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Effects of limited enzymatic hydrolysis with trypsin on the functional properties of hemp (*Cannabis sativa* L.) protein isolate

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Abstract

Effects of limited enzymatic hydrolysis induced by trypsin on the physicochemical and functional properties of hemp (*Cannabis sativa* L.) protein isolate (HPI) were investigated. The enzymatic hydrolysis was confirmed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion chromatography (SEC). SEC and differential scanning calorimetry (DSC) analyses confirmed the presence of aggregates in the corresponding hydrolysates (with the degree of hydrolysis of 2.3–6.7%). Functional properties, including protein solubility (PS), thermal properties, emulsifying and foaming properties, and water holding and fat adsorption capacities (WHC and FAC) were evaluated. The PS was remarkably improved by the limited enzymatic hydrolysis at all tested pH values. However, the enzymatic hydrolysis led to the marked decreases in emulsifying activity index, foaming capacity and foam stability, WHC and FAC. These decreases were to a great extent related to the presence of aggregates in the hydrolysates.

Keywords: Hemp protein isolate (HPI); *Cannabis sativa* L.; Enzymatic hydrolysis; Functional property; Trypsin

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. As the by-product during commercial utilization of the valuable fiber, hempseed contains over 30% oil and about 25% protein of high quality. The hempseed oil is rich in polyunsaturated fatty acids (PUFAs), especially linoleic (ω-6) and α-linolenic (ω-3) acids, while the high-quality storage proteins, with superior essential amino acid composition, are easily digested and rich in all essential amino acids (Callaway, 2004). Most of essential amino acids contained in hemp protein are sufficient for the FAO/WHO suggested requirements of infants or children (Tang, Ten, Wang, & Yang, 2006). Hemp proteins mainly consist of edestin (globulin) and albumin. Like the hexamer of soy glycinin, the edestin molecule is also composed of six identical subunits, and each subunit consists of an acidic subunit (AS) and a basic subunit (BS) linked by one disulfide bond (Patel, Cudney, & McPherson, 1994). In the hemp protein isolate (HPI), isolated from defatted hempseed, the edestin approximately accounts for 80% total hemp protein content (Tang et al., 2006). Although HPI has good potential to be applied as a source of protein nutrition, it shows much poorer functional properties, especially protein solubility, as compared to soy protein isolate (SPI) (Tang et al., 2006). The poor functional properties might greatly limit the application of this protein in many food formulations.

Many physical, chemical and enzymatic treatments have been widely applied to modify the functional properties of plant proteins, through changing the protein structure. Usually, the enzymatic modification is more preferable

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due to milder process conditions required, easier control of the reaction and minimal formation of by-products (Mannheim & Cheryan, 1992). In most enzymatic modification cases, enzymatic hydrolysis has been most widely used to improve the functional properties of proteins, such as solubility, emulsification, gelation, water- and fat- holding capacities, and foaming ability, or to tailor the functionality of certain proteins to meet specific needs (Arzu, Mayorga, Gonzalez, & Rolz, 1972; Kim, Park, & Rhee, 1990; Kuehler & Stine, 1974). However, in some cases, extensive enzymatic modification would on the contrary impair some functional properties of food proteins, or even cause off-flavors in the corresponding hydrolysates. Hence, the degree of hydrolysis (DH) should be controlled to obtain reproducible and optimum peptide size distribution, when the enzymatic hydrolysis is applied to modify the functional properties. To date, limited proteolysis has been used to modify the functional and physicochemical properties of soy protein (Jung, Murphy, & Johnson, 2005; Surówka, Żmudzinski, Fik, Macura, & Lasocha, 2004; Surówka, Żmudziński, & Surówka, 2004), sunflower protein (Martineza, Baeza, Millán, & Pilosof, 2005), rapeseed protein isolates (Vioque, Sanchez-Vioque, Clemente, Pedroche, & Millán, 2002), whey protein (Chobert, Bertrand-Harb, & Nicolas, 1988), oat bran protein concentrate (Guan, Yao, Chen, Shan, & Zhang, 2007), and legumin from faba bean (*Vicia faba*) (Dudek, Horstmann, & Schwenke, 1996). No the literature has been reported about this technique to modify hemp proteins, possibly due to the scarcity of commercial hempseed products. This situation may be changing, since hemp seeds have been obtained at a large scale during the commercial utilization of the valuable hemp fiber.

Trypsin (EC 3.4.21.4) is a member of a large family of serine proteases and cleaves the peptide bond on the carboxyl side of arginine and lysine (Kishimura et al., 2007). Studies on limited hydrolysis using trypsin to improve the properties of proteins have been carried out extensively (Barac, Jovanovic, Stanojevic, & Pesic, 2006; Giardina, Pelizzola, Avalli, Iametti, & Cattaneo, 2004; Guan et al., 2007; Henning, Mothes, Dudek, Krause, & Schwenke, 1997; Mutilangi, Panyam, & Kilara, 1996). On the whole, the protein solubility is one of the most outstanding functional properties that are improved by the limited hydrolysis. In some plant protein cases, the emulsifying and foaming properties are also improved, while in other cases, these properties are impaired. In fact, the modification by limited enzymatic hydrolysis is largely dependent upon the nature of protein and the DH. For example, Guan et al. (2007) showed that the enzymatic hydrolysis in a DH-dependent mode increased the solubility, water-holding capacity, emulsifying activity and foaming ability of oat bran protein concentrate. However, Henning et al. (1997) pointed out that the hydrolysis with a rather low DH led to the best emulsifying activity of legume while extensive hydrolysis on the contrary impaired the emulsifying properties.

It is well known that hemp proteins are a kind of easily digested proteins. Therefore, it will be a good choice to apply trypsin to modify the properties of HPI. Thus, one main objective of the present work was to investigate the effects of limited enzymatic hydrolysis by trypsin on some physicochemical and functional properties of HPI, including protein solubility, emulsifying and foaming properties, water holding and fat adsorption capacities, and thermal properties. The effects of thermal treatment on the enzymatic hydrolysis and properties of HPI were also evaluated.

2. Materials and methods

2.1. Materials

Defatted hempseed protein meal, a byproduct during the commercial utilization of the valuable hempseed oil and fiber, was kindly supplied by YUNNAN Industry Hemp Co. Ltd. (China). This meal had been obtained on a large scale from hemp (*Cannabis sativa* L.) seeds, through de-hulling, disintegrating and de-fatting 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). Trypsin powder (from porcine pancreas; catalog no. T4799, 1000–5000 BAEE units/mg solid) and Folin and Ciocalteu's Phenol Reagent (F-9252) were from sigma (St. Louis. MO, USA). All other chemicals used in the present study were of analytical or better grade.

2.2. Preparation of hemp protein isolate (HPI)

HPI was produced from the defatted hempseed meal according to Tang et al. (2006), with a slight modification. Defatted hempseed meal was dispersed in deionized water (1:20, w/v), and the pH of the dispersion was adjusted to 10.0 with 2 N NaOH. The resultant dispersion was gently stirred at 37 °C for 2 h, then centrifuged at 8000*g* at 20 °C for 30 min in a CR22G high-speed centrifuge (Hitachi Co., Japan). The pellet was discarded, and the supernatant was adjusted to pH 5.0 with 2 N HCl and then centrifuged at 5000*g* (Hitachi Co., Japan) at 20 °C for 20 min. The obtained precipitate was re-dispersed in deionized water. The dispersion was homogenized and adjusted to pH 7.0 with 2 N NaOH, then followed by freeze-drying to produce HPI product. The protein content of obtained HPI was 91.2% (determined by Kjeldahl method, $N \times 6.25$, wet basis).

2.3. Thermal pretreatment

To investigate the heat treatment on the enzymatic hydrolysis of HPI by trypsin, the HPI dispersions were prepared and thermally pretreated as follows. The 2% (w/v) HPI dispersions in deionized water were adjusted to pH