

1-1-2009

Molecular associations in acacia gums

S Al-Assaf

Glyndwr University, s.lassaf@glyndwr.ac.uk

M Sakata

C McKenna

H Aoki

G O. Phillips

Follow this and additional works at: <http://epubs.glyndwr.ac.uk/phrc>

 Part of the [Materials Chemistry Commons](#)

© Springer 2009 This is an electronic version of an article that was originally published in *Structural Chemistry*, published by Springer Netherlands in 2009. The original publication is available at <http://http://www.springerlink.com>

Recommended Citation

Al-Assaf, S., Sakata, M., McKenna, C., Aoki, H., & Phillips, G. O. (2009) 'Molecular associations in acacia gums'. *Structural Chemistry*, 20(2), 325-336

This Article is brought to you for free and open access by the Materials Science at Glyndŵr University Research Online. It has been accepted for inclusion in Phillips Hydrocolloids Research Centre by an authorized administrator of Glyndŵr University Research Online. For more information, please contact d.jepson@glyndwr.ac.uk.

Molecular associations in acacia gums

Saphwan Al-Assaf*, Makoto Sakata, Catherine McKenna, Hiromitsu Aoki and Glyn O. Phillips

Glyn O. Phillips Hydrocolloids Research Centre, Glyndwr University (formally NEWI), Mold Road, Plas Coch Campus, Wrexham LL11 2AW, UK.

*Corresponding Author: s.lassaf@glyndwr.ac.uk

Tel ++44 (0)1978 293321

Fax ++44 (0)1978 293370

Running title: Molecular association in acacia gums

Keywords: Gum arabic, spray drying, radiation cross-linking, filtration, homogenisation

ABSTRACT

The tendency of polysaccharides to associate in aqueous solution has long been recognised. Molecular associations can profoundly affect their performance in a given application due to its influence on the molecular weight, shape and size. This will ultimately determine how the molecules will interact with each other, with other molecules and with water. There are several factors, such as hydrogen bonding, hydrophobic association, ion mediated association, electrostatic interaction, concentration dependence and the presence of proteinaceous components, which affect this behaviour. Our objective is to highlight the role of the proteinaceous component, present in acacia gum, to promote associations when the gum is subjected to various processing treatments such as maturation, spray drying and irradiation. The results demonstrate the ability of the proteinaceous component to promote hydrophobic associations which influence the size and proportion of the arabinogalactan high molecular weight component (AGP). Heat treatment in solid state (maturation) increases the hydrophobic character of the gum and hence its emulsification performance. Spray drying also involves aggregation through hydrophobic association but changes the surface properties of peptide moieties to become more hydrophilic compared to the association promoted by the maturation treatment in the solid state. Irradiation induced cross-linking, in the presence of unsaturated gas, was used to introduce C-C bonds into the carbohydrate moiety and thus confirms the hydrophobic association prompted by the heat used in the maturation and spray drying. This association can be reversed by treatments such as filtration or high pressure homogenisation. The results reported here reconcile the contradiction about structure of gum arabic proposed by the wattle blossom and twisted hairy rope models and shows that the AGP fraction is basically an aggregated fraction made up of AG units stabilised by low molecular weight highly proteinaceous components.

1. INTRODUCTION

Gum arabic (*Acacia senegal*) is predominately carbohydrate made up of approximately galactose (44%), rhamnose (13%), arabinose (27%), glucuronic acid and 4-O-methyl glucuronic acid (16%) [1]. It also contains 2-3% peptide moieties as an integral part of the structure. Three major fractions were identified following fractionation by hydrophobic interaction chromatography [2]. These are arabinogalactan protein (AGP), arabinogalactan (AG) and glycoprotein (GP). In *A. senegal* the AGP ~ 10% of the total gum which contains 10% of the protein and its Mw $\sim 1.5 \times 10^6$ g/mol. The protein was shown to be rich in hydroxyproline, proline and serine [3, 4]. The AG (Mw 2.8×10^5 g/mole) makes up 88% of the total weight and <1% protein with similar composition to that found in the AGP fraction. While the GP (Mw 2.5×10^5 g/mol) makes up only 1.24% of the total and contains ~50% of the total protein but with different amino acid compositions found in the AGP and AG fractions [5]. The neutral sugars present in the three fractions were reported to be similar but the GP fraction contained significantly less glucuronic acid compared to the AGP and AG fractions [6, 7]. All three fractions interact with Yariv's reagents and therefore, can be classified as arabinogalactan-protein complexes [8-10].

Enzyme digestion by pronase treatment of gum arabic, when monitored by gel permeation chromatography (GPC), showed that the high molecular weight peak was degraded resulting in the two peaks of the whole gum being converted into a single peak of lower molecular weight and similar to that for the AG fraction alone [3]. This indicates that the AGP was degraded to AG with a corresponding decrease in molecular weight from 4.9 to 2.0×10^5 [11]. This led to the suggestion of the wattle blossom structure for gum arabic, also proposed by Fincher [12], with a number of AG units of molecular weight 2×10^5 (g/mol) attached to a polypeptide chain via hydroxyproline. The hairy twisted rope model proposed

by Qi et al suggests a core rod-like protein (150nm long) with a highly repetitive amino acid sequence and the carbohydrate blocks (30 sugar residues) attached via hydroxyproline [13].

We have recently shown that molecular associations in gum arabic can lead to an increase in the molecular weight by maturation in the solid state under controlled heat and humidity [14]. The process does not involve any change in the basic structural components, and it is evident that as maturation proceeds to increasing levels, there is a regular aggregation/association of lower molecular weight components, which themselves contain protein, to yield more high molecular weight arabinogalactan protein. The process thus mimics the biological process, which produces more arabinogalactan protein as the tree grows to 15 years, and the maturation, which continues upon storage of the gum after harvesting [4]. Thus, all the original structural features of the harvested gum are retained by the maturation process. The change is completely associated with the increased formation of the arabinogalactan protein component by a physical aggregation process. An indication of the extent of re-organisation necessary to form the amount AGP in the matured sample of $M_w > 2$ million with complete solubility would require 2-3 units of AGP in the original gum to associate with 10-15 arabinogalactan and glycoprotein moieties in the control gum as shown previously [15].

The objective of this paper is to determine the role of proteinaceous component as a catalyst to promote association in acacia gum when subjected to various processing treatments. These include: maturation, enzyme degradation, filtration treatment, irradiation, high shear mixing, high pressure homogenisation and spray drying were used to identify the nature of associations.

2. EXPERIMENTAL

2.1. Materials

Gum arabic samples were obtained from the Gum Arabic Company (Sudan) and the spray dried gums were obtained commercially from various suppliers.

2.2. Methods

Gel permeation chromatography coupled online to a multi angle laser light scattering, refractive index and UV detectors (GPC-MALLS) was used to determine the molecular weight and distribution and has already been described [14, 16].

Protease EC 3.4.24.31 Type XIV Bacterial from *Streptomyces griseus* (Lot No. 99H0709) was obtained from Sigma, UK. Samples were made by dissolving 0.045gm (based on dry weight) in 4.5 ml of 0.2M NaCl containing 1mM sodium phosphate buffer at pH7.5. 0.5ml of protease (1.6×10^{-3} g/ml) added to the above solutions while 0.5ml of solvent was added to the control. The control and test samples were then incubated for 24hrs at 37°C. 2mg/ml solutions were prepared by diluting 2ml of the test sample or control to a total volume of 4.5 ml using 0.2M NaCl at pH7.5. 100 μ l filtered through 0.45 μ m nylon filter was then injected into the GPC-MALLS system for Mw determination.

Irradiation of gum arabic to doses of 6.1, 14 and 50kGy, in solid state, under different atmosphere conditions was carried out as described previously [17, 18].

Filtration of gum arabic solution in 0.2M NaCl at 2mg/ml was carried out using membrane filters of various pore-sizes. Fresh filter was used for each filtration step.

Gum arabic solutions at 20% in deionised water were homogenised using a laboratory scale high pressure homogeniser (Nano-Mizer NM2-L200-D10-Collision type S generator) obtained from Yoshida Kikai Co. Ltd., Japan. The respective solution was subjected to 1 or 3

passes at 25, 50, 75 and 150 MPa. The solution was then diluted to 2mg/ml made up to 0.2M NaCl and filtered through a 0.45mm filter prior to injection into the GPC-MALLS system.

3. RESULTS AND DISCUSSIONS

3.1. Maturation process

The maturation process to produce higher molecular weight materials by controlled heat treatment of gum arabic in the solid state has been already described [14, 15] and is summarised here. Table 1 shows the molecular weight parameters of control and a series of matured gums. The gum (control) was matured to different levels, and the resulting products were designated (A–C). The changes illustrated in the UV elution profile where the aggregation of the proteinaceous components through hydrophobic associations is evident in Figure 1. The UV profile was chosen to illustrate the difference since it clearly shows the presence of three major fractions identified by hydrophobic fractionation mentioned earlier. The latter Figure shows that with increasing the maturation treatment there is an increase in AGP fraction resulted from incorporating the AG units while the GP fraction is completely absent. The matured (aggregated) samples were completely soluble and had exactly the same specific optical rotation, sugar moieties and amino acids in the same proportions as control (untreated) gum [15]. The conformational changes of peak 1 (AGP) and peak 2 (AG+GP), monitored using the plot of $\log M_w$ versus $\log R_g$, showed that the slope of peak 1 changes from 0.4 for the control to values ~ 0.3 . This indicates the formation of more compact spherical molecules as the maturation process proceeds. On the other hand, the slope of peak 2 changes from 1.1 (typical of rod shape molecules) to values approaching 0.66 (typical of random coil or globular shape). Furthermore, the increase in the molecular weight is accompanied by an increase in viscosity, both in water and salt solution and the polyelectrolyte character of the matured samples is retained as demonstrated by the increase in the hydrodynamic volume (intrinsic viscosity) with lowering the salt concentration

[15]. The maturation process involves at least three stages to achieve the desired modification/changes in the solid state. The first stage involves breaking hydrogen bonds and is typically associated with short period (1-2 hours) of heating at temperatures $> 110^{\circ}\text{C}$ (data not shown). The same effect is also observed when the gum is heated in the solid state at temperature of 50°C or greater for longer period. This initial stage is usually associated with a reduction in the molecular weight. The reduction of the molecular weight and viscosity is directly related to the disassociation of low molecular weight proteinaceous component (short peptide chains) which appears at the total volume of the column ($\sim 20\text{ml}$) and indicates its crucial role in stabilising the AGP (high molecular weight) fraction. It is also possible that the reduction in the molecular weight could be due to elimination of the intermolecular hydrogen bonds reported to be present in the numerous polysaccharides substituents and the polypeptide proposed in the twisted hairy robe model [13]. With further increase in the heating time (>2 hours at 110°C) hydrophobic associations become dominant and subsequently lead to increasing the molecular weight and complete disappearance of the GP (glycoprotein) peak (Figure 1). The third stage involves, heating longer than 24 hours at 110°C , the formation of insoluble (hydrogel) material with great capacity to absorb and retain water many times its own weight [14]. All these changes are almost completely controlled by the small amount of protein ($\sim 2\%$) in relation to the total (carbohydrate) mass. Disaggregation of the highly associated hydrogel network can be achieved by heating, filtration through small pore size filters and the application of high shear mixing to gum arabic dispersion. The resultant product has increased solubility and high molecular weight.

The effect of filtration on polysaccharide solutions greatly influences the apparent molecular weight when determined by an absolute method such as light scattering [19]. Table 1 shows the effect of filtration through different pore size filters (0.1, 0.2, 0.45 and $0.8\ \mu\text{m}$) on diluted solutions (2mg/ml) of the control and matured (aggregated) samples. The control sample was a conventional *A. senegal* gum in the spray dried form, with a weight

average molecular weight (M_w) of 622K g/mol when filtered through the standard pore size filter (0.45 μ m). Table 1 shows the progressive reduction in molecular weight, radius of gyration and % of the high molecular weight fraction (AGP) as the pore size of the filter is reduced which can be largely explained by the effect of treatment on the high molecular weight fraction (AGP) and its radius of gyration (Table 1). Filtration using smaller pores size filters leads to the disassociation of the AGP peak into the smaller AG units, so decreasing the M_w for the whole gum, and increasing the M_w of the AG peak (Table 1). The extent of molecular weight reduction is greater the higher molecular weight is of the initial sample. The filtration treatment is the reverse process to the maturation process which indicates the role of hydrophobic associations. Although the mass recovery of the whole gum is always in the region of ~100% we cannot rule out the possibility that a very small percentage of high molecular weight material is retained on the filter. The disassociation in matured samples is accompanied also by the production of low molecular weight highly proteinaceous materials, possibly short peptide chains, which appear in the total column volume (20-24ml) as shown in Figure 2. The UV response of this peak illustrates the role of this low molecular weight fraction in stabilizing the high molecular fraction (AGP). This peak is very similar to that initially produced during the 1st stage of maturation. It, therefore, suggests that it participates in hydrophobic association and possibly further stabilised by hydrogen bonding.

High pressure homogenisation is another treatment that has been shown to influence the solution properties [20, 21]. Three main areas where this treatment has been used are to:

- (i) Inactivate microorganisms by inducing changes in morphology, of biochemical reactions, genetic mechanisms, cell membranes and the walls of microorganisms.
- (ii) Disturb non-covalent interactive forces which stabilize the structure of proteins and polysaccharides and induce denaturation, aggregation and gel formation.

- (iii) Inactivation of enzymes due to changes in conformation of enzyme and substrate.

Proteins are well known to be denatured when subjected to high pressure homogenisation[21]. The extent of denaturation depends on the native protein structure, magnitude of applied pressure and solvent conditions (composition, ionic strength, pH and temperature [22]. The role of the above factors was highlighted in a recent study by Puppo et al who reported an increase of adsorbed proteins and a decrease of flocculation following high pressure treatment of soybean protein emulsions [23]. However, no such improvement in the emulsification properties was found of β -lactoglobulin and whey protein concentrate (Galazka et al, 1996, 1995) after the same treatment.

Applying high pressure has been also used to study the disassociation or possibly degradation of polysaccharides such as tragacanth [24], xanthan [25], methyl cellulose [20], chitosan [26], modified starch [27]. The previous reports suggested that depending on the pressure treatment the functional properties can be greatly affected due to disassociation or disruption of the covalent bonds. However, the effect of high pressure homogenisation on gum arabic solutions has not been investigated.

Gum arabic is used mainly in confectionary, thickener, stabiliser and emulsifier in variety of foods. One of its most widely used applications is in the beverage industry as an emulsifier of citrus flavours. Gum arabic adsorbs on to the surface of freshly formed droplets during homogenisation, forming a protective membrane which prevents the droplets from flocculation and coalescence through electrostatic and steric repulsion forces [2]. Of particular importance is the amount of the high molecular weight AGP component which is now been established as the component which controls its efficiency in emulsification and adhesion [2, 14, 15]. However, the presence of other fractions was reported to be necessary for the AGP to function in this manner. Gum arabic's role as an emulsifier is achieved as a consequence of its amphiphilic character due to the presence of protein and polysaccharide

moieties. It reduces the oil–water interfacial tension, thereby facilitating the disruption of emulsion droplets during homogenization. The peptide moieties (~2% present in gum arabic) are hydrophobic and strongly adsorb on to the surface of oil droplets, while the polysaccharide chains are hydrophilic and extend out into the solution.

Food oil-in-water emulsions are generally produced using either colloid mills or high pressure homogenisers [28]. The colloid mill (high shear mixing) is used to manufacture mayonnaises etc., in which stability depends less on the presence of small droplets than on the overall composition and the high viscosity of the formulation. On the other hand, high pressure homogeniser is usually used to make liquid emulsions such as beverage emulsions, because smaller droplets are required to prevent creaming and coalescence [21, 29]. Previous investigations on gum arabic emulsions either employed high shear mixing or high pressure treatment. None considered the differences between these two methods.

We have subjected similar samples used for the filtration treatment to high pressure homogenisation in order to determine the effect on the molecular parameters particularly on the high molecular weight fraction (AGP). The changes following various high pressure homogenisation treatments were monitored using GPC-MALLS (Table 2). Mw of the control sample (untreated) was 0.72 million and the matured sample was 2.36 million, with AGP contents of 9.9 % and 18.0 % respectively. We prevented causing changes during the high shear mixing step using the polytron, which can take place, by placing the solutions in an ice water bath and confirmed this by measurement. High shear mixing is typically used during preparing the pre-emulsion and also industrially to dissolve the gum. However, a decrease in molecular weight and AGP content was evident on increasing the pressure and on increasing the number of passes (Figure 3 and Table 2). For the control sample pressures up to 150MPa led to not more than 20% reduction in the weight average molecular weight and the proportion of the AGP fraction after one pass. Only the Rg value is decreased by this

pressure treatment. The same treatment reduced the Mw of the matured sample from 2.3 million to 900K (g/mol). The Rg of AGP fraction decreased also (from 95 nm to 38 nm). Repeated homogenisation (3 passes) for the control sample changed Mw by 28% after three passes at 150MPa. The change in all molecular parameters was considerably greater for the matured gum (Figure 3 and Table 2) due to higher aggregation (through hydrophobic associations) compared to the control. As after the filtration treatment, the decrease in the proportion of the AGP and Mw for the whole gum was also accompanied by an increase in the proportion of the AG fraction and formation of low molecular weight proteinaceous components. It should be mentioned here that high pressure treatment up to 150MPa for 3 passes could result in breaking some of the covalent bonds. The high elongational flow the polymer encounters during the passage through the value of the pressure homogeniser provides sufficient energy to disrupt covalent bonds and then degrade the polymer as demonstrated with methyl cellulose [20]. The results obtained at low pressure treatment (25 and 50MPa), which is relevant to the pressure typically used in a large scale high pressure homogenisation, support the disassociation mechanism possibly due to changing the surface functional properties of the proteinaceous components. As a consequence there is a reduction in the hydrophobic associations which is reflected by the reduction in apparent molecular weight. The results given above explain the superior emulsification of the matured sample compared to the control as demonstrated previously [14, 30].

3.2. Spray drying process

Spray drying is a technique widely used in dairy industry and for polysaccharides such as gum arabic. It involves the elimination of water from the solutions by spraying the product in a current of hot air. Prior to spray drying the gum undergoes dissolution procedure and can be subjected to temperatures up to 70-80°C. Removal of the impurities (bark, sand and

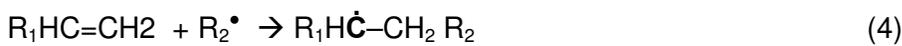
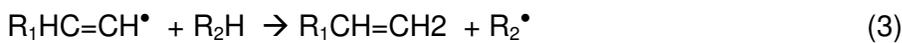
foreign matters) is achieved through the clarification process such as filtration and centrifugation or both. Subsequently, the gum solution is pasteurised and typical temperature used in this step could be up to 90°C for 10-30 sec. All these heating steps can have considerable impact on the quality product as we have demonstrated previously [16, 31]. The main difference between the raw material and the spray dried gum is the presence of larger proportion of an aggregate peak at the start of the elution volume as shown in Figure 4. The fresh raw material collected from recent harvest in certain locations in Sudan also show the presence of aggregate peak which is believed due to the presence of green gum [31]. The proportion of the aggregate peak in raw material is considerably lower than that usually detected in spray dried gum. The presence of the aggregate peak in the spray dried samples has been attributed to the processing conditions which employs harsh treatments [16, 31] particularly at the dissolution and pasteurisation steps. Additionally we have demonstrated that upon controlling the spray drying conditions the presence of the aggregate peak can be almost eliminated. Aggregation due to harsh temperature treatment during pasteurisation where the glycoprotein (GP) peak (elution volume 14-16ml) is greatly reduced and sometimes completely absent from the elution profile was also reported previously [31]. Detailed investigation on the structure, molecular weight parameters and emulsification performance of several gum arabic samples subjected to spray drying will be published elsewhere. The objective here is to use one of the spray dried product which showed presence of aggregate peak and subject it to similar filtration treatments used for the matured samples given above (see Table 1). The sample we selected has an apparent molecular weight of 1.7×10^6 (g/mol) which included the aggregate peak (see Table 3). Upon the removal of the aggregate peak contribution electronically (i.e. excluded from the calculation) the molecular weight is reduced to 8.5×10^5 (g/mol). The latter value approaches the molecular weight parameters obtained from standard gum arabic in spray dried form which does not show aggregation. Repeated filtration through 0.45 μm filter resulted in a significant

decrease in the molecular weight to 5.4×10^5 and the molecular weight of the AGP peak reduced from 3.4×10^6 to 2.5×10^6 . Filtration through smaller pore size filters (0.2 and 0.1 μm) also showed a decrease in the molecular weight. Repeated filtration through 0.2 μm filter resulted in similar weight average molecular weight to that observed after four filtration steps through 0.45 mm filter (Table 3). Here also there was also an increase in the molecular weight of the AG peak similar to observed for the matured samples when subjected to similar filtration treatments. There seems to be a common feature between the spray dried and matured samples which is the production of low molecular weight highly proteinaceous materials at the total column volume as shown in Figure 5. The results obtained here also suggest the role of these proteinaceous components in increasing the AGP peak proportion following the spray drying process.

3.3. Radiation cross-linking

We have previously shown that gum arabic and other polysaccharides can be modified in the solid state when irradiated in the presence of acetylene. The process also applicable to a range of polysaccharides (charged or uncharged) and proteins systems [32]. The proposed mechanism to increase the molecular weight by the addition of C-C bond between two chains is illustrated in the following equations (1-5).

Note: R_1H and R_2H designate two polymeric chains





The direct radiation action forms a free radical (designated here as R_1^\bullet) which then adds to the acetylene to give a radical with a double bond. This addition to the acetylene is slow and the reactive radical with a double bond abstracts hydrogen atom from a nearby polysaccharide chain to give two radicals, one on the original acetylene adduct and one on a nearby polysaccharide chain (R_2^\bullet). These recombine to give a cross-linked stable radical. This radical has fair degree of mobility and either recombines with acetylene, radical generated as a result of the action of ionizing radiation or another similar radical. This process to increase the molecular weight and produce hydrogel form has been recently described [17, 18]. Table 4 gives the molecular weight parameters of control and irradiated sample for 6kGy. The molecular weight of the starting material is 5.9×10^5 g/mol with Mw of 2.3×10^6 and proportion of 12% for the first peak (AGP). These parameters are typical of standard *A. senegal* in the spray dried form [16] and similar to that used in the maturation section above. Irradiation of *A. senegal* in the solid state in the presence of acetylene results in increasing the molecular weight to 2.9×10^6 (g/mol) while retaining complete solubility as indicated by the mass recovery. The Mw of the first peak (AGP) is 9.4×10^6 g/mol with proportion of 26% (Table 4). The molecular weight parameters of the irradiated sample are comparable to that obtained for sample C produced by the maturation treatment (see Table 1). Our objective here is to show the difference between the maturation process and irradiation induced cross-linking, which both result in increasing the molecular weight but through different mechanisms as outlined above. Filtration of the irradiated sample through 0.45 μ m filter three times gave very comparable M_w parameters to that obtained after one filtration step. The results are indicative of stable cross-linked network structure which can not be disassociated. Furthermore the GP peak is intact after irradiation treatment which

resulted in yielding the highest molecular weight. It is also not affected by the filtration treatment which indicates that it does not play part in increasing the molecular weight of irradiated samples. Here the addition of the C-C bond is 98% more favourable to take place on the carbohydrate moiety compared to the protein which makes up about 2% of the total mass. Further filtration through smaller pore size filter (0.20 μ m) also gives very comparable M_w parameters to that obtained after filtration through 0.45 μ m filter. The results are further indication of stable cross-linked network structure which can not be disaggregated. Filtration through even smaller pore size filter (0.10 μ m) reduces the Mw of the whole gum to 1.7×10^6 (g/mol) compared to the starting Mw of 2.9×10^6 g/mol. The reduction here is mainly due to the removal of ultra high molecular weight from peak 1 which resulted also in reducing the Rg 67 to 50nm and a reduction in the mass recovery (see Table 4). Even when the smallest pore size filter is used the molecular weight does not reduce to original molecular as demonstrated for the matured and spray dried samples.

3.4. Enzyme digestion

Enzyme degradation of gum arabic by pronase treatment has been crucial to elucidate its structure as highlighted earlier in the introduction. Additionally it has also been used to distinguish between gum species and identify the similarities and difference, for example, between *A. senegal* and *A seyal* [33]. We have used this method here to show the difference between the matured samples produced by heat treatment and those prepared by irradiation under different atmosphere conditions. Our first objective is to show that the matured samples are produced purely by aggregation of the proteinaceous components and not by Miallard type reaction. Table 5 give the molecular weight parameters obtained by GPC-MALLS for the test samples before and after treatment with protease for 24 hours at 37°C.

Enzyme digestion of control *A. senegal* (sample D) reduced the Mw of the whole gum from 8.3×10^5 to 3.73×10^5 (g/mol) and is consistent with previous reports [34]. The main effect of enzyme digestion is the reduction of the Mw of the first peak (AGP) and a slight increase in the Mw of the second peak as outlined in the introduction. Another control sample (Sample E), with starting Mw of 5.96×10^5 g/mol, used for the irradiation experiment was also subjected to the same treatment and showed the same effect. Note the reduction after enzyme treatment for control samples is dependent upon the starting Mw and thus the difference between the molecular weights of the enzyme treated control samples. Heating sample D, in the solid state, at 110°C for 5 and 24 hrs resulted in increasing the molecular weight from 8.3×10^5 to 1.0 and 1.7×10^6 g/mol respectively as shown earlier (Samples D5 and D24). The mass recovery for Sample D24 was 72% indicating that 28% of the material is converted into a hydrogel form, mentioned earlier as the third stage of maturation treatment, which can not pass through $0.45 \mu\text{m}$ pore size filter. Enzyme digestion of the matured samples (D5 and D24) decreased the molecular weight to 4.1 and 5.1×10^5 g/mol (samples D5-24 and D24-24 respectively) and show almost 100% mass recovery. The results given above demonstrate that the maturation process is aggregation through hydrophobic associations and not Maillard type reaction. The difference between the molecular weight of digested control and matured samples could be due to the aggregation of GP component and its subsequent transfer to higher molecular weight fraction. The GP fraction is not affected by the protease treatment as shown previously [10]. Additionally, the results obtained for Sample D24 following enzyme digestion (Sample D24-24) shows that the enzyme can access the hydrogel form as demonstrated by the complete mass recovery (see Table 5).

Irradiation of *A. senegal*, in the kibbled form (Sample E), for 6.1 and 14kGy results initially in increasing the molecular weight and subsequently hydrogel formation (Samples E6 and E14 respectively, see Table 5). We have previously shown (Phillips et al, 2003) that irradiation to

6kGy results in the maximum increase in the molecular and higher doses gives comparable molecular weight but with less mass recovery compared to the starting material. Enzyme digestion of irradiated samples (E6 and E14) resulted in increasing the molecular weight as a result of increasing the % mass recovered for peak 1 (high molecular weight peak). It is possible that the presence of ultra high molecular weight materials in both irradiated samples which is normally retained on the filter is released back into solution following enzyme digestion as demonstrated by the increase in the mass recovery. The results given above demonstrate a clear difference between the modification of gum arabic by maturation and radiation cross-linking. The latter produces stabilised network structure by the addition of C-C bonds between the carbohydrate moieties since it makes up 98% of the whole gum. On the other hand, the aggregation process achieved by maturation mainly involves hydrophobic association of the proteinaceous components.

Furthermore *A. senegal* in the kibbled form (sample E) was also irradiated for 50kGy in the presence of nitrogen (Sample E50N) and butane (Sample E50B). Nitrogen is typically used to reduce the effect of irradiation by removing the oxygen and thus eliminating the formation of peroxy radicals which are the precursors to strand breakage [35]. On the other hand, the saturated gas (butane) does not contribute directly to the cross-linking mechanism proposed above. The objective here is to account for the heat generated during irradiation to such a high radiation dose and to investigate its effect on the molecular weight parameters. The results of irradiation for 50kGy in the presence of nitrogen and butane are tabulated in Table 5. Irradiation in the presence of nitrogen resulted in comparable Mw to that of the control. The only identifiable difference is the slight reduction in the mass of the first peak (AGP). Irradiation in the presence of butane seems to have a greater effect compared to nitrogen and resulted in increasing the molecular to 8×10^5 compared to 5.9×10^5 (g/mol). Again the difference is mainly in the first peak (AGP). Possible reason for this slight increase is cross-

linking due to radical-radical association as shown recently when gum arabic is irradiated in paste-like state [36] as a result of a greater radical mobility. Gum arabic contains on average ~10% moisture and it is possible that there some cross-linking takes place. Enzyme digestion of irradiated samples (E50N and E50B) resulted in a decrease in the Mw to 3.5 and 3.7×10^5 (g/mol) respectively compared to the control of 3.4×10^5 (g/mol). It is, therefore, evident that irradiation to high doses that there is also a minimal modification due to heat generated during irradiation since the resultant products seem to degrade in a similar manner to that obtained after maturation.

4. CONCLUSIONS

Two models have been proposed to represent AGP and 'AGP-like' molecules. The first, proposed by Fincher [12] predicts a spherical wattle-blossom model for monomers of AGP. The second suggests a twisted hairy rope model [13]. The two models were based on results obtained from various techniques and methods which led to the proposal of either model. Subsequently, there have been several studies to support either model and provide feasible explanation of the remarkable properties of gum arabic in terms of its low viscosity at higher concentration and excellent emulsification performance and stability. The wattle blossom model depicts the AGP structure as a continuous peptide core with carbohydrate units attached to it and suggests a spherical type structure which contradicts the 150nm long rod-like structure proposed in the twisted hairy robe model by Qi et al [13]. It is possible now to reconcile the two models using our results which provide sufficient evidence that the high molecular weight in gum arabic we identified here using GPC as the first peak and referred to as the AGP is not composed of single polypeptide chain with various carbohydrate units attached to it. The AGP is mainly composed of AG units associated through hydrophobic association and this is why the protein sequence and sugar contents is similar in both fractions but different to that in the GP fraction as reported previously [6, 7, 37]. However,

key to the stability and coherent of the AGP structure is a small proportion of low molecular weight highly proteinaceous components often reported in the literature as the GP fraction [5]. Recent circular dichroism study on the three fractions (AG, AGP and GP), obtained by HIC fractionation, reported that the AGP and GP fractions have polyproline II, B-sheet, and random coil secondary structures whereas no secondary structure was identified in the AG fraction [7]. Furthermore, Renard et al also subjected the GP fraction to further size separation technique coupled to an absolute detector, HPSEC-MALLS, and determined its molecular weight. Their results identified three populations, with low polydispersity index, within this fraction. The three populations had M_w of 2.6×10^6 , 7.9×10^5 and 2.9×10^5 g/mol respectively. This clearly demonstrated the tendency of this proteinaceous fraction to aggregate possibly promoted by the freeze-drying process it was subjected to prior to HPSEC-MALLS measurements. Another relevant study to this investigation was recently reported by Mahendran et al [37]. In this study deglycosylation of gum arabic was performed by treatment with anhydrous hydrogen fluoride and revealed the presence of two putative core proteins with M_w of 3×10^4 and 5×10^3 (g/mol) which respectively correspond to protein of approximately 250 and 45 amino acids in length. It is interesting to note that Renard et al [7] estimated the polypeptide backbone for the AG fraction to be 43 amino acid residues in agreement with the value reported for the one of the protein cores identified by Mahendran et al [37]. On the other hand, based on biochemical analyses and molecular weight determination and assuming a single chain, Renard et al [7] estimated the peptide backbone length for the high molecular weight fraction (AGP) to be 2253 amino acid residues but suggested that several glycoprotein domains could account for their value compared to the previous value of >400 reported by Qi et al [13]. Our results provide evidence that the high molecular weight fraction (AGP) in gum arabic is indeed highly associated molecular structure. When subjected to treatments such as filtration or high pressure homogenisation it can be disassociated to yield the basic molecular units which make up the gum (i.e. the AG)

and a low molecular weight fraction highly proteinaceous as we demonstrated. Further evidence to support our proposal came from enzyme digestion of gum arabic which was shown to be dependent on the starting molecular weight. The reduction of the molecular weight following enzyme digestion can be as low as 1.8×10^5 or 5.0×10^5 g/mol. With increased associations the enzyme can not access all sites to induce the reduction which is not uncommon in AGPs [1]. It is reasonable, therefore, to suggest that exudation of the gum could be regulated through factors such as location or age of the tree since it is widely known that trees five years and older can produce the gum. Depending on these factors the aggregation or association of the gum can proceed to different extent that is greatly influenced by the location (i.e. temperature, humidity) and possibly subsequent treatments following harvest which result in variable gum quality.

References

1. Williams PA, Phillips GO (2006) Food and Food Ingredients Journal of Japan 211:181.
2. Islam AM, Phillips GO, Sljivo A, Snowden MJ, Williams PA (1997) Food Hydrocoll 11:493.
3. Osman ME, Menzies AR, Williams PA, Phillips GO, Baldwin TC (1993) Carbohydr Res 246:303.
4. Idris OHM, Williams PA, Phillips G O (1998) Food Hydrocoll 12:379.
5. Randall RC, Phillips GO, Williams PA (1989) Food Hydrocoll 3:65.
6. Williams PA, Phillips GO, Stephen A M (1990) Food Hydrocoll 4:305.
7. Renard D, Lavenant-Gourgeon L, Ralet MC, Sanchez C (2006) Biomacromolecules 7:2637.
8. Akiyama Y, Eda S, Kato K (1984) Agri Biol Chem 48:235.
9. Osman ME, Menzies AR, Martin BA, Williams PA, Phillips GO, Baldwin TC (1995) Phytochemistry 38:409.
10. Williams PA, Phillips GO (2000) In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids, Woodhead Publishing Limited, Cambridge, pp 155--168.
11. Connolly S, Fenyo JC, Vandeveld MC (1978) Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales 181:683.
12. Fincher GB, Stone BA, Clarke AE (1983) Ann Rev Plant Physiol Plant Mol Biol 34:47.
13. Qi W, Fong C, Lamport DTA (1991) Plant Physiol 96:848.
14. Al-Assaf S, Phillips GO, Aoki H, Sasaki Y (2007) Food Hydrocoll 21:319.
15. Aoki H, Al-Assaf S, Katayama T, Phillips GO (2007) Food Hydrocoll 21:329.
16. Al-Assaf S, Phillips GO, Williams PA (2005) Food Hydrocoll 19:647.
17. Al-Assaf S, Phillips GO, Williams PA, du Plessis TA (2007) Nucl Instr Meth Phys Res B 265:37.
18. Al-Assaf S, Phillips GO, Williams PA (2006) Food Hydrocolloids 20:369.
19. Al-Assaf S, Phillips GO, Gunning A P, Morris VJ (2002) Carbohydr Polym 47:341.
20. Flourey J, Desrumaux A, Axelos MAV, Legrand J (2002) Food Hydrocoll 16:47.
21. Flourey J, Desrumaux A, Legrand J (2002) J Food Sci 67:3388.
22. Galazka VB, Smith D, Ledward DA, Dickinson E (1999) Food Hydrocoll 13:81.
23. Puppo MC, Speroni F, Chapleau N, de Lamballerie M, Anon MC, Anton M (2005) Food Hydrocoll 19:289.
24. Silvestri S, Gabrielson G (1991) Int J Pharm 73:163.
25. Lagoueyte N, Paquin P (1998) Food Hydrocoll 12:365.
26. Kasaai MR, Charlet G, Paquin P, Arul J (2003) Innovat Food Sci Emerg Tech 4:403.
27. Modig G, Nilsson L, Bergenstahl B, Wahlund KG (2006) Food Hydrocoll 20:1087.
28. Dalgleish DG (2004) In: Friberg SE, Larsson K (eds) Food emulsions, Marcel Dekker Inc, New York, pp 1--44.
29. Pandolfe WD, Kinney RR (1998) Chem Processing 61:39.
30. Aoki H, Katayama T, Ogasawara T, Sasaki Y, Al-Assaf S, Phillips GO (2007) Food Hydrocoll 21:353.
31. Al-Assaf S, Phillips GO (2006) Foods & Food Ingredients Journal of Japan 3:189.
32. Phillips GO, Du Plessis TA, Al-Assaf S, Williams P (2003) New biopolymers obtained by solid state irradiation in an unsaturated gaseous atmosphere, US Pat 6841644.
33. Hassan EA, Al-Assaf S, Phillips GO, Williams PA (2005) Food Hydrocoll 19:669.

34. Elmanan M, Al-Assaf S, Phillips GO, Williams PA (2008) Food Hydrocoll 22:682.
35. Al-Assaf S, Hawkins CL, Parsons BJ, Davies MJ, Phillips GO (1999) Carbohydr Polym 38:17.
36. Katayama T, Nakauma M, Todoriki S, Phillips GO, Tada M (2006) Food Hydrocoll 20:983.
37. Mahendran T, Williams PA, Phillips GO, Al-Assaf S, Baldwin TC (2008) J Agri Food Chem 56:9269.

Table1. Summary of the effect of filtration on the molecular weight (g/mol) determined by GPC-MALLS of different matured (supergum) samples compared with the control. The value in brackets is the Rg in nm followed by the % mass recovery of the peak. dn/dc value of 0.141 was used for the calculation.

Fraction	Filter pore size (μm)	Control	A	B	C
Whole	0.80	7.15×10^5 (28)	1.58×10^6 (97)	2.50×10^6 (85)	3.32×10^6 (106)
	0.45	6.22×10^5 (30)	1.22×10^6 (67)	1.61×10^6 (62)	2.56×10^6 (88)
	0.20	6.14×10^5 (25)	1.10×10^6 (56)	1.31×10^6 (54)	1.59×10^6 (62)
	0.10	5.56×10^5 (33)	6.26×10^5 (31)	6.33×10^5 (29)	6.67×10^5 (28)
AGP	0.80	3.84×10^6 (41) / 9.6	9.17×10^6 (107) / 13.3	1.19×10^7 (91) / 17.9	1.48×10^7 (112) / 19.9
	0.45	2.64×10^6 (43) / 10.2	6.74×10^6 (77) / 12.8	8.59×10^6 (76) / 14.4	1.20×10^7 (94) / 18.4
	0.20	2.49×10^6 (33) / 10.4	5.86×10^6 (65) / 12.7	7.44×10^6 (60) / 12.7	8.66×10^6 (69) / 13.9
	0.10	2.26×10^6 (28) / 8.6	2.99×10^6 (35) / 9.0	3.00×10^6 (32) / 9.2	3.13×10^6 (37) / 10.1
AG+GP	0.80	3.80×10^5 (8) / 90.4	4.09×10^5 (41) / 86.7	4.76×10^5 (35) / 82.1	4.38×10^5 (31) / 80.1
	0.45	3.89×10^5 (12) / 90.4	4.02×10^5 (22) / 87.2	4.02×10^5 (17) / 85.6	4.29×10^5 (32) / 81.6
	0.20	3.96×10^5 (17) / 89.6	3.98×10^5 (33) / 87.3	4.12×10^5 (31) / 87.3	4.46×10^5 (30) / 86.1
	0.10	3.95×10^5 (36) / 91.4	3.88×10^5 (27) / 91	3.92×10^5 (26) / 90.8	3.87×10^5 (17) / 89.9

Table 2. Molecular weight parameters of control and matured gum samples determined by GPC-MALLS following various pressure treatments of gum arabic solutions.

Condition for pressure treatment			GA (control)			GA enhanced by maturation		
			Average Mw x 10 ⁶	AGP (%)	AGP Rg/nm	Average Mw x 10 ⁶	AGP (%)	AGP Rg/nm
Control (No-treatment)			0.72	9.9	74	2.36	18.0	95
Homogenisation conditions	1pass	25MPa	0.66	10.0	45	1.62	17.9	64
		50MPa	0.64	9.8	47	1.31	17.7	53
		75MPa	0.59	9.3	31	1.13	16.8	42
		150MPa	0.57	7.8	41	0.94	14.7	38
	3pass	25MPa	0.63	9.8	37	1.31	17.7	50
		50MPa	0.59	9.3	37	1.04	16.8	41
		75MPa	0.56	8.1	29	0.89	15.0	34
		150MPa	0.52	5.9	25	0.75	11.7	29

Table 3. Molecular weight parameter of spray dried gum arabic subjected to various filtration treatments. Molecular weight parameters were determined by electronically excluding the contribution of the aggregate peak unless otherwise stated.

Pore size (mm)		<i>0.45x1*</i>	0.45x1	0.45x2	0.45x3	0.45x4	0.2x1	0.1x1	0.1x2
Whole gum	Mw x 10 ⁵	<i>16.69</i>	8.48	6.746	5.63	5.450	7.06	6.52	5.469
	Mw/Mn	<i>4.07</i>	2.09	1.98	2.86	2.28	2.56	2.19	1.86
	% mass	<i>106</i>	99.2	106	103	99.6	109	99.8	100
	Rg / nm	<i>62.8</i>	46.6	41.4	13.8	55.5	24	35.4	38.6
AGP (peak 1)	Mw x 10 ⁶	<i>9.74</i>	3.41	2.84	2.68	2.53	2.77	2.49	2.54
	% mass	<i>12.7</i>	11.9	10.36	9.05	9.37	12.19	12.26	7.10
	Rg/nm	<i>67.6</i>	46.9	40.9	33.9	19.5	36.1	35.3	34.3
AG+GP (peak 2)	Mw x 10 ⁵	<i>4.96</i>	4.96	4.22	3.52	3.38	3.91	3.91	3.92
	Rg / nm	<i>46.3</i>	46.3	41.8	nd.	72.0	nd.	35.4	40.6
	% mass	<i>87.3</i>	87.3	89.28	90.6	90.30	86.36	87.5	92.91

* Third column in italic gives the molecular weight parameters when the gum was processed by including the contribution of the aggregate peak.

Table 4. Effect of filtration treatment on the molecular weight parameters of irradiated *A. senegal* for 6kGy in the spray dried form. Molecular weight (g/mol) determined for the whole gum means the three fractions. Processed as two peaks means the peak 1 (AGP) and peak 2 (AG+GP). Rg is the root mean square radius of gyration. % mass obtained by integration of the area under the peak.

Sample	Whole gum		Processed as two peaks			Comments
	Mw* 10 ⁶	Rg/ nm	Mw *10 ⁶	% mass	Rg /nm	
Control	0.59	25	2.34 0.32	12.0 88.0	35 17	Control, un-irradiated, filtered through 0.45mm filter
6kGy-0.45x1	2.92	67	9.48 0.54	26.68 73.34	71 39	Irradiated for 6kGy, filtered through 0.45 micron filter
6kGy-0.45x3	3.12	72	10.4 0.55	26.20 73.82	75 40	6kGy, filtered three times through 0.45 micron filter
6kGy-0.20	3.25	74	10.9 0.60	25.88 74.15	78 43	6kGy, filtered through 0.20 μ m filter
6kGy-0.10	1.74	50	6.62 0.44	20.91 79.11	54 28	6kGy, filtered through 0.10 μ m filter

Table 5. Molecular weight parameters of control and treated *A senegal* samples by enzyme digestion with Protease for 24 hours at 37°C. Molecular weight (g/mol) determined for the whole gum means the three fractions. Processed as two peaks means the peak 1 (AGP) and peak 2 (AG+GP). Rg is the root mean square radius of gyration. P is the polydispersity index (Mw/Mn). Enzyme digested samples are given in bold.

Sample	M _w processed as one peak	% mass	P	Rg/nm	M _w processed as two peaks	% mass	P	Rg	Samples code / Figure
D	8.34 ± 0.32 × 10 ⁵	106	2.23	21.6	Peak 1 3.06 ± 0.10 × 10 ⁶ Peak 2 4.73 ± 0.19 × 10 ⁵	14.85 91.9	1.29 1.43	29.6 -	Control sample, <i>A. senegal</i> in the kibbled form
D-24	3.73 ± 0.02 × 10 ⁵	102	1.49	-	Peak 1 1.62 × 10 ⁶ ± 0.04 Peak 2 3.11 × 10 ⁵ ± 0.05	4.85 97.5	1.12 1.29	21.5 -	Sample D digested with Protease for 24hrs
D5	1.02 ± 0.03 × 10 ⁶	118	2.59	29.1	Peak 1 3.93 ± 0.13 × 10 ⁶ Peak 2 4.93 ± 0.16 × 10 ⁵	18.1 100	1.47 1.45	35.2 -	Sample D heated for 5 hrs at 110C in the solid state.
D5-24	4.19 ± 0.03 × 10 ⁵	98.2	1.566	-	Peak 1 1.76 × 10 ⁶ ± 0.03 Peak 2 3.39 × 10 ⁵ ± 0.05	5.4 92.5	1.12 1.32	18.4 -	Sample D5 digested with Protease for 24hrs
D24	1.72 ± 0.07 × 10 ⁶	71.8	3.34	38.6	Peak 1 6.35 ± 0.25 × 10 ⁶ Peak 2 5.92 ± 0.26 × 10 ⁵	14.05 57.7	1.74 1.39	42.1 27.2	Sample D heated for 24 hrs at 110C in the solid state
D24-24	5.17 ± 0.06 × 10 ⁵	102	1.88	-	Peak 1 1.91 × 10 ⁶ ± 0.04 Peak 2 3.39 × 10 ⁵ ± 0.04	11.67 90.7	1.18 1.35	20.2 -	Sample D24 digested with Protease for 24 hrs
E	5.96 ± 0.09 × 10 ⁵	101	2.05	19.8	Peak 1 2.41 × 10 ⁶ ± 0.22 Peak 2 3.48 × 10 ⁵ ± 0.11	12.04 89.6	1.23 1.34	32.9 -	Sample E, Control <i>A senegal</i> in the kibbled form
E24	3.38 ± 0.05 × 10 ⁵	100	1.46	-	Peak 1 1.63 × 10 ⁶ ± 0.06 Peak 2 2.97 × 10 ⁵ ± 0.04	3.06 97	1.09 1.32	19.7 -	Sample E digested with Protease for 24h
E6	1.12 ± 0.05 × 10 ⁶	99	4.07	71.0	Peak 1 6.77 × 10 ⁶ ± 0.26 Peak 2 3.58 × 10 ⁵ ± 0.15	11.85 88.0	1.75 1.46	83.4 -	Sample E irradiated for 6.1kGy.
E6-24	1.81 ± 0.12 × 10 ⁶	102	6.41	132.5	Peak 1 1.07 × 10 ⁷ ± 0.21 Peak 2 3.66 × 10 ⁵ ± 0.18	14.1 88.2	2.44 1.49	145.5 18.7	Sample E6 digested with protease for 24hrs
E14	8.57 ± 0.03 × 10 ⁵	88	3.43	57.4	Peak 1 5.61 × 10 ⁶ ± 0.48 Peak 2 3.34 × 10 ⁵ ± 0.11	8.7 80	1.56 1.47	71.4 -	Sample E irradiated for 14kGy.
E14-24	1.24 ± 0.01 × 10 ⁶	101	4.82	106	Peak 1 8.16 × 10 ⁶ ± 0.61 Peak 2 3.24 × 10 ⁵ ± 0.08	10.62 78.7	2.36 1.40	121 -	Sample E14 digested with protease for 24hrs.
E50N	6.62 ± 0.06 × 10 ⁵	112	2.81	38.7	Peak 1 3.37 × 10 ⁶ ± 0.12 Peak 2 3.44 × 10 ⁵ ± 0.09	11.5 100	1.35 1.61	54.0 -	Sample E irradiated for 50kGy, N2 saturated
E50N-24	3.56 ± 0.06 × 10 ⁵	99.5	1.62	-	Peak 1 1.76 × 10 ⁶ ± 0.07 Peak 2 2.97 × 10 ⁵ ± 0.08	3.97 95.6	1.10 1.40	20.6 -	Sample E50N digested with protease for 24hrs
E50B	8.18 ± 0.10 × 10 ⁵	98	2.96	52.9	Peak 1 4.17 × 10 ⁶ ± 0.44 Peak 2 3.68 × 10 ⁵ ± 0.12	11.2 86.6	1.49 1.49	68.3 -	Sample E irradiated for 50kGy, butane saturated
E50B-24	3.78 ± 0.10 × 10 ⁵	94.7	1.75	-	Peak 1 2.06 × 10 ⁶ ± 0.07 Peak 2 3.12 × 10 ⁵ ± 0.07	3.42 91.2	1.10 1.49	18.6 -	Sample E50B digested with protease for 24hrs

Figure legends

Figure 1. Elution profile monitored UV at 214nm of control and matured samples.

Figure 2. Elution profile of High molecular (matured FR-2879) gum arabic sample monitored by UV detector at 214nm following different filtration treatment through 0.2 μ m filter (Grey line) and 0.1 μ m filter (dark line).

Figure 3. Effect of pressure treatment on change of average molecular weight (A) and AGP content (B) in gum arabic solutions. Control gum (circles) and matured gum labelled GA-H (squares). Filled symbols (1 pass) and open symbols (3 passes).

Figure 4. Elution profile of gum arabic in the spray dried form monitored by light scattering, refractive index and UV. The aggregate peak is marked on the plot by two dotted lines.

Figure 5. Elution profile of gum arabic in the spray dried form, monitored by UV at 214nm, following repeated filtration through 0.45 μ m filters.

