

Effect of high-hydrostatic pressure and pH treatments on the emulsification properties of gum arabic

Article

Accepted Version

Ma, F., Bell, A. E. and Davis, F. J. ORCID:
<https://orcid.org/0000-0003-0462-872X> (2015) Effect of high-hydrostatic pressure and pH treatments on the emulsification properties of gum arabic. *Food Chemistry*, 184. pp. 114-121. ISSN 0308-8146 doi:
<https://doi.org/10.1016/j.foodchem.2015.03.075> Available at
<https://centaur.reading.ac.uk/40142/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.foodchem.2015.03.075>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Effect of High-hydrostatic Pressure and pH treatments on the Emulsification Properties of Gum Arabic

Fanyi Ma,¹ Alan E. Bell,¹ & Fred, J. Davis²

¹Department of Food and Nutrition Science, and ²Chemistry, University of Reading, Whiteknights, Reading RG6 6AP, UK

Abstract

This study investigated the emulsification properties of the native gums and those treated at high pressure (800 MPa) both at their “natural” pH (4.49 and 4.58 respectively) and under “acidic and basic” pH (2.8 and 8.0). The emulsification behaviour of KLTA gum was found to be superior to that of the GCA gum. High pressure and pH treatment changed the emulsification properties of both gums. The acidic amino acids in gum arabic were shown to play an important role in their emulsification behaviour, and mechanism of emulsification for two “grades” gums were suggested to be different. The highly “branched” nature of the carbohydrate in GCA gum was also thought to be responsible for the “spreading” of droplet size distributions observed. Coomassie brilliant blue binding was used to indicate conformational changes in protein structure and Ellman’s assay used to estimate any changes in levels of free thiol present.

Key words: Gum arabic, arabinogalactan protein complex (AGP), high-hydrostatic pressure, emulsification properties, thiol

23 **1. Introduction**

24 Gum arabic (GA, E414) is one of the most extensively used exudate gums from the
25 various species of *Acacia* tree, and a food hydrocolloid that displays both emulsifying
26 and emulsion stabilising properties (Nakauma et al., 2008; Yadav et al., 2007;
27 Williams & Phillips, 2009). About 80% of the commercial gum arabic supplied is
28 derived from *Acacia senegal* (*A. senegal*), with majority of the remaining gum arabic
29 is from *Acacia seyal* (*A. seyal*) (Tan, 1990; Dickinson, 2003). Gum arabic is
30 considered to be a “heterogeneous” material with good emulsification properties,
31 playing an important role in stabilising the dispersed system (Nakauma et al., 2008).

32
33 Gum arabic is most extensively used for flavour encapsulation and emulsification of
34 flavour oils in the carbonated beverage industries due to its ability to form an
35 adsorbed film at the oil-in-water interface (Dickinson et al., 1989). The main
36 ingredient of most flavoured soft drinks is the insoluble essential oils, such as the
37 orange oil. Therefore, the industry is trying to convert essentially insoluble oil into a
38 stable beverage emulsion (Tan, 1990). In the beverage emulsions, the gum is
39 required to stabilise a concentrated oil emulsion (about 20%v/v oil) for long periods
40 and to continue to stabilise these following dilution prior to bottling (Islam et al.,
41 1997). Gum arabic has shown an impeccable stability in the flavour oil system both
42 at the “concentrated” stage and after the final dilution of the beverage. These
43 effective emulsifying properties are due to the solubility and the affinity to the oil
44 phase over a wide pH range (Tan, 1994; Glicksman, 1969).

45
46 An average molecular weight (Mw) of *Acacia senegal* is about 380,000 Da, whereas
47 a typical molecular weight for *Acacia seyal* sample is about 850,000 Da (Mahendran

48 et al., 2008). Gum arabic is a complex branched heteropolysaccharide with a
49 backbone of 1,3-linked β -galactopyranose units and side-chains of 1,6-linked
50 galactopyranose units terminating in glucuronic acid or 4-O-methylglucuronic acid
51 residues (Dickinson, 2003). Gum arabic consists of three main groups (Elmanan et
52 al., 2007; Idris et al., 1998; Montenegro et al., 2012; Randall et al., 1989; Akiyama, et
53 al., 1984; Conolly et al., 1988; Williams et al., 1990):

- 54 i) Arabinogalactan (AG, $M_w \approx 280\text{kDa}$), the main component, which consists of
55 about 88%w/w of the gum and contains the least protein (0.44%w/w);
- 56 ii) Arabinogalactan protein complex (AGP, $M_w \approx 1450\text{kDa}$), 10%w/w of the total
57 gum and contains about 9%w/w protein, in which the backbone chain links to the
58 arabinogalactan chains through serine and hydroxyproline groups;
- 59 iii) Glycoprotein (GP, $M_w \approx 250\text{kDa}$) which is the smallest fraction, 1%w/w of the gum
60 overall but having the highest protein content (55%w/w, about 4000 amino acid
61 residues containing all of the cysteine and methionine) .

62

63 The most widely accepted structural model for the arabinogalactan protein complex
64 (AGP) is “wattle blossom model” suggested by Fincher et al. (1983), containing
65 several polysaccharide units linked to a common protein core (Dickinson, 2003). The
66 “blocks” of carbohydrate are linked to a polypeptide chain through either serine or
67 hydroxyproline residues (Williams & Phillips, 2009). This model suggests how gum
68 arabic used in oil-in-water emulsion acts as an emulsifier. Recent studies on *A.*
69 *senegal* have suggested a repeating “backbone” protein structure of [ser-hyp-hyp-
70 hyp-thr-leu-ser-hyp-ser-hyp-thr-hyp-thr-hyp-hyp-hyp-gly-pro-his] with the attached
71 arabinogalactan (α -1-3) linked and with short protein side chains also attached to
72 “backbone” at intervals. It is likely that the “availability” of this protein “backbone” is

73 related to its eventual emulsifying capacity of the gum (Mahendran et al., 2008;
74 Goodrum et al., 2000).

75

76 The structure of *A. seyal* was investigated by Jurasek et al. (1995), Hassan et al.
77 (2005), Flindt et al. (2005), Siddig et al (2005) and Nie et al. (2013). It is suggested
78 that the sugar and amino acid composition were essentially same as the *A. senegal*
79 and that the architecture of AGP structure is also similar. However, Siddig et al (2005)
80 suggested that there was also a “second” high molecular fraction in the AGP of *A.*
81 *seyal*, and Nie et al (2013) stated that the polysaccharides in *A. seyal* were more
82 highly “branched”.

83

84 High-hydrostatic pressure (range of 100 MPa to 1GPa), is commonly used in food
85 industry for both food processing and food preservation (Hite, 1899). High-
86 hydrostatic pressure treatment is a novel technology and multifactorial process which
87 includes the destruction of micro-organisms, the alteration of enzyme activity, the
88 control of phase changes and the altered conformation of biopolymers leading to
89 changes in their functional properties (Farr, 1990; Galazka & Ledward, 1995). An
90 important aspect of the use of pressure treatment is that the food material can be
91 processed with minimal effects on the natural colour, flavour, and taste of the
92 products with little or no loss of vitamin content (Heremans, 1992; Galazka et al.,
93 1995 & 2000). Not only can this pressure be used to kill vegetative cells and reduce
94 spore numbers, it can be used to modify and alter the properties and structure of any
95 proteins present (Galazka & Ledward, 1995). The effects of pressure on protein are
96 wide ranging and a continuing area for further investigation. Researchers have
97 shown that high-hydrostatic pressure can make changes in the hydrophobic

98 associations, hydrogen bonding and electrostatic interactions in proteins (Ledward,
99 1995). Therefore, high pressure treatment does not appear affect primary structure,
100 but changes the secondary, tertiary, and quaternary structures (Galazka et al., 2000).

101

102 In many protein tertiary structures, disulphide “bridges” were found to be some of the
103 major stabilising interactions. Disulphide “bridges” (SS) can be formed when two
104 cysteine residues (thiol group, -SH) which are adjacent in the 3D structure are
105 oxidised (Branden & Tooze, 1999). It has been suggested that such disulphide
106 “bridges” can rearranged under high pressure (Phillips et al., 1994; Galazka et al.,
107 2000; Kieffer et al., 2007). Due to limitations in assay sensitivity little or no cysteine
108 and methionine can be detected in the crude gum arabic (Phillips & Williams, 2009;
109 Biswas et al., 1995). However significant levels can be detected in the purified GP
110 fraction (about 200 residues in the 4000 peptides, Renard, et al., 2006).

111

112 Therefore, detecting the protein dye binding and changes in the sulphhydryl (thiol, SH)
113 in gum could indicate protein conformational changes after high pressure treatment
114 at varying pH levels. The aim of this study was to investigate the effect of high-
115 hydrostatic pressure and pH on the emulsification properties of KLTA (“premium”
116 grade) and GCA (“secondary” grade) gum samples.

117 **2. Materials & Methods**

118 **2.1 Materials**

119 The spray dried gum samples of “food grade” used in the study were supplied by
120 Kerry Ingredients, Bristol, UK. KLTA gum is a spray dried preparation of Kordofan
121 gum light type A (*A. senegal*), and is generally recognised as “good” gum. GCA is

122 gum commercial *Acacia* (*A. seyal*) also spray dried preparation and is considered to
123 be “poor” gum. The protein content of KLTA is about 3%w/w and GCA is about
124 2%w/w respectively. All chemicals, reagents and dialysis tubing used were
125 purchased from Fisher Scientific (Loughborough, UK) and Sigma-Aldrich (Dorset, UK).
126 All chemicals were of analytical grade unless specified.

127

128 **2.2 Sample preparation**

129 The gum arabic dispersions (40%w/v) were made by adding the required amount of
130 gums to deionised water (pH 7, conductance: 18mΩ), with gentle stirring at room
131 temperature (20°C) overnight to allow dispersed. The solutions were further
132 degassed under a vacuum to remove any entrapped air bubbles. The gum samples
133 were prepared in duplicate (both for the KLTA and GCA) and were either dialysed
134 overnight at 4°C (native gums) or dialysed against the various phosphate buffer
135 solutions (0.3 M) overnight at 4°C to equilibrate to the required pH (2.8 and 8.0). The
136 samples were then pressurised at 800MPa for 10 minutes using a prototype
137 Stansted “food lab” high pressure apparatus (Stansted Fluid Power, Essex, UK). The
138 pH treated and native samples were then dialysed against several changes of
139 deionised water for 24h at 4°C. No change in samples volume was observed.
140 Materials were also freeze dried and stored in vacuum desiccators over P₂O₅ for
141 further study.

142

143 **2.3 Droplet distribution measurements**

144 The emulsification properties were examined by measuring the droplet size
145 distribution of emulsions made using native, pH 2.8 and pH 8.0 non-pressurise and
146 pressure treatment (simplified native non pressure (NP), pressure treated (P), pH 2.8

147 (superscript 2.8), pH 8.0 (superscript 8), for example, pH 2.8 pressurised KLTA gum
148 simplified as KLTA P^{2.8}).

149

150 Each sample was added to an oil-in-water model system, 0.1g of freeze dried gum,
151 0.5ml orange oil and 99.4ml deionised water. The emulsions were measured using a
152 Malvern Mastersizer 2000 particle size analyser (1 kHz, particle size: 0.02--2000µm).
153 Deionised water (99.4g) was added to a circulating water system passing through
154 the optical cell (total volume 100ml stirrer/circulator 1000 rpm) and measured the
155 background. And then, the gum materials (0.1g) were added and circulated using
156 small volume dispersion unite for about 2 min at 1000rpm. The cold-pressed, orange
157 oil from California (Sigma Aldrich Chemicals, UK) was then added (0.5ml) and then
158 mixed for a further 2.5 hours to allow the system to equilibrate. The samples were
159 measured after addition (time=0), and then measured every 30 minutes until the
160 emulsion stabilised in the prevailing shear conditions (2.5 hours, data not shown).
161 The droplet distribution profile of the unstabilised (no gum) oil emulsion was
162 measured after 2.5 hours, and the mean droplet diameter at peak fraction was found
163 to be about 300µm.

164

165 **2.4 Coomassie brilliant blue assay**

166 The method used was that of Bradford (1976). The reagent used was a solution of
167 100mg of brilliant blue. G. dye (Coomassie Blue G) in 50 ml of 95% v/v ethanol to
168 which was added 100mls of 85% w/v phosphoric acid, the total volume being
169 adjusted to 1000ml with distilled water. Sample containing between 10 and 100ug of
170 protein in 0.1ml of deionised water were added to 5muls of the freshly prepared dye
171 reagent and mixed. After 5 minutes the absorbance was read at 595nm and

172 compared with a standard curve of bovine serum albumin, 1-100ug protein. The
173 colour produced by this assay was found to be stable for up to one hour after mixing.
174 The standard curve was using a serial dilution technique using bovine serum
175 albumin (BSA) as a protein standard, and a linear function:

$$176 \quad \mathbf{y = 0.0007x + 0.0059}$$

177 Where: y: absorbance at 595 nm; x: amount of protein contained (μg)

178

179 **2.5 Ellman's assay**

180 Analysis of the effect on the thiol groups was carried out using the Ellmans' Assay
181 (Ellman, 1959). All of the spray dried gum samples were hydrated in pH 8 phosphate
182 buffer solutions (1g in 10ml). At this pH thiol groups are ionized thus making them
183 more reactive towards the Ellman's reagent, 5-5'-dithiobis-(2-nitrobenzoic acid).
184 From this solution 3ml was the mixed with 2ml of pH 8 phosphate buffer and 5ml
185 deionised water. 3ml of this solution was added to a 3ml photocell. The absorbance
186 was adjusted to zero. Once the absorbance was adjusted to zero 20 μl of Ellman's
187 reagent (3mM in 0.1M phosphate buffer pH 8) was added. This allows the formation
188 of the 2-nitro-5-thiobenzoate anion (Ratio of 1:1) which is yellow in colour and has a
189 molar concentration of 14,150M⁻¹cm⁻¹ at wavelength 412nm. The absorbance
190 peaked after 2 minutes. After the 2 minutes the absorbance 412nm was read from
191 the spectrophotometer (Cecil 1000 series UV-VIS ectrophotometer). The following
192 equation was then applied to determine the sulphhydryl content (mmoles/g).

$$193 \quad \mathbf{C_0 = (A/\acute{\epsilon}) D}$$

194 Where C₀ = Original concentration;

195 A = Absorbance at 412nm;

196 $\acute{\epsilon}$ = Extinction coefficient (14, 150 M⁻¹cm⁻¹);

197 D = Dilution Factor

198

199 3. Results & Discussion

200 3.1 Emulsification properties of native, pressurised and pH (2.8 and 8.0) 201 treated gum arabic

202 Fig. 1 shows the droplet size distribution of emulsions made using both the native
203 non-pressure treated (NP) and pressure treated (P) KLTA and GCA gums (pH≈4.5,
204 n=6). The peaks of KLTA NP and KLTA P were tightly distributed at about 16µm,
205 and 18µm respectively (fig. 1 (a) and (b)). No significant differences in values
206 between the native materials and those for the pressurised samples were observed.

207

208 Fig. 1 (c) and (d) show the droplet size distributions of native and pressurised GCA
209 gums. In this case, although the mean of the droplet size distribution in the untreated
210 GCA gum was only slightly greater than the untreated KLTA gum (19.60µm and
211 15.78µm respectively). The overall variability of the GCA untreated replicates also
212 increased. This “variability” was further enhanced by the pressure treatment of the
213 GCA gum samples, with an overall increase in the mean droplet size to 33.53µm.

214 Assuming that the increase in droplet size is an indicator of the gums decreased
215 ability to stabilise a given surface area, then the GCA “poor” gums would seem to
216 have “reduced” emulsification power, and be more detrimentally affected by any
217 pressure treatment, than the equivalent KLTA “good” gum.

218

219 It has been reported that the “poor” GCA (*A. seyal*) has a different distribution of the
220 protein throughout, and there may be more than one high molecular weight AGP

221 fraction, which may also contribute to the overall emulsification properties (Hassan et
222 al., 2005; Flindt et al., 2005; Siddig et al., 2005). In addition, the pressure treatment
223 may act directly on the carbohydrate chains and cause some “interdigitation” of the
224 sugar chains leading to a molecule with a reduced “hydrodynamic volume” (Whistler
225 & Daniel, 1990). This “interdigitation” effect may also be more marked for the more
226 highly “branched” structure of the GCA (*A. seyal*) gum (Nie et al., 2013).

227

228 Fig. 2 shows the droplet size distributions of emulsions made using pH 2.8 treated
229 gums (non-pressurised (NP) and pressurised (P) KLTA and GCA gums). The pre-
230 treatment (pH 2.8) of KLTA gum significantly increased the mean droplet size of the
231 model emulsions (15.78 μm to 59.92 μm , fig. 1 (a) and fig. 2 (a) respectively). The
232 individual non-pressurised profiles however, remain reasonably reproducible (little
233 spread of measurements). After pressure treatment (fig. 2 (b)), the ability of the
234 KLTA to consistently produce an emulsion of similar mean droplet sizes, was lost
235 (mean increased from 59.92 μm to 302.34 μm for KLTA NP^{2.8} and KLTA P^{2.8}
236 respectively). A similar pattern of behaviour was observed for the pre-treated pH 2.8
237 GCA gums with the mean droplet size increasing from 19.60 μm to 261.39 μm to
238 359.49 μm for GCA NP, GCA NP^{2.8} and GCA P^{2.8} respectively (fig. 1 (c), fig. 2 (c) and
239 fig. 2 (d)). The emulsions again were showing an increased “spread” of the means
240 and a general “broadening” of the individual distributions.

241

242 The most common use of KLTA “good” (*A. senegal*) gum in the food industry is the
243 stabilisation of emulsions of flavour oil in soft drinks at low pH (2.5 -- 4, Harnsilawat
244 et al., 2006; Friberg, 1997; Tan, 1990). Treating the KLTA at the low pH 2.8
245 produced a significant increase in the mean droplet size, indicating the decrease in

246 the emulsification power. Treatment of the “poor” GCA gum under the same
247 conditions produced an even more pronounced increase in the mean droplet size.
248 Effectively, after the “acid treatment” the GCA gum has almost no remaining
249 emulsifying ability (Mean droplet size of the oil emulsion only (with no gum) was
250 about 300µm, data not shown). Since hydrolysis of any part of the gum arabic
251 structure (KLTA or GCA) is very unlikely at pH 2.8 (Su et al., 2008; Chanamai &
252 McClements, 2002), any difference in behaviour is presumably as a result of
253 conformational changes in the proteins present.

254

255 Fig. 3 shows the droplet size distribution of emulsions using gums pre-treated at pH
256 8.0. While both gums (KLTA and GCA) follow the general trend ($NP < NP^8 < P^8$), the
257 increased mean droplet size and the data spread (distribution of curves) are not as
258 great as those observed for gums pre-treated at pH 2.8. For KLTA gum, the mean
259 droplet sizes from KLTA NP to KLTA NP^8 and KLTA P^8 were 15.78µm to 32.46µm to
260 45.20µm respectively (fig. 1 (a), fig. 3 (a) and fig. 3 (b) respectively). For GCA gum,
261 the equivalent sequence of droplet sizes was from 19.60µm, to 44.06µm and to
262 57.15µm (fig. 1 (c), fig. 3 (c) and fig. 3 (d) respectively). The emulsification data for
263 the gums treated at pH 8.0 differs substantially from that observed at pH 2.8 for both
264 types of gum.

265

266 It is interesting to note that the KLTA is rich in acidic residues (127/94 residues per
267 1000 and 103/80 residues per 1000 for the acid/basic amino acid ratio for the KLTA
268 and GCA respectively, Williams & Phillips, 2009). Given that the pKa of any basic (-
269 NH_2^+) groups present is about 10.7 (Silverman, 2002), these groups are going to be
270 fully protonated at any of the pH conditions used in this study and are unlikely to play

271 a significant role in changing the conformation of the protein (fig. 4). On the other
272 hand, changing the pH is likely to have considerable effect on any acidic groups
273 (COO^-) present as they usually have pKa values in the region of 4.8 (Silverman,
274 2002).

275

276 A treatment at pH 8.0 would lead to any acidic groups becoming fully ionised (both
277 the protein and the carbohydrate present). The subsequent electrostatic repulsion of
278 these groups would then denature the protein and “expand” the carbohydrate
279 moieties (fig. 4 (b)), leading to less surface activity (lower hydrophobicity of the
280 AGP). Returning the material to its original pH would reverse the ionisation of the
281 acid groups (restore the hydrodynamic volume of the carbohydrate part), but it would
282 not cause the protein to “refold”, leaving a material that is less hydrophobic and
283 prone to aggregation (McClements, 2004; Dickinson & Pawlowsky, 1998; Dickinson,
284 2009^{a&b}, fig. 4 (c)).

285

286 Conversely, treatment at pH 2.8 would cause the acid groups to become fully
287 protonated and to become less hydrophilic, both in terms of the “compression” of the
288 protein and the reduced repulsion of the carbohydrate side chains (fig. 4(d)). This
289 would lead in terms to a both a reduction in the surface area “covered” and “thinning”
290 of the surface carbohydrate larger. Subsequent dialysis would again not necessarily
291 fully reverse this denaturation process, and such changes would result in reduced
292 emulsifying activity.

293

294 The results suggested that high pressure treatment inhibited the “improvement” of
295 emulsification of gum arabic. This may be caused by “interdigitation” of

296 carbohydrates, and also by the protein denaturation in the gum. Such denaturation
297 may occur due to the pH changing, or during the high pressure processing. If such
298 protein denaturation happened during high pressure processing, the tertiary structure
299 was the most likely to be affected, the most labile linkages likely to be any disulphide
300 bonds present (Creighton, 1989). Therefore, the protein “content” and free thiol
301 groups present were followed to indicate any conformation changes in the proteins
302 present.

303

304 **3.2 Estimation of protein “content” in gum samples (Coomassie brilliant blue)**

305 Table 1 (2) shows the protein “content” of the gum samples as assayed using
306 coomassie brilliant blue as reagent. While the native (“natural” pH, 4.49 and 4.58 for
307 KLTA and GCA respectively) and the gums pre-treated on pH 8.0 all showed “dye
308 binding” (blue colour development during assay), samples pre-treated at pH 2.8 did
309 not. This suggested that the acid pre-treatment may have in some way
310 changed/denatured any protein present or altered the overall gum structure, such
311 that the protein is no longer “accessible” during the assay. “Calculated” protein
312 content is an indicator of changes in “accessibility” of the protein to the dye (note no
313 detectable protein was found in the final dialysis liquids, suggesting no significant
314 hydrolysis had occurred). These changes were subsequently reflected in the
315 emulsification behaviours (fig. 2).

316

317 The final protein values in KLTA “good” and GCA “poor” gums show significant
318 differences in their ability to bind the dye (measured as “protein content” 5.99% and
319 0.63% respectively). High pressure treatment alone did not affect significantly
320 change the dye binding levels in both types of gums. Treatment at pH 8.0 also

321 showed a similar pattern of differences between the gum types and pressure
322 treatments.

323
324 Coomassie brilliant blue is used in detection and quantification of proteins as the dye
325 has the ability to form complex structures in solution by electrostatic and hydrophobic
326 interactions (Banik et al., 2009). The “nominal” protein content is 3% for KLTA and 2%
327 for GCA, however the calculated results obtained using BSA as a standard
328 suggested that the assay is unreliable in terms of the absolute levels of protein
329 present.

330
331 The “Bradford” reagent depends on the amphoteric nature of the proteins with
332 Arginine (Arg) and Lysine (Lys) residues being the primary binding sites for the dye
333 (Wei & Li, 1996). Since Arg and Lys are both considered “basic” amino acids, it is
334 perhaps not supposing that after the gums were treated at pH 2.8, conformational
335 changes were such that no protein was detected (i.e. no binding). KLTA and GCA
336 gums would be expected to bind the dye differently because of the relative different
337 amounts of Arg and Lys and the total levels of protein in each gum (42 and 29
338 residues/1000, KLTA and GCA respectively)). Simplistically, GCA should bind $\frac{29}{42} \times \frac{2}{3}$

339 less dye than KLTA, this should give a “calculated” protein content of 2.76% all other
340 conditions being equal. The recorded value of 0.63% suggests that there is a
341 conformational difference in the GCA protein moiety of the GCA gum when
342 compared with the KLTA material with respect to its binding of coomassie brilliant
343 blue. Previous authors have suggested that the protein structures of gum *A. senegal*
344 “good” and *A. seyal* “poor” are different despite compositional similarities (Flindt et al.,
345 2005; Siddig, et al., 2005). Subsequent the high pressure treatment of both types

346 (KLTA and GCA) native gums shows no significant change in the dye binding
347 (calculated %w/w protein) for the KLTA or GCA gums (5.99% to 6.74%, and 0.63%
348 to 0.99% for native and pressurised KLTA and GCA gums respectively).

349

350 **3.3 Estimation of “free” sulphhydryl content in gum samples (Ellman’s assay)**

351 Table 1 (3) shows the calculated “free” sulphhydryl content of the various gums tested
352 (combination of pH and pressure treatment). The thiol group was barely detected
353 since the calculated results is $\text{mmoles} \times 10^{-5} / \text{g}$. However, the calculated results still
354 can indicate the difference of gum samples. The sulphhydryl contents of the KLTA
355 “good” gum and the GCA “poor” gum were $2.22 \text{ mmoles} \times 10^{-5} / \text{g}$ and 1.93
356 $\text{mmoles} \times 10^{-5} / \text{g}$ for respectively. The native untreated KLTA and GCA gums had
357 significant differences in sulphhydryl level, and the high pressure treatment of native
358 KLTA and GCA gums showed significant changes in sulphhydryl levels. This again
359 indicated the conformation changes after the pressure treatment.

360

361 Once pressurised KLTA gum showed no further changes at any of the pH treatment
362 used (KLTA P is not significant different from KLTA P^{2.8}, KLTA P⁸). This suggested
363 that the statistical differences observed between these gums and “native” KLTA gum
364 (*A. senegal*), is simply a pressure effect on the gum, i.e. conformational change in
365 the protein exposing more sulphhydryl groups. The various pH treatments on both
366 types of gums without applied pressure only produced a significant increase in
367 measured thiol levels at pH 8.0 for the GCA “poor” gum. This may suggest the
368 different conformation of two types of gums, and/or may be as a result of “extension”
369 of the protein structure at pH 8.0.

370

371 Previous studies have suggested that high pressure treatment can denature proteins
372 and this may result in an altered protein conformation consequently changing its
373 functional properties (Galazka et al., 1995). For example, egg white protein has been
374 formed to have improved foaming properties and a changed conformation after high
375 pressure treatment (Plancken et al., 2007). In this study, we are using the “exposure”
376 of thiol groups as an indicator of changes in the protein conformation.

377

378 High pressure treatment alone caused a significant increase in available free thiol
379 groups for both gums, suggesting the protein conformational changes, which was
380 consistence with protein “content” measured. pH 8.0 treated alone of the GCA “poor”
381 gum produced a significant changes in the measured thiol levels. This is presumably
382 as a result of the “opening” of the protein structure caused by the increased
383 repulsion of the acidic amino acids under these conditions (fig. 4). (Creighton, 1989;
384 Ludwig & Macdonald, 2005).

385

386 All pressurised pH treated gums (KLTA P^{2.8}, KLTA P⁸, and GCA P^{2.8}, GCA P⁸)
387 showed no statistical differences in free thiol levels over their respective, pressure
388 treated only controls (KLTA P and GCA P). For both gums (KLTA and GCA), a
389 combination of pH treatments with pressure produced significant changes in all
390 samples with respect to the thiols “available” to the Ellman’s assay,. Overall the
391 results indicate that with the exception of the pH treatment at pH 8.0, the major
392 determinant of protein conformational change is the high pressure treatment.
393 Hydrophobicity of protein was found to increase after the high pressure treatment
394 (Messens et al., 1997; Galazka et al., 2000). Previous studies (Fauconnier et al.,
395 2000; Panteloglou et al., 2010) have suggested that GCA (*A. seyal*) was a poorer

396 emulsifier due to having a protein moiety which was “less elastic” and had a “tighter
397 structure” compared to KLTA (*A. senegal*). The different responses to various
398 treatments again suggested different conformational arrangements in the two types
399 of gums.

400

401 **4. Conclusion**

402 This study was carried out to investigate the effect of high hydrostatic pressure
403 (800MPa) and pH changes on the emulsification properties of KLTA “good” and GCA
404 “poor” gums. The emulsification properties of native/untreated KLTA gum were
405 superior to native GCA gum. High pressure treatment had little effect on KLTA gum,
406 but affects the GCA “poor” gums significantly, suggesting the protein distribution and
407 conformation of these two gums are different. High pressure treatment may also
408 change the overall gum structure by causing the carbohydrate to “interdigitate”, and
409 reducing its hydrodynamic volume.

410

411 The “natural” pH value of native gum solutions was about 4.49 and 4.58 for KLTA
412 and GCA respectively, and pre-treatments at both pH 2.8 and pH 8.0 significantly
413 reduced the overall emulsification properties. The results suggested that the ratio of
414 the acidic and basic amino acids in gum arabic plays an important role in the
415 emulsification abilities of the gums. At pH 2.8, the basic groups in amino acids were
416 protonated, and at pH 8.0, the acid groups became ionised. Therefore, the protein
417 and carbohydrates had been “compressed” and “expanded” respectively. The highly
418 “branched” nature of the carbohydrate in GCA was also thought to be responsible for
419 the “spreading” of droplet size distribution. Both the dye binding and “available” thiol

420 residues suggested conformational differences between the protein fractions of the
421 two types of gums.

422

423 In conclusion in order to improve the emulsification properties of “poor” gums it may
424 be necessary to investigate methods which chemically modify the carboxylic acid
425 groups in both the protein and carbohydrate parts of the gum to reduce their
426 electrostatic repulsion of each other.

427

428 **References**

429 **Akiyama, Y., Eda, S. & Kato, K.** (1984). Gum Arabic is a kind of Arabinogalactan-
430 Protein. *Agricultural Biology and Chemistry*, 48, 235-237.

431 **Banik, S.P., Pal, S., Ghorai, S., Chowdhury, S. & Khowala, S.** (2009). *Interference*
432 *of sugars in the Coomassie blue G dye binding assay of proteins.* Elsevier,
433 *Analytical Biochemistry*, 386, 113-115.

434 **Biswas, S., Biswas, B. & Phillips, G.O.** (1995). Classification of natural gums Part
435 VIII. Chemometric assignment of commercial gum exudates from Africa using
436 cluster analysis on the protein amino acid compositions. *Food Hydrocolloids*, 9,
437 151-163.

438 **Bradford, M.M.** (1976). A rapid and sensitive method for the quantitation of
439 microgram quantities of protein utilizing the principle of protein-dye binding.
440 *Analytica biochemistry*, 72, 248-254.

441 **Branden, C. & Tooze, J.** (1999). *Introduction to Protein Structure.* New York:
442 Garland Publishing.

443 **Chanamai, R. & McClements, D.J.** (2002). Comparison of gum arabic, modified
444 starch, and whey protein isolate as emulsifiers: influence of pH, CaCl₂ and

445 temperature. *Journal of Food Science*, 67, 120-125.

446 **Conolly, S., Fenyo, J.C. & Vandeveld, M.C. (1988).** Effect of a Proteinase on the
447 Macromolecular Distribution of *Acacia Senegal* Gum. *Carbohydrate Polymers*, 8,
448 23-32.

449 **Creighton, T.E. (1989).** Disulphide Bonds between Cysteine Residues. In: Creighton,
450 T.E., eds. *Protein Folding*. New York: W.H. Freeman and Company, 301-353.

451 **Dickinson, E. (2003).** Hydrocolloids at Interfaces and the Influence on the
452 Properties of Dispersed Systems. *Food Hydrocolloids*, 17, 25-39.

453 **Dickinson, E. (2009a).** Hydrocolloids and emulsion stability. In: Phillips, G.O. &
454 Williams, P.A. (Eds). *Handbook of hydrocolloids*. Oxford: Woodhead Publishing
455 Limited and CC Press LLC.

456 **Dickinson, E. (2009b).** Hydrocolloids as emulsifiers and emulsion stabilizers. *Food*
457 *Hydrocolloids*, 23, 1473-1482.

458 **Dickinson, E., Murry, B.S. & Elverson, D.J. (1989).** On the film forming and
459 emulsion stabilizing properties of gum arabic: dilution and flocculation aspects.
460 *Food Hydrocolloids*, 3, 101-115.

461 **Dickinson, E. & Pawlowsky, K. (1998).** Influence of κ -carrageenan on the
462 properties of a protein-stabilised emulsion. *Food Hydrocolloids*, 12, 417-423.

463 **Ellman, G.L. (1956).** Tissue sulphhydryl groups. *Archive of Biochemistry and*
464 *Biophysics*, 82, 70-76.

465 **Elmanan, M., Al-Assaf, S., Phillips, G.O. & Williams, P.A. (2008).** Studies on
466 Acacia Exudate Gums: Part VI. Interfacial Rheology of *Acacia Senegal* and
467 *Acacia Seyal*. *Food Hydrocolloids*, 22, 682-689.

468 **Farr, J.D. (1990).** High Pressure Technology in the Food Industry. *Trends in Food*
469 *Science and Technology*, 1, 14-16.

470 **Fauconnier, M. -L., Bleckey, C., Groyne, L., Razafindralambo, H., Vanzeveren,**
471 **E., Marrier, M. & P.** (2000). Characterization of Two Acacia Gum and Their
472 Fractions Using a Langmuir Film Balance. *Journal of Agricultural and Food*
473 *Chemistry*, 48, 2709-2712.

474 **Fincher, G.B., Stone, B.A. & Clarke, A.E. (1983).** Arabinogalactan-Protein:
475 Structure, Synthesis and Function. *Annual Review of Plant Physiology*, 34, 47-
476 70.

477 **Flindt, C., Al-Assaf, S., Phillips, G.O. & Williams, P.A.** (2005). Studies on *Acacia*
478 exudate gums. Part V. structural features of *Acacia seyal*. *Food Hydrocolloids*.
479 19, 687-701.

480 **Friberg, S.E.** (1997). Emulsion stability. Friberg, S.E. & Larsson, K. (Eds). *Food*
481 *Emulsions*, (3rd Edition). New York: Marcel Dekker Inc.

482 **Galazka, V.B. & Ledward, D.A.** (1995). Developments in High Pressure Food
483 Processing. *Food Technology*, 123-125.

484 **Galazka, V.B., Ledward, D.A., Dickinson, E. & Langley, K.** (1995). High Pressure
485 Effects on the Emulsifying Behaviour of Whey Protein Concentrate. *Journal of*
486 *Food Science*, 60, 6, 1341-1343.

487 **Galazka, V.B., Dickinson, E. & Ledward, D.A.** (2000). Influence of High Pressure
488 Processing on Protein Solutions and Emulsions. *Current Opinion in Colloid and*
489 *Interface Science*, 5, 182-187.

490 **Glicksman, M.** (1969). *Gum Technology in Food Industry*. USA: Academic Press.

491 **Goodrum, L.J., Patel, A., Leykam, J.F. & Kieliszewski, M.J.** (2000). Gum arabic
492 glycoprotein contains glycomodules of both extension and arabinogalactan-
493 glycoproteins. *Phytochemistry*, 54, 99-106.

494 **Harnsilawat, T., Pongsawatmanit, R. & McClements, D.J.** (2006). Stabilisation of

495 model beverage cloud emulsions using protein-polysaccharide electrostatic
496 complexes formed at the oil-water interface. Cooperative State Research,
497 Extension, Education Service, US Department of Agriculture.

498 **Hassan, E.A., Al-Assaf, S., Phillips, G.O. & Williams, P.A.** (2005). Studies on
499 *Acacia* gums: Part III molecular weight characteristics of *Acacia seyal* var. *seyal*
500 and *Acacia seyal* var *fistula*. *Food Hydrocolloids*. 19, 669-677.

501 **Heremans, D.A.** (1995). High Pressure Effects in Biomolecules. In: Ledward, D.A.,
502 Johnston, D.E., Earnshaw, R.G. & Hasting, A.P.M. Ed. *High Pressure*
503 *Processing of Foods*. Leicestershire: Nottingham University Press, 81-97.

504 **Hite, B.H.** (1899). The Effect of Pressure on the Preservation on Milk. *Bulletin of*
505 *West Virginia Agricultural Experimental Station*, 58, 15-35.

506 **Idris, O.H.M., Williams, P.A. & Phillips, G.O.** (1998). Characterisation of gum from
507 *Acacia senegal* trees of different age and location using multidetection gel
508 permeation chromatography. *Food Hydrocolloids*. 12, 379-388.

509 **Islam, A.M., Phillips, G.O., Sljivo, A., Snowden, M.J. & Williams, P.A.** (1997). A
510 review of recent developments on the regulatory, structural and functional
511 aspects of gum arabic. *Food Hydrocolloids*, 11, 4, 493-505.

512 **Jurasek, P., Varga, S. & Phillips, G.O.** (1995). Classification of natural gums VII.
513 Relationships between the series Vulgares (*Acacia senegal*) and Gummiferae
514 (*Acacia seyal*). *Food Hydrocolloids*, 9,1, 17-34.

515 **Kieffer, R., Schurer, F., Köhler, P. & Wieser, H.** (2007). Effect of hydrostatic
516 pressure and temperature on the chemical and functional properties of wheat
517 gluten: studies on gluten, gliadin and glutenin. *Journal of Cereal Science*, 45,
518 285-292.

519 **Ledward, D.A.** (1995). High Pressure Processing – The Potential. In: Ledward, D.A.,
520 Johnston, D.E., Earnshaw, R.G. & Hasting A.P.M. Ed. *High Pressure*
521 *Processing of Foods*. Leicestershire: Nottingham University Press, 1-5.

522 **McClements, D.J.** (2004). Protein-stabilised emulsions. *Current opinion in Colloid &*
523 *Interface Science*, 9, 305-313.

524 **Mahendran, T., Williams, P.A., Phillips, G.O., Al-Assaf, S. & Baldwin, T.C.** (2008).
525 New Insights into the Structural Characteristics of the Arabinogalactan-Protein
526 (AGP) Fraction of Gum Arabic. *Journal of Agriculture and Food Chemistry*, 56,
527 9269-9276.

528 **Messen, W., Van Camp, V. & Huyghebaert, A.** (1997). The use of high pressure to
529 modify the functionality of food proteins. *Trends in Food Science & Technology*,
530 8, 4, 107-112.

531 **Montenegro, M.A., Boiero, M.L., Valle, L. & Borsarelli, C.D.** (2012). Gum arabic:
532 more than an edible emulsifier. In: Verbeek, C.J.R (Ed). *Products and*
533 *Applications of Biopolymers*. Croatia: InTech.

534 **Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K., &**
535 **Phillip, G.O.** (2008). Comparison of Sugar Beet Pectin, Soybean Soluble
536 Polysaccharide, and Gm Arabic as Food Emulsifiers. 1. Effect of Concentration,
537 pH and Salts on the Emulsifying Properties. *Food Hydrocolloids*, 22, 1254-1267.

538 **Nie, S., Wang, C., Cui, S.W., Wang, Q., Xie, M. & Phillip, G.O.** (2013). The core
539 carbohydrate structure of *Acacia seyal var seyal* (gum arabic). *Food*
540 *Hydrocolloids*. 32, 221-227.

541 **Panteloglou, A.G., Bell, A.E. & Ma, F.** (2010) Effect of high-hydrostatic pressure
542 and pH on the rheological properties of gum arabic. *Food Chemistry*, 122, 4,
543 972-979.

- 544 **Phillips, L.G., Whitehead, D.M. & Kinsella, J.** (1994). Structure-Function
545 Properties of Food Proteins. USA: Academic Press, INC.
- 546 **Plancken, I., Loey, A.V. & Hendrickx, M.X.** (2007). Foaming Properties of Egg
547 White Proteins Affected by Heat or High Pressure Treatment. *Journal of Food*
548 *Engineering*, 78, 1410-1426.
- 549 **Randall, R.C., Phillips, G.O. & Williams, P.A.** (1989). Fractionation and
550 Characterization of Gum from Acacia Senegal. *Food Hydrocolloids*, 3, 1, 65-75.
- 551 **Renard, D., Lavenant-Gourgeon, L., Ralet, M. & Sanchez, C.** (2006). *Acacia*
552 *senegal* gum: continuum of molecular species differing by their protein to sugar
553 ratio, molecular weight, and charges. *Biomacromolecules*, 7, 2637-2649.
- 554 **Siddig, N.E., Osman, M.E., Al-Assaf, S., Phillips, G.O. & Williams, P.A.** (2005).
555 Studies on acacia exudate gums, part IV. distribution of molecular components
556 in *Acacia seyal* in relation to *Acacia senegal*. *Food Hydrocolloids*. 19, 679-686.
- 557 **Silverman, R.B.** (2002). *The Organic Chemistry of Enzyme Catalyzed Reactions*.
558 UK: Elsevier Science.
- 559 **Su, J., Flanagan, J. & Singh, H.** (2008). Improving encapsulation efficiency and
560 stability of water-in-oil-in-water emulsions using a modified gum arabic (*Acacia*
561 (sen) SUPER GUM™). *Food hydrocolloids*, 22, 112-120.
- 562 **Tan, C.T.** (1990). Beverage Emulsions. In: Larsson, K. & Friberg, S.E. *Food*
563 *Emulsions*, 2nd Ed. New York: Dekker, 445-478.
- 564 **Tan, C.T** (1994). Beverage emulsions. In: Phillips, G.O. & Williams, P.A. (Eds). *Gums*
565 *and Stabilisers for the Food Industry 7*. Oxford: Oxford University Press Inc.
- 566 **Wei, Y. & Li, K.** (1996). Linear regression method for the study of the Coomassie
567 brilliant blue protein assay, *Talanta*, Elsevier. 44, 413-420.
- 568 **Williams, P.A. & Phillips, G.O.** (2009) Gum arabic. In: *Handbook of Hydrocolloids*,

569 *2nd Edition*. Oxford: Woodhead Publishing Limited, 252-273.

570 **Whistler, R. & Daniel, R.** (1990). Function of polysaccharides in foods. In: Branen,

571 A.L., Davidson, P.M. & Salminen, S. (Eds). *Food Additives*. New York: Marcel

572 Dekker.

573 **Yadav, M.P., Igartuburu, M., Yan, Y. & Nothangel, E.A.** (2007). Chemical

574 investigation of the structural basis of the emulsifying activity of gum arabic.

575 *Food Hydrocolloids*, 21, 297-300.

576

577

578

579

580 **Table 1.** Mean droplet diameters at the peak volume fraction of the emulsions,
581 calculated %w/w protein “content”, and “free” sulphhydryl content
582 Paired symbols (a, b, c, d, e, f, g, h, i, j, k, l) show significant difference (P<0.05)

		(1) Mean Droplet Diameters (µm)±SD	(2) Calculated % w/w protein ±SD	(3) “Free” sulphhydryl content (mmole×10 ⁻⁵ /mg) ±SD
(i) Native	a) KLTA NP	15.78±4.19 ^a	5.99±0.71 ^a	2.22±0.35 ^a
	b) KLTA P	18.19±2.93 ^b	6.74±1.13 ^b	2.81±0.20 ^{ab}
	c) GCA NP	19.60±3.56 ^c	0.63±0.43 ^{abc}	1.93±0.24 ^{abc}
	d) GCA P	33.54±13.85 ^{abcd}	0.99±0.76 ^{abd}	2.27±0.01 ^{bcd}
(ii) pH 2.8	e) KLTA NP ^{2.8}	59.92±24.99 ^{abcde}	0	2.26±0.29 ^{be}
	f) KLTA P ^{2.8}	302.34±75.11 ^{abcdef}	0	3.00±0.53 ^{acdef}
	g) GCA NP ^{2.8}	261.39±71.94 ^{abcdeg}	0	2.01±0.20 ^{bdfg}
	h) GCA P ^{2.8}	359.49±145.21 ^{abcdeh}	0	2.71±0.27 ^{cdeg}
(iii) pH 8.0	i) KLTA NP ⁸	32.46±5.30 ^{abcefg}	5.74±0.57 ^{cdi}	2.47±0.27 ^{cfgi}
	j) KLTA P ⁸	45.20±7.24 ^{abcfgh}	5.72±0.37 ^{cdj}	2.67±0.29 ^{acdgi}
	k) GCA NP ⁸	44.06±7.19 ^{abcfghij}	0.82±0.65 ^{abij}	2.55±0.26 ^{cfgk}
	l) GCA P ⁸	57.15±11.62 ^{abcdfghij}	0.47±0.44 ^{abij}	3.01±0.30 ^{acdeghjk}

583

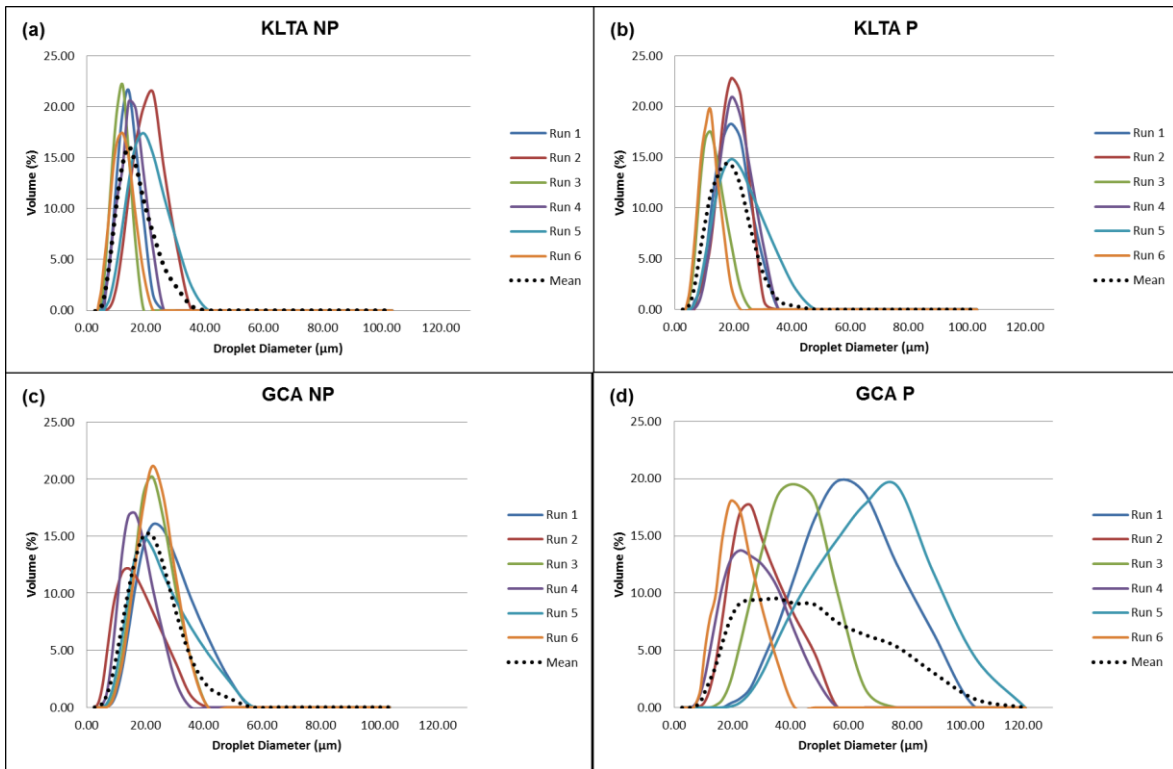
584

585

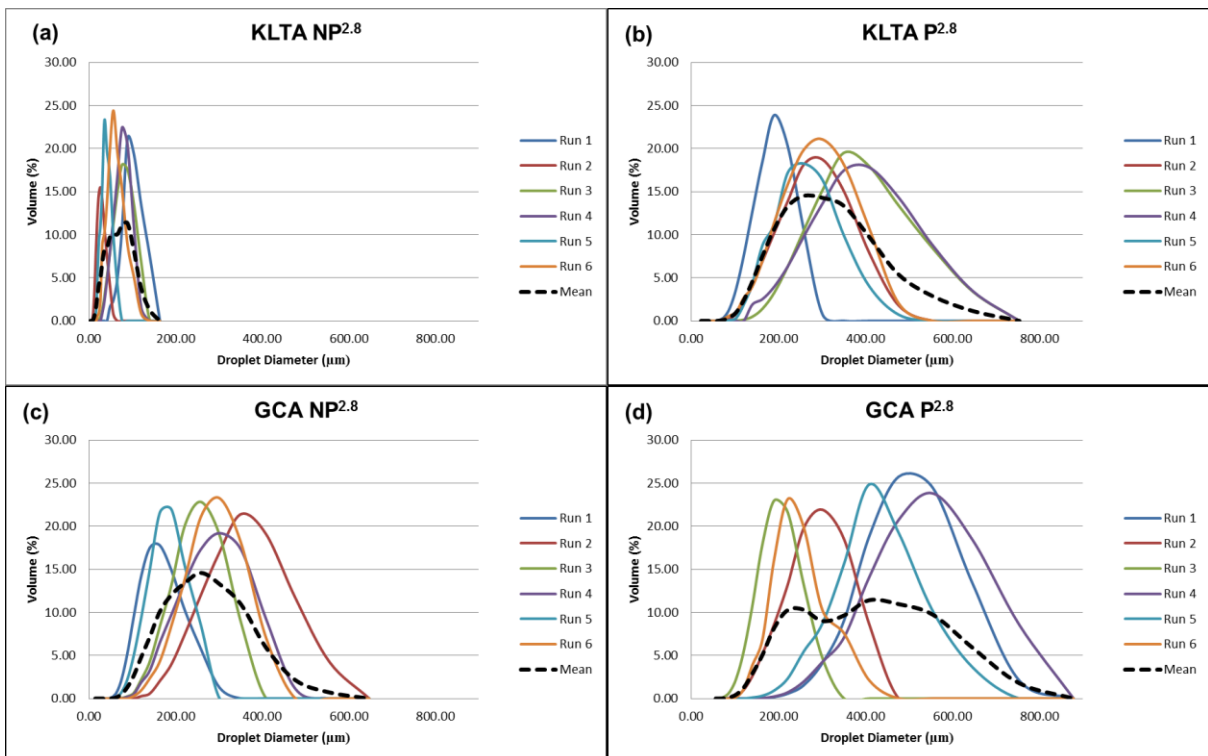
586

587

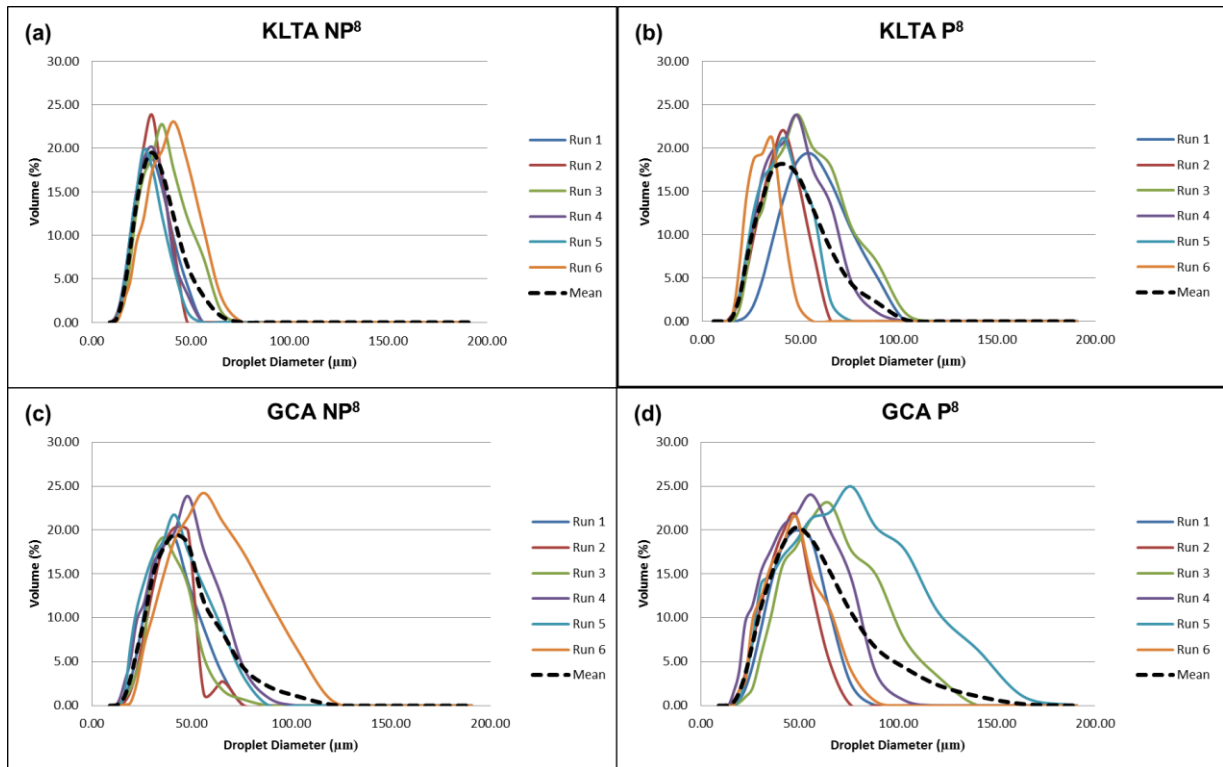
588 **Figure 1.** Droplet size distributions of emulsions made using KLTA NP (a), KLTA P
 589 (b), GCA NP (c) and GCA P (d) gum arabic



590
 591 **Figure 2.** Droplet size distributions of emulsions made using KLTA NP^{2.8} (a), KLTA
 592 P^{2.8} (b), GCA NP^{2.8} (c) and GCA P^{2.8} (d) gum arabic



594 **Figure 3.** Droplet size distributions of emulsions made using KLTA NP⁸ (a), KLTA P⁸
595 (b), GCA NP⁸ (c) and GCA P⁸ (d) gum arabic



596

597

598

599

600

601

602

603

604

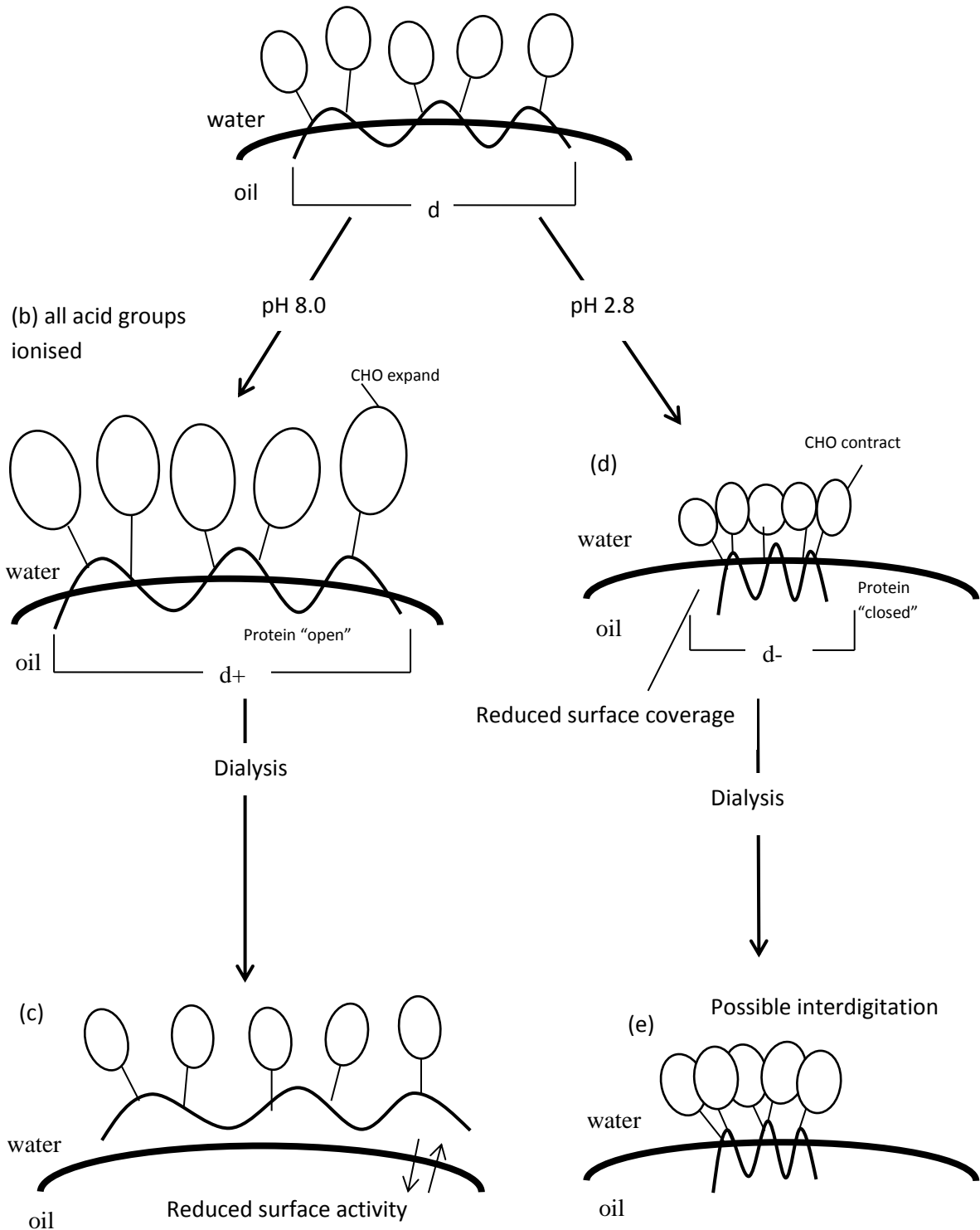
605

606

607

608 **Figure 4.** Possible mechanisms for changes in conformation which may affect gum
 609 emulsification properties after pH treatment

(a) Native untreated gum, natural balance of ionised/non-ionised carboxylic acid groups



610