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Improvement of emulsifying properties of soy protein through selective hydrolysis: Interfacial shear rheology of adsorption layer

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ABSTRACT

The interfacial viscoelastic behavior of adsorption layers at the oil/water interface for soy protein isolate (SPI), β-conglycinin (7S) and two hydrolysates of soy protein were investigated using a rotational rheometer equipped with Du Noüy ring geometry. Two kinds of soy protein hydrolysates were prepared by selective and limited hydrolysis. The limited hydrolysis products of soy protein (soy protein hydrolysates, SPH) and sodium caseinate, with flexible structures, quickly adsorbed at the oil/water interface and formed fluid-like interface with low interfacial shear modulus. The SPI, 7S and selective hydrolysis products of soy protein (reduced-glycinin, RG) exhibited the viscoelastic properties of globular proteins and formed interfacial films with high elastic and viscous moduli. The dynamic time sweep experiments showed that the adsorption of RG was faster than that of the SPI and 7S, which could help to prevent coalescence during the formation of emulsion, and was expected to result in the formation of smaller droplet size. Moreover, RG exhibited the highest adsorbed amount, indicating that it was less spread out at the surface and thus reduced the chance of protein unfolding. The microstructures of the emulsions suggested that the emulsifying properties of RG and 7S were comparable to those of sodium caseinate. This study indicates that selective hydrolysis may significantly improve the emulsifying and rheological properties of soy protein, and provides useful information for the preparation of high emulsifying soy protein products.

1. Introduction

Soy proteins are widely used in food products, such as beverages, infant formulas, ice cream and dairy products. The interfacial and emulsifying properties of soy proteins have been investigated in recent years (Martinez, Sánche, Patino, & Pilosof, 2009; Piazza, Dürr-Auster, Gigli, Windhab, & Fischer, 2009; Wang et al., 2012; Xu, Liu, & Zhang, 2015). Due to their large molecular weight and globular structure, soy proteins exhibit poor surface activity compared to low molecular weight surfactants and flexible proteins, such as sodium caseinate. Many studies have demonstrated that proteolysis may improve the emulsifying properties of soy protein (Jung, Murphy, & Johnson, 2005; Qi, Hettiarachchy, & Kalapathy, 1997). Glycinin (11S) and β-conglycinin (7S) are two major components of soy protein, and it has been demonstrated that the emulsifying activity and emulsion stability of 7S are much stronger than those of 11S (Aoki, Taneyama, & Inami, 1980). Therefore, it is assumed that selectively decomposing only 11S and keeping 7S will greatly improve the emulsifying properties of soy protein.

Proteins adsorb at the interface and form highly viscoelastic films, which provides steric and electrostatic repulsion for emulsion stability (Humblet-Hua, van der Linden, & Sagis, 2013). Due to the pressure difference between droplets of difference size, molecules of the dispersed phase diffused from small to large droplets, which might result in the creep and collapse of the adsorbed layer (Meinders & van Vliet, 2004; Murray, 2011). Therefore, it is of vital important to study the interfacial viscoelastic behavior of adsorbed layer for protein stabilized emulsion. Protein adsorption is a

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complex process, and it is difficult to determine whether protein charge, hydrophobicity, structural flexibility or molecular weight is important for interfacial adsorption. Recently, the study of structural and mechanical properties of proteins at the interface using surface shear rheological measurements has become very popular (Radulova, Danov, Kralchevsky, Petkov, & Stoyanov, 2014; Radulova, Golemanov, Danov, Kralchevsky, Stoyanov, Arnaudov, et al., 2012; Rühs, Scheuble, Windhab, Mezzenga, & Fischer, 2012; Wyss et al., 2007), offering insight into the mechanism of the formation and breakage of the interfacial film, which can be used to predict the shelf-life of foods.

Protein adsorption can be divided into three steps (Baldursdottir, Fullerton, Nielsen, & Jorgensen, 2010; Langevin, 2014). The first step involves protein diffusion, in which the interfacial tension and complex viscosity are unchanged over time and are only detectable for dilute protein solutions (ca. 10 μg/mL). The second step is the formation of the protein monolayer, which includes structural deformation, interfacial tension reduction and the rapid increase in complex viscosity. The last step is interfacial gelification, especially for globular proteins. In this step, the multilayer is formed, with a slight drop in surface tension and a slow increase in complex viscosity. Previous studies have confirmed that the protein adsorption process can be well described based on the variation of viscoelastic moduli G' and G'', and complex viscosity, η (Freer, Yim, Fuller, & Radke, 2004b). The magical rod (Reynaert, Brooks, Moldenaers, Vermant, & Fuller, 2008), bicone geometry (Maestro, Deshmukh, Mugele, & Langevin, 2015) and the du Noüy ring (Bertelli, Dip, Pires, Albuquerque, & Lucas, 2014; Wang, Xie, Qiao, Goffin, Hodgkinson, Yuan, et al., 2012) were used to measure the surface shear rheology. Many proteins have been studied using these instruments, such as β-lactoglobulin (Jung, Gunes, & Mezzenga, 2010), β-casein (Bantchev & Schwartz, 2003; Partanen, Forssell, Mackie, & Blomberg, 2013), hydrophobins (Milani, Monogioudi, Baldrighi, Cavallo, Arima, Marra, et al., 2013) and silk fibroin (Qiao, Wang, Shao, Sun, & Miller, 2015). Works have been done on dilatational rheology of soy protein and its hydrolysates by Martinez et al. (2009) and Ruiz-Henestrosa et al. (2007a, 2007b). However, there are few studies on shear rheology of soy proteins and its hydrolysates, especially for the oil/water interface.

The selective hydrolysis products of soy protein (RG) had relatively small molecular size and flexible structure, was expect to show improved emulsifying properties. In order to verify the hypothesis, the interfacial viscoelastic behavior of adsorption layers at the oil/water interface for soy protein isolate (SPI), β-conglycinin (7S) and two hydrolysates of soy protein were investigated using a rotational rheometer equipped with Du Noüy ring geometry. Meanwhile, sodium caseinate, which is a widely used commercial emulsifier, was chosen as a reference. Two mechanisms for the protein stabilized emulsions were also discussed.

2. Materials and methods

2.1. Materials and chemicals

Soybeans were obtained from a local market. Soy protein isolate (SPI) was prepared following the procedure of Diftis and Kiosseoglou (2006). The protein content was determined to be approximately 92% ($N\times6.25$) by the Kjeldahl method. Papain (EC 3.4.22.2) was purchased from Ruji Biotechnology Co. (Shanghai, China). Pepsin (EC 3.4.23.1) was purchased from Sangon Biotech Co. (Shanghai, China). Soybean oil was purchased from a local supermarket. Sodium caseinate was purchased from Murray Goulburm (Australia). All other chemicals used in the study were of analytical grade unless otherwise specified. All of the aqueous solutions were prepared using deionized water.

2.2. Preparation of soy protein and the hydrolysates

The 7S fraction was prepared from the soybeans according to the method of Ruíz-Henestrosa et al. (2007a), with slight modifications. Soy protein was extracted from defatted soybean flours by making a slurry with 15-fold volumes of water adjusted to pH 7.5 with 2 M NaOH. This slurry was centrifuged at 10,000× *g* for 20 min. Dry sodium bisulfite (0.98 g L^{-1}) was then added to the supernatant, the pH was adjusted to 6.4 with 2 M HCl, and the mixture was stored overnight at 4 °C. This preparation was centrifuged (10,000× *g* for 20 min), and the supernatant was collected and treated using 0.25 M of NaCl. The pH was adjusted to 5.0 with 2 M HCl. The mixture was stirred for 1 h at 4 °C and then centrifuged at 10,000× *g* for 20 min. The supernatant was diluted with 2 volumes of distilled water, adjusted to pH 4.8 with 2 M HCl, and centrifuged at 10,000× *g* for 20 min. The precipitate (7S) was washed 3 times with distilled water, resuspended in distilled water, adjusted to pH 7.0 with 2 M NaOH and freeze-dried.

A 5% (w/v) SPI dispersion was hydrolyzed at 50 °C and a pH of 7.0 with papain. The E/S ratio was 0.5 wt%. The degree of hydrolysis of the hydrolyzed protein was determined to be 1.0% using the pH-stat method (Alder-Nissen, 1986). The hydrolysates were then heated at 90 °C for 10 min to inactivate the enzyme and cooled in ice water at room temperature for 1 h. After centrifugation (10,000× *g* for 10 min), the supernatant was freeze-dried to form the soy protein hydrolysates (SPH).

A 5% (w/v) soy protein isolate dispersion was adjusted to the pH of 2.0 with 2 M HCl. Pepsin was added to the SPI dispersion, and the mixture was incubated at 37 °C for 1 h while gently stirring. The E/S ratio was 0.05 wt%. Immediately after the enzyme reaction had taken place, the mixture was adjusted to pH 7.0 with 2 M NaOH, pasteurized and freeze-dried to yield a powdered reduced-glycinin (RG).

2.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a discontinuous buffered system with mercaptoethanol using 12% separating gel and 4% stacking gel. Lyophilized SPI samples (2 mg mL^{-1} in buffer containing 0.0625 M of Tris—HCl, 10% glycerin, 2% SDS, 5% β-mercaptoethanol and 0.0025% bromophenol blue) were incubated for1 h at room temperature, and then heated at 95 °C for 5 min and centrifuged at 5000× *g* for 10 min using a TGL-16G 144centrifuge (Anting Scientific Instrument Co. Ltd., Shanghai, China). Aliquots (15 μL) of the prepared samples were loaded onto the gels.

2.4. Interfacial rheology measurements

Measurements of the interfacial rheology of proteins at the oil/water interface were obtained using a HAAKE MARS III rheometer (Thermo Scientific, Germany) equipped with a Du Noüy ring (platinum ring) geometry. The Du Noüy ring used had a diameter of 19.450 mm. The thickness of the wire was 0.379 mm. Twenty milliliters of the soy peptides-sugar conjugates solution was placed in a beaker (50 mm in diameter) and the ring was lowered to make contact with the surface. In order to increase the repeatability and avoid the destruction of the platinum ring, the gap was zeroed with a cone geometry and then the gap was kept constant at the position of 48.723 mm. Subsequently, 20 mL of the soybean oil was carefully poured on top of the aqueous phase. The measurement was conducted according to Baldursdottir et al. (2010). In order to get optimal measurement results, the inertia determination and MSC (Micro Stress Control) calibration was performed every time before starting a series of measurements.