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Experimental Evaluation of Procedures to Quantify Carbohydrate Carbon in Some Calcareous Soils

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ABSTRACT
Carbohydrates are an important component of soil organic matter, and a method is needed to quantify them, which would be efficient in terms of time and cost. Different extractants and methods were examined in this work for their efficiency to extract carbohydrate C from four calcareous soils. Four extractants (distilled water, 0.5 M potassium sulfate (K₂SO₄), and 0.25 and 0.5 M sulfuric acid (H₂SO₄)) and three incubation methods (shaking for 16 h, heating in an oven (85 °C) for 16 h, and heating in a water bath (85 °C) for 2.5 h) were compared. The results show that significantly more carbohydrate C was extracted from all four soils with oven and water bath heating of the soil–extractant suspensions than with shaking them at room temperature. The efficiency of the extractants decreased in this order: 0.5 M H₂SO₄ > 0.25 M H₂SO₄ > 0.5 M K₂SO₄. The combination of the heated–water bath incubation method with 0.5 M H₂SO₄ as extractant was the most efficient method.

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KEYWORDS
Extractant; extraction method; hydrolysis; phenol–sulfuric acid; soil carbohydrate

Introduction
Soil carbohydrates comprise 5–25% of the total soil organic matter (Cheshire 1979). They make an important contribution to soil quality via formation and stabilization of soil structure (Haynes and Francis 1993; Puget, Angers, and Chenu 1999). Hydrolysis and extraction from soil is a prerequisite to quantify them. Various procedures have been proposed for this purpose, differing particularly in the extractants used, temperature and shaking time. There is no standard protocol, but heating and rotary shaking methods along with sulfuric acid (H₂SO₄) or distilled water extractants have been widely used to extract soil carbohydrates. Hot water (Debosz, Vognsen, and Labouriau 2002; Haynes and Francis 1993; Puget, Angers, and Chenu 1999) and 0.5 M H₂SO₄ (Puget, Angers, and Chenu 1999) have been proposed to extract the most labile fractions of soil carbohydrates. Piccolo, Zena, and Conte (1996), Spaccini et al. (2004) and Fallahzade and Hajabbasi (2012) used dilute acid and rotary shaking to extract soil carbohydrates. Adesodun, Mbagwu, and Oti (2001) used plane rotary shaking (16 h) and heating (2.5 h) in combination with distilled water and 0.25 M H₂SO₄ as extractants, while Caravaca, Lax, and Albaladejo (2004) and Bongiovanni and Lobartini (2006) used 0.5 M H₂SO₄.

Although thermal methods have been widely applied to hydrolyze soil carbohydrates, the heating procedures were not clearly defined. Furthermore, little research has been performed on methods and extractants suited to extract carbohydrates from calcareous soils. Addressing this knowledge gap, the objective of our study here was to compare different extraction procedures for the determination of total carbohydrates in four calcareous soils of central Iran.
Materials and methods

Soil sampling

Samples of four calcareous soils from different site of central Iran (Figure 1) were collected for this study. Soil A (Typic Haplocalcids) was collected from a wheat (*Triticum aestivum* L.) field at an altitude of about 1500 m above sea level in the southwest of the province Yazd. The climate of this region is arid with a mean annual rainfall of 60 mm and potential evapotranspiration of 2800 mm. Prior to 1980s the field has not been used in any way and this field was desert. The cultivation (land preparation for cropping) started about 30 years ago. A flood-irrigation system was developed to reduce salinity levels in the root zone and also to provide water for cropping. Wheat was in rotation with fallow and barley and in this field. Soil B (Typic Haplocambids) was collected from a sugar beet (*Beta vulgaris* L.) field at an elevation of around 1510 m above sea level in the southeast of Isfahan province. Also, the climate of this region is arid, with annual rainfall below 100 mm and potential evapotranspiration above 1500 mm. In this soil, due to salinity problems of soil and water, the main farming practices are limited to salt-tolerant crops such as wheat, barley, and sugar beet. Sugar beet was in rotation with wheat and barley. Soil C (Calcic Haploxeralfs) was collected from another wheat (*Triticum aestivum* L.) cultivation field with heavy textured soils and with an altitude of around 1700 m, southwest of Chaharmahal Bakhtiari province. Winter wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.) cropping systems are common practices in this soil. This area is a disturbed rangeland site converted to farmland about 35 years ago. Prior to cultivation, the rangeland site was covered by native species including *Astragalus cyclophyllus* and *Bromus tomentellus*. Soil D (Typic Calciixerpts) was collected from a field on which tomato (*Lycopersicum esculentum* Mill.) and snap bean (*Phaseolus vulgaris* L.) were cultivated at an elevation of about 2100 m above sea level in the Zagros Mountains, in the southwest of Chaharmahal Bakhtiari province. The mean annual precipitation is around 500 mm in this region. Prior to cultivation, in this area forest lands

![Figure 1. Sampling locations of the four soils used in this study.](image-url)
were mainly covered with Oak (*Quercus brantii* Lindl.). But, forest degradation and cultivation (wheat farming) have started almost 40 years ago. Tomato and snap bean were in rotation with wheat and barley. In four selected soils, the crop residues during land preparation were not burned. The locations of the studied soils in central Iran are shown in Figure 1.

For each calcareous soil, three different sites were selected and from each site three samples were taken, then mixed as a composite soil. The sampling depth was 0–20 cm (plow layer) for soil D and 0–30 cm (plow layer) for the others. After air-drying, the samples were sieved through a 2 mm mesh-size sieve. Soil electrical conductivity and pH of the resulting fine earth fraction were measured in a saturation extract. The saturation extract was prepared by filtration of water-saturated soil. Calcium carbonate was determined using titrimetry (Loeppert and Suarez 1996). Soil organic carbon was analyzed using the Walkley and Black method (Nelson and Sommers 1996), and soil texture by means of the pipette method (Gee and Bauder 1986). The soils’ physical and chemical characteristics are listed in Table 1.

### Soil carbohydrate extraction

Carbohydrates were extracted from 1 g of the fine earth samples, after suspension in 10 mL of either distilled water, 0.5 M potassium sulfate (K₂SO₄), 0.25 or 0.5 M H₂SO₄ as extractants, by incubation of the suspensions (i) for 16 h at 85 °C in an oven, (ii) for 16 h under shaking on a plane rotary shaker at room temperature, or (iii) for 2.5 h at 85 °C in a water bath. Thus, 12 extraction schemes (4 extractants × 3 incubation conditions) were tested for each soil. Each extraction was performed in three replicates.

### Chemical analysis of soil carbohydrate

After incubation, the suspensions were centrifuged for 30 min at 3000 rpm and the supernatants were analyzed for total soluble soil carbohydrates using the phenol–sulfuric acid method of Dubois et al. (1956). In brief, 2 mL of each supernatant solution were mixed with 0.05 ml phenol solution (80% (w/w)) in a 15 mL tube. The mixture was then instantly acidified by adding 5 mL sulfuric acid (97%), stirred for 10 s, left to stand for 1 h at room temperature (25 °C), and analyzed using a spectrophotometer at a wavelength of 490 nm. The calibration curve was obtained using glucose standard. Glucose solutions (0, 20, 40, 60, 80, and 100 mg L⁻¹) in distilled water were prepared. In calibration curve, the concentrations of glucose solutions were 0, 5.67, 11.35, 17.02, 22.70, and 28.37 mg L⁻¹, respectively (Figure 2). If the absorbance was not within the range of these standards (see below), the supernatant solution was diluted with distilled water and analyzed again.

The carbohydrate concentration of soil sample was calculated from the carbohydrate concentration of the supernatant solution using the following equation:

\[
\text{Carbohydrate (mg kg}^{-1}\text{)} = \frac{\text{Carbohydrate (mg L}^{-1}\text{)}}{V_s + V_a + V_p} \times \frac{V_e}{V_s} \times Df
\]

where \(V_s\) is the volume of supernatant solution (2 mL), \(V_a\) is the volume of concentrated sulfuric acid (5 mL), \(V_p\) is the volume of phenol (0.05 mL), \(V_e\) is the volume of extractant (10 mL), \(m_s\) is the

### Table 1. Some physical and chemical characteristics of the four study soils.

<table>
<thead>
<tr>
<th>Soils</th>
<th>Location (province)</th>
<th>Texture (-)</th>
<th>pHₑᵃ (-)</th>
<th>ECₑᵇ (dS m⁻¹)</th>
<th>CCEᶜ (g kg⁻¹)</th>
<th>OCᵈ (%)</th>
<th>Soil classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil A Yazd</td>
<td>Clay loam</td>
<td>7.7</td>
<td>3.4</td>
<td>340.0</td>
<td>0.29</td>
<td>Typic Haplocalcids</td>
<td></td>
</tr>
<tr>
<td>Soil B Isfahan</td>
<td>Silty clay</td>
<td>8.4</td>
<td>14.3</td>
<td>432.5</td>
<td>0.52</td>
<td>Typic Haplocambids</td>
<td></td>
</tr>
<tr>
<td>Soil C Chaharmahal Bakhtiari</td>
<td>Clay</td>
<td>8.5</td>
<td>0.5</td>
<td>481.0</td>
<td>0.75</td>
<td>Calic Haploxeralfs</td>
<td></td>
</tr>
<tr>
<td>Soil D Chaharmahal Bakhtiari</td>
<td>Silty clay loam</td>
<td>8.1</td>
<td>0.6</td>
<td>330.0</td>
<td>0.78</td>
<td>Typic Calcixerepts</td>
<td></td>
</tr>
</tbody>
</table>

ᵃpH of the saturated extract.
ᵇElectrical conductivity of the saturated extract.
ᶜCalcium carbonate equivalent.
ᵈOrganic carbon.
mass of the soil sample (1 g), and $Df$ is the dilution factor. Soil carbohydrate carbon (C) was assumed to correspond to 40% of the mass of the measured glucose equivalents.

**Variability and statistical analysis**

Analysis of variance (ANOVA) was used to determine extractant and incubation treatment effects using the software package SAS 9.1 for Windows (Cary, North Carolina). Means were compared by using the Duncan test and considered significantly different at $p < 0.05$.

**Results and discussion**

Both the incubation method and the extractant had significant influence on total carbohydrate C extraction from all four soils, and there was also a significant interaction between these factors (Table 2).

**Role of incubation method**

More carbohydrate C was extracted from all soils when the suspensions were heated (oven or water bath) than with shaking at room temperature (Table 2). This result agrees with that of Adesodun, Mbagwu, and Oti (2001), who found a similar heating effect. The water bath method extracted less carbohydrate C than the oven method from soils C and D, while there was no difference between these two incubation methods for the other soils, although the duration of incubation was much shorter in the water bath. The lower efficiency of the shaking method as compared to the two other incubation methods indicates that temperature was a much more important factor than mechanical mixing during incubation. Heating may also have increased carbohydrate extractability by deprotonation of functional groups.

**Role of extractant**

The concentrations of extracted carbohydrate C were highest for all soils when 0.5 M $\text{H}_2\text{SO}_4$ was used as extractant (Table 2). Except for soil D, the extraction efficiency of 0.5 M $\text{H}_2\text{SO}_4$ was almost two times as high (soil A) or more (soils B and C) than that of 0.25 M $\text{H}_2\text{SO}_4$, which was second in overall extraction efficiency, followed by $\text{K}_2\text{SO}_4$ and distilled water. The latter two extractants did not differ for soils B and C, and for soil C also the difference between these two and 0.25 M $\text{H}_2\text{SO}_4$ was not significant. The results suggest that acid hydrolysis of hemicellulose and possibly also other polysaccharides was a key factor responsible for the differences, in

![Standard curve of light absorbance at 490 nm for aqueous glucose solutions.](image-url)
It is likely that the carbohydrates extracted with 0.5 M K$_2$SO$_4$ and distilled water were mostly polysaccharides, deriving from plant exudates and microbial origin (Haynes and Francis 1993). Even with heating, water was not found to solubilize plant structural carbohydrates (Angers, Nadeau, and Mehuys 1988; Cheshire 1979). The generally higher extraction efficiency of potassium can be attributed to the displacement of calcium from cation exchange sites of colloidal carbohydrates, resulting in a colloid dispersion effect due to an enlarged diffuse double layer (Haney et al. 1999). In line with our findings, Haney et al. (1999) observed that distilled water at 22 °C extracted about 17% less extractable C than 0.5 M K$_2$SO$_4$ in the high soil pH (>7.7). Similar results were obtained by Matlou and Haynes (2006), whereas Bolan, Baskaran, and Thiagarajan (1996) found that distilled water extracted more organic C from soils than 0.5 M K$_2$SO$_4$.

**Interaction between extractants and incubation conditions**

Comparing the differences in extraction efficiency between methods with different incubation temperatures and to the differences between extractants of different acidity, it appears that acidity had more influence on extraction efficiency than temperature. Also, other studies found that acid hydrolysis was more effective in solubilizing soil organic C than hot water (Chan and Heenan 1999; Silveira et al. 2008). However, it is important to note that heating and acidification together had a more than additive effect (Figure 3). This means that they amplified each other’s effect. Thus, the combination of 0.5 M H$_2$SO$_4$ as extractant with incubation at 85 °C was overall by far the most efficient extraction method. Given that the water bath method was as efficient as the oven method for three out of four soils but still much more efficient than the shaking method also in the case of the fourth soil, we recommend the combination of 0.5 M H$_2$SO$_4$ extractant with heated-water bath incubation as method of choice, at least for these calcareous soils, because of the substantial saving in time.

**Conclusions**

The distilled water extractant resulted in the lowest carbohydrate C concentration for most soils, indicating the lowest efficiency of this method. The ability of 0.5 M H$_2$SO$_4$ solution to extract soil carbohydrates was clearly superior to that of the other extractant solutions. With heating more soil carbohydrates were solubilized than with shaking. The water bath method (along with 0.5 M H$_2$SO$_4$
extractant) was most efficient due to the much shorter time required than by the oven method. It is thus recommended as the method of choice for the determination of total carbohydrate C in the studied calcareous soils.

References


Figure 3. The concentration of extracted carbohydrate C from soils A, B, C, and D using different incubation methods and extractants. Bars indicate standard errors (n = 3).


