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Gelatin increases the coarseness of whey protein gels and impairs water exudation from the mixed gel at low temperatures

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ABSTRACT

To understand the origin of water holding of mixed protein gels, a study was performed on water exudation from mixed whey protein (WP)-gelatin gels upon applied pressure. Mixed gels were prepared with varying WP and gelatin concentration and gelatin type to obtain gels with a wide range of gel properties. Gels were characterized for their water holding (maximum of exuded water, A_{max}, and ease with which water can be exuded, k), gel coarseness (from CLSM image analysis) and gel stiffness (Young's modulus) at 20 and 40 °C, below and above the melting temperature of gelatin. Gelatin caused an increase in gel coarseness of the WP network, as induced by phase separation between WP and gelatin. The effect of gel coarseness and gel stiffness on A_{max} was found to be intertwined but above all, dictated by the gelatin concentration and gelatin network. At 20 °C, a transition point in gelatin concentration was observed above which stiffness surpassed coarseness in importance for A_{max}. Above this concentration, gelatin dominates the mechanical response of the mixed system. At 40 °C, when gelatin is melted, coarser and less stiff networks, as set by the WP network, lead to higher A_{max}. Tailoring of the coarseness and stiffness and therefore A_{max} and k, can be achieved by selective mixing in terms of protein concentrations, and type of gelatin. By varying gelatin type from A to B, altered phase behavior leads to gels with higher coarseness and lower stiffness but similar A_{max}.

1. Introduction

Most semi-solid food products are complex mixtures of hydrated biopolymers that, depending on the conditions (temperature, time, pH), form a gel in which water is entrapped by the matrix. Under applied pressure, e.g. during oral processing, water is exuded from the gel and the amount of exuded water affects sensory perception by release of tastants or juiciness. Therefore, understanding the origin that determines water holding (WH) in mixed biopolymer gels is essential to tailor oral perception of complex food products.

For gels made from globular proteins, the ability to hold water was found to be determined by the gel coarseness (Hermansson & Lucisano, 1982; Urbonaite, de Jongh, van der Linden & Pouvreau, 2015a) and gel stiffness (Urbonaite, de Jongh, van der Linden & Pouvreau, 2015b). Typically, coarseness sets the maximum amount of water exuded where stiffness affects water exudation kinetics. With regard to coarseness, upper and lower limiting length scales were defined for water exudation from protein gels. The (lower) length scale at which no water can be exuded from globular protein gels was reported to be generic and below 0.1 μm. The upper limiting length scale was found to be protein specific and, for example, for 14% whey protein was determined to be > 1.8 μm (Urbonaite et al., 2015a,b). The role of gel stiffness on water exudation was found to be gel morphology specific: stiffness was overruled by coarseness in fine gels but counteracted coarseness in water exudation kinetics from coarse gels (Urbonaite, van der Kaaij, et al., 2016).

For mixed biopolymer gels, WH has received little attention in literature. During gelation of biopolymer mixtures, i.e.

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protein–polysaccharide or protein–protein mixtures, (segregative) phase separation may occur depending on the type of biopolymers used. This partitioning was found to affect texture properties as shown for whey protein/κ-carrageenan gels (Çakir et al., 2012; Çakir & Foegeding, 2011; Çakir, Khan & Foegeding, 2012) and for acid induced gels of whey protein and different polysaccharides (van den Berg, et al., 2008; van den Berg, van Vliet, van der Linden, van Boekel & van de Velde, 2007a; van Den Berg, Van Vliet, van Der Linden, van Boekel & van De Velde, 2008). Enhanced gel coarseness, as induced by increasing the gellan gum concentration in mixed gels with whey protein, was related to higher serum release during oral processing causing wet or watery mouthfeel by sensory panelists (van den Berg, van Vliet, van der Linden, van Boekel & van de Velde, 2007b). Similar to protein–polysaccharide mixtures, segregative phase separation and therefore the formation of bicontinuous networks can also occur in mixtures of proteins. In mixtures of gelatin and globular proteins, both proteins are, at sufficiently high concentrations, able to form a gel, and depending on their ratio and whether gelatin or globular proteins are gelled first, different microstructures and rheological responses can be obtained (Ersch, ter Laak, van der Linden, Venema & Martin, 2015; Ersch, Meinders, et al., 2016; Walkenström & Hermansson, 1994, 1996, 1997; Ziegler & Rizvi, 1989; Ziegler, 1991). Although selective mixing of proteins can be used to obtain a wide range in gel hardness and stiffness (Ersch, ter Laak, et al., 2015), the release of serum upon applied pressure (= water exudation) has not been covered in literature yet. Only Pang, Deeth, Sharma, and Bansal (2015) report on the improvement of water holding by addition of gelatin to milk protein acid induced gels but detailed explanations on the role of gel properties were not provided.

The aim of this study is to understand how gelatin affects the water holding of whey protein gels, and more specifically the effect of gel coarseness and stiffness on water holding of mixed whey protein-gelatin gels. When gelatin concentration is increased at constant whey protein concentration, segregative phase separation is induced and hence enhanced heterogeneity on micrometer length scale (Ersch, Meinders, et al., 2016). In addition, gelatin may affect the gel stiffness of the whey protein gel.

To allow comparison of water holding of gels similar in coarseness but different in gel stiffness, gel properties were evaluated at 20 and 40 °C, below and above the coil-helix transition temperature for gelatin. This study on water holding leads to insights on whether coarseness or stiffness is more dominant in determining water holding characteristics of mixed protein gels allowing control and design of water exudation from protein gels and hence oral perception of complex foods by selective mixing of globular proteins and gelatin.

2. Material & methods

2.1. Materials

Whey protein (WP) isolate was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA) product name BIPRO (94% protein determined by Kjeldahl). Gelatin (type A and type B) were provided by Rousselot BVBA (Ghent, Belgium). Type A (from porcine skin) had a bloom number of 290, an isoelectric point (pI) around 8 and a protein content of 89.6% w/w; type B (from bovine bones) had a bloom number of 260, pI of around 5 and a protein content of 88.8% w/w (determined by manufacturer). Chemicals such as NaOH and HCl were of analytical grade and purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Methods

2.2.1. Preparation protein solutions and gels

Stock solutions of proteins were prepared with deionized water (Merck Millipore, Darmstadt, Germany, 18.2 MΩ cm) at pH 7.0 and ionic strength of 150 mM via addition of NaCl. Stock solutions were mixed to prepare samples with different protein concentrations and protein ratios. During sample handling temperature was kept at 40 °C, above the melting temperature of gelatin. The protein concentration was based on the available amount of water, given in grams of protein per gram of water ($g_{protein}/g_{water}$) as previously described and discussed (Ersch, 2015; Ersch, Meinders, et al., 2016). Gels were prepared by heating the protein mixtures in 20 ml syringes (20 mm in diameter), lubricated with paraffin oil, closed airtight to reduce air bubble formation, and heated for 30 min at 95 °C in a water bath. Subsequently the samples were cooled overnight at room temperature. Every sample was prepared in duplicate and three samples per syringe were obtained.

2.2.2. Water holding

Water holding of protein gels was measured using a centrifugation procedure adapted from Kocher and Foegeding (1993). A microcentrifuge filtration unit was composed of an inner spin tube and a 2 ml Eppendorf tube (Axygen Biosciences, Inc., Union City, USA). Gels were cut in 10 mm height and 4.8 mm diameter cylinders using a cork borer and carefully placed on the bottom of the spin tube. Centrifugation was performed at different g-force ranging from 100 g to 1400 g for 10 min at 20 and 40 °C. Exuded serum from the gel was collected at the bottom of the Eppendorf tube and weighed. Water holding (WH) was calculated as the percentage remaining water in the gel after centrifugation,

$$WH = \frac{W_T - W_g}{W_T} * 100\% \quad (1)$$

where W_T is the total amount of water in the sample and W_g is removed water from the sample at a given centrifugal force (g). Measurements were performed in duplicate, and both at 20 and 40 °C, below and above the coil to helix transition temperature for gelatin.

Obtained WH (%) values at various centrifugal forces were fitted using an exponential decay

$$WH = A_{max} \cdot e^{-k\,(g - g_{min})} + B \quad (2)$$

where A_{max} represents the maximum amount of water that can leave the system when a high force is applied, B (equals $100 - A_{max}$) refers to the amount of water remaining in the gel, g_{min} is the minimal g-force needed for exudation of water and k represents the coefficient reflecting how easy water leaves the system under applied force (Urbonaite et al., 2015b). Parameters A_{max}, k and g_{min} were obtained by least-squares fitting of the experimental data. At the conditions used in this study, for most protein gels, there appears to be no threshold for water exudation and g_{min} is zero. Only in a limited amount of samples, the fitting of Eq. (2) improved using g_{min} as an additional regression parameter indicating the existence of a threshold force. No physical interpretation to this parameter has, however thus far been proposed.

2.2.3. Gel stiffness

Gels were cut into cylindrical specimens (2 cm × 2 cm) using a steel wire. Gels were compressed in a single compression test to 90% of their initial height at a compression speed of 1 mm/s using a texture analyzer (TA-XT plus, Stable Micro Systems Itd., Godalming U.K.). The holder plates had a much larger diameter than the