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Impact of surface deposition of lactoferrin on physical and chemical stability of omega-3 rich lipid droplets stabilised by caseinate

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ABSTRACT

There is considerable interest in developing delivery systems to encapsulate and protect chemically labile lipophilic food components, such as omega-3 rich oils. In this study, multilayer emulsion-based delivery systems were prepared consisting of omega-3 rich oil droplets coated by either caseinate (Cas) or lactoferrin–caseinate (LF–Cas). Surface deposition of LF onto Cas-coated oil droplets was confirmed by ζ-potential measurements. Emulsions containing lactoferrin and caseinate had better physical stability to pH changes and salt addition (pH 3–7, 0–50 mM $CaCl_2$ at pH 7) than those containing only caseinate (pH 5–7, 0–2 mM $CaCl_2$ at pH 7). The addition of LF also retarded the formation of lipid oxidation markers (hydroperoxides and thiobarbituric acid reactive substances) in the emulsions. The ability of LF to enhance both the physical and chemical stability of protein-stabilised emulsions is useful for the fabrication of delivery systems designed for utilisation within the drug and food industries.

1. Introduction

A number of health benefits have been associated with an increased consumption of omega-3 rich oils, *e.g.,* reduced risks of heart disease, hypertension, depression and arthritis (Shahidi & Miraliakbari, 2004, 2005). However, their high susceptibility to lipid oxidation poses a major technological challenge to incorporating them into a wide variety of foods. An effective means of addressing this challenge is to utilise delivery systems that encapsulate, stabilise and deliver omega-3 rich oils. Emulsion-based delivery systems are particularly suited for this purpose because they can be fabricated from food-grade ingredients using common food processing operations (McClements, 2005a; McClements, Decker, & Weiss, 2007). In addition, there is considerable scope to design emulsion-based delivery systems to inhibit lipid oxidation processes (McClements & Decker, 2000). For example, the physicochemical properties of the interfacial coating surrounding oil droplets can be designed to retard lipid oxidation by controlling its composition, thickness or charge. This approach may be particularly effective because lipid oxidation reactions are believed to occur primarily at the oil–water interface, a location where lipophilic (*e.g.,* unsaturated triacylglycerols), amphiphilic (*e.g.,* lipid peroxides) and hydrophilic (*e.g.,* transition metals) reactants can come into close proximity (McClements & Decker, 2000). Previous studies have shown that various food proteins can retard lipid oxidation in oil-in-water emulsions, including whey protein, lactoferrin, caseinate, and soy protein (Hu, McClements, & Decker, 2003a, 2004; Huang, Satue-Gracia, Frankel, & German, 1999; McClements & Decker, 2000; Nielsen, Petersen, Meyer, Timm-Heinrich, & Jacobsen, 2004). These proteins are surface active molecules that can accumulate at oil–water interfaces and therefore be located at the site where lipid oxidation usually occurs. There are appreciable differences between the ability of different proteins to retard lipid oxidation and to physically stabilise emulsions. Ideally, one would like to utilise a single protein or a combination of different proteins that provides good physical and chemical stability of encapsulated lipids at the conditions existing within a particular food product (*e.g.,* pH, ionic composition, and temperature history).

One of the most versatile methods of controlling the physicochemical properties of protein coatings around colloidal particles is the layer-by-layer (LbL) electrostatic deposition approach (Caruso & Mohwald, 1999; Guzey & McClements, 2006, 2007; McClements et al., 2005). In this approach, the composition, structure, charge, and thickness of interfacial protein coatings can be controlled by depositing layers of charged protein molecules onto oppositely charged particle surfaces. This approach was recently utilised by Ye and co-workers to fabricate emulsions containing lipid droplets coated by β-lactoglobulin (β-Lg) and lactoferrin (LF) (Ye & Singh, 2006, 2007a, 2007b). LF is particularly suited for build-

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ing multi-layer protein coatings because it has an appreciably higher isoelectric point (pI) than most other commonly used food proteins: the pI of LF is around pH 8, whereas that of whey proteins, soy proteins and caseinate is around pH 5 (Tomita et al., 2009; Wakabayashi, Yamauchi, & Takase, 2006). At neutral pH, LF is therefore positively charged while these other proteins are negatively charged. Ye and co-workers used this principle to deposit cationic LF molecules onto anionic β-Lg-coated lipid droplets.

Previous studies have shown that adsorbed caseinate (Cas) coatings are more effective antioxidants than adsorbed β-Lg coatings, but that emulsions stabilised by caseinate have different physical stability under certain similar conditions, *e.g.,* salt concentrations and pH (Hu, McClements, & Decker, 2003a, 2003b). The current study therefore focused on developing oil-in-water emulsions containing omega-3 rich oil droplets coated by Cas–LF electrostatic complexes, and then testing their chemical and physical stability. We postulated that lipid droplets coated by Cas–LF complexes would have different physicochemical properties than those coated by caseinate alone. Previous studies have shown that cationic droplets are more stable to lipid oxidation than anionic ones because they are able to prevent cationic iron ions (Fe^{2+} or Fe^{3+}) from coming into close contact with the emulsified lipids due to electrostatic repulsion (Mei, McClements, & Decker, 1999). LF has a positive charge at neutral pH, whereas casein has a negative charge. Hence, one would expect droplets coated with cationic LF–Cas complexes would have higher oxidative stabilities than droplets coated by anionic caseinate. In addition, LF is capable of chelating transition metals, which may also improve the oxidative stability of emulsions by preventing these lipid-oxidation catalysts from interacting with the emulsified lipids (Huang et al., 1999; Nielsen et al., 2004; Shiota, Uchida, Oda, & Kawakami, 2006). On the other hand, LF adsorbed to an oil–water interface could promote lipid oxidation by drawing the iron ions closer to the droplet surface previously noted for other whey proteins (Kellerby, McClements, & Decker, 2006). The formation of multilayer coatings around lipid droplets has also been shown to be able to greatly improve their physical stability to pH changes, high salt concentrations, freezing, drying and heating as well as their oxidative stability (Guzey et al., 2006; Katsuda, McClements, Miglioranza, & Decker, 2008) .

This study therefore focused on elucidating how physicochemical properties of emulsions stabilised by sodium caseinate and bovine lactoferrin (LF) impact the physical and chemical stability of omega-3 rich oil droplets.

2. Materials and methods

2.1. Materials

2.1.1. Food grade reagents

Unstabilised, deodorised, refined and bleached menhaden oil (eicosapentaenoic acid, 10–17%; docosahexenoic acid, 7–12%) was donated by Omega Protein (Reedville, VA) and stored in the dark at −80 °C until gently thawed prior to emulsification. Food grade sodium caseinate (Extra grade, Lot#4098) was donated by the American Casein Company (Burlington, NJ), and the manufacturer reported that it contained 96.2% protein, 3.5% ash and 0.5% fat. Food grade lactoferrin (Lot 10373317) was supplied by DMV International (Delhi, NY) and the manufacturer reported that it contained 97.7% protein and 0.12% ash. Typically, the iron-binding sites of natural lactoferrin are less than 10% saturated (Baker & Baker, 2005).

2.1.2. Chemical reagents

Monobasic phosphate, dibasic phosphate, $CaCl_2$, ammonium cyanate, $BaCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, Trichloroacetic acid, Thiobarbituric acid and 2,6-di-*tert*-butyl-4-methylphenol were purchased from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). All other solvents and reagents were of analytical grade.

2.2. Methods

2.2.1. Production of emulsions

This study evaluated two types of menhaden oil emulsions. The first was a primary emulsion stabilised solely with sodium caseinate and the second was a secondary emulsion produced from the primary emulsion through the surface deposition of lactoferrin, as illustrated in Fig. 1. Specifically, 5 g of sodium caseinate were dissolved overnight under gentle stirring in 195 g buffer solution (10 mM phosphate, pH 7.0). This solution was then coarsely homogenised under chilling for 60 s with 50 g of oil using a hand blender (Tissue Tearor, Model 985379-395, Biospec Products Inc.). This coarse emulsion was then passed five times through a high pressure homogeniser (Microfluidiser M-110L processor, Microfluidics Inc., Newton, MA) operating at 82 MPa. A "primary emulsion" was formed by mixing a portion of the stock emulsion with phosphate buffer, while a "secondary emulsion" was formed by mixing stock emulsion with lactoferrin solution (10% w/w protein in phosphate buffer). Both types of emulsions had equal concentrations of oil and caseinate.

2.2.2. Physical characterisation

The influence of pH changes (3–7) and mineral addition (0–100 mM $CaCl_2$) on the physical stability of the primary and secondary emulsions was tested. Emulsion particle size distribution was measured by injecting controlled sample volumes into the analysis chamber of a static laser light scattering instrument (Malvern Mastersizer, Malvern Instruments, Worcestershire, UK). A refractive index of 1.43 was used for the disperse phase (oil) and 1.33 for the continuous phase (water). Background measurement and system alignment were preformed for each sample while the sample chamber was filled with the corresponding buffer and salt concentrations. Particle sizes were reported as the surface-averaged (d_{32}) and volume-averaged (d_{43}) mean diameters as well as the full particle size distribution (PSD). The electrical charge (ζ-potential) of the particles in the emulsions was calculated through measuring the electrophoretic mobility in a capillary electrophoresis device inserted into a dynamic laser light scattering instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). Emulsion samples were diluted in 10 mM phosphate buffer (pH = 7.0) at a ratio of 1:500 (v/v) and then placed in a capillary test tube that was loaded into the instrument operating with predefined parameters (accounting for sample RI and salt content). Samples were equilibrated for 1 min inside the instrument before data was collected over at least 10 sequential readings and processed using the Smoluchowski model. Emulsions were also viewed under a light microscope to help visualise structural changes caused by varying environmental conditions, *i.e.,* pH and $CaCl_2$. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The slides were then observed with a 60× oil immersion objective lens on a conventional optical microscope (ECLIPSE 80i, Nikon Instruments, Tempe, AZ) equipped with a CCD camera connected to digital image processing software (NIS-Elements Basic Research, Nikon Instruments). Images were recorded using image analysis software (Scion Image, Frederick, MD, USA). At least three pictures were taken of each sample.

Pure protein solutions were also tested under various $CaCl_2$ concentrations. These experiments focused on measurements of surface charge and PSD made in dynamic laser light scattering instrument and processed with the Mark-houwink model. Also, optical transmission at 600 nm was measured using UV/visible