

## SHORT COMMUNICATION

# QUANTIFICATION OF SOIL SACCHARIDES BY SPECTROPHOTOMETRIC METHODS

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Extraction and accurate determination of mono- and oligo-saccharides in soil is an important parameter in elucidating the role of saccharides in stabilization of soil aggregates. Partial hydrolysis with mild sulfuric acid ( $H_2SO_4$ ) conditions (0.25–0.5 M) is frequently used for determination of soil saccharides (Cheshire and Mundie, 1966; Barker *et al.*, 1967; Cheshire *et al.*, 1969; Oades *et al.*, 1970; Doutre *et al.*, 1978; Murayama, 1984). Other extractants including water, *N,N*-dimethylformamide, sodium ethylene diaminetetraacetate (EDTA) and urea have been utilized with limited success (Cheshire, 1977; Barker *et al.*, 1967).

Various methods including colorimetric assays, and gas and liquid chromatography, have been utilized for determination of soil saccharides. Of those methods, colorimetric procedures are popular because they are simple, rapid, and do not require elaborate instrumentation other than a spectrophotometer. However, colorimetric methods do not reveal the composition of saccharides present in soil. Recent advances in ion chromatography and detector methodology allows rapid, sensitive and highly selective determination of saccharides using pulsed amperometric detection (Hughes and Johnson, 1981; Martens and Frankenberg, 1990).

Our objective was to evaluate the effectiveness of water, acid concentration and heat on extraction of soil saccharides. The viability of using colorimetric methods for soil saccharide quantification was also assessed.

The soil used in this study was an Arlington coarse-loamy, mixed, thermic Haplic Durixeralf (pH 7.9; 670 g sand, 80 g silt, 250 g clay  $kg^{-1}$  soil; C/N 11). Water extraction and acid hydrolysis employed for determination of soil saccharides were conducted as follows: 1 g soil samples were treated with 10 ml of water and refluxed at 80°C or shaken at room temperature for 16 h. Acid hydrolysis was conducted with 1 g soil samples treated with  $H_2SO_4$  concentrations ranging from 0.05 to 2.5 M and were either refluxed at 80°C or shaken at room temperature for 16 h. The acid extracts were then treated with 0.1 M EDTA to prevent co-precipitation of the saccharides with di- and trivalent cations, and titrated to pH 3.5–4.0 with 5.0 M KOH. Samples were then transferred to centrifuge tubes and centrifuged at 10,000  $rev\ min^{-1}$  for 10 min. The supernatant was removed and filtered through a 0.22  $\mu m$  Millipore (GS) membrane filter (Bedford, Mass). The ionic interferences present in the extracts were removed by filtration through a Supelco™ (Bellefonte, Pa) solid phase extraction system composed of a 3 ml strong cation (3-propylsulfonic acid,  $H^+$ ) (SCX) column and a 3 ml strong anion (3-quaternary propylammonium  $Cl^-$ ) (SAX) column.

The phenol-sulfuric acid colorimetric procedure was performed as described by Dubois *et al.* (1956) and the anthrone-sulfuric acid method was carried out as described by Brink *et al.* (1960). Standard curves were obtained by using D-glucose as a reference standard for both colorimetric analyses.

The high performance anion chromatographic (HPAC) analysis was performed on a Dionex (Sunnyvale, Calif.) LC gradient pump module equipped with a Model PAD-2 detector. Sample injection was via a Dionex Autosampler equipped with a 200  $\mu l$  sample loop. Saccharides were separated on a CarboPac PA1 pellicular anion-exchange resin (250  $\times$  4 mm) and a CarboPac guard column (25  $\times$  3 mm) at a flow rate of 0.8  $ml\ min^{-1}$  at ambient temperatures. Mono- and disaccharides were separated with the following gradient. Eluant A was 0.22  $\mu m$  filtered water (18 Mohm) at 93% for 15 min. Eluant B consisted of 50 mM sodium hydroxide containing 1.5 mM sodium acetate at 7% for 15 min then linearly ramped to 100% at 25 min. The signal response was monitored by pulsed amperometric detection (PAD) with a gold-working electrode and triple-pulsed amperometry (Hughes and Johnson, 1981). The following working pulse potentials and durations were used for detection of saccharides:  $E_1 = 0.05\ V$  ( $t_1 = 300\ ms$ );  $E_2 = 0.60\ V$  ( $t_2 = 120\ ms$ );  $E_3 = -0.80\ V$  ( $t_3 = 300\ ms$ ). The CHOH groups were oxidized at  $E_1$ ,  $E_2$  removes the reaction products, while  $E_3$  cleans the electrode at a negative potential. Cyclic voltammetry was used to choose the three potentials. The response time of the PAD was set as 1 s. Chromatographic data were collected and plotted using Dionex AutoIon 300 software. Constituent peaks were confirmed through the use of external standards and lactose as an internal standard.

Water was ineffective in extracting saccharides from soil with only trace amounts of inositol and ribitol detected (Table 1). Heating the water to 80°C did little to increase the effectiveness in extraction of the saccharides. Cheshire (1977) reported that hot water (80°C) extracted < 3% of the soil saccharides when compared to a mild  $H_2SO_4$  hydrolysis. When  $H_2SO_4$  at 25°C was used for extraction, quantitative amounts of inositol, ribitol, and arabinose were detected. Sulfuric acid concentrations ranging from 0.25 to 2.5 M were more effective in extracting the total saccharide content than 0.05 M. The combination of heat (80°C) with  $H_2SO_4$  was much more effective in extracting the saccharides, giving ca 10  $\times$  more total saccharides. Arabinose, galactose, glucose, and xylose were the main monosaccharides detected. There was little difference in extraction of saccharides when comparing 0.13 and 0.25 M  $H_2SO_4$  at 80°C. The need for heat with mild  $H_2SO_4$  extraction suggest that many of the soil saccharides are present as compounds containing acid-labile hexopyranosyl glycosidic linkages.

The saccharides extracted with 0.25 M  $H_2SO_4$  refluxed at 80°C and detected by HPAC-PAD are shown in Fig. 1. The saccharides detected included inositol, ribitol, fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose. Lactose was used as an internal standard. The total saccharide content as determined by the phenol-sulfuric acid and anthrone-sulfuric acid colorimetric procedures was compared to that detected by HPAC-PAD (Table 2). In

Table 1. Effect of water and sulfuric acid with heating on the release of saccharides from soil as detected by HPAC-PAD

Treatment	Saccharides extracted									Total
	Inositol	Ribitol	Fucose	Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose	
Water (25°C, shaken, 16 h)	Trace	Trace	ND	ND	ND	ND	ND	ND	ND	Trace
Hot water (80°C, 16 h)	Trace	Trace	ND	ND	ND	ND	ND	ND	ND	Trace
H <sub>2</sub> SO <sub>4</sub> (25°C)										
0.05 M	60	22	ND	ND	ND	ND	ND	ND	ND	82
0.25 M	78	77	ND	18	ND	ND	ND	ND	ND	173
0.50 M	35	36	ND	32	ND	ND	ND	ND	ND	104
1.00 M	32	32	ND	101	ND	ND	ND	ND	ND	165
2.50 M	13	25	ND	133	ND	ND	ND	ND	ND	171
H <sub>2</sub> SO <sub>4</sub> (80°C)										
0.13 M	35	53	20	279	48	265	192	242	62	1196
0.25 M	38	45	32	301	46	254	233	244	66	1259

<sup>a</sup>ND, not detected.

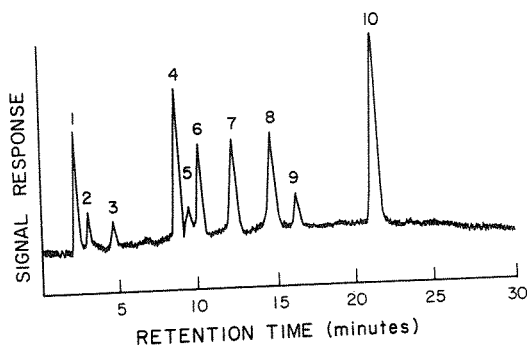


Fig. 1. Chromatogram of H<sub>2</sub>SO<sub>4</sub> (80°C) extract of soil saccharides as detected by high performance anion exchange chromatography coupled with pulsed amperometric detection: 1 = inositol, 2 = ribitol, 3 = fucose, 4 = arabinose, 5 = rhamnose, 6 = galactose, 7 = glucose, 8 = xylose, 9 = mannose, 10 = lactose (internal standard).

evaluating the colorimetric assays to the standard HPAC-PAD method, various extraction procedures were employed including water extracts at ambient temperature, water extracts heated to 80°C and H<sub>2</sub>SO<sub>4</sub> (80°C). The phenol-sulfuric acid and anthrone-sulfuric acid methods gave comparable results of the water extract (25°C) without pretreatment with the ion-exchange resins. However, when subject to the SAX and SCX resins to remove ionic interferences, the HPAC-PAD revealed no saccharides were present in these extracts. Upon pretreatment with the SAX and SCX resins, the phenol-sulfuric acid method confirmed the HPAC-PAD results, but the anthrone-sulfuric acid method gave erroneous results detecting as much as 413 mg of saccharides kg<sup>-1</sup> of soil. Similar observations were noted with the water extracts subjected to 80°C, but the magnitude of total saccharides detected by the anthrone-sulfuric

Table 2. Quantification of soil saccharides by colorimetric assays and ion chromatography

Extraction method	SAX and SCX <sup>a</sup>	Phenol-sulfuric acid <sup>b</sup> (mg saccharides kg <sup>-1</sup> soil)	Anthrone-sulfuric acid
Water (25°C)	—	201	180
Water (25°C)	+	0 (0)	413
Water (80°C)	—	1119	289
Water (80°C)	+	0 (0)	597
Acid extract (0.25 M)	—	1841	848
Acid extract (0.25 M)	+	1204 (1259)	468

<sup>a</sup>Strong anion-exchange (SAX) and strong cation-exchange (SCX) resins.

<sup>b</sup>Values in parentheses are total saccharides obtained by high performance anion chromatography with pulsed amperometric detection.

acid method was considerably higher 597 mg kg<sup>-1</sup>. The phenol-sulfuric acid method overestimated (1.53-fold) the total saccharide content of the acid extracts (0.25 M H<sub>2</sub>SO<sub>4</sub>, 80°C) without the clean-up (ion-exchange resins) while the anthrone-sulfuric acid method gave an underestimate. Both colorimetric procedures are subject to interferences. The phenol-sulfuric acid assay is subject to interference by the presence of NO<sub>3</sub><sup>-</sup> and Fe<sup>3+</sup> while the anthrone method is affected by Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and Fe<sup>3+</sup>. When the acid extract was subjected to SAX and SCX resins, the total saccharide content detected by the phenol-sulfuric acid method (1204 mg kg<sup>-1</sup>) was very similar to the HPAC-PAD method (1259 mg kg<sup>-1</sup>). The anthrone-sulfuric method only recovered 37% of the total saccharide content detected by the HPAC-PAD method. Dautre *et al.* (1978) compared the phenol-sulfuric acid and anthrone-sulfuric acid methods to gas chromatography (GC) in detection of soil saccharides and concluded that the phenol-sulfuric acid method was significantly superior to the anthrone-sulfuric acid method when compared with GC analysis. We confirm the conclusions of Dautre *et al.* (1978) with liquid chromatography.

Because of the simplicity of the phenol-sulfuric acid method, we recommend that this colorimetric method be used for the determination of total saccharide content, only when soil extracts are subjected to anion- and cation-exchange resins to eliminate interfering compounds. However, chromatography procedures including either GC in determination of alditol acetates or HPLC should be used to quantitate the individual saccharide composition. There are obviously serious problems with the anthrone-sulfuric acid method in determination of saccharides in soil extracts even when subjected to ion exchange resins.

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