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Preparation, structure and stability of sodium caseinate and gelatin micro-particles

Alev Emine Ince Coskun, Dilek Sağlam, Paul Venema, Erik van der Linden, Elke Scholten*

Physics and Physical Chemistry of Foods, Department of Agrotechnology and Food Sciences, Wageningen University, P.O. Box 17 6700AA, Wageningen, The Netherlands

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ABSTRACT

Protein particles are promising candidates for texturing food products and can be produced in several ways. Here, we produced protein particles using a two-step emulsification method. This method is suitable to change the size of the particles and to control the protein concentration inside the particles. In this study, we prepared protein particles from two different protein sources, sodium caseinate (NaCas) and gelatin, that are gelled by acidification and cooling, respectively. The size and the internal protein concentration of the particles, their stability against heating and pH changes were studied. Although similar emulsification conditions were used to prepare the particles, NaCas particles were found to be 10 times smaller (average diameter 400 nm) than the gelatin particles (average diameter 4 μm). The internal protein concentration of the NaCas particles (16.8% w/w) is approximately twice as high compared to that of gelatin particles (7.6% w/w) (using an initial protein concentration of the solution of 10% (w/w)). The NaCas particle dispersions were found to be stable between pH 3 and pH 4. The particles disintegrated at pH values further away from the iso-electric point. Upon heating the dispersions at 90 °C, the NaCas particles were shown to be heat stable. Dispersions of gelatin particles were stable against aggregation at all pH values studied, except at pH 6, while the particles melted above 40 °C. Swelling of both particles was observed for both acidic and alkaline pH values. We conclude that emulsification method is robust for different protein sources used.

1. Introduction

Recently there is an increasing interest in the formulation of protein particles (Donato, Schmitt, Bovetto, & Rouvet, 2009; Nicolai & Durand, 2013; Phan-Xuan et al., 2013; Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011; Schmitt, Bovay, Vuilliomenet, Rouvet, & Bovetto, 2011; Zhang & Zhong, 2010). Several properties of food systems can be influenced by the addition of protein particles with different size, morphology and internal protein concentrations. The addition of protein particles can be used for texturing food products, e.g. to control the hardness of a solid product or the rheological behaviour of a liquid product (Purwanti, Peters, & van der Goot, 2013).

To prepare protein particles, several methods are described in literature, such as heat-induced gelation, pH-induced gelation, micro-particulation and micro-emulsification. The most commonly studied method involves heat-induced aggregation of globular protein solutions close to the iso-electric point (Donato et al., 2009; Le Bon, Durand, & Nicolai, 2002; Mehalebi, Nicolai, & Durand, 2008; Moitzi et al., 2011; Nicolai & Durand, 2013; Schmitt et al., 2011). In these studies, pH adjustment is followed by heating above the denaturation temperature of the protein, which induces the aggregation of protein and thereby create protein particles with diameters of a few hundred nm. Heat-induced gelation of whey protein creates a protein network by the formation of hydrogen bonds, hydrophobic interactions and di-sulfide bonds. The di-sulfide bonds are mostly responsible for irreversible aggregation at pH values away from the iso-electric point of the protein (Nicolai & Durand, 2013; Schmitt et al., 2011). To control the size of the protein aggregates, shear is applied during the heat-induced denaturation. This method is often referred to as micro-particulation (Dissanayake & Vasiljevic, 2009; Lieske & Konrad, 1994; Singhal, Gupta, & Kulkarni, 1991). Using different conditions for blending or shearing, a broad range of particle size distribution can be achieved, typically ranging from 0.1 μm to 10 μm.

* Corresponding author. Tel.: +31 317 482288.
E-mail addresses: alev.ince@wur.nl (A.E. Ince Coskun), dilek.bsaglam@gmail.com (D. Sağlam), paul.venema@wur.nl (P. Venema), erik.vanderlinden@wur.nl (E. van der Linden), elke.scholten@wur.nl (E. Scholten).

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To increase the range of pH values at which the formation of protein particles occurs, salt has been used to control the aggregation process (Ako, Durand, Nicolai, & Becu, 2009; Ako, Nicolai, Durand, & Brotons, 2009; Nicolai & Durand, 2013; Phan-Xuan et al., 2013). Above a critical salt concentration, a decreased electrostatic repulsion between the proteins has been shown to result in more extensive heat-induced aggregation of smaller protein aggregates, which is accompanied by micro-phase separation, leading to the formation of dense protein domains with a specific size of a few microns. The microstructure of these final particles can be tuned by varying temperature, heating time, protein concentration, pH and ionic strength (Durand, Gimel, & Nicolai, 2002; Nicolai & Durand, 2013). The dense protein domains, which can be harvested as protein particles, can also be obtained by the addition of polysaccharides. Addition of kappa-carrageenan to whey protein solutions has been shown to lead to phase separation into a protein-rich and a polysaccharide-rich phase. By gelation of the protein phase during the phase separation, gelled protein micro particles can be obtained (Durand et al., 2002).

Another method to obtain protein particles is via gelation of the protein in a confined geometry, such as in the micro-emulsification method used by Zhang and Zhong (2010). In this method, they prepared a protein solution in oil by emulsification, followed by heat-induced denaturation of the proteins. In one of their other studies, they used enzymatic crosslinking together with the micro-emulsification method to increase the heat stability of the whey protein isolate (WPI) gels (Zhang & Zhong, 2009).

Similar to the micro-emulsification method, a two-step emulsification method has been used to prepare dense protein particles (Sağlam et al., 2011). In this method, a WPI solution was first emulsified in oil and the gelation was induced by heating the emulsion at temperatures above the denaturation temperature of WPI. Afterwards the oil was removed and the particles were re-dispersed in an aqueous phase. Since the gelation takes place in a confined geometry, the diameter of particles can be better controlled. In addition, the microstructure of the particles can be influenced by adjusting the interactions between the proteins during gelation. When the particles are prepared at pH values close to the iso-electric point (pH 5.5), the particles are high in protein concentration and have a rough surface, whereas particles prepared at pH values further away from the iso-electric point (pH 6.8) are less dense and have a smoother surface. Both particles swell or shrink upon changing the pH of the continuous phase, however they maintained their internal network structure (Sağlam, Venema, de Vries, & van der Linden, 2013). This method, which combines emulsification and gelation, is expected to be also flexible relative to protein source and gelation method used. The flexibility relative to the protein source has received limited attention in the literature (Ding, Norton, Zhang, & Pacek, 2008; Xu, Li, Tao, Cui, & Xia, 2013). Therefore, we have used the emulsification method to prepare protein particles from protein sources other than globular proteins and using gelation mechanisms other than heat-induced aggregation. The proteins sodium caseinate (NaCas) and gelatin were chosen as examples for acid-induced and cold-set gelation, respectively. In this paper, we discuss the preparation, the pH- and heat-stability of both particles. In addition, the rheological properties of the protein particle dispersions as a function of shear rate are studied.

2. Materials and methods

2.1. Materials

Sodium caseinate (EM7-A9040445) was obtained from DMV International (Veghel, The Netherlands). The composition was stated as 86.3% protein, 6.1% maltodextrin and lactose, 3.8% ash, 2.9% moisture, and 0.8% fat by the manufacturer. The iso-electric point of NaCas is at pH 4.6 (Ruis, Venema, & van der Linden, 2007). Gelatin was kindly supplied by Rousselot (Gent, Belgium, 250 Bloom). The iso-electric point of gelatin (Type A) was slightly above pH 8 as stated by the manufacturer. Whey protein isolate (BiPro, Lot 198-1-420) was obtained from Davisco Foods International Inc. (Minnesota, U.S.A.). The composition of WPI was 90.25% protein, 0.95% fat, 2.85% ash, 0.95% lactose and 5% moisture. Polyglycerol polyricinoleate (PGPR) was purchased from Danisco DuPont (GRINDSTED® PGPR, Denmark). D-(+)-Gluconic acid δ-lactone (GDL) and Rhodamine B were supplied by Sigma–Aldrich (Steinheim, Germany). Sunflower oil (Reddy, NV Vandemoortele, Breda) was purchased from a local supermarket.

2.2. Preparation of protein particles

2.2.1. NaCas protein particle preparation

A two-step emulsification method (Sağlam et al., 2011) with small modifications was used to prepare NaCas particles. A 10% (w/w) NaCas solution was prepared by dissolving the powder in distilled water (initially at pH 7). The solution was stirred overnight at room temperature to allow for complete hydration and subsequently 3.8% (w/w) of glucono-δ-lactone (GDL) was added and stirred for 10 min. To prepare the oil phase, 2.5% (w/w) PGPR was dissolved in sunflower oil by stirring at room temperature for at least 2 h. A 20% (w/w) water-in-oil emulsion was prepared by pouring the NaCas solution slowly into the oil phase while mixing at 6000 RPM (10 min) using a high speed blender (Ultraturrax, T25 Digital, IKA Werke, Germany). To allow for acidification of the aqueous phase to pH 3.5 (through hydrolysis of GDL), the emulsion was kept at room temperature for 24 h. Subsequently, the emulsion was centrifuged (34,000 RCF, Avanti J-26 XP, Beckman Coulter, USA) for 1 h to remove the oil. The pellet was collected and washed using a 1% (w/w) WPI solution at pH 3.5 in a 1:2 (w/w) pellet to WPI solution ratio. To re-disperse the pellet, a high speed blender (8000 RPM for 5 min) was used and the dispersion was further homogenized (LabhoScope Homogeniser, Delta Instruments, Drachten, The Netherlands) at 100 bar for 6 cycles. The washing and centrifugation steps were repeated twice. Final dispersions of NaCas particles in 1% (w/w) WPI at pH 3.5 were prepared at the desired volume fractions. A 1% (w/w) WPI solution was used in both the washing and dispersing steps. WPI acts as a good emulsifier to remove residual oil in the washing steps and as a dispersing agent it retains the equilibrium in the dispersions (Sağlam, Venema, de Vries, van Aelst, & van der Linden, 2012).

2.2.2. Gelatin protein particle preparation

A gelatin solution was prepared by heating a 10% (w/w) gelatin solution (initially at pH 4.8) in a water bath at 60 °C for 30 min to allow for complete dissolution. The sunflower oil containing 2.5% (w/w) PGPR was also heated to 60 °C. A primary 20% (w/w) water-in-oil emulsion was prepared by pouring the gelatin solution into the oil phase, while mixing at 6600 RPM for 10 min. The temperature was kept at 60 °C during mixing. To allow for the gelation of gelatin, the emulsion was cooled to and kept at 4 °C for 1 h. After removal of oil by centrifugation (34,000 RCF at 10 °C for 1 h), the pellet containing the gelatin particles was re-dispersed by adding a 1% (w/w) WPI solution and using a high speed blender at 9000 RPM for 5 min and a homogenizer at 100 bar for 6 cycles. The dispersion was kept below 40 °C during the homogenization to prevent melting of gelatin particles. The washing and centrifugation steps were repeated twice. Final dispersions were prepared in a 1% (w/w) WPI solution (pH 7) at the desired volume fractions. A 1% (w/w)