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Hempseed meal protein isolates prepared by different isolation techniques. Part I. physicochemical properties

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ABSTRACT

Protein isolates from hemp seed meal were prepared using alkaline extraction/isoelectric precipitation (HPI) and micellization (HMI) procedures and compared in terms of their physicochemical properties and functionality. The micellization technique resulted in lower protein recovery than the isoelectric precipitation technique. Both HPI and HMI proteins had protein contents higher than 90%. The HMI protein powders were lighter in colour than the corresponding HPI isolates due to higher content of co-extracted polyphenols for the latter. The electrophoretic mobility and subunit composition, as well as amino acid composition of the isolates were not affected by the extraction procedure, indicative of similar protein composition. The HPI exhibited minimum protein solubility at pH 5.0, while for HMI it was shifted to pH 6.0. Differential scanning calorimetry indicated that highly alkaline conditions during HPI extraction led to partial protein denaturation which is reflected in lower transition enthalpy of HPI than HMI. FTIR spectra have also confirmed changes in HPI protein secondary structure, i.e. lower intensity of the peak (1634 cm^{-1}) corresponding to native protein structural elements such as intramolecular β-sheets and higher intensities of peaks (1618 cm^{-1}, 1683 cm^{-1} and 1694 cm^{-1}) indicating enhanced protein aggregation compared to HMI. Protein conformational changes during alkali extraction resulted in higher water retention capacities of HPI in comparison to HMI.

1. Introduction

Although animal-based proteins have a competitive edge over plant-based proteins in terms of their nutritional and functional properties, plant protein ingredient market is forecast to record faster growth in order to satisfy the demands of consumers with different ethnic, religious, dietary and moral preferences associated with consumption of animal-based products. Proteins recovered from oilseeds, cereals and legumes waste and by-products represent economically attractive, sustainable and renewable alternatives to animal-derived proteins, especially if they are characterized with high content of sulfur-containing amino acids (Teh, Bekhit, Carne, & Birch, 2014). Hemp seed meal, a by-product after oil extraction, also represents an excellent alternative plant protein source.

Hemp (*Cannabis sativa* L.) has been an important source of food, fibre and medicinal remedies for thousands of years. The phloem fibre from the stalk is still used as raw material in industries for durable fabrics and specialty papers production, while the seeds are used as food or as raw material for oil production. Hempseed oil is an especially rich source of linoleic acid (18:2 *omega*-6) and *alpha*-linolenic acid (18:3 *omega*-3) and has a favourable ω-6 to ω-3 fatty acid ratio (3:1) (Callaway, 2004; Pojić et al., 2014). Hemp seed meal can be separated into protein rich and fibre rich fractions by dry milling and sieving (Pojić et al., 2014). While the high protein fraction contains 41–44% protein and 5–7% crude fibre, the fibre-rich fraction is mostly composed of crude fibre 21–29% and contains only 10–20% protein (Pojić et al., 2014).

The two main proteins in hempseed are albumin and edestin, globular and legumin type proteins, respectively. The nutritional value of hempseed protein isolates has been reported to be comparable to egg white and soybean proteins (Callaway, 2004; Wang, Tang, Yang, & Gao, 2008). Hempseed protein has a high content of

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sulfur-containing amino acids, methionine and cysteine, and very high levels of arginine and glutamic acid (Callaway, 2004).

Protein composition and functionality are influenced by the isolation method and purification conditions (Karaca, Low, & Nickerson, 2011; Krause, Schultz, & Dudek, 2002; Mwasaru, Muhammad, Bakar, & Man, 1999; Papalamprou, Doxastakis, Biliaderis, & Kiosseoglou, 2009; Rodríguez-Ambriz, Martínez-Ayala, Millán, & Davila-Ortiz, 2005). The most widely used procedure to prepare protein isolates from oilseeds involves alkaline extraction/isoelectric precipitation. Following alkaline solubilization of the protein components, removal of the insoluble material is carried out by centrifugation and protein precipitation in the extracts takes place by pH adjustment to the isoelectric point. However, isoelectric point isolated vegetable proteins have limited food applications due to their off-flavour and colour, associated with the co-extracted non-proteinaceous material (e.g. phenolic compounds). It was already reported that hemp seed proteins obtained using alkali extraction/isoelectric precipitation have a non-appealing greenish colour (Girgih et al., 2014; Isinguzo, 2011), mostly associated with phenolics and their polymerization products. The other characteristic that limits hemp protein utilization, despite its favourable nutritional profile, is related to its poor solubility, which was reported to be lower in comparison to soy protein (Tang, Ten, Wang, & Yang, 2006) due to edestin aggregation at low pH values.

In order to preserve protein native state, Murray, Myers, and Barker (1979) introduced the micellization technique for seed proteins isolation. Micellization involves protein extraction (mostly of globulin type) with a salt solution, centrifugation to remove insoluble material, precipitation from a salt extract by dialysis or dilution in cold water, followed by protein recovery by centrifugation (Arntfield, Ismond, & Murray, 1985; López & Ordorica-Falomir, 1986); i.e. essentially a “salting in-salting out” process. According to Murray et al. (1979) strong protein-protein interactions during protein micellar mass formation exclude non-proteinaceous material.

Since investigations concerning the influence of micellization (salt extraction) on hemp protein functionality are limited, this study aimed at recovering hemp protein isolates from hemp seed meal using both the isoelectric and micellization procedures and studying the effects of extraction procedures on the physico-chemical and functional properties of the obtained isolates.

2. Materials and methods

2.1. Materials

Hemp meal was supplied by the company Svet Konoplje (Kisač, Serbia). Hemp flour was obtained by grinding hemp meal in a laboratory mill, Foss Knifetec 1095 (Foss, Hillerød, Denmark), fitted with tubing to allow circulation of water to cool the sample during milling. Ground hemp meal was separated into two fractions of different particle size by use of a universal laboratory sifter (Bühler AG, Uzwil, Switzerland) equipped with a stack of sieves of decreasing mesh size. The fraction of <250 μm was used as a raw material for protein isolation, since it had significantly higher protein content in comparison to fraction with particle sizes larger than 250 μm (Pojić et al., 2014). All chemicals used were of reagent grade and double distilled water was used to prepare all solutions used in the experimental work.

2.2. Preparation of protein isolates

Initially, defatting of hemp meal was performed using a triple hexane extraction at a 1:3 meal to hexane ratio (w/v) for 2 h each, followed by air-drying in a fume hood at room temperature (23 °C) for 16 h.

2.2.1. Isoelectric precipitation

Isoelectric-precipitated isolates were prepared according to the method of Tang et al. (2006) with minor modifications. Defatted hemp meal was suspended in water at 1:20 ratio (w/v) and the pH was adjusted to 10.0 by adding 1.0 M NaOH under constant stirring for 2 h at 35 °C and then centrifuged for 20 min at 6000×*g*. The supernatant was collected and adjusted to pH 5.0 with 1 M HCl and then the suspension was left at 4 °C overnight to facilitate protein precipitation. The precipitate was recovered by centrifugation (7500×*g*, 20 min), followed by washing three times with water to remove salts. The protein precipitate was collected and re-suspended in water (pH was adjusted to 7.0). The suspension was freeze dried and stored at −20 °C for further analysis.

2.2.2. Micellization

Defatted hemp meal was dispersed in 0.8 M NaCl solution (pH = 7.0) at 1:10 ratio (w/v) and stirred at 35 °C for 2 h. The suspension was centrifuged at 6000×*g* for 20 min and the supernatant was dialysed against water (pH = 7.0) at 4 °C for 72 h using ultrafiltration membranes of cellulose (Sigma-Aldrich, MW cut off 12000–14000 Da). The precipitated proteins were recovered by centrifugation (7500×*g*, 20 min), freeze-dried and stored at −20 °C till further analysis.

2.3. Chemical analysis

Protein content of the protein isolates was analyzed using AOAC Official method 992.23 (AOAC, 1998). The determined nitrogen content was converted to protein concentration by multiplying it with the factor 5.7.

2.4. Colour of protein powders

Colour measurements of hemp meal isolates were carried out in triplicates using a Minolta Chroma Meter CR-400 (Sensing Inc., Japan) colorimeter equipped with light protection tube CR-A33f (8 mm–diameter specimen area). Before conducting the tests, the instrument was calibrated using a standard light white reference tile and the measurements were performed under standard illuminant D65. The results were expressed in terms of L^* (lightness), a^* (redness to greenness-positive to negative values, respectively), and b^* (yellowness to blueness-positive to negative values, respectively) values.

2.5. Total phenolic content

Determination of total phenolic content was performed on protein extracts prepared by extracting 2 g of sample sequentially with 40 mL of methanol:water (50:50, v:v) and 40 mL of acetone: water (70:30, v:v) at room temperature for 60 min. After centrifugation at 2500×*g* for 15 min, combined supernatants were made up to 100 mL with distilled water.

Total phenolic content of protein extract was determined spectrophotometrically at 750 nm (Jenway, 6405UV/Vis) by using the Folin-Ciocalteu's reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999); gallic acid was used as the reference standard compound and the obtained results were expressed as mg gallic acid equivalents (GAE)/100 g of fresh weight.

2.6. Electrophoresis

SDS-PAGE was performed according to Laemmli (1970) at 5%