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Carbohydrate Polymers 54 (2003) 327–333
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

# Mesquite gum: fractionation and characterization of the gum exuded from *Prosopis laevigata* obtained from plant tissue culture and from wild trees

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Received 11 April 2003; revised 23 June 2003; accepted 24 June 2003

**Abstract**

The effect of abiotic and biotic stresses in the in vitro gum production from *Prosopis laevigata* has been studied. The presence of autoclaved microbial biomass (MB) in combination with an incubation temperature of 35 °C induced the greatest gum production. In vitro and in situ gum samples from mesquite were fractionated by hydrophobic affinity chromatography. The results indicated that both gums consist of five principal fractions. The protein content of the fractions varied greatly (0.04–50.05%). For both gums, the protein content in the fractions (F) increased in the following order F2a < F1 < F2b < F3a < F3b. The monosaccharide compositions, protein contents and other analyses of whole gums also were performed. The molecular mass distribution was determined by gel permeation chromatography (GPC). Showing the existence of five components varying in molecular mass from $3.5 \times 10^4$ to $9.4 \times 10^5$.


*Keywords:* Mesquite gum; Gummosis; In vitro; In situ

## 1. Introduction

The mesquite tree (*Prosopis* spp) has been since Pre-Columbian time one of the main natural resources and common cultural denominators for the inhabitants of desert regions in Southern USA and Mexico (Felger, 1977; Maldonado-Aguirre & De la Garza, 2000).

Each part of the plant is used as a source for human and animal food, wood and charcoal, building material, medicine, nectar for apiculture, shadow, and several other uses (NAS, 1979; Felker, 1981). Furthermore, this species plays an important role in the arid regions as it helps to retain and stabilize sand dunes, develop ground-carpet vegetation, stabilize water tables, retain moisture, fix nitrogen and improve soils (Felker & Clark, 1980; Felker, Clark, Lagg, & Pratt, 1981; Figuereido, 1990; Signoret, 1970).

The *Prosopis* genus has been subjected to an irrational anthropogenic action since Columbian times that has resulted in the disappearance and/or deterioration of their natural populations, causing an increased desertification and poverty in the surrounding regions (Maldonado-Aguirre & De la Garza, 2000; Salih, 1998; Vernon-Carter, Beristain, & Pedroza-Islas, 2000).

Researchers have sought to establish lines of action that promote the generation and validation of technologies that will allow the use, conservation and restoration of this valuable species, providing it with an economical added value (Felker & Bandurski, 1979), as is use of the gum exuded by the mesquite tree (Vernon-Carter et al., 2000). Exudate polysaccharides are produced in *Prosopis* and many other trees as a response to abiotic and biotic factors such as mechanical wounding, heat and water physiological stress (Greenwood & Morey, 1979).

Natural gums are extensively used in a variety of industrial applications due to their emulsifying, micro-encapsulation, thickening and stabilizing properties, among other. In general terms, it may be stated that mesquite gum is the neutral salt of a complex acidic branched polysaccharide formed by a core of β-D-galactose residues, comprising a (1,3)-linked backbone with (1,6)-linked branches, bearing L-arabinose (pyranose and furanose ring forms), L-rhamnose, β-D-glucuronate and 4-*O*-methyl-β-D-glucuronate as single sugar or oligosaccharide side chains. It also contains a small amount of protein (0.7–5.8%), which may be central

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0144-8617/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0144-8617(03)00187-5

to the overall primary structure (Anderson & Farquhar, 1982; Anderson & Weiping, 1989; Goycoolea, Calderón de la Barca, Balderrama, & Valenzuela, 1997; Vernon-Carter et al., 2000). There exist points of chemical similarity with samples of gum arabic (Islam, Phillips, Slijvo, Snowden, & Williams, 1997).

Mesquite gum has been reported as being a very efficient microencapsulating agent of cardamom essential oil (Beristain, García, & Vernon-Carter, 2001) and of orange peel oil (Beristain & Vernon-Carter, 1994) over a wide range of water activities (Beristain, Azuara, & Vernon-Carter, 2002). It also has been found to provide better stabilization against droplet coalescence to oleoresin-in-water emulsions (Vernon-Carter, Beristain, & Pedroza-Islas, 1998; Vernon-Carter, Gomez, Beristain, Mosqueira, Pedroza-Islas, & Moreno-Terrazas, 1996) and better protection against pigment degradation than gum arabic, but reported as having a slightly lower encapsulating capacity of orange peel oil than gum arabic (Beristain & Vernon-Carter, 1995). Use of a blend of both gums, is compatible and reported to have a synergistic effect providing a higher stability against drop coalescence in oil-in-water emulsions (Vernon-Carter et al., 1996), achieving the same amount of orange peel oil encapsulation as pure gum arabic (Beristain & Vernon-Carter, 1995), or rendering shrimp feedstuff microcapsules with enhanced morphology, microstructure and particle size (Pedroza-Islas, Vernon-Carter, Duran-Dominguez, & Trejo-Ramirez, 1999) and dissolution and floatability kinetics (Pedroza-Islas, Alvarez-Ramírez, & Vernon-Carter, 2000) than either gum on its own. Good wall materials for lipid encapsulation are those which exhibit efficient thermal properties (low effective diffusivity and high activation energy) that act as barriers to oxygen transfer, and mesquite gum has been reported as having lower effective diffusivity and higher activation energy than gum arabic, and that blends of mesquite gum–gum arabic–maltodextrin DE 10 where a high proportion of gum arabic or mesquite gum in the blend prevails exhibit better activation energy than maltodextrin DE 10 (Pérez-Alonso, Baéz-González, Beristain, Vernon-Carter, & Vizcarra--Mendoza, 2003) which provides excellent oxidative stability to encapsulated lipids (Kenyon, 1985).

The main reason for not using mesquite gum in industrial applications is that its supply cannot be guaranteed, as all the gum is collected from wild trees. Orozco, Meráz, Lechuga, Cruz, and Vernon-Carter (2000) have established that under certain experimental in vitro conditions, mesquite trees may produce an exudate.

Thus, the objectives of this work were to: (a) evaluate the effect of abiotic and biotic stresses in the in vitro gum production, and (b) chemically characterize, fractionize and compare the in vitro exuded gum with that collected in situ from the *Prosopis laevigata* wild trees.

It is hoped that this study effectively contributes to promote the research and commercial use of mesquite gum.

## 2. Material and methods

### 2.1. Chemicals

Water from an Easy Pure LF compact ultrapure water system (Barnstead, USA) was used exclusively. All reagents were of analytical grade and were purchased from Sigma, USA.

### 2.2. Plant material

Legume specimens from *P. laevigata* H. & B. were collected from a mesquite tree forest located around the village of San Bartolo in the Mexican State of San Luis Potosí, during June–October 1994. The legumes were collected from mesquite trees that had been previously marked as exuding copious gum. The legumes were sun-dried in order to reduce moisture content and avoid infestation by insects and pathogens. Seeds withdrawn from the legumes were sown in a mixture (1:1) of garden soil and peat contained in plastic pots (five seeds per pot), placed and kept in a greenhouse. The identification of voucher specimens was confirmed by Professor María Edith López Franco, Botanical taxonomist of the Universidad Nacional Autónoma de México, Campus Iztacala (UNAM-Iztacala). Botanical vouchers from this species were also collected and deposited in the Herbarium, Biology Department, UNAM-Iztacala.

### 2.3. In vitro gum production

Stem segments of 5–6 cm in length were removed from 3 to 4 year-old plants growing in the greenhouse. The explants free of leaves were disinfected by soaking in 70% ethanol for 1 min, followed by 10 min immersion in commercial sodium hypochlorite solution with 0.1% Tween 20 and rinsed five times in sterile, distilled water.

Explants were immersed singly at a depth of 1 cm in culture tubes (25 × 150 mm) containing 20 ml of MS medium (Murashige & Skoog, 1962) supplemented with 30 g/l sucrose, 1.6 g/l L-glutamine, 100 mg/l citric acid, 50 mg/l ascorbic acid and 2 g/l Phytagel (agar substitute gelling agent, Sigma Chemical Co., St Louis, MO). The pH of the medium was adjusted to 5.8 before autoclaving (15 min at 121 °C). One hundred cultures per treatment were maintained in continuous darkness conditions and subjected to several stressfull factors in order to promote gum exudation.

#### 2.3.1. Abiotic and biotic stresses promoting gummosis

The effects on the production of gum of three incubation temperatures (25, 35 and 40 °C), two mechanical wounding levels (wounded and non-wounded) and two MB levels (MB and non-MB) were tested in a 3 × 2 × 2 factorial arrangement of treatments (see Table 1). Mechanical wounding involved removing 2 cm of bark from the explants by

Table 1
Milligrams of mesquite gum produced in vitro in response to several stress factors

| | 25 °C | 35 °C | 40 °C |
|---|---|---|---|
| Mechanical wounding (Mech. Wnd.) | 1.3 ± 0.02[a] | 5.4 ± 0.04[d] | 1.3 ± 0.04[a] |
| Microbial biomass (MB) | 1.3 ± 0.05[a] | 13.5 ± 0.07[e] | 2.6 ± 0.04[b] |
| Mech. Wnd. + MB | 2.7 ± 0.02[b] | 13.5 ± 0.04[e] | 4.08 ± 0.02[c] |
| Temperature only | 1.3 ± 0.01[a] | 2.6 ± 0.02[b] | 2.7 ± 0.02[b] |

Data are means of three replicates per treatment. Values with same letter within the same column or the same row are not statistically different.

cutting longitudinally before they were placed in the medium. The wounded part of the explant was not immersed in the medium. A strain of *Pseudomonas pseudoalcaligenes* was isolated from stem segment cultures of *P. laevigata*; the isolated bacterium was grown in MS medium and incubated on a rotary shaker (110 rpm) at 37 °C; after 10 days, the cultures were autoclaved, the bacterial biomass separated by filtration. A suspension of 30 mg of dry bacterium (no live cells) in 2.0 ml of water was autoclaved and layered on top of the medium (Rojas, Alba, Magaña-Plaza, Cruz, & Ramos-Valdivia, 1999). The experiment was evaluated after 14 days. The explants were taken out from the culture tubes and the exudate was collected with the help of capillary tubes and stored at − 30 °C until required.

### 2.4. Origin and purification of the gums

*In vitro mesquite gum*. The in vitro exudate gum was dissolved in deionized water, filtered through nitrocellulose membranes with 0.45 μm pore diameter, dialyzed against running tap deionized water for 24 h, and freeze-dried.

*In situ mesquite gum*. Exuded mesquite gum samples from *P. laevigata* were collected by the authors during the dry season (October 1999–May 2000) from trees growing at the same locations where the mesquite legumes had been collected. The gum tears were pulverized, dissolved in warm water, activated charcoal was added. The mixture was filtered through Whatman No. 1 filter paper with the help of a filter-aid (Celite), dialyzed against running tap deionized water for 24 h, and freeze-dried.

Gum samples used in previous works (Beristain et al., 2002, 2001; Beristain & Vernon-Carter, 1994, 1995; Pedroza-Islas et al., 2000, 1999; Pérez-Alonso et al., 2003; Vernon-Carter et al., 1998, 1996) were obtained from the same geographical locations, and reported as originating from *Prosopis juliflora* species, whereas actually they are from *P. laevigata* species (Vernon-Carter et al., 2000).

### 2.5. Analytical methods

#### 2.5.1. Optical rotation

The specific rotation of the gum samples was determined in 1.0% (w/w) aqueous solutions using a Shibuya 400 Mitschrlich Polarimeter with a cell path length of 10 cm.

#### 2.5.2. Nitrogen, ash and moisture estimations

The percentage nitrogen in the gums was determined by Kjeldahl method using a Büchi system (Scrubber 412, Digestion unit 426 and Distillation unit K-314). The protein content was calculated using a conversion factor of 6.25 as proposed by Anderson and Farquhar (1982). The moisture content was estimated by heating to constant weight at 105 °C, and ash content by heating (muffle furnace) to constant weight at 550 °C.

#### 2.5.3. Analysis of sugars

The content of the total sugars of the gums was determined using the phenol–sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

The neutral sugar compositions were determined following hydrolysis (Randall, Phillips, & Williams, 1989) with $H_2SO_4$ by high performance liquid chromatography (Agilent 1100 Series) and peaks integrated with a coupled Agilent Chem Station software (Agilent, USA) using a Zorbax (5 μm) carbohydrate analysis column, 4.6 mm ID × 250 mm (Agilent, USA) with a refractive index detector (Agilent, 1100 Series, G1362A). Samples were eluted using a 75/25 acetonitrile/water mobile phase at a flow rate of 1.4 ml/min and a temperature of 30 °C.

#### 2.5.4. Determination of glucuronic acid

The glucuronic acid content was determined using the carbazole method (Randall et al., 1989). D-glucuronic acid (free acid) was used as standard.

### 2.6. Fractionation using hydrophobic affinity chromatography

A defined volume of a 10% (w/v) solution of the in situ and in vitro gums in 4.2 M NaCl was applied to a phenyl-Sepharose CL-4B column (Pharmacia) and eluted successively with 4.2 M NaCl, 2.0 M NaCl, and deionized water. A flow rate of 40 $cm^3$/h was used. The absorbance was monitored at 280 nm using a low-pressure chromatographic system (Biologic LP, Bio-Rad, CA, USA). The recovered fractions were dialyzed against deionized water and then freeze-dried. Protein and sugar contents of each fraction were determined (Randall et al., 1989).

Table 2
Analytical data in situ and in vitro gums from *P. laevigata*

| | In situ gum | In vitro gum |
|---|---|---|
| Moisture (%) | 10.6 ± 0.5 | 11.1 ± 0.05 |
| Ash (550 °C, %) | 2.2 ± 0.1 | 2.1 ± 0.2 |
| Specific rotation (°) | +77° | +85° |
| Nitrogen (Kjeldahl, %) | 0.4 ± 0.07 | 0.48 ± 0.02 |
| Hence protein (%) (N × 6.25) | 2.7 ± 0.06 | 3.01 ± 0.1 |
| Total sugars (%) | 87.3 ± 6.3 | 90.4 ± 3.9 |
| *Sugar composition after hydrolysis* (%) | | |
| Glucuronic acid | 16.2 ± 1.3 | 5.7 ± 0.1 |
| Galactose | 43.3 ± 1.4 | 26.8 ± 2.8 |
| Arabinose | 40.4 ± 2.04 | 52.2 ± 3.3 |
| Rhamnose | 1.3 ± 0.2 | 11.9 ± 0.5 |

All percentages expressed were corrected for moisture content.

### 2.7. Molecular mass distribution

The molecular mass distributions were determined by gel permeation chromatography (GPC) using a 2.5 × 90 $cm^2$ column Sephacryl S-500-HR (Pharmacia). For eluent 0.5 M NaCl, at a flow rate of 34.5 $cm^3$/h was used. Elution was monitored by UV absorption at 280 nm; using a low-pressure chromatography system (Biologic LP, Bio-Rad, CA, USA). The volume of sample injected was 1 $cm^3$ at a concentration of 50 mg/$cm^3$. The column was calibrated using protein and dextran standards (Pharmacia Biotech) in the range of 13,700–669,000 Da (Randall et al., 1989).

### 2.8. Statistical analysis

For the experiment of in vitro gum production, statistical analysis was carried out using the Tukey's test of NCSS statistical software (2001). Significance was determined at the 5% level.

## 3. Results and discussion

### 3.1. In vitro gum production

Gummosis is widespread in taxonomically diverse plants and is affected by environmental factors and infectious agents (Clarke, Anderson, & Stone, 1979; Greenwood & Morey, 1979). The application of different physical, biological and mechanical stresses stimulated gummosis in the mesquite cultures (Table 1). The cultures incubated at 25, 35 and 40 °C (in absence of another source of stress) and combination of MB, mechanical wounding (Mech. Wnd.), and incubation temperatures of 25 and 40 °C did not result in an important gum production, obtaining only from ~1 to 4 mg of gum per treatment. The greatest gum production was achieved when the cultures were incubated at 35 °C in presence of MB (13.5 mg per treatment). Mechanical wounding combined with MB and incubation at 35 °C, did not increase significantly the gum production. These results suggest a synergism between MB and temperature inducing gummosis. Bacteria are frequently associated with *Acacia* gums even within the plant itself where their presence may stimulate gum production (Clarke et al., 1979).

### 3.2. Gum analysis

Both, the in vitro and in situ exudates, produced solutions clear brown in color, of a slightly darker shade than those yielded by *Acacia senegal*, but exhibiting comparable solubility (>50%) (Anderson & Dea, 1971), and gave a positive response to the gum arabic identification test (Food Chemicals Codex, 1981).

The chemical and physical chemical characteristics of the in vitro and in situ mesquite gums are given in Table 2.

Table 3
Characteristics of fractions of in situ and in vitro gums from *P. laevigata* isolated by hydrophobic affinity chromatography

| Fraction | Eluent | Gum recovery (%) | Total sugars (%) | Protein (%) | Protein recovery (%) |
|---|---|---|---|---|---|
| *In situ gum* | | | | | |
| 1 | 4.2 M NaCl | 85.4 | 99.9 | 0.04 | 3.7 |
| 2a | 2.0 M NaCl | 5.4 | 99.9 | 0.01 | 0.02 |
| 2b | 2.0 M NaCl | 5.3 | 94.9 | 5.01 | 24.5 |
| 3a | $H_2O$ | 2.1 | 86.1 | 13.8 ± 0.1 | 26.6 |
| 3b | $H_2O$ | 1.6 | 69.1 | 30.8 ± 1.6 | 45.0 |
| | | 99.8 (total) | | | 99.8 (total) |
| Whole gum | | | 87.3 ± 6.3 | 2.7 ± 0.06 | |
| *In vitro gum* | | | | | |
| 1 | 4.2 M NaCl | 85.6 | 97.6 | 2.3 | 2.8 |
| 2a | 2.0 M NaCl | 5.4 | 98.5 | 1.4 | 0.01 |
| 2b | 2.0 M NaCl | 5.5 | 95.6 | 4.3 | 18.01 |
| 3a | $H_2O$ | 1.1 | 64.2 | 35.7 | 35.0 |
| 3b | $H_2O$ | 0.7 | 49.6 ± 0.8 | 50.05 ± 4.5 | 43.9 |
| | | 98.3 (total) | | | 99.7 (total) |
| Whole gum | | | 90.4 ± 3.9 | 3.01 ± 0.1 | |

All percentages expressed in dry basis.

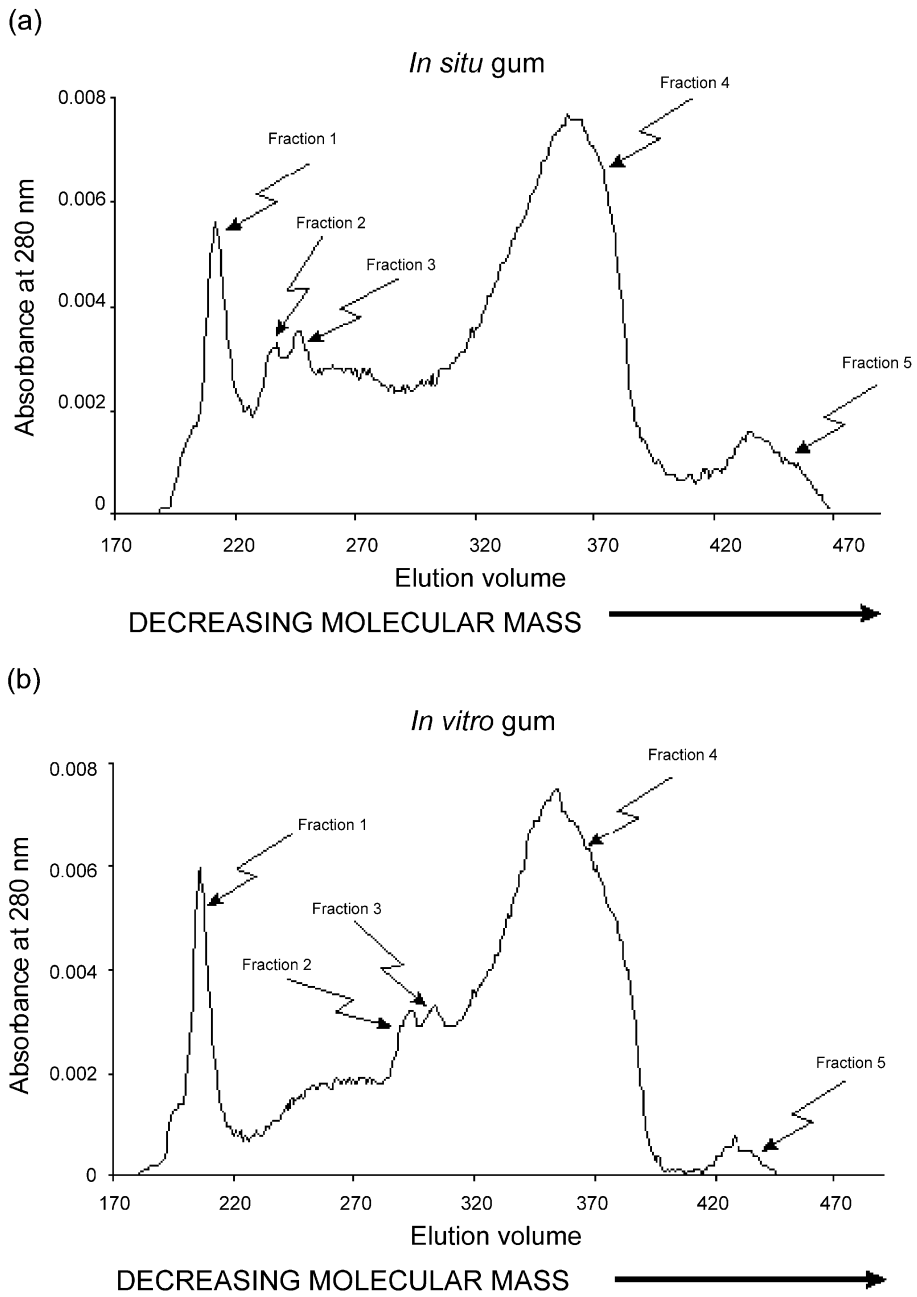


Fig. 1. Gel permeation chromatograms of gums from *P. laevigata*: (a) in situ and (b) in vitro.

The specific rotation values of both gums are similar and in accordance with reported values for gum from *P. laevigata* (Anderson & Farquhar, 1982). Most *Prosopis* gums have been found to be dextrorotatory (Anderson & Weiping, 1989) with the exception of *P. juliflora* which is laevorotatory (Anderson & Farquhar, 1982).

The polysaccharides isolated from the two *Prosopis* gums contained galactose, arabinose and rhamnose, the content of galactose predominating in the in situ gum and arabinose in the in vitro gum. The in vitro gum showed a higher rhamnose content in comparison with values found for the in situ gum and for gums from *Prosopis* species (Anderson & Farquhar, 1982; Anderson & Weiping, 1989). The sugar composition of the in situ gum is very close to that reported by Anderson and Farquhar (1982) for *P. laevigata*.

The glucuronic acid content of the two gums was also very different. For the in situ gum it was found to be ca. 16%, consistent with values reported for *Prosopis* species (Anderson & Farquhar, 1982), but for the in vitro gum was found to be much lower, ca. 6%.

The values quoted for the nitrogen contents of both gums are comparable and slightly lower than those reported for *P. laevigata* (Anderson & Farquhar, 1982).

Moisture and ash contents for both gums were similar (Table 2) and within the range for samples from other *Prosopis* gums (Anderson & Farquhar, 1982; Anderson & Weiping, 1989).

### 3.3. Chromatographic analysis

#### 3.3.1. Fractionation

The in vitro and in situ gums were separated into five fractions by hydrophobic affinity chromatography (HAC). Gum recovery, % protein and total sugars data for the fractions are given in Table 3. In both gums, fraction 1 represented more than 85% of the total gum but was found to be particularly low in protein, accounting for 3.7 and 2.8% of the total protein for the in situ and in vitro gum, respectively. Fractions 2a and 2b, accounted for ~11% of the total gum in both samples, and contained ~25% of the total protein for the in situ gum and ~18% for the in vitro gum. Fractions 3a and 3b accounted for ~72% of the total protein for the in situ gum and ~80% of the in vitro gum but comprise only 3.7 and 1.8% of the total gum, respectively.

Ray, Bird, Iacobucci, and Clark (1995) studied the emulsification properties of the two largest fractions of *A. senegal* (F1 and F2, representing ~99% of the recovered gum) in orange oil emulsions. They found that the protein rich fraction F2 produced much better emulsions than fraction F1 and the whole gum. The fractions obtained for both exuded mesquite gums in this work are similar to those obtained by Ray et al. (1995), so that it is possible that the combined fractions 2a and 2b, are responsible for the good emulsifying properties of whole mesquite gum.

#### 3.3.2. Gel permeation

In situ and in vitro gums from *P. laevigata* were also fractionated by GPC. Here the elution is in order of decreasing molecular size rather than by increasing hydrophobicity as in HAC. The resulting chromatograms showed five main peaks (Fig. 1), indicating that the gums contained fractions, which differed significantly in molecular mass. The values obtained for the projected average molecular mass of the fractions are given in Table 4. Molecular species with a molecular mass from $9.3 \times 10^5$ to $3.5 \times 10^4$ were obtained for the in situ gum and of $9.4 \times 10^5$ to $3.6 \times 10^4$ for the in vitro gum. The values obtained for molecular mass of fractions 1, 4 and 5 are similar for both samples, but there were significant differences observed in molecular mass of fractions 2 and 3. The molecular mass of fraction 2 and 3 for the in situ gum were of $6.9 \times 10^5$ and $5.9 \times 10^5$, respectively, while those for in vitro gum fraction 2 had a molecular mass of $3.1 \times 10^5$ and fraction 3 of $2.6 \times 10^5$. Idris, Williams, and Phillips (1998) showed that the chemical, physical chemical and molecular characteristics of *A. senegal* gum exhibit some variations depending on location and age of the tree where the gum is collected.

Table 4
Average molecular mass from GPC data (g/mol)

| Sample | Fraction 1 | Fraction 2 | Fraction 3 | Fraction 4 | Fraction 5 |
|---|---|---|---|---|---|
| In situ gum | $9.3 \times 10^5$ | $6.9 \times 10^5$ | $5.9 \times 10^5$ | $1.1 \times 10^5$ | $3.5 \times 10^4$ |
| In vitro gum | $9.4 \times 10^5$ | $3.1 \times 10^5$ | $2.6 \times 10^5$ | $1.2 \times 10^5$ | $3.6 \times 10^4$ |

## 4. Conclusions

This work shows that the chemical and physical chemical characteristics of in vitro gum from *P. laevigata* exhibit some variations in comparison with gum obtained in situ, but both gums show that they are made up of five fractions, of which fractions 2a and 2b, are very similar for the in vitro and in situ gums and may be responsible for the reportedly good emulsifying properties of whole mesquite gum. Also both gums have a wide molecular mass distribution that consists of five molecular mass components of significantly different molecular characteristics. From the results it may be inferred that mesquite gum might be a suitable replacement of other gums such as gum arabic and gum tragacanth. Moreover, it is demonstrated that the stem segments of *P. laevigata* retained in vitro their ability to exude true gum.

## Acknowledgements

The authors are grateful for the financial support of this research from the Consejo Nacional de Ciencia y Tecnología (CONACyT) through grants G-33565-B and 5-34147-B.

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