Food Hydrocolloids 29 (2012) 272–279

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

Effect of acid treatment on structural and foaming properties of soy amaranth protein mixtures

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ARTICLE INFO

Article history:
Received 19 December 2011
Accepted 20 March 2012

Keywords:
Amaranth protein
Soy proteins
Acid-neutralization treatment
Foaming properties

ABSTRACT

To obtain a food ingredient composed of soybean and amaranth proteins with better functionality, the proteins were subjected to an acid treatment followed by neutralization. The native and treated proteins, amaranth (A and TA), soybean (S and TS) and the 1:1 mixture (M and TM) were studied. The structural characteristics and surface tension and foaming properties of the proteins were analyzed.

The acid-neutralization treatment caused structural modifications on all the proteins. The soybean proteins suffered some conformational changes and dissociation whereas the amaranth proteins were partially hydrolyzed by an endogenous aspartic protease, activated at acid pH. M showed the equivalent presence of S and A proteins, but TM, presented characteristics more similar to the TA proteins suggesting that the amaranth protease acted on the soybean proteins.

The acid treatment did not modify the S tensioactivity while TA and TM increased their tensioactivity compared to A and M. Amaranth proteins showed to be faster and more efficient than S in decreasing the surface tension, and present the higher velocity of foam formation. The acid treatment improved the foam formation capacity of all samples. Foam stabilization was also enhanced by the acid treatment, though in this case S proteins were better foam stabilizers than A. Although M showed an intermediate behavior between S and A, the TM showed a foam stability nearer the TS.

The mixture of amaranth and soybean proteins subjected to acid treatment make up an ingredient with improved surface and foam properties compared with its non treated components.

1. Introduction

Among seed proteins, those of soybean and amaranth stand out because of their high nutritional quality. Their amino acid compositions are close to the human diet requirements and are complementary (Bressani, 1994; Liu, 2000). Concerning their food functionality, it was reported that soybean proteins exhibit high functional properties compared to other plant proteins (Hettiarachchy & Kalapathy, 1998, chap. 6; Zayas, 1997). On the other hand, more recently the functional properties of amaranth proteins have begun to be studied, and it has been informed that although they show a lower solubility than soy proteins, they present good functional properties (Abugoch, Martinez & Añón, 2010; Avanza, Puppo, & Añón, 2005; Bejano-Luján, Lopez da Cunha & Netto, 2010; Fidantsi & Doxastakis, 2001; Mahajan & Dua, 2002; Marcone & Kakuda, 1999; Ventureira, Martinez & Añón, 2010). On the basis of these characteristics it seems reasonable to propose the use of these two protein sources to prepare a mixed food ingredient, which is expected to present excellent functional and nutritional qualities.

Many foods, most of them composed of foams and/or emulsions, require ingredients with good surface properties. The capacity of a protein to form and stabilize foams and emulsions depends on its structural characteristics and its physicochemical properties; e.g., a high solubility favors foaming and emulsifying properties. Flexible protein molecules showing an important surface hydrophobicity present a higher foaming capacity, whereas foam stability depends on the protein capacity to develop intermolecular bonds forming a viscoelastic film (Damodaran, 1997; Utsumi, Matsumura, & Mori, 1997).

Many plant storage proteins present a packed and scarcely flexible globular conformation that limits their foaming capacity, which may be improved by means of structural modifications. Chemical, physical and enzymatic modifications have been shown to improve functional properties. Amaranth proteins have turned more soluble with better foaming properties upon enzymatic hydrolysis (Condes, Scilingo, & Añón, 2009; Scilingo, Molina Ortiz, Martinez, & Añón, 2002), and chemical and enzymatic treatments have been shown to increase the foaming capacity of soybean

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doi:10.1016/j.foodhyd.2012.03.013

proteins (Molina Ortiz & Wagner, 2002; Wagner & Gueguen, 1999a,b).

HCl addition is an easy treatment to reduce the pH of protein solutions without adding other chemicals but Cl^-; upon this treatment the protein molecules unfold, expose more hydrophobic patches and become more flexible. This favors their adsorption in the interface and decreases the surface tension. Although these characteristics favor foam formation, foam stability is poor when completely unfolded proteins are used. Partial unfolding may be reached by mild acid treatments or by acid treatment followed by neutralization. It was demonstrated that after this pH-shifting process, soy proteins adopted a molten globule-like conformation that led to a markedly improved emulsifying activity and emulsion stability (Jiang, Chen, & Xiong, 2009).

The purpose of this work was to obtain a food ingredient with foaming properties, composed of soybean and amaranth proteins. Considering the possibility of hybrid structures formation with better functionality, the mixture of proteins was subjected to an acid treatment followed by neutralization. The structural and surface properties of the mixture and its isolated components were studied.

2. Materials and methods

2.1. Flours

Amaranth flour was prepared from seeds of *Amaranthus hypochondriacus* (Mercado commercial cultivar) kindly provided by Estación Experimental del Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Chapingo, México. They were ground in an Udy mill (UDY Corp., USA) equipped with a 1 mm mesh and screened through a 10 xx mesh (92 μm) (Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina). The flour was defatted with hexane (10% w v^{-1} suspension) at room temperature during 24 h, under continuous stirring during the first 5 h. The defatted flour was dried at room temperature and stored at 4 °C until used.

Commercially defatted soybean flours were donated by Sanbra S. A. (Brazil).

2.2. Protein isolates

The amaranth isolate was prepared according to Martínez and Añón (1996). The flour was dispersed in water in a 1:10 w v^{-1} ratio and the pH was adjusted to 9.0 by adding 2 M NaOH. The dispersion was stirred during 1 h and then centrifuged at 9000 *g* for 20 min at 10 °C. Proteins were precipitated by adjusting the supernatant to pH 5.0 with 2 M HCl, and were separated by centrifugation at 9000 *g* for 20 min at 4 °C. The pellet was dispersed in a small volume of water, neutralized with 0.1 M NaOH, and freeze-dried. The soybean protein isolate was prepared according to the method described by Petruccelli and Añón (1994). A 1:10 w v^{-1} dispersion of flour in water was adjusted to pH 8.0 with 2 N NaOH and stirred for 1 h. Then it was centrifuged at 10,000 *g* for 30 min at 4 °C, and the supernatant was adjusted to pH 4.5 to precipitate proteins. After centrifugation at 10,000 *g* for 20 min at 4 °C the precipitate was dissolved in water, neutralized and freeze-dried.

2.3. Acid treatment of the isolates

Each soybean and amaranth isolate and a 1:1 mixture of both isolates were dispersed in 0.01 M HCl at a concentration of 10 mg mL^{-1}, and the pH was adjusted to 2.0 with 2 M HCl. After stirring at room temperature for 3 h dispersions were diluted to 1 mg mL^{-1} with 35 mM phosphate buffer pH 7.5, 0.4 M NaCl (buffer A). These preparations were stirred for 3 h at room temperature and stored for 18 h at 4 °C before their use in different assays. The protein content of the isolates, as determined by the Kjeldahl method, was 83.1% ± 0.5 (db) amaranth (factor 5.85, Becker et al., 1981) and 85.2% ± 0.3 (db) soybean (factor 5.71, Wilson, 1995, chap. 22). The samples used for the assays were: native soybean isolate (S); amaranth isolate (A); 1:1 mixture of native soybean and amaranth isolates (M); treated soybean isolate (TS); treated amaranth protein isolate (TA); treated 1:1 mixture of soybean and amaranth isolates (TM).

2.4. Solubility

The native and treated samples were dispersed in buffer A (1 mg mL^{-1}) and stirred for 1 h at 25 °C. These dispersions and aliquots of diluted (1 mg mL^{-1}) treated and native samples were centrifuged at 15,000 *g* during 15 min at 20 °C. Protein content in the supernatant (P_s) was determined by the Lowry method and this value was used to calculate percent protein solubility in relation to the initial protein content in the sample (P_{in}), as follows:

$$S\% = P_s \times 100/P_{in}$$

Determination of solubility of the treated samples was carried out at the end of the acid treatment detailed before.

2.5. Gel filtration chromatography (FPLC)

Native and treated samples dissolved in buffer A were analyzed in a Pharmacia LKB, FPLC System (Uppsala, Sweden). Samples (200 μL) were injected in a Superose 6B HR 10/30 column and eluted with buffer A. The optical density at 280 nm was recorded. The column was calibrated with blue dextran (V_0), thyroglobulin (669 kDa). α-amylase (200 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), cytochrome C (12.4 kDa) and aprotinin (6.5 kDa). The calibration curve obtained from duplicate measurements was:

$$\log MM = A - (B \times K_{AV}) \quad \text{and} \quad K_{AV} = (V_e - V_0)/(V_T - V_0)$$

where MM is the molecular mass in kDa; V_e is the elution volume in mL; V_T is the total volume of the column (25 mL) and V_0 is the void volume. Curves were processed and data were evaluated using Pharmacia AB, FPLC director and FPLC assistant software.

2.6. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in minislabs (Bio-Rad Mini Protean II Model) according to Laemmli's method (1970). Runs were carried out with 12% (w v^{-1}) acrylamide gels and the following continuous buffer system: 0.375 M Tris–HCl, pH 8.8, 0.1% (w v^{-1}) SDS for the separating gel; 0.025 M Tris–HCl, 0.192 M glycine and 0.1% (w v^{-1}) SDS, pH 8.3 for the running buffer, and 0.125 M Tris–HCl, pH 6.8, 20% (v v^{-1}) glycerol, 1% (w v^{-1}) SDS, and 0.05% (w v^{-1}) bromophenol blue as sample buffer. For runs under reducing conditions the sample buffer contained 5% 2-mercaptoethanol (2-ME) and samples were heated for 1 min in a boiling-water bath. Samples containing 40–50 μg of protein were loaded. The following protein molecular mass standards were used: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); a-lactalbumin (14.4 kDa). Gels were fixed and stained with Coomassie Brilliant Blue Stain or Silver Stain as indicated in the figures. Gels images were scanned.

2.7. Differential scanning calorimetry

DSC measurements were performed in a TA Q100 (TA-Instruments, USA) calorimeter. The equipment was calibrated at a heating