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The effect of calcium on the composition and physical properties of whey protein particles prepared using emulsification

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ABSTRACT

Protein microparticles were formed through emulsification of 25% (w/w) whey protein isolate (WPI) solutions containing various concentrations of calcium (0.0–400.0 mM) in an oil phase stabilized by polyglycerol polyricinoleate (PGPR). The emulsions were heated (at 80 °C) and the microparticles subsequently re-dispersed in an aqueous phase. Light microscopy and scanning electron microscopy (SEM) images revealed that control particles and those prepared with 7.4 mM calcium were spherical and smooth. Particles prepared with 15.0 mM calcium gained an irregular, cauliflower-like structure, and at concentrations larger than 30.0 mM, shells formed and the particles were no longer spherical. These results describe, for the first time, the potential of modulating the properties of dense whey protein particles by using calcium, and may be used as structuring agents for the design of functional food matrices with increased protein and calcium content.

1. Introduction

The development of functional foods such as high protein drinks is of growing interest in the field of clinical nutrition, as protein malnutrition is a common problem for many (hospitalized) patients and elderly people, for whom the continuation of a balanced oral diet and intake of sufficient amounts of proteins is not possible (Beattie, Prach, Baxter, & Pennington, 2000; Potter, Roberts, McColl, & Reilly, 2001; Sullivan, Sun, & Walls, 1999). A low intake of proteins would lead to chronic wasting (loss of muscle mass) (Campbell & Leidy, 2007) and deterioration of bone health (Wolfe, Miller, & Miller, 2008). Foods high in protein would therefore be beneficial for such patients and the elderly.

Besides protein, calcium is also of nutritional importance, and may aid in the prevention of loss in bone mass. The recommended daily intake of calcium for elderly people is 1200 mg/day, but often not more than two thirds is consumed (Guéguen & Pointillart, 2000). However, the design of products high in proteins and calcium is still a challenge, as processing, storage and temperature fluctuations often lead to undesirable textural attributes such as chalkiness, hardening and off-flavour taste (Drake, Chen, Tamarapu, & Leenanon, 2000; McMahon, Adams, & McManus, 2009).

Recently it has been shown that protein gelation in a confined space to form dense protein particles can be employed as means to design high protein foods (Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011; Sağlam, Venema, de Vries, & van der Linden, 2013). Protein particles of about 3 μm of diameter were obtained by heat-set gelation of a concentrated whey protein (25% w/w) solution emulsified in oil. In this work it was hypothesized that this may be a valid approach to design particles rich in both protein and calcium by incorporation of the calcium within the protein particles. The resulting protein particles can be designed with different microstructures. Changes in pH to values further from the iso-electric point of the proteins showed swelling of the particles upon re-dispersion in aqueous phase, due to their polyelectrolyte nature (Sağlam et al., 2013). Changes in microstructure affect the permeability of the protein network and may cause changes in the diffusivity of molecules encapsulated within (Sağlam, Venema, de Vries, van Aelst, & van der Linden, 2012; Sağlam et al., 2013). The design of protein particles containing calcium may lead to similar structure changes and therefore be promising for development of functional foods with tailored controlled release of microencapsulated drugs or (bioactive) compounds. However, the effect of calcium on the preparation of such protein particles has yet to be evaluated.

It is known that calcium can affect the properties of whey protein gels, such as rheological properties and water holding capacity (Nicolai, Britten, & Schmitt, 2011). The presence of calcium induces

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progressive aggregation of whey protein, and promotes gelation upon heating, thereby affecting the protein network structure (Phan-Xuan et al., 2013, 2014). Aggregation of the proteins in the presence of positively-charged calcium is a result of the decreased electrostatic repulsion between positively charged proteins or of the increased electrostatic attraction for negatively-charged protein to form neutral protein–calcium complexes (Chi, Krishnan, Randolph, & Carpenter, 2003). At low calcium levels, the protein aggregation leads to the formation of a more fine stranded network (Mulvihill & Kinsella, 1988), while at higher calcium levels a more course stranded network is formed. Furthermore, denaturation and aggregation of the proteins in the presence of calcium might be affected by confined spaces. Previous research has shown that interactions between proteins situated at an oil-water interface, can lead to the formation of different microstructures of the resulting protein network (Öhgren, Lorén, Altskär, & Hermansson, 2011). The incorporation of calcium within the protein solutions may affect both the aggregation of the proteins and the affinity for the interface, and thereby new microstructures might be obtained.

The aim of this research was to investigate the effect of calcium on the microstructure and physical properties of whey protein micro-particles prepared by emulsification. Different calcium concentrations may result in protein particles with different microstructures and accompanying physical properties, and may show new processing and nutritional functionalities.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, Fonterra, 895) was obtained from Caldic Int. (Mississagua, ON). The composition of WPI as stated by the manufacturer was 94% protein, 0.3% fat, 1.5% ash and 4.7% moisture. PGPR was obtained from Palsgaard (Palsgaard® 4150, Juelsminde, Denmark). Sunflower oil (Unico Inc., Concord, Canada) was obtained from the local supermarket. In all experiments, demineralized water (Milli-Q Ultrapure Water Purification Systems, Billerica, MA) was used.

2.2. Preparation of the dispersed and continuous phase

Whey protein isolate (WPI) solutions (28% w/w) were prepared by dispersing WPI powder in demineralized water. The solutions were stirred for 2 h at room temperature. Solutions were then diluted with demineralized water and/or a calcium chloride solution ($CaCl_2{\cdot}2\ H_20$) of either 1 M or 4 M to reach the desired calcium concentration. The solutions had a final protein concentration of 25% w/w.

A 1% (w/w) WPI solution was used as a washing liquid to remove the remainder of the oil, and the solution was prepared similar as described above.

PGPR (2.5% w/w) was dissolved in sunflower oil by stirring for at least for 2 h at room temperature, and the mixture was kept from light exposure to avoid oxidation.

2.3. Preparation of whey protein micro-particles

Whey protein micro-particles were prepared according to the method described by Sağlam et al. (2011), with some modifications. A water-in-oil (w/o) emulsion with a 30:70 ratio of aqueous phase to oil phase was prepared by adding a 25% (w/w) WPI solution to sunflower oil containing PGPR (2.5% w/w). Pre-emulsification was carried out with a high speed mixer (POLYTRON® System PT 1300 D, Kinematica AG, Switzerland) set at a speed of 6500 rpm for 5 min. The emulsification step was completed by passing the emulsion through a valve homogenizer (2 passes, Emulsiflex C5, Avestin, Ottawa, Canada), at low pressure (35 bar). The emulsion was then heated in an 80 °C water bath for 20 min and subsequently centrifuged (30,000*g*), Thermo Scientific Sorvall® WX Ultra 80) for 1 h to separate the particles from the oil phase. The pellet, which contained the particles, was re-dispersed in a 1% WPI solution with a 1:2 (w/w) ratio of pellet to solution, using the high speed mixer (Polytron) at a speed of 20,000 rpm for 10 min. The washing and dispersing step was repeated 3 times. As a final step, the dispersion was passed through the valve homogenizer (6 passes, 150 bar).

2.4. Protein and calcium ions determinations

Total protein content was determined by the Dumas method using Leco FP-528 (Leco Corp., St. Joseph, MI, USA) and a conversion factor of 6.38. EDTA (Leco Corp., St. Joseph, MI, USA) was used as a standard for calibration of the equipment.

Protein leakage from the particles during their preparation was measured by determining the protein content of the supernatant after centrifugation, using the Bio-Rad DC protein assay with albumin as a standard.

The concentration of total released calcium was determined using ion chromatography (Rahimi-Yazdi, Ferrer, & Corredig, 2010). Analysis was performed on the supernatant collected after centrifugation of protein particles at different stages of the production process. Analysis was also performed on the final particle dispersions and on their aqueous phase immediately after preparation and during storage (1 week at 4 °C). The chromatography system used consisted of an 861 Advanced Compact IC, 838 Advanced Sample Processor, 833 IC Liquid Handling Unit and ICNet version 2.3 SR5 Metrodata software (Metrohm AG, Switzerland). The column (Metrosep C4-250 packed with 7 μm silica gel, Metrohm AG, Switzerland) was kept at 30 °C. A standard curve was prepared using a standard calcium solution (1.000 g/L, Fluka) in nitric acid. Samples were diluted to calcium concentrations of 1–10 mg/L. 12.5 mL of each diluted sample was used for analysis. All measurements were done at least in duplicate.

2.5. Particle size measurements

The average apparent diameters of the droplets in the water-in-oil emulsions both before and after heating where measured using dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments, Worcestershire UK). To avoid multiple scattering, emulsions were diluted 30× in *n*-tetradecane (Sigma Aldrich, St Louis, MO, USA). Values of 2.0780 mPa s and 1.420 for viscosity and refractive index at 25 °C, respectively, were used to calculate the particle size. All particle size distributions reported are apparent diameters determined on the basis of volume frequency (d_{43}) which is calculated from the z-average, and are an average of at least 7 measurements.

The particle size distribution of the protein micro-particles was determined using light scattering (Mastersizer 2000, Malvern Instruments Ltd., UK) equipped with a small volume sample dispersion unit (Hydro 2000SM). The particles where added to the demineralized water in the dispersion unit. Stirring was set at 2800 rpm. The sample amount was chosen to obtain 10–20% obscuration during measurement. A 300 RF lens was used. The light source was a He–Ne laser with a wavelength of 632.8 nm. The particle size distribution was calculated based on the Mie theory, and an average value of 1.38 ± 0.01 was used as the refractive index of the particles, as determined by the Abbe refractometer. Unless differently indicated, sizes are reported as volume-averaged diameters (d_{43}) and are an average of at least 10 measurements.