Innovative Food Science and Emerging Technologies 31 (2015) 151–159

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Conversion of a low protein hemp seed meal into a functional protein concentrate through enzymatic digestion of fibre coupled with membrane ultrafiltration

Sunday A. Malomo, Rotimi E. Aluko *

The Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

ARTICLE INFO

Article history:
Received 4 July 2015
Received in revised form 5 August 2015
Accepted 11 August 2015
Available online 1 September 2015

Keywords:
Hemp seed
Protein solubility and digestibility
Protein isolate
Membrane ultrafiltration
Foaming
Emulsion

ABSTRACT

Industrial hemp seed meal (HPM) containing ~37% protein content was digested with carbohydrases and a phytase. The digest was then passed through a 10 kDa ultrafiltration membrane and the retentate freeze-dried as the membrane protein concentrate (mHPC), which had 74% protein content. Protein digestibility (89%) was significantly ($P < 0.05$) higher than the ~85% obtained for HPM, traditional isoelectric protein isolate (iHPI), and a commercial protein concentrate (cHPC). The mHPC also had significantly ($P < 0.05$) higher protein solubility than other hemp products in the pH 3–9 range with a minimum of 76% at pH 4.0. In contrast the HPM and iHPI had <25% maximum protein solubility. Foaming capacity was highest for mHPC (55–98%) when compared to 10–70% for the other protein products. However, mHPC and cHPC emulsions had bigger oil droplet sizes (4.5–15.5 μm) when compared with <1 μm for HPM and iHPI emulsions.
Industrial relevance: Hemp seed oil remains the most valuable product of the hemp seed industry but the proteins are also becoming very popular due to the high arginine content. Arginine is perceived as a heart-healthy amino acid because it serves as a precursor of the vasodilatory agent, nitric oxide. The traditional method of protein isolation involves isoelectric precipitation, which damages protein functionality and reduces performance as an ingredient below the required level for high quality food products manufacture. Therefore, in this research work, a novel, industrially scalable method that uses food-grade enzymes to digest most of the polysaccharides in a defatted low protein hemp seed meal was developed. The digested polysaccharide fragments were then removed by ultrafiltration/diafiltration to leave behind a protein product with twice the original protein content of the starting raw material. Most importantly, the work confirmed that the membrane-isolated protein possessed superior functionality, especially protein solubility and digestibility in comparison with existing similar hemp seed protein products. Since the membrane ultrafiltration setup is available on a commercial scale, work could be adapted by the hemp seed processing industry to manufacture a new line of high protein products with desirable functionality in food systems. However, spray-drying must be used instead of freeze-drying in order to make the process economically feasible.

1. Introduction

The increased utilization of hemp plant for various industrial purposes has led to abundant amounts of protein-rich seed residue, which can be converted into various forms of powdered protein products. In Canada, hemp seed processing primarily involves the cold-pressing method to expel oil and leave behind a high protein (30–50%) residue/meal (Malomo, He, & Aluko, 2014). The hemp seed protein (HSP) products have been shown as promising raw materials for the production of peptides with potential human health applications (Girgih, Alashi, et al., 2014; Girgih, He, Alashi, Malomo, & Aluko, 2014). However, the hemp seed protein materials are poorly utilized in applications with added value. For instance, the current commercial HSP products are mainly in the form of protein concentrates that have less than 70% protein content and contain high levels of fibre and phytate (Lu et al., 2010). The high fibre and phytate contents of these HSP products have been reported to cause protein functionality reduction, which limits their use as new food ingredients (Yin, Tang, Wen, & Yang, 2009). Moreover, current HSP isolates are generally prepared by alkaline extraction, which is then followed by isoelectric precipitation and is the most commonly used traditional procedure for plant protein isolate production (Malomo et al., 2014). The harsh conditions used in the traditional procedure have negative effects on protein functionalities, especially protein solubility and foaming properties (Liu, Chen, Wang, & Wang, 2013). The poor food functionality of current HSP products can also be attributed to the high phytate content, which can cause protein

* Corresponding author.
E-mail address: rotimi.aluko@umanitoba.ca (R.E. Aluko).

http://dx.doi.org/10.1016/j.ifset.2015.08.004
1466-8564/

cross-linking and reduce protein solubility. Therefore, in order to produce novel protein isolates with improved food functionalities, a method that involves fibre and phytate digestion followed by removal of non-protein materials by membrane filtration was developed. The use of membrane ultrafiltration obviates the need for harsh protein precipitation protocols while ensuring high yield of proteins with minimal conformational denaturation. Recent studies have confirmed the successful application of this novel method of protein isolate production with high quality properties. For instance, Wang, Chen, Hua, Kong, and Zhang (2014) reported a soy protein isolate with higher protein contents (91–93%) and better in vitro digestibility from phytase-assisted processing method. A better functional (foam capacity) protein isolates with high protein content (92%) and yield (75%) was produced from rice bran using a combination of xylanase and phytase predigestion process (Wang, Hettiarachchy, Qi, Burks, & Siebenmorgen, 1999). Another study (Udenigwe, Lin, Hou, & Aluko, 2009) had shown that pre-digestion of the fibre with cellulase resulted in up to 23% higher protein content of flaxseed protein isolate. However, pre-digestion with carbohydrases and phytases coupled to membrane ultrafiltration has not been previously reported for protein isolate production. During industrial hemp seed processing, the defatted meal is usually milled and passed through several sieves to obtain high-value protein products. The milling and sieving process leaves behind a high-fibre product that has less economic value than the mainstream protein products. Therefore, this study aimed to convert this high-fibre but low protein industrial side-stream of hemp seed processing into a high protein product through enzymatic digestion of the main non-protein materials (fibre and phytate) followed by removal of the low molecular weight digests by membrane ultrafiltration. The membrane protein isolate was then compared with the traditional protein isolate from isoelectric pH precipitation and a commercial hemp seed protein concentrate to determine protein digestibility and potential functionality in food systems.

2. Materials and methods

2.1. Hemp seed protein products and chemical reagents

Hemp seed protein meal (HPM), a 37% protein content (~9% residual oil) product of the hemp seed oil processing industry was kindly provided by Hemp Oil Canada (St. Agathe, Manitoba, Canada). To produce HPM, the hemp seeds were mechanically pressed to remove the oil. The defatted hemp seed cake was then milled in a classifier milling system to the desired particle size and sifted through various screens to remove seed coat and other coarse materials. Hemp Pro70, a commercial hemp seed protein concentrate (cHPC) produced through proprietary methods was purchased from Manitoba Harvest Hemp Foods (Winnipeg, MB, Canada). All enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other analytical-grade reagents were obtained from Fisher Scientific (Oakville, ON, Canada).

2.2. Preparation of hemp seed protein concentrate (mHPC) by ultra- and diafiltration

mHPC was produced from HPM according to the method previously described by Udenigwe et al. (2009) with modifications. Approx. 5% (*w*/*v*) slurry of HPM was adjusted to the following conditions: 4.5% (*w*/*w*) cellulase, 0.75% (*w*/*w*) hemicellulase, 0.75% (*w*/*w*) xylanase, and 0.5% (*w*/*w*) phytase at 37 °C and pH 5.0. The digestion was allowed to proceed for 4 h, cooled to room temperature and then subjected to membrane ultrafiltration/diafiltration processing using a 10 kDa molecular weight cut-off membrane. The digested fibre and phytate fragments were removed in the permeate while the target proteins remained in the retentate. Three rounds of ultrafiltration/diafiltration were used in order to ensure that most of the digested non-protein materials were removed, after which the retentate was freeze-dried as the mHPC. Protein concentration of the mHPC was determined using the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.3. Preparation of traditional (isoelectric pH precipitated) hemp seed protein isolate (iHPI)

iHPI was produced from HPM according to the previously described method of Malomo et al. (2014) with slight modifications. HPM was dispersed in deionized water (1:20, *w*/*v*) and the dispersion adjusted to pH 8.0 using 2 M NaOH to solubilize the proteins while stirring at 37 °C for 2 h; this was followed by centrifugation (7000 x *g*, 60 min at 4 °C). The precipitate was discarded and the supernatant filtered with cheese-cloth; adjusted to pH 5.0 with 2 M HCl to precipitate the proteins and thereafter centrifuged (7000 x *g*, 60 min at 4 °C). The resultant precipitate was re-dispersed in deionized water, adjusted to pH 7.0 with 2 M NaOH and freeze-dried to obtain the iHPI. Protein concentration of the iHPI was determined using the modified Lowry method (Markwell et al., 1978).

2.4. Amino acid composition

The amino acid profiles of HSP products 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) and the tryptophan content was determined after alkaline hydrolysis (Landry & Delhaye, 1992).

2.5. In vitro protein digestibility method

The in vitro protein digestibility of the HSP products was carried out according to a previously described method (Hsu, Vavak, Satterlee, & Mill, 1977) with slight modifications using an enzyme system consisting of trypsin and chymotrypsin. A 10 mL aliquot of aqueous protein suspension (6.25 mg protein/mL) in double distilled water was adjusted to pH 8.0 with 0.1 M NaOH while stirring at 37 °C. The enzyme solution (containing 1.6 mg trypsin and 3.1 mg chymotrypsin/mL) was maintained in an ice bath and 1 mL of the solution was then added to the protein suspension. The pH drop was recorded automatically over a 10 min period using a Metrohm 842 Titrando system (Mississauga, ON, Canada). The % protein digestibility of each protein sample was calculated using the regression equation predicted by Hsu et al. (1977) as follows:

$$\%\ \text{Protein digestibility (Y)} = 210.46 - 18.10X_f$$

where X_f is the final pH value of each sample after a 10 min digestion.

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

The HSP products were subjected to SDS-PAGE (reducing and non-reducing) according to the method of Aluko and McIntosh (2004) with some 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 (reducing buffer), followed by heating at 95 °C for 10 min, cooled and centrifuged (10000 × *g*, 15 min). After centrifugation, 1 μL of supernatant was loaded onto 8–25% gradient gels and electrophoresis 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.