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Comparison of Biopolymer Emulsifier Performance in Formation and Stabilization of Orange Oil-in-Water Emulsions

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Abstract The effectiveness of various biopolymer emulsifiers at forming and stabilizing model beverage emulsions was examined: β-lactoglobulin (BLG); gum arabic (GA); conventional modified starch (MS-old); new modified starch (MS-new). Orange oil-in-water emulsions (5% oil) were prepared using high pressure homogenization. For BLG, MS-new, MS-old and GA, the minimum droplet diameters produced were 171, 254, 222 and 497 nm, while the minimum mass ratio of emulsifier-to-oil required to produce small droplets were 0.5:5, 1:5, 3:5 and 5:5, respectively. The influence of pH (3–8), ionic strength (0–500 mM NaCl, 0–50 mM $CaCl_2$) and thermal treatment (30–90 °C) on the stability of the emulsions was examined. Extensive droplet aggregation occurred in BLG-stabilized emulsions around their isoelectric point (pH $\approx$ 5), at high salt concentrations ($\geq$300 mM NaCl, $\geq$10 mM $CaCl_2$, pH 7) and at high temperatures (>70 °C, 200 mM NaCl, pH 7) due to changes in electrostatic and hydrophobic interactions. There was little effect of pH, ionic strength and temperature on emulsions stabilized by GA or MS due to strong steric (rather than electrostatic) stabilization. The new type of modified starch used in this study was capable of forming stable emulsions with small droplet sizes at low concentrations.

Keywords Emulsions · Nanoemulsions · Orange oil · Flavor oils · Whey protein · Gum arabic · Modified starch · Emulsion stability

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Introduction

Many soft drinks and beverages come in the form of oil-in-water emulsions that consist of small oil droplets dispersed within an aqueous medium [1–4]. The type of oil present within the droplets depends mainly on whether the beverage emulsion is of the "cloud" or "flavor" type [4]. The droplets in cloud emulsions are comprised primarily of non-flavor oils (e.g., vegetable oils or terpene oils), whereas the droplets in flavor emulsions are comprised of flavor oils (e.g., citrus oils) or mixtures of flavor oils and non-flavor oils. In this study, we used orange oil-in-water emulsions as model beverage emulsions. Orange oils have been used as flavoring agents for many centuries [4, 5]. Recent studies have demonstrated that phytochemicals found in orange oil may also have health-promoting effects, such as anti-carcinogenic and anti-inflammatory activities [6–8]. Emulsifying orange oil may be a convenient means of incorporating it into a wide variety of food products, thereby allowing consumers to benefit from its potential health promoting effects.

The droplets in beverage emulsions are stabilized by emulsifiers, which are surface-active molecules that rapidly adsorb to the surfaces of the oil droplets created during homogenization. The emulsifiers play two key roles in beverage emulsions: (1) facilitation of emulsion formation; (2) improvement of emulsion stability [2, 9, 10]. The size of the droplets produced during homogenization depends on how quickly the emulsifiers adsorb to the droplet surfaces formed during homogenization, how effectively they reduce the interfacial tension, and how good they are at preventing droplet aggregation within the homogenizer. The long-term stability of the resulting emulsions depends on how effective the adsorbed emulsifier layer is at

preventing droplet aggregation during storage, transport, and utilization of the final product.

A variety of emulsifiers are available for application in the food and beverage industries, including small molecule surfactants, phospholipids, proteins, and polysaccharides [11–13]. Previous studies have examined the influence of different types and combinations of synthetic surfactants on the formation and stability of model beverage emulsions [14]. Nevertheless, there is particular interest in the utilization of biopolymer emulsifiers rather than synthetic emulsifiers, since these are often perceived to be more "label friendly." Two amphiphilic polysaccharides, gum arabic and modified starch, are the most common biopolymer emulsifiers currently used in beverage emulsions [4, 15–17]. Nevertheless, research has also shown that proteins and protein/polysaccharide complexes can also be used to successfully form and stabilize model beverage emulsions [18–20].

Each type of biopolymer emulsifier has its own advantages and disadvantages for forming and stabilizing emulsions. Typically, globular proteins are capable of creating smaller emulsion droplets during homogenization than polysaccharides, but the droplets formed are more susceptible to aggregation when environmental conditions are changed, such as pH, ionic strength, and temperature [19]. Conversely, polysaccharides are capable of producing emulsions that have excellent stability to changes in environmental conditions, but they tend to form larger droplets during homogenization and have to be used at appreciably higher concentrations than proteins [19, 21]. For instance, it was shown that >12% gum arabic was required to stabilize 20% orange oil-in-water emulsions and that it was the high molecular mass protein-rich fraction that preferentially adsorbed to the oil-water interface [22, 23]. Since only a fraction of the gum arabic was involved in the emulsification process, the total concentration required to produce a stable emulsion was much higher than for protein emulsifiers [24, 25].

Recently, there have been a number of technical innovations in the production of modified starches, which has led to the commercial availability of biopolymer emulsifiers with improved functional performance. The purpose of the current research is to compare the effectiveness of globular protein, gum arabic, and two modified starch emulsifiers at forming and stabilizing model beverage emulsions. One of the modified starch emulsifiers has been commercially available for some time (PURITY GUM^{TM} 2000), whereas the other has only been introduced recently (PURITY GUM^{TM} Ultra). The information produced by this study will be useful for the selection of the most appropriate biopolymer emulsifiers for application in food and beverage emulsions.

Materials and Methods

Materials

Orange oil was supplied by a food ingredient manufacturer (Givaudan Flavors Corporation, Cincinnati, OH). Food grade β-lactoglobulin was obtained from Davisco Foods International Inc. (Le Sueur, MN). Gum arabic (GA) was donated by TIC Gums (Philadelphia, PA). Two OSA modified starch (MS) samples were donated by the National Starch and Chemical Company (Bridgewater, NJ). The modified starch (PURITY GUM^{TM} 2000) produced by the traditional approach was referred to as "MS-old," whereas the modified starch (PURITY GUM^{TM} Ultra) produced using the new approach was referred to as "MS-new." Sodium chloride, calcium chloride, sodium citrate, and sodium azide were purchased from Sigma Chemical Company (St. Louis, MO). Double-distilled water was used to prepare all solutions and emulsions.

Methods

Preparation of Oil-in-Water Emulsions

Aqueous phases were prepared by dispersing BLG, GA or MS in aqueous buffer solutions (10.0 mM sodium citrate, 0.01 wt% sodium azide, pH 3.0). Orange oil-in-water emulsions were prepared by homogenizing 5.0 wt% oil phase with 95.0 wt% aqueous phase at ambient temperature ($\approx$25 °C). An emulsion pre-mix was prepared using a high-speed blender (2 min, Biospec Products Inc., Bartlesville, OK), which was then passed through a high pressure microfluidizer (Model 101, Microfluidics, Newton, MA) three times at 9,000 psi. The influence of initial emulsifier concentration (0.1–10 wt%) on the size of the droplets produced using these homogenization conditions was determined to compare the relative efficiency of different emulsifier types on emulsion formation. After these experiments, we selected BLG, GA, and one MS (PURITY GUM^{TM} Ultra) for further study.

The Influence of Environmental Stresses on Emulsion Stability

The stability of the various emulsions to pH, ionic strength, and temperature was tested. Three 5 wt% orange oil-in-water emulsions were prepared using different emulsifier types and concentrations determined in preliminary experiments described in the "Efficiency of Emulsion Formation": BLG (0.5 wt%); GA (10.0 wt%); MS (1.0 wt%).

- *pH stability* Emulsion samples were prepared in aqueous buffer solutions, and then the pH was adjusted to the desired final value (pH 2–8) using either NaOH and/or HCl solution. Emulsion samples (10 ml) were then transferred into glass test tubes (160 × 15 mm) and stored at ambient temperature (≈25 °C) overnight prior to analysis.
- *Salt stability* Emulsions (pH 7.0) were diluted with different amounts of NaCl and buffer solution to form a series of samples with the same droplet concentration, but different salt concentrations (0–500 mM NaCl or 0–50 mM $CaCl_2$). The emulsions were stirred for 30 min and then transferred into glass test tubes (160 × 15 mm) and stored at ambient temperature overnight prior to analysis.
- *Stability to heating* Emulsions (pH 7.0) were prepared containing either 0 or 200 mM NaCl, and then 10-ml samples were transferred into glass test tubes (160 × 15 mm), which were stored in a water bath for 30 min at a fixed temperature ranging from 30 to 90 °C. The emulsion samples were then immediately placed at room temperature and stored overnight prior to analysis.

It should be noted that the salt and thermal stability of the model emulsions was tested at neutral pH in this study, which is representative of some food and beverage emulsions, e.g., nutritional beverages and non-dairy creamers. Nevertheless, it would be useful to examine the salt and thermal stability of the model emulsions at other pH values in future studies so as to better represent other categories of food products, e.g., using an acidic pH for soft drinks.

Measurement of Emulsion Stability

Particle size The particle size distribution and mean droplet diameter of diluted emulsions were measured by a commercial dynamic light-scattering device (Nano-ZS, Malvern Instruments, Worcestershire, UK). As stated by the manufacturer, this instrument measures the speed at which particles move through the solution due to Brownian motion and then uses the Stokes-Einstein equation to convert this speed into a particle size. Mean particle sizes were reported as "*Z*-average" diameters (the scattering intensity-weighted mean diameter), which were calculated from the signal intensity versus particle diameter data. Samples were diluted prior to making the particle size measurements to avoid multiple scattering effects with sodium citrate buffer (at the same pH and ionic composition as the initial sample) using a dilution factor of 1:100 sample to buffer to reach an instrument attenuation factor of ~6.

ζ-potential The electrical charge (ζ-potential) of lipid droplets in the emulsions was determined using a particle electrophoresis instrument (ZEN3600, Nano-series, Zetasizer, Malvern Instruments, Worcestershire, UK). The manufacturer states that this instrument uses a combination of laser Doppler velocimetry and phase analysis light scattering to measure particle electrophoretic mobility. A mathematical model known as the "Henry equation" is then used to convert the measured electrophoretic mobility into a ζ-potential based on the Smoluchowski approximation. Emulsions were diluted until they gave an instrument attenuation factor of ~6 using buffer solution at the same pH and NaCl concentration as the initial sample. The emulsions were agitated prior to analysis to ensure that they were homogeneous.

Creaming index Emulsion samples (10 ml) were placed in glass test tubes (16 × 150 mm) and then stored at ambient temperature for 7 days before analysis. The susceptibility of the emulsions to creaming was ascertained by measuring the height of the boundary layer between the opaque droplet-rich layer at the top and the transparent or turbid droplet-depleted layer at the bottom of the test tubes. Creaming results are reported as the Creaming Index (CI) = 100 × (height of interface)/(height of total emulsion).

Statistical analysis All measurements were performed on three freshly prepared samples and are reported as means and standard deviations.

Results and Discussion

Efficiency of Emulsion Formation

The purpose of our initial experiments was to establish the relative efficiency of the different types of biopolymer emulsifier at forming emulsions during homogenization. In particular, we aimed to determine the minimum droplet diameter (d_{min}) that could be produced by a given emulsifier under standardized homogenization conditions and to determine the minimum amount of emulsifier (C_{min}) needed to achieve a relatively small particle size. The value of d_{min} was defined as the lowest droplet size that could be produced in the emulsifier range used, whereas C_{min} was defined as the minimum emulsifier concentration required, attaining a droplet diameter within 10% of d_{min}.

Model beverage emulsions were prepared by homogenizing 5% orange oil with 95% aqueous phases containing different emulsifier types and concentrations under standardized homogenization conditions. The dependence of the mean droplet diameter (*Z*-average) of the resulting emulsions on initial emulsifier concentration was then measured 3 h after homogenization. The mean droplet diameter tended to decrease as the emulsifier concentration was increased (Fig. 1), which can be attributed to the fact

that interfaces become saturated more rapidly at higher emulsifier concentrations, and there was more emulsifier available to cover all the oil-water interfaces formed during homogenization [9, 10, 26]. Typically, the dependence of the mean droplet diameter after homogenization on the initial emulsifier concentration can be divided into two regimes: *region I*—the droplet size progressively decreases with increasing emulsifier concentration, because there is insufficient emulsifier present to cover all the newly created droplet surfaces; *region II*—the droplet size remains fairly constant, because there is more than enough emulsifier present to cover all of the droplets formed [2]. In *region I*, the droplet diameter is limited by the total amount of emulsifier available to cover the oil-water interface formed, but in *region II* the droplet diameter is limited by the maximum disruptive forces generated by the homogenizer [27, 28]. The BLG, MS-new, and MS-old appeared to follow this kind of behavior. The emulsifier concentration demarked regions I and II, and was found to be about 0.1% for BLG, 1% for MS-new, and 3% for MS-old (Fig. 1). It corresponded to the calculated C_{min} values. On the other hand, the decrease in the mean droplet diameter with increasing emulsifier concentration was more gradual for gum arabic, and no clear distinction could be made between regions I and II. In this case, C_{min} corresponded to the highest level (10%) of gum arabic used. The minimum droplet diameter that could be produced also depended on emulsifier type, e.g., $d = 171$, 254, 222, and 497 nm for BLG, MS-new, MS-old, and GA, respectively. In the remainder of the experiments we prepared model beverage emulsions using emulsifier concentrations capable of producing small droplet sizes without having too much excess emulsifier present in the aqueous phase, i.e., 0.5% BLG, 1% MS-new, and 10% GA. We did not carry out further experiments with the MS-old sample, since this required about three times more emulsifier than the MS-new sample.

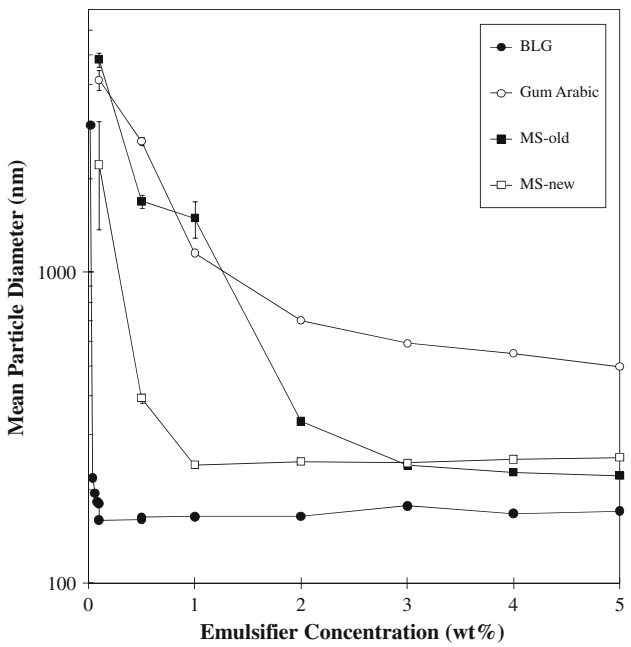

Fig. 1 Influence of emulsifier concentration on the mean particle diameter of orange oil-in-water emulsions produced by homogenization under standardized homogenization conditions

Influence of pH on Emulsion Stability

Commercially, beverage emulsions may have aqueous phases that vary in pH—although soft drinks tend to be acidic, some nutritional beverages may have pH values close to neutral. We therefore examined the influence of pH on the physicochemical properties of orange oil emulsions stabilized by BLG, GA, and MS. The mean droplet diameter (*Z*-average) of the BLG-stabilized emulsions was around 180 nm at relatively low (pH 2 and 3) and high (pH 6–8) pH values (Fig. 2a). Nevertheless, a large increase in mean particle diameter was observed around the isoelectric point ($4 < pH < 5$) of the BLG. On the other hand, the mean droplet diameter of GA-stabilized emulsions ($d \approx 600$–650 nm) and MS-stabilized emulsions ($d \approx 230$–270 nm) remained relatively constant from pH 2–8 (Fig. 2a). The particle size measurements were supported by creaming stability measurements, which indicated that BLG-stabilized emulsions were highly unstable to creaming at pH values around their p*I* but stable at higher and lower pH values, whereas GA- and MS-stabilized emulsions were stable across the entire pH range studied (data not shown). These results are in agreement with earlier studies of the pH-stability of biopolymer-stabilized oil-in-water emulsions [19, 29, 30]. The poor stability of the BLG-stabilized emulsions around the proteins isoelectric point can be attributed to a reduction in the electrostatic repulsion between the droplets [2]. The good pH-stability of the MS- and GA-stabilized emulsions can be accounted for by the fact that the lipid droplets are coated by a relatively thick layer of hydrophilic polysaccharide molecules that protrude into the aqueous phase, and hence they are largely stabilized by steric repulsion rather than electrostatic repulsion [2, 19]. In addition, the presence of the thick polysaccharide layer decreases the magnitude of the attractive van der Waals forces between the droplets [31].

The pH-dependence of the droplet ζ-potential for the three types of emulsion is shown in Fig. 2b. The ζ-potential of the BLG-stabilized droplets went from highly positive at low pH to highly negative at high pH, with a point of zero charge between pH 4 and 5. This pH dependence of the droplet charge is due to the fact that the isoelectric point (p*I*) of the adsorbed layer of BLG molecules is around pH 5 [19, 29, 30, 32]. At relatively high H^+ concentrations

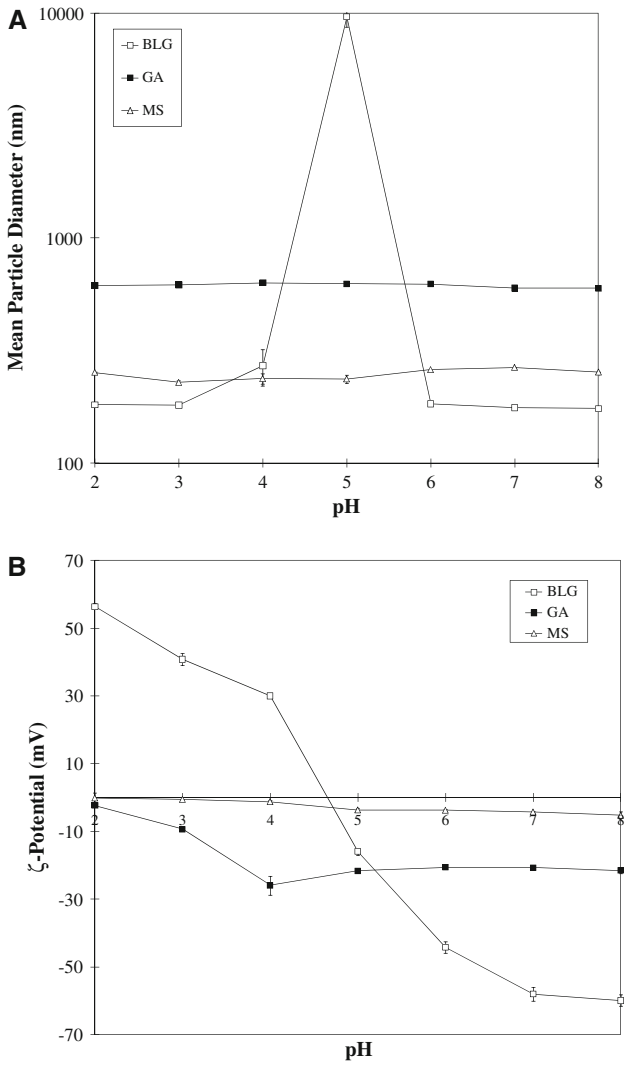

Fig. 2 **a** Influence of pH on the mean particle diameter of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or MS-new (0 mM salt). **b** Influence of pH on the droplet charge (ζ-potential) of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (0 mM salt)

(pH $\ll$ p*I*), the amino groups are positively charged ($-NH_3^+$), and the carboxyl groups are neutral (–COOH), so the net protein charge is positive. At relatively low H^+ concentrations (i.e., pH $\gg$ pI), the carboxyl groups are negatively charged ($-COO^-$), and the amino groups are neutral ($-NH_2$) so the net protein charge is negative. At the p*I*, the number of positively and negatively charged groups on the protein is balanced, and so the protein has no net charge. The interfacial layers formed by globular proteins tend to be relatively thin (a few nm thick), and so this type of biopolymer emulsifier tends to stabilize emulsions mainly by electrostatic (rather than steric) repulsion. Hence, when the protein loses its net charge around the p*I*, the stability of the droplets to aggregation is greatly reduced since the attractive van der Waals forces then dominate. The ζ-potentials of the lipid droplets coated by GA and MS were negative at all pH values (Fig. 2b), which can be attributed to the presence of some negatively charged side groups ($-COO^-$) on these polysaccharide molecules [3]. Interestingly, the GA had a much higher negative charge than the MS at all pH values, which suggests that the linear charge density of the gum arabic was higher than the modified starch. This may have important consequences for the interactions of biopolymer-coated lipid droplets with other charged species in food and beverage systems, such as transition metals that promote lipid oxidation. For example, it has been shown that negatively charged droplets attract positively charged transition metals to lipid droplet surfaces, which promotes lipid oxidation [33, 34]. There was an appreciable reduction in the negative charge on the GA- and MS-coated lipid droplets when the pH was reduced below about 5, which can be attributed to the fact that this solution pH moved around and below the pK_a values of the carboxyl groups so that they lost some of their negative charge. This result is in agreement with previous studies of the electrical charge of amphiphilic polysaccharides, which also found that their negative charge increased with increasing pH [35].

Influence of Ionic Strength on Emulsion Stability

The ionic strength of emulsified foods and beverages may vary considerably depending on the nature of the food products in which the oil droplets are present. We therefore examined the influence of ionic strength (0–500 mM NaCl; 0–50 mM $CaCl_2$) on the stability of orange oil emulsions stabilized by the three different kinds of biopolymer emulsifier. In the absence of salt, the mean droplet diameters (*Z*-average) of the emulsions were initially $\approx$180, 270, and 600 nm for BLG, MS, and GA, respectively. There was little change in the particle size of the emulsions stabilized by gum arabic or modified starch with increasing ionic strength for both NaCl (Fig. 3a) and $CaCl_2$ (Fig. 4a), which can be attributed to the fact that these emulsions are primarily stabilized by steric repulsion rather than electrostatic interactions [2]. On the other hand, there was an appreciable increase in the mean particle diameter of the BLG-stabilized emulsions at NaCl $\geq$300 mM (Fig. 3a) and $CaCl_2$ $\geq$10 mM (Fig. 4a). This droplet aggregation observed at higher salt concentrations is due to a reduction in the electrostatic repulsion between the protein-coated droplets caused by electrostatic screening and ion binding effects [2]. Above a critical salt level, the electrostatic repulsion is no longer strong enough to overcome the attractive interactions (van der Waals and hydrophobic) acting between the droplets. Visual observations of the emulsions containing different salt levels indicated that a

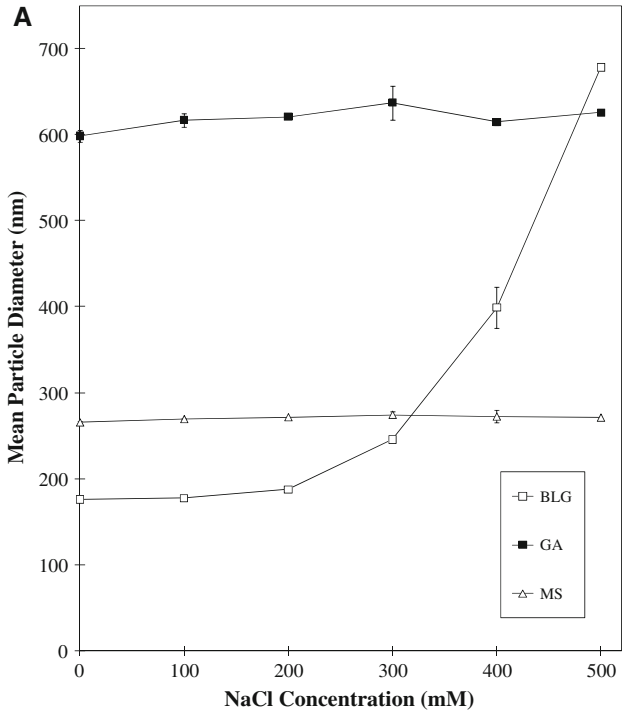

Fig. 3 **a** Influence of NaCl concentration on the mean particle diameter of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (pH 7.0). **b** Influence of NaCl concentration on the droplet charge (ζ-potential) of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (pH 7.0)

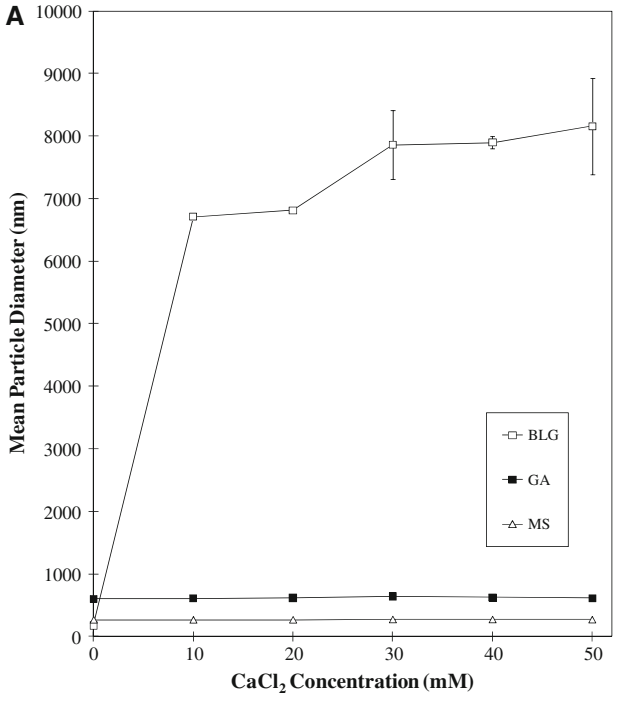

Fig. 4 **a** Influence of $CaCl_2$ concentration on the mean particle diameter of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (pH 7.0). **b** Influence of $CaCl_2$ concentration on the droplet charge (ζ-potential) of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (pH 7.0)

distinct cream layer formed on top of the BLG-stabilized emulsions at higher salt levels, but that the rest of the emulsions were relatively stable to gravitational separation (data not shown).

For the BLG stabilized emulsions, there was a decrease in the magnitude of the negative ζ-potential on the droplets with increasing salt concentration (Figs. 3b, 4b), which can be attributed to electrostatic screening and ion-binding

effects [36]. Counter-ions (Na^+ and Ca^{2+}) in the aqueous phase accumulate loosely around the negatively charged groups ($–COO^-$) on the protein surface due to electrostatic attraction, thereby screening their net charge. In the case of multivalent counter-ions (such as Ca^{2+}), the surface charge may also be reduced due to ion-binding effects, which would account for the larger initial decrease in ζ-potential observed in the BLG-stabilized emulsions for Ca^{2+} ions (Fig. 4b) than for Na^+ ions (Fig. 3b). Interestingly, there was little change in the ζ-potential of the emulsions stabilized by GA or MS with increasing salt concentration (Figs. 3b, 4b), which may be attributed to charge compensation effects, such as a change in the interfacial structure or composition with increasing ionic strength [36].

Influence of Thermal Processing on Emulsion Stability

Many emulsified food products go through some type of thermal process during their manufacture or utilization, e.g., sterilization, pasteurization, or cooking. We therefore investigated the influence of heat treatment (30–90 °C, 20 min) and salt concentration (0 or 200 mM NaCl) on the particle size (d_{43}), ζ-potential, and creaming stability of model beverage emulsions stabilized by BLG, GA, and MS at pH 7. A concentration of 200 mM NaCl was selected since the BLG-stabilized emulsion was just stable to droplet aggregation at this salt level at ambient temperature (see Fig. 3b). We postulated that these emulsions would become unstable after heating because of the resulting increase in hydrophobic attraction between the droplets. The NaCl was added to the emulsions before they were subjected to heat treatment, since this has previously been shown to have the biggest negative impact on emulsion stability for globular protein-stabilized emulsions [37, 38].

In the absence of added salt, all the emulsions were relatively stable to droplet aggregation and creaming after heat treatments with little change in mean particle diameter, and no visible evidence of phase separation was detected (data not shown). For example, after heat treatments ranging from 30 to 90 °C, the mean droplet diameters (d_{43}) of the emulsions were $\approx 169 \pm 2$, 265 ± 3, and 622 ± 9 nm for BLG, MS, and GA, respectively. In the presence of added salt (200 mM), the BLG-stabilized emulsions became unstable to droplet aggregation when they were heated above 60 °C, as demonstrated by an appreciable increase in mean particle diameter (Fig. 5) and evidence of creaming (data not shown). The instability of the BLG-emulsions to heating in the presence of salt can be attributed to thermal denaturation of the globular proteins adsorbed to the lipid droplet surfaces [37–39]. When the BLG molecules unfold, they expose non-polar groups to the surrounding aqueous phase, which increases the surface hydrophobicity of the droplets and promotes aggregation through hydrophobic attraction [38]. In addition, sulfhydryl groups are also exposed when the protein is heated above its thermal denaturation temperature, which promotes droplet-droplet aggregation through covalent disulfide bonds [40]. In the absence of salt, the electrostatic repulsion is strong enough to overcome the hydrophobic and van der Waals attraction, but in the presence of salt, the additional hydrophobic attraction associated with protein unfolding promotes droplet aggregation [2]. The GA-and MS-stabilized emulsions did not exhibit extensive droplet aggregation or creaming at either 0 or 200 mM NaCl (Fig. 5), which can be attributed to the fact that they are stabilized primarily by polysaccharides that do not unfold to expose non-polar groups at higher temperatures.

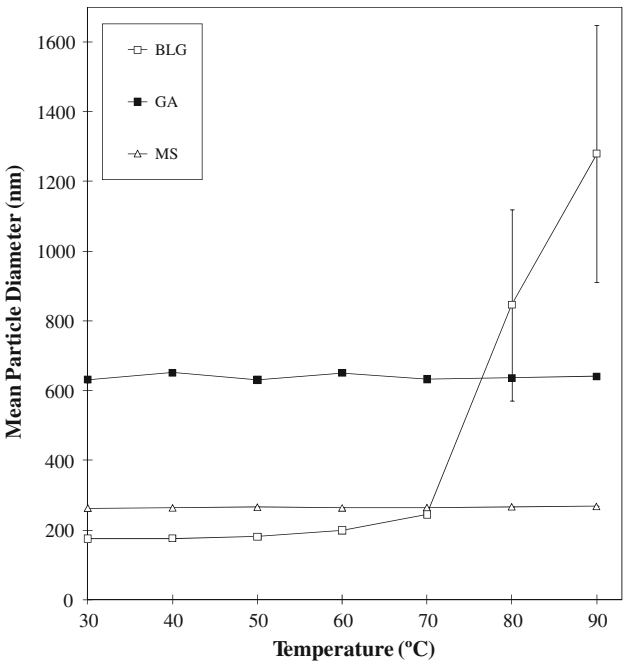

Fig. 5 Influence of holding temperature on the mean particle diameter of orange oil-in-water emulsions stabilized by β-lactoglobulin, gum arabic, or modified starch (200 mM NaCl, pH 7.0)

Conclusions

This study has characterized the influence of biopolymer emulsifier type (BLG, MS, GA) on the formation and stability of model beverage emulsions. The minimum droplet size achievable (d_{min}) and the minimum amount of emulsifier-to-oil (R_{min}) required to produce small droplets depended on emulsifier type. For BLG, MS-new, MS-old, and GA: d_{min} values were 171, 254, 222, and 497 nm and R_{min} values were 1:10, 1:5, 3:5, and 1:1, respectively. Although the protein emulsifier (BLG) was better at

producing small droplets at low emulsifier concentrations during homogenization than the two polysaccharides (MS and GA), it was much more susceptible to droplet aggregation when environmental or solution conditions were altered. Extensive droplet aggregation occurred in the BLG-stabilized emulsions around their isoelectric point (pH 5), at high salt concentrations ($\geq$300 mM NaCl, $\geq$10 mM $CaCl_2$, pH 7), and at high temperatures (>70 °C, pH 7, 200 mM NaCl), which was attributed to changes in electrostatic and hydrophobic interactions between droplets. On the other hand, there was little change in particle size when emulsions stabilized by either GA or MS were exposed to changes in pH, ionic strength, or temperature, which was attributed to strong steric (rather than electrostatic) stabilization. This study showed that model beverage emulsions with good stability to a range of environmental stresses and solution conditions could be formed using food grade biopolymers. In particular, we demonstrated that a new form of modified starch can form stable emulsions with small droplet diameters ($d < 300$ nm) at relatively low emulsifier-to-oil levels (1:5). The information generated in this study will be useful for the selection of the biopolymer emulsifiers for use in food and beverage emulsions.

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References

1. Given PS (2009) Encapsulation of flavors in emulsions for beverages. Curr Opin Colloid Interface Sci 14:43–47
2. McClements DJ (2005) Food emulsions: principles, practice, and techniques. CRC Press, Boca Raton
3. Tan C (1998) Beverage flavor emulsion—a form of emulsion liquid membrane microencapsulation. In: Contis ET (ed) Food flavors: formation, analysis and packaging influences. Elsevier, New York, p 29
4. Tan C (2004) Beverage emulsions. In: F. S, L. K and S. J Food emulsions. Marcel Decker, New York
5. Perez-Cacho PR, Rouseff RL (2008) Fresh squeezed orange juice odor: a review. Crit Rev Food Sci Nutr 48:681–695
6. Dong P, Qiu PJ, Zhu Y, Li SM, Ho CT, McClements DJ, Xiao H (2010) Simultaneous determination of four 5-hydroxy polymethoxyflavones by reversed-phase high performance liquid chromatography with electrochemical detection. J Chromatogr A 1217:642–647
7. Xiao H, Yang CS, Li SM, Jin HY, Ho CT, Patel T (2009) Monodemethylated polymethoxyflavones from sweet orange (*Citrus sinensis*) peel inhibit growth of human lung cancer cells by apoptosis. Mol Nutr Food Res 53:398–406
8. Manthey JA, Bendele P (2008) Anti-inflammatory activity of an orange peel polymethoxylated flavone. 3′,4′,3,5,6,7,8-heptamethoxyflavone, in the rat carrageenan/paw edema and mouse lipopolysaccharide-challenge assays. J Agric Food Chem 56:9399–9403
9. Walstra P (1993) Principles of emulsion formation. Chem Eng Sci 48:333
10. Walstra P (2003) Physical chemistry of foods. Marcel Decker, New York
11. Kralova I, Sjoblom J (2009) Surfactants used in food industry: a review. J Dispers Sci Technol 30:1363–1383
12. Brazel CS (1999) Microencapsulation: offering solutions for the food industry. Cereal Food World 44:388–393
13. Stauffer S (1999) Emulsifiers. Eagen Press, St Paul
14. Edris AE (1998) Preparation and stability of a protein stabilized orange oil-in-water emulsion. Nahrung Food 42:19–22
15. Trubiano PC (1995) The role of specialty food starches in flavor encapsulation. Flavor Technol 610:244–253
16. Garti N, Leser ME (2001) Emulsification properties of hydrocolloids. Polym Adv Technol 12:123–135
17. Garti N (1999) Hydrocolloids as emulsifying agents for oil-in-water emulsions. J Dispers Sci Technol 20:327–355
18. Klein M, Aserin A, Svitov I, Garti N (2010) Enhanced stabilization of cloudy emulsions with gum Arabic and whey protein isolate. Colloids Surf. B Biointerfaces 77:75–81
19. Chanamai R, McClements DJ (2002) Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, $CaCl_2$ and temperature. J Food Sci 67:120–125
20. Harnsilawat T, Pongsawatmanit R, McClements DJ (2006) Stabilization of model beverage cloud emulsions using protein–polysaccharide electrostatic complexes formed at the oil–water interface. J Agric Food Chem 54:5540–5547
21. Tse KY, Reineccius GA (1995) Methods to predict the physical stability of flavor—cloud emulsion. In: Ho CT, Tan CT, Tong CH (eds) Flavor technology—physical chemistry, modification, and process, pp 172–182
22. Randall RC, Phillips GO, Williams PA (1988) The role of the proteinaceous component on the emulsifying properties of gum arabic. Food Hydrocolloids 2:131–140
23. Williams PA, Phillips GO, Randall RC (1990) Structure–function relationships of gum arabic. In: Gums and stabilisers for the food industry. Oxford University Press, Oxford, p 25
24. Phillips GO, Williams PA (2000) Gum arabic. In: Handbook of hydrocolloids. CRC Press, p 165
25. Stephen AM, Phillips GO, Williams PA (2005) Gums and mucilages. In: Food polysaccharides and their applications. Taylor & Francis, London, p 469
26. Jafari SM, Assadpoor E, He YH, Bhandari B (2008) Re-coalescence of emulsion droplets during high-energy emulsification. Food hydrocolloids 22:1191–1202
27. Tcholakova S, Denkov ND, Danner T (2004) Role of surfactant type and concentration for the mean drop size during emulsification in turbulent flow. Langmuir 20:7444–7458
28. Tcholakova S, Denkov ND, Sidzhakova D, Ivanov IB, Campbell B (2003) Interrelation between drop size and protein adsorption at various emulsification conditions. Langmuir 19:5640–5649
29. Kulmyrzaev A, Chanamai R, McClements DJ (2000) Influence of pH and $CaCl_2$ on the stability of dilute whey protein stabilized emulsions. Food Res Int 33:15–20
30. Demetriades K, Coupland JN, McClements DJ (1997) Physical properties of whey protein stabilized emulsions as related to pH and NaCl. J Food Sci 62:342–347
31. Guzey D, McClements DJ (2007) Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by beta-lactoglobulin–pectin complexes. J. Agric. Food Chem. 55:475–485
32. Demetriades K, McClements DJ (1998) Influence of pH and heating on physicochemical properties of whey protein-stabilized emulsions containing a nonionic surfactant. J Agric Food Chem 46:3936–3942

33. Hu M, McClements DJ, Decker EA (2003) Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. J Agric Food Chem 51:1435–1439
34. McClements DJ, Decker EA (2000) Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci 65:1270–1282
35. Acedo-Carrillo JI, Rosas-Durazo A, Herrera-Urbina R, Rinaudo M, Goycoolea FM, Valdez MA (2006) Zeta potential and drop growth of oil in water emulsions stabilized with mesquite gum. Carbohydr Polym 65:327–336
36. Israelachvili J (1992) Intermolecular and surface forces, 2nd edn. Academic Press, London
37. Kim HJ, Decker EA, McClements DJ (2002) Role of postadsorption conformation changes of beta-lactoglobulin on its ability to stabilize oil droplets against flocculation during heating at neutral pH. Langmuir 18:7577–7583
38. Kim HJ, Decker EA, McClements DJ (2005) Influence of protein concentration and order of addition on thermal stability of beta-lactoglobulin stabilized *n*-hexadecane oil-in-water emulsions at neutral pH. Langmuir 21:134–139
39. Kim HJ, Decker EA, McClements DJ (2004) Comparison of droplet Flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin at pH 3 and 7. Langmuir 20:5753–5758
40. Monahan FJ, McClements DJ, German JB (1996) Disulfide-mediated polymerization reactions and physical properties of heated WPI-stabilized emulsions. J Food Sci 61:504–509