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Thermal aggregation of whey proteins under shear stress

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ABSTRACT

Processed food protein such as whey protein, used as hydrocolloid, is a possibility to impart specific structural and physical properties of food. The specific protein properties can be achieved by controlled denaturation or rather aggregation. The resulting aggregate structure and thereby the functional properties at the macroscopic scale depend strongly on the process conditions. Heating conditions, shear stress and material composition, determine the reaction kinetics as well as the resulting particle size distribution and the structure of the aggregates. These conditions often cannot be investigated separately in industrial processes. Therefore, the impact of shear rate, heating time and protein concentration on the particle characteristics was investigated by a rotational rheometer at 80 °C. By increasing the protein concentration (from 5 to 30% w/w), smaller more compact and stable aggregates were produced. This is due to the higher viscosity and the higher shear stress. The aggregates appearance changes from long and crystalline like structure to a spherical shape. The influence of shear rate is dependent on protein concentration. In suspension with 5% protein the aggregate size initially increases with increasing shear rates because of the predominant effect of increasing number of collisions; and decreases subsequently due to limitation of the particle growth. At high concentrations the size of the aggregates decreases with the shear rate, because of increasing shear stress. The size of whey protein aggregates can thus be regulated by the applied shear rate during processing.

1. Introduction

Whey proteins are often heat treated to change their physical and functional characteristics in food industry. The variation whey protein properties can be achieved by controlled denaturation. The principle mechanism of the irreversible denaturation reaction is a two-step reaction. In the first step the whey proteins unfold during heating and in the second step the unfolded protein molecules aggregate mainly by disulfide bonds and hydrophobic interactions. Processing conditions, such as temperature, heating time and shear stress determine the reaction kinetics as well as the size and the structure of the aggregates (Havea, Singh, & Creamer, 2001; Tolkach & Kulozik, 2007; Zuniga, Tolkach, Kulozik, & Aguilera, 2010). Depending on the properties of the aggregates they can be used as food hydrocolloids for different applications: as fat replacer in food products (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004), as stabilizer in foams, as functional ingredient for viscosity and structure modification in many food products (Çakır-Fuller, 2015; Damodaran and Paraf, 1997) or to enhance the yield of cheese (Hinrichs, 2001). The knowledge about the unfolding and aggregation of whey proteins during concurrent heating and shearing is also fundamental for the fouling mechanism in heat exchanger during the manufacturing of whey protein concentrates and powders (Davies, Henstridge, Gillham, & Wilson, 1997; Simmons, Jayaraman, & Fryer, 2007).

Whey proteins behavior during concurrent thermal and mechanical treatment was studied by Steventon (1992), Erabit, Flick, and Alvarez (2014) and Simmons et al. (2007) using a couette apparatus. By Spiegel and Huss (2002) with a scraped surface heat exchanger. Paquin, Lebeuf, Richard, and Kalab (1992) and Dissanayake and Vasiljevic (2009) used a high pressure system, but in this case heating and shearing was not applied at the same time. Various factors such as heating conditions, shear stress, protein concentration, pH, Calcium etc. affecting the aggregation of whey proteins have been already identified (Donovan & Mulvihill, 1987; Onwulata, Phillips, Tunick, Qi, & Cooke, 2010; Plock, Spiegel, & Kessler, 1998; Quéguiner, Dumay, Salou-Cavalier, & Cheftel,

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1992b; Steventon, 1992). This study focused on protein concentration, heating time and shear rate.

Protein concentration: The reaction kinetics strongly depend on the protein concentration. The higher the concentration the faster is the total reaction (Kessler & Beyer, 1991; Spiegel & Huss, 2002; Verheul, Roefs, Kruif, & de, 1998; Wolz & Kulozik, 2015). Aggregate size is also influenced by the protein concentration. At low protein concentrations (<5–10%) an increase of concentration results in an increase of aggregate size under conditions without shear stress (Boulet, Britten, & Lamarche, 2000; Elofsson, Dejmek, & Paulsson, 1996; Hoffmann & Mil, 1997). At higher protein concentrations (>5–10%) and under shear stress a decrease of the aggregate size can be measured (Steventon, 1992). However, molecular crowding was reported in cell biology works as an effect in contrast to our experience in whey protein technology. Molecular crowding was reported to even stabilize proteins against external stress factors (Ellis, 2001; Mittal, Chowhan, & Singh, 2015). These contrary observations will be discussed in conjunction with the experimental results.

Heating: Heating time and temperature are one of the main process parameters influencing the temperature depending denaturation reaction. With increasing temperature and constant heating time the degree of denaturation increases. Using the Arrhenius approach for the concentration independent rate constant of the β-Lg denaturation, a two-step reaction could be identified. The bend temperature separates the Arrhenius plot into two parts. At temperatures below the bend temperature unfolding is the rate dominating step. At temperatures above the bend temperature the aggregation reaction is rate limiting (Wit, 2009; Wolz & Kulozik, 2015). Additionally, structure and size of aggregates built during heating are influenced by the temperature (Giroux, Houde, & Britten, 2010; Zuniga et al., 2010). In the unfolding limited temperature area, smaller and more porous aggregates are achieved. In the aggregation limited area, bigger and more rigid aggregates are formed (Spiegel, 1999).

Shear rate: Studies on the aggregation of whey proteins during concurrent heating and shearing show a decrease of the aggregate diameter with increasing shear stress (Cheftel, Kitagawa, & Quéguiner, 1992; Quéguiner, Dumay, Salou-Cavalier, & Cheftel, 1992a). But Steventon (1992) also ascertained an opposite tendency at short periods of heating. A possible reason for this is a reduced efficiency of the protein collision at high shear rates. In principle shearing can have different effects on the protein structure. 1. It is possible to denature proteins by mechanical forces, but for that to occur extremely high shear rates have to be applied (Thomas & Geer, 2011). 2. The aggregates can break up due to increasing shear stress. This can be attributed to three mechanisms: (i) Deformation and fragmentation caused by pressure fluctuation in fluid flows. (ii) Erosion, i.e. abrasion of primary aggregates from the particle surface (iii) Fragmentation of the aggregates in large fragments caused by the mechanisms (i) and (ii) (Steventon, 1992; Taylor & Fryer, 1994). 3. Due to the enforced rate of collision during shearing, an increased aggregation can be achieved. The growth of the aggregates is first induced by the Brownian motion (thermal motion) and is independent of shear stress. This collision of particles causes the formation of primary aggregates. If the primary aggregates are large enough (0.25–1 μm), the particle motion is influenced by fluid flow and is hydrodynamically shear controlled. In consequence, the number of collisions between primary aggregates increase. The probability of particle interaction increase and the aggregation is intensified (Ker & Toledo, 1992; Simmons et al., 2007; Taylor & Fryer, 1994).

The frequency of collisions and thereby the probability of aggregation can be mathematically described by the coagulation theory of Smoluchowski (1916). As a simplification all particles are assumed to be spherical. The theory is only valid for laminar shear flow. The number of collisions J_1 per volume and time is limited by the Brownian motion and thereby by diffusion in the first step (perikinetic aggregation).

$$J_1 = \frac{4 \cdot k_B \cdot T \cdot N^2}{3 \cdot \eta_k} \quad (1)$$

With the constant of Boltzmann k_B, the absolute temperature T, the number of particles per volume unit N and the viscosity of the continuous phase η_k. Thus, in this case the aggregation is independent of the particle size and only depends on the particle number. In the second step of the aggregation process, the number of collisions strongly depends on the particle size. Shear stress promotes the probability of collision and thereby the aggregation of the whey proteins (orthokinetic aggregation). The number of collisions in this second step J_2 can be described by eq. (2).

$$J_2 = \frac{2 \cdot d^3 \cdot N^2 \cdot \dot{\gamma}}{3} \quad (2)$$

The number of collisions J_2 depends on the particle diameter d, the number of particles N and the shear rate γ (Walstra, Jenness, & Badings, 1984). If Brownian motion or hydrodynamic shear forces are dominant they can be described by the ratio between the numbers of collisions of both steps (J_2/J_1). J_2/J_1 means number of collisions induced by shear proportional to the number of collisions induced by thermal diffusion (Fischer, Pollard, Erni, Marti, & Padar, 2009). Next to the frequency of collision also the intermolecular bonds can only be created, if the energy of the collision is high enough to overcome the shear stress of the fluid. Additionally, the particles must face each other correctly and the kinetic energy must be sufficient. At higher shear rates the encounter time between particles is also reduced. Hence, under these conditions there is insufficient contact time for successful primary particle attachment and thus these particles are more likely to remain as individual particles in the submicron range (Steventon, 1992; Zumaeta, Byrne, & Fitzpatrick, 2006).

The mentioned studies already gave deeper insights to control aggregate size and structure during the shear process. While most research has focused on low protein concentration (<10% (w/w)), rather little attention has been paid to higher protein concentration. Concentrates with high protein contents are generated or applied in various processes. The influence of process conditions during shear treatment has not been investigated so far at protein concentrations as high as up to 30%, which is of relevance for evaporation processes and extrusion cooking.

Therefore, the objective of the current study was to investigate the structure formation during thermal aggregation of whey proteins under shear stress as a function of protein concentration, heating time and shear stress with a particular interest at high protein concentrations.

2. Material and methods

2.1. Preparation of protein solutions

Whey protein concentrate (WPC80, GermanProt, Sachsenmilch, Leppersdorf, Germany) with a protein concentration of 80% was diluted in deionized water to obtain solutions with protein concentrations of 5%, 10%, 20% and 30% (w/w). Thus, the relation between protein and ions was constant. For this reason the ionic strength and the pH was not adjusted. The pH changes slightly from pH7.0 for 5% protein content up to pH6.7 for 30% protein concentration. Analysis of the main the components in the WPC80 yielded