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Structural changes in casein aggregates under frozen conditions affect the entrapment of hydrophobic materials and the digestibility of aggregates

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HIGHLIGHTS

- Freeze-drying a caseinate with β-carotene successfully entrapped this dispersion.
- The entrapped β-carotenes were spatially distributed in the dried powder.
- Freezing modified casein aggregate structures due to the interactions with ice.
- The structural change during freezing affected the encapsulation efficiencies.
- The structural change during digestion was dependent on the freezing conditions.

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ABSTRACT

Freeze-dried casein nanoparticles that could entrap β-carotene were produced after aging under frozen conditions. Structural changes that occurred during aging and simulated digestion were investigated. Freeze-dried specimens of casein particles prepared from sodium caseinate solutions containing dispersed β-carotene successfully entrapped this dispersion in the resulting freeze-dried powders. The entrapped β-carotenes were distributed between the surface (surface load) and interior (inner load) of these dried powders. Because of aging, the amount of the inner load decreased while the surface load simultaneously increased. The hydrophobicity of rehydrated casein particles indicated that a change, caused by the aging process, occurred in particle structure. These structural modifications increased the hydrophobicity in the dried specimens. When rehydrated, these hydrophobic surfaces reassociated with each other to cancel the net gain in hydrophobicity. SAXS measurements on freeze-thawed casein nanoparticles also suggested the formation of modified nano- and microstructures. These modified structures were formed by freezing and thawing, along with interactions between clusters or between clusters and ice. The kinetics of the proteolytic reactions of the freeze-thawed specimens in a simulated gastric fluid were measured. The degree of aggregation and processing conditions did not significantly affect the digestion kinetics of the casein clusters. The SAXS analyses, however, suggested that these conditions affected nano- and microstructure formation during digestion. When the aggregated casein clusters were exposed to the gastric conditions, the fractured clusters produced by the proteolytic reactions produced 2–3 times larger aggregates with hollow networks and rough surfaces created by the crosslinked casein clusters. Processing conditions, such as pH and aging time, likely affected these nano- and microstructure formations under gastric conditions. These results may provide exciting future research opportunities if the structural modifications can impact the bioavailability of entrapped materials.

1. Introduction

Protein-based nanoparticles are being recognized as potential delivery vehicles for nutraceutical and pharmaceutical materials (Chen et al., 2006; Hawkins et al., 2008). Nanoparticles designed to

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encapsulate food additives or supplements should protect the sensitive core materials during storage and consumption, then carry them to a designated position in the gastrointestinal tract, and release them at an appropriate rate to maximize bioavailability. These advanced features given by the bioencapsulation need to be designed such that GRAS food materials can be used and safety standards are met. In pursuit of this objective, the physicochemical processes involved in matrix formation are a crucial early step for optimizing bioencapsulation process. The process of formation (e.g. by gelation, complexation, or aggregation) of the matrix that entraps core materials and the characteristics of the chemical compounds that form the shell matrix must be elucidated in our research. From an engineering perspective, it is also important to develop a processing route for obtaining products with desirable features.

We have previously reported that a freezing step could be an interesting processing tool for controlling the properties of an encapsulated system (Nakagawa, 2013; Nakagawa and Nagao, 2012; Nakagawa et al., 2013; Sowasod et al., 2013; 2012). During freezing, the growth of ice crystals in an aqueous solution concentrates in the liquid phase. This low-temperature phase is called a cryoconcentrated (freeze-concentrated) phase. The concentration of solutes in the cryoconcentrate is controlled by the phase equilibrium. Freezing enables a highly concentrated microspace to form without the involvement of any other materials. In addition, the concentration of the microspace can be adjusted by controlling the temperature.

Gelatin and gum acacia form a complex coacervate under appropriate pH conditions, and this complexation can be used to entrap oil droplets (Schmitt and Turgeon, 2011; Turgeon et al., 2007). We previously applied the freezing step to a solution of gelatin and gum acacia, but at a pH insufficient to induce complexation; here the complexes were formed by freeze-concentration (Nakagawa, 2013; Nakagawa and Nagao, 2012). This earlier work suggested that the freezing process facilitated the production of core–shell type nanoparticles with an oil core. The conditions for release from the nanoparticle core could be tuned by changing the freezing conditions. Based on this concept, an attempt to produce core–shell nanoparticles was carried out with a ternary system of polysaccharides (chitosan, *kappa*-carrageenan, and carboxymethylcellulose sodium salt). At certain proportions of this polysaccharide mixture, a gel was formed upon freezing, even though no gel formed under ambient conditions (Nakagawa et al., 2013; Sowasod et al., 2013; 2012). This method seemed applicable to the production of core–shell nanoparticles simply by freeze-drying, or spray-drying via freeze-thawing. The freezing process tended to retard the release of soluble fractions in the oil phase, via the polysaccharide-complex coating layer, to the external solvent phase. The freezing rate and aging time were the most important parameters in this process (Nakagawa and Fujii, 2015).

Sodium caseinate is widely used in food industry as a food ingredient. It is commercially produced from skim milk by acid precipitation and re-suspension of the precipitate by alkali conditions. The mean radius of rehydrated caseinate at neutral condition, measured by light scattering technique, has been reported in the range of 20–120 nm depending on the mean molecular weight (Lucey et al., 2000). Caseinate salts are known to form aggregated clusters under low pH conditions. The degree of aggregation is dependent on pH. The aggregation is visible at a pH of 5.0–6.0, and an obvious precipitate is seen at a pH of 4.5. These aggregated clusters were shown to be nanoparticles that can load lipophilic molecules through hydrophobic interactions. Casein-based nanoparticles could be a potential delivery vehicle for nutraceuticals and pharmaceuticals such as vitamins, polyphenols, and lipophilic drugs (Baracat et al., 2012; Elzoghby et al., 2013; Jarunglumlert and Nakagawa, 2013; Pan et al., 2013; Semo et al., 2007; Shapira et al., 2012; Zhang and Zhong, 2013). Aggregate formation upon freezing is of particular interest in the present work. A previously published study employed in situ small angle X-ray scattering (SAXS) measurements to confirm the formation of casein aggregates in a cryoconcentrated phase formed by freezing (Nakagawa and Kagemoto, 2013). This work also suggested that the freezing process affected the structural properties of the aggregates, and, as a result, it could alter the reaction rate of proteolysis. The sensitivity of protein-based nanoparticles to proteolytic enzymes in the gastrointestinal tract is a vitally important parameter because it could have a direct and/or indirect impact on the bioavailability of entrapped materials (Acosta, 2009; Livney, 2010; McClements and Xiao, 2014). If the freezing process could control both the encapsulation and digestive properties of the protein nanoparticles, it would be advantageous for producing functional delivery systems for loading sensitive nutraceutical and pharmaceutical materials. It would allow us to encapsulate, for example, heat-sensitive ingredients by applying low temperatures, and acid-sensitive ingredients by shielding them from low pH conditions.

In this study, casein-based nanoparticles were produced by a freezing process. The resulting structural changes were then investigated under simulated gastric conditions. A sodium caseinate solution mixed with β-carotene, whose pH was adjusted with acetic acid, was prepared and freeze-dried. The encapsulation efficiencies of β-carotene in the obtained dried powders were measured and compared to the surface hydrophobicities. Here, β-carotene was selected as a model core material that has low solubility in water, and expected to be associated to the hydrophobic part of caseins. The hydrophobility was an indicator of the entrapment ability of a particle for lipophilic substances, for instance, lower surface hydrophobicity could entrap larger amount of lipophilic materials in the inner part of a particle. The nano- and microstructures of the aggregated nanoparticles before and after freezing were analyzed by SAXS. The dried powders were added to samples of simulated gastric fluid (SGF) to assess the kinetics of the digestion reaction. SAXS was again used to evaluate the structural changes that occurred during simulated digestion.

2. Materials and methods

2.1. Materials

Sodium caseinate (SC) from bovine milk, β-carotene (type I, synthetic, ≥93%), and pepsin from porcine gastric mucosa (≥2500 units/mg protein) were purchased from Sigma-Aldrich (St Louis, MO, USA). Acetic acid, hydrochloric acid, hexane, acetone, sodium acetate, sodium tetrahydroborate, dimethyl sulfoxide (DMSO), 1-anilinonaphthalene-8-sulfonic acid (ANS), and trichloroacetic acid (TCA) were purchased from Wako Pure Chemical Industries, Osaka, Japan. All chemicals used in this work were analytical grade.

2.2. Preparation of aggregated casein clusters and freeze-dried specimens

A SC solution was prepared by adding 25 g of SC to 400 mL of distilled water (resulting in a 5 w/v% solution). Rehydration was accomplished by stirring overnight at ambient temperature (20–25 °C). β-Carotene in acetone was added to the SC solution at a 1:400 weight ratio (β-carotene:SC) with stirring in a thermostatic bath at 37 °C for 2 h. Two samples of the β-carotene-SC mixture were used for the preparation of aggregated casein clusters. The samples were adjusted to pH 6.0 and 5.5, using a pH meter (SK-620PH, Sato Keiryoki Mfg Co., Tokyo, Japan), by adding 1% (v/v)