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Modulation of physicochemical properties of emulsified lipids by chitosan addition

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ABSTRACT

Electrostatic interactions between polysaccharides and proteins at oil–water interfaces alter the physicochemical properties and stability of emulsions. In this research, we studied the influence of chitosan addition on the properties of oil-in-water emulsions containing whey protein-coated lipid droplets. Experiments were carried out under conditions where the protein and polysaccharide had similar charges (pH 3.0) or opposite charges (pH 6.5). At pH 3.0, chitosan addition (0–0.025%) had little influence on droplet charge, aggregation, creaming stability or shear viscosity of whey protein emulsions, which was attributed to the fact that the cationic chitosan molecules did not adsorb to the cationic droplet surfaces due to electrostatic repulsion. At pH 6.5, chitosan addition caused a decrease in particle negative charge, an increase in particle size, a decrease in creaming stability, and an increase in viscosity. These effects were attributed to droplet aggregation caused by charge neutralization and bridging resulting from attraction of cationic chitosan molecules to anionic patches on the protein-coated droplet surfaces. Addition of cationic polyelectrolytes to protein-stabilized emulsions may be utilized to control their physicochemical properties, stability and biological fate, which may be useful for developing commercial products with novel or improved functional properties.

1. Introduction

There is increasing interest within the food industry to utilize "all-natural" or "label friendly" ingredients to fabricate products with desirable functional and nutritional properties. Proteins and polysaccharides are natural polymers that are widely used as functional ingredients in foods and beverages (Schmitt et al., 1998). Proteins and polysaccharides are often used in isolation as emulsifiers, foaming agents, thickeners, or gelling agents (Imeson, 2010). They can also be utilized in combination with each other to create novel functional properties in foods (Schmitt and Turgeon, 2011). For example, the texture and stability of certain kinds of food products can be controlled by manipulating the molecular interactions between proteins and polysaccharides to form novel structures (Benichou et al., 2002; Dickinson, 1995, 2003; Syrbe et al., 1998). One of the most important types of molecular interaction between proteins and polysaccharides are the electrostatic interactions that occur between charged groups (Schmitt and Turgeon, 2011; Turgeon et al., 2007). For proteins, the electrical charge goes from positive, to neutral, to negative as the pH is raised from below to above the isoelectric point (Das and Kinsella, 1990). For polysaccharides, the electrical charge depends on their functional groups (Imeson, 2010): those with no ionizable groups are neutral (e.g., amylose and amylopectin); those with sulfate or phosphate groups are anionic (e.g., xanthan, carrageenan, pectin); and, those with amino groups are cationic (e.g., chitosan).

Under appropriate solution conditions (pH and ionic strength), proteins and ionic polysaccharides may be electrically attracted or repelled depending on the sign, magnitude, and distributions of their charged groups (Cooper et al., 2005). These electrostatic interactions can be utilized to rationally create novel structures and functional properties in foods (Matalanis et al., 2011; Schmitt et al., 1998; Schmitt and Turgeon, 2011). In this study, we focus on the utilization of electrostatic interactions between a cationic polysaccharide and protein-coated lipid droplets to modulate emulsion properties. Previous studies have shown that electrostatic interactions between ionic polysaccharides and protein-coated lipid droplets may either increase or decrease emulsion stability depending on the precise nature of the system involved. Emulsions that are stable to droplet aggregation can be produced by adsorbing ionic polysaccharides onto the surfaces of oppositely charged protein-coated lipid droplets to form multilayer interfacial coatings (Gu et al., 2005; Guzey and McClements, 2006, 2007; McClements, 2010). These coatings improve emulsion stability by altering the electrostatic, steric, and van der Waals interactions operating between the droplets (Guzey and McClements, 2007). Nevertheless, preparation conditions and system composition must be carefully controlled during the formation of multilayer emulsions so as to avoid droplet aggregation (Cho and McClements, 2009;

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McClements, 2005b). Extensive droplet aggregation may occur in emulsions formed under conditions where there is insufficient polysaccharide to completely cover the protein-coated lipid droplets due to charge neutralization and bridging flocculation (Dickinson, 1992, 2003). Droplet aggregation may also be induced under conditions where there is an excess of non-adsorbed polysaccharides in the continuous phase, through a depletion mechanism (Dickinson, 1992, 2003). The tendency for droplet aggregation to occur in emulsions depends on the precise nature of the polymers involved, and so it is important to carry out studies on particular protein–polysaccharide combinations.

In this study, we examined the influence of chitosan on the stability and textural properties of whey protein stabilized emulsions. Whey proteins are widely used in the food industry to form and stabilize emulsion-based foods and beverages (Dalgleish, 1996). Whey protein-coated lipid droplets tend to be stable to droplet aggregation under conditions where there is a strong electrostatic repulsion between the droplets, i.e., pH far from isoelectric point, and low ionic strengths (McClements, 2004). Chitosan is a polysaccharide that is isolated from crab and shrimp waste to produce functional ingredients suitable for application in the food and other industries (Kurita, 2006). Chitosan is a linear polysaccharide with amino side groups that is obtained by partial deacetylation of chitin after treatment with strong alkali at elevated temperatures (Shahidi et al., 1999). The fact that chitosan is the only major polysaccharide that is cationic has meant that its application for modifying the functional properties and physiological activity of foods and other materials has been widely studied (Aider, 2010; Friedman and Juneja, 2010; Muzzarelli, 1996).

A number of previous studies have investigated the influence of chitosan on the physicochemical and biological properties of emulsions. The formation of cationic chitosan layers around anionic lipid droplets has been shown to improve their stability to environmental stresses (Aoki et al., 2005; Hong and McClements, 2007; Mun et al., 2008; Zinoviadou et al., 2012), enhance their chemical stability (Gudipati et al., 2010; Lomova et al., 2010), and modulate their lipase digestibility (Hu et al., 2011; Li et al., 2010). The influence of pH, ionic strength, chitosan level, and chitosan type on the stability of protein-stabilized emulsions has been investigated (Laplante et al., 2002, 2005a,b). Emulsions with novel structural and textural properties were recently produced by mixing chitosan–gum arabic complexes with whey-protein coated lipid droplets (Moschakis et al., 2010).

The focus of the current study was to investigate the nature of the electrostatic interactions between chitosan and whey-protein coated lipid droplets, and to determine the influence of these interactions on the physicochemical properties and stability of oil-in-water emulsions. This information may prove useful for formulating food and beverage products with novel rheological properties, for controlling the behavior of ingested emulsions in the gastrointestinal tract, or for developing methods of removing fat droplets from waste-water during food processing.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (Le Sueur, MN). Medium chain triglyceride (MCT) oil (Miglyol 812) was purchased from Coletica (Northport, NY). Chitosan, acetic acid, and other analytical grade reagents were obtained from Sigma–Aldrich Co. (St. Louis, MO). A buffer solution was prepared by dissolving acetic acid in distilled and deionized water (10 mM) and adjusting the pH to the required value using NaOH or HCl solutions.

2.2. Emulsion formation

The oil (medium chain triglycerides) and aqueous phase (0.5% WPI in buffer solution) were mixed together in a beaker, and then blended using a high-shear mixer (M133/1281-0, Biospec Products Inc., ESGC, Switzerland) for 2 min to form a coarse emulsion. This coarse emulsion was then passed through a high pressure homogenizer (Microfluidics, Newton, MA) three times at 10,000 psi at ambient temperature.

2.3. Particle size and charge measurements

Samples were first diluted with buffer solutions of the appropriate pH value (pH 2–7) to avoid multiple scattering effects. The particle size distributions of the samples were then measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK), and the ζ-potential was determined using a particle electrophoresis instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK) as described previously (Mao and McClements, 2011).

2.4. Rheology measurements

The rheological behavior of samples was measured using a dynamic shear rheometer (Kinexus Rotational Rheometer, Malvern Instruments, Malvern, UK). A cup and bob geometry consisting of a rotating inner cylinder (diameter 25 mm) and a static outer cylinder (diameter 27.5 mm) was used in viscosity and oscillation measurements. The samples were loaded into the rheometer measurement cell and allowed to equilibrate at 25 °C for 5 min before beginning experiments. Shear viscosity (η) measurements were then carried out at different shear rates (0.01–10 s^{-1}). The storage modulus (G') and loss modulus (G'') were measured using an oscillation experiment. The influence of frequency on the shear modulus of mixed samples was assessed by varying the oscillation frequency (0.01–10 Hz of the inner cylinder, strain = 1%). This strain was within the linear viscoelastic region of the material as established by a preliminary stress sweep test.

2.5. Optical microscopy

An optical microscope (Nikon Eclipse 80i, Nikon Instrument Inc., Melville, NY) with a 60× objective lens (NA 0.75) was used to capture images of the emulsions. A small aliquot of emulsion was placed on a microscope slide below a cover slip prior to analysis.

2.6. Statistical analysis

All measurements were performed on at least two freshly prepared samples (i.e., new samples were prepared for each series of experiments) and were reported as means and standard deviations.

3. Results and discussion

3.1. Properties of protein-coated lipid droplets and chitosan molecules

3.1.1. Optimization of emulsion preparation conditions

Initially, a series of preliminary experiments was carried out to determine the optimum conditions for producing emulsions with little free protein remaining in the continuous phase. This was achieved by examining the influence of initial WPI concentration on the mean particle diameter (d_{32}) of 10 wt% MCT oil-in-water emulsions produced using standardized homogenization conditions i.e., 10,000 psi, three passes (Fig. 1). As expected, there was