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

Fanyi Ma,[1] Alan E. Bell,[1] & Fred, J. Davis[2]

[1]Department of Food and Nutrition Science, and [2]Chemistry, 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

1. Introduction

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

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

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

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

i) Arabinogalactan (AG, Mw ≈ 280kDa), the main component, which consists of about 88%w/w of the gum and contains the least protein (0.44%w/w);

ii) Arabinogalactan protein complex (AGP, Mw ≈ 1450kDa), 10%w/w of the total gum and contains about 9%w/w protein, in which the backbone chain links to the arabinogalactan chains through serine and hydroxyproline groups;

iii) Glycoprotein (GP, Mw ≈ 250kDa) which is the smallest fraction,1%w/w of the gum overall but having the highest protein content (55%w/w, about 4000 amino acid residues containing all of the cysteine and methionine) .

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

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

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

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

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

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

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

2. Materials & Methods

2.1 Materials

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

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

2.2 Sample preparation

The gum arabic dispersions (40%w/v) were made by adding the required amount of gums to deionised water (pH 7, conductance: 18mΩ), with gentle stirring at room temperature (20°C) overnight to allow dispersed. The solutions were further degassed under a vacuum to remove any entrapped air bubbles. The gum samples were prepared in duplicate (both for the KLTA and GCA) and were either dialysed overnight at 4ºC (native gums) or dialysed against the various phosphate buffer solutions (0.3 M) overnight at 4ºC to equilibrate to the required pH (2.8 and 8.0). The samples were then pressurised at 800MPa for 10 minutes using a prototype Stansted “food lab” high pressure apparatus (Stansted Fluid Power, Essex, UK). The pH treated and native samples were then dialysed against several changes of deionised water for 24h at 4ºC. No change in samples volume was observed. Materials were also freeze dried and stored in vacuum desiccators over P_2O_5 for further study.

2.3 Droplet distribution measurements

The emulsification properties were examined by measuring the droplet size distribution of emulsions made using native, pH 2.8 and pH 8.0 non-pressurise and 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

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

$$y = 0.0007x + 0.0059$$

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

2.5 Ellman's assay

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

$$C_0 = (A/\varepsilon)\, D$$

Where C_0 = Original concentration;

A = Absorbance at 412nm;

ε = Extinction coefficient (14, 150 $M^{-1}cm^{-1}$);

D = Dilution Factor

3. Results & Discussion

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

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

Fig. 1 (c) and (d) show the droplet size distributions of native and pressurised GCA gums. In this case, although the mean of the droplet size distribution in the untreated GCA gum was only slightly greater than the untreated KLTA gum (19.60µm and 15.78µm respectively). The overall variability of the GCA untreated replicates also increased. This “variability” was further enhanced by the pressure treatment of the GCA gum samples, with an overall increase in the mean droplet size to 33.53µm. Assuming that the increase in droplet size is an indicator of the gums decreased ability to stabilise a given surface area, then the GCA “poor” gums would seem to have “reduced” emulsification power, and be more detrimentally affected by any pressure treatment, than the equivalent KLTA “good” gum.

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

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

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

The most common use of KLTA “good” (*A. senegal*) gum is the food industry is the stabilisation of emulsions of flavour oil in soft drinks at low pH (2.5 -- 4, Harnsilawat et al., 2006; Friberg, 1997; Tan, 1990). Treating the KLTA at the low pH 2.8 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

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

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

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

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

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

3.2 Estimation of protein "content" in gum samples (Coomassie brilliant blue)

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

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

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

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

The “Bradford” reagent depends on the amphoteric nature of the proteins with Arginine (Arg) and Lysine (Lys) residues being the primary binding sites for the dye (Wei & Li, 1996). Since Arg and Lys are both considered “basic” amino acids, it is perhaps not supposing that after the gums were treated at pH 2.8, conformational changes were such that no protein was detected (i.e. no binding). KLTA and GCA gums would be expected to bind the dye differently because of the relative different amounts of Arg and Lys and the total levels of protein in each gum (42 and 29 residues/1000, KLTA and GCA respectively)). Simplistically, GCA should bind $\frac{29}{42}\times\frac{2}{3}$ less dye than KLTA, this should give a “calculated” protein content of 2.76% all other conditions being equal. The recorded value of 0.63% suggests that there is a conformational difference in the GCA protein moiety of the GCA gum when compared with the KLTA material with respect to its binding of coomassie brilliant blue. Previous authors have suggested that the protein structures of gum *A. senegal* “good” and A. seyal “poor” are different despite compositional similarities (Flindt et al., 2005; Siddig, et al., 2005). Subsequent the high pressure treatment of both types

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

3.3 Estimation of "free" sulphydryl content in gum samples (Ellman's assay)

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

Once pressurised KLTA gum showed no further changes at any of the pH treatment used (KLTA P is not significant different from KLTA $P^{2.8}$, KLTA P^{8}). This suggested that the statistical differences observed between these gums and "native" KLTA gum (*A. senegal*), is simply a pressure effect on the gum, i.e. conformational change in the protein exposing more sulphydryl groups. The various pH treatments on both types of gums without applied pressure only produced a significant increase in measured thiol levels at pH 8.0 for the GCA "poor" gum. This may suggest the different conformation of two types of gums, and/or may be as a result of "extension" of the protein structure at pH 8.0.

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

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

All pressurised pH treated gums (KLTA $P^{2.8}$, KLTA P^{8}, and GCA $P^{2.8}$, GCA P^{8}) showed no statistical differences in free thiol levels over their respective, pressure treated only controls (KLTA P and GCA P). For both gums (KLTA and GCA), a combination of pH treatments with pressure produced significant changes in all samples with respect to the thiols "available" to the Ellman's assay,. Overall the results indicate that with the exception of the pH treatment at pH 8.0, the major determinant of protein conformational change is the high pressure treatment. Hydrophobicity of protein was found to increase after the high pressure treatment (Messens et al., 1997; Galazka et al., 2000). Previous studies (Fauconnier et al., 2000; Panteloglou et al., 2010) have suggested that GCA (*A. seyal*) was a poorer

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

4. Conclusion

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

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

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

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

References

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

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

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

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

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

Chanamai, R. & McClements, D.J. (2002). Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: influence of pH, $CaCl_2$ and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Phillips, L.G., Whitehead, D.M. & Kinsella, J. (1994). Structure-Function Properties of Food Proteins. USA: Academic Press, INC.

Plancken, I., Loey, A.V. & Hendrickx, M.X. (2007). Foaming Properties of Egg White Proteins Affected by Heat or High Pressure Treatment. *Journal of Food Engineering, 78,* 1410-1426.

Randall, R.C., Phillips, G.O. & Williams, P.A. (1989). Fractionation and Characterization of Gum from Acacia Senegal. *Food Hydrocolloids, 3, 1,* 65-75.

Renard, D., Lavenant-Gourgeon, L., Ralet, M. & Sanchez, C. (2006). *Acacia senegal* gum: continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges. Biomacromolecules, 7, 2637-2649.

Siddig, N.,E., Osman, M.E., Al-Assaf, S., Phillips, G.O. & Williams, P.A. (2005). Studies on acacia exudate gums, part IV. distribution of molecular components in *Acacia seyal* in relation to *Acacia senegal*. *Food Hydrocolloids*. *19,* 679-686.

Silverman, R.B. (2002). *The Organic Chemistry of Enzyme Catalyzed Reactions.* UK: Elsevier Science.

Su, J., Flanagan, J. & Singh, H. (2008). Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUM^{TM}). *Food hydrocolloids, 22*, 112-120.

Tan, C.T. (1990). Beverage Emulsions. In: Larsson, K. & Friberg, S.E. *Food Emulsions, 2nd Ed*. New York: Dekker, 445-478.

Tan, C.T (1994). Beverage emulsions. In: Phillips, G.O. & Williams, P.A. (Eds). *Gums and Stabilisers for the Food Industry 7.* Oxford: Oxford University Press Inc.

Wei, Y. & Li, K. (1996). Linear regression method for the study of the Coomassie brilliant blue protein assay, Talanta, Elsevier. 44, 413-420.

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.
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580 **Table 1.** Mean droplet diameters at the peak volume fraction of the emulsions,

581 calculated %w/w protein "content", and "free" sulphydryl 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" sulphydryl 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^{abcefghi}	5.74±0.57^{cdi}	2.47±0.27^{cfgi}
	j) KLTA P^{8}	45.20±7.24^{abcfghi}	5.72±0.37^{cdj}	2.67±0.29^{acdgj}
	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}

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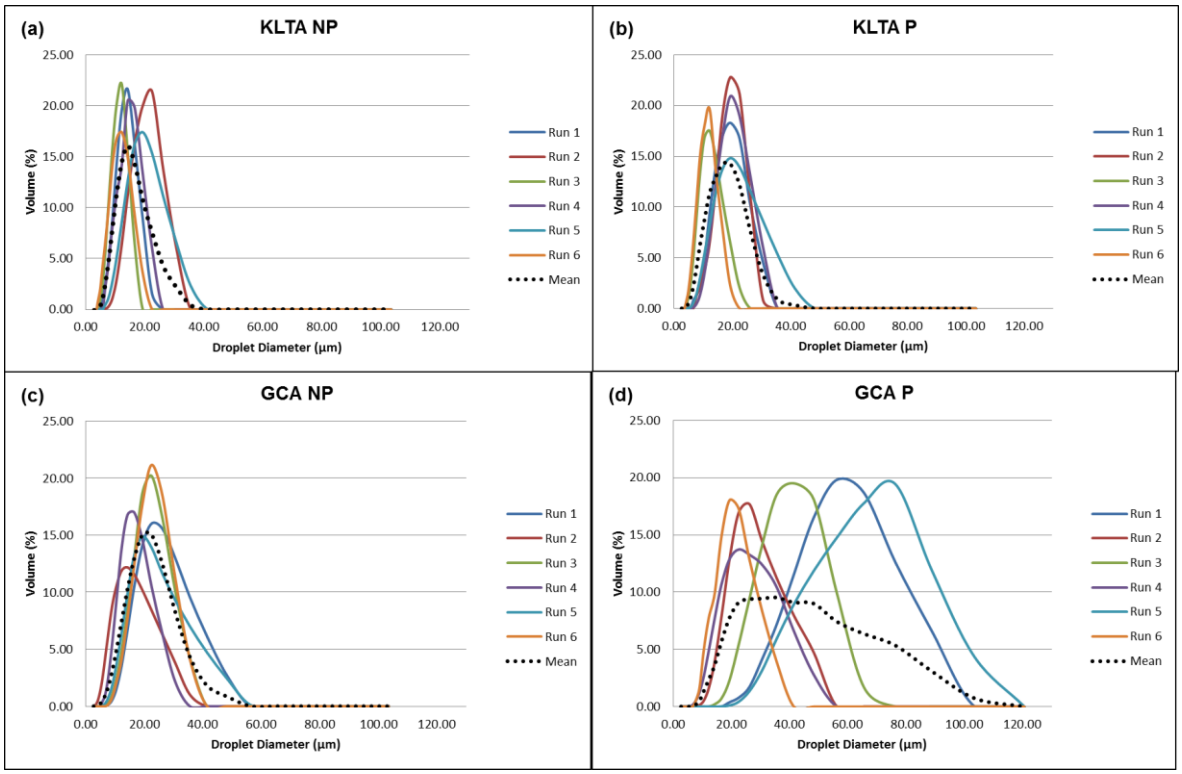
588 **Figure 1.** Droplet size distributions of emulsions made using KLTA NP (a), KLTA P

589 (b), GCA NP (c) and GCA P (d) gum arabic



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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

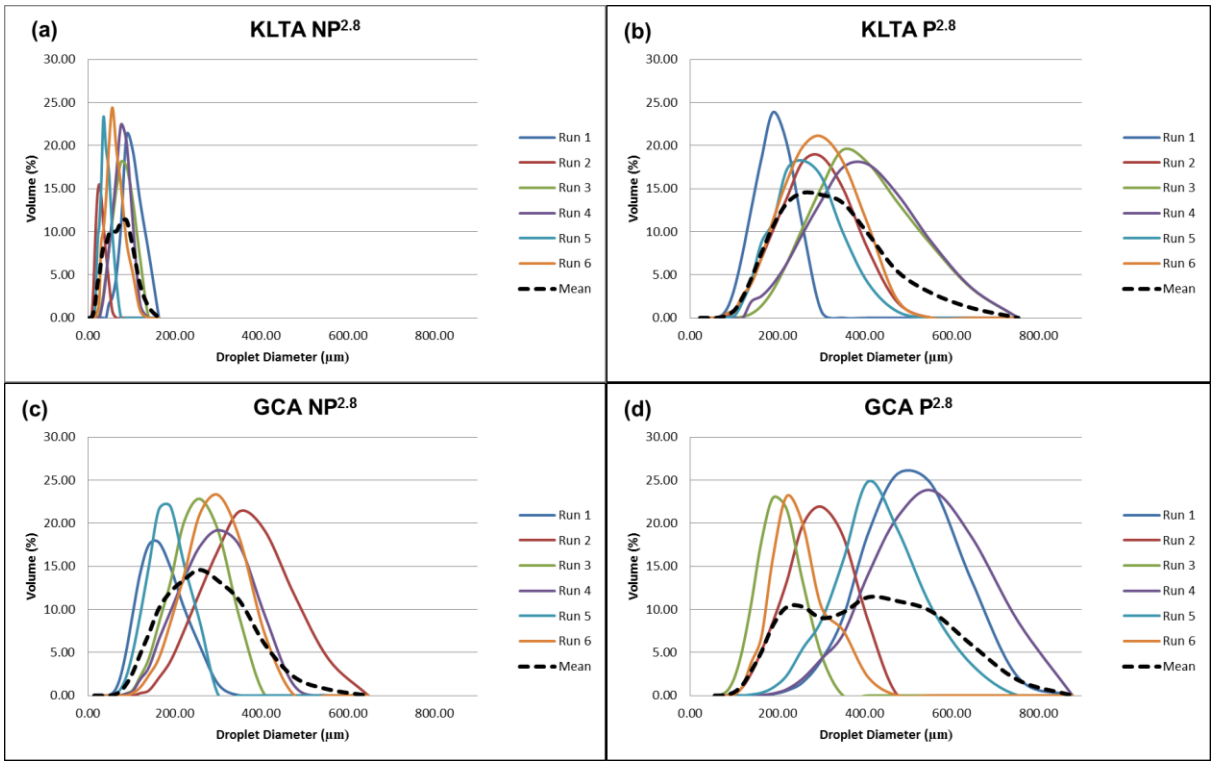

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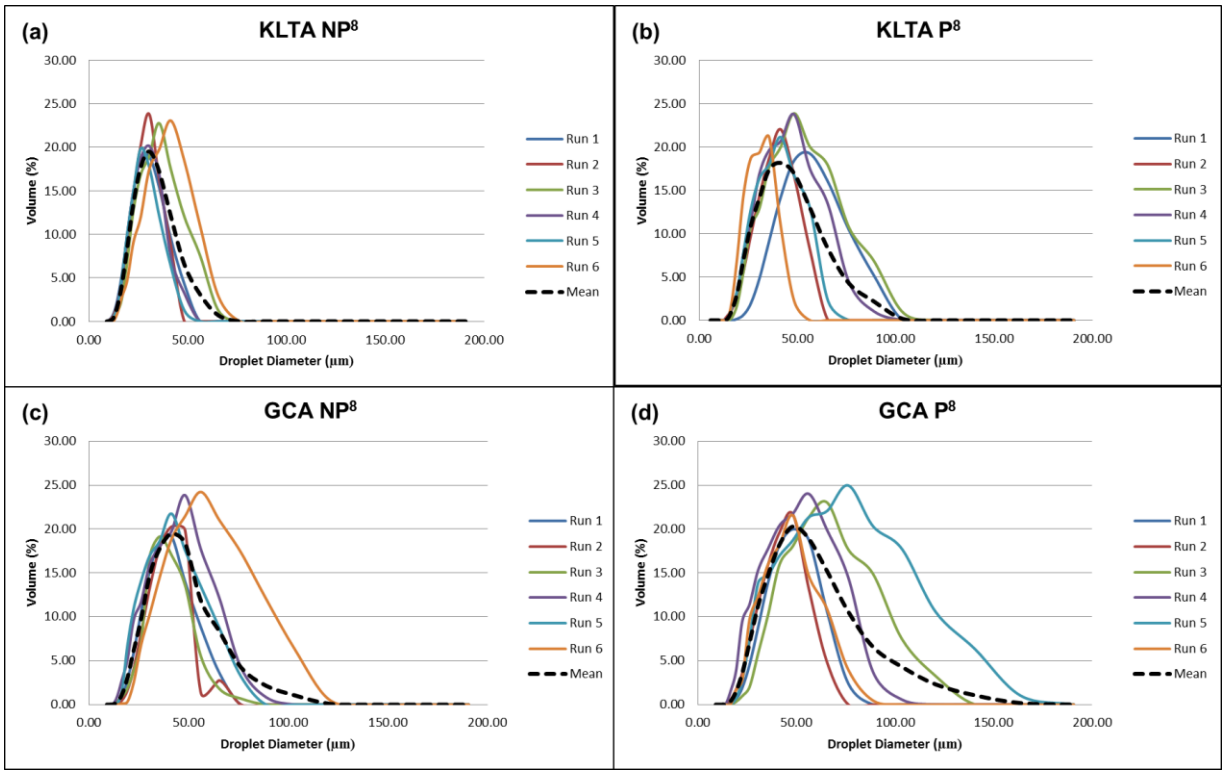
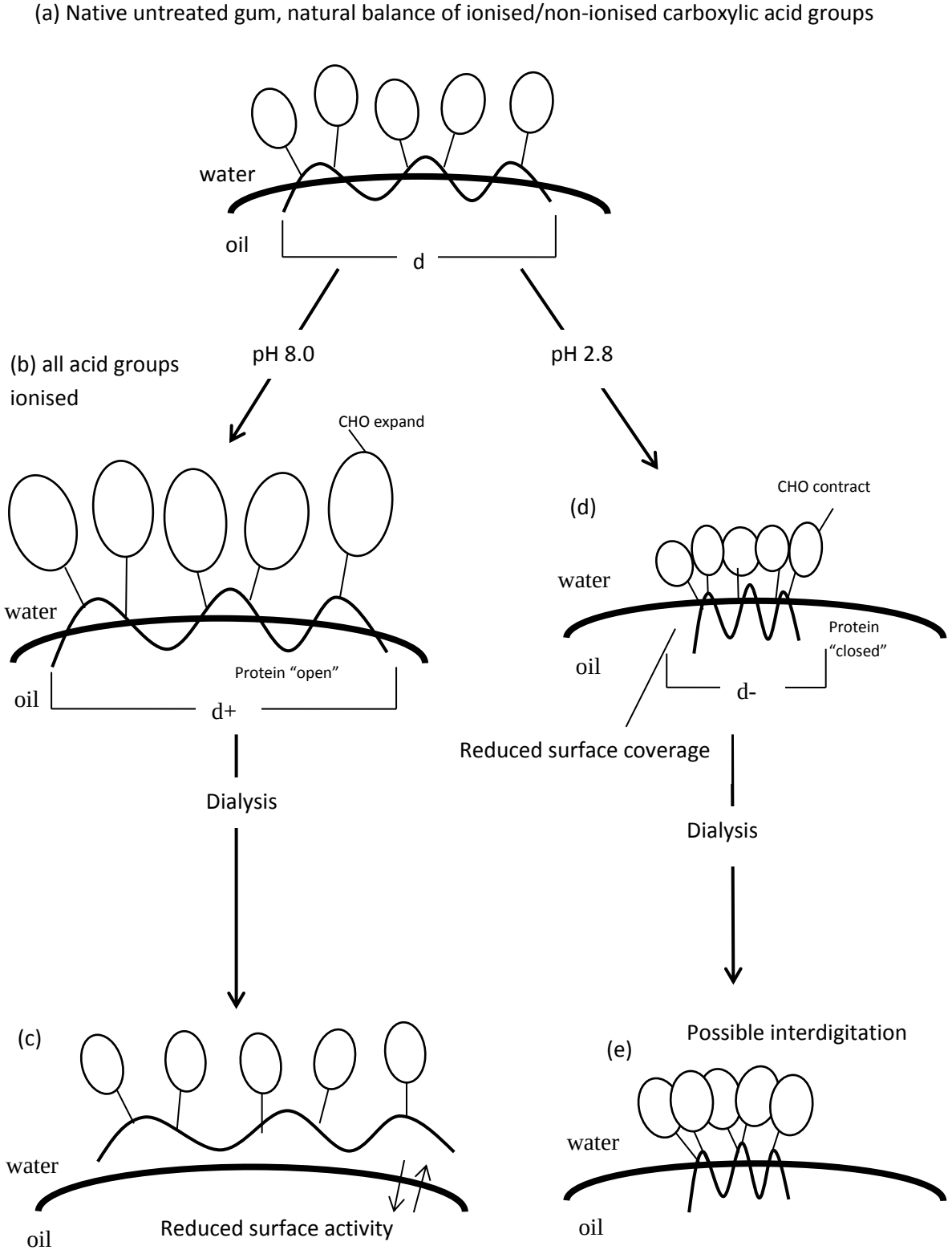
Figure 3. Droplet size distributions of emulsions made using KLTA NP[8] (a), KLTA P[8] (b), GCA NP[8] (c) and GCA P[8] (d) gum arabic



608 **Figure 4.** Possible mechanisms for changes in conformation which may affect gum

609 emulsification properties after pH treatment



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