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Edible foam based on Pickering effect of probiotic bacteria and milk proteins

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ABSTRACT

We report the preparation and characterization of aqueous Pickering foams using bio-particles constituted by lactic acid bacteria surface modified by oppositely charged milk proteins. Cell surface modification was shown by zeta potential measurements. Foams stabilized by bacterial Pickering bio-particles showed improved stability compared to purely milk protein stabilized foams. The stability of foams increased with the bacterial concentration whereas the foam volume (foamability) decreased. On the other hand, protein concentration was correlated with foamability but not with the foam stability. Optical and fluorescence microscopy revealed organized cell structures around and in between the air bubbles providing for an internal network that effectively stabilizes the foam. Therefore, entirely food grade stable foams can be produced by using modified health promoting bacterial cells and surface active milk proteins. Such Pickering systems can potentially be utilized in bottom up construction of more complex hierarchical food structures and further improve properties such as foam stability.

1. Introduction

Many foods are colloidal multiphase materials created out of building blocks like protein and fat particles, air bubbles and food polymers suspended in aqueous media. Such dispersions cover a variety of structures and textures that can be appreciated as food. A main concern of such colloidal dispersions used for industrially produced food is structural stability over the shelf life. Especially, foams are known to be notoriously unstable (Dickinson, 2010).

Many food foams are stabilized by surface active proteins. Protein-stabilized foams were investigated in terms of foamability and foam stability using surface active milk proteins such as flexible caseins (Marinova et al., 2009; Saint-Jalmes, Peugeot, Ferraz, & Langevin, 2005; Sánchez & Patino, 2005), globular whey protein isolates (Marinova et al., 2009), and barley protein isolates (Yalçın, Çelik, & Ibanoğlu, 2008).

Pickering stabilization of emulsions and foams is achieved by using partly hydrophobic solid particles smaller than the droplets or air bubbles. Such particles typically adsorb irreversibly to interfaces due to a large binding energy. In this way, they form a steric protection against coalescence hindering the reduction of interfacial area (Dickinson, 2010). Pickering particles of biological origin such as spores (Ballard & Bon, 2011; Binks, Clint, Mackenzie, Simcock, & Whitby, 2005), viruses (Kaur et al., 2009; Russell et al., 2005) and bacterial cells (Dorobantu, Yeung, Foght, & Gray, 2004; Wongkongkatep et al., 2012) were used to prepare emulsions. Inorganic solid particles such as aluminum oxide, calcium carbonate (Binks, Campbell, Mashinchi, & Piatko, 2015; Cui, Cui, Cui, Chen, & Binks, 2010; Gonzenbach, Studart, Tervoort, & Gauckler, 2006) were shown to stabilize aqueous foams in the presence of short amphiphilic molecules or surfactants. These foams have interesting properties in terms of ultra-stability against disproportionation, coalescence, drainage, and creaming thanks to the dense layer of surface modified particles formed at the air bubble surfaces.

Micron-sized, partly crystalline fat particles are important structuring agents, and traditional Pickering stabilizers of foam bubbles in foods such as ice cream and whipped cream. A major nutritional concern of such fat particles is their high energy density and the high content of saturated fatty acids in solid fat particles. From a colloidal point of view, bacteria can be thought of as particles with micron-range size just as fat particles in food. Bacteria can easily be produced by fermentation of simple carbohydrate and nitrogen sources. Some bacteria of the genus lacto bacillus possess

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probiotic properties (Illanes & Guerrero, 2016), and are as well a natural part of milk products such as yoghurt and cheese. The size, availability and heath properties of bacteria make them an interesting candidate for Pickering particles and structural element of food. Still only a few examples exist for Pickering stabilization of foams using bacterial cells. As an example in non-food applications, bacteria are found to stabilize foam in waste water systems (Heard, Harvey, Johnson, Wells, & Angove, 2008; Lam, Velikov, & Velev, 2014).

In this paper, we present a concept for stabilization of foam by bacterial cell of the species *Lactobacillus acidophilus* (La5). As such, the surface of cells is hydrophilic and not suitable for Pickering stabilization. We show that surface active proteins such as β-casein (βCas), or proteins mixtures such as sodium caseinate (NaCas), can be adsorbed to the bacterial surface and in this way stable foams can be produced where bacterial cell are associated to air surfaces.

2. Materials and methods

2.1. Materials and chemicals

βCas from bovine milk (BioUltra, ≥98%), chitosan (CH, extracted and/or purified from Pandalus borealis shell, low molecular weight, deacetylation ≥ 75%), acetic acid (glacial, ≥ 99.85%), citric acid, and 4′,6-Diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich, Steinheim, Germany. Tri-sodium citrate dihydrate was bought from Merck, Darmstadt, Germany. NaCas was obtained from DMV International, Veghel, The Netherlands. *Lactobacillus acidophilus* (La5) was a kind gift from Chr. Hansen, Copenhagen, Denmark. De Man, Rogosa, Sharpe (MRS) broth and atmosphere generation system (AnaeroGen sachets) were bought from Oxoid, Basingstoke, England and broth was sterilized in an autoclave (115 °C, 15 min). All the chemicals were used as received. MilliQ water (18.2 MΩcm at 25 °C) was used in all the experiments.

2.2. Preparation of buffer, protein and chitosan solutions

Desired amounts of NaCas and βCas were first dissolved in MilliQ water and then the corresponding amounts of citric acid and tri-sodium citrate dihydrate were added to reach a pH of 3.7 and total cirate/citric acid concentration of 10 mM (referred to as citrate buffer in the following text). Due to the buffering capacity of proteins, the actual amount of base form and acid form depended on the protein concentration. Both of protein solutions were prepared and used within 24 h in all the experiments. 1 mg/mL chitosan solution was prepared using 1% v/v glacial acetic acid at pH adjusted to 5.6 using 0.1 M HCl and filtered through a 0.2 μm pore size membrane. MilliQ water was adjusted to pH 5.6 (referred to as "MilliQ, pH 5.6") using 0.1 M HCl and used for washing and zeta-potential measurements of chitosan containing samples.

2.3. Dynamic light scattering and morphology of proteins

The particle size of the βCas solution at pH 3.7 was determined at 25 °C by dynamic light scattering (DLS), using a Zetasizer Nano ZSP (Malvern Instruments, Worcestershire, UK) and the data were expressed in terms of hydrodynamic diameter (d_H) (also known as z-diameter) and polydispersity index. Each measurement was presented with the means and the standard deviations from triplicated measurements.

The micro-structure of βCas solution was observed by transmission electron microscopy (TEM) using a typical procedure for milk protein micelles (Jung, Savin, Pouzot, & Schmitt, 2008; Liu, Yang, & Guo, 2013). 5 mg/mL βCas solution at pH 3.7 was centrifuged twice (4000 × g for 15 min at 4 °C) and filtered through 0.45 μm pore membrane prior to analysis. The filtered sample was gently vortexed, and 6 μL of the sample was placed on a form/carbon film grid (300 meshes, Axlab A/S, Denmark) for 3 min, followed by blotting with filter paper. The grid was subsequently washed once by dipping into MilliQ water and the excess water was blotted by filter paper. Staining was done by adding 1% (w/w) uranyl acetate to the grid for 1 min followed by blotting. In total, two grids were prepared with the same sample and examined with a Philips CM-100 electron microscope (PW 3020, Philips Electron Optics, Netherlands) operated at 80 kV. ImageJ software was used to process the obtained images.

2.4. Growth of L. acidophilus, dry biomass determination and enumeration of cells

L. acidophilus was grown in MRS media broth at 37 °C, aerobically for 16 h in a flask. Preculture was produced by propagating 100 μL culture from frozen stock for 24 h in 10 mL MRS broth. Pellets of 3 sizes were produced by incubating a predetermined amount of preculture (25, 250 or 500 μL) in a known amount of MRS broth (10, 50 or 100 mL) for 16 h. After growth, the cells were harvested by centrifugation at 4000 × g for 5 min at 4 °C. The supernatant was discarded, and the pellet was suspended in MilliQ water and centrifuged again. This washing procedure was repeated twice, and then the cell pellets were used for coating or for zeta-potential measurements. In order to quantify the dry weight of the pellets corresponding to incubation of 25, 250 or 500 μL preculture, the above procedure was used, but instead of harvesting and washing, filtering and washing them in filter was done in this case. Briefly, after incubation the suspension of bacteria was filtered through pre-weighted 0.2 μm pore size cellulose nitrate membrane filters and washed by passing through 2 times 10, 50 or 100 mL sterile MilliQ. The cell containing filters were oven-dried at 105 °C for several days until a stable weight was measured. The bacterial concentrations (given as dry biomass) corresponding to the pellet's resuspension in 10 mL foaming solution were 0.6 ± 0.1, 3.8 ± 0.2, 7.0 ± 0.3 mg/mL. The procedure was done in triplicates and was found to give reproducible pellets sizes.

2.5. Coating of bacteria

The deposition of macromolecules such as proteins or chitosan on bacteria was adapted from a previous report (Priya, Vijayalakshmi, & Raichur, 2011). This deposition is driven by electrostatics. Since both cells and proteins (βCas and NaCas) are negatively charged at neutral pH, the deposition of the protein coating was done at pH 3.7 just below the pI of the proteins (see Supporting Information, SI, Fig. S1) and above the neutrality point of the cells, which is approx. pH 2.5 (Hairden & Harris, 1953). After harvesting and washing the cell culture twice with MilliQ water by centrifuging at 4000 × g, 5 min, 4 °C, the pellet was suspended in either 10 mL βCas or NaCas solutions in citrate buffer, with protein concentrations ranging between 1 and 5 mg/mL, and the mixtures were incubated for 20 min at 37 °C and 225 rpm using an orbital shaker. For samples used for zeta potential determination, the excess proteins were removed by washing twice with citrate buffer or MilliQ, pH 5.6 and centrifugation at 4000 × g, 5 min, 4 °C whereas this washing step was avoided for samples which were used for foaming experiments. Chitosan coated cells were produced similarly by suspending bacterial pellets into 50 mL chitosan solution and using MilliQ, pH 5.6 for washing. The surface modification of cells was confirmed by zeta potential measurements.