1 **Formation and Stabilization of Nanoemulsion-Based Vitamin E**
2 **Delivery Systems using Natural Biopolymers: Whey Protein**
3 **Isolate and Gum Arabic**

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24 **Abstract**

25 Natural biopolymers, whey protein isolate (WPI) and gum arabic (GA), were
26 used to fabricate emulsion-based delivery systems for vitamin E-acetate. Stable
27 delivery systems could be formed when vitamin E-acetate was mixed with
28 sufficient orange oil prior to high pressure homogenization. WPI ($d = 0.11\ \mu\text{m}$,
29 1% emulsifier) was better than GA ($d = 0.38\ \mu\text{m}$, 10% emulsifier) at producing
30 small droplets at low emulsifier concentrations. However, WPI-stabilized
31 nanoemulsions were unstable to flocculation near the protein isoelectric point (pH
32 5.0), at high ionic strength ($> 100\ \text{mM}$), and at elevated temperatures ($> 60\ ^\circ\text{C}$),
33 whereas GA-stabilized emulsions were stable. This difference was attributed to
34 differences in emulsifier stabilization mechanisms: WPI by electrostatic repulsion;
35 GA by steric repulsion. These results provide useful information about the
36 emulsifying and stabilizing capacities of natural biopolymers for forming food-
37 grade vitamin-enriched delivery systems.

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39 **Keywords:** nanoemulsions; Vitamin E; whey protein isolate; gum arabic; orange
40 oil; stability

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43 **1. Introduction**

44 There is considerable interest in fortifying foods and beverages with oil-
45 soluble vitamins so as to improve human health and wellness (Velikov & Pelan,
46 2008). Vitamin E refers to a group of oil-soluble bioactive compounds that
47 includes various tocopherols and tocotrienols (Zigoneanu, Astete, & Sabliov,
48 2008). The most nutritionally important form of vitamin E is α -tocopherol, and so
49 there has been great interest in incorporating this compound into food products.
50 Nevertheless, there are numerous challenges associated with incorporating α -
51 tocopherol into functional food and beverage products, due to its poor water-
52 solubility, chemical instability (to oxygen, light, and heat), and variable oral
53 bioavailability. The poor chemical stability of α -tocopherol can be largely
54 overcome by using the esterified form, *i.e.* α -tocopherol acetate (Mayer, Weiss, &
55 McClements, 2013; Zigoneanu, Astete, & Sabliov, 2008). Many of the other
56 challenges associated with using α -tocopherol can be overcome by incorporating
57 it into colloidal delivery systems that can be dispersed into aqueous-based food
58 and beverage products (Garti & Yuli-Amar, 2008; Saberi, Fang, & McClements,
59 2013; Velikov & Pelan, 2008).

60 Emulsion-based delivery systems, such as emulsions and nanoemulsions, are
61 good candidates to deliver lipid-soluble bioactive compounds since they can be
62 produced using natural food ingredients using simple production methods, and
63 may be designed to increase water-dispersion and bioavailability (Huang, Yu, &
64 Ru, 2010). For applications in the food industry, emulsion-based delivery systems
65 must be stabilized by some form of food-grade emulsifier, such as proteins,
66 polysaccharides, phospholipids, or surfactants (Chu, et al., 2007; Kralova &
67 Sjoblom, 2009; McClements, 2011; Mirhosseini, et al., 2008). Each of these

68 emulsifiers has its own advantages and disadvantages for particular applications,
69 and therefore it is important to establish the most appropriate emulsifier for
70 specific products.

71 Consumers are increasingly demanding “clean” labels on food and beverage
72 products, and therefore it is important to develop emulsion-based delivery systems
73 using natural (rather than synthetic) emulsifiers. In a previous study, we
74 characterized the ability of two natural small molecule surfactants (lecithin and
75 quillaja saponin) to form and stabilize emulsion-based delivery systems
76 containing Vitamin E (Ozturk, et al., 2014). In this manuscript, we focus on the
77 ability of two natural biopolymers, gum arabic (GA) and whey protein isolate
78 (WPI), to produce emulsion-based delivery systems for the same vitamin.

79 Gum arabic is the most widely used amphiphilic polysaccharide to stabilize
80 beverage emulsions (Tan, 2004). It is derived from the acacia Senegal tree and
81 contains at least three high molecular weight fractions (Williams & Phillips,
82 2009). The emulsifying activity of gum arabic at oil-water interfaces can be
83 attributed to the hydrophobic protein fraction that is covalently linked to
84 hydrophilic polysaccharide structures. The hydrophobic part anchors the gum
85 arabic molecules to the droplets surfaces, while the hydrophilic part provides
86 stability against droplet aggregation through steric and electrostatic repulsion
87 (Chanamai & McClements, 2002; Charoen, et al., 2011; Williams & Phillips,
88 2009). Whey protein isolate is frequently used as a natural emulsifier in foods and
89 beverages to facilitate the formation and stabilization of oil-in-water emulsions
90 (Adjonu, et al., 2014). WPI is a mixture of different globular proteins, with β -
91 lactoglobulin being the most dominant followed by α -lactalbumin (Morr & Ha,

1993). WPI forms relatively thin interfacial layers that mainly stabilize emulsions against flocculation through electrostatic repulsion, and is therefore particularly sensitive to pH and ionic strength (McClements, 2004). WPI-stabilized emulsions are also highly sensitive to thermal processing due to interfacial protein denaturation at elevated temperatures. Whey protein isolate is therefore only suitable for use in products where the composition and environmental conditions favor a stable product (Chanamai & McClements, 2002; Charoen, et al., 2011).

Two of the most important types of emulsion-based delivery system are nanoemulsions ($d < 200$ nm) and emulsions ($d > 200$ nm), which are distinguished in terms of their particle dimensions (McClements, 2012). For certain applications there are advantages to using nanoemulsions over emulsions. Nanoemulsions tend to have high optical clarity, greater physical stability, and better oral bioavailability (Hatanaka, et al., 2010; Huang, Yu, & Ru, 2010; Salvia-Trujillo, et al., 2013). Nanoemulsions and emulsions can be produced using either low-energy (*e.g.* spontaneous emulsification) or high-energy methods (*e.g.* high pressure homogenization) (McClements, 2012). A major advantage of high-energy methods is that they can produce fine droplets at much lower emulsifier-to-oil ratios than low-energy methods (Yang, et al., 2012).

Food biopolymers have different emulsifying abilities due to differences in their molecular structures that affect their surface adsorption characteristics during homogenization, and their ability to stabilize droplets once formed. We therefore examined the major factors influencing the initial size of the droplets in oil-in-water nanoemulsions produced by high pressure homogenization, *i.e.*, oil phase composition, emulsifier type, and emulsifier concentration. Since foods may be exposed to harsh conditions during food processing, storage, transportation, and

utilization we also examined the influence of various environmental stresses on their stability, *i.e.*, pH, ionic strength, and temperature. Finally, we compared the emulsifying and stabilizing properties of the two biopolymers (Gum arabic and whey protein isolate) to two small molecule surfactants (Lecithin and Q-Naturale) characterized previously (Ozturk, et al., 2014). In this research, the oil phase consisted of a mixture of vitamin E acetate and orange oil, which would be suitable for utilization in the beverage industry. This flavor oil was used to reduce the interfacial tension and viscosity, and thereby facilitate emulsion formation (Piorkowski & McClements, 2014). This study has important implications for the utilization of natural emulsifiers in the development of label-friendly emulsion-based delivery systems for oil-soluble vitamins and other lipophilic nutraceuticals.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was kindly provided by Davisco Foods International Inc. (Le Sueur MN). Gum arabic (GA) was provided by TIC Gums (Belcamp, MD). Orange oil (10×) was provided by International Flavors and Fragrances (Union Beach, NJ). Vitamin E acetate was obtained from BASF (Florham Park, NJ). Sodium phosphate monobasic, sodium phosphate dibasic, and sodium chloride were purchased from Sigma-Aldrich Co. (St. Louis, MO). Double distilled water was used to prepare all solutions and emulsions.

2.2. Emulsion preparation

Oil-in-water nanoemulsions were prepared by homogenizing 10% (w/w) of lipid phase (orange oil and vitamin E-acetate) with 90% (w/w) of aqueous phase using the well-established two-step procedure (McClements, 2015). The aqueous

141 phase consisted of emulsifier (WPI or GA) and buffer solution (10 mM sodium
142 phosphate, pH 7.0). The oil phase consisted of different ratios of orange oil and
143 vitamin E-acetate (0 – 100% vitamin). Emulsifiers were dissolved in double
144 distilled water to ensure complete solubilization and then 80 mM sodium
145 phosphate (pH 7.0) buffer was added to attain 10 mM buffer in the final volume.
146 Coarse emulsions were prepared by blending both lipid and aqueous phase
147 together using a high-speed blender (Bamix, Switzerland) for 2 min at room
148 temperature and then they were passed through a high pressure homogenizer
149 (Microfluidics M110L, Newton, MA, USA) for 3 cycles at 12,000 psi in order to
150 obtain fine emulsions.

151 2.3. *Emulsion stability testing*

152 The stability of the emulsion-based delivery systems to environmental
153 stresses that might occur during industrial utilization was tested.

154 2.3.1. *Influence of pH*

155 Emulsions containing 10% (w/w) oil phase (5% VE and 5% orange oil) and
156 90% (w/w) aqueous phase (2% WPI, 10% GA in 10 mM Na-phosphate buffer, pH
157 7.0) were prepared as described in *Section 2.2*. Freshly produced emulsions were
158 placed in 20 ml beakers and each sample was adjusted to a different pH value (2
159 to 8) using HCl or NaOH solutions at various concentrations. Finally, the samples
160 were transferred to small test tubes and stored at ambient temperature for 24 h
161 prior to particle size, charge, and creaming stability measurements.

162 2.3.2. *Influence of ionic strength*

163 Freshly produced emulsion-based delivery systems containing 2% WPI or
164 10% GA (pH 7) as emulsifier were transferred into glass test tubes and

165 appropriate amounts of 1M NaCl solution or buffer solution were added to each
166 tube to obtain samples with a range of different final salt concentrations (100,
167 200, 300, 400 and 500 mM). The samples were then vortexed and stored for 24 h
168 at ambient temperature prior to particle size, charge, and creaming stability
169 measurements.

170 2.3.3. *Influence of temperature*

171 Freshly produced nanoemulsions containing 2% WPI or 10% GA (pH 7)
172 emulsifier were transferred into glass test tubes that were then placed into water
173 baths set at different temperatures (30-90 °C) for 30 min, and then cooled to room
174 temperature. They were then mixed and stored for 24 h at ambient temperature
175 prior to particle size measurements and creaming stability observations.
176 Emulsions containing salt (150 mM NaCl) were also treated at the same
177 temperatures to observe the effect of salt addition on the thermal stability of the
178 emulsions.

179 2.4. *Determination of particle size*

180 The particle size of the delivery systems was measured using a static light
181 scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK). The
182 particle size of each sample was represented as the surface-weighted mean
183 diameter (d_{32}), which was calculated from the full particle size distribution.

184 2.5. *Determination of ζ -potential*

185 The electrical charge (ζ -potential) on the droplets in the delivery systems was
186 measured using particle microelectrophoresis (Zetasizer Nano ZS-90, Malvern
187 Instruments, Worcestershire, UK). Samples were diluted with buffer solutions at
188 appropriate pH prior to measurements so as to avoid multiple scattering effects.

189 2.6. Statistical analysis

190 Each experiment was performed at least twice with three measurements per
191 sample, and all results are reported as the average and standard deviation of these
192 six measurements.

193 3. Results and discussion

194 3.1. Influence of emulsifier type and concentration on particle size

195 Manufacturers of emulsion-based food products have to choose the most
196 appropriate emulsifier for specific applications. We therefore examined the
197 influence of emulsifier type and concentration on the formation of emulsion-based
198 delivery systems for two natural biopolymers: gum arabic and whey protein
199 isolate. Initially, a series of aqueous phases were prepared by dissolving different
200 amounts (0.1 to 10 % (w/w)) of either GA or WPI in aqueous buffer solutions (10
201 mM sodium phosphate buffer, pH 7.0). Emulsions were then formed by
202 homogenizing 90% (w/w) aqueous phase with 10% (w/w) lipid phase (5% VE +
203 5% orange oil). The mean particle diameter and particle size distributions were
204 then measured after the samples were stored for 1 week at ambient temperature.

205 As expected, mean particle diameter (d_{32}) decreased with increasing
206 emulsifier concentration for both WPI- and GA-stabilized emulsions (Fig. 1a).
207 The amount of emulsifier needed to create small droplets was considerably higher
208 for GA than for WPI, *e.g.*, about 10% GA was needed to achieve $d_{32} = 384$ nm,
209 but only 1% WPI was needed to achieve $d_{32} = 110$ nm. WPI- and GA-stabilized
210 emulsions formed at the same homogenization conditions (12,000 psi, 3 passes)
211 have different particle sizes at all emulsifier concentrations, which suggests that

212 the droplet size was limited by emulsifier type (rather than homogenization
213 conditions).

214 The observed differences in the minimum amount of emulsifier required to
215 attain small droplets ($c_{\min}$), and in the minimum diameter of the droplets produced
216 ($d_{\min}$) can be attributed to differences in the nature of the two emulsifiers. GA
217 leads to relatively high $c_{\min}$ and $d_{\min}$ values because (i) it contains large molecules
218 that adsorb slowly to the droplet surfaces; (ii) it has a relatively low surface-
219 activity; and (iii) it forms thick interfacial layers (Chanamani, & McClements,
220 2001; Djordjevic, Cercaci, Alamed, McClements, & Decker, 2008). In contrast,
221 WPI gives relatively low $c_{\min}$ and $d_{\min}$ values because (i) it contains small
222 molecules that adsorb rapidly to the droplet surfaces; (ii) it has a relatively high
223 surface-activity; and (iii) it forms thin interfacial layers (Charoen, Jangchud,
224 Jangchud, Harnsilawat, Naivikul & McClements 2011). Multimodal particle size
225 distributions were observed below 1% WPI and 1% GA, but monomodal ones
226 were observed at higher emulsifier concentrations (Figs. 1b and 1c). The observed
227 decrease in droplet diameter with increasing emulsifier concentration can be
228 attributed to various factors: faster emulsifier adsorption to oil droplet surfaces
229 during homogenization leads to lower interfacial tensions thereby facilitating
230 droplet breakup; more emulsifier available to cover the droplet surfaces formed
231 during homogenization (Jafari, et al., 2008; Ziani, et al., 2011). From a practical
232 point of view, it is usually advantageous to use the least amount of emulsifier
233 required to form stable emulsions, since this reduces costs, off-flavors, and
234 toxicity. In addition, high concentrations of non-adsorbed emulsifier may
235 decrease emulsion stability by promoting Ostwald ripening or droplet flocculation

236 (Klang & Valenta, 2011; McClements, 1994; Weiss, Cancelliere, & McClements,
237 2000).

238 At low emulsifier concentrations, we observed visible phase separation of the
239 emulsions after storage, which was attributed to droplet aggregation and
240 creaming. A distinct cream layer was observed on top of emulsions containing <
241 5 % (w/w) GA and < 0.5 % (w/w) WPI (data not shown), which is due to the
242 relatively large dimensions of the droplets in these systems. However, negligible
243 or no creaming was observed at higher emulsifier concentrations in both
244 emulsions, which can be attributed to the small droplet size in these systems
245 (McClements, 2012). The creaming rate is proportional to the square of the
246 droplet diameter, and so a reduction in droplet size decreases the susceptibility of
247 an emulsion to gravitational separation. In addition, the gravitational forces
248 become comparable to Brownian motion effects for sufficiently small droplets
249 (McClements, 2012). Brownian forces favor an even distribution of droplets
250 throughout the system, and therefore can counteract the gravitational forces that
251 favor an uneven vertical distribution of droplets.

252 When the size of the droplets is limited by the amount of emulsifier present
253 (rather than homogenization conditions), the minimum emulsifier concentration
254 required to cover all the droplets produced is given by (McClements, 2007):

$$c_s = \frac{6 \cdot \Gamma \cdot \phi}{d_{32min}} \quad (1)$$

255 Here, c_s is the concentration of surfactant in the emulsion (kg m^{-3}), Γ is the
256 surface load of the emulsifier at saturation (kg m^{-2}), ϕ is the disperse phase
257 volume fraction (dimensionless), and d_{32min} is the smallest droplet diameter that

can be produced when the droplets are saturated with emulsifier (m). Equation 1 indicates that the amount of emulsifier required to produce small droplets increases as the surface load of the emulsifier increases. This equation was used to calculate the effective surface load of the two emulsifiers based on the known droplet concentration ($\phi \approx 0.1$), and measured mean particle diameters $d_{32min} \approx 110$ and 384 nm at 1% WPI and 10% GA, respectively. These calculations suggest that the surface load of WPI ($\Gamma \approx 1.83$ mg m⁻²) is about 35 times smaller than that of GA ($\Gamma \approx 64$ mg m⁻²), which may be due to differences in interfacial thickness and packing of the molecules at the oil-water interface. Gum arabic forms much thicker layers than whey protein at oil-water interfaces. It should also be noted that this equation assumes that all of the emulsifier adsorbs to the droplet surfaces during homogenization, and that adsorption occurs extremely quickly so re-coalescence is avoided. In the case of gum arabic, it is likely that an appreciable fraction of the emulsifier did not adsorb to the droplet surfaces due to its low surface activity, and that adsorption was not fast enough to prevent re-coalescence within the homogenizer. Thus, the calculated surface load for gum arabic should be treated with some caution.

These experiments indicated that WPI could be used to form food-grade nanoemulsions ($d < 200$ nm), whereas GA could only be used to form emulsions.

3.2. Influence of oil composition on particle size

In this series of experiments we examined the influence of oil phase composition on the droplet size of vitamin-fortified emulsion-based delivery systems prepared using WPI and GA. Oil phase composition was varied by mixing different ratios of VE and orange oil prior to homogenization. This

282 procedure was carried out because VE is a highly viscous liquid that is difficult to
283 homogenize alone, and so we blended it with a low viscosity food-grade oil
284 (orange oil) to facilitate homogenization. Emulsions containing 10 % oil phase
285 and 90 % aqueous phase (2 % WPI or 10% GA, 10 mM sodium phosphate buffer,
286 pH 7.0) were produced by high pressure homogenization (12,000 psi, 3 passes).

287 Mean particle diameters (Fig. 2) and particle size distributions (not shown)
288 were measured after 1 week storage of the emulsions at ambient temperature. In
289 WPI-stabilized emulsions, the mean particle diameter (d_{32}) initially decreased
290 with increasing VE, reached a minimum value around 50% VE, and then
291 increased upon further VE addition. In GA-stabilized emulsions, d_{32} initially
292 decreased with increasing VE, reached a minimum value around 80% VE, but
293 then increased for pure VE (**Fig. 2**). In addition, a distinct cream layer was
294 observed on top of the emulsions containing 100% VE after storage, whereas the
295 other emulsions appeared homogeneous.

296 The observation of a minimum droplet size at a particular vitamin E
297 acetate concentration can be attributed to two effects: Ostwald ripening and
298 droplet disruption within the homogenization. At low VE levels (high orange oil
299 levels), the emulsions are susceptible to Ostwald ripening due to the appreciable
300 water-solubility of orange oil, leading to rapid droplet growth after
301 homogenization (Shimazaki, et al., 1993). Adding a highly water-insoluble
302 substance, such as VE, to the oil phase prior to homogenization inhibits droplet
303 growth due to a compositional ripening effect that opposes Ostwald ripening
304 (Ziani, et al., 2011). Thus, increasing the VE level in the oil phase initially leads
305 to the formation of smaller droplets. However, if the VE level is increased too
306 much, then the viscosity of the oil phase becomes so great that droplet disruption

307 within the homogenizer becomes inefficient, leading to larger droplets that are
308 susceptible to creaming (Jafari, et al., 2008). Consequently, an intermediate oil
309 phase composition is required to form small and stable oil droplets.

310 Monomodal particle size distributions were observed when the oil phase
311 contained ≤ 80 % (w/w) vitamin E acetate for the GA-stabilized emulsions and
312 from 20 to 60 % (w/w) VE for the WPI-stabilized emulsions (data not shown). On
313 the other hand, broad bimodal distributions were observed at other oil phase
314 compositions. The emulsions that contained relatively large droplets were highly
315 susceptible to creaming. Indeed, they separated into a white cream layer at the top
316 and a cloudy serum layer at the bottom after 1 week storage.

317 In summary, we have shown that oil phase composition strongly affected the
318 formation and stability of the emulsion-based delivery systems, and that small
319 stable droplets can be produced at relatively high vitamin E acetate levels (60-
320 80%), which means that a high vitamin loading capacity can be achieved.

321 *3.3. Influence of environmental conditions on emulsion stability*

322 Emulsion-based delivery systems may experience a variety of environmental
323 stresses (*e.g.* pH, ionic strength, or temperature changes) during food processing,
324 storage, transportation, and utilization. It is therefore important to establish the
325 influence of these environmental stresses on emulsion stability. In this section, we
326 examined the influence of pH, ionic strength, and temperature on the physical
327 stabilities of 2% WPI-stabilized nanoemulsions and 10% GA-stabilized
328 emulsions. All the systems contained 5% VE and 5% orange oil in the lipid
329 phase, which was a level where small stable droplets could initially be fabricated.

330 3.3.1. *Influence of pH*

331 The influence of pH on mean particle diameter, ζ -potential, and stability of
332 the emulsions were examined. The WPI-stabilized nanoemulsions contained
333 relatively small droplets that were visibly stable to creaming at pH values from 2
334 to 5 and from 6 to 8 (**Fig. 3a**). The mean particle diameter increased appreciably
335 at pH 5 and the nanoemulsion became highly unstable to phase separation with a
336 transparent serum layer being observed at the bottom of the test tubes after
337 storage. The 10% GA-stabilized emulsions were stable to droplet aggregation and
338 creaming across the entire pH range.

339 To provide some insight into the origin of emulsion instability we measured
340 the electrical characteristics of the oil droplets as a function of pH (Fig. 3b). For
341 WPI-stabilized emulsions, the electrical charge was highly positive well below the
342 isoelectric point ($pI \approx 5$) and highly negative well above it. WPI forms thin
343 interfacial layers and therefore primarily stabilizes emulsions by electrostatic
344 repulsion (rather than by steric repulsion). Thus, a decrease in the magnitude of
345 the droplet charge when the pH is close to the isoelectric point of the adsorbed
346 proteins causes instability. At this pH, the van der Waals attraction dominates the
347 electrostatic repulsion thereby leading to droplet flocculation. Conversely, gum
348 arabic stabilizes emulsions primarily by steric repulsion (rather than electrostatic
349 repulsion) due to its ability to form thick hydrophilic layers around the oil
350 droplets. The charge of the GA-coated oil droplets was highly negative at pH 8,
351 but became increasingly less negative as the aqueous phase pH was reduced to 2
352 (**Fig. 3b**). The reduction of negative charge from pH 5 to 2, can be attributed to
353 protonation of the carboxylic acid groups on the polysaccharide ($-\text{COOH}$). Even
354 though droplet charge may not be critical for the stability of the GA-coated oil

355 droplets, it may be important for their chemical stability, as anionic droplets can
356 attract cationic transition metals (*e.g.*, Fe^{2+}) to their surfaces, which can promote
357 oxidation reactions.

358 3.3.2. *Influence of ionic strength*

359 Food and beverages may include different concentrations of salt, therefore we
360 examined the influence of ionic strength on the stability of vitamin E
361 nanoemulsions formed using WPI and GA as emulsifiers.

362 Visible observation of the WPI-stabilized nanoemulsions indicated that a thin
363 cream layer was present at the top of the samples at > 200 mM NaCl. On the other
364 hand, no cream layer was observed on top of the 10% GA-stabilized emulsions
365 (data not shown). Surprisingly, particle sizes determined by static light scattering
366 indicated that there was little particle aggregation in WPI-stabilized
367 nanoemulsions or GA-stabilized emulsions across the entire range of salt
368 concentrations studied (**Fig. 4a**). Nevertheless, there was a slight increase in
369 droplet diameter (d_{32}) for the WPI-stabilized emulsions, from around $0.110\ \mu\text{m}$ at
370 0 mM NaCl to $0.118\ \mu\text{m}$ at 500 mM NaCl. The fact that we did observe some
371 creaming of the droplets at high salt concentrations in this system, suggests that
372 the droplets may have been weakly flocculated and that these flocs were disrupted
373 when the emulsions were diluted and stirred during the particle size
374 measurements.

375 The addition of salt caused a slight decrease in the magnitude of the negative
376 charge on the WPI-coated droplets (**Fig. 4b**), which may be attributed to
377 electrostatic screening effects (Israelachvili, 2011). However, salt addition had
378 little impact on the magnitude of the electrical charge on the GA-coated droplets

(Fig. 4b), which suggests that there may have been a charge regulation process occurring. For example, the addition of salt may have changed the thickness and internal structure of the adsorbed gum arabic layer, as well as promoting electrostatic screening effects (Israelachvili, 2011).

3.3.3. *Influence of temperature*

Emulsion-based delivery systems may be exposed to temperature variations during processing, storage, transport, and utilization. We therefore examined the influence of temperature on the stability of Vitamin E emulsions and nanoemulsions (10% GA-stabilized and 2% WPI-stabilized) by placing them in water baths set at different temperatures (30-90°C) for 30 minutes. After all thermal treatments, we did not observe any increase in mean particle diameter or creaming in any of the samples (data not shown). We then investigated the effect of salt addition (150 mM NaCl) on the thermal stability of the 2% WPI-stabilized nanoemulsions and the 10% GA-stabilized emulsions. For WPI-stabilized nanoemulsions, there was an appreciable increase in mean particle diameter for samples held at $\geq 60^{\circ}\text{C}$, and visible gelation was observed at $\geq 70^{\circ}\text{C}$ (Fig. 5a). Presumably, the instability of WPI-stabilized systems to heating in the presence of salt was related to thermal denaturation of adsorbed proteins on the surface of the oil droplets (Kim, Decker, & McClements, 2002). Hydrophobic and sulfhydryl groups are exposed when denaturation occurs which favors droplet-droplet aggregation through hydrophobic attractions and covalent disulfide bonds (Charoen, Jangchud, Jangchud, Harnsilawat, Naivikul & McClements 2011). In the absence of salt, the electrostatic repulsion is strong enough to overcome these attractive interactions, but in the presence of salt the electrostatic repulsion is reduced and the attractive interactions (van der Waals and hydrophobic)

dominate, leading to aggregation (Kim, Decker, & McClements, 2002). We observed no particle aggregation or creaming in the GA-stabilized emulsions after holding at any of the temperatures, which indicated that this system had good thermal stability in the absence and presence of salt. The origin of this effect can be attributed to the strong steric repulsion between the droplets, and the lack of exposed hydrophobic and sulfhydryl groups at elevated temperatures.

3.4. Comparison of emulsifying and stabilizing parameters

Finally, we compared the ability of the four natural emulsifiers studied here and previously (WPI, GA, lecithin, and Q-Naturale) to form stable emulsion-based delivery systems for Vitamin E-acetate. The emulsion formation and stabilizing capabilities of these emulsifiers are compared in **Table 1**. The biopolymer WPI could form droplets that were as small as those produced by the small molecule surfactants (lecithin and Q-Naturale). On the other hand, the biopolymer GA could not form very fine droplets ($d > 300$ nm), and was therefore unsuitable for fabrication of nanoemulsions. In addition, the amount of WPI required to form stable nanoemulsions was fairly similar to that of the small molecule surfactants, with the emulsifier-to-oil ratio (EOR) required to form small droplets being around 1:10. Conversely, the EOR was much higher for GA than any of the other natural emulsifiers studied, being around 10:10.

There were also appreciable differences between the ability of the four natural emulsifiers to stabilize the emulsion-based delivery systems under different environmental conditions. Despite being the least effective emulsifier at forming small droplets, GA was the most effective emulsifier at stabilizing against environmental stresses. It produced emulsions that were stable to a wide range of

pH values (2 – 8), ionic strengths (0 to 500 mM), and temperatures (30 to 90 °C). Conversely, the WPI was the emulsifier that was most sensitive to changes in environmental conditions, exhibiting instability at intermediate pH values (pH 5), elevated salt concentrations (> 100 mM), and higher temperatures (> 60 °C). whereas lecithin and Q-Naturale-based nanoemulsions were stable at wide range of pH. All the systems except WPI-stabilized ones showed great stability against the entire range of temperatures (30-90 °C). The natural small molecule surfactants had relatively good stability to pH and temperature, but exhibited some aggregation at high salt levels, which can be attributed to electrostatic screening.

4. Conclusions

In this study, we examined some of the major factors influencing the formation and stability of Vitamin E-fortified emulsion-based delivery systems produced using two natural food-grade biopolymers: gum arabic and whey protein isolate. WPI was a much more effective emulsifier at forming emulsions containing fine droplets, with a lower amount of emulsifier required and smaller droplets being produced. This effect was attributed three major differences between the protein and polysaccharide emulsifiers: (i) the faster adsorption of the small protein molecules to the droplet surfaces within the homogenizer; (ii) the higher surface activity of the protein; and, (iii) the lower surface load (thinner interfacial layers) of the protein.

Conversely, the gum arabic was much more effective at stabilizing the droplets against environmental stresses than the protein, which can be attributed to differences in the stabilizing mechanism of these two biopolymers. WPI

452 nanoemulsions were highly unstable to droplet aggregation at their isoelectric
453 point (pH 5) and at moderate ionic strengths (>100 mM NaCl), which can be
454 attributed to the fact that they are mainly stabilized by electrostatic repulsion. GA
455 emulsions were stable over a wider range of pH values (2 to 8) and salt
456 concentrations (0 to 500 mM NaCl), which is due to the fact that they are mainly
457 stabilized by steric repulsion. Both emulsifiers formed droplets that were resistant
458 to aggregation after being held at temperatures ranging from 30 to 90 °C for 30
459 minutes (pH 7, no added salt). However, WPI nanoemulsions became unstable to
460 heating above 60 °C in the presence of salt (150 mM NaCl), which can be
461 attributed to an increase in the hydrophobic attraction between the droplets when
462 the adsorbed protein molecules become thermally denatured. GA-stabilized
463 emulsions were not affected by thermal treatment in the absence or presence of
464 salt because of the strong steric repulsion between the droplets. The results of this
465 study are useful to food and beverage manufacturers who would like to utilize
466 natural emulsifiers within their products. WPI may be most suitable for
467 applications where low levels of biopolymer are required, however, the pH of the
468 sample would have to be in an appropriate range (< 4 or > 6). On the other hand,
469 can be used to form stable emulsions over a wide range of pH conditions, but
470 relatively high amounts are required to form stable emulsions.

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583

Figure 1a

Figure 1b

Figure 1c

Figure 2

Figure 3a

Figure 3b

Figure 4a

Figure 4b

Figure 5

Table 1: Comparison of influence of natural emulsifier type on the formation and stability of vitamin E-acetate emulsions. Key: QN = Q-Naturale (quillaja saponin); L = Lecithin; WPI = whey protein isolate; GA = gum arabic. Here $d[3,2]_{min}$ is the smallest droplets that could be produced under standardized homogenization conditions, and EO_{min} is the minimum emulsifier-to-oil ratio to produce small droplets.

Emulsifier	Formation		Stability		
	$d[3,2]_{min}$	EO_{min}	pH	NaCl (mM)	T (°C)
QN	0.116	0.5:10	>3	≤300	≤90
L	0.132	2.0:10	>3	≤100	≤90
WPI	0.11	1.0:10	<5; >5	<100	<60 ^a
GA	0.384	1.0:1.0	>2 (2%GA)	≤500	≤90
			>2 (10%GA)	≤500	≤90

Figure Captions

Figure 1a Influence of emulsifier concentration on the mean particle diameter (d_{32}) of emulsions (10 % (w/w) of oil phase (5% VE + 5% orange oil) and 90 % (w/w) aqueous phase (gum arabic or whey protein isolate) in 10 mM sodium phosphate buffer, pH 7.0)) produced using standardized homogenization conditions (12,000 psi, 3 passes).

Figure 1b Influence of emulsifier concentration on the particle size distribution of emulsions containing 10% oil phase (5% VE + 5% orange oil) and 90 % aqueous phase (10% GA, 10 mM sodium phosphate buffer, pH 7.0) produced using standardized homogenization conditions (12,000 psi, 3 passes).

Figure 1c Influence of emulsifier concentration on the particle size distribution of emulsions containing 10% oil phase (5% VE + 5% orange oil) and 90 % aqueous phase (2% WPI, 10 mM sodium phosphate buffer, pH 7.0) produced using standardized homogenization conditions (12,000 psi, 3 passes).

Figure 2 Influence of oil composition on the mean particle diameter (d_{32}) of emulsions (10% (w/w) oil phase (5% VE + 5% orange oil) and 90 % (w/w) aqueous phase (10% GA; 2% WPI in 10 mM sodium phosphate buffer, pH 7.0)) produced using standardized homogenization conditions (12,000 psi, 3 passes).

Figure 3 Influence of pH on the mean particle diameter (d_{32}) (a) and ζ -potential (b) of emulsions (10 % (w/w) oil phase (5% VE + 5% orange oil) and 90 % (w/w) aqueous phase (10% GA; 2% WPI in 10mM sodium phosphate buffer, pH 7.0)) produced using standardized homogenization conditions (12,000 psi, 3 passes) and stored at ambient temperature for 24 h.

Figure 4 Influence of ionic strength (0-500 mM NaCl) on the mean particle diameter (d_{32}) (a) and ζ -potential (b) of emulsions (10 % (w/w) oil phase (5% VE + 5% orange oil) and 90 %

(w/w) aqueous phase (10% GA; 2% WPI in 10mM sodium phosphate buffer, pH 7.0)) produced using standardized homogenization conditions (12,000 psi, 3 passes) and stored at ambient temperature for 24 h.

Figure 5 Influence of temperature (30-90 °C for 30 min) in the presence of salt (150 mM NaCl) on the mean particle diameter (d_{32}) of emulsions (10 % (w/w) oil phase (5% VE + 5% orange oil) and 90 % (w/w) aqueous phase (10% GA; 2% WPI in 10mM sodium phosphate buffer, pH 7.0)) produced using standardized homogenization conditions (12,000 psi, 3 passes) and stored at ambient temperature for 24 h.

Table 1: Comparison of influence of natural emulsifier type on the formation and stability of vitamin E-acetate emulsions. Key: QN = Q-Naturale (quillaja saponin); L = Lecithin; WPI = whey protein isolate; GA = gum arabic. Here $d_{[3,2]_{\min}}$ is the smallest droplets that could be produced under standardized homogenization conditions, and $EOR_{\min}$ is the minimum emulsifier-to-oil ratio to produce small droplets.