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A comparative study of the structural and functional properties of isolated hemp seed (*Cannabis sativa* L.) albumin and globulin fractions

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ABSTRACT

The aim of this work was to determine the structural and functional characteristics of two major hemp seed proteins, the water-soluble albumin (ALB) and the salt-soluble globulin (GLB). The 0.5 M NaCl extract of hemp seed protein flour was dialyzed against water to obtain the two fractions: ALB in the water phase and GLB in the insoluble precipitate. The protein fractions were subjected to structural (gel electrophoresis, intrinsic fluorescence and circular dichroism) and functional (protein solubility, foaming, and emulsion) tests. Amino acid composition data showed the presence of higher contents of aromatic and hydrophobic residues in GLB. Gel electrophoresis indicated that the ALB has less disulfide bonds and hence a more open (flexible) structure; this was confirmed by the intrinsic fluorescence and circular dichroism data showing greater exposure of tyrosine residues when compared to GLB. ALB had significantly ($p < 0.05$) higher protein solubility and foaming capacity than the GLB at all the pH or sample concentration values but emulsion forming ability was similar for both protein fractions. Differences in emulsion stability were observed mostly at 10 mg/mL sample concentration; these differences were minimized at 25 mg/mL and eliminated at 50 mg/mL. We conclude that the ALB fraction will serve as excellent ingredient for food foam formulation while the GLB may be slightly more useful than the ALB in the formulation of food emulsions.

1. Introduction

The amount and structure of proteins present in a food system have been reported to play an important role in making such ingredients useful for novel food formulations (Mundi & Aluko, 2013; Yin et al., 2011). Barac, Pesic, Stanojevic, Kostic, and Bivolarevic (2014) studied and reported the influence of the functional properties of a food or its components (in addition to its nutritional properties) in enhancing utilization for novel food product development. This functionality is dependent on changes in structural properties of proteins, which determine the type of interactions with other food components (Tang, Sun, & Foegeding, 2011). An example is the tendency of hydrophobic portions of a protein to interact with lipids and lipid soluble compounds (Karaca, Low, & Nickerson, 2011). Critical efforts have been aimed at effective utilization of inexpensive plant proteins as food hydrocolloids for nutritional and functional purposes under different pH conditions. Proteins from plants like canola (Tan, Mailer, Blanchard, & Agboola, 2014), peanut (He, Liu, et al., 2014), kidney bean (Mundi & Aluko, 2012, 2013), lentils (Avramenko, Low, & Nickerson, 2013), pinto bean (Tan, Ngoh, & Gan, 2014), adzuki, pea and soy bean (Barac et al., 2014), amaranth (Shevkani, Singh, Rana, & Kaur, 2014) and cowpea (Oduro-Yeboah, Onwulata, Tortoe, & Thomas-Gahring, 2014) have been examined for their structural and functional properties in model food systems.

Food-grade hemp seed is a new but widely cultivated plant of industrial importance and requires detailed functional characterization of component proteins. This is very important because availability of non-drug varieties of hemp seed possessing low δ-9-tetrahydrocannabinol (THC) contents (Lu et al., 2010) has increased industrial utilization for food product manufacture. The legal use of the seeds from low-THC plants as human food has been reported in Canada and other North American countries due to its considerable amounts of over 30% oil and about 25% easily digestible proteins (Teh & Birch, 2013). The uniqueness of hemp seed protein (HSP) has been reported (Park, Seo, & Lee, 2012) to contain 65% globulin called edestin and 33% albumin; edestin is composed of six identical subunits having an acidic subunit (AS) and a basic subunit (BS) linked by one disulfide bond. Previous works have reported the

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functional (Tang, Ten, Wang, & Yang, 2006; Teh, Bekhit, Carne, & Birch, 2013; Yin et al., 2008) or bioactive (Girgih, He, Malomo, & Aluko, 2014; Girgih, Udenigwe, & Aluko, 2011; Wang, Tang, Chen, & Yang, 2009) properties of HSP isolates and hydrolyzates but there is scanty information on the structural and functional properties of the seed globulin and albumin fractions. Therefore, this study was aimed at determining the structural and functional properties of albumin (ALB) and globulin (GLB) fractions of HSP. Specifically, pH-dependent changes in protein solubility, foaming and emulsifying properties were studied relative to protein conformational changes. The information from this work may provide opportunities for industrial application of HSP as a useful food ingredient and a suitable alternative source of functional proteins to traditional ingredients.

2. Materials and methods

2.1. Materials

Defatted (through solvent-free mechanical press extraction) hemp seed protein meal (HPM) containing ~40% (w/w) protein content, the by-product obtained from commercial oil extraction was purchased from Manitoba Harvest Fresh Hemp Foods Ltd (Winnipeg, MB, Canada) and stored at −20 °C until used for protein extraction. Spectra/Por dialysis membrane with 6–8 kDa molecular weight cut-off (MWCO), gel electrophoresis protein markers as well as other analytical grade reagents were purchased from Fisher Scientific (Oakville, ON, Canada).

2.2. Protein fraction extraction and isolation

The method of Aluko (2004), with slight modifications was employed in the extraction and fractionation of HPM protein into ALB and GLB. The HPM was extracted (1:10 w/v) with 0.5 M NaCl solution for 1 h at 24 ± 2 °C under continuous stirring followed by centrifugation (7000 *g*, 60 min at 4 °C) and the supernatant clarified with Whatman No 1 filter while the precipitate was discarded. The supernatant was dialyzed against water at 4 °C for 5 days using the 6–8 kDa MWCO dialysis tubing (with dialysis water changed 3 times daily). The content of the dialysis bag was then centrifuged (7000 *g*, 60 min at 4 °C) and the supernatant collected as the albumin protein fraction. The precipitate was washed with 100 mL of distilled water, centrifuged again under similar conditions as above and the precipitate collected as the globulin protein fraction. The two protein fractions were individually freeze-dried followed by storage at −20 °C until required for subsequent analyses. Protein concentration of the fractions was determined using the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.3. Amino acid composition

The amino acid profiles of ALB and GLB were determined using the HPLC Pico-Tag system according to the method previously described after samples were digested with 6 M HCl for 24 h (Bidlingmeyer, Cohen, & Tarvin, 1984). The cysteine and methionine contents were determined after performic acid oxidation (Gehrke, Wall, Absheer, Kaiser, & Zumwalt, 1985) while the tryptophan content was determined after alkaline hydrolysis (Landry & Delhaye, 1992).

2.4. Protein solubility (PS)

PS of ALB and GLB was determined according to the method described by Adebiyi and Aluko (2011) with slight modifications. Briefly, 10 mg of sample was dispersed in 1 mL of 0.1 M phosphate buffer solutions (pH 3.0–9.0) and the resulting mixture was vortexed for 2 min and centrifuged at 10,000 *g* for 20 min. Protein content of the supernatant was determined using the modified Lowry method (Markwell et al., 1978). Total protein content was determined by dissolving the protein samples in 0.1 M NaOH solution. PS was expressed as percentage ratio of supernatant protein content to the total protein content.

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

The freeze-dried ALB and GLB were subjected to SDS-PAGE according to the method of Aluko and McIntosh (2004) with minor modifications. The protein samples were each dispersed (10 mg/mL) in Tris/HCl buffer, pH 8.0 containing 10% (w/v) SDS only (non-reducing buffer) or SDS + 10% (v/v) β-mercaptoethanol (β-ME) as the reducing buffer followed by heating at 95 °C for 10 min, cooled and centrifuged (10,000 *g*, 15 min). After centrifugation, 1 μl of supernatant was loaded onto 8–25% gradient gels and electrophoresis was performed with Phastsystem Separation and Development units according to the manufacturer's instructions (GE Healthcare, Montréal, PQ, Canada). A mixture of protein standards (14.4–116 kDa) was used as the molecular weight marker and the gels were stained with Coomassie brilliant blue.

2.6. Intrinsic fluorescence emission

The method described by Li and Aluko (2006) was used to record intrinsic fluorescence spectra on the JASCO FP-6300 spectrofluorimeter (JASCO, Tokyo, Japan) at 25 °C using a 1 cm path length cuvette. Protein stock solution (10 mg/mL) was prepared in 0.1 M sodium phosphate buffer and adjusted to pH 3.0, 5.0, 7.0 or 9.0; each buffer was then used to dilute the respective stock solution to 0.002% (w/v) and fluorescence spectra recorded at excitation wavelengths of 275 nm (tyrosine and tryptophan) with emission recorded from 280 to 450 nm. Emissions of the buffer blanks were subtracted from those of the respective samples to obtain fluorescence spectra of the sample.

2.7. Measurements of circular dichroism (CD) spectra

CD spectra of samples was measured at 25 °C in a J-810 spectropolarimeter (JASCO, Tokyo, Japan) using the spectral range of 190–240 nm (far-UV) for secondary structure determinations and 250–320 nm (near-UV) for tertiary structure according to the method described by Omoni and Aluko (2006). Protein stock solutions were diluted to required concentration in 10 mM phosphate and the secondary structure determined using a cuvette with path length of 0.05 cm containing 2 mg/mL protein solution while the tertiary structure was measured in a 0.1 cm cuvette containing 4 mg/mL protein concentration. All the CD spectra were obtained as the average of three consecutive scans with automatic subtraction of the buffer spectra.

2.8. Foam capacity (FC)

FC was determined according to the method described by Adebiyi and Aluko (2011) using slurry that were prepared as 20, 40, or 60 mg/mL (protein weight basis) sample dispersions in 50 mL graduated centrifuge tubes containing 0.1 M phosphate buffer, pH 3.0, 5.0, 7.0, and 9.0. Sample slurry was homogenized at 20,000 rpm for 1 min using a 20 mm foaming shaft on the polytron PT 3100 homogenizer (Kinematica AG, Lucerne, Switzerland). The capacity of the continuous phase to include air (FC) was determined as follows using the mean of three measurements;