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Modulation of emulsion rheology through electrostatic heteroaggregation of oppositely charged lipid droplets: Influence of particle size and emulsifier content

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ABSTRACT

The influence of electrostatically-induced heteroaggregation of oppositely charged lipid droplets on the rheology and stability of emulsions has been studied. 20 wt.% oil-in-water emulsions (pH 6) containing oppositely charged droplets were fabricated by mixing cationic lactoferrin-coated lipid droplets with anionic β-lactoglobulin-coated lipid droplets. Emulsions containing mixtures of droplets with different charges (positive or negative) and sizes (large or small) were prepared, and then their overall particle characteristics (ζ-potential and size) and rheology were measured. Emulsions formed by mixing positive droplets and negative droplets that were both relatively small ($d_{43} \approx 0.3$ μm) exhibited extensive flocculation and had paste-like properties at intermediate positive-to-negative particle ratios. On the other hand, emulsions formed by mixing positive droplets and negative droplets that were both relatively large ($d_{43} \approx 3$ μm) exhibited little aggregation and had relatively low viscosities at all particle ratios. Emulsions with small negative droplets and large positive droplets (or *vice versa*), exhibited some aggregation and viscosity enhancement at intermediate particle ratios. The presence of relatively high levels of protein in the aqueous phase of mixed emulsions reduced the level of droplet aggregation and viscosity enhancement observed, which was attributed to the ability of protein molecules to bind to droplet surfaces and neutralize their charges. Electrostatically-induced heteroaggregation of lipid droplets may be a useful means of controlling the physicochemical properties of emulsion-based products in the food, personal care, pharmaceutical and cosmetic industries.

1. Introduction

There is growing interest in the utilization of soft matter physics principles to fabricate commercial materials with novel physicochemical and functional properties, e.g., foods, cosmetics, and pharmaceuticals [1,2]. Recent studies have reported that controlled heteroaggregation of oppositely charged lipid droplets can be used to manipulate the characteristics of emulsion-based products [3–5]. These studies have shown that products with high viscosities or paste-like characteristics can be prepared by mixing an emulsion containing positive droplets with another one containing negative droplets. The oppositely charged droplets interact with each other through electrostatic attraction leading to the formation of microclusters. At sufficiently high particle concentrations, a three-dimensional network of aggregated droplets extends throughout the volume of the material leading to elastic-like behavior. The microstructure and rheological properties of these mixed systems have been shown to depend on total particle concentration, the ratio of positive-to-negative particles, and aqueous phase properties that modulate electrostatic interactions, such as pH and ionic strength [4,5]. The novel materials produced by controlled heteroaggregation of lipid droplets may be useful for commercial applications, such as pharmaceutical, food, and cosmetic products. For example, this principle can be used to produce reduced-fat food products with similar textures as high-fat products. Indeed, previous research has shown that foods with novel textural attributes can be formed by mixing anionic polysaccharide-coated fat droplets with cationic protein-coated fat droplets [3].

In this article, we focus on the influence of particle size on microcluster formation and rheology of mixed emulsions created by electrostatic heteroaggregation of oppositely charged lipid droplets. We also examine the influence of initial protein content on the formation and properties of these systems, since free protein may bind to any charged sites on lipid droplet surfaces and alter their ability to interact with other droplets. The knowledge gained from this study will facilitate the rational design of emulsion-based products where highly viscous or gel-like characteristics.

2. Experimental methods

2.1. Materials

Corn oil was purchased from a commercial food supplier (Mazola, ACH Food Companies, Inc., Memphis, TN) and stored at 4 °C until

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use. Lactoferrin powder (LOT #10404498) was supplied by Friesland-Campinanin (Delhi, NY) and was reported to contain 97.7% protein and 0.12% ash. β-Lactoglobulin powder (BioPURE, LOT #JE-001-0-415) was supplied by Davisco Foods International (Eden Prairie, MN) and was reported to contain 97.4% total protein, 92.5% β-lactoglobulin (β-Lg), and 2.4% ash. Glacial acetic acid, sodium acetate, and Bradford assay agents were purchased from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO). Double distilled water was used to prepare all solutions.

2.2. Emulsion preparation

2.2.1. Formation of single-protein emulsions

Aqueous emulsifier solutions were prepared by dispersing either β-Lg powder or LF powder into acetic acid buffer (pH 6, 10 mM), and then stirring for at least 3 h at room temperature to ensure complete dispersion. The pH of the protein solutions was then adjusted to 6.0 using 1 M NaOH or HCl. Oil-in-water emulsions containing a single protein type were prepared by blending 20 g of corn oil and 80 g of aqueous protein solution for 2 min using a hand blender (M133/1281-0, 2 speed, Biospec Products Inc., ESGC, Switzerland) and then recirculating them four-times through a two-stage homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA) at a first-stage/second-stage pressure of 5400/600 psi (to produce small droplets) and 400/10 psi (to produce large droplets). β-Lg emulsions were then heated to 90 °C for 30 min to cross-link the adsorbed proteins, so as to prevent any competitive adsorption effects with the lactoferrin mixed systems. All emulsions were then stored for 24 h prior to utilization.

Preliminary experiments established that a β-Lg-to-oil mass ratio of 0.05-to-1 and a LF-to-oil mass ratio of 0.3-to-1 were suitable to form single-protein emulsions with relatively little free protein [4]. Based on this knowledge, two different levels of protein were used to prepare 20 wt.% oil-in-water emulsions with different non-adsorbed protein contents: (i) 1% β-Lg and 6% LF; (ii) 0.1% β-Lg and 0.6% LF. 1.0% β-Lg and 6% LF are suitable for producing emulsions with relatively small droplet diameters ($d_{43} \approx 0.35$ μm) with little free protein. Hence, when droplets containing relatively large droplets are produced at low homogenization pressures using this amount they will contain an appreciable amount of protein that is not directly adsorbed at the oil–water interface.

2.2.2. Formation of mixed-protein emulsions

Mixed emulsions containing 0–20 wt.% β-Lg-coated droplets (pH 6.0) and 20–0 wt.% LF-coated droplets (pH 6.0) were prepared by mixing different ratios of the two single-protein 20 wt.% oil-in-water emulsions together, stirring for 10 min, and then allowing them to stand for 24 h prior to analysis to enable structure formation to occur. After this time, some of emulsions separated into a thin white creamed layer on top of a clear or slightly turbid serum layer, and so all samples were gently stirred prior to analysis to ensure they were homogeneous. It should be noted that stirring colloidal dispersions containing aggregated particles may have caused some disruption of their structure, and it would be useful in future experiments to examine the impact of mechanical forces on the structure and physicochemical properties of mixed systems.

We prepared a series of mixed emulsion samples containing droplets with different sizes and charges: (i) Small Cationic–Small Anionic, S(+):S(−); (ii) Large Cationic–Small Anionic, L(+):S(−); (iii) Small Cationic–Large Anionic, S(+):L(−); and, (iv) Large Cationic–Large Anionic, L(+):L(−). For most experiments, the small anionic and cationic droplets were produced by homogenizing at high pressure using 1.0% β-Lg and 6.0% LF (respectively), while the large anionic and cationic droplets were produced by homogenizing at low pressure using 0.1% β-Lg and 0.6% LF. However, in some experiments we also produced anionic and cationic emulsions with high levels of additional protein by homogenizing at low pressure using 1.0% β-Lg and 6.0% LF.

2.3. Emulsion characterization

2.3.1. Particle charge measurements

The ζ-potential of emulsions was determined using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). The ζ-potential of any particles in the system was measured after 200-fold dilution with an acetic acid buffer solution (pH = 6.0). After loading the samples into the instrument they were equilibrated for about 120 s before particle charge data were collected over 20 continuous readings.

2.3.2. Particle size analysis

The particle size distribution of emulsions was measured using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, UK). To avoid multiple scattering effects the emulsions were diluted approximately 200-fold using pH-adjusted acetic acid buffer (pH = 6.0). The emulsions were stirred continuously throughout the measurements to ensure they were homogenous. Dilution and stirring may alter the structure of any aggregated particles in a colloidal dispersion, and therefore the particle size data on mixed systems should only be used as a qualitative guide to the extent of aggregation in the systems. Even so, laser diffraction measurements can still provide some valuable information about aggregation in colloidal dispersions, since any strongly flocculated particles will tend to remain intact. It should also be noted that the theory used to calculate the particle size distribution (Mie theory) assumes that the particles are spherical and homogeneous, and therefore data obtained on aggregated emulsions should be treated with caution because flocs are non-spherical and non-homogenous. Measurements are reported as the volume–length mean diameter: $d_{43} = \sum d_i n_i^4 / \sum d_i n_i^3$, where n_i is the number of droplets of diameter d_i.

2.3.3. Rheological properties

The rheological behavior of samples was measured using a dynamic shear rheometer (Kinexus Rotational Rheometer, Malvern, UK). A cup and bob geometry consisting of a rotating inner cylinder (diameter 25 mm) and static outer cylinder (diameter 27.5 mm) was used in viscosity measurements. The samples were loaded into the rheometer measurement cell and allowed to equilibrate at 25 °C for 5 min before beginning all experiments. Samples underwent a constant shearing treatment (1 s^{-1} for 10 min) prior to analysis to remove history effects. The shear stress of the emulsions was then measured over a range of shear rates (0.01–10 s^{-1}), and the apparent viscosity was calculated from this data.

2.3.4. Microstructure analysis

The microstructure of the mixed emulsions was assessed by optical microscopy. Mixed emulsions were diluted 30-fold and then gently agitated in a glass test tube before analysis to ensure that they were homogeneous and that the particles could be easily distinguished from one another. A drop of diluted sample was then placed on a microscope slide and covered by a cover slip, and then the microstructure was determined using optical microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan). Images were acquired using a CCD camera (CCD-300-RC, DAGE-MTI, Michigan City, IN) connected to digital image processing software (Micro Video Instruments, Inc., Avon, MA) installed on a computer. All microscopic examinations were carried out on the same day as the ζ-potential and particle size measurements.