A Comparison of Acid Hydrolyses for the Determination of Carbohydrate Content in Soils

A. Piccolo, A. Zena, and P. Conte

Dipartimento di Scienze Chimico-agricole, Università di Napoli "Federico II", via Università 100, I-80055 Portici, Italy

ABSTRACT

Four Italian surface soils were used to compare four different acid hydrolyses for the determination of the total content of carbohydrates in soils. Soil hydrolyses in 0.25M and 1M H₂SO₄ and by mechanical shaking for 16 h released carbohydrates as efficiently as the 8 h soil hydrolysis under reflux. Degradation of released carbohydrates was probably the cause of the low values given by the hydrolysis in 72% H₂SO₄ for 15 min followed by a 16 h shaking in 0.5M H₂SO₄. This study confirms that the carbohydrate determination based on the colorimetric phenoil-sulphuric acid method is more accurate than the colorimetric anthrone-sulphuric acid method. Moreover, soil characteristics may influence the accuracy and precision of results depending on the hydrolysis procedure as it was shown by the soils rich in organic carbon and clay.

INTRODUCTION

Extraction and accurate determination of soil carbohydrates is essential to assess the role of carbohydrates in stabilizing soil aggregates (Piccolo, 1996). Various colorimetric methods have been used over the years to determine soil total carbohydrates. Lynch et al. (1957) adopted carbazole to form a colorimetric

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complex with uronic acids. Dubois et al. (1956) employed a phenol-sulphuric acid mixture 
that, by complexing saccharides, produces a green color whose intensity is proportional 
to the carbohydrate content. Brink et al. (1960) introduced the use of the anthrone-sulphuric acid 
method to measure the hexose sugar content in soil hydrolysates. However, various interfering 
substances were recognized to be present in the soil hydrolysates and to yield erroneous 
results in the colorimetric determination of carbohydrates (Stevenson, 1994). Most of the 
interferences are soil inorganic components coextracted with carbohydrates in the sulphuric acid 
solution used to hydrolyse soil polysaccharides.

Oades et al. (1970) have already pointed out that the sulphuric acid concentration, 
and the duration and temperature of soil hydrolysis, must be a compromise between 
maximum release and minimum degradation of soil sugars. Maximum hydrolysis 
of soil polysaccharides was obtained by the popular method of Cheshire and 
Mundie (1966) that implies a soil pre-soaking period in 24N H₂SO₄, followed by 
a soil reflux in 1N H₂SO₄. However, this method was found to underestimate 
pentoses and deoxyhexoses, being far too severe for these classes of saccharides 
(Oades et al., 1970).

Soil hydrolysis of polysaccharides were further improved by removing 
interfering inorganic ions with the use of anion- and cation-exchange resins 
(Mundie, 1976; Doutre et al., 1978). Recently, Martens and Frankenberg (1990) 
have shown, for one soil sample only, that the purification step with exchange 
resins improves analytical results when the hydrolysis is conducted at a milder 
0.25M H₂SO₄ concentration under reflux for 8 h. They also reported that the 
phenol-sulphuric acid method gave values for total sugar content, comparable to 
that obtained by summing up the values obtained for single monosaccharides by 
ion chromatography and pulse amperometric detector (PAD). The same 
equivalence could not be found when the anthrone-sulphuric acid method was 
used for total carbohydrate measurements.

The objective of this work was to further simplify the acidic hydrolysis for soil 
carbohydrate determination by substituting shaking procedures to the refluxing 
method. Moreover, the soil total carbohydrates released by the different hydrolytic 
procedures were determined by both phenol-sulphuric acid and anthrone-sulphuric 
acid methods, in order to assess the accuracy and precision of both method.

MATERIALS AND METHODS

Soils

Four Italian surface (0-20 cm) soils (a sandy-clay-loam andisol; a sandy andisol; 
a clay-loam vertisol; a sandy-clay-loam entisol) were sampled in areas near Naples. 
Soil samples were air dried, ground, and passed first through a 2-mm sieve and 
then through a 0.15-mm sieve to remove plant remains. The soils were analyzed 
for their chemical and physico-chemical properties by common procedures (Black 
et al., 1982). The analytical values found for the four soils are reported in Table 
1.
TABLE 1. Some chemical and physico-chemical properties of the soils studied.

<table>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>Sand (%)</td>
<td>42.5</td>
<td>60.1</td>
<td>7.0</td>
<td>40.3</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>29.8</td>
<td>27.9</td>
<td>45.4</td>
<td>30.1</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>27.7</td>
<td>12.0</td>
<td>47.6</td>
<td>29.6</td>
</tr>
<tr>
<td>pH-H$_2$O</td>
<td>7.2</td>
<td>6.2</td>
<td>7.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Total CaCO$_3$ (%)</td>
<td>11.1</td>
<td>0.1</td>
<td>7.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>1.2</td>
<td>4.3</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>CEC (meq/100 g)</td>
<td>17.1</td>
<td>18.5</td>
<td>28.6</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Methods of Soil Hydrolysis

Four different hydrolysis methods were performed on the above soils in order to solubilize carbohydrates.

1. One g of soil was refluxed with 0.25M H$_2$SO$_4$ in a Soxhlet apparatus for 8 h at 120°C as by the procedure recommended by Martens and Frankenberger (1990).
2. One g of soil was added with 10 mL of 0.25M H$_2$SO$_4$ and shaken in a rotary shaking apparatus for 16 h.
3. One g of soil was added with 10 mL of 1M H$_2$SO$_4$ and shaken in a rotary shaking apparatus for 16 h.
4. One g of soil was added with 3.7 mL of a 72% H$_2$SO$_4$ and left at 50°C for 15 min. Distilled water was then added to bring the acid concentration to 0.5M and the soil mixture shaken in a rotary shaking apparatus for 16 h.

Treatment of Hydrolysate and Carbohydrate Determination

The acidic soil extracts were treated with 0.1M EDTA to prevent coprecipitation of saccharides with divalent and trivalent cations and the pH adjusted to 4 with 5M KOH. Samples were then centrifuged at 10,000 rpm for 10 min and about 9 mL of supernatant was separated from the soil residue and filtered through and the soil mixture shaken in a rotary shaking apparatus for 16 h.

Treatment of Hydrolysate and Carbohydrate Determination

The acidic soil extracts were treated with 0.1M EDTA to prevent coprecipitation of saccharides with divalent and trivalent cations and the pH adjusted to 4 with 5M KOH. Samples were then centrifuged at 10,000 rpm for 10 min and about 9 mL of supernatant was separated from the soil residue and filtered through
a 0.2-μm filter. To remove inorganic ions interferences, the extract was passed first through a cation exchange resin (Dowex 50X2-400) and then through an anion exchange resin (Dowex 1X2-200). Two mL of this purified extract were used to determine the total carbohydrate content by the phenol-sulphuric acid method of Dubois et al. (1956) and by the anthrone-sulphuric acid method of Brink et al. (1960). Color intensity was determined by a Perkin Elmer Lambda 3B UV/VIS Spectrophotometer while calibration curves were obtained by using increasing concentrations of D-glucose as standard. Each hydrolysis and carbohydrate determination were performed in triplicates.

RESULTS AND DISCUSSIONS

The total content of soil carbohydrates found in the four soils according to the adopted hydrolytic methods are reported in Table 2. For all hydrolytic procedures, the anthrone-sulphuric acid method appeared to constantly determine a significantly lower amount than that found by the phenol-sulphuric acid method. The differences in values between the two methods varied from one to two orders of magnitude. These results confirm the report of Martens and Frankenberg (1990) who showed that, despite the elusion of the neutralized soil hydrolysates through anion and cation exchange resins, the anthrone-sulphuric acid determination method was less accurate than the phenol-sulphuric acid method. Higher amounts of total soil carbohydrates by the phenol-sulphuric acid method were also reported in earlier works (Dubois et al., 1956; Ivarson and Cowden, 1962). Moreover, the relatively low standard deviations measured for the two determination methods (Table 2) indicate the precision of the values obtained for both methods. This suggests that the generally poor accuracy of the anthrone-sulphuric acid method must be due to negative interferences which cannot be eliminated by the treatment with exchange resins. The values for total soil carbohydrates determined by the phenol-sulphuric acid method (Table 2) show that the adopted hydrolytic procedures produced different analytical results. The lowest values were found for procedure IV that combined the harsh soil treatment of concentrated \( \text{H}_2\text{SO}_4 \) after Cheshire and Mundie (1966) with a milder shaking treatment for 16 h in a 0.5M \( \text{H}_2\text{SO}_4 \). The latter mild shaking treatment was also adopted for procedures II and III by using 0.25M and 1M \( \text{H}_2\text{SO}_4 \) respectively. However, despite the milder conditions, total carbohydrates resulted generally higher than those found for procedure IV. This indicates that procedure IV is too severe a hydrolysis and some of the carbohydrates released from the soil polysaccharides by hydrolysis are further degraded in concentrated \( \text{H}_2\text{SO}_4 \). These may well be the pentoses and deoxyhexoses which were reported by Oades et al. (1970) to be the least stable in acid solutions.

The 16 h shaking treatments (II and III) were both able to give total carbohydrate values, by the phenol-sulphuric acid method, comparable to those obtained by procedure I that was based on an 8 h reflux of the soil sample in 0.25M \( \text{H}_2\text{SO}_4 \).
### Comparison of Acid Hydrolysates

**Table 2.** Mean values (μg/g) of total content of soil carbohydrates determined by the phenol- and anthrone-sulphuric acid methods after different soil hydrolysates.

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<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>846(±2)</td>
<td>1943(±1)</td>
<td>950(±2)</td>
<td>1300(±2)</td>
</tr>
<tr>
<td>B</td>
<td>109(±1)</td>
<td>289(±2)</td>
<td>139(±0.6)</td>
<td>138(±0.6)</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>A</td>
<td>1540(±4)</td>
<td>1365(±1)</td>
<td>929(±7)</td>
<td>1748(±5)</td>
</tr>
<tr>
<td>B</td>
<td>19(±2.5)</td>
<td>42(±0.6)</td>
<td>43(±0.6)</td>
<td>23(±0.6)</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1466(±6)</td>
<td>1440(±4)</td>
<td>1602(±8)</td>
<td>1907(±7)</td>
</tr>
<tr>
<td>B</td>
<td>26(±2)</td>
<td>74(±4)</td>
<td>26(±2)</td>
<td>36(±1)</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>836(±11)</td>
<td>787(±0.6)</td>
<td>771(±7)</td>
<td>839(±7)</td>
</tr>
<tr>
<td>B</td>
<td>47(±2)</td>
<td>219(±1)</td>
<td>103(±1)</td>
<td>84(±0.6)</td>
</tr>
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</table>

*Standard deviations are indicated in parentheses.

*Phenol-sulphuric acid method.

*Anthrone-sulphuric acid method.

However, some differences among the hydrolytic procedures were noted depending on the soil properties. Soil A and D, with similar textural properties, organic carbon, and CaCO₃ content (Table 1), showed an increase in carbohydrate content when passing from hydrolysis I to hydrolysis II (Table 2). Conversely, the sandy, organic carbon-rich B soil gave a lower value when a sample was shaken for 16 h, in respect to the one refluxed for 8 h in H₂SO₄ at the same concentration. No significant difference between hydrolysis I and II was shown by the carbohydrate values of the clay-rich C soil. However, this soil showed an increased carbohydrate value when the 16 h shaking was performed in a 1M H₂SO₄ as in procedure III. This result may be attributed to the higher clay dispersing capacity of a more concentrated H₂SO₄ solution and a more efficient hydrolysis of polysaccharides.
when they are adsorbed on clay particles. The increased H$_2$SO$_4$ concentration of procedure III produced a carbohydrate value for soil D higher than for hydrolysis II, whereas values for soil A and B did not change significantly.

Our results indicate that soil hydrolysis by 16 h shaking in 0.25M H$_2$SO$_4$ can more effectively release soil polysaccharides and produce less degradation than 8 h reflux in H$_2$SO$_4$ at the same concentration. The only exception appears to pertain to the soil B, particularly rich in organic matter (4.3%), from which a higher carbohydrate value was found by the reflux hydrolysis. The use of a 1M H$_2$SO$_4$ solution did not affect the results for soil B, but significantly increased the carbohydrate release from the clay rich soil.

CONCLUSIONS

This study confirms that the anthrone-sulphuric acid method for the determination of total content of carbohydrates in soils is not accurate. This method gave values consistently lower than the phenol-sulphuric acid method for the four different hydrolysis procedures which were tested.

Moreover, among the hydrolytic methods, the soil acidic hydrolysis based on soil pre-treatment with concentrated H$_2$SO$_4$ appeared too severe and led to the degradation of part of the released carbohydrates. Conversely, a mild 0.25M H$_2$SO$_4$ solution and a period of 16 h shaking gave values comparable to those of a reflux procedure. Because of its simplicity and sample handling capacity, a shaking method is preferable to the reflux method except for soils particularly rich in organic matter. A 1M H$_2$SO$_4$ concentration and a sample shaking method is, instead, more suitable for clay-rich soils because of the higher clay dispersing power of a more concentrated H$_2$SO$_4$.

REFERENCES


COMPARISON OF ACID HYDROLYSES


