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Relating water holding of ovalbumin gels to aggregate structure

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ABSTRACT

To increase understanding of water holding in heat-set ovalbumin gels, the relation between aggregate structure (both in solution and in gels) and gel coarseness or gel stiffness is studied. The aggregate morphology obtained by preparation of ovalbumin gels between pH 5.8 and 6.8 differs, as shown by a combination of light scattering, confocal microscopy, electron microscopy and neutron scattering techniques. It is shown that with decreasing pH larger aggregates are formed that are more compact, indicated by the fractal dimension that increased from 1.9 to 2.6. Furthermore, larger, more compact aggregates at pH 5.8 as compared to pH 6.8 resulted in more coarse gels with a less merged microstructures. In addition, a lower gel stiffness is observed at lower pH. The combination of a higher coarseness and lower gel stiffness explains both the lower total water holding and the easier exudation of water from the gel upon applied forces.

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1. Introduction

The ability of protein-based food products to entrap water and prevent its exudation upon mechanical deformation (the so-called water holding) is important for the perception of juiciness (Northcutt, Foegeding, & Edens, 1994; Van Oeckel, Warnants, & Boucque, 1999) and for the release of tastants from food products. Understanding the structural origins that determine water holding is therefore essential to allow design of food products with controlled oral perception. Water holding of protein gels is generally determined by gel stiffness and gel microstructure. The relation between gel stiffness and water holding was found to be system specific and to be either positive or negative.(Chantrapornchai & McClements, 2002; Croguennec, Nau, & Brule, 2002; Furukawa & Ohta, 1982; Maltais, Remondetto, Gonzalez, & Subirade, 2005; Urbonaite, de Jongh, van der Linden, & Pouvreau, 2015; Urbonaite, de Jongh, van der Linden, & Pouvreau, 2014) Gels with a fine microstructure are reported to have a higher water holding capacity than more coarse gels.(Chantrapornchai & McClements, 2002; Croguennec et al., 2002; Handa, Takahashi, Kuroda, & Froning, 1998; Kitabatake, Shimizu, & Doi, 1988; Maltais et al., 2005; Pares, Saguer, Saurina, Sunol, & Carretero, 1998; Verheul & Roefs, 1998) However, protein networks with similar microstructure were found to differ in water holding due to the interplay between gel stiffness and microstructure.(Urbonaite et al., 2015, 2014).

To obtain understanding of the mechanism behind water holding, up to now information was retrieved on microstructure length scale in combination with macroscopic (mechanical) properties. The mechanism by which protein aggregates cluster and develop into space-filling networks is also expected to play a role in the microstructure and stiffness and thus in water holding. However, little is known about the relation between aggregate structure and water holding.

To obtain information on protein aggregate structure, scattering techniques such as light scattering and X-ray diffraction have been deployed. With the assumption of a fractal gel model, the fractal dimension (d_f) was studied in a number of food protein aggregates in solution.(Mehalebi, Nicolai, & Durand, 2008; Pouzot, Nicolai, Durand, & Benyahia, 2004; Weijers, Visschers, & Nicolai, 2004) The d_f directly relates to the compactness of aggregates, where a lower d_f represents a less compact structure.(Mehalebi et al., 2008;

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Pouzot et al., 2004; Weijers et al., 2004; Weitz, Huang, Lin, & Sung, 1985). Light scattering to elucidate aggregate structure and fractal dimensions is however only suitable for aggregates in solution. Therefore, fractal dimension of aggregates in gels were deduced from rheology or gel permeability.(Hagiwara, Kumagai, & Nakamura, 1998; Ikeda, Foegeding, & Hagiwara, 1999; Ould Eleya, Ko, & Gunasekaran., 2004; Verheul & Roefs, 1998; Verheul, Roefs, Mellema, & de Kruif, 1998) The fractal dimension as measured with rheology is higher in gels than in solution. This is reported to be due to the fact that the results of rheology measurements are determined not only by the fractal dimension, but also by longer range interactions (the aggregate superstructure) in the gel.(Hagiwara et al., 1998) Therefore, notwithstanding the higher measured d_f, the aggregate structure is reported to be similar in solution and gel.(Mehalebi, Nicolai, & Durand, 2008; Pouzot et al., 2004).

Besides applying rheology or gel permeability, spin echo small angle neutron scattering (SESANS) can be used to directly measure aggregate size and density in turbid protein gels. SESANS has been developed in material science where e.g. silica spheres in solution and aligned fibres in solid state were investigated.(Bouwman et al., 2004) The few studies in food-related systems dealt with droplet clusters of vegetable oil-in-water emulsions stabilized with whey protein (Bot, Duval, Duif, & Bouwman, 2007) and aggregation of casein micelles in yoghurt production.(Tromp & Bouwman, 2007) In a recent study it was shown that SESANS could also be applied to study the relative density of aggregates in a protein gel network.(Nieuwland et al., 2015).

This study aims at understanding how aggregate structure sets gel stiffness and gel microstructure and with that relate to water holding. A link between aggregate structure and macroscopic properties has not yet been made, with the exception of one study (Mine, 1996) that relates aggregate structure to gel elasticity. We have used ovalbumin as a model system, as its aggregation (Ianeselli et al., 2010; Koike, Takada, & Nemoto, 1998; Mine, 1995; Pouzot, Nicolai, Visschers, & Weijers, 2005; Sun & Hayakawa, 2002; Tani et al., 1995; Weijers, Visschers, & Nicolai, 2002; Weijers et al., 2004) and gelation (Croguennec et al., 2002; Doi & Kitabatake, 1989; Doi, 1993; Koike et al., 1998; Koike, Nemoto, & Doi, 1996; Mine, 2002; Sun & Hayakawa, 2002; Tani et al., 1995; Visschers & de Jongh, 2005) mechanisms are well-described. Information on aggregate size and density was obtained by light scattering, SESANS and scanning electron microscopy (SEM). Gel structure was visualized on nanometer scale and micrometer scale with respectively SEM and confocal laser scanning microscopy (CLSM). Furthermore, gel stiffness was determined.

2. Materials and methods

2.1. Materials

Albumin from chicken egg white (Grade III, > 90% proteins; Product number A5378) was purchased from Sigma–Aldrich (St Louis, MO, USA). 2-(N-morpholino)ethanesulfonic acid hydrate (MES hydrate) and sodium chloride were obtained from Sigma–Aldrich. Hydrochloric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany).

2.2. Gel preparation

MES buffer stock solution (200 mM) was added to an ovalbumin stock solution (13.3 wt% protein) in a 1:9 ratio to yield a protein solution with 12 wt% protein, pH 5.8, 6.3 or 6.8, a MES concentration of 20 mM and an ionic strength of 21 mM (set with NaCl). The protein solution was heated (30 min, 95 °C), cooled to room temperature and stored at 4 °C prior to use. Gels were analyzed by SESANS, and imaged by SEM and CLSM. Furthermore, the Young's moduli was determined from large deformation studies and water holding measurements were performed.

2.3. Light scattering (SLS and DLS)

For light scattering experiments, a concentration series of ovalbumin aggregates was prepared for all three pH values. The ovalbumin solutions (12 wt%, pH 5.8; 6.3; 6.8) and buffer solutions were filtered over a 0.1 μm filter (Sartorius stedim biotech, Goettingen, Germany) prior to dilution and heating. For pH 6.8 a concentration range of 5–25 g/L was prepared, for pH 6.3 a range of 0.05–5 g/L and for pH 5.8 a range of 0.1–2 g/L. After heating, the solutions were diluted if necessary to exclude interaction between particles and the aggregate solutions were filtered over a 5 μm filter (Millex SV, low protein binding, Merck millipore, Darmstadt, Germany) prior to the measurements.

Light scattering measurements were made using an ALV Compact Goniometer System with four detector units (ALV/CGS-4, Langen, Germany) and two ALV-5000/E multiple tau digital correlators. This setup allowed to perform simultaneously angular dependent static and dynamic light scattering. A Coherent Verdi V2 diode-pumped laser was used operating with vertically linearly polarized light with wavelength $\lambda = 532$ nm. The range of scattering wave vectors covered was $4.65 \times 10^{-3} < q < 3.09 \times 10^{-2}$ nm^{-1} ($q = 4\pi \cdot n_s \cdot \sin(\theta/2)/\lambda$, with n_s the refractive index of the solution and θ the angle of observation). The temperature of the cuvette holder was controlled by a thermostat bath to 20 ± 0.1 °C.

2.4. Data analysis SLS

The relative excess scattering intensity (I_r) was calculated as the measured intensity minus the solvent scattering divided by the scattering intensity of toluene at 20 °C. In dilute solutions I_r is related to the weight-average molar mass (M_w) and the structure factor ($S(q)$) of the solute.(Pouzot et al., 2004).

$S(q)$ may be written in terms of the z-average radius of gyration (R_{gz}) of the solute if $qR_{gz} < 1$:

$$S(q) = \left(1 + \frac{q^2 R_a^2}{3}\right)^{-1} \quad (1)$$

At higher concentrations $S(q)$ is the structure factor of the solution, and M_w and R_{gz} should be replaced by an apparent molar mass (M_a) and radius (R_a).

The q dependence of the scattering signal (I_r/HC) was determined for a range of protein concentrations at the different pH values. Extrapolation of I_r/HC to $q = 0$ yields an apparent molar mass, M_a, which is inversely proportional to the osmotic compressibility. The structure factor is determined by plotting

$$S(q) = I_r/HCM_a \quad (2)$$

as a function of qR_a. R_a was obtained by fitting the initial q dependence to equation (1).(Weijers et al., 2004)

2.5. Spin echo small angle neutron scattering (SESANS)

Ovalbumin gels for SESANS experiments were prepared as described above, but D_2O was used instead of H_2O.

SESANS experiments were carried out using the set-up as described by Rekveldt et al.(Rekveldt et al., 2005) with a neutron wavelength, λ, of 0.203 nm. With a spin-echo sequence changes

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