Modulation of Food Texture Using Controlled Heteroaggregation of Lipid Droplets: Principles and Applications

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ABSTRACT: This article reviews the development and potential application of novel food-grade materials based on heteroaggregation of oppositely charged particles. These particles typically consist of a hydrophobic lipid core and an electrically charged emulsifier shell. Heteroaggregation leads to the formation of emulsion-based products that are highly viscous or gel-like at much lower droplet concentrations than in nonaggregated systems. Heteroaggregation may therefore be useful for developing food products with novel textural characteristics or for creating reduced-calorie versions of full fat foods. We give an overview of the principles of heteroaggregation, the relationship between emulsion microstructure and texture, and possible commercial applications of this method. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 3833–3841, 2013

INTRODUCTION

The recent increase in the number of overweight or obese individuals in many countries has become a major health and economic issue because of the increase in associated chronic diseases and associated health care costs.[1,2] In response to this problem the food industry is developing reduced-calorie versions of many of its products. Dietary fat has the highest energy density of all the major macronutrients found in foods (i.e., fat, protein, and carbohydrates). Consequently, the removal of fat from food products has been a major target of the food industry. However, the development of high quality reduced fat products has proved to be challenging because fats play complex roles in determining the overall appearance, flavor profile, texture, and biological response (such as satiety) of foods.[3,4] These desirable attributes of a product may be lost once the fats are removed, which leads to consumer rejection. Therefore, there is a need to develop new strategies to create reduced fat foods that maintain their desirable characteristic sensory attributes, such as creaminess, richness, or smoothness.

In this article, we focus on the development of reduced fat emulsion-based foods. Emulsions consist of the two of immiscible liquids (usually oil and water) and are formed by application of vigorous stirring or more intense mechanical forces.[5] The most common emulsions used in the food industry are oil-in-water (O/W) and water-in-oil (W/O) emulsions: O/W emulsions consist of oil droplets dispersed in water (e.g., milk, cream, dressings, sauces, and desserts), whereas W/O emulsions consist of water droplets dispersed in oil (e.g., butter and margarine). The optical properties, rheology, and stability of these products depend on the concentration, size, and interactions of the droplets they contain. Typically, the lightness, viscosity, and stability of emulsions increases as their fat content increases.[6] Consequently, removing some or all of the fat droplets from a product (such as a dessert, dressing, or sauce) will alter its desirable sensory properties.[7,8] For this reason, fat replacers are typically added to reduced-fat foods to replace the physicochemical and sensory attributes normally provided by the fat droplets. For example, food-grade polymers or particles may be added to the aqueous phase of reduced-fat O/W emulsions to increase their texture or provide opacity.

Heteroaggregation has proved to be a useful technique for creating novel structures and material properties in nonfood applications, such as ceramics, water purification, and delivery systems (see next section). Recently, it has been shown that controlled heteroaggregation can also be used to formulate food-grade emulsions that have high viscosities or gel-like properties at reduced fat contents.[9–12] These aggregated systems may be used to create reduced fat food products that can mimic the desirable textural and sensory properties of their high fat counterparts. In this article, we provide an overview of the physical principles of heteroaggregation, the factors that affect the formation and physicochemical properties of heteroaggregates, and their potential applications within the food industry. This information should prove useful for the development of novel food
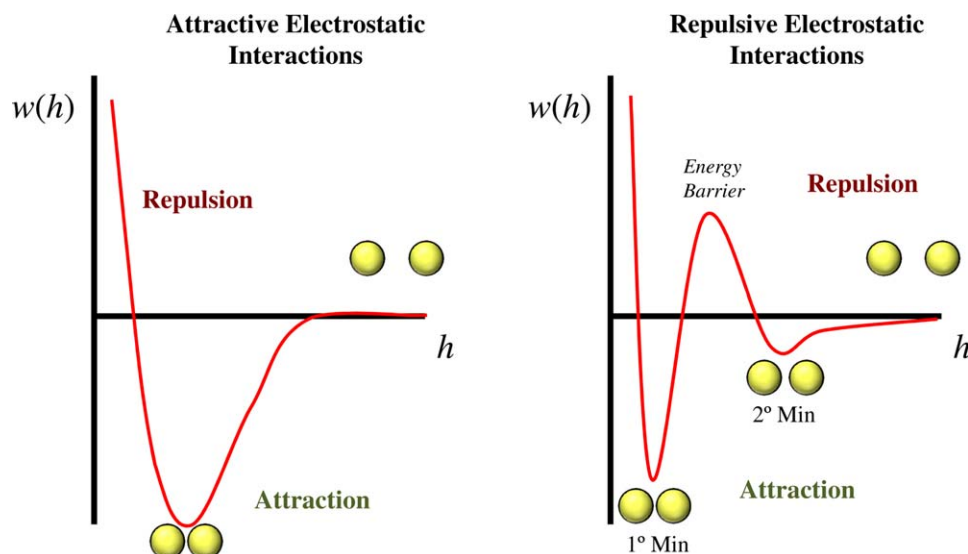
Figure 1. Schematic representation of the interaction potential between two emulsion droplets: (a) extended DLVO theory with van der Waals attraction, electrostatic *attraction*, and steric repulsion; (b) extended DLVO theory with van der Waals attraction, electrostatic *repulsion*, and steric repulsion. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

products, as well as other emulsion-based commercial products, such as cosmetics, health-care products, and pharmaceutical preparations.

PRINCIPLES OF HETEROAGGREGATION

In general, heteroaggregation is defined as the aggregation of dissimilar particles, which may differ in their size, shape, charge, chemical composition, and other properties.[13,14] Heteroaggregation has been widely used in nonfood science applications for a variety of reasons, such as controlling the rheological properties of ceramics,[15] creating ion-exchange columns,[16] removing colloidal particles from aqueous solutions,[17] and encapsulating and targeting biomolecules.[18–21] Heteroaggregation of oppositely charged particles through electrostatic attraction is the most commonly used method for most applications, and therefore this method will be the focus of this review.

There can be considered to be two different stages in the heteroaggregation of oppositely charged particles. First, the particles are attracted to each other through electrostatic attraction, which leads to the formation of heteroaggregates. Second, the continued growth of the heteroaggregates is inhibited because of electrostatic repulsion between similarly charged particles. The dimensions and structural organization of the droplets within a heteroaggregated system depend on the sign, strength, and range of the electrostatic interactions, as well as the collision mechanism (e.g., Brownian motion, gravitational separation, or shearing).

Colloidal Interactions

One of the most widely utilized models for describing the interactions between charged colloidal particles is the extended DLVO theory. In this theory, the overall interaction between colloidal particles is taken to be the sum of three contributions: $V_T = V_V + V_E + V_S$, where V_T is the total interaction energy, V_V is the energy resulting from van der Waals attractive forces, V_E is the energy associated with electrostatic interactions, and V_S is the energy associated with steric repulsion forces.[22] The electrostatic interactions may be either attractive (oppositely charged particles) or repulsive (similarly charged particles), which leads to different colloidal interaction profiles (Figure 1). In addition, their sign, magnitude, and range may change appreciably if solution conditions (such as pH and ionic strength) are changed. In particular, the strength of the electrostatic interactions tends to decrease appreciably when the ionic strength of the aqueous phase increases, or the pH changes so that the charges on the two kinds of droplets have a similar sign. Mathematical models are available to predict the influence of different properties on the various types of interactions between colloidal particles, and these have been used in theoretical models and computer simulations of heteroaggregation.[23]

Modeling Aggregate Formation

Ideally, one would like to predict the structural organization of the colloidal particles within any aggregates formed when a suspension of positive and negative particles are mixed together. A number of different approaches have been developed to provide insights into the structure of the aggregates formed, for example, statistical thermodynamics and computer simulations.

Statistical thermodynamics has recently been used to model aggregate formation when two types of equal-sized particles (A and B) are mixed together.[24] This theory relates the number of particles per aggregate to the total particle concentration, the ratio of the two particle types, and the strength of the attractive forces between the different particle types. It is assumed that the interactions between similar particle types (i.e.,A-A or B-B) are repulsive (hard shell model), while the interactions between different particle types (i.e., A-B) are attractive ("sticky" hard shell model). This theory relates the average aggregate size (S) to the strength of the attraction between the unlike particles and the ratio of unlike particles.[24] A major limitation of
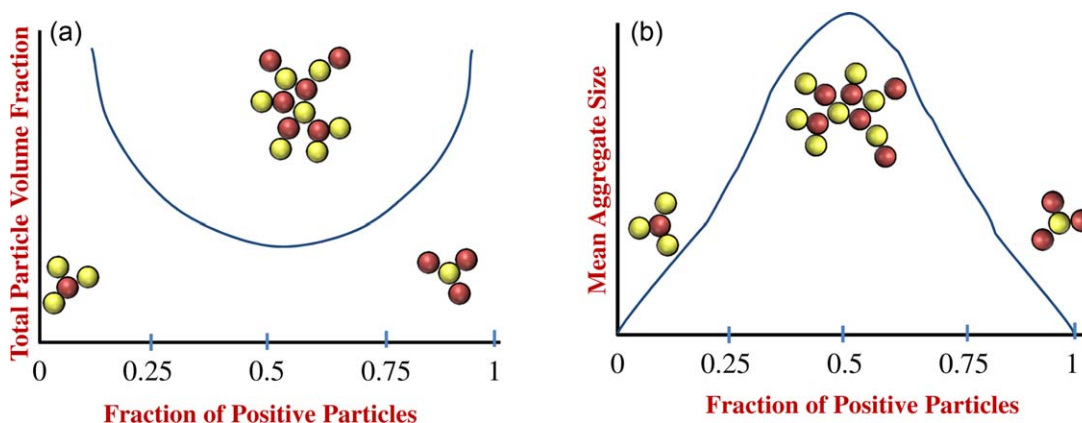
Figure 2. Schematic representation of a phase diagram (a) and average cluster size (b) for a heteroaggregated system containing particles that are attracted to each other. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

statistical thermodynamic approaches is that they do not take into account nonequilibrium effects, which are known to be important in heteroaggregation.

More sophisticated approaches are required to take into account these nonequilibrium effects, such as those based on computer simulations.[13,25,26] For example, heteroaggregation can be modeled using diffusion-limited cluster–cluster aggregation simulations.[27] These simulation methods have been used to examine the influence of various factors on the structural organization of mixed colloidal systems, such as total particle concentration, unlike particle ratio, particle size ratio, and interaction strength.[13,26,28–30] Computational approaches have shown that various kinds of heteroaggregates with different structures can be formed depending on the initial characteristics of the system. A highly schematic representation of the nature of the aggregates formed under different conditions is shown in Figure 2. When one kind of particle dominates, then small heteroaggregates tend to be formed that consist of one kind of particle surrounded by the other kind of particle. On the other hand, when the two types of particles are present in similar concentrations, then large heteroaggregates are formed that contain a mixture of both particles. At relatively high total particle concentration, heteroaggregation may lead to the formation of a three-

dimensional (3D) network of particles that give the system solid-like characteristics.

CONVENTIONAL EMULSIONS

Initially, we consider the formation and properties of conventional emulsions so as to contrast their behavior with mixed emulsions in which heteroaggregation has been induced. A conventional O/W emulsion consists of fat droplets dispersed within an aqueous medium. Each fat droplet is coated by a thin layer of emulsifier molecules that normally protects it from aggregation. There are many methods available for preparing O/W emulsions, including various low-energy and high-energy methods, which have been reviewed elsewhere.[31,32] In the food industry, the most common method of making fine emulsions is to use high-pressure homogenization (Figure 3). In this method, an oil phase is blended with an aqueous phase that contains a water-soluble emulsifier. The resulting coarse emulsion is then passed through the high-pressure homogenizer to further reduce the particle size. The size of the droplets present within an emulsion can be controlled by varying the homogenizer type or operating conditions, as well as sample composition, such as the type and amount of emulsifiers present.
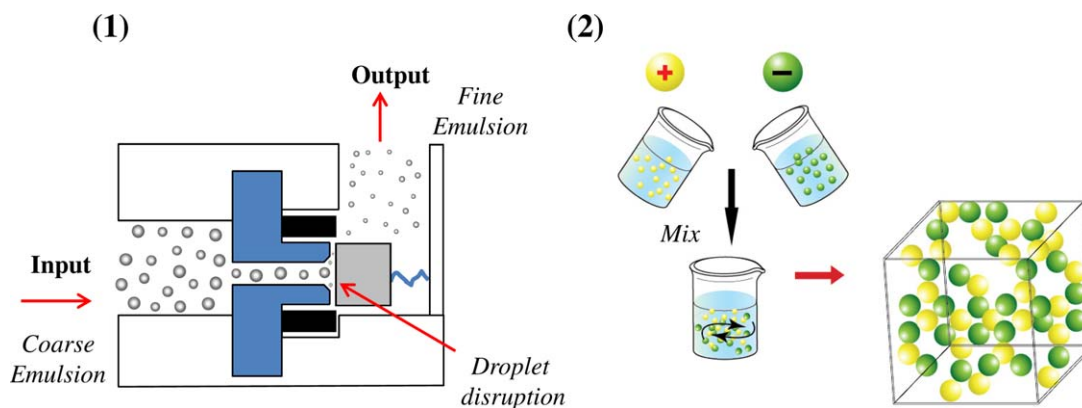

Figure 3. Schematic representation of principle of heteroaggregation. First, a mechanical device is used to produce two conventional emulsions with opposite charges, such as a high-pressure valve homogenizer. Second, the two emulsions with opposite charges are mixed together to form a heteroaggregated emulsion. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
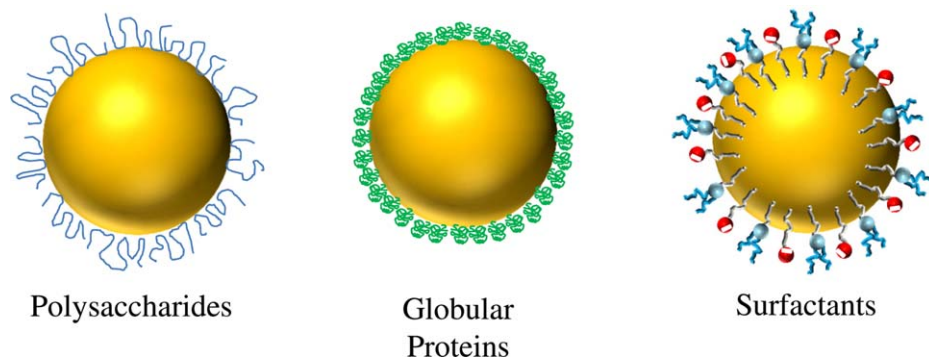
Figure 4. Highly schematic representation of protein-, polysaccharide-, and surfactant-coated fat droplets that can be used as building blocks to induce heteroaggregation. In reality, the thickness of the interfacial layers usually decreases in the following order: polysaccharides > proteins > surfactants. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The long-term stability of the emulsions can be controlled by selecting different types of emulsifiers to coat the fat droplets. The nature of the emulsifier selected determines interfacial characteristics such as charge, thickness, polarity, and chemical reactivity. In turn, these interfacial characteristics influence the physiochemical properties, sensory attributes, and biological fate of emulsions.[33,34]

The tendency for fat droplets to aggregate with each other, or to remain as individual entities, depends on the balance of attractive and repulsive interactions operating between them (Figure 1). When the attractive interactions dominate, then the droplets will tend to associate and form aggregates. In the case of conventional emulsions, this process can be referred to as *homoaggregation* because there is only one type of particle involved. Homoaggregation can be induced in conventional emulsions in a variety of ways, for example, decreasing electrostatic repulsion by increasing the ionic strength, or increasing attraction by adding absorbing or nonadsorbing polymers to the aqueous phase.[31] The size, shape, and deformability of the aggregates formed depend on the strength of the attractive interactions between droplets.

The aggregation state of the droplets within an emulsion plays a major role in determining the overall texture and stability.[31] In dilute emulsions, droplet aggregation tends to lead to an increase in the creaming rate because of an increase in effective particle size. Conversely, in concentrated emulsions, droplet aggregation may decrease the creaming rate because the droplets are trapped within a 3D network that inhibits droplet movement. The textural characteristics of emulsions are also strongly dependent on the droplet aggregation state. Droplet aggregation leads to an increase in shear viscosity in relatively dilute systems and to gel formation in relatively concentrated systems.

HETEROAGGREGATED EMULSIONS

In this section, we focus on heteroaggregation in O/W emulsions because these are normally the most suitable systems for utilization within the food industry. Nevertheless, it should be noted that heteroaggregation can also be induced in W/O emulsions containing oppositely charged water droplets,[35] or in other

types of colloidal suspensions, for example, air bubbles or biopolymer particles in water.

Heteroaggregated emulsions are typically formed by mixing together two conventional emulsions.[9,10,36] Each of these emulsions contains fat droplets coated by a layer of electrically charged emulsifier molecules, but the type of emulsifier used is different. The two emulsions are then mixed together under conditions where one of them contains negatively charged droplets, and the other contains positively charged droplets (Figure 3). The structure of the heteroaggregates formed and the subsequent functional performance of the overall emulsion depends strongly on the electrical characteristics of the different fat droplets. One of the most important factors determining the formation of heteroaggregated emulsions is therefore the nature of the emulsifiers used.

Emulsifiers

A variety of different emulsifiers can be used to prepare O/W emulsions containing electrically charged droplets, such as proteins, polysaccharides, and ionic surfactants (Figure 4). Each type of emulsifier has its own advantages and disadvantages for particular applications.[31] Selection of the most appropriate emulsifier is one of the most important factors influencing the formation of heteroaggregated emulsions. In this section, we provide an overview of a number of electrically charged foodgrade emulsifiers that can be used.

Proteins. Protein-based emulsifiers can be isolated from various natural sources, including various animal, plant, and marine products.[31] However, the most commonly used protein-based emulsifiers in the food industry are those derived from bovine milk because of their relatively low cost and ease of isolation, that is, caseins and whey proteins.[37] Caseins are relatively flexible and disordered proteins that make up about 80% of milk proteins, and include four main fractions: α_{S1}-casein, α_{S2}-casein, β-casein, and κ-casein.[38] Whey proteins are compact globular proteins that make up about 20% of milk proteins, and also include a number of different fractions, such as β-lactoglobulin (β-Lg), α-lactalbumin, bovine serum albumin (BSA), lactoferrin, and various other minor components.[39]

These proteins are amphiphilic molecules that have both nonpolar and polar groups on the same molecule, and can therefore

Table I. Summary of the Isoelectric Points and Acid Dissociation Constants (pK_a) of Some Common Food-Grade Biopolymers that can Be Used to Form Electrically Charged Emulsion Droplets

Types of emulsifiers							
Proteins (isoelectric points)				Polysaccharides (acid-dissociation constants)			
Casein	β-Lg	Lactoferrin	WPI	Modified starch	Gum arabic	Pectin	Chitosan
4.5	5	8.5	5	2.3	3.5	3.5	6.3–7

β-Lg, β-lactoglobulin; WPI, whey protein isolate.

adsorb to oil–water interfaces.[40] They normally provide stabilization against droplet aggregation by a combination of electrostatic and steric repulsion.[41] The electrical characteristics of different proteins are determined by their primary sequence, especially the type, number, and location of ionizable amino acid side groups and other charged groups (such as phosphates) along the polypeptide backbone. Each type of protein can be characterized by its isoelectric point (pI), which is the pH where the net charge on the protein is zero (that is, the number of positive and negative charges are balanced). Below the pI the electrical charge on the proteins is positive, but above this pH it is negative. The isoelectric points and acid dissociation constant point of some common biopolymers are summarized in Table I. The variation of the electrical charge on protein-coated fat droplets stabilized by different proteins (lactoferrin and β-Lg) is shown in Figure 5.

When the adsorbed proteins form a relatively thin interfacial coating around the fat droplets, the primary stabilization mechanism is electrostatic repulsion.[40,41] For this type of system, the stability of the emulsion is particularly sensitive to changes in pH and ionic strength. Droplet aggregation tends to occur

Figure 5. Change in electrical charge (z-potential) of protein-coated fat droplets with pH: β-Lg, β-lactoglobulin; LF, lactoferrin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

when the pH is close to the isoelectric point (low net droplet charge) or at high salt concentrations (strong electrostatic screening).[42] β-Lg is a commonly used globular protein that forms thin interfacial coatings around fat droplets, and is therefore highly sensitive to solution pH and ionic strength. However, when the adsorbed proteins form a relatively thick hydrophilic coating around the fat droplets, the stabilization mechanism is a combination of electrostatic and steric repulsion. Emulsions stabilized by this kind of protein are much more resistant to alterations in pH and ionic strength. Lactoferrin is an example of a globular protein that forms thick interfacial coatings around fat droplets because of its high molecular weight and the fact it contains hydrophilic carbohydrate side chains that protrude into the aqueous phase.[43,44] Experimental studies have shown that lactoferrin-coated fat droplets are highly stable to changes in pH and salt concentration, provided there is sufficient protein present to fully coat the droplet surfaces. If there is insufficient surface coverage, the lactoferrin-coated droplets do aggregate.[45]

Another factor that is important for determining the functional performance of globular protein-coated fat droplets is their response to temperature changes.[46] Globular proteins (such as β-Lg, BSA, and lactoferrin) unfold when they are heated above their thermal denaturation temperature (T_m). These conformational changes expose reactive amino acid groups, such as those containing nonpolar or sulfhydryl groups, which promote protein–protein interactions.[40] As a result, fat droplets coated by these proteins may aggregate at elevated temperatures because of increases in the hydrophobic attraction or disulfide bond formation between proteins on different droplets. On the other hand, there are no major changes in the conformation of caseins when they are heated, and therefore they are more stable to thermal processing.[47]

Previous studies have shown that heteroaggregation can be induced by mixing together two protein-stabilized emulsions: one containing β-Lg-coated droplets and one containing lactoferrin-coated droplets.[9] β-Lg has an isoelectric point around pH 4.5, whereas lactoferrin has a pI around 8.5 (Figure 5).[44] Consequently, there is a range of intermediate pH values where the two types of droplets have opposite charges and will tend to associate with each other through electrostatic attraction.

Polysaccharides. A number of natural and modified polysaccharides are amphiphilic molecules that are capable of stabilizing
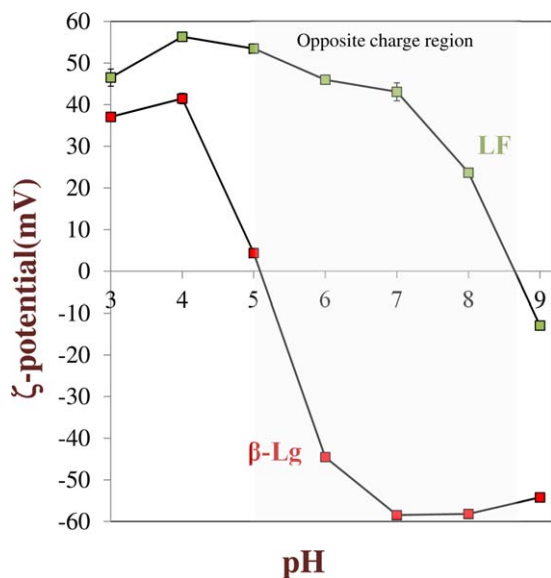
O/W emulsions, such as gum arabic (GA) and modified starch (MS).[48] MS is produced by chemically modifying natural starches so that they gain some nonpolar groups.[49] This is normally achieved by covalently attaching nonpolar octenyl succinic anhydride (OSA) side groups to the polar starch backbone. This leads to an amphiphilic biopolymer molecule that can adsorb to oil–water interfaces and stabilize fat droplets against aggregation.[49,50] The non-polar OSA groups tend to penetrate into the oil droplets, while the polar starch molecules protrude into the surrounding aqueous phase.[51] Previous studies have shown that MS-coated fat droplets are negatively charged over a wide pH range (pH 2–9) because of the presence of anionic groups on the OSA side chains.[52] MS molecules form a relatively thick hydrophilic layer at the droplet surfaces and therefore can prevent droplet aggregation through a combination of steric and electrostatic repulsion.

GA is isolated from the exudate of a shrub (acacia tree) and is surface active because of the presence of polysaccharide and protein moieties on the same molecule.[53] The protein part is believed to be nonpolar and anchors the molecule to the fat droplet surface, whereas the polysaccharide part is polar and protrudes into the aqueous phase.[54] GA is negatively charged form around pH 2–9 and can therefore be used to create anionic droplets suitable for fabricating heteroaggregates.[52] The fact that GA forms a thick negatively charged interfacial coating around fat droplets means that it mainly provides stabilization against aggregation through a combination of steric and electrostatic repulsion. In general, polysaccharides-based emulsifiers tend to be more stable to pH, ionic strength, and thermal treatment than protein-based emulsifiers.[55]

Surfactants. There are a number of food-grade surfactants that can also be used to form electrically charged fat droplets in O/W emulsions.[56,57] These surfactants consist of a hydrophilic head group that protrudes into the aqueous phase, and a hydrophobic tail group that protrudes into the oil phase. Most of the ionic surfactants available for utilization in the food industry are negatively charged, such as DATEM, CITREM, and lysolecithin.[56,57] Nevertheless, lauric arginate is a cationic surfactant that is capable of producing stable positively charged droplets at relatively low pH values (pH < 7).[58] Ionic surfactants can be used in isolation, or they can be mixed with nonionic surfactants to improve emulsion stability.

Emulsifier Exchange. A potential problem with using two different kinds of fat droplets stabilized with different emulsifiers is the exchange of emulsifiers between them. When one mixes droplets coated by different emulsifiers together then the emulsifier from one droplet may exchange with the emulsifier from a different droplet. This process is likely to occur via the bulk aqueous phase that separates the droplets, that is, an adsorbed emulsifier exchanges with a nonadsorbed emulsifier in the surrounding aqueous phase. As a result, the electrical charge on the two kinds of droplets will become more similar. If complete mixing of the emulsifiers occurs at the droplet interfaces, then all the droplets will eventually have the same charge, which may prevent heteroaggregation. Emulsifier exchange may limit the types of emulsifiers that can be used to form heteroaggregated

systems. Polymeric emulsifiers tend to be more resistant to exchange than small molecule surfactants,[59,60] particularly if they can be cross-linked at the interface, for example, by thermal, chemical, or enzymatic treatment.[61]

Preparation of Heteroaggregated Emulsions

In principle, various kinds of oppositely charged particles can be used to induce particle–particle heteroaggregation, including fat droplets, air bubbles, starch granules, and biological cells. Previous studies have largely been carried out using oppositely charged fat droplets and therefore we will focus on these systems. In this case, heteroaggregation is induced by mixing an O/W emulsion containing positively charged droplets with another one containing negatively charged droplets.[9,36] This process can be carried out using either a one-step or two-step method depending on the charge characteristics of the fat droplets:

- *One-step method*: The two emulsions are mixed together under conditions where the two types of droplets have opposite charges so that heteroaggregation occurs immediately (Figure 3).
- *Two-step method*: The two emulsions are mixed together under conditions where the two types of droplets have similar charges, and then the solution conditions (pH) are altered so that the droplet charges become opposite and then heteroaggregation occurs.

The nature of the heteroaggregates formed and the resulting physicochemical properties of the mixed system depend on a number of factors, including the total particle concentration, the positive-to-negative particle ratio, the size of the two types of particles, and the mixing method.[9] Recent studies have been carried out in our laboratory using two different surface-active globular proteins to coat the fat droplets: lactoferrin (LF) and β-Lg.[9,11,12,36,43,44] LF has an isoelectric point around 8.5, whereas β-Lg has an isoelectric point around 4.5 and so there is a wide range of pH values where they have opposite charges (Figure 5). Some of the major factors influencing the heteroaggregation of this system are highlighted below:

Particle Ratio. Mixed emulsions were prepared by mixing together different mass ratios of LF-coated droplets ($d \approx 300$ nm) and β-Lg coated droplets ($d \approx 300$ nm). Small aggregates were formed at low- and high-particle ratios, but very large aggregates were formed at intermediate particle ratios, which is in agreement with theoretical predictions and computer simulations.[26,28,29] The apparent shear viscosity of the mixed systems was orders of magnitude higher than that of the two original emulsions (Figure 6). Indeed, the samples at intermediate particle ratios were paste-like materials that did not flow to the bottom of the test tubes when they were inverted, whereas the original emulsions were fluid-like. Consequently, heteroaggregation may be able to produce highly viscous or gel-like food materials at much lower fat contents than is required normally.

Total Particle Concentration. The influence of total particle concentration on the apparent shear viscosity of emulsions containing only positive droplets, only negative droplets, or a mixture of positive and negative droplets has been studied.[9,11]
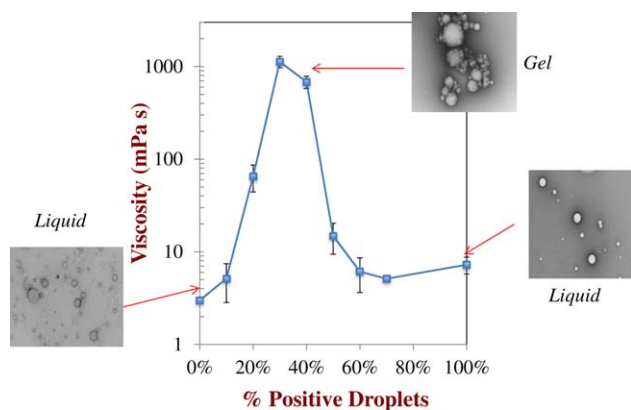
Figure 6. Influence of particle ratio on the apparent shear viscosity of mixed emulsions containing LF-coated droplets (positive) and b-LG coated droplets (negative). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

There was only a moderate increase in the viscosity of the emulsions containing a single type of droplet with increasing particle concentration from 0% to 40%. On the other hand, there was a steep increase in viscosity of mixed emulsions because of the fact that they were highly aggregated. At sufficiently high particle concentrations, a 3D network of aggregated droplets was formed that had paste-like characteristics. These systems could be characterized by a yield stress, consistency index, and flow index.[9,11]

Particle Size. The size of the particles in mixed emulsions also has a major influence on cluster formation and bulk physicochemical properties.[12] We recently carried out studies containing oppositely charged droplets with different sizes: $L(+)$, $L(-)$, $S(+)$, and $S(-)$, where L and S refer to large and small particles, respectively. Emulsions containing small droplets ($d \approx 300$ nm) were much more susceptible to heteroaggregation and gelation than those containing larger droplets ($d \approx 3000$ nm) (Figure 7). This effect was attributed to the fact that the small droplets could form a 3D network that could fill the entire volume of the container, and thereby give elastic-like properties. In addition, the droplet–droplet collision frequency will be appreciably higher for smaller droplets because of their faster diffusion rates.

Emulsifier Type. Most of the early studies on heteroaggregation were carried out using highly purified globular proteins: β-Lg and lactoferrin.[9–12] However, these proteins are too expensive for most commercial applications and therefore it is important to identify commercially viable alternatives. Recent studies have shown that heteroaggregation can be induced using widely used commercial ingredients, such as whey protein isolate (WPI), MS, and GA.[62] For example, heteroaggregation has been induced by mixing WPI-coated droplets with either MS- or GA-coated droplets at low pH. At pH values below the isoelectric point of WPI (pI $\approx$ 5), the WPI-coated droplets are positive, whereas the MS- or GA-coated droplets are negative, leading to an electrostatic attraction between them. The possibility of using these emulsifiers in commercial food applications has been examined.[62]

Emulsion Type. We recently showed that heteroaggregation could also be induced in W/O emulsions.[35] In this case, two
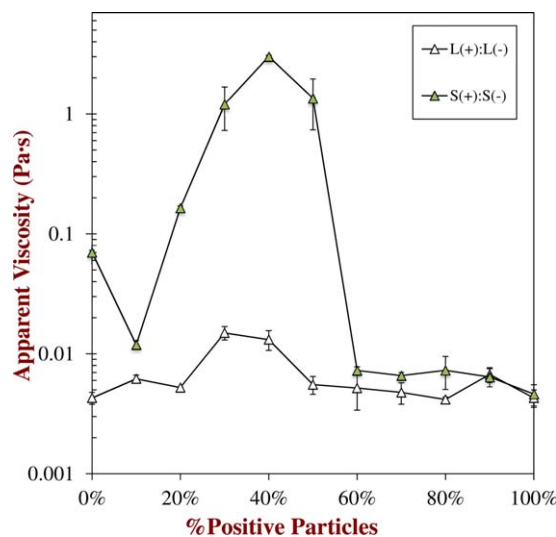

Figure 7. Influence of droplet size on the apparent shear viscosity of mixed emulsions containing small ($d \approx 300$ nm) or large ($d \approx 3000$ nm) droplets. L, Large droplets; S, Small droplets; $(+)$, cationic droplets; $(-)$, anionic droplets. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

W/O emulsions were prepared, one containing β-Lg-rich water droplets and the other containing LF-rich oil droplets. These two emulsions were then mixed together, which induced heteroaggregation between the negatively charged β-Lg-rich water droplets and the positively charged LF-rich oil droplets. As in W/O emulsions, heteroaggregation caused a pronounced increase in the apparent shear viscosity of the W/O emulsions, particularly after the mixed emulsions were heated to induce thermal denaturation and gelation of the globular proteins. This approach may therefore be suitable to application in commercial products that have an oily continuous phase, such as margarines and spreads.

PHYSICOCHEMICAL PROPERTIES OF HETEROAGGREGATED EMULSIONS

In commercial applications, a product may experience various environmental stresses during its production, storage, transport, and utilization. It is therefore important to understand how the physicochemical properties of heteroaggregated emulsions are impacted by environmental stresses such as pH, ionic strength, temperature, and mechanical stresses.

pH

The pH of the aqueous solution surrounding the droplets in mixed emulsions plays a critical role in determining their physicochemical properties. The sign and magnitude of the electrical charge on protein-based and polysaccharide-based emulsifiers usually depends on pH.[43,45,52] For example, the change in charge with pH is shown in Figure 5 for two emulsions containing protein-coated droplets with different isoelectric points. At low or high pH values, both types of emulsion droplets are either positively charged or negatively charged and therefore no heteroaggregation occurs because of the electrostatic repulsion between them. At intermediate pH values, the two types of

emulsion droplets are oppositely charged and therefore hetero-aggregation occurred, which led to a large increase in viscosity.

Ionic Strength

The ionic strength of the aqueous phase surrounding the droplets also has a major influence on the properties of heteroaggregated systems because of its influence on electrostatic interactions. As the ionic strength increases both attractive and repulsive electro-static interactions will be reduced because of electrostatic screen-ing effects.[24] The addition of low or intermediate levels of salt to a mixed emulsion containing oppositely charged droplets has been shown to reduce the viscosity of the system by promoting dissociation of heteroaggregated droplets.[43] On the other hand, the viscosity may increase again at high salt levels because homo-aggregation occurs because of reduction of the electrostatic repul-sion between similarly charged droplets.[43]

Temperature

Commercial products are often subjected to thermal processes, such as sterilization, pasteurization, or cooking, and therefore it is important to understand how temperature influences their properties. Heating protein-coated fat droplets can influence the overall physicochemical properties because of thermally induced alterations in the interfacial properties of the proteins. For example, globular proteins unfold above their thermal denatura-tion temperature, which increases the hydrophobic and disulfide bonds between proteins. As a result, the strength of droplet–droplet interactions may increase, which leads to increases in viscosity or gel formation.[11] On the other hand, fat droplets coated by random-coil proteins or polysaccharides (such as casein, GA, or MS) are relatively stable to heating, and are therefore less influenced by thermal processing.

Mechanical Stresses

The structural organization of the aggregated droplets within a mixed emulsion may be permanently altered when a mechanical stress (such as a compressive or shear force) is applied, thereby resulting in irreversible changes in physicochemical properties, such as rheology and appearance. At present, there have been few reported studies on the influence of mechanical stresses on the microstructure or rheology of heteroaggregated emulsions, but this would be an important area for future work, as this has con-siderable commercial implications for their practical application.

CONCLUSIONS

The use of heteroaggregation to control the properties of food products is relatively new, but it has great potential for certain applications. In the food area, controlling the textural characteris-tics of products is one of the main potential applications of het-eroaggregation. Inducing droplet aggregation in a product through heteroaggregation causes a large increase in viscosity and may lead to gel-like or paste-like properties. This phenomenon can be used to create food products with novel textural charac-teristics, or it can be used to reduce the fat-content of high fat food products. Another potential application of heteroaggregates in the food industry is to encapsulate multiple oil soluble com-ponents within a single system. For example, positively charged emulsions can encapsulate one kind of lipophilic component, whereas the negatively charged emulsions can be used to encap-sulate another kind of lipophilic component. These two oppo-sitely charged emulsions could then be mixed together to form microclusters. This may be useful if the two agent components would normally interact with each other, or if they needed to be released at different times or locations. Future research on the sensory evaluation of heteroaggregates and their potential biologi-cal fate would be useful for their practical applications.

REFERENCES

1. Thow, A. M.; Jan, S.; Leeder, S.; Swinburn, B. *Bull. World Health Organ.* **2010**, *88*, 609.

2. Jacobson, D.; Gance-Cleveland, B. *Obes. Rev.* **2011**, *12*, e244.

3. Stanley, W. C.; Dabkowski, E. R.; Ribeiro, R. F.; O'Connell, K. A. *Circ. Res.* **2012**, *110*, 764.

4. Abumrad, N. A.; Piomelli, D.; Yurko-Mauro, K.; Merrill, A.; Clandinin, M. T.; Serhan, C. N. *Adv. Nutr.* **2012**, *3*, 60.

5. McClements, D. J. *Curr. Opin. Colloid. Interterface* **2012**, *17*, 235.

6. Thurgood, J. E.; Martini, S. *J. Sens. Stud.* **2010**, *25*, 861.

7. Giroux, H. J.; Perreault, V.; Britten, M. *J. Food Sci.* **2007**, *72*, S125.

8. Bayarri, S.; Taylor, A. J.; Hort, J. *J. Agric. Food Chem.* **2006**, *54*, 8862.

9. Mao, Y. Y.; McClements, D. J. *Food Hydrocoll.* **2011**, *25*, 1201.

10. Mao, Y. Y.; McClements, D. J. *Food Funct.* **2012**, *3*, 1025.

11. Mao, Y. Y.; McClements, D. J. *Food Chem.* **2012**, *134*, 872.

12. Mao, Y. Y.; McClements, D. J. *J. Colloid Interface Sci.* **2012**, *380*, 60.

13. Lopez-Lopez, J. M.; Schmitt, A.; Moncho-Jorda, A.; Hidalgo-Alvarez, R., *Soft Matter.* **2006**, *2*, 1025.

14. Yates, P. D.; Franks, G. V.; Biggs, S.; Jameson, G. *J. Colloid Surf. A* **2005**, *255*, 85.

15. Piechowiak, M. A.; Videcoq, A.; Ferrando, R.; Bochicchio, D.; Pagnoux, C.; Rossignol, F. *Phys. Chem. Chem. Phys.* **2012**, *14*, 1431.

16. Han, S. J.; Daniels, E. S.; Sudol, E. D.; Dimonie, V. L.; Klein, A. *J. Appl. Polym. Sci.* **2013**, *127*, 3601.

17. Findlay, A. D.; Thompson, D. W.; Tipping, E. *Colloid Surf. A* **1996**, *118*, 97.

18. Spruijt, E.; Bakker, H. E.; Kodger, T. E.; Sprakel, J.; Stuart, M. A. C.; van der Gucht, J. *Soft Matter.* **2011**, *7*, 8281.

19. Lemmers, M.; Sprakel, J.; Voets, I. K.; van der Gucht, J.; Stuart, M. A. C. *Angew. Chem. Int. Edit.* **2010**, *49*, 708.

20. Ohsugi, A.; Furukawa, H.; Kakugo, A.; Osada, Y.; Gong, J. P. *Macromol. Rapid. Comm.* **2006**, *27*, 1242.

21. McParlane, J.; Dupin, D.; Saunders, J. M.; Lally, S.; Armes, S. P.; Saunders, B. R. *Soft Matter.* **2012**, *8*, 6239.

22. Islam, A. M.; Chowdhry, B. Z.; Snowden, M. *J. Adv. Colloid Interface* **1995**, *62*, 109.

23. Norio Ise, I. S. S. Structure Formation in Solution: Ionic Polymers and Colloidal Particles; Springer: New York, **2005**.

24. Dickinson, E. *J. Colloid Interface Sci.* **2011**, *356*, 196.

25. Piechowiak, M. A.; Videcoq, A.; Rossignol, F.; Pagnoux, C.; Carrion, C.; Cerbelaud, M.; Ferrando, R. *Langmuir* **2010**, *26*, 12540.

26. Lopez-Lopez, J. M.; Schmitt, A.; Moncho-Jorda, A.; Hidalgo-Alvarez, R. *Adv. Colloid Interface* **2009**, *147–148*, 186.

27. Meakin, P. *J. Colloid Interface Sci* **1986**, *112*, 187.

28. Cerbelaud, M.; Ferrando, R.; Videcoq, A. *J. Chem. Phys.* 1322010.

29. Cerbelaud, M.; Videcoq, A.; Abelard, P.; Pagnoux, C.; Rossignol, F.; Ferrando, R. *Soft Matter.* **2010**, *6*, 370.

30. Jia, J. J.; Jia, Z. H.; Iwata, S. *J. Colloid Interface Sci.* **2011**, *362*, 633.

31. McClements, D. J. Food Emulsions: Principles, Practice, and Techniques; CRC Press: Boca Raton, **2005**.

32. McClements, D. J.; Rao, J. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 285.

33. Muschiolik, G. *Curr. Opin. Colloid. Interterface* **2007**, *12*, 213.

34. McClements, D. *J. Annu. Rev. Food Sci.* **2010**, *T 1*, 241.

35. Iqbal, S.; Hameed, G.; Baloch, M. K.; McClements, D. J. *J. Food Eng.* **2013**, *115*, 314.

36. Mao, Y.; McClements, D. *J. Food Funct.* **2012**.

37. Dickinson, E. *Food Hydrocoll.* **2003**, *17*, 25.

38. Dalgleish, D. G.; Agboola, S. O. *Abstr. Pap. Am. Chem. Soc.* **1994**, *207*, 78.

39. Hu, M.; McClements, D. J.; Decker, E. A. *J. Agr. Food Chem.* **2003**, *51*, 1696.

40. Dickinson, E. Introduction to Food Colloids; Royal Society of Chemistry: Cambridge, **1992**.

41. McClements, D. *J. Curr. Opin. Colloid Interface* **2004**, *9*, 305.

42. Demetriades, K.; Coupland, J. N.; McClements, D. J. *J. Food Sci.* **1997**, *62*, 342.

43. Mao, Y. Y.; McClements, D. *J. Food Hydrocoll.* **2012**, *27*, 80.

44. Mao, Y.; McClements, D. J. *J. Food Sci.* **2012**, *77*, E144.

45. Tokle, T.; McClements, D. *J. Food Hydrocoll.* **2011**, *25*, 976.

46. Kim, H. J.; Decker, E. A.; McClements, D. *J. Langmuir* **2002**, *18*, 7577.

47. Dickinson, E. *Colloids Surf. B Biointerfaces* **2010**, *81*, 130.

48. Nakauma, M.; Funami, T.; Noda, S.; Ishihara, S.; Al-Assaf, S.; Nishinari, K.; Phillips, G. O. *Food Hydrocoll.* **2008**, *22*, 1254.

49. Trubiano, P. C. *ACS Sym. Ser.* **1995**, *610*, 199.

50. McClements, D. J.; Decker, E. A.; Weiss, J. *J. Food Sci.* **2007**, *72*, R109.

51. Shogren, R. L.; Viswanathan, A.; Felker, F.; Gross, R. A. *Abstr. Pap. Am. Chem. Soc.* **2000**, *220*, U111.

52. Charoen, R.; Jangchud, A.; Jangchud, K.; Harnsilawat, T.; Naivikul, O.; McClements, D. J. *J. Food Sci.* **2011**, *76*, E165.

53. Phillips, G. O.; Williams, P. A. Handbook of Hydrocolloids; CRC PressI Llc, **2000**.

54. McNamee, B. F.; O'Riordan, E. D.; O'Sullivan, M. *J. Agric. Food Chem.* **1998**, *46*, 4551.

55. Chanamai, R.; McClements, D. J. *J. Food Sci.* **2002**, *67*, 120.

56. Kralova, I.; Sjoblom, J. *J. Dispers. Sci. Technol.* **2009**, *30*, 1363.

57. Stauffer, S. E., Emulsifiers; Eagen Press: St Paul, **1999**.

58. Ziani, K.; Chang, Y. H.; McLandsborough, L.; McClements, D. J. *J. Agric. Food Chem.* **2011**, *59*, 6247.

59. Dickinson, E. *Colloids Surf. B Biointerfaces* **1999**, *15*, 161.

60. Pugnaloni, L. A.; Dickinson, E.; Ettelaie, R.; Mackie, A. R.; Wilde, P. *J. Adv. Colloid Interface* **2004**, *107*, 27.

61. Roth, S.; Murray, B. S.; Dickinson, E. *J. Agric. Food Chem.* **2000**, *48*, 1491.

62. Schmitt, C.; Kolodziejczyk, E. Protein—Polysaccharide Complexes from Basics to Food Applications, **2010**.