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Mixing whey and soy proteins: Consequences for the gel mechanical response and water holding

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ABSTRACT

To design food products based on mixtures of proteins from animal and plant sources, understanding of how the structural and mechanical properties of mixed protein systems can benefit from selectively mixing is essential. Heat-induced gels were prepared from mixtures of whey proteins (WP) and soy proteins (SP) at different ratios and constant total protein concentration (10 w/w %). The effect of mixing on the aggregation phenomena (light scattering), mechanical response, and microstructure (CLSM, SEM) was investigated at ionic strengths of 0.1 and 0.3 M. Having similar gelation mechanisms, whey and soy proteins formed one network with both proteins contributing to the gel properties. With an increasing fraction of SP in the mixed protein gel, gel strength and stiffness decreased and water holding increased. In addition, a decrease in gel coarseness was observed, which was most significant for 0–3 w/w % SP fraction. A similar transition was also observed in the aggregation kinetics and aggregate size in dilute solutions.

1. Introduction

Proteins are desirable food ingredients owing to their nutritional value as well as their impact on texture. Recently, there has been a growing interest in designing food products based on mixtures of proteins, especially animal and plant proteins (Comfort & Howell, 2002; Ersch, ter Laak, van der Linden, Venema, & Martin, 2015; Grygorczyk, Alexander, & Corredig, 2013; Roesch & Corredig, 2005, 2006; Walkenström & Hermansson, 1997; Wong, Vasanthan, & Ozimek, 2013). By mixing of proteins, food products with novel texture and sensory attributes can be developed (Polyakov, Kireyeva, Grinberg, & Tolstoguzov, 1985). Furthermore, it is an excellent way of introducing plant proteins into existing formulations (Drake, Chen, Tamarapu, & Leenanon, 2000; Schmidt, Sistrunk, Richter, & Cornell, 1980). A partial or total replacement of animal proteins with plant proteins however, can have a negative effect on physical (texture) and sensory properties (Drake et al., 2000; Gökce & Gürsoy, 2003). Therefore, understanding of the structural and mechanical properties of protein mixtures containing plant proteins, and understanding how these properties can benefit from selectively mixing is essential.

Among plant proteins, soy protein (SP) is one of the most researched and readily available plant protein, and therefore commonly used as a partial replacer of traditional animal proteins (Comfort & Howell, 2002; Ersch et al., 2015; Roesch & Corredig, 2005, 2006). Typical examples for SP incorporated in mixed protein products include nutritious beverages, yogurts, coffee creamers, whipped toppings and yellow fat spreads (Chronakis & Kasapis, 1993; Kolar, Cho, & Watrous, 1979; Patil, Patel, Gupta, & Rajor, 1984). Although gelling behavior, microstructure and mechanical properties of SP in single systems under different physiochemical conditions were extensively studied (Kangll, Matsumura, & Mori, 1991; Puppo, Lupano, & Anon, 1995; Renkema, 2004; Renkema & van Vliet, 2002; Urbonaite, de Jongh, van der Linden, & Pouvreau, 2014), there are only a limited number of studies on the effects of SP in mixed protein systems. One such study includes sequential gelation of mixed systems of SP and gelatine (Ersch et al., 2015) that lead to the formulation of a wide variety in gel mechanical properties. Other studies deal with the simultaneous (acid-induced) gelation of SP-casein (Roesch &

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Corredig, 2006) or SP-sodium caseinate (Martin, de los Reyes Jimenez, & Pouvreau, 2016) and report on the impact of SP on the tailoring of mechanical properties of mixed protein gels. To date, very little research has been done on mixed globular–globular protein systems (Comfort & Howell, 2002; Ngarize, Adams, & Howell, 2004, 2005; Roesch & Corredig, 2005), and even fewer with plant proteins (Comfort & Howell, 2002; Roesch & Corredig, 2005). Comfort and Howell (2002) studied the heat induced gelation of a mixture of whey proteins and commercial soy proteins. They observed phase separation during heating at certain concentrations and ratios of WP:SP mixtures. This phase separation could be a result of the presence of large aggregates and possibly insoluble particles that are often present due to the processing history of the SP, and may affect the protein compatibility in mixtures. On the other hand, Roesch and Corredig (2005) used commercial SP with mild processing history and in contrast to phase separation they reported the formation of aggregates or complexes of WP and SP, depending on their ratio. The discrepancy between studies is likely due to the difference in the soy protein quality employed. Therefore, to minimize the effect of large aggregates present and possibly insoluble particles, in this study, we have used a self-extracted SP and avoided drying. Nevertheless, mechanical properties in terms of fracture behavior, or water holding of these types of globular protein mixtures have not been dealt with so far.

The aim of the present study is to understand the consequences of mixing WP and SP in different ratios on macroscopic properties like mechanical response and water holding of mixed heat-set gels while keeping the final protein concentration constant. The differences in the macroscopic properties are then related to the gel microstructure, network formation and aggregation phenomena.

2. Materials and methods

2.1. Materials

Defatted soybean flour was obtained from Cargill B.V (Amsterdam, The Netherlands). Whey protein isolate (BiPro) was obtained from Davisco (Le Sueur, Minnesota, US). 3-(N-morpholino)propanesulfonic acid (MOPS), sodium chloride and Rhodamine B were purchased from Sigma–Aldrich (Steinheim, Germany). Reagents were of analytical grade and were used without further purification. Demineralized water was used in the preparation of all samples and for cleaning of glassware.

2.2. Methods

2.2.1. Sample preparation

Soy proteins (SP) were extracted from defatted soybean flour by an isoelectric precipitation method described by Urbonaite et al. (2014). Extracted SP solution had a total protein content of 11.56 w/w % (as determined by Kjeldahl, calculated using N x 6.25). The solution was adjusted to a pH 7.0 by 5 M NaOH and stored at 4 °C in the presence of 0.02 w/w % sodium azide to prevent microbial growth until use. Whey protein (WP) stock solution was prepared by dissolving whey protein isolate powder in demineralized water containing 0.02 w/w % sodium azide to reach a final concentration of 14 w/w %. From the stock solutions of WP and SP, 7 samples were prepared, including single systems of WP and SP which are used as a reference and mixed fractions of WP:SP (9:1, 7:3, 5:5, 3:7 and 1:9) with total protein concentration 10 w/w % in a 20 mM MOPS buffer at a pH 7.0 containing 0.1 M or 0.3 M NaCl. Samples were stirred overnight at 4 °C and were equilibrated to room temperature by immersing in a water bath at 25 °C prior to measurements.

The two salt concentrations were chosen to obtain self-supporting gels and to be able to discern differences in gel microstructure, with increasing SP concentration. The concentration of 0.1 and 0.3 M NaCl were chosen based on our own experience with whey protein gels and available knowledge (Ako, Nicolai, Durand, & Brotons, 2009; Foegeding, Bowland, & Hardin, 1995; Langton & Hermansson, 1992; Urbonaite, van der Kaaij, et al., 2016).

2.2.2. Protein aggregation and aggregate characterization

Dilute solutions of different ratios of WP:SP were prepared by mixing of WP and/or SP to a final concentration of 1 w/w %, with 0.01 or 0.03 M NaCl. Prior to the mixing, all solutions were filtered with disposable syringe filters (Machery–Nagel Chromafil RC-20/25, pore size 0.2 μm and for WP also 0.1 μm). For turbidity measurements, the solutions were heated at 95 °C for 60 min. Absorbance was measured during heating at a wavelength of 400 nm using a Cary 4000 UV–vis spectrophotometer (Varian, The Netherlands BV). Quartz cuvettes with a path length of 1 cm were used. Size of protein aggregates were determined at 25 °C using zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire,UK). Protein aggregates were prepared by heating at 95 °C for 60 min (the same conditions as for the turbidity measurement). Measurements were performed 5 times per sample and z-average size, which represents the mean of weighted intensity distribution calculated from the cumulant fit (Koppel, 1972), was determined. For all the samples, polydispersity index (PDI, width of the intensity distribution) was in the range 0.2–0.5.

2.2.3. Small deformation rheology

Dynamic rheology measurements were performed in an Anton Paar MC502 rheometer (Graz, Austria) with a sand-blasted cup-bob geometry (CC17). Samples were subjected to an oscillatory shear at constant strain of 0.5% and a frequency of 1 Hz throughout the experiment. Samples were covered with a thin layer of paraffin oil to prevent evaporation. All the samples were heated from 20 °C to 95 °C and were kept at 95 °C for 30 min before cooling back to 20 °C. The temperature was then kept at 20 °C for 15 min. Heating and cooling rates were set at 5 °C/min. Storage modulus (G′) and loss modulus (G″) were determined. To investigate the contribution of glycinin to the gel network in mixed protein gels, the samples were heated to 85 °C (temperature below the denaturation temperature of glycinin) in a similar manner as described above. Strain sweep (in the range 1–100%) was performed at the end of heating – cooling cycle, at 20 °C.

2.2.4. Large deformation rheology

Gels for compression test were prepared by heating protein solutions in 20 mL syringes (of diameter 20 mm), lubricated with paraffin oil, at 95 °C for 30 min in the water bath. Afterward the samples were cooled and stored overnight at room temperature. Every sample was prepared in duplicate and six measurements were performed for each sample. Gel pieces were cut into cylinders of height and diameter 20 mm and compressed to 90% of their initial height at a constant deformation rate of 1 mm/s with a texture analyzer (TA-XT plus, Stable Micro Systems Ltd., Godalming, U.K.) equipped with a 50 kg load cell. Paraffin oil was applied to both sides of the gel for sufficient lubrication. True fracture stress, true fracture strain and Young's modulus were calculated as described by de Jong and van de Velde (2007).

2.2.5. Water holding

Water holding of protein gels was measured using a centrifugation procedure previously described by Urbonaite et al. (2014). A micro-centrifuge filtration unit was composed of an inner spin tube and a 2 mL Eppendorf tube (Axygen Biosciences, Inc., Union City, CA, USA) in which a protein gel cylinder (10 mm height and 4.8 mm diameter) was placed. The bottom of the spin tube was covered