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Gradual disaggregation of the casein micelle improves its emulsifying capacity and decreases the stability of dairy emulsions

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ABSTRACT

The casein micelle is a highly aggregated colloid consisting of phosphoproteins and minerals, in particular calcium and phosphate. Its properties are affected by physico-chemical changes which provide possibilities for the development of new casein aggregates (CAs) with novel functionalities. The aim of this study was to investigate the emulsifying and emulsion-stabilizing capacity of gradually demineralized CAs in model dairy emulsions. Tri sodium citrate (TSC) was used to remove calcium and inorganic phosphate from pure casein micelles in order to produce four suspensions of differently demineralized CAs. Two types of milkfat-in-suspension (30:70 v/v) emulsions were then prepared to study the emulsifying and emulsion-stabilizing capacity of these CAs separately. Casein micelles were progressively demineralized (from 24 to 81% calcium reduction) and dissociated with the increase in TSC concentration. Three distinct populations of particles (micelle-like aggregates, sodium caseinate-like aggregates and casein monomers) were present in every suspension in different proportions. The smaller CAs had better emulsifying capacity and similar surface activity according to interfacial studies. The state of aggregation of the CAs was thus the main factor that controlled their emulsifying capacity. However, the emulsions formed with these smaller aggregates were less stable against creaming and flocculation, but still resisted coalescence under our storage conditions (21 days at 50 °C). The properties of the interfacial casein layers did not depend on the aggregation state of the CAs used to form the emulsions. The differences in instability were attributed to the nature of the non-adsorbed CAs and storage conditions.

1. Introduction

The casein micelle consists of a highly aggregated particle of 150–200 nm diameter constituted of proteins (*i.e.* the four casein molecules αs_1, αs_2, β, κ), and minerals (mainly calcium phosphate) that ensure its colloidal stability (Dalgleish & Corredig, 2012; Holt & Horne, 1996; Holt, Carver, Ecroyd, & Thorn, 2013; Marchin, Putaux, Pignon, & Léonil, 2007; Schmidt & Payens, 1976; Trejo, Dokland, Jurat-Fuentes, & Harte, 2011; Walstra, 1990). The casein micelle has a key role in food products, especially dairy products, as it often contributes to their functional properties (*i.e.* the ability to form and/or stabilize networks such as gels, foams and emulsions, etc) (Foegeding & Davis, 2011).

The colloidal properties of the casein micelle (structure, composition, charge, hydration, etc) can be modified by controlling environmental factors such as pH, salt and chelating agent addition, temperature, etc (de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011; Gaucheron, 2004; Silva et al., 2013). However, only a few studies have described the link between the colloidal organization and the functional properties of the modified casein micelle (Broyard & Gaucheron, 2015). Of all their functional properties, the capacity of the casein micelle to emulsify and stabilize oil in water emulsions is of great interest for the food industry, especially for the dairy industry. Indeed, many dairy products are edible emulsions (*e.g.* cream and ice-cream, infant formulae, etc) (Barbosa-Cánovas, Kokini, Ma, & Ibarz, 1996; Guzey & McClements,

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2006).

Emulsions consist of mixtures of two immiscible liquids (such as oil and water), one of the liquids being dispersed as droplets in the other (McClements, 2005). These systems are thermodynamically unstable. The two phases will separate as a result of creaming, flocculation (agglomeration) and/or coarsening (fusion by coalescence or Oswald ripening) of the droplets. It is crucial to control both their formation and their stability during manufacture and storage to ensure the final quality of food emulsions.One way to improve the formation and the stability of emulsions is to use emulsifying agents that adsorb at the oil-water interface and lower its tension. This results in the formation of smaller droplets that are less prone to creaming. The adsorbed layer formed by the emulsifying agents at the droplet surface can also protect the emulsion against flocculation and coalescence. Emulsifying agents can be assessed according to two main characteristics: their ability to facilitate the blending of the emulsion phases (*i.e.* emulsifying capacity) and their ability to stabilize the emulsion (*i.e.* emulsion-stabilizing capacity). Caseins are known to adsorb at the interface, either in individual or aggregated form (Dickinson, 1999), and are therefore able to fulfill the role of emulsifying agent.

The emulsifying and stabilizing capacity of caseins is associated with their chemical nature and conformation at the interface and also depend on their aggregation state. Poorly aggregated casein systems such as sodium caseinate (30–50 nm diameter – formed by extreme acid demineralization of native casein micelle) (Pitkowski, Durand, & Nicolai, 2008) have enhanced emulsifying properties but are less effective for the stabilization of emulsions than highly aggregated casein micelles (Courthaudon et al., 1999; Mulvihill & Murphy, 1991). However, little information is available on the emulsifying properties of the intermediate aggregation states of casein micelles. Ye (2011) contributed to this information by studying different milk protein concentrates (MPCs) containing both casein and whey proteins as well as lactose in soya oil-based emulsions. Demineralization of the MPCs was induced by cation exchange but did not control the diffusible phase.

The aim of our study was to investigate the effects of the gradual disaggregation of pure casein micelles on their colloidal properties and on their emulsifying and stabilizing capacity in model dairy emulsions. Tri sodium citrate (TSC), a calcium chelating salt, was used to remove calcium and inorganic phosphate from the casein micelle and to produce four suspensions of differently demineralized casein aggregates (CAs). Dialysis was performed on each suspension to control their diffusible phases. The CAs in these suspensions were characterized physico-chemically and used to form two types of emulsion to study their emulsifying and emulsion-stabilizing capacity separately. In addition, emulsions containing large droplets were produced to facilitate the creaming during storage and foster the appearance of flocculation and coalescence.

2. Materials and methods

2.1. Chemicals

All chemicals used for this study, hydrochloric acid (HCl) and tri sodium citrate (TSC) (Carlo Erba reagent, Val de Reuil, France), sodium azide (NaN_3) (Riedek-de Haën, Seelze, Germany), sodium hydroxide (NaOH), sodium dodecyl sulfate (SDS), D(+)-saccharose (saccharose) (VWR international, Leuven, Belgium), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) (Sigma-Aldrich, St. Louis, USA), sodium di-hydrogen phosphate 2-hydrate ($NaH_2PO_4 \cdot 2H_2O$) (Panreac, Barcelona, Spain), Fast Green FCF (FG) (Sigma-Aldrich, St. Louis, USA) and Nile Red (NR) (5H-Benzo α-phenoxazine-5-one, 9-diethylamino, Sigma-Aldrich, St. Louis, USA) were of analytical grade.

2.2. Materials

Purified casein micelles were used to monitor our system. They were supplied by Gillot SAS (Saint Hilaire de Briouze, France) and obtained by microfiltration (0.1 μm pore size membrane) of raw skimmed milk followed by diafiltration against milli-Q water and spray dried according to Pierre, Fauquant, Le Graet, and Maubois (1992) and Schuck et al. (1994) on Bionov facilities (Rennes, France). The powder comprised 96% (w/w) proteins - especially caseins (97%) (w/w). Residual whey proteins (3%) (w/w), lactose and diffusible calcium were present in the powder.

Anhydrous milkfat (AMF, melting point 32 °C) was supplied by Corman (Limboug, Belgium).

2.3. Preparation of different CA suspensions

Casein micelle powder was suspended in milli-Q water at a concentration of 28 g kg^{-1} and NaN_3 (1.6 g kg^{-1}) was added for conservation (Fig. 1A). To ensure good resuspension of the powder, the suspension was stirred at 900 rpm for 6 h at 40 °C in a water bath and then for 16 h at room temperature. The rehydration of the casein micelle powder was checked by laser light diffraction as defined by Schuck, Dolivet, and Jeantet (2012). The results expressed in volume showed that more than 90% of the particles were of size of casein micelles (150 nm diameter). This suspension was used to prepare four CA suspensions (S1, S2, S3 and S4). In S2, S3 and S4 varying amounts of a stock solution of TSC (0.85 mol kg^{-1} in milli-Q water, pH 7.0) were added to reach final concentrations of 4, 13 and 34 mmol kg^{-1}, respectively. S1 was kept as a control

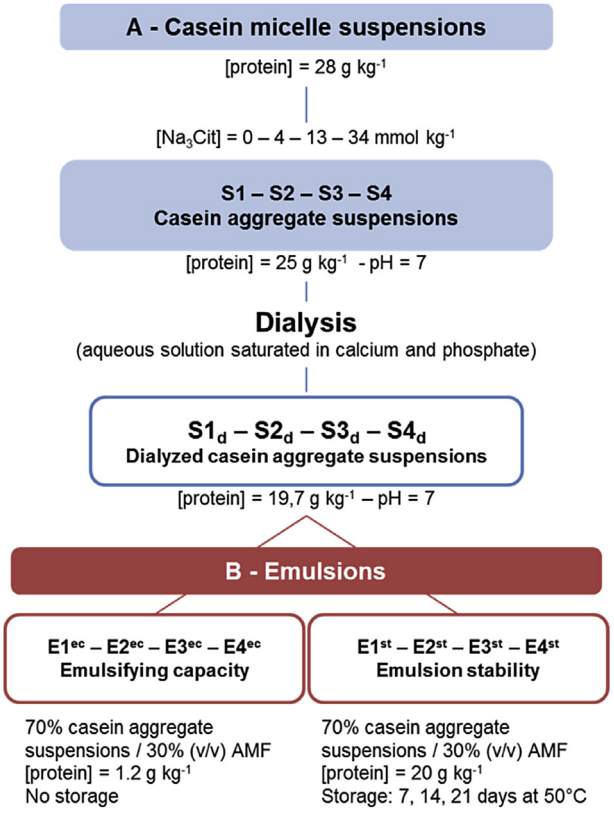

Fig. 1. Preparation of CA suspensions and emulsions. d, ec and st represent « dialyzed », « emulsifying capacity » and « stability », respectively.