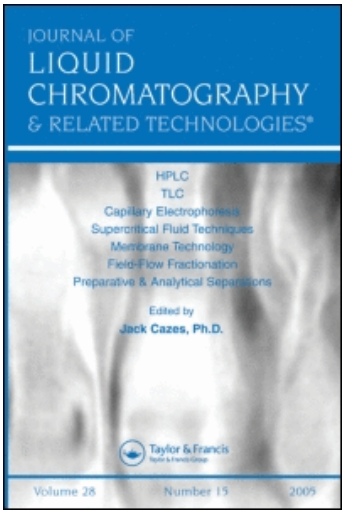

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Fractionation and Characterization of the Monosaccharides from Mesquite Prosopis spp. and Arabic Gum by Normal, Bonded Phase, HPLC

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Abstract: A sensitive and selective assay of galactose, arabinose, and rhamnose is presented for its determination in mesquite and Arabic gums. Gum samples from mesquite and Arabic were fractionated by hydrophobic affinity chromatography, hydrolyzed, and analyzed by normal bonded phase high performance liquid chromatography with refraction index measurement. The assay demonstrated that it was fast and with good linear and high precision response. The column and detector performance showed good retention, selectivity, and efficiency. Based on replicated analysis of standards over a range of 0–13.33 mg/mL, the method is accurate (95–100%), and precise (CV of 3% daily). The results indicated that both gums consist of three principal fractions. The mesquite tears and native showed higher total carbohydrates content (75.73 and 76.26%) in comparison with Arabic gum (61.81%). The saccharides isolated from the Arabic gum contained galactose, arabinose, and rhamnose. Fraction I of Arabic gum showed a higher rhamnose content (7.24%) in comparison with mesquite (0.89%). Only the hydrolyzed fraction I showed the rhamnose content in mesquite. In the fractions, the content of galactose predominating in the Arabic gum was 25.21–26.35% and arabinose in the mesquite gum was 34.43–53.49%.

Keywords: Monosaccharides, Mesquite, Prosopis, Arabic Gum, HPLC

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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.[1,2] Researchers have promoted the generation and validation of technologies that will allow the use of the gum exuded by the mesquite tree.[3,4] Exudates polysaccharides are produced in Prosopis and many other trees as a response to mechanical wounding, heat, and water physiological stress.[5]

Natural gums are extensively used in a variety of industrial applications due to their emulsifying, microencapsulation, thickening, and stabilizing properties. Although, there are over one thousand species known, only gum from Acacia Senegal or of related species of Acacia have been approved for food applications. The gum finds wide use as a flavor encapsulator in dry mix products such as puddings, desserts, cake mixes, and soup mixes, and is also used to emulsify essential oils in soft drinks and to prevent sugar crystallization in confectionary products.[6,7]

In general terms, it may be stated that mesquite gum and Arabic gum is heteropolymolecular, consisting of molecules that differ in their sugar composition and their mode of linkage. Experimental investigations concentrated on the elucidation of the carbohydrate structure using fractionated samples concluded that Arabic and mesquite gums consist of a β1-3 linked galactopyranose backbone with branches of galactopyranose linked β1-6 containing arabinopyranose, arabinofuranose, and rhamnopyranose, with glucuronic acid and 4-0-methylglucuronic acids as terminating groups.[4,8,9]

The aim of the present investigation is to determine galactose, arabinose, and rhamnose in mesquite and Arabic gums by normal-bonded-NH_2, high performance liquid chromatography, in samples that have been separated previously by sequential fractionation hydrophobic affinity chromatography and hydrolysis with H_2S0_4 10%. The technique has the advantage that it is relatively quick, and very reproducible.

EXPERIMENTAL

Fractionation Using Hydrophobic Affinity Chromatography

A glass column, dimensions 2.6 $\times$ 70 cm, was racked with phenyl-Sepharose CL-4B gel (pharmacia) using degassed 4.2 M NaCI as the effluent solution. A 250 cm^3 volume of a 10% w/w solution of gum Arabic in 4.2 M NaCI was prepared and filtered through a Whatman no.1 filter paper.

The solution was passed down the column under gravity at a flow rate of 40 cm^3/h. The elution volume was passed through a UV spectrophotometer fitted with a 10 mm flow cell and the absorbance monitored at 280 nm.

Most of the gums passed straight through the column and were collected (fraction I). A proportion of the gum that has been adsorbed on the Sepharose gel was desorbed and collected by passing 2.0 M NaCl.

The NaCI down the column, and the remainder, was desorbed by eluting with distilled water, yielding two further fractions (fractions II and III).

The recovered fractions were exhaustively dialysed against distilled water until the dialysate was free from chloride ions and were then freeze-dried.

Details of the fractionation using hydrophobic affinity chromatography in the gums samples used in this study are given in Figure 1.

Sample Preparation and Hydrolysis

The galactose, arabinose, and rhamnose ratios in the whole gum and the various fractions were determined using HPLC. Aliquots of 0.03–0.05 g of the gum were weighed out accurately into tare 15 cm^3 stopped Pyrex test tubes 10 cm^3, 4% w/w sulphuric acid was added to each tube. The tubes were placed in a water bath at 100°C for 4 h and were then reweighed and made up to the own weight by addition of distilled water. The solutions were neutralized by adding 2.0 g $BaCO_3$ and shaking overnight.[10] The filtered hydrolyzates (0.22 μm) were analyzed by the HPLC system.

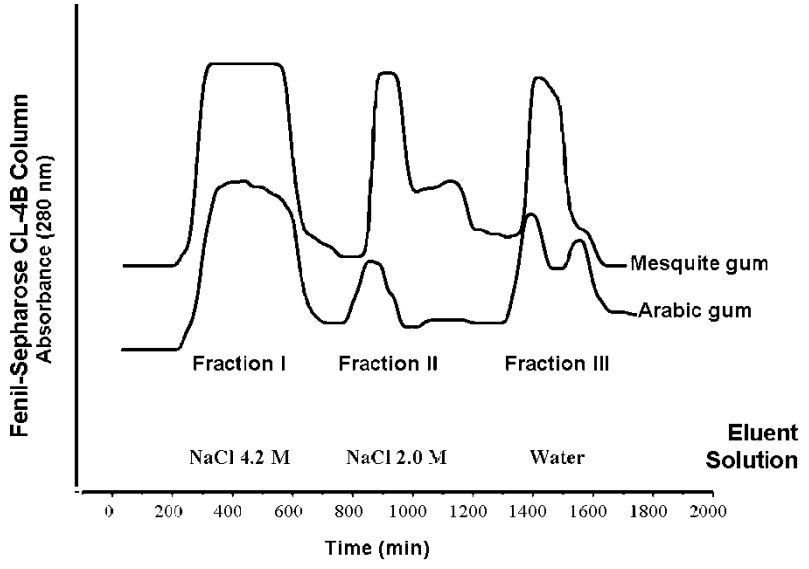

Figure 1. Fractionation of mesquite and Arabic gums using hydrophobic affinity chromatography.

Analytical Method

HPLC analyses were carried out using a Varian Solvent Delivery System pump Model 9012 and a Rheodyne Model 7125 injector (Rheodyne Inc., Cotati CA) fitted with a 10 μL loop. For detection a Varian Model 350 differential refractometer (RI) detector was used. A Star Chromatography Workstation Version 5.51 was used (Varian Associates, Inc. USA). A Supelcosil LC-NH_2, 5 μm (4.6 mm ID × 25 cm) column was used with a 3 cm guard column (Supelco, Mex.) with 80/20 acetonitrile-water as the mobile phase, at a flow rate of 1.0 mL/min and a temperature of 30°C. The mobile phase was filtered with 0.2 μm prior to use.

Quantitative determinations were done by comparison of three successive analyses on each of the samples and the standard mixtures of sugars (galactose, arabinose, rhamnose). Descriptive statistics and regression analysis were applied to the data. The monosaccarides concentrations were determined with reference to the calibration standards mixture of 13.33 mg/mL (Sigma-Aldrich de México).

RESULTS AND DISCUSSION

Detector Performance

The sensitivity of a component in the detector must be determined before quantitation. A sensitivity plot is shown in Figure 2, for galactose, arabinose, and rhamnose. The sensitivity for the three carbohydrates were 387.30, 384.22, and 460.78, respectively, in a range of 0 to 13.33 mg/mL.

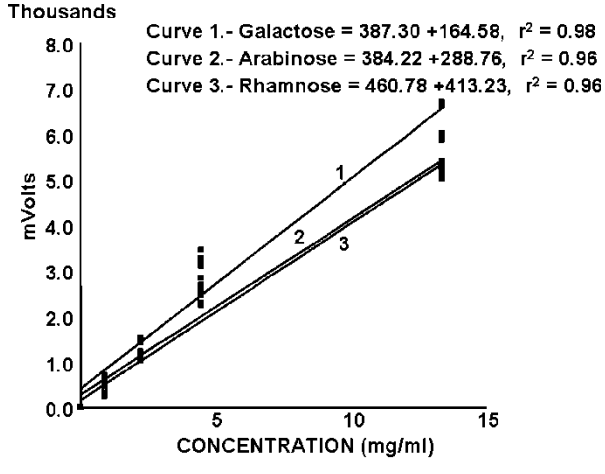

Figure 2. Linearity and sensitivity plot for galactose, arabinose and rhamnose.

The calibration curve constructed exhibited good linearity by plotting the variation of peak area as a function of concentration; four injections of standard solutions ($n = 20$) were assayed. The correlation coefficients ranged from 0.96 to 0.98, and the intercept was not significantly different from zero ($p = 0.05$). This allows use of only one standard solution for routine analysis. Detection limits were evaluated from the calibration graph at 0.1 mg/mL (signal-to-noise ratio, $S/N = 3$). Limit of quantitation was evaluated at 0.6 mg/mL ($S/N = 10$). Validation tests confirmed the suitability of the proposed method for routine analysis of carbohydrates samples.

High pressure liquid chromatography analysis showed peaks at rhamnose (5.7 min), arabinose (7.2 min), and galactose (8.8 min) retention times that corresponded to purified standard and samples. To confirm these peaks, the extract was spiked with standard mixture, resulting in the peak identification. Representative chromatograms of native fractions of mesquite and Arabic gum are shown in Figures 3 and 4, respectively.

The detector selectivity response of three carbohydrates standards by the refraction index detection system is shown in the Figure 3. Note the baseline noise and the peak size for the refraction response. The chromatogram shows a baseline separation. This injection contained approximately 0.80 mg of carbohydrates. Samples tested did not exhibit interfering peaks with carbohydrates to prevent quantification. The sample and refraction detection system is selective over the matrix sample effect.

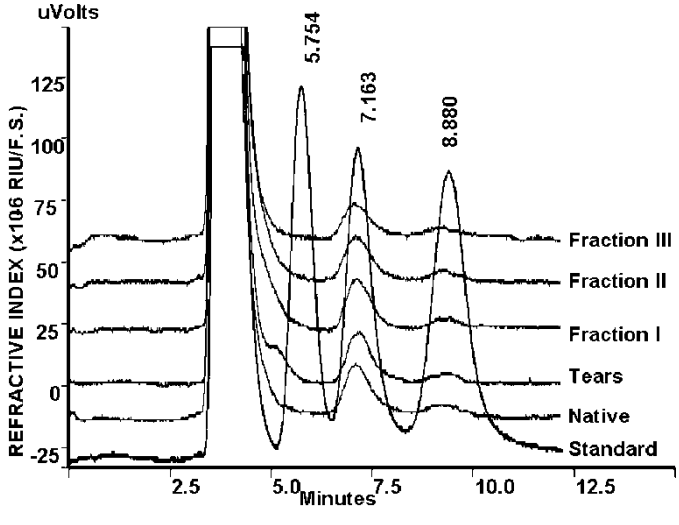

Figure 3. The detector selectivity response of three carbohydrates standards, Galactose (5.754 min), Arabinose (7.163 min), and Rhamnose (8.880 min) by refraction index detection system in mesquite sample and standard.

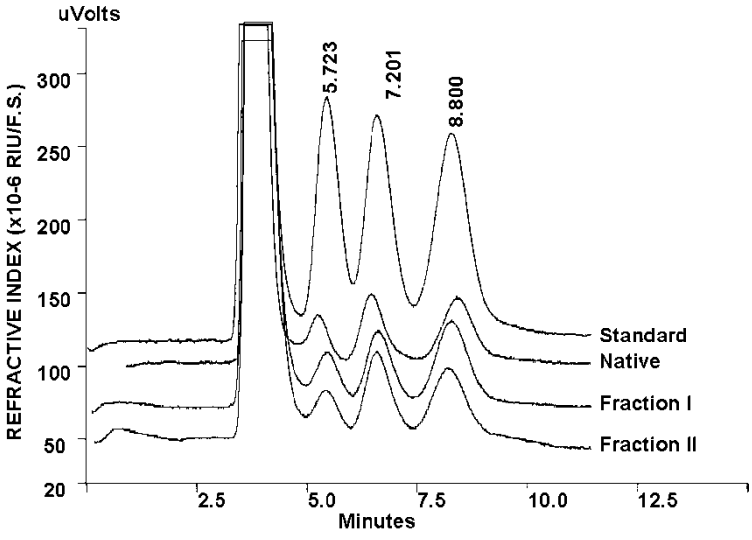

Figure 4. Chromatograms of native and fractions of Arabic gum.

Column Performance

The total analysis time was 15 min. Figure 4 and Table 1, show the column performance, which corresponds to a capacity factor of 0.56–1.69, selectivity = 1.74–3.03, and plate count was about 1262–1626. The column shows good resolution = 2.16–5.14. Theoretically, absolute resolution indicates that the peaks are 99.7% separated to baseline. The theoretical plates indicate that the good resolution is due to column selectivity and efficiency. In our laboratory, we probed a more efficient column, Microsorb-NH_2, 3 um (4.6 mm × 10 cm) that gave sharper peaks, but the retention time was long, and the analysis time was 30 min.

Table 1. Column performance. Column chromatographic parameters

Carbohydrate	Rhamnose	Arabinose	Galactose
Retention time	5,95	7,537	10,283
Capacity factor	0,56	0,97	1,69
Column efficiency	1,74	1,74	3,03
Teoretical plates	1261,84	1420,16	1626,15
Equivalent plates	0,024	0,021	0,018
Resolution	2,16	4,76	5,14

Table 2. Rhamnose, arabinose, galactose, relation galactosa/arabinose percentages and total sugars data for the fractions of the Arabic and mesquite gums.

	Carbohydrates (%)				
Samples	Rhamnose	Arabinose	Galactose	Relation Gal/Arab	Total (%)
Mesquite tears	ND	61.18 2.08 ± 3.39	14.55 3.67 ± 5.25	0.24	75.73
Mesquite native	ND	62.26 4.7 ± 7.56	14 0.69 ± 4.96	0.22	76.26
Mesquite fraction I	0.89	53.49 0.57 ± 1.06	21.66 0.41 ± 1.90	0.41	76.04
Mesquite fraction IIa	ND	44.67 1.34 ± 3.00	2.14 0.49 ± 2.93	0.05	46.81
Mesquite fraction III	ND	34.43 0.60 ± 1.74	1.79 0.08 ± 4.56	0.05	36.22
Arabic native	10.01	25.44 0.13 ± 0.53	26.35 0.18 ± 0.67	1.04	61.81
Arabic fraction I	7.24	20.94 1.21 ± 5.77	31.69 1.17 ± 3.76	1.49	59.37
Arabic fraction II	5.85	23.38 0.63 ± 2.71	25.21 0.81 ± 3.23	1.08	54.44

Precision and Accuracy

The precision and repeatability of the method and the chromatographic system were assayed by the analysis of 23 replicate standard samples in 15 days, which contained a standard mixture at 13.33 mg/mL. The calculated coefficient of variation was lower by 5% for the standard areas. Daily variability for 10 replicate standards were 3% (calibration factor $= 2-3 \times 10^{-3}$, standard deviation $= <0.9 \times 10^{-5}$). Repeatability also was tested on whole and fractioned samples. The results are given in Table 2.

Chromatographic Analysis (Fractionation)

The Arabic and mesquite gums were separated into two and three fractions by hydrophobic affinity chromatography. Rhamnose, arabinose, galactose, relation galactosa/arabinose percentages, and total sugars data for the fractions are given in Table 2.

The sugar composition of the arabic and mesquite gums were very close to that reported by Anderson and Farquhar[11] for P. laevigata.

The values quoted for the sugar composition for the whole gum are typical for samples from A. senegal as reported by other workers.[10,12]

The mesquite tears and native (75.73% and 76.26%) showed higher total carbohydrate content in comparison with arabic gum (61.81%). The saccharides isolated from the arabic gum contained galactose, arabinose, and rhamnose.

Fraction I, was the material which passed down the hydrophobic column without adsorbing. Arabic gum showed a higher rhamnose (7.24%) content in comparison with mesquite (0.89%). Only the hydrolyzed fraction I showed rhamnose content in mesquite. Fraction II, which had initially adsorbed onto the column packing but was desorbed on changing the eluent from 4.2 to 2.0 M NaCI, and Fraction III, which were desorbed from the column on elution with water, showed that the content of galactose predominated in the arabic gum and arabinose in the mezquite gum. The assay demonstrated to be fast and with good linear and high precision response. The results showed that the linear range of assay was suitable for routine analysis and applicable to the entire range of monosaccharides levels normally found in mesquite and arabic gums.

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