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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