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Dilatational properties of soy globulin adsorbed films at the air–water interface from acidic solutions

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Abstract

In this paper we present surface dilatational properties of soy globulins (β-conglycinin, glycinin, and reduced glycinin with 10 mM of dithiothreitol (DTT)) adsorbed on the air–water interface, as a function of adsorption time. The surface rheological parameters (surface dilatational modulus, E, its elastic and viscous components, and phase angle) were measured as a function of protein concentration (ranging from 1 to 1×10^{-3}%, wt/wt) at pH 2.0 and 5.0. We found that the surface dilatational modulus, E, increases with time, θ. This phenomenon has been related to protein adsorption, unfolding, and/or protein–protein interactions (at long-term adsorption). The dilatational properties of the adsorbed films depend on the molecular structure of the protein, the pH, and on the protein concentration in the aqueous phase. Soy globulins are adsorbed at the air–water interface with different degrees of association at different concentrations in the bulk phase and at different aqueous phase pH.

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1. Introduction

Proteins due to their amphiphilic character can adsorb at fluid interfaces. The adsorption of proteins at interfaces and other dynamic surface properties—such a the film viscoelasticity—are known (Dickinson, 1992; Halling, 1981) to play an important role in the formation and stability of food dispersed systems (foams and emulsions). Due to the adsorption at fluid interfaces, protein molecules prevent the recoalescence of previously created bubbles or droplets. In addition, during the protein adsorption the surface or interfacial tension of the air–water or oil–water interface decreases (Dickinson, 1992), which is an important attribute to optimise the input of energy involved in the foaming or emulsification process (Walstra, 1993), and for the production of smaller bubbles or droplets, which is an important factor for the stability of the dispersion (Dickinson, 1992). On the other hand, emulsification and foaming involve interfacial deformation and the response of the adsorbed layer to such deformations (measured by the surface dilatational properties) is crucial for understanding the role of proteins in food systems (Benjamins, 2000).

Vegetable proteins from legumes (Kinsella, 1979; Utsumi, Matsumura, & Mori, 1997) are being used successfully for the formation and stabilization of new food products, most of them presented commercially as dispersions (emulsions and foams). Soy proteins act as a macromolecular emulsifier to stabilize food dispersions such as confectionary products, coffee

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whitener, dressings, sausages, and soups (Utsumi et al., 1997). The use of soy proteins as functional ingredients is gaining increasing acceptance in food manufacturing from the standpoints of human nutrition and health. The applicability of soy proteins in foods is based on their functionality. Despite their commercial importance, fundamental knowledge about the dispersion properties (emulsifying or foaming) of soy proteins is scarce. Very little progress has been made so far in understanding the mechanical properties of soy globulin adsorbed films.

This paper presents experimental information on surface dynamic properties (time-dependent viscoelastic characteristics) of β-conglycinin (7S), glycinin (11S), and glycinin reduced with DTT (11S + 10 mM DTT) adsorbed films at the air–water interface, at 20 °C and at pH 2.0 and 5.0. The concentration of protein in the bulk phase was the variable studied. Globulins 7S and 11S are two major storage proteins in soybeans with a molecular weight of about 180 and 360 kDa, respectively. Native soy glycinin because of its compact tertiary structure, which is stabilized by disulfide cross-linking, has limited foaming and emulsifying (Kinsella, 1979; Utsumi et al., 1997) properties. However, reduction of some disulfide bonds may improve their foaming and emulsifying ability by allowing greater conformational flexibility (German, O'Neill, & Kinsella, 1985). The 7S globulin is a glycoprotein which does not contain disulfide bonds. The strong pH dependence of the molecular conformation and the associated functional properties (Kinsella, 1979; Utsumi et al., 1997) mean that the optimum functionality of soy proteins occurs at pH < 5, which limits their application as food ingredients. Thus, more research is required to resolve this and other issues related to the use of soy proteins in food formulations.

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 2.0 and 5.0. Analytical-grade acetic acid and sodium acetate were used for adjusting the pH 5.0 as supplied by Sigma (>95). HCl (analytical-grade, Panreac) and KCl (analytical-grade, Merck) were used for adjusting the pH 2.0 and the ionic strength (I) of the aqueous solutions, respectively. The isolation of β-conglycinin and glycinin soy globulins, solubility, and structural characterization have been described elsewhere (Molina, Carrera, Rodríguez Niño, Añón, & Rodríguez Patino, 2003). Glycinin was reduced using 10 mM DTT (German et al., 1985; Kim & Kinsella, 1986).

2.2. Methods

For surface pressure (π) and surface dilatational properties measurements of adsorbed protein films at the air–water interface an automatic drop tensiometer (TRACKER, IT Concept, Longessaigne, France) was utilized, as described elsewhere (Rodríguez Patino, Rodríguez Niño, & Carrera, 1999). Briefly, the method involved a periodic automatically controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume, at the desired amplitude ($\Delta A/A$) and angular frequency (ω). The surface dilatational modulus (E) (Eq. (1), its elastic (Ed) and viscous (Ev) components, and the phase angle (ϕ) were derived from the change in surface pressure (π) resulting from a small change in surface area (A). The surface dilatational properties were measured as a function of time, θ. The percentage area change was determined (data not shown) to be in the linear region.

$$E = \frac{\mathrm{d}\sigma}{\mathrm{d}A/A} = -\frac{\mathrm{d}\pi}{\mathrm{d}\ln A} \tag{1}$$

$$E = (\sigma_0/A_0)\cdot(\cos\phi + \mathrm{i}\sin\phi) = Ed + \mathrm{i}Ev \tag{2}$$

where σ_0 and A_0 are the strain and stress amplitudes, respectively, ϕ is the phase angle between stress and strain, $\pi = \sigma^0 - \sigma$ is the surface pressure, and σ and σ^0 are the surface tension in the presence and in the absence of protein, respectively.

The dilatational modulus is a complex quantity and is composed of real and imaginary parts (Eq. (2). The real part of the dilatational modulus or storage component is the dilatational elasticity, $Ed = |E|\cdot\cos\phi$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, $Ev = |E|\cdot\sin\phi$. The ratio (σ_0/A_0) is the absolute modulus, $|E|$, a measure of the total unit material dilatational resistance to deformation (elastic + viscous). For a perfectly elastic material the stress and strain are in phase ($\phi = 0$) and the imaginary term is zero. In the case of a perfectly viscous material $\phi = 90°$ and the real part is zero.

The experiments were carried out at 20 °C. The temperature of the system was maintained constant within ±0.1 °C by circulating water from a thermostat. Protein solutions ranging from 1% to 10^{-3}%, wt/wt were prepared freshly and stirred for 30 min. The solution was placed in the syringe and then in the compartment and was allowed to stand for 30 min to reach the desired constant temperature. Then a drop of protein solution was delivered and allowed to stand for 180 min at 20 °C to achieve protein adsorption at the air–water interface. The sinusoidal oscillation for surface dilatational measurements started after 15 min of adsorption time. Afterwards, the drop was subjected to repeated measurements with five oscillation cycles followed by a time corresponding to 50 cycles without any oscillation up

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