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Physicochemical and functional properties of cowpea protein isolates treated with temperature or high hydrostatic pressure

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ABSTRACT

The effect of thermal (TT, 70 and 90 °C) and high hydrostatic pressure (HHPTs, 200, 400 and 600 MPa) treatments on physicochemical and functional properties of cowpea protein isolates (CPIs) extracted at pH 8.0 (A8) and pH 10.0 (A10) was analyzed. The pH of protein extraction affected some physicochemical properties (surface hydrophobicity (Ho) and denaturation temperature), without affecting the functional properties. Treatments led to the formation of soluble protein aggregates stabilized by disulfide bonds, especially with TT at 90 °C. TT and HHPTs shifted the wavelengths of maximum emission to red and to blue, respectively. All treatments induced unfolding and denaturation. HHPTs was more efficient than TT to enhance gelation and water holding capacities. Interestingly, treated and untreated CPIs exhibited high values of solubility (72–97%). TT and HHPT induced greater changes in physicochemical and functional properties of A8 than in those of A10. Remarkably, functional properties were improved from the less energetic treatments (70 °C, 200 MPa).

Industrial relevance: The comparison between treatments (one traditional and one corresponding to an emerging technology) gives information about the possibility of obtaining modified proteins for different functional purposes. The modified cowpea protein isolates may be used in beverages because of high solubility, in desserts because of gel formation capacity and/or as additives in other foodstuff because of improved water holding capacity. This knowledge would increase the added value of a local production currently marketed without processing.

1. Introduction

Cowpea (*Vigna unguiculata*) is a legume that belongs to the Fabaceae family and is commonly known as black-eyed pea, alubia, caupí, tape or frijole. In the Northeast of Argentina cowpeas are frequently produced by small and medium scale farmers for either personal consumption (human or animal) or trade. Cowpeas are also used as green manure, employed in a rotary scheme with other annual crops or in fruit plantations to increase or sustain soil fertility. In previous studies Avanza, Acevedo, Chaves, and Añón (2013) found protein contents ranging from 24.3 to 27.1 g/100 g (d.b.) for flours of different varieties of cowpea which make it an attractive source of proteins in replacement of animal proteins. Cowpea proteins, as other vegetable ones, are less expensive and their production requires less energy, land and water resources than animal protein production. Thus, the emphasis in vegetable food proteins may also result in ecological benefits.

The use of cowpea as nourishment has been limited due to the beany flavor, the long time needed to cook it, and the presence of certain antinutritional factors (polyphenols, tannins and phytic acid). By isolating the proteins from cowpea flours, the nutritional properties could be preserved and the negative effects of antinutritional factors could be avoided. Cowpea protein isolates (CPIs) can be used as ingredients and supplements. Their value as ingredients in food products is determined by their nutritional characteristics and functional properties. Such properties are influenced by environmental variables such as temperature, pH and ionic strength during protein isolation and, also, during food processing, manufacturing, storage and preparation (Kinsella & Melachouris, 1976; Petruccelli & Añón, 1994; Mwasaru, Muhammad, Bakar, & Che Man, 1999).

Abbreviations: CPIs, cowpea protein isolate; A8, cowpea protein isolate, protein extraction carried out at pH 8.0; A10, cowpea protein isolate, protein extraction carried out at pH 10.0; TT, thermal treatments; HHPTs, high hydrostatic pressure treatments; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MW, molecular weight; 2-ME, 2-mercaptoethanol; FI, fluorescence intensity; λ_{max}, maximum emission wavelength; Ho, surface hydrophobicity; ANS, 1,8-aniline–naphthalene-sulfonate; T_d, denaturation temperature; ΔH, enthalpy change of transition; DD, degree of protein denaturation; So, solubility; WHC, water holding capacity; η^*, apparent viscosity; LGC, least gelation concentration.

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The CPIs are prepared by alkaline extraction from defatted flour followed by isoelectric precipitation. The protein structure may be modified during extraction; the standard pH of extraction for storage proteins from different seeds is 8.0 (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004; Petruccelli & Añón, 1995). Mwasaru et al. (1999) tested harsh conditions (agitation at 8500 rpm, and pH up to 12.5) of protein extraction and observed protein denaturation as a consequence of those treatments, even at the lowest pH tested. Therefore, the effects described by Mwasaru et al. (1999) might be due to a combination of shear stress and high OH^- concentrations. Moreover, extremely high pHs induce the formation of lysinoalanine, a toxic cross-linked amino acid. Thus, the relationship between protein quality and processing parameters is worthy of extensive investigation (Rivas, Dench, & Caygill, 1981).

Thermal treatment (TT), one of the most traditional in food processing, affects the native structure of food proteins (Kinsella & Melachouris, 1976). Changes in the secondary, tertiary or quaternary structure are usually referred to as denaturation. Thermal denaturation leads to dissociation of proteins into their constituent subunits, to unfolding of their structure, and to exposure of their hydrophobic groups (Privalov, 1979). The association–dissociation and aggregation because of heating have been widely studied in storage proteins from soybean (Petruccelli & Añón, 1995), oat (Ma & Harwalkar, 1988), and kidney bean (Tang & Mab, 2009), among other seeds.

Over the last decades high hydrostatic pressure treatments (HHPTs) have been shown to constitute an adequate option for satisfying the high demand of high quality and minimally processed, free of additives and microbiologically safe foods. The HHPTs can preserve small molecules (vitamins, free amino acids) and significantly modify secondary, tertiary, and quaternary structures, affecting non-covalent bonds (O'Reilly, Kelly, Murphy, & Beresford, 2001). In particular, HHPTs produces a variable degree of protein denaturation that depends mainly on the applied pressure level and media composition, leading to aggregation and dissociation of polypeptides and modifying their surface hydrophobicity, solubility and other functional properties.

The aim of this study was to analyze the effects of different treatments that may modify the protein structure: one of them during protein isolation, e.g. increase in the pH during protein extraction, and other procedures applied on CPIs, e.g. TTs and HHPTs. Those effects were evaluated on physicochemical and functional properties of CPIs. The comparison between treatments (one traditional and one corresponding to an emerging technology) gives information about the possibility of obtaining modified proteins for different functional purposes. The knowledge about the effects of treatments on structural properties of CPIs proteins and the consequences in their functionalities may be useful and would increase the added value of a local production currently marketed without processing.

2. Materials and methods

2.1. Material

Cowpea seed variety Cuarentón was obtained from Estación Experimental El Sombrero-Corrientes (Instituto Nacional de Tecnología Agropecuaria-INTA) (crop 2012). Shrunken, discolored and insect-infested seeds were eliminated. Seeds were sun-dried and stored in a hermetic vessel at 10 °C until use.

2.2. Preparation of cowpea protein isolates

The preparation of CPIs was carried out according to Qi, Hettiarachchy, and Kalapathy (1997)) with slight modifications. Cowpea seeds were ground (Braun KSM2, coffee grinder, Mexico) and passed through an 80 ASTM (177 μm). A 10 g/100 mL suspension of the obtained flour was defatted with hexane for 24 h at 4 °C under continuous stirring. After fat extraction, most of the hexane was separated by filtration and the residual hexane was evaporated at room temperature for 24 h. The defatted flour was dispersed in distilled water (10 g/100 mL) and pH was adjusted to 8.0 or 10.0 using 2 mol/L NaOH for protein extraction. The dispersion was stirred for 60 min at room temperature and then centrifuged at 10,000 ×*g* for 30 min at 20 °C. The pH of supernatants was adjusted to 4.5 and then centrifuged at 10,000 ×*g* for 20 min at 5 °C. Proteins were dispersed in distilled water and pH was adjusted to 7.0 using 2 mol/L NaOH. Samples were then freeze-dried, and stored at 4 °C. The protein content of the flour and the CPIs was determined by the Kjeldhal method (N × 6.25) (AOAC, Official methods of analysis, 1990). Ash percentage was determined according to AOAC, Official methods of analysis (1990). The CPIs obtained were termed A8 and A10 according to their pH of extraction.

2.3. Protein dispersions and treatments

Prior to thermal (TT) and high hydrostatic pressure (HHPT) treatments, aqueous dispersions of A8 and A10 at 10 mg protein/mL (pH 7.0) were prepared. For TT, the protein dispersions were heated in a water bath at 70 or 90 °C for 5, 10, 20 or 30 min. The time of treatment was recorded once the dispersion reached the desired temperature and it was monitored during all treatment by using a thermocouple ±1 °C (model Tes-1317R, RTD DATA Logger Thermometer, Taiwan). After heating, dispersions were immediately cooled by immersion in an ice bath. Heating and cooling rates were ca. 50 and 45 °C/min, respectively. For HHPTs, the protein dispersions were vacuum-conditioned in polyethylene flasks and were subjected to 200, 400, or 600 ± 5 MPa for 5 min in a High Pressure System Stansted Fluid Power Ltd. model FPG 9400:922 (Stansted, UK) with a vessel working volume of 2 L, equipped with temperature and pressure regulation. A mixture of propylene glycol and water (30:70) was used as compression fluid. The target pressure was reached at 6.5 MPa/s and released at 20 MPa/s. Conditioning temperature of vessel and initial temperature of compression fluid were 20 °C. The adiabatic heating was manifested as an increase in temperature that was maximal for 600 MPa (maximal temperature = 38 °C). The treated A8 and A10 were freeze-dried and stored at 4 °C until analysis.

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

All gels were run in minislabs (Bio-Rad Mini Protean Tetra Cell Model). SDS-PAGE was performed according to Laemmli's (1970) using continuous gels (12 g/100 mL acrylamide). Treated and untreated A8 and A10 were dispersed (1 mg/mL protein) in 0.125 mol/L Tris–HCl, pH 6.8, 20 mL/100 mL glycerol, 0.1 g/100 mL SDS, and 0.05 g/100 mL bromophenol blue and centrifuged at 15,800 ×*g* for 5 min at 4 °C. Supernatants were loaded on to the gel (30–40 μg protein per lane). Samples to be run under reducing conditions were boiled for 1 min in sample buffer containing 5 mL/100 mL 2-mercaptoethanol (2-ME) before centrifugation. Electrophoresis was performed at a constant current of 30 mA per gel for approximately 45 min. Molecular weight standards provided by Pharmacia Hepar Inc., (Franklin, OH, USA) were used. Gels were fixed and stained with Coomassie Brilliant Blue dye solution (2 g/L) in water/methanol/acetic acid (5:5:2) overnight and destained with 25% *v*/*v* methanol and 10% *v*/*v* acetic acid. Gels images were acquired with a HP Scanjet G2710 scanner.

2.5. Fluorescence spectroscopy

Treated and untreated A8 and A10 were dispersed (1 mg/mL protein) in buffer Tris–HCl 0.05 mol/L pH 7.5 and stirred for 1 h at room temperature; all dispersions were centrifuged at 10,000 ×*g* for 30 min at room temperature, the supernatants were analyzed. The intrinsic fluorescence was determined at 25 °C, with a Perkin-Elmer LS 50B fluorescence spectrophotometer at an excitation wavelength of 280 nm (slit width, 5 nm), an emission wavelength of 300–450 nm (slit width, 5 nm), and a scanning speed of 300 nm/min (Perkin-Elmer, Waltham,