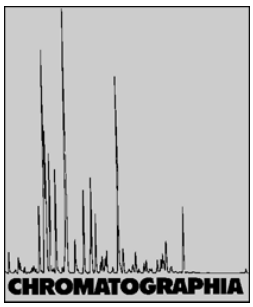
Determining Sugar Composition of Food Gum Polysaccharides by HPTLC



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Key Words

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Summary

Most commercially important food gums, including gum arabic, guar gum, and carrageenan are polysaccharides which consist of multiple sugars, including uronic acids. For the first time, the sugars in these and other gums were determined by comparison of R_F values with standards by HPTLC on Si 50000 plates after acid hydrolysis of the polysaccharides. The solvent system consisted of npropanol:water:triethylamine:30% NH_3 (80:20:0.2:4). Analysis was rapid, as the separations were accomplished using a single plate development and the sugars were located by simple charring. Sugar separations on Si 50000 with this solvent system are more efficient than others and provide the additional advantage over bonded-phase silicas in that sugars can be detected using aggressive methods.

Introduction

A novel synthetic porous silica, Si 50000, has proven very useful for separating polar analytes such as amino acids [1] and sugars [1–3] by HPTLC. Si 50000 has an extremely large pore diameter (5000 nm) and a low surface area of about 0.5 m^2/g [1]. Other HPTLC quality silica gels have a pore diameter of about 6 nm and a surface area of about 500 m^2/g [4]. The low surface activity [1] of Si 50000 results in minimal spot tailing of polar compounds such as carbohydrates.

Optimal HPTLC resolution of monosaccharides on Si 50000 was obtained using a single development with a solvent system consisting of n-propanol/water/25% NH_3 [1]. When other silica gels were used [5–8] as stationary phases, multiple plate developments were required in order to achieve comparable resolution of sugars. Impressive separations of branched cyclodextrins [9] and of homologous glucans up to DP 30 [10] have also been achieved. These separations [9, 10] used multiple plate developments with n-butanol/pyridine/water mixtures.

A rapid procedure was required to monitor sugar composition of potentially useful polysaccharides isolated from underutilized and abundant agricultural processing wastes. Corn fiber gum hemicellulose [11, 12] for example, contains D-xylose, Larabinose, D,L-galactose, and D-galacturonic acid. The presence of glucose in corn fiber gum hydrolyzates would indicate contamination with starch. Also, a procedure to monitor sugar composition of polysaccharides isolated by various methods from flaxseed meal was required. In the present study I applied separations on Si 50000 plates to monitor these processes, and also to conveniently assay the sugar composition of several important industrial polysaccharides.

Experimental

Materials

Si 50000 HPTLC plates, 10 H 20 cm (E. Merck, Darmstadt, Germany, cat. no. 15135) without fluorescent indicator, were purchased from EM Science (Gibbstown, NJ, USA). The plates were cut to appropriate widths as needed. Sugars, the polysaccharides pectin, gum arabic, xanthan gum, gellan gum, guar gum and carrageenan, and triethylamine and N-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Sigma (St. Louis, MO, USA). Corn fiber gum [12] and flaxseed mucilage [13] were prepared as described earlier.

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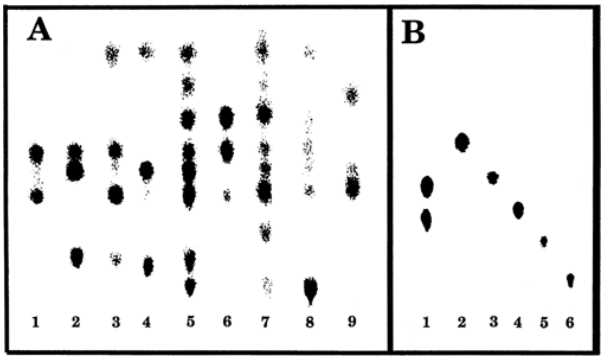

Figure 1. A) HPTLC chromatogram of sugars in food gum polysaccharide hydrolyzates using single development with n-propanol:water:triethylamine:30% NH_3 (80:20:0.2:4). **1** = guar gum; **2** = xanthan gum; **3** = gum arabic; **4** = gellan gum; **5** = standard mixture of rhamnose, fucose, xylose, arabinose, glucose, galactose, glucuronic acid, and galacturonic acid (listed in order of decreasing R_F value); **6** = corn fiber gum; **7** = flax mucilage; **8** = pectin; **9** = carrageenen. **B)** HPTLC chromatogram of standard di- and tri- and tetrasaccharides using two developments with n-propanol:water:-triethylamine:30% NH_3 (85:15:0.2:4). **1** = mixture of maltose and maltotriose; **2** = sucrose; **3** = trehalose; **4** = raffinose; **5** = stachyose.

HPTLC Separations of Sugars

For HPTLC, solutions of sugars and polysaccharide hydrolyzates were prepared at levels of about 10 mg mL^{-1}. About 0.5 µL were spotted, and plates were typically developed to about 8 cm, which required about 60 min. The solvent system consisted of n-propanol:water:-triethylamine: 30% NH_3 (80:20:0.2:4) unless otherwise specified. Sugars were detected after spraying with a solution of 5% H_2SO_4 and 6.5 mM N1(1naphthyl)ethylenediamine dihydrochloride in methanol and heating on a hot plate or placing in a 100 °C oven [14].

Polysaccharide Hydrolysis

Pectin, gum arabic, xanthan gum, and gellan required relatively strong acid conditions for hydrolysis to constituent sugars. These polysaccharides (10 mg) were weighed into screw-cap vials and mixed with 150 µL 12N H_2SO_4, vortexing periodically over 45 min at room temperature. The acid was then diluted to 1N H_2SO_4 by adding water (1.65 mL) and the mixture heated in an oven at 100 °C for 1.5 hr. After cooling to room temperature, $BaCO_3$ was gradually added until solution pH was neutral by pH paper. The $BaSO_4$ was removed by vacuum filtration, and the sugar-containing filtrate was dried under a stream of nitrogen. The syrups were dissolved in water (1 mL), and the solution was filtered through a 0.2 µ Anotop 10 plus membrane filter (Whatman, Maidstone, England, cat. no. 6809 3022). Corn fiber gum, flaxseed mucilage, guar gum and carrageenan (10 mg) were hydrolyzed with N H_2SO_4 (1 mL) at 100 °C for 1.5 hr. After cooling, solutions were neutralized by addition of $BaCO_3$ and then treated as above.

Results and Discussion

The sugars most commonly found in food gum polysaccharides are L-arabinose, D-xylose, D-glucose, D- and L-galactose, D-galacturonic acid, D-glucuronic acid, L-rhamnose, and L-fucose. Conditions for their separation on Si 50000 HPTLC plates were optimized, using a solvent system similar to that used earlier [1], consisting of n-propanol/water/25% NH_3. It was observed that the addition of a small quantity of triethylamine to the mixture resulted in chromatographic mobility and highly efficient resolution of the two uronic acids. Many combinations were tested and optimal results for the separation of the eight sugars was achieved using n-propanol:water:triethylamine:30% NH_3 (85:15:0.2:4) as solvent system. Their separation using a single development is shown in Figure 1A, lane 5, and the R_f values of these and other mono-, di-, tri-, and tetra-

Table I. R_F values of standard sugars on Si 50000 HPTLC plates using single elution with n-propanol : water : triethylamine : 30% NH_3 (80 : 20 : 0.2 : 4).

Sugar	R_F	Sugar	R_F	Sugar	R_F	Sugar	R_F
Arabinose	.497	Gulose	.471	Sorbose	.491	Sucrose	.474
Ribose	.566	Mannose	.490	Psicose	.531	Laminaribiose	.484
Xylose	.591	Fucose	.686	Isomaltose	.237	Turanose	.486
Lyxose	.604	Rhamnose	.784	Gentiobiose	.259	Lactitol	.270
2-deoxy-Ribose	.850	2-deoxy-Glucose	.800	Lactose	.267	Raffinose	.225
Galactose	.361	Galacturonic Acid	.111	Cellobiose	.367	Melezitose	.277
Glucose	.435	Glucuronic Acid	.200	Maltose	.387	Maltotriose	.344
Talose	.460	Fructose	.467	Trehalose	.391	Stachyose	.082

Table II. Proportions of sugars in food gums, calculated from previously published structural information on corn fiber gum [12] and other gums [15]. Ar = L-arabinose; Xy = D-xylose; Ga = D or D,L-galactose; Gl = D-glucose; GaA = D-galacturonic acid; GlA = D-glucuronic acid; Ma = D-mannose; Rh = L-rhamnose; Fu = L-fucose; 3,6-anhydro-D-Ga = 3,6-anhydro-D-Galactose.

	Ar	Xy	Ga	Gl	GaA	GlA	Ma	Rh	Fu	3,6-anhydro-D-Ga
Guar gum			1				2			
Xanthan gum				2		1	2			
Gum arabic	2		4			1		1		
Gellan gum				2‘		1		1		
Corn fiber gum	7	10	1			1				
Flaxseed mucilage	3	6	4		2			5	2	
Pectin					20			1		
Carrageenan			1							1

saccharides are listed in Table I. Figure 1B is a chromatogram indicating possible separations among the disaccharides maltose, trehalose, and lactose, the trisaccharides maltotriose and raffinose and the tetrasaccharide stachyose. These higher saccharides were effectively mobilized by using two developments with the solvent system n-propanol:water:triethylamine: 30% NH_3 (85:15:0.2:4).

The sugar composition of corn fiber gum [12] and the other food gum polysaccharides [15] that were evaluated are listed in Table II. All the expected sugars were resolved and all but one were detected, as shown in Figure 1A. In corn fiber gum, the relatively low level of glucuronic acid was not detected (Figure 1A, lane 6). Flax mucilage (Figure 1A, lane 7) contained all but D-glucuronic acid of the eight sugars in the standard mixture (Figure 1A, lane 5). We found that uronic acids undetected by simple charring were revealed when plates were heated at 100 °C after spraying with 6.5 mM N(1naphthyl)ethylenediamine dihydrochloride in methanol containing 5% H_2SO_4 [14]. Similarly, L-rhamnose was then detected in pectin, along with traces of xylose, arabinose, glucose, and galactose. Use of this spray reagent also allows for sensitive quantitation of sugars by densitometry [14]. In the flax mucilage (Figure 1, lane 7) hydrolyzate, a component with a mobility less than galactose was detected, whose Rf did not correspond to any of the monosaccharides tested (Table I). Work is underway to characterized this material.

In conclusion, we have modified a mobile phase described earlier [1] for separating sugars by HPTLC on Si 50000 plates by addition of triethylamine. This allows efficient resolution of the sugars, including uronic acids, which constitute the important food gum polysaccharides. The use of our solvent system and Si 50000 plates is the most effective means by which to separate sugars by HPTLC.

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