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Water holding of soy protein gels is set by coarseness, modulated by calcium binding, rather than gel stiffness

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ABSTRACT

This work aims to differentiate between the contributions to water holding (WH) by gel microstructure and network stiffness of soy protein (SP) gels. SP were succinylated to increase calcium binding affinity, and the presence of different calcium salts were used to generate gels with different morphologies while keeping ionic strength and protein concentration constant. It was found that not gel stiffness, but coarseness (gel microstructure inhomogeneity) is more dominant in setting the WH ability. A higher energy dissipation of applied stress onto the protein network was related to inability of a gel network to retain water.

1. Introduction

Population growth requires a more intense exploration and implementation of alternative protein sources in production, especially a wider application of plant proteins is desired for the structuring of food products. Particularly the use of soy proteins as structuring – gelation agent in food products has gained a lot of attention as this protein source is known to form gels with a high water holding (WH) capacity.

Two gel characteristics, microstructure and stiffness, are reported to determine this water holding propensity. The microstructural (morphological) aspects of the gel network appear relevant. These are set by e.g. the type of protein, protein concentration, pH, ionic strength and type of salts (Foegeding, Bowland, & Hardin, 1995; Hermansson, 2008; Maltais, Remondetto, Gonzalez, & Subirade, 2005; Molina, Defaye, & Ledward, 2002; Renkema & van Vliet, 2002). The impact of morphology on the water holding capacity has been reported for different protein systems and attempts to link it to typical length scales in the network have been made (Chantrapornchai & McClements, 2002; Hermansson, 1982; Puppo & Añón, 1998; Puppo, Lupano, & Anon, 1995; Stevenson, Dykstra, & Lanier, 2013). It has been reported that pore size diameters in the range of 0.1–2 μm are relevant for WH (Hermansson, 1986) and more recently, a dissection to identify the most relevant length scale that sets WH in soy gels was presented, showing that the structure contributions at the supra-micron level were most important (Urbonaite, de Jongh, van der Linden, & Pouvreau, 2014). However, as modulation of coarseness of gels typically coincides with variation in, for example, osmotic pressures within the network, a conclusive picture has not emerged yet.

Alternatively, when WH is evaluated under an applied external force, like during oral processing, WH becomes also a function of the deformability of the network. Van den Berg and colleagues were able to demonstrate the deformation of the porosity of the continuous serum phase and how this affects the exudation of liquid (van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2007). The impact of gel stiffness represented by the Young's modulus on the ease of water removal from the gel was reported for soy gels more recently (Urbonaite et al., 2014).

The measurement of water holding capacity comprises two distinct outcomes: (i) the maximum amount of water that is

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exuded from the protein gel, given an applied force, and (ii) the kinetics of this exudation (Kocher & Foegeding, 1993). The variation in how the water holding capacity is assessed experimentally, together with variation in microstructure and deformability when comparing different gels, has prohibited thus far a complete understanding of how WH can be set by controlling gel network formation.

This work aims to differentiate between the contributions to WH by microstructure and network stiffness for soy protein gels. To this end, the approach has been taken to keep protein concentration, ionic strength and pH constant to derive a series of different types of gel morphologies. The obtained gels are then analyzed for their coarseness, their mechanical deformability and their WH capacity.

2. Material and methods

2.1. Material

Defatted soy flour was provided by Cargill BV (Amsterdam, The Netherlands). Calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and succinic anhydride (dihydro-2,5-furandione) were obtained from Sigma–Aldrich (Steinheim, Germany). Reagents were of analytical grade and used without further purification.

2.2. Preparation of enriched soy protein solution

Defatted soy flour was suspended in reverse osmosis (RO) water at a ratio 1:10 (w/w) at 45 °C and stirred for 30 min. Then the pH was adjusted to 8.0 with 5 M NaOH and stirred for an additional 30 min. The supernatant was collected by centrifugation (30 min, 6000 g, 13 °C). Isolation of the globulins (predominantly glycinin and β-conglycinin) was achieved by isoelectric precipitation (pH 4.5, 6 M HCl). After mild stirring of precipitate overnight at 4 °C, the suspension was centrifuged (30 min, 6000 g, 7 °C). The pellet was resuspended 3 times at pH 4.5 in RO water at a ratio 1:3 (w/w) to remove any remaining insoluble material followed by centrifugation (30 min, 6000 g), and was finally suspended in RO water at ratio 1:4 (w/w) at pH 7.0 using 5 M NaOH. This material was denoted as enriched soy protein solution (SP). Protein content of SP was determined by Kjeldahl (calculated using N x 6.25), where typical isolation procedure yielded of 11–12% (w/w). The ratio of predominant proteins glycinin:β-conglycinin was estimated to be 50:50 by gel electrophoresis and reversed phase high-performance liquid chromatography (results are not shown). SP was stored at 4 °C in solution in the presence of 0.02% sodium azide and used within a month after preparation. The enthalpy change, indicative for the protein nativity, was 15.2 J/g protein measured by differential scanning calorimetry (DSC) (Q1000, TA Instrument, New Castle, USA).

2.3. Succinylation

Succinylation of SP was performed by diluting the protein solution to 5% w/w with phosphate buffer at pH 8.0 to a final buffer concentration of 75 mM. Samples were prepared in 50 ml aliquots. Succinic anhydride (10, 40 and 100 mg/g protein) was added in portions of 5–10 mg while constant stirring and maintaining the pH at 8.0 ± 0.2 by manual addition of 2 M NaOH. Between each succinic anhydride addition, the solution was allowed to equilibrate for at least 5 min. After the final addition samples were stirred for another 15 min. Succinylated samples were dialyzed in a dialysis tube (MW cut off: 12–14 kDa) against demi water in 100 times excess 4 times for at least 3 h. After dialysis, pH was adjusted to 7.0, samples were freeze-dried followed by resolubilization in demi water to 11% w/w protein. Protein content of succinylated SP was checked by Kjeldahl (calculated using N × 6.25). Succinylated SP was stored at 4 °C in solution with 0.02% azide and used within a month.

2.4. Degree of succinylation

The degree of succinylation was determined by OPA (o-Phthaldialdehyde) assay adapted from Church et al. (Church, Swaisgood, Porter, & Catignani, 1983). OPA reacts with primary amine groups (N-terminus and lysine ε-amino groups) in the presence of DMA (2-(dimethyl amino) ethanethiol hydrochloride) and results in the formation of alkyl-iso-indole fluorescent moieties. The OPA reagent was freshly prepared in a 50 ml volumetric flask by dissolving 40 mg OPA dissolved in 1 ml methanol, 25 ml 0.1 M borax solution, 200 mg DMA, 5 ml 10% SDS solution and demi water. Presence of alkyl-iso-indole derivatives were measured at 340 nm in a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) of 1 ml OPA reagent mixed with 100 μl 0.05% succinylated SP, equilibrated for 1 h at room temperature in the dark. A linear calibration curve was obtained by measuring absorbance at 340 nm of 0.08–0.6 mM L-leucine as described above. Using this calibration curve the amount of NH_2 (mM) of protein prior and after modification was obtained. Degree of modification was expressed as % of modified lysine residues.

2.5. Apparent isoelectric point (IEP)

The zeta-potential was determined at 25 °C by a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Reagents and protein solutions were filtered using 0.2 μm pore size syringe filter (Chromafil RC-20/25, Macherey-nagel, Düren, Germany). To determine the zeta-potential, 2.5 mg/ml (modified) SP was mixed with 5 mM $NaNO_3$ buffer previously adjusted to a series of pH from 3.0 up to 5.0. Measurements were performed in triplicates. The apparent IEP was determined by plotting a linear regression line across measured zeta potential values (mV) over defined pH range and taking the x-axis intercept as apparent IEP.

2.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements of (modified) SP were performed using DSC Q1000 (TA Instruments, New Castle, USA). Samples containing 10% w/w (modified) SP were heated from 20 to 120 °C with a heating rate of 0.5 °C/min. The enthalpy change and denaturation temperature was determined using standard software (TA Universal analysis) provided by the supplier. Measurements were performed in duplicates.

2.7. Intrinsic tryptophan fluorescence

Fluorescence spectra of 0.2 mg/ml SP (modified and non-modified) at pH 7.0 were recorded on a Varian Eclipse fluorescence spectrophotometer (Agilent Technologies, Amstelveen, The Netherlands). Excitation was performed at 295 nm and the resulting emission spectrum was recorded from 305 to 405 nm, using a scan speed of 10 nm/min. Both the excitation and the emission slit were set at 5 nm. Spectra were recorded 3-fold and averaged. Spectra were corrected by subtracting the spectrum of a protein free sample, obtained under identical conditions.