)	moisture (%)	ash (%)	others (%)
hemp meal	50.2(0.5) b	6.7(0.4) b	3.2(0.2)	39.9
soy meal	43.3(0.5) c	8.0(0.3) a	3.3(0.1)	45.4
HPI	86.9(1.2) a	3.9(0.3) d	2.6(0.1)	6.6
SPI	89.0(0.8) a	5.0(0.4) c	2.3(0.2)	3.7

[a] All data were based on the wet basis. Each value was the mean and standard deviation of duplicate measurements. The letters (a–d) indicate significant ($P < 0.05$) difference within the same column.

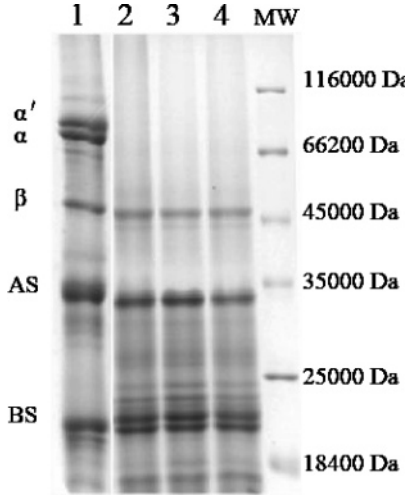

Figure 1. SDS–PAGE profiles of hemp and soy proteins: lane 1, SPI; lane 2, defatted hemp meal; lane 3, whole HPI; lane 4, the precipitate of HPI at pH 7.0. AS and BS within the figure indicate the acidic and basic subunits, respectively.

the sealed pans containing protein isolate samples and buffers were equilibrated at 25 °C for more than 6 h.

Statistics. An analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) test with a confidence interval of 95% or 99% was used to compare the means.

RESULTS AND DISCUSSION

Proximate and SDS−PAGE Analyses. The composition of the defatted hempseed meal used in this study was as follows (% w/w): protein, 50.2; moisture, 6.7; ash, 3.2; and others (mainly carbohydrate), 39.9 (**Table 1**). The protein content of this meal was significantly ($P < 0.05$) higher than that of defatted soy meal (43.3%). According to the process described in Materials and Methods, the recovery of hemp protein isolate (HPI) and its protein content were about 73% (relative to the total protein content in meal) and 86.9% (w/v) respectively. The chemical composition of HPI was similar to that of soy protein isolate (SPI), except that the moisture content of the former was significantly ($P < 0.05$) lower than that of the latter (**Table 1**). The difference in moisture content may be attributed to the water-holding ability difference of the proteins.

The SDS−PAGE profiles of HPI and SPI are shown in **Figure 1**. In the presence of reducing agent β-mercaptoethanol (2-ME), defatted hemp meal and HPI showed similar protein constituents, which were separated in SDS−PAGE profiles (**Figure 1**, lanes 2 and 3). Like the case of soy proteins, in which glycinin (a legume) consisting of acidic (AS) and basic (BS) subunits is a major component, hempseed proteins also include a kind of legumin, so-called “edestin”. The edestin is composed of AS and BS with molecular weight (MW) of about 33.0 and 20.0 kDa, respectively, and the BS was more heterogeneous than the acidic subunits (**Figure 1**). Patel and others (*14*), using crystallographic technique, showed that like the hexamer of soy glycinin, the edestin molecule is also composed of six identical subunits, and each subunit consists of an AS and a BS linked by one disulfide bond.

Table 2. Amino Acid Composition of Hemp Protein Isolate (HPI),[a] Soy Protein Isolate (SPI),[a] and Casein[b]

	content (mg/g of protein)[c]		
amino acids	HPI	SPI	casein
Asp	98.0	118.1	63
Glu	168.1	212.9	190
Ser	54.0	54.8	46
Gly	41.7	38.6	16
His[d]	29.3	29.0	27
Arg	103.2	75.7	33
Thr[d]	47.6	41.0	37
Ala	47.0	38.3	27
Pro	47.2	52.9	
Tyr	38.2	37.1	55
Val[d]	51.8	44.1	60
Ile[d]	41.5	44.8	49
Leu[d]	69.0	70.0	84
Met[d]	14.5	9.3	26
Cys	1.7	0.6	0.4
Phe[d]	49.6	53.0	45
Lys[d]	43.3	53.9	71

[a] Data from this study. [b] Data from Wang et al. (*14*). [c] Duplicate analysis. [d] Essential amino acids.

Besides the bands of acidic and basic subunits of edestin, an obvious band at about 48.0 kDa and some peptides with MW less than 18.4 kDa were observed (**Figure 1**, lanes 2 and 3). The band at about 48.0 kDa was similar to the β-subunit of β-conglycinin; however, other major subunits similar to α and α'-subunits of β-conglycinin were completely absent in HPI. The peptides with MW of less than 18.4 kDa may correspond to the albumin components. The relative content of edestin and other components was approximately estimated by the densitometric scanning technique. The result showed that edestin (including AS and BS) is the major protein component in hemp proteins, constituting about 82% of total protein, while the protein component of 48.0 kDa and the others were about 5.0% and 13%, respectively (data not shown). This is distinctly different from the case of SPI, in which it is usually composed of glycinin (legumin) and β-conglycinin (vicilin) with a similar content. Thus, it is expected that the properties of HPI might be remarkably different from those of SPI.

Our preliminary experiments showed that the solubility of HPI under neutral pH conditions was low. In the present study, the precipitate of HPI at pH 7.0 was obtained by centrifuging the HPI dispersion at 10 000*g* for 20 min and analyzed by SDS−PAGE (**Figure 1**, lane 4). The protein constituents of the precipitate were almost the same as those of whole protein isolates, suggesting a strong interaction between individual proteins of HPI. The component of about 48.0 kDa seems to not be a kind of water-soluble glycoprotein, previously reported by Hillestad and Wold (*15*), since it also coprecipitates with edestin to a similar extent. The property of this component may resemble that of the β-subunit of β-conglycinin, and the latter consists of highly hydrophobic residues (*16*).

Amino Acid Composition. The amino acid composition (mg/g of protein) of HPI is given in **Table 2**. Since casein and SPI are considered good sources of amino acid nutrition for infants, the amino acid compositions of these two proteins were

Table 3. Comparison of Essential Amino Acid Content of HPI,[a] SPI,[a] and Casein[b] to FAO/WHO Suggested Requirements[c]

	amino acid content[f]			FAO/WHO suggested requirements[f]			
amino acids	HPI	SPI	casein	1 year old	2–5 year old	10–12 year old	adult
His	29.3	29.0	27	26	19	19	16
Ile	41.5	44. 8	49	46	28	28	13
Leu	69.0	70.0	84	93	66	44	19
Lys	43.3	53.9	71	66	58	44	16
SAA[d]	16.2	9.9	26	42	25	22	17
ARM[e]	87.8	90.1	100	72	63	22	19
Thr	47.6	41.0	37	43	34	28	9
Trp			14	17	11	9	5
Val	51.8	44.1	60	55	35	25	13

[a] Data from this study. [b] Data from Wang et al. (*14*). [c] Data from Friedman and Brandon (*16*). [d] Sulfur-containing amino acids, Met and Cys. [e] Aromatic amino acids, Phe and Tyr. [f] All values are mg of amino acid per g of protein.

also included for comparison. HPI had similar or higher levels of aspartic acid, glutamic acid, serine, arginine, leucine, phenylalanine, and lysine, which is consistent with the data for these amino acids listed by Callaway (*3*). In comparison to SPI, HPI had higher levels of arginine, methionine, and cystine and lower levels of aspartic acid, glutamic acid, and lysine, while the content of other amino acids was similar. The higher methionine and cystine contents are related to the presence of a methionine- and cystine-rich seed protein in hemp seeds (*5*). Except lysine, HPI had higher levels of other essential amino acids than SPI. In comparison to casein, HPI had similar or higher levels of all amino acids except tyrosine, valine, leuine, methionine, and lysine. The results indicated that HPI also had good profiles of essential amino acids required for infants similar to those of casein and SPI. In some regards, the nutrition of HPI is superior to that of SPI.

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: threonine, valine, leucine, isoleucine, lysine, tryptophan, phenylalanine, methionine, and histidine. Arginine and cystine are also essential for low birth weight infants (*17*). According to the FAO/WHO suggested requirements for 1 year old infants, HPI had high histidine (29.3 mg/g of protein), aromatic amino acids (including phenylanine and tyrosine; 87.8 mg/g of protein), and threonine (47.6 mg/g of protein) contents, and similar isoleucine and valine contents (**Table 3**). By comparison, only lysine and sulfur-containing amino acids (methionine and cystine) in HPI are limiting amino acids for 1−5 year old infants or children. As for 10−12 year old children, all the essential amino acids in HPI are sufficient for the FAO/WHO suggested requirements, except that the content of sulfur-containing amino acids is slightly lower. To date, there is no report concerning the amino acid limitations of hemp proteins for human consumption. In early literature, edestin as the sole protein in the diet of young rats is inadequate for growth, and cystine and lysine at least are two amino acids responsible for this deficient quality (*18*).

Protein Solubility. The PS of HPI was minimum at pH in the range of 4.0−6.0 and increased gradually below pH 4.0 and above pH 6.0 (**Figure 2**). At pH 7.0, only about 38% of protein was solubilized in 0.01 M phosphate buffer. This may be due to the occurrence of edestin aggregation at pH less than 7.0 (*19*). However, at above pH 8.0, the PS increased up to more than 90%. The data suggest that HPI is a kind of typical alkali-soluble protein. The underlying mechanism of solubilization at

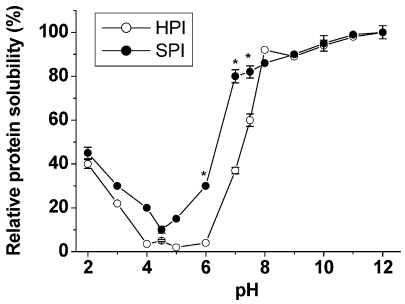

Figure 2. Protein solubility profiles of HPI (○) and SPI (●) at different pH values. Each value is the mean and standard deviation of triplicate measurements. The ∗ above the HPI curve within the figure presents the significant ($P < 0.05$) difference as compared with SPI.

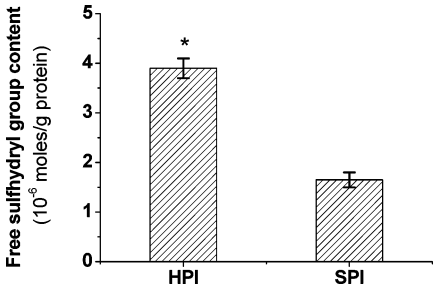

Figure 3. Free SH contents of HPI and SPI at pH 8.0. Results are means and standard deviations of triplicate measurements. The ∗ on the top of the column indicates significant ($P < 0.01$) difference.

alkaline pH (especially at pH > 10.0) may be related to the dissociation of edestin molecules (*20*).

In comparison, SPI had a similar PS profile, but the PS of SPI was higher than that of HPI at pH less than 8.0 (**Figure 2**). SPI also had a distinct isoelectric point at about pH 4.5. At pH above 8.0, the PS of HPI was similar to that of SPI. The difference in PS at pH < 7.0 may be attributed to differences of protein constituents and aggregation extent of hexamers (glycinin or edestin). The high content of methionine and cystine residues in HPI (**Table 2**) may result in increases in the formation of covalent disulfide bonds between individual molecules, thus increasing the extent of aggregation.

Free Sulfhydryl (SH) Content. Figure 3 shows the free SH contents of HPI and SPI at pH 8.0. The free SH content of HPI (about 3.9×10^6 mol/g of protein) is significantly higher than that of SPI ($P < 0.01$). The data is consistent with the relative methionine and cystine contents of HPI and SPI (**Table 2**). Typically, proteins with high SH contents have stronger ability to associate or aggregate each other, due to the formation of covalent disulphide bonds. Thus, the differences in free SH content between HPI and SPI mainly account for the PS difference at neutral and acidic pH values.

DSC Characteristics. Figure 4 shows the DSC profiles of HPI in the absence and presence of protein perturbants, for example, SDS and dithiothreitol (DTT), and the DSC characteristics are summarized in **Table 4**. HPI presented a typical endothermic peak with the thermal denaturation temperature (T_d) of about 95 °C in the absence of SDS or DTT. Like the case of glycinin in SPI, this peak clearly corresponded to the edestin component, especially the hexamer form. The on-set temperature (T_o) and thermal peak temperature (T_d) of the endotherm were insignificantly ($P > 0.05$) affected by the presence of 20−40 mM SDS, while the enthalpy change (ΔH) was significantly ($P < 0.05$) decreased (**Table 4**). The T_d and ΔH represent the thermal stability and the extent of ordered structure of a protein, respectively (*21*, *22*). On the other hand, SDS is an anionic

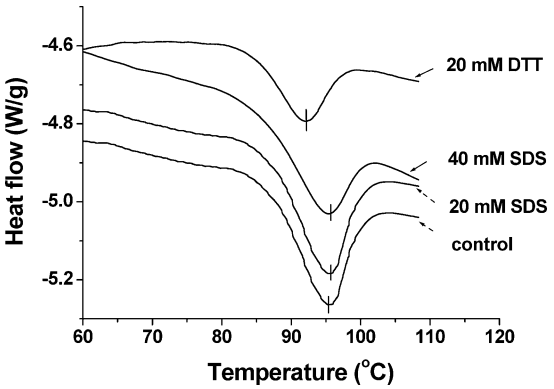

Figure 4. Typical DSC profiles of HPI in the absence and presence of various levels of SDS and DTT.

Table 4. DSC Characteristics of HPI in the Absence and Presence of SDS and DTT[a]

	T_o[b] (°C)	T_d[c] (°C)	ΔH[d] (J g^{-1})	$\Delta T_{1/2}$[e] (°C)
control	86.7(1.20) a	95.1(0.31) a	11.9(0.85) a	8.1(0.59) a
20 mM SDS	87.2(0.56) a	94.9(0.40) a	7.0(0.50) c	7.2(0.40) a
40 mM SDS	86.9(0.95) a	94.7(0.30) a	7.8(0.45) c	7.9(0.54) a
+20 mM DTT	85.2(0.83) b	92.3(0.90) b	9.0(0.78) b	7.5(0.37) a

[a] Means ± standard deviations of triplicate analyses. HPI sample (with 2.0–2.5 mg of protein content) was dispersed in 10 μL of 0.05 M phosphate buffer (pH 7.0). Different letters (a–c) indicate significant ($P < 0.05$) difference within the same column. [b] On-set temperature of denaturation. [c] Thermal denaturation temperature. [d] Enthalpy changes of the endotherm. [e] Width at half peak height of endothermic peak.

detergent, which can interact with the hydrophobic regions of protein molecules through its dodecyl hydrocarbon chain, thus causing unfolding and destabilization (*23*). Thus, the data suggest that the thermal stability of edestin is nearly unaffected by the interaction with SDS, while the extent of its ordered structure is decreased. The decrease in extent of the ordered structure may be attributed to the dissociation of protein tertiary structure of edestin or its aggregate molecules by SDS. Similar effects of SDS on the thermal properties of the globulins from oat (*24*), faba bean (*25*), red bean (*13*), and flaxseed (*26*) have been observed.

To ascertain the main interactions maintaining the thermal stability, we also investigated the influence of DTT on the DSC characteristics of the edestin component, as shown in **Table 4**. DTT is a reducing agent and can reduce the disulfide bond of cystinyl residues to sulfhydryl groups in the proteins, causing destabilization. The presence of 20 mM DTT led to considerable decreases in T_o, T_d and ΔH of the endotherm (**Table 4** and **Figure 4**), indicating that the thermal stability and the extent of the ordered structure were remarkably affected by the reduction of the disulfide bonds by DTT. The data are consistent with the amino acid composition and free SH content analyses (**Table 2** and **Figure 3**). However, the extent of decrease in ΔH by DTT was significantly lower than that by SDS ($P < 0.05$), reflecting that the ordered tertiary structure of edestin is more affected by the dissociation of hydrophobic interactions than the disruption of disulfide bonds.

Emulsifying Activities. The emulsifying activity index (EAI) and emulsion stability index (ESI) of HPI and SPI as a function of pH are shown in **Figure 5**. At any tested pH value (pH 3.0−8.0), the EAI of HPI was significantly lower than that of SPI ($P < 0.05$). The EAI profiles of HPI (and SPI) at different pH values are similar to the PS profiles of these two proteins (**Figure 2**), suggesting a possible relation between the EAI and

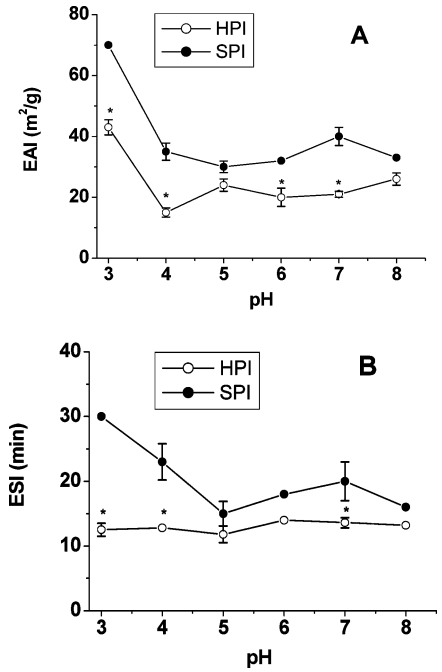

Figure 5. Emulsifying activity index (A) and emulsion stability index (B) profiles of HPI (○) and SPI (●) as a function of pH. Results are mean values and standard deviations of triplicate measurements. The ∗ above the HPI curve within the figure presents the significant ($P < 0.05$) difference as compared with SPI.

Table 5. Water Holding and Fat Absorption Capacities of HPI and SPI[a]

samples	WHC	FAC
HPI	3.37(0.15) b	5.27(0.07) a
SPI	4.36(0.06) a	5.32(0.15) a

[a] Values are means ± SD ($n = 3$) in units of g/g of sample. Within the same column, different letters (a, b) indicate significant difference ($P < 0.05$).

the PS. Although the PS values at pH higher than 7.0 were remarkably higher than those at pH in the range of 4.0−7.0, the EAI values of HPI were not significantly higher ($P > 0.05$). Thus, besides the PS, the EAI of HPI may be affected by other parameters, such as surface hydrophobicity and aggregation state of proteins. The ESI of HPI was nearly unchanged in the range of pH 3.0−8.0. However, the ESI values at most pH values were significantly lower than those of SPI ($P < 0.05$). The data indicate that the emulsifying activities of HPI are poor, when compared with those of SPI.

Water Holding and Fat Absorption Capacities (WHC and FAC). The WHC of HPI was significantly lower than that of SPI ($P < 0.05$), but the FAC of HPI was almost the same as that of SPI (**Table 5**). SPI had superior WHC in agreement with the results of the previous study (*27*). The relative poor WHC of HPI may be attributed to the severe extent of protein aggregation at neutral pH, since the polar groups of proteins would be buried in the interior of the aggregates. The comparative FAC values of HPI and SPI suggest that these two proteins have similar surface hydrophobicity, since FAC can reflect the ability of the hydrophobic groups of proteins to interact with the lipids.

In conclusion, HPI has superior essential amino acid composition, and most of essential amino acids are sufficient for the FAO/WHO suggested requirements of infants or children. However, it shows much poorer protein solubility, emulsifying

activities, and water holding capacity in comparison with SPI. Therefore, this protein should be modified to achieve some targeted functional properties before it can be applied in the food industry as a good source of nutrition for infants and children.

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