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High hydrostatic pressure improves protein solubility and dispersion stability of mineral-added soybean protein isolate

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ABSTRACT

The influence of ion type, ion concentration and pH on the effect of high hydrostatic pressure (HHP) on solubility and dispersion stability of soybean protein isolate (SPI) was analyzed. Solubilizing effect of HHP was detected for calcium-, magnesium- and iron- added SPI, the magnitude of this effect was dependent on ion type, ion concentration and pH. The solubilizing effect was highest for calcium, followed by magnesium and iron at pH 7.0. The pH value affected the levels of solubility and the range of calcium concentration where solubility was increased. HHP-denatured soybean proteins may coexist with different minerals and at different pHs in the form of soluble species. For a given calcium concentration, pH may affect the structure of HHP-induced aggregates, leading to different solubilities and dispersion stabilities. HHP improved the stability of insoluble proteins in calcium-added SPI dispersions, avoiding their settling. Our results confirm that thermal treatment and HHP differentially affect protein–protein interactions. A transient dissociation of calcium from proteins during HHP is postulated. This dissociation would play a role in the structure of aggregates. When calcium is present during denaturation, different aggregates may be formed if calcium is bound to (thermal treatment) or transiently dissociated from (HHP) SPI proteins.

1. Introduction

Soybean protein isolates (SPI) represent a low cost additive with high biological value, interesting functional properties and health-improving compounds. Their major proteins are glycinin (an 11S globulin of about 360 kDa) and β-conglycinin (a 7S globulin of about 180 kDa) (Badley et al., 1975; Thanh & Shibasaki, 1977). Minerals such as calcium, magnesium and iron are vital micro-nutrients due to their physiological functions. Mineral-enriched SPIs are an excellent source of macro- and micro-nutrients necessary to meet recommended dietary requirements of a growing population (Messina, Melina, & Mangels, 2003; Pimentel & Pimentel, 2003). The addition of minerals to SPI-containing food products is an outstanding issue from a nutritional point of view and also because of the changes in functional properties of proteins that they induce (Scilingo & Añón, 2004). Aggregation process and solubility are determinants of functional properties of proteins, and they are very sensitive to denaturing treatments and ions addition. The addition of minerals to soy-based drinks is limited by settling, because protein solubility is decreased when mineral concentration exceeds certain values (Tang, Wang, Yang, & Li, 2009).

High hydrostatic pressure (HHP) is an emerging technology that has been applied in the processing of soy-based food (Lakshmanan, de Lamballerie, & Jung, 2006; Puppo et al., 2004). HHP denatures soybean proteins, achieving denaturation degrees of about 75% for β-conglycinin and of 100% for glycinin, after treatments at 600 MPa (Speroni, Añón, & de Lamballerie, 2010). The effects of HHP on soybean protein solubility depend on the composition of the media

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in which proteins are dispersed. In complex systems as soy-milk, a decrease in protein solubility (approximately to a half of initial values) was observed at 500 and 600 MPa (Lakshmanan et al., 2006). Calcium-induced insolubilization of soybean proteins (SPI, glycinin and β-conglycinin partially purified fractions) was reverted at pH 8.0 (Tris–HCl 0.050 mol L^{-1} – buffer) in samples treated at 400 or 600 MPa (Añón, de Lamballerie, & Speroni, 2012).

The pH of media is one of the most critical parameters, among factors that affect structure and solubility of proteins. Calcium addition decreases pH of medium because calcium ions compete with hydrogen ions for the same binding sites on protein molecule (Kroll, 1984). Moreover, HHP-induced denaturation carried out at different pHs could differentially affect the association between calcium and soybean proteins.

The aim of this work was to analyze the influence of ion type, ion concentration and pH on the effect of HHP on solubility and dispersion stability of SPI, and to deepen the knowledge about the mechanisms involved in these effects. This knowledge will be useful to incorporate soybean protein dispersions, supplemented with minerals, as food additives with tailor-made properties.

2. Materials and methods

2.1. Experimental design

The effect of mineral type was evaluated by adding calcium, magnesium or iron at pH 7.0. The effect of pH was evaluated in calcium-added samples for values 5.9, 6.4, 7.0 and 8.0. Ion concentrations ranged between 0.0015 and 0.0075 mol L^{-1}. Denaturation treatments evaluated were: HHP (600 MPa – 10 min) and thermal (95 °C – 15 min). Treatments were carried out in triplicate.

2.2. Sample preparation

2.2.1. Preparation of soybean protein isolate

SPI was prepared from defatted soybean flour manufactured by The Solae Company (Brazil). Alkaline extraction (pH 8.0 – 90 min – 20 °C) was followed by isoelectric precipitation (pH 4.5 – 15 min), as described by Speroni et al. (2010). The isoelectric precipitate was dispersed in distilled water and its pH was adjusted to 7.0 with 2 mol L^{-1} NaOH. Then dispersion was freeze-dried. The same batch of SPI was used for the whole study.

2.2.2. Preparation of mineral-added SPI dispersions

Dispersions of SPI were prepared at 10 g L^{-1} in bi-distilled water. Minerals were added at different concentrations between 0.0015 and 0.0075 mol L^{-1}, from stock solutions of 1.0 mol L^{-1} ($CaCl_2$ and $MgCl_2$) or 0.1 mol L^{-1} ($FeSO_4$). Stock solution of $MgCl_2$ was purchased (Sigma, St Louis, USA), while stock solutions of $CaCl_2$ and $FeSO_4$ were prepared from $CaCl_2$ dihydrate (Sigma, St Louis, USA) and $FeSO_4$ heptahydrate (Sigma, St Louis, USA). After mineral addition, pH was accurately adjusted with 1 mol L^{-1} NaOH or HCl to different values between 5.9 and 8.0.

2.2.3. High hydrostatic pressure treatment

Prior to HHP, SPI dispersions were vacuum packed in polyethylene bags (Cryovac BB2800, Sealed Air, Buenos Aires, Argentina). Then they were treated at 600 ± 5 MPa for 10 min in a high pressure system Stansted Fluid Power Ltd model FPG 9400:922 (vessel capacity: 2 L; maximum working pressure: 900 MPa) (Stansted, United Kingdom). The compression fluid used was a mixture of propylene glycol and water (30:70). The working pressure was reached at 5 MPa s^{-1} and released at 20 MPa s^{-1}. Conditioning temperature of vessel and initial temperature of samples were 20 °C. The adiabatic heating produced an increase of temperature up to 33.5 °C at the end of compression stage.

2.2.4. Thermal treatment

Dispersions of SPI underwent a thermal treatment at 95 ± 1 °C for 15 min in a thermostatic bath (Vicking, Buenos Aires, Argentina). Conditions were chosen to thermally denaturate β–conglycinin and glycinin (Speroni et al., 2010).

2.3. Sample analysis

2.3.1. Determination of protein content of SPI

Protein content of SPI was determined by the Kjeldahl method (AOAC, 1990). Digester and distillation unit were from BÜCHI (Flawil, Switzerland). Conversion factor was 5.8.

2.3.2. Determination of pH of SPI dispersions

A combination pH electrode S200CIT Sensorex (Garden Grove, CA, USA), connected to an Ion 510 series benchtop meter OAKTON Instruments (Vernon Hills, IL, USA) was used to measure pH of protein dispersions.

2.3.3. Molecular characterization of SPI using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Untreated SPI was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using a separating gel (120 g L^{-1} polyacrylamide) and a stacking gel (40 g L^{-1} polyacrylamide). These gels were prepared in a 0.375 mol L^{-1} Tris–HCl, 1.0 g L^{-1} SDS buffer, pH 8.8, in a mini slabs system (Bio-Rad Mini-Protean II Model). Protein molecular weights were estimated using low MW markers in the range 14.4–94 kDa (Pharmacia, Amersham, England). Gels were fixed and stained with R-250 Coomasie blue (2 g L^{-1}) in water/methanol/acetic acid (5:5:2) overnight and distained with 25% v/v methanol and 10% v/v acetic acid.

2.3.4. Determination of protein solubility

Samples were centrifuged at 10,000*g* for 20 min at 4 °C in an Aircooled Microlitre Centrifuge Z 233 MK-2 Hermle (Gosheim, Germany). Protein concentration was determined in the supernatants. Bicinchoninic Acid Protein Assay (BCA Sigma Kit) (Sigma Chemical Co., St. Louis, MO, USA) was used for quantification of soluble proteins in samples added with calcium or magnesium, while Bradford method was used for samples added with iron, since iron interferes with BCA (data provided by Sigma). Bovine serum albumin was used as standard (Sigma Chemical Co., St. Louis, MO, USA). Absorbance was measured at 562 nm (BCA) or 595 nm (Bradford) in a Synergy HT™ Multi-mode Microplate Reader (BIO TEK Instruments, Winooski, VT, USA).

Results were expressed as:

$$\text{Solubility}(\%) = \text{protein concentration in the supernatant}(\mu g/\mu L) \times 100/\text{initial protein concentration}(\mu g/\mu L)$$

$$\text{Relative increase of solubility}(\%) = (\text{solubility after HHP treatment} - \text{solubility before HHP treatment}) \times 100/\text{solubility before HHP treatment}.$$

2.3.5. Settling of insoluble protein

The sedimentation of insoluble proteins was analyzed through the use of a vertical dynamic light scan analyzer Quick Scan