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Interfacial and foaming characteristics of soy globulins as a function of pH and ionic strength

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Abstract

Soy proteins exhibit high functional properties compared with other plant proteins. However, a notable feature of soy proteins is the strong pH and ionic strength (*I*) dependence of the molecular conformation and associated functional properties. In this work we have studied the effect of pH (5.0 and 7.0) and ionic strength (0.05 and 0.5 M) on the adsorption, surface dilatational properties and foaming characteristics (foam power and foam stability) of soy globulin (β-conglycinin (7S) and glycinin (11S)) solutions. The protein concentration in solution and temperature were maintained constant at 0.1 wt% and 20 °C, respectively. The rate of adsorption and surface dilatational properties (surface dilatational modulus, *E*, and loss angle) of soy globulins at the air–water interface depend on the protein and, especially, on the pH and *I*. The adsorption decreased drastically at pH 5.0, close to the isoelectric point of the protein, because of the existence of a lag period and a low rate of diffusion. The interfacial characteristics of soy globulins are much improved at the high ionic strength (even for acidic solutions). At pH 5 the foam capacity is zero for 7S globulin at *I* 0.05 M and for 11S at every *I*. At pH 7 foaming and foam stability are higher for β-conglycinin than for glycinin. We have observed that there exist close relationships between foaming and the rate of diffusion of soy globulins to the air–water interface, and between the foam stability (against drainage and disproportionation/collapse) and the surface pressure (π) and *E* at long term adsorption. The last section deals with a comparison between interfacial and foaming characteristics of the soy globulins with those of β-lactoglobulin aqueous solutions.

Keywords: Food dispersion; Food colloids; Foam; Emulsifier; Soy protein; Beta-conglycinin; Glycinin; Adsorption; Surface tension; Air–water interface

1. Introduction

Soy proteins are employed in food products because of their highly nutritive value and ability to stabilize food dispersions (emulsions, foams, gels, etc.), as well as their ability to improve texture, water absorption, and act as a thickening agent [1,2]. Soy proteins exhibit high functional properties compared to other plant proteins [2–4]. The demand for safe, high quality health foods with good nutritional value has increased the use of soy proteins. Although they have been studied extensively, the optimum use of soy protein under conditions present in foods requires much more research at a molecular level.

Globulins account for about 50–90% of seed proteins. Storage globulins are grouped into two types according to their sedimentation coefficients, β-conglycinin (a 7S globulin) and glycinin (an 11S globulin). The ratio of 11S to 7S globulins, which varies among cultivars, is about 0.5–1.7 in soybean [2,5]. The 7S globulins of soybean are classified into three major fractions with different physicochemical properties, β-conglycinin being the most prevalent of these fractions accounting for 30–50% of the total seed protein. β-Conglycinin is a glycoprotein present as a trimer with a molecular mass of 150–200 kDa. It is composed of a combination of three subunits, α (≈67 kDa), α' (≈71 kDa) and β (≈50 kDa) stabilized by non-covalent bonds. Glycinin (11S globulin), which represents ≈30% of total protein in soybeans, is a hexamer with a molecular mass of 300–380 kDa, organized in a close packed globular conformation. It is composed of an acidic (A, ≈38 kDa) and a basic polypeptide (B, ≈20 kDa) linked by a single disulfide bridge

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[6–8]. A notable feature of soy proteins is the strong pH and ionic strength (I) dependence of the molecular conformation and the associated functional properties [1,2,9–12]. Optimum functionality occurs at pH < 5 and $I > 0.5$ M, which limits their application as food ingredients [8]. In fact, the pH of food products ranges from pH 3 to 7, and the ionic strength varies from 0.02 to 0.2, whereas soy proteins are soluble at neutral or basic pH and at high ionic strengths (≈0.5 M). High protein solubility generally correlates with good foaming and emulsifying capacity [1]. Thus, research is required to resolve this and other issues related to the use of soy proteins in food formulations (emulsions, foams, gels, etc.).

The role of proteins in the formation and stabilization of food dispersions (emulsions and foams) has been extensively studied [13–18]. Foaming and emulsifying characteristics and the stability of the resulting dispersion depend on the properties of these proteins at fluid interfaces [19,20]. The interfacial behaviour of proteins (adsorption, structure, mechanical properties, etc.) depends on their physical, chemical, and conformational properties (size, shape, amino acid composition and sequence, charge and charge distribution, etc.), which are affected by extrinsic factors (pH, ionic strength, temperature, etc.) [21,22].

The aim of this work is to study the effect of pH (5.0 and 7.0) and ionic strength (0.05 and 0.5 M) on the adsorption, surface dilatational properties and foaming characteristics (foam power and foam stability) of soy globulin (β-conglycinin and glycinin) at a representative protein concentration in solution of 0.1 wt%. The temperature was maintained constant at 20 °C. Although systematic studies dealing with protein adsorption at the water–fluid interface have been published recently [23,24], the kinetics of soy globulins adsorption in solution have not been systematically studied so far [25–30]. In this work we complement previous studies on static [29–31] and dynamic [28,30,32,33] properties of spread and adsorbed soy globulin films and foaming characteristics of soy globulin aqueous solutions [1,26,27]. For interfacial pressure and surface dilatational property measurements of adsorbed films an automatic drop tensiometer was used. The foaming properties were characterized through their foam formation and stability measured in a commercial instrument.

2. Materials and methods

2.1. Materials

Samples for interfacial characteristics of soy protein films were prepared using Milli-Q ultrapure water and were buffered at pH 5.0 and 7.0. Analytical-grade acetic acid and sodium acetate, and Trizma [$(CH_2OH)_3CNH_2$/$(CH_2OH)_3CNH_3Cl$] for buffered solutions at pH 5.0 and 7.0, respectively, were used as supplied by Sigma (>95%) without further purification.

2.2. Isolation of 7S and 11S globulins

7S and 11S globulins were isolated as Nagano et al. [34] with slight modifications (Fig. 1). Proteins were extracted from defatted soybean meal by making a slurry with 15-fold volumes of water adjusted to pH 7.5 with 2N NaOH. This slurry was centrifuged at 10,000 × g for 15 min. Dry sodium bisulfite (SBS) was then added to the supernatant (0.98 g SBS/l), the pH was adjusted to 6.4 with 3N HCl, and the mixture was kept at 4 °C overnight. This preparation was centrifuged (10,000 × g for 15 min), obtaining two fractions:

(i) The insoluble fraction, which turned out to be the 11S globulin fraction, was washed once again with SBS (0.98 g SBS/l), the pH was adjusted to 6.4 with 3N HCl, and the mixture was kept at 4 °C overnight. This preparation was centrifuged (10,000 × g for 15 min). The insoluble fraction of 11S globulin was, finally, dissolved in water at pH 8.0 and lyophilized.
(ii) The salt concentration of the supernatant, which turned out to be the 7S globulin fraction, was then adjusted with NaCl 0.25 M solution and the pH was adjusted at pH 5 with HCl 4N, stirred during 1 h and then centrifuged (10,000 × g for 15 min). Afterwards, the supernatant was diluted with cold water (≅2000 ml) at pH 4.8 (HCl 3N) and centrifuged (10,000 × g for 15 min). The whole process was repeated once again and finally, the insoluble fraction of purified 7S globulin was dissolved in water at pH 8.0 and lyophilized.

The protein content of 7S and 11S fractions, determined by Kjeldahl method, was 96.1 and 97.9%, respectively.

2.3. Methods

2.3.1. Solubility

Soy globulins (10 g) were extracted twice with 200 ml of 1N NaOH stirring for 2 h at room temperature. Aliquots were taken for precipitation of the proteins at different pH values adjusted with HCl. The samples were centrifuged at 7500 × g for 15 min at 16 °C and the nitrogen content determined in the supernatant. Percentages of protein solubility were calculated as the percent distribution of soluble nitrogen in the supernatants in relation to the total nitrogen extracted versus pH.

2.3.2. Gel filtration chromatography

Molecular masses of soy globulins were determined by FPLC. Samples were passed through a PD-10 column (Amersham Pharmacia) to remove non-protein components. Gel filtration was carried out in a fast protein liquid chromatography system (FPLC) equipped with a Superose 12 HR 10/30 column (Armsham Pharmacia). Injection volume was 200 μl. The eluent was 20 mM phosphate buffer, 0.5 M sodium chloride buffer, pH 8.3 at a flow rate of 0.4 ml/min. Elution was monitored at 214 nm to detect small peptides lacking aromatic residues. The molecular masses were determined with a calibration curve made with blue dextran (2000 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards. All these reagents were obtained from Sigma.

2.3.3. Amino acid analysis

Amino acids were determined by high-performance liquid-chromatography (HPLC), according to the method of Alaiz et al.