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# Fractionation and characterization of gum from *Acacia tortuosa*. Effect of enzymatic and alkaline treatments

Olga Beltrán[a,*], Gladys León de Pinto[a], Maritza Martínez[a], Luc Picton[b], Céline Cozic[b], Didier Le Cerf[b], Guy Muller[b]

[a]*Centro de Investigaciones en Química de los Productos Naturales, Facultad de Humanidades y Educación, Universidad del Zulia, Apartado 526, Maracaibo, Venezuela*
[b]*Laboratoire 'Polymères, Biopolymères et Membranes', UMR 6522 CNRS Université de Rouen, 76 821 Mont Saint Aignan Cedex, France*

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**Abstract**

The polysaccharide from *Acacia tortuosa* (Gummiferae ssp) has been characterized using size exclusion chromatography (SEC) with multiangle laser light scattering (MALLS). Comparison of the elution profile of the native gum with those exhibited by the gum after basic and enzymatic hydrolyses showed interesting modifications. Four distinct populations have been observed. The polysaccharide consists of a mixture of arabinogalactan (AG) and a complex arabinogalactan–protein (AGP)as has been reported for *Acacia Senegal* gum (Arabic gum).


*Keywords:* Acacia tortuosa; Gum exudate; Molecular weight distribution; Arabinogalactan–protein

## 1. Introduction

*Acacia tortuosa*, a tropical American Gummiferae species, produces a clear gum highly soluble in water. Analytical data and the relevant structural features of the polysaccharide isolated from Venezuelan *Acacia* gum have been reported (León de Pinto, Martínez, Ortega, Villavicencio, & Borjas, 1993; León de Pinto, Martínez, Galindo de Bolaño, & Igartuburu, 1997). Many analytical data were basically similar to other Gummiferae *Acacia* gums but differed in the sugar composition. It was demonstrated that the presence of traces of xylose and rhamnose was not observed. A combination of chemical methods with $^{13}C$ NMR spectroscopy supported that the core of the structure is mainly a branched β (1→3) galactan. Arabinose and the uronic acid residues could not be totally removed from the core. Arabinose (as furanose and pyranose residues) exists as terminal and 3-*O*-linked, while xylose is present as terminal residues.

Characterization of water-soluble polymers mixture has become important in a number of scientific and technological disciplines. Traditionally, size exclusion chromatography (SEC) has been applied to separate various mixtures of proteins, nucleic acids and polysaccharides (Chmelík, Chmelíková, & Novotny, 1997). Nevertheless, proper analysis of SEC requires using adequate polymer standards with chemical structure similar to that of the analyte. This condition is no more necessary by using SEC coupled on line with a multiangle laser light scattering (MALLS) detection.

This work deals with the molecular characterization of *Acacia tortuosa* gum using SEC-MALLS analysis. We have particularly focused the attention on specific enzymatic and alkaline treatments of such gum.

## 2. Materials and methods

### 2.1. Origin and purification of gum sample

Gum from *Acacia tortuosa* wild, known in Venezuela as uveda, was collected in the location of 'Los Puertos de Altagracia', East of Maracaibo lake, Zulia state, Venezuela,

* Corresponding author. Tel.: +58 261 7596269; fax: +58 261 7596204.
*E-mail address:* obeltran@luz.edu.ve (O. Beltrán).

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by the authors in no rainy season (February–April 2002). Taxonomic identification was done by Dr Lourdes Cardenas, Botanical taxonomist. The gum exudates very soluble in water, were purified by dissolution, dialysis and freeze-dried.

### 2.2. Alkaline treatment

The original gum (5 g) was hydrolyzed with a saturated barium hydroxide solution (200 ml) at 100 °C for 8 h. The hydrolyzed gum was neutralized with sulfuric acid (1 M), filtrated and freeze-dried.

### 2.3. Enzymatic treatment

The gum sample (250 mg) was dissolved in deionized water (25 ml) and the pH was adjusted (7.5) with NaOH (0.1 M). It was added pronase (1 ml, 3.4%) to the gum solution (9 ml) and incubated overnight at 37 °C.

### 2.4. Analytical techniques

The absolute determination of molecular weight and size distributions (MWD and RHD, respectively) were performed by coupling on line a SEC to a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI). The light scattering signal is proportional to the product of concentration and molecular weight whereas the DRI signal is proportional only to the concentration.

### 2.5. Size exclusion chromatography

The solution of 0.1 M $LiNO_3$, used as carrier, was filtered through 0.1 μm filter (Millipore) degassed (ERC 413) previously, eluted at 0.5 ml $min^{-1}$ flow rate (Flom HPLC pump 301) and clarified through a 0.45 μm filter unit upstream columns. The sample was injected through a 100 μl full loop. The size exclusion chromatography (SEC) line consisted of an OHPAK SB-G guard column as protection and two OHPAK SB 804 and 806 HQ columns (Shodex) in series. The column packing is a polyhydroxymethylmethacrylate gel.

### 2.6. Multi angle laser light scattering (MALLS)

The MALLS photometer, a DAWN—Enable Optical System (EOS) from Wyatt technology Inc. (Santa Barbara, USA) is fitted with a K5 cell with 18 photodiodes and He–Ne laser ($\lambda = 690$ nm). The QELS detector from Wyatt technology is connected to 115° angle of the MALLS detector. The collected data were analyzed using the Astra V-4.85 software package. The SEC-MALLS technique has been described previously (Picton, Bataille, & Muller, 2000). The concentrations of each eluted fraction have been determined with the DRI (ERC 7515A) according to a classical value used for polysaccharides of $dn/dc$ (0.15 ml $g^{-1}$). The samples were dissolved (about 5 g $l^{-1}$) in the 0.1 μm filtered carrier ($LiNO_3$ 0.1 M + $NaN_3$ 0.02%, water from Milli-Q water reagent system) and gently stirred during 5 h, then filtered through 0.45 μm type membrane (Millipore).

Molar masses (from static light scattering) and hydrodynamic radii ($R_h$ from quasi elastic light scattering) have been reported in the following results. The use of QELS apparatus is fully justified by the fact that quite the whole studied samples presents gyration radius ($R_g$) not large enough to be measured. Effectively, the low dimensions of tortuosa gum species ($R_g < \lambda/20$, where $\lambda$ is the wavelength of the laser) lead to light scattering angular dependences which are too small to measure the slope thus the $R_g$.

## 3. Results and discussion

The elution profile of *Acacia tortuosa* gum is shown in Fig. 1 wherein are represented the distribution of both molar masses (MWD) and hydrodynamic radii (RHD) as a function of elution volume together with light scattering (The signal from 90° LS photodiode detector) and DRI response. Four distinct populations are detected. The first population (13.0–15.4 ml) is representative of low concentrated and high molar masses species as indicated by DRI and LS responses.

The second (15.4–17.7 ml) and third (17.7–19.0 ml) populations have high DRI but low LS responses. These features indicate high concentration of lower masses species than those present in the first population. The fourth population (19.0–21.0 ml) shows low DRI and low LS responses as indication of both low concentration and molar masses.

Physicochemical characteristics of *Acacia tortuosa* native gum, obtained by the application of SEC/MALLS are shown in Table 1. It is important to know that the given results in terms of molar masses and proportions have to be considered with attention and caution as the separation was not very efficient. Nevertheless, the whole gum results indicated values of 410,000 and 170,000 g $mol^{-1}$ for weight average molar mass ($\overline{M_w}$) and number average molar mass ($\overline{M_n}$), respectively. The high polydispersity index (2.4) of the native gum suggests a very large spectrum of molar masses species (Fig. 1), which is probably consistent with a very complex system. This observation is fully confirmed by the detailed average molar masses of each population given in Table 1.

Studies of the well-known *Acacia senegal* (arabic gum) and *Acacia seyal* gum have attributed high molar masses fraction to an arabinogalactan-protein (AGP) complex (Picton et al., 2000; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). A way to evidence AGP consists in a pronase treatment leading to a destruction of such species (Connolly, Fenyo, & Vandevelde, 1987). Elution profiles

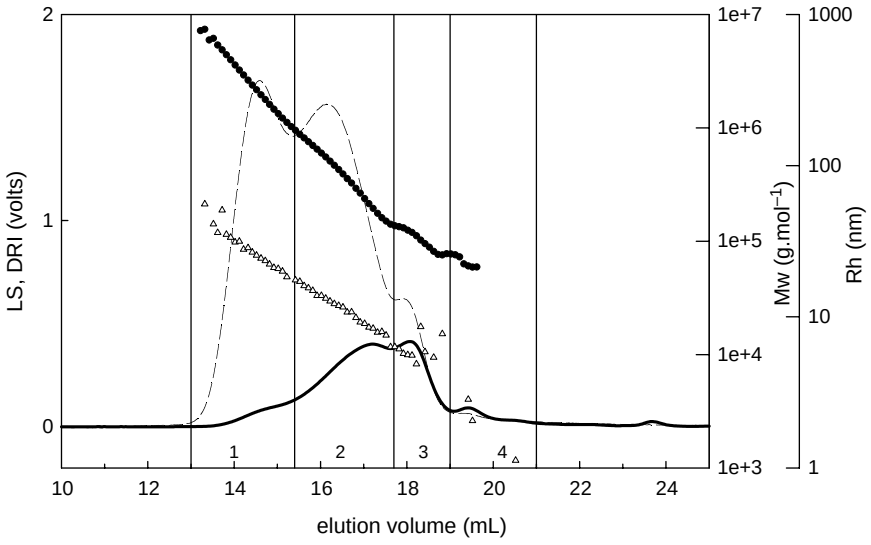


Fig. 1. MWD (full circle) and RHD (open triangle) versus elution volume for the *Acacia tortuosa* gum (native gum). Bold line: DRI, dotted line: LS.

(LS and DRI) together with both molar masses (M w) and hydrodynamic radii ($R_h$) distributions of the pronase treated native gum are shown in Fig. 2. Four populations are again observed as in the native gum (Fig. 1). The whole integration of the gum gives only 70% of the total injected sample (Table 2). This result is easily explained by residual pronase clearly identified at lower eluted volume (i.e. Fig. 2, between 21.0 and 23.0 ml). However, there are interesting modifications notably the strong decrease of LS and DRI responses of the higher fraction in molar masses (i.e. population 1). The population 1, of high molar mass, decreases strongly in concentration from 10 to less than 1% (Tables 1 and 2). It is also observed that the proportion of population 2 decreases by a factor of 1.44. In contrast, the populations of lower molecular weight (3 and 4) increase. Consequently, the polydispersity index is lower for pronase treated gum (2) than for native gum (2.4) but the molecular system is still very complex. The whole modification in the species composition of the *Acacia tortuosa* gum, after pronase treatment, following the example of arabic gum, suggests that the selective enzyme will hydrolyze the peptide bonds but not those involved with carbohydrate–protein. These results suggest that the fractions 1 and 2 may contain the molecular complex arabinogalactan–protein; the treatment by pronase led to do the removal of the proteic fractions which are moved to the population 3 and 4, of lower molar masses.

Alkaline treatment has been also conducted on *Acacia tortuosa* gum. Fig. 3 presents SEC/MALLS analysis with LS and DRI responses together with both molar masses and $R_h$ distributions. The four populations that have been detected on the native gum (Fig. 1) are again observed. As the pronase treatment, the alkaline hydrolysis induces also a large decrease of the first population concentration from 10% in the native gum to 3%. On the contrary, after pronase treatment, the second population of basic treated gum seems less or not affected in proportion as shown in Tables 1 and 3 (52% before treatment and 47% after treatment). Moreover, the average molar masses ($M_w$ and $M_n$) of the second population are higher for the gum after basic treatment than for native gum.

The enzymatic and alkaline hydrolyses may have similar behaviour, they both break peptidic linkages except those involved in carbohydrate–protein linkages. The glycosidic linkages to amino acids are quite stable to alkaline conditions when hydroxiproline or hydroxilysine is involved, but serine or threonine favours the β-alkoxielimination (Clarke, Anderson, & Stone, 1979). Although, pronase and alkaline treatments seem do not degrade drastically carbohydrate according to the variation observed

Table 1
Physico-chemical characteristics of *Acacia tortuosa* native gum obtained by SEC/MALLS experiments

| Fraction (elution volume, ml) | | | | | |
|---|---|---|---|---|---|
| | 1 (13.0–15.4) | 2 (15.4–17.7) | 3 (17.7–19.0) | 4 (19.0–21.0) | Whole gum (13.0–21.0) |
| $M_w$ (g mol$^{-1}$) | 1,800,000 | 365,000 | 115,000 | 77,000 | 410,000 |
| $M_n$ (g mol$^{-1}$) | 1,500,000 | 270,000 | 111,000 | 74,000 | 170,000 |
| Polymer recovery (%) | 10 | 52 | 30 | 8 | 87 |

Polydispersity index: 2.4.

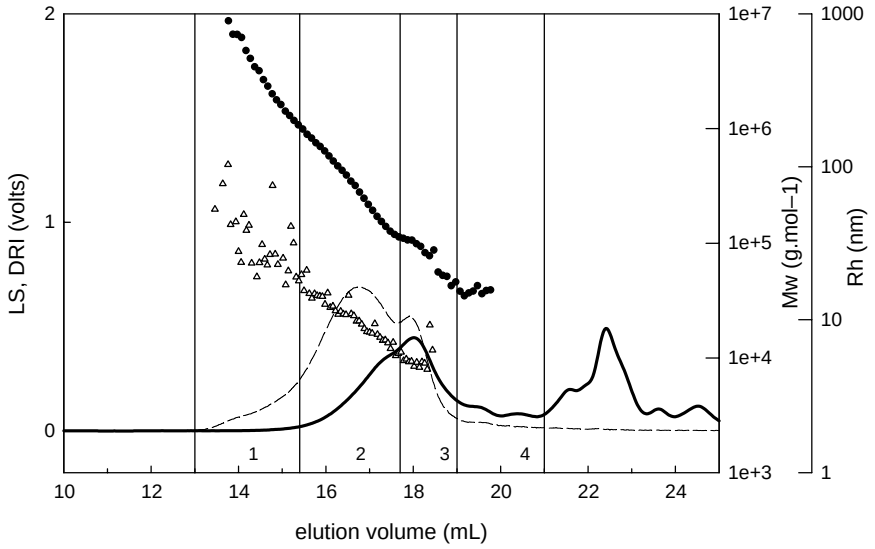


Fig. 2. MWD (full circle) and RHD (open triangle) versus elution volume for the *Acacia tortuosa* gum after pronase tratment. Bold line: DRI, dotted line: LS.

Table 2
Physico-chemical characteristics of *Acacia tortuosa* gum, after enzymatic treatment, obtained by SEC/MALLS experiments

| Fraction (elution volume, ml) | | | | | |
|---|---|---|---|---|---|
| | 1 (13.0–15.4) | 2 (15.4–17.7) | 3 (17.7–19.0) | 4 (19.0–21.0) | Whole gum (13.0–21.0) |
| $M_w$ (g mol$^{-1}$) | 2,000,000 | 280,000 | 86,000 | 40,000 | 380,000 |
| $M_n$ (g mol$^{-1}$) | 1,500,000 | 205,000 | 77,000 | 37,000 | 165,000 |
| Polymer recovery (%) | >1 | 36 | 49 | 14 | 70 |

Polydispersity index: 2.0.

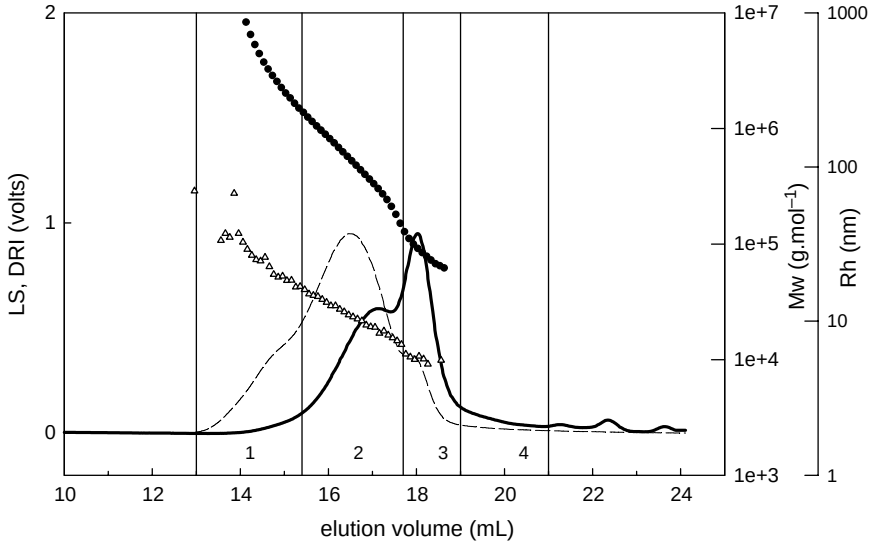


Fig. 3. MWD (full circle) and RHD (open triangle) versus elution volume for the *Acacia tortuosa* gum after alkaline treatment. Bold line: DRI, dotted line: LS.

in the population 2 and 3, in molar mass and proportion, after the treatment.

It has been shown that both pronase and alkaline treatments lead to a degradation of the native gum, which affects mainly the higher molar masses species. Comparison between pronase and alkaline treated gums together with the original gum are shown in the Figs. 4 and 5 which represent, respectively, the cumulative and differential weight fraction of molar masses. Differences between both treatments are evidenced. It clearly appears that the degradation is more

Table 3
Physico-chemical characteristics of *Acacia tortuosa* gum, after alkaline treatment, obtained by SEC/ MALLS experiments

| Fraction (elution volume, ml) | | | | | |
|---|---|---|---|---|---|
| | 1 (13.0–15.4) | 2 (15.4–17.7) | 3 (17.7–19.0) | 4 (19.0–21.0) | Whole gum (13.0–21.0) |
| $M_w$ (g mol$^{-1}$) | 2,500,000 | 460,000 | 93,000 | 91,000 | 350,000 |
| $M_n$ (g mol$^{-1}$) | 1,900,000 | 360,000 | 87,000 | 91,000 | 140,000 |
| Polymer recovery (%) | 3 | 47 | 44 | 6 | 89 |

Polydispersity index: 2.5.

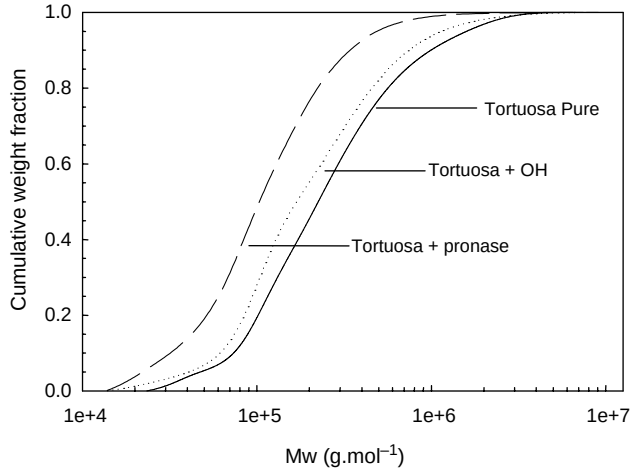


Fig. 4. Cumulative distributions of molar masses for *Acacia tortuosa* gum before and after basic and pronase treatments.

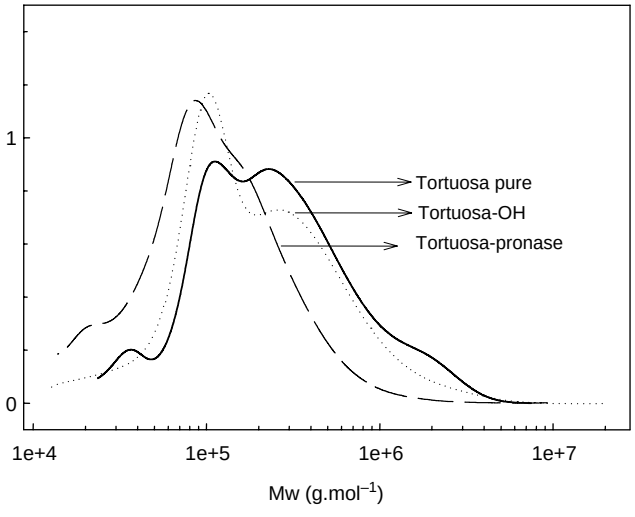


Fig. 5. Differential distributions of molar masses of *Acacia tortuosa* gum before and after basic and pronase treatments.

efficient with pronase than with alkaline treatment (Fig. 4). The differential weight fraction mainly for the smaller molar masses (Fig. 5) evidences that pronase leads to very small species as compared to the native gum whereas alkaline degradation does not much modify the distribution of lower mass of native gum.

The results showed that the gum from *Acacia tortuosa* is a polydisperse system, which contains a wide range of molar mass species. Comparison of relevant physico-chemical characteristics, obtained by SEC/MALLS experiments of the native gum and the gum after pronase and basic hydrolyses indicated the possible presence of arabinogalactan–protein complex together with arabinogalactan. The results support the new conception that the gum is a source of two structural complexes, which has also been reported for *A. senegal* gum (Connolly et al., 1987), *Acacia glomerosa* (León de Pinto, Sanabria, Martínez, Beltrán, & Igartuburu, 2002) and for other analogue materials (Jiang & Ramsden, 1999; Qin, Yamauchi, Aizawa, Inakuma, & Kato, 2001).

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## References

Chmelík, J., Chmelíková, J., & Novotny, M. (1997). Characterization of dextrans by size—exclusion chromatography on unmodified silica gel columns, with light—scattering detection, and capillary electrophoresis with laser—induced fluorescence detection. *Journal of chromatography A*, *790*, 93–100.

Clarke, A. E., Anderson, R. L., & Stone, B. (1979). Form and function of arabinogalactans and arabinogalactan-proteins. *Phytochemistry*, *18*, 521–540.

Connolly, S., Fenyo, J., & Vandevelde, M. (1987). Heterogeneity and homogeneity of an arabinogalactan—protein: *Acacia Senegal* gum. *Food Hydrocolids*, *1*(5), 477–480.

Jiang, G., & Ramsden, L. (1999). Characterization and yield of the arabinogalactan - protein mucilage of taro corms. *Journal of Science and food Agriculture*, *79*, 671–674.

León de Pinto, G., Martínez, M., Galindo de Bolaño, L., Rivas, C., & Ocando, R. (1997). The polysaccharide gum from *Acacia tortuosa*. *Phytochemistry*, *47*(1), 53–56.

León de Pinto, G., Martínez, M., Ortega, S., Villavicencio, N., & Borjas, L. (1993). Comparison of gum specimens from acacia tortuosa and other gummiferae species. *Biochemical Systematic and Ecology*, *21*(8), 795–797.

León de Pinto, G., Sanabria, L., Martínez, M., Beltrán, O., & Igartuburu, M. (2002). Structural elucidation of proteic fraction isolated from *Acacia glomerosa* gum. *Food Hydrocolids*, *16*, 599–603.

Picton, L., Bataille, I., & Muller, G. (2000). Analysis of a complex polysaccharide (gum arabic) by multi—angle laser light scattering coupled on—line to size exclusion chromatography and flow field flow fractionation. *Carbohydrate Polymer*, *42*, 23–31.

Qin, X., Yamauchi, R., Aizawa, K., Inakuma, T., & Kato, K. (2001). Structural features of arabinogalactan—proteins from the fruit of *lycium chinense* Mill. *Carbohydrate Research*, *333*, 79–85.

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