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Formation and stability of single and bi-layer nanoemulsions using WPI and lactoferrin as interfacial coatings under different environmental conditions

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ABSTRACT

Electrostatic deposition of lactoferrin (LF) on the stability of nanoemulsions stabilized by whey protein isolate (WPI) was investigated as a function of pH (2–10) and LF concentration (0.25–5% w/w). The WPI-stabilized nanoemulsions prepared by emulsification and solvent evaporation were diluted with LF solutions to form a series of bi-layer emulsions at different pH levels. At pH 2 and 4, the bi-layer nanoemulsions were stable regardless of LF concentrations but underwent droplet aggregation at pH 5 near to the pI of WPI. At pH 6, electrostatic interactions were favored for the deposition of LF onto the WPI-coated droplets. However, the emulsions were prone to droplet aggregation at low concentrations of LF (0.5–1% w/w), presumably due to bridging flocculation. Thus, a sufficiently high level of LF was necessary to stabilize the emulsions. At pH 7, 8, 9 and 10, there was sufficient electrostatic attraction between the protein molecules to form WPI-LF bi-layer at the droplet surface and the formed emulsions were stable. Based on the results, a stable bi-layer nanoemulsion can be formed using 3% (w/w) LF at ≥ pH 6. The incorporation of LF in bi-layer emulsions improved the stability of single layer WPI-stabilized emulsions at pH near the pI of WPI (pH 4–6) and salt addition (0–90 mM $CaCl_2$) but the bi-layer emulsions were susceptible to droplet aggregation at temperatures above 60 °C. This study showed the overall potential benefits of having of a WPI-LF bi-layer to improve the stability of WPI-stabilized nanoemulsions.

1. Introduction

In recent years, it has been shown that WPI-stabilized nanoemulsions containing triglyceride oil droplets smaller than 100 nm in diameter were able to be prepared by emulsification and solvent evaporation method (Lee, Choi, Li, Decker, & McClements, 2011). Although nanoemulsions are kinetically stable, they are also susceptible to destabilization under certain environmental conditions, such as thermal treatment, pH changes and salt addition. It is therefore important to improve the environmental stability of these nanoemulsions. This can be achieved by controlling the characteristics of the interfacial coatings around the small oil droplets. Specifically, the interfacial layer can be designed to contain one or more biopolymers around oil droplets using a layer-by-layer (LBL) electrostatic deposition method (Guzey & McClements, 2006; McClements, 2010). This can be achieved by depositing oppositely charged biopolymers onto the surfaces of electrically charged oil droplets through electrostatic interactions. The procedure can be repeated several times to form multi-layer emulsions with two or more biopolymers.

Many previous studies have already reported the assembling of multi-layer emulsions by proteins and/or polysaccharides, e.g. β-lactoglobulin, caseinate, lactoferrin, sodium alginate and pectin (Fioramonti et al., 2015; Salminen & Weiss, 2013; Lesmes, Baudot, & McClements, 2010; Schmelz, Lesmes, Weiss, & McClements, 2011). However, few studies have been carried out on the formation of multi-layer emulsions at the nanometric scale using WPI and LF as interfacial coatings. In particular, LF is an iron-binding glycoprotein and contains a high concentration of positive charges that can interact with other milk proteins that are mostly negatively charged (Ye & Singh, 2007). This results from the pI of WPI and LF that differs being around pH 5 and 9, respectively (Salminen & Weiss, 2013; Shimazaki, 2000). It can therefore be expected that these two proteins are oppositely charged of each other and can interact electrostatically when the solution conditions are favourable such as at pH values between their pI. Furthermore, our previous studies have indicated that WPI and LF molecules can be deposited successively to form protein bi-layers at certain pH levels on surfaces when monitored by quartz crystal microbalance with dissipation (QCM-D) (Teo, Dimartino et al., 2016).

However, one of the challenges in producing a stable emulsion containing two or more layers is due to droplet aggregation

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(Guzey & McClements, 2006; Pallandre, Decker, & McClements, 2007). In this case, the biopolymer concentration used must be sufficient to cover the droplet surface to prevent bridging flocculation but not excessive to avoid depletion flocculation (Fioramonti et al., 2015; Guzey & McClements, 2006). Although several studies have been carried out to produce multi-layer emulsions and investigated on their physicochemical stability, few have investigated thoroughly on the effects of pH and biopolymer concentration to determine optimal conditions for making protein by-layer emulsions. These two variables have important implications on the physicochemical properties of the interfacial layer formed on the droplets of bi-layer emulsions.

The objective of this work was therefore to study the effects of pH and LF concentration in order to optimise the conditions to produce stable, bi-layer nanoemulsions containing WPI and LF. The effect of environmental stresses on the stability of nanoemulsions was also studied. This research aimed to provide insight into the design of nanoemulsions with customised interfacial structure for its potential application in encapsulation and delivery of high value food ingredients.

2. Materials and methods

2.1. Materials

WPI (Alacen™ 895, typical composition analysis 93.9% protein, 0.3% fat, 0.4% carbohydrate, 1.5% ash and 4.7% moisture) was supplied by Fonterra Co-operative Group Limited (Auckland, New Zealand). According to the manufacturer, the WPI powder is manufactured by ion exchange and ultra-filtration techniques and contains un-denatured, soluble whey protein. Bovine LF with an iron saturation of 13% and free from lipopolysaccharide (LPS) was obtained from Tatua Co-operative Dairy Company Limited (Morrinsville, New Zealand). The LF powder contained 97.7% protein (of which 93.5% was lactoferrin), 1.1% ash and 0.3% moisture. Corn oil was purchased from a local food supplier (Davis Trading Company, Palmerston North, New Zealand). Ultrapure water purified by treatment with a Milli-Q apparatus (Millipore Corp., Bedford, MA, USA) was used to prepare all the solutions used in all experimental works. Ethyl acetate (HPLC grade) was purchased from Fischer Scientific (New Jersey, USA). Hydrochloric acid (HCl), sodium hydroxide (NaOH), calcium chloride ($CaCl_2$) and sodium azide were of analytical grade and purchased from Thermo Fischer Scientific (Victoria, Australia) or BDH Chemicals (Poole, England).

2.2. Preparation of nanoemulsions

Two types of nanoemulsions consisting of a single layer or bi-layer structure were prepared and studied. The nanoemulsions were initially prepared with 2% (w/w) WPI solution using emulsification and solvent displacement method as described in Teo, Goh et al. (2016). Briefly, an organic phase (90% w/w ethyl acetate and 10% w/w corn oil) was mixed with an aqueous phase (2% w/w WPI) at a ratio of 10:90 and subjected to high pressure homogenization (M-110P, Microfluidics, Westwood, MA, USA) for 4 cycles at 80 MPa (12000 psi). Ethyl acetate was then removed from the nanoemulsion by rotatory evaporation (Buchi Rotavapor R-215, Vacuum Controller V850 and Heating Bath B-491, BUCHI Labortechnik AG, Flawil, Switzerland) under reduced pressure at 50 °C for 20 min. After which, the nanoemulsions were mixed with an equal amount of LF solution (1:1 ratio) at different concentrations (0–10% w/w) and pH values (2–10) to form a series of bi-layer emulsions. As control, the nanoemulsion referred to as a single layer emulsion was also mixed with Milli-Q water at 1:1 ratio under the same pH values as bi-layer emulsions. Prior to mixing, the pH of the WPI-stabilized nanoemulsions and LF solution were individually adjusted to the desired value using solutions of HCl or NaOH. The final concentration of LF presented in the bi-layer emulsions after mixing was 0, 0.25, 0.5, 0.75, 1, 2, 3, 4 and 5% (w/w). In this way, the single layer emulsion was stabilized by WPI while the bi-layer emulsion was formed by depositing LF molecules on the surfaces of WPI-coated droplets.

2.3. Effect of environmental conditions on nanoemulsions

The physical stability of the single layer and bi-layer emulsions to environmental stresses including the effects of temperature, pH and ionic strength was carried out. To study the effect of heat treatment on the emulsion stability, the emulsions were heated in water baths at different temperatures (30–90 °C) for 15 min. The emulsions were then immediately cooled to room temperature by placing them in a chilled ice-water bath. For pH stability, the emulsions were adjusted to different pH levels (2–10) using different concentrations of HCl or NaOH solutions. The addition of salt on the stability of emulsions was studied by mixing the emulsions with different concentrations of $CaCl_2$. The final concentration of $CaCl_2$ presented in the emulsions ranged from 0 to 90 mM.

2.4. Characterization of nanoemulsions

2.4.1. Particle size and morphology

The particle size and size distribution of nanoemulsions were measured by dynamic light scattering technique using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) equipped with a helium/neon laser at a wavelength of 633 nm and analyzed at a backscattering of 173°. The emulsion samples were measured without further dilution during the size measurement. The particle size results were reported as the Z-Average mean diameter. For those aggregated samples (particularly, single layer emulsions at pH 5 and salt concentrations above 5 mM $CaCl_2$), they were measured using a Mastersizer instrument (Mastersizer 2000 Hydro MU, Malvern Instruments Ltd, Worcestershire, UK). Their particle sizes were reported as the volume mean diameter, $D_{4,3} = \frac{\Sigma_i n_i d_i^4}{\Sigma_i n_i d_i^3}$ where n_i is the number of droplets with diameter d_i.

The microstructure of selected emulsions was examined by transmission electron microscopy (TEM). Prior to analysis, the emulsions were embedded in resin according to the method as described by Gallier, Tate and Singh (2013). After resin embedding, the samples were viewed using a transmission electron microscope (FEI Tecnai™ G2 Spirit BioTWIN, Czech Republic) operated at 60 kV and equipped with a LaB_6 filament. The microscopic images were captured with a 2 K × 2 K Veleta Camera (14 bit) (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

2.4.2. Zeta potential (ζ-potential)

The ζ-potential of nanoemulsions was measured using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) and disposable ζ-potential cells ("Size & Zeta" folded capillary cell; DTS1070). The samples were used without further dilution during the measurement.

2.5. Data analysis

All experimental works for sample preparation and analysis were carried out at least in duplicates and the results are reported as averages and standard deviations of the measurements.

3. Results and discussion

3.1. Effects of pH and lactoferrin concentration on the adsorption at the droplet surface of WPI nanoemulsions

The interfacial characteristics of emulsions can be modified by surface deposition of oppositely charged biopolymers to form bi-layer