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# Molecular weight distribution and emulsification properties of the gum from *Acacia oerfota*

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**Abstract**
The molecular weight distribution of the gum exudate from *A. oerfota* was investigated using Gel Permeation Chromatography (GPC-MALLS). Emulsification properties of the gum were also examined, both results were compared to those from *A. Senegal* var. *senegal* gum. Molecular weights of *A. oerfota* gum, from two different location in Sudan, were $6.23x10^5$ and $9.59x10^5$ with the same radius of gyration (Rg) 178. Both gums show three fractions, AGP, (AG + GP) of molecular weights $4.77x10^5$ and $5.5x10^5$ respectively. The mass% of AGP is very small (1.11 and 0.84) in comparison to 98.89 and 99.16 for AG and GP respectively. Emulsification studies of *A. oerfota* show that it possesses a large droplet size, hence poor emulsification performance of a lower stability than that of *A. Senegal*.

**Keywords:** *Acacia oerfota*, Molecular Weight distribution, Emulsion

## Introduction
*Acacia oerfota* gum molecules possess a highly branched D-galactan framework, with attached D-glucuronic acid residues and side-chains of L-arabinose some of which are at least, six units long. The gum contains the highest proportion of L-arabinose than any other *Acacia* gum exudate [1]. The polysaccharide from *Acacia oerfota* has a, high, positive specific optical rotation, low methoxyl and L-rhamnose contents, and contains D-galactose, L-arabinose, and D-glucuronic acid, which is present as aldobiouronic acids, 6-O-(β-D-glucopyranosyluronic acid) and 4-O-(α-D-glucopyranosyluronic acid. Autohydrolysis gave 3-O-β-L-arabinofuranosyl-L-arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose, β-(1 → 3)-linked-L-arabinose trisaccharides, which was studied by linkage and methylation analysis. Partial acid hydrolysis, gave 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose. An examination of the O-methyl derivative of degraded gum A gave 2,3,4,6-tetra-, 2,3,4-, 2,3,6-, and 2,4,6-tri-, and 2,4-di-O-methyl-D-galactose; 2,3,4-tri-O-methyl-L-arabinose; and 2,3,4-tri-O-methyl-D-glucuronic acid. Degraded gum A was subjected to a Smith degradation, and the product was examined by linkage and methylation analysis [2].

## 2. Material and methods
### 2.1 Materials
Thirty samples were collected from two locations from around Senga city, Sinnar state and Wadel hadad, Aljazeera state Sudan (15 sample from each location). Several *A. oerfota* trees were tapped by making incisions about 15cm long and 3 cm wide using an axe. 10 to 20 incisions were made, on branches of the trees Fig. 1. Fifteen samples were collected from each location. Gum nodules, collected, were dried at roaom temperature, cleaned by hand, ground using mortar and pestle, and kept in labeled plastic containers for analysis.

**Fig 1:** *Acacia oerfota* gum samples

## 2.2 Methods

### 2.2.1 Determination of number average molecular weight by Osmotic pressure

Different concentrations ( 0.25%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) of *A. oerfota* gum were injected into Osmometere 050 and the osmotic pressure was determined at 25 $C^0$. The number average molecular weight was calculated using [3].

### 2.2.2 Gel Permeation Chromatography (GPC-MALLS)

The system utilizes Waters (Division of Millipore, USA) Solvent Delivery System Model 6000A connected to a 10x300mm Superose 6 column (Amersharm Biosciences), and a, manual, Rheodyne Model 7125 syringe. The column eluent, was monitored by, refractive index, a multi-angle laser light scattering photometer using a 690 nm He–Ne laser (Wyatt Technology Corporation, USA), and an Agilent 1100 series G1314A ( Agilent Technologies) UV at 214 nm [4]. RI provides an accurate concentration profile, MALLS measures absolute molecular mass and radius of gyration (Rg), and the UV detects the proteinaceous components of the gum [5]. The data was processed by Astra for Windows software (version 4.90.07, Wyatt Technology Corporation, USA).

1.0 mg/ml gum samples were prepared (based on dry weight) in 1mM phosphate buffer pH 7 containing 0.2M NaCl, and hydrated by roller (SRT9. Stuart Scientific, UK) mixing, the solutions were left overnight to ensure full dissolution, centrifuged for 10 minutes at 3000 rpm using Megafuge 1.0R (Heraeus SEPATECH, Germany) centrifuge and filtered using 0.45-µm nylon filter (Whatmann, 13 mm) prior to injection into the GPC-MALLS system.

### 2.2.3 Emulsion preparation

A sample of 20% (w/w) gum solution(on dry weight basis) was roller mixed overnight until the sample is completely dissolved. 20 g of the prepared gum solution were filtered using 100 µm mesh, mixed with 0.52 ml of 10 % (W/V) sodium benzoate solution as a preservative, and 0.48 ml of 10 % (W/V) citric acid solution to adjust the pH to 4. 15.71 and 15.73 mls of distilled water were added, followed by 4.2 g of ODO oil to give a total weight of 40 g and a 10% final concentration.

The solution was homogenized for 3 minutes using a POLYTRON(PT 2100, KINEMA TICA AC) homogenizer at 22000 rpm. (PTDA21) 9 mm tip diameter was used To achieve small particle size < 1 micron, the pre-emulsified mixture was homogenized using a high-pressure Nano Vater(NV30-FA, MITSUBISHI GOT1000.). In order to achieve effective disaggregation of the gum which was passed twice at 75MPa.

The final emulsion was kept in closed glass universals. Particles size of emulsion, as prepared, was noted. The emulsions were, then, placed in a Vacuum Oven (GALLENKAMP. OVA031.XX1.5) at 60 **C**. Droplet sizes were measured after 3 and 7 days using a Mastersizer 3000, a laser diffraction particle size analyzer (Malvern Instruments). Distilled water was used as dispersant and a value of 1.45 was used for the refractive index for oil phase (ODO). Emulsification stability of samples kept at 60°C was evaluated by particle size change after accelerated stability test for 3 and 7 days. The particle size of the emulsions was described by the volume median diameter (VMD).

### 2.2.4 Emulsion stability index of *Acacia oerfota*

Emulsification stability was evaluated by the change in the particle size of emulsion after acceleration test. Emulsion stability index **(**ESI) was calculated according to PHRC grading system using the equation:

$ESI = d0.5_{as\ prepared} + (d0.5_{3\ days@60C} - d0.5_{as\ prepared}) + (d0.5_{7\ days@60C} - d0.5_{as\ prepared})$

## 3. Results and discussion

### 3.1 Determination of number average molecular weight by Osmotic pressure

Fig 2. shows that the values of number average molecule weight (Mn) of *Acacia oerfota* gum obtained by osmotic pressure is $1.68x10^5$ and $1.80x10^5$ g/mol for the two total composite of *Senga* and *Wadel hadad* areas respectively. Osman, et, all. 1993 reported that A. *senegal* has (2 - 3 $x10^5$ g/mol) as a values of number average molecule weight.

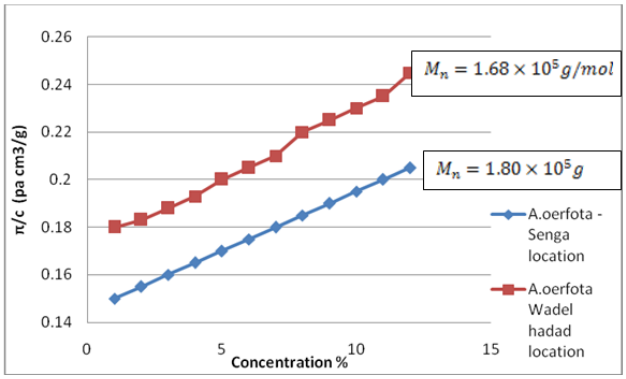


**Fig 2:** A plot of $\frac{\pi}{C}$ versus C for *Acacia oerfota* gum at 25 C

### 3.2 Gel permeation chromatography

Table 1. shows the molecular weight distribution for *A. oerfota* gum from the two locations vary between $6.23x10^5$ and $9.59x10^5$ with a similar radius of gyration (Rg) of 178. It also show three fractions of AGP of Mw $4.77x10^5$, AG and GP of Mw $5.5x10^5$ and Rg of AGP is50. The mass% of AGP

is very small 1.11 and 0.84 in comparison to 98.89 and 99.16 for AG and GP.

**Table 1: GPC** molecular weight distribution of *A. oerfota* gum.

| function/sample | *A. oerfota* Senga | *A. oerfota Wadelhadad* | *A. senegal* |
|---|---|---|---|
| $M_w$ whole gum (x$10^5$) | 6.23 | 9.59 | 5.95 |
| $M_w$/ $M_n$ | 3.71 | 5.33 | - |
| Rg (whole gum)/nm | 178 | 178 | 50.6 |
| Mw AGP | 4.77 | 14.19 | 1.68 |
| % mass (AGP) | 1.11 | 0.84 | 17.73 |
| Rg-AGP | 50 | 187 | 38.0 |
| Mw (AG+GP) (x$10^5$) | 5.76 | 8.24 | 3.59 |
| % mass (AG+GP) | 98.89 | 99.16 | 82.27 |
| % Mass recovery | 108.685 | 105.78 | 113.97 |

### 3.3 GPC MALLS - RI

Fig 3. shows RI profiles of *A. Oerfota* and *A. senegal* gums. The profile is composed of three peaks AGP, AG and GP. There is quite different in the first peak of AGP. The mass % of AGP of the two *A. Oerfota* gums are almost similar, but very low in comparison with the AGP of *A. senegal* gum.

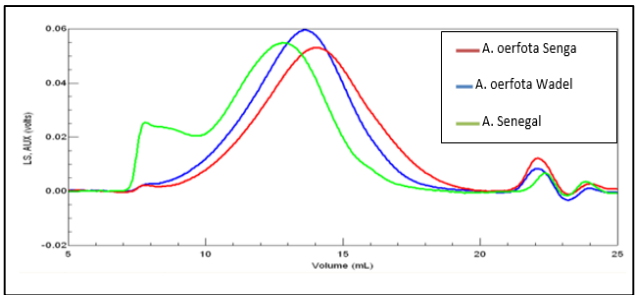


**Fig 3:** The RI of two Compos samples of *A. Oerfota* and *A. Senegal*

### 3.4 GPC MALLS – LS

Fig 4. shows that light scattering profiles for AGP and AG of the two *A. Oerfota* and *A. senegal* gums. (GP is not detected due to small $M_w$). The first peak reflects the significant difference in molecular weights of Arabinoglactan protein components in *A. Oerfota* and *A. senegal* gums. The molecule weight of the Arabinoglactan protein in *A. oerfota* is very low in Comparison to that of *A. senegal* gum [6]. However, The AG+GP molecular weight of *A. oerfota* is higher than that of *A. senegal* gum.

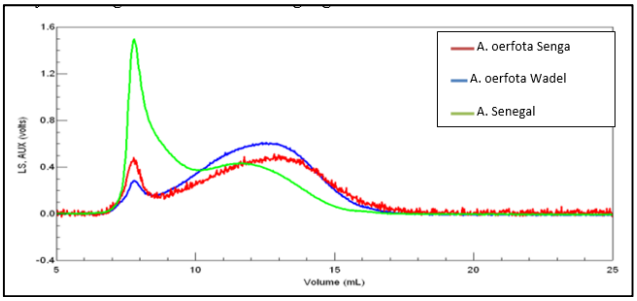


**Fig 4:** The LS of two Composite samples of *A. Oerfota* and *A. senegal* gums

### 3.5 Gpc Malls - Uv

Fig 5. shows the UV, GPC profiles for *A. oerfota* and *A. senegal* gums. They, clearly, indicate the significant difference in protein content. The AGP signal of *A. oerfota* gum is very small in comparison to that of *A. senegal* gum [7]. The AG and GP signals are almost identical, indicating similar protein composition.

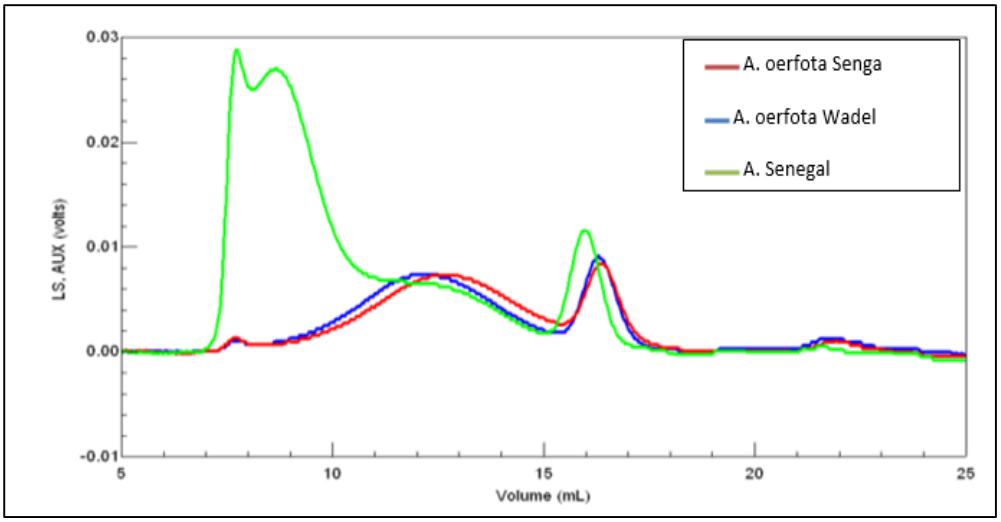


**Fig 5: The** UV of two Composite samples of *A. oerfota* and *A. senegal* gums

### 3.6 The emulsification properties of *Acacia oerfota* gum

Good emulsions are characterized by small particle diameter size between 0.1 and 1 micron and a narrow band [8]

The emulsification properties of *A. oerfota* gum were studied by determination of the emulsion particle size at zero time and after incubation for 3 and 7 days at 60 $^0$C.

Table 2. shows that the location have no significant effect in the emulsification properties of *A. oerfota* gum. The surface weighted mean D(3,2) and volume weighted mean D(4,3) was increasing and the span% was decreasing with time.
d(4,3) is more sensitive to the existence of large particles in an emulsion compared to d(3,2). Thus, d(4,3) more sensitive to the phenomena of flocculation. This might explain the higher value of d(4,3) compared to d(3,2) since all emulsions showed large droplets.
The gum is designed as grade 3 According to emulsion stability index value that taken as a parameter to evaluated the grade of the gum sample (Table 2.)

**Table 2:** The emulsification properties of *A. oerfota* gum

| | Senga location | | | Wadel hadad location | | |
|---|---|---|---|---|---|---|
| **Characters** | **As prepared** | **After 3 days** | **After 7 days** | **As prepared** | **After 3 days** | **After 7 days** |
| D(3,2) | 0.674 | 1.81 | 1.99 | 0.364 | 2.08 | 1.79 |
| D(4,3) | 1.26 | 4.43 | 4.34 | 1.56 | 5.42 | 4.46 |
| span% | 2.32 | 1.95 | 1.9 | 1.98 | 1.74 | 1.68 |
| Dx(10) | 0.40 | 0.69 | 0.74 | 0.166 | 0.63 | 0.58 |
| Dx(20) | 0.49 | 0.96 | 1.16 | 0.238 | 2.95 | 2.17 |
| Dx(50) | 0.72 | 4.26 | 4.08 | 0.477 | 3.23 | 4.42 |
| Dx(80) | 1.1 | 7.3 | 6.91 | 3.51 | 8.1 | 6.63 |
| Dx(90) | 2.1 | 8.98 | 8.49 | 5.4 | 9.71 | 7.98 |
| Grade | 3 | 3 | 3 | 3 | 3 | 3 |

Fig. 6 shows more variable particles size distributed in wide range ( 0.1 - 10) micron and most particle diameter size is large, this indicate instability of *Acacia oerfota* gum emulsion [3] The emulsions exhibited a typical bimodal droplet size distribution with a pronounced shoulder reflecting a two groups of the droplet with the small and largest diameter. The small one in the range of good emulsion particle size, but it decreasing with time according to flocculation and coalescence process. The pig droplet size and low viscosity (3.4 - 11.4 $cm3g^{-1}$) and pig rate of gyration of *A. oerfota* gum supported instability of the emulsions.

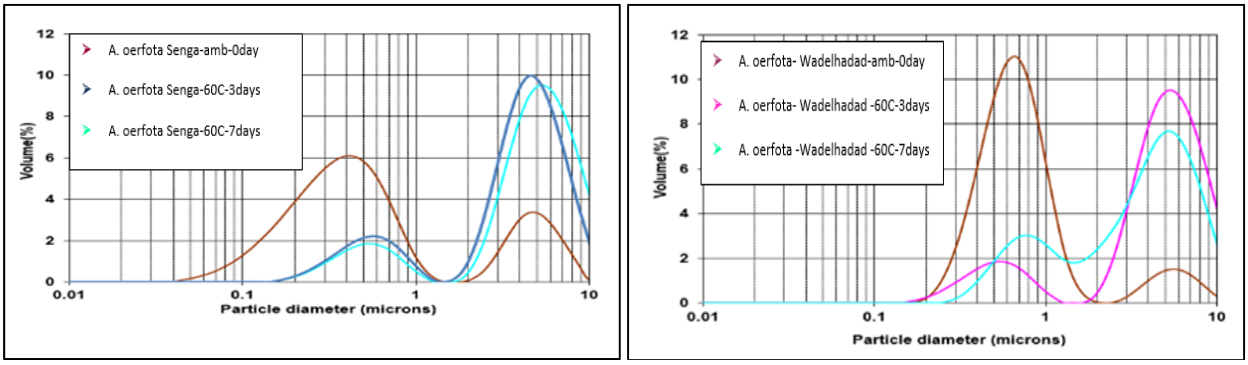


**Fig 6:** The emulation particle size profile of *A. oerfota* from two locations

Figs. 7. shows high value of poly dispersipility index (span%) for the fresh emulsion and after storage for 3 and 7 days at 60ºC respectively. in two locations indicating a bad uniformity of the droplet size. These values reflecting the instability of the emulsions by lowering the amount of protein that asociated with surface of the emulsion droplet.

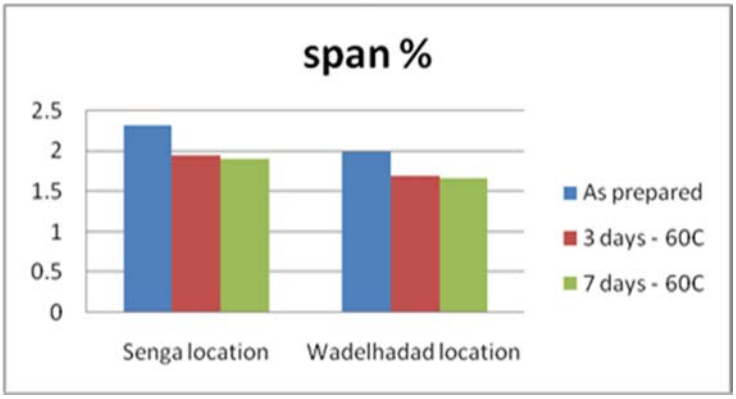


**Fig 7:** The span% of *A. oerfota* gum from two location

Figs. 8. Show specific surface area ($m^2/g$) of cumulative droplet distributions of (D.5, D.9, >1 microns, and >2 microns). The results clearly showed that extreme changing was found in emulsions during the incubation for 3 and 7 days at 60ºC. Also the instability of the emusiun inhanced by decreasing of D(0.5) area to less than 6%, and increasing the droplet particles size with the diameter of more than one microns to more than 80%.

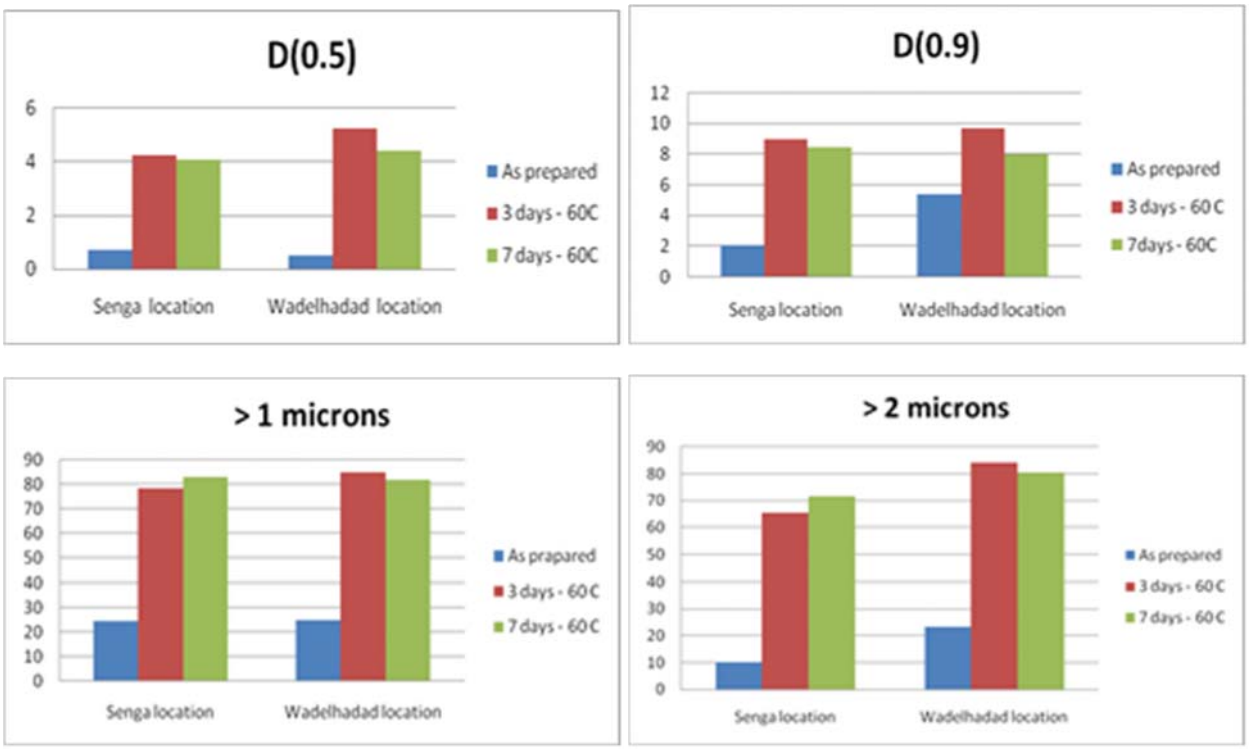


**Fig 8:** The volume weighted mean diameter of *A. oerfota* gum emulsion from two location

*Acacia oerfota* gum is a poor emulsifier In spite of high a molecular weight ($6.23x10^5$ - $9.59x10^5$ g/mole). This fact can be explained by small mass (Fig. 3) and small molecular weight appear in the first beak (Fig. 4) and small concentration (Fig. 5) of AGP compared with Acacia senegal. The less amount of AGP lead to less amount of protein surface around to the oil droplet required to inhibit flocculation and coalescence through electrostatic and steric repulsions.

## 4. Conclusion

The molecular weight of *A. oerfota* gum ranges between ($6.23x10^5$ and $9.59x10^5$ g/mole) with the same radius of gyration. The *Arabinoglactan* protein has only 1% mass and low molecular weight in Comparison to *A. senegal.*
*A. oerfota* gum forms poor unstable emulsion.

## 5. Acknowledgement

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