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Barley protein concentrates: Extraction, structural and functional properties

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ABSTRACT

Protein concentrates were prepared from defatted barley (*Hordeum vulgare* L.) flour using alkaline and enzymatic treatments. Milder enzymatic treatments included (i) a bi-enzymatic method involving the use of starch-hydrolyzing enzymes, and (ii) a tri-enzymatic method using the former bi-enzymatic treatment followed by digestion with glucanase. The concentrate obtained through alkaline extraction (AI-BP) was comprised mainly of low molecular weight fractions of proteins. Bi-enzymatic treatment produced a protein concentrate with the highest protein content (49.0%), while those obtained by the tri-enzymatic treatment followed by an isoelectric precipitation step (TEI-BP) gave the highest protein recovery yield (78.3%). In both of the latter concentrates, 35 kDa B-hordeins were the major protein fraction. Divergence in secondary/tertiary structure elements (AI-BP; TEI-BP) was obtained and attributed to the difference in the protein profiles. Further characterization of protein concentrates indicated that they exhibit pseudoplastic behavior. Emulsifying capacity of concentrates was comparable to that of whey protein isolate.

1. Introduction

The increasing demand for health-promoting foods has fueled the development of functional protein ingredients. Furthermore, there is an ever-increasing interest in exploring new sources of plant-derived proteins, based on health, environmental, and economical rationales. Among the currently available plant-derived proteins, soy and wheat proteins remain dominant. Although the use of pulse proteins has recently grown dramatically, the demand for ancient grains has also spiked. One grain that has yet to be thoroughly investigated for its protein characteristics and functionality is barley (*Hordeum vulgare* L). Barley has been domesticated for over 10,000 years and is now the fourth most grown cereal globally after wheat, rice and corn. Globally, 70% of barley production used in livestock feeding, 16% transformed into malt for beer-making, and 14% is directed for human consumption (Zhou, 2010). However, barley has gained popularity in recent years owing to the association of the soluble β-glucan fiber and phytochemical compounds it contains with various health benefits (Alu'datt et al., 2012). Additionally, barley contains 10–20% proteins, dominated by the prolamin hordeins (52%) which function as the main storage proteins (87%) of the endosperm layer. Glutelin is another major storage protein of the endosperm, which represents 23% of the total barley proteins. Two other cytoplasmic proteins, albumins and globulins, represent minor storage proteins of the grain aleurone and embryo (Sullivan, Arendt, & Gallagher, 2013). Barley is intensively used as a raw material in the starch industry generating large quantities of protein as a by-product that can be valorized in the food industry (Yalçın, Çelik, & İbanoğlu, 2007). Indeed, barley proteins are good candidates for value-added application as food supplements owing to their functional properties, including emulsifying capacity and stability, foaming, elasticity, cohesiveness, and water holding capacity that enhance rheological food properties (Wang, et al., 2010; Yalçın, et al., 2007). In addition, barley proteins constitute a good source for essential and non-essential amino acids, such as threonine, valine, phenylalanine, and arginine (Sullivan, et al., 2013). Therefore, barley proteins have a potential for application in food development with a competitive advantage compared to the trend-leading whey and soya proteins. This is especially timely to address the increased consumer preference for the plant-based proteins sourced from alternative whole grains, having the ability to enhance the nutritional profile and to add to the health attributes of familiar foods. The overall objective of the study was to optimize the isolation of barley proteins and characterize their structural properties. This was achieved by the following specific objectives: (1) Developing an enzymatic approach for the isolation of barley proteins and comparing its efficiency with conventional extraction methods under alkaline conditions; (2) Characterizing the primary, secondary and tertiary structural properties of two protein concentrates, obtained by using enzymatic and alkaline extractions; and (3) Assessing their functional properties along with pea protein concentrate (PPC) and whey protein isolate (WPI) as standards.

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2. Materials and methods

2.1. Source of materials

Sodium hydroxide (NaOH), hexane (C_6H_{14}), tris base, and sodium citrate dihydrate [$HOC(COONa)(CH_2COONa)_2{\cdot}2H_2O$] were obtained from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid was purchased from Acros (Fair Lawn, NJ). Citric acid anhydrous ($C_6H_8O_7$) was obtained from Debro (On, Ca). Potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), glycerol trioleate, α-amlyase from *Bacillus* sp., α-amylase from *Bacillus licheniformis* (Termamyl™), amyloglucosidase from *Aspergillus niger*, and β-1,3,4 glucanase from *Trichoderma longibrachiatum* were purchased from Sigma Aldrich (St. Louis, MO). In addition to β-glucanase activity, β-1,3,4 glucanase from *T. longibrachiatum* may contain other carbohydrate hydrolyzing enzyme activities, such as xylanase, cellulose, β-glucosidase, β-xylosidase and α-L-arabinofuranosidase activities. Deionized water (Millipore) was used in all experiments. Barley flour was manufactured by Meunerie Milanaise (Qc, Ca). SDS-PAGE broad molecular weight standard (6.5–200 kDa) was obtained from Bio-Rad (On, Ca). PPC was obtained from Roquette (Nord-Pas-de-Calais, FR), and WPI from Hilmar (Hilmar, CA).

2.2. Preparation of defatted barley flour (DBF)

Barley flour of the 2-row variety was defatted with hexane using a solvent-to-flour ratio of 1:10 (w/v) by shaking at 200 rpm for 1 h at 25 °C. The slurry was centrifuged at 8500*g* for 15 min at 4 °C in a Beckman Avanti centrifuge, model J25-I (Beckman Coulter, CA, USA). The precipitate was then air-dried at room temperature as a thin layer and stored at 4 °C until needed.

2.3. Methods for the preparation of barley protein extract

2.3.1. Alkaline extraction

An alkaline extraction was performed by mixing the DBF with 0.5 M NaOH (pH 11.0) at a solvent-to-flour ratio of 10:1 (w/v); e.g., 50 g DBF in 500 mL NaOH solution, for 2 h at 23 °C. The mixture was centrifuged at 4000*g* for 15 min and the supernatant was dialyzed for 48 h at 4 °C against Millipore water using a 2000-Da benzoylated dialysis tubing to remove low molecular weight sugars. This preparation, designated alkaline barley protein (A-BP) extract, was freeze dried to be used for further analyses.

2.3.2. Sequential alkaline/isoelectric precipitation extraction

The alkaline extraction was repeated as explained above (Section 2.3.1) and followed by an isoelectric precipitation (IEP). After centrifugation of the alkaline-treated defatted flour, the pH of the supernatant was adjusted to 4.5 with 0.5 M HCl and left to precipitate overnight at 4 °C. The pH was re-determined to confirm that it remained stable; the preparation was then centrifuged at 4000*g* for 20 min, and the pellet was re-suspended in distilled water (1:1), dialyzed, and freeze-dried. This preparation was designated alkaline and IEP barley protein (AI-BP) extract.

2.3.3. Barley protein extraction by enzymatic treatment

To prevent excessive protein degradation during the extraction process by the alkaline methods, milder enzymatic techniques to hydrolyze barley carbohydrates (starch reserves and cell-wall glucans) and recover extracts enriched in proteins were assayed (Waglay, Karboune, & Khodadadi, 2016). To this end, DBF was subjected to digestion with an α-amylase (mono-enzymatic treatment), with an α-amylase followed by an amyloglucosidase (bi-enzymatic treatment), or with an α-amylase followed by an amylo-glucosidase and a β-glucanase (tri-enzymatic treatment).

2.3.3.1. Mono- and bi-enzymatic digestion approach for protein extraction. In the mono-enzymatic treatment approach, DBF was digested with each of two α-amylases, one from *Bacillus* sp. and another, Termamyl™, from *B. licheniformis*. DBF was re-suspended in 10 mM potassium phosphate buffer (pH 6.5) at a 1:10 ratio (w/v), and either the α-amylase from *Bacillus* sp. or Termamyl™ was added to the final concentrations of 10,000U/g or 5000 U/g, respectively. The reaction mixture involving the amylase from *Bacillus* sp. was incubated at 65 °C for 1 h, while the reaction mixture using Termamyl™ was incubated at 40 °C for 16 h. After incubation, each reaction mixture was centrifuged at 8500*g*, and the supernatant was recovered, and freeze-dried to be stored until needed for further analyses.

In the bi-enzymatic treatment, DBF was successively digested with the α-amylase from *Bacillus* sp. followed by amyloglucosidase. Two different combinations of enzyme/flour ratios for the treatment with the α-amylase and amyloglucosidase were used. In the first combination, the α-amylase was added to a suspension (1:10) of DBF in 10 mM potassium phosphate buffer (pH 6.5) at the final ratio enzyme/flour of 5000 U/g and incubated for 1 h at 40 °C. The amyloglucosidase was then added to the reaction mixture at the ratio enzyme/flour of 330 U/g and the incubation continued at the same temperature for an additional period of 16 h. For the second combination, two aliquots of DBF suspension were digested with 10,000 U/g of α-amylase for 1 h at 40 °C, then 660 U/g amyloglucosidase was added to each of them; then incubation continued at 40 °C for 4 h for one aliquot and 16 h for the other. After incubation, the reaction mixtures were centrifuged at 4000*g* for 15 min and the supernatants were dialyzed, freeze-dried, and stored for further analyses. The resulting product was designated bi-enzymatic barley protein (BE-BP) extract.

2.3.4. Tri-enzymatic digestion approach for protein isolation

The isolation of proteins from barley upon hydrolysis of starch and the cell-wall glucan was carried out by the tri-enzymatic approach. This approach was based on the sequential digestion with the α-amylase from *Bacillus* sp. (10,000 U/g, 65 °C, 1 h, pH 6.5), the amyloglucosidase from *A. niger* (660 U/g, 40 °C, 16 h, pH 6.5), and the β-1,3,4-glucanase from *T. longibrachiatum* (8 U/g, 37 °C, 1 h, pH 5.0). The mixture was centrifuged at 4000*g* for 15 min and the supernatant dialyzed and freeze-dried. This preparation was designated as the tri-enzymatic barley protein (TE-BP) extract.

2.3.5. Combined tri-enzymatic digestion and isoelectric precipitation (IEP)

A tri-enzymatic starch and glucan removal was carried out as described above (Section 2.3.4) and followed by the IEP, whereby the pH of the mixture was adjusted to 3.7 with 0.5 M HCl and kept undisturbed overnight at 4 °C. It was then centrifuged at 4000*g* for 20 min and the pellet was recovered, dialyzed, freeze-dried, and its protein content determined. This preparation was designated tri-enzymatic and IEP barley protein (TEI-BP) extract.

2.4. Protein determination

Nitrogen content of the barley protein extracts was determined by using a Leco TruSpec Micro CHNS (Leco Corporation, MI), following the Dumas method (Kirsten and Hesselius 1983). Protein content was obtained by multiplying the nitrogen content by the barley protein nitrogen conversion factor of 5.83. The protein recovery yield (%) and the purification factor (w/w) were calculated for all extraction methods according to the Eqs. (1) and (2), respectively:

$$\text{Yield } (\%) = \frac{Protein\ content_{precipitate}}{Protein\ content_{initialflour}} \times 100 \quad (1)$$

$$\text{Purification factor} = \frac{Protein\ proportion_{initialflour}}{Protein\ proportion_{extract}} \quad (2)$$