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Unravelling of the water-binding capacity of cold-gelated whey protein microparticles

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ABSTRACT

Whey protein microparticles (CG MPs) were made with a cold gelation method. Without shearing or mixing during gelation spherical CG MPs were formed, while shearing or mixing resulted in smaller irregularly shaped CG MPs. The water-binding capacity of pellets (WBC-P) that were obtained after centrifuging CG MP dispersions was remarkably large (11–18 g water/g dry matter), though this value decreased at larger centrifugation speeds.

Microscopy images hinted at the presence of two water domains in the CG MP pellets: water within and between the CG MPs. The images also imply that the amount of water within the CG MPs was determined by the centrifugation speed. The amount of water between CG MPs seemed to be defined by the amount of particle-particle interactions that were present, as suggested by the effects of the particles' size and the inhibition of the disulphide bridge formation on the WBC-P.

Although microscopy images showed two water domains, only one main peak was found with time domain nuclear magnetic resonance. This was explained by water diffusion from one water domain to the other within the measuring time. This fast diffusion implies that the CG MPs had a relatively open structure. Overall, it was concluded that water-binding by CG MPs was affected by various factors and that a good understanding of the water-binding requires the use of a range of measurements.

1. Introduction

The water-binding capacity (WBC; "the ability of a protein sample present in excess water to bind water when subjected to an external force" (Peters et al., 2016)) of protein particles is an important property of the particles, giving insight into the ability of the particles to interact with water. Often, the WBC is determined by centrifuging a particle dispersion and calculating the WBC from the pellet weight. This results in one value that shows both the ability of the particles to swell and to include water between them. It is hypothesized that the amount of water bound by the particles is governed by their internal network, while water inclusion between particles is determined by particle–particle interactions. Time domain nuclear magnetic resonance (TD NMR) was shown to be a useful tool to better understand the WBC of protein particle pellets (WBC-P), because a distinction could be made between water inside the particles and water between the particles (Peters et al., 2016).

Here, we studied the WBC-P of pellets obtained from dispersions that contained whey protein microparticles made via cold gelation (CG MPs). Cold gelation is a two step-process. In the first step, whey proteins are turned into reactive aggregates by mildly heating a protein solution with a concentration below the critical gelation concentration (11–12%) (Anker, Stading, & Hermansson, 1999; Mahmoudi, Mehalebi, Nicolai, Durand, & Riaublanc, 2007); a low ionic strength; and a pH above or below its isoelectric point (pH 5.2–5.4) (Gentès, St-Gelais, & Turgeon, 2010; Turgeon & Beaulieu, 2001). This procedure results in the formation of protein aggregates with a hydrodynamic diameter of about 80 nm (Alting, Hamer, de Kruif, & Visschers, 2000; Alting et al., 2004). Repulsive forces between the aggregates prevent further aggregation and gelation, yielding a stable solution of these aggregates. The second step in this process is inducing gelation of the protein aggregate suspension, which can be accomplished by reducing the repulsive

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forces between the aggregates through lowering the pH. Often, glucono–delta–lactone (GDL) is used to form a regular gel by gradually decreasing the pH (Alting et al., 2000). The addition of a polysaccharide prior to addition of GDL results in phase separation during gelation, and the creation of a water-in-water emulsion with protein being the dispersed phase, which may result in the formation of CG MPs (De Jong, Klok, & van de Velde, 2009; De Jong & van de Velde, 2007; Van Riemsdijk, Snoeren, van der Goot, Boom, & Hamer 2011; Van Riemsdijk, Sprakel, van der Goot, & Hamer, 2010).

CG MPs are investigated because we hypothesize that CG MPs have the potential to swell to a greater extent than directly heat-gelated whey protein gels, which might lead to a larger and possibly distinct WBC-P range. This is suspected because a protein solution with a lower protein concentration was used during cold gelation than during heat gelation. Increased swelling of protein gels can be achieved by creating a gel with a reduced crosslink density (Gunasekaran, Ko, & Xiao, 2007), by utilizing a lower protein concentration, resulting in fewer covalent and non-covalent interactions between the proteins (Katsuta, Rector, & Kinsella, 1990).

In addition, Van Riemsdijk et al. (2011) reported that the degree of particle–particle interactions could be influenced via the size and shape of the CG MPs. They changed the size and shape of CG MPs by applying a simple shear flow during gelation. This resulted in spherical particles that became smaller at larger shear rates. An increase of the shear rate to either 54 s^{-1} or 108 s^{-1} resulted in irregularly shaped particles that were smaller than the spherical ones. A decrease in size caused an increase in viscosity of the CG MP dispersions. This was explained by increasing particle–particle interactions as the CG MPs became smaller and more irregular, resulting in more water inclusion between the particles and therefore a higher viscosity. Thus, by applying shear, the size and shape of CG MPs can be altered as well as the interaction with water, without changing the composition of the CG MPs. Another route Van Riemsdijk et al. (2011) utilized to control particle interaction was through the addition of a sulphhydryl blocking agent during gelation. It was found that the viscosity of the resulting dispersion of CG MPs was significantly lower than the viscosity of an untreated CG MP-dispersion.

In this study, pellets of CG MPs are investigated to gain a better understanding of their water-binding properties and the water-binding properties of CG MPs themselves. To investigate a range of WBC-Ps, the size and shape of CG MPs is altered by applying a shear or mixing force during gelation. From these pellets the water-binding properties are studied by measuring the weight of the CG MP pellet obtained after centrifugation, and examining the pellets with microscopy and TD NMR. In addition, the effect of particle–particle interactions on the WBC-P of CG MPs is studied by blocking the sulphhydryl groups of the proteins.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI; BiPRO) with lot no. JE 034-7-440 (Davisco Food International Inc., Le Sueur, MN) was used to prepare cold-gelated whey protein microparticles (CG MPs). WPI has a reported protein content of 97.7% on dry basis. In the preparation process of CG MPs, locust bean gum (LBG; Danisco Holland BV, The Netherlands) was used as polysaccharide, and glucono–delta–lactone (GDL; Sigma–Aldrich, Germany) was used as acidifier. *N*-ethylmaleimide (NEM; Fluka, The Netherlands) was used to block the sulphhydryl groups of the proteins, and Rhodamine B (Sigma–Aldrich, Germany) was used to visualize the proteins with confocal laser scanning microscopy (CLSM). Milli-Q water (resistivity of 18.2 MΩ cm at 25 °C, total oxidizable carbon <10 ppb, Merck Millipore, France) was used in all experiments, unless stated otherwise.

2.2. Methods

2.2.1. Preparation of cold-gelated whey protein microparticles

For the preparation of the CG MPs the same method was used as described by Van Riemsdijk et al. (2011), with some additions. First, WPI- and LBG stock solutions were prepared. To make a WPI stock solution, 9% w/w WPI was dissolved in water and mixed with an overhead mixer at room temperature for at least 2 h, resulting in a solution with a pH of about 6.9. The solution was then heated at 68.5 °C for 2.5 h to obtain reactive aggregates. The aggregate solution was cooled under tap water for 2–3 min, placed in ice water for 10 min, and further cooled at room temperature until it reached room temperature.

An LBG stock solution was made by dissolving 1% w/w LBG in water. This solution was heated in a water bath of 80 °C for 1 h. Then the solution was cooled in the same way as the WPI stock solution.

To prepare the CG MPs, the WPI stock solution; LBG stock solution; GDL; and water were added together, such that a 3% w/w WPI; 0.45% w/w LBG; and 0.2% w/w GDL solution was obtained, which was subsequently mixed. Part of this solution was placed in an in-house made Couette shear device, while another part was placed in a mixing device. The Couette shear device has two cylinders: a stationary inner- and a rotating outer cylinder with diameters of respectively 40 and 42 mm. The bottom of the shear device has a cone/plate geometry with an angle of 2.75°. To mix the solution during incubation, a 200 mL double-walled glass vessel was used with an anchor stirrer. The vessel was closed with a clasp to prevent evaporation. During acidification and gelation, the rotational speed of the cylinder and the stirrer was set at 0, 50, 100 or 300 rpm, corresponding to a shear rate in the Couette shear device of 0, 107, 215 or 644 s^{-1} respectively, while the devices were heated with water at 25 °C from a water bath. In addition, CG MPs were made in the Couette shear device at 25 rpm (54 s^{-1}). This was not done in the mixer due to a practical limitation: it cannot rotate at this speed. CG MPs formed at 0 rpm (static conditions) in the Couette shear device were called unsheared CG MPs, and CG MPs formed at 0 rpm in the mixing device were called unmixed CG MPs. Every kind of CG MP was made twice. The addition of GDL caused a gradual lowering of the pH of the solution of 7.1 (±0.0) before incubation to 5.2 (±0.0) after 16 h of incubation, for all samples.

After 16 h of incubation, the CG MP dispersion was diluted 1:1 and centrifuged at 2000*g* for 15 min to remove the LBG from the continuous phase as much as possible. The formed supernatant was discarded, while the pellet was re-dispersed in an amount of water equal to the amount of discarded supernatant, and centrifuged at 2000*g* for another 15 min. Part of the pellet was then re-dispersed, resulting in a 6.5% w/w pellet dispersion. This dispersion and the rest of the pellet were analyzed within one day.

To test the effect of blocking of the sulphhydryl groups on the size and shape of CG MPs, and the water-binding capacity of a pellet of these CG MPs (WBC-P), the same procedure was used as described above, with the difference that 30 min before incubation, NEM was mixed into the reactive protein aggregates solution (2.25 mM). During incubation, the solution was sheared at 50 rpm.

2.2.2. Size, shape and water-binding capacity of cold-gelated whey protein microparticles

CLSM was used to study the size and shape of the CG MPs present in the dispersions obtained after gelation, the 6.5% w/w pellet dispersions of CG MPs, and the pellets of CG MPs obtained