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Short communication

Determination of neutral sugars in soil by capillary gas chromatography after derivatization to aldononitrile acetates

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Abstract

We have quantified ribose, rhamnose, arabinose, xylose, fucose, mannose, glucose, and galactose in soil by gas chromatography (GC) simultaneously after converting to aldononitrile acetate derivatives. A recommended single-hydrolytic step by 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h was more effective for releasing soil neutral sugars from non-cellulosic carbohydrates and better suited to our purification procedure compared with the sulphuric acid hydrolysis. Linearity of the GC detection for each neutral sugar was in the range of 10–640 $\mu g\,ml^{-1}$ and the recovery of neutral sugars from the spiked soil samples ranged from 76% to 109.7%. The coefficients of variation of the neutral sugars in four soils were lower than 2.0% for the instrument and 4.6–7.6% for the whole determination procedures. Compared with the trimethylsilyl (TMS) derivatization, the recovery of our newly modified method was more satisfactory and the reproducibility of ribose was improved significantly. Moreover, the aldononitrile acetate derivative was more stable than TMS derivative. Therefore, it is a promising approach suitable for a routine use in the quantitative analysis of soil neutral sugars, since it is fast, sensitive, and reproducible.

Keywords: Aldononitrile acetate; Gas chromatography; Soil neutral sugars; TFA hydrolysis; Determination

1. Introduction

Soil carbohydrates, including neutral and amino sugar, represent 5–25% of soil organic matter (SOM) (Angers et al., 1988; Murata et al., 1999). In the carbon cycling in terrestrial system, neutral sugars constitute a significant part of the labile pool of SOM and provide a major source of energy for microbial processes in soils (Martens and Loeffelmann, 2002). Moreover, they are the most crucial binding agents for soil aggregation (Cheshire, 1979; Oades and Waters, 1991; Puget et al., 1999). The origin and relative contribution of individual monosaccharides to the total carbohydrate pool may provide an indicator of organic matter origin (Hu et al., 1995). For instance, arabinose (A) and xylose (X) are considered to be mostly originated from plant, whereas mannose (M) and galactose (G) are mainly of microbial origin in soil (Chantigny et al., 2000), and thus, the ratio of (G + M)/(A + X) is often used to characterize the origin of soil carbohydrates. When the ratio is lower than 0.5, the contribution is considered mainly from plant materials, whereas the ratio higher than 2 is a clue of microbial origin (Oades, 1984). Therefore, it is important to quantify accurately the neutral sugar compositions of soil carbohydrates.

Capillary gas chromatography (GC) is a very universal tool to analyse carbohydrates in soil. In this case, the derivatization must be conducted before GC determination because saccharides are non-volatile. Alditol acetates derivatization have been used to derivatize soil neutral sugars (Oades, 1967; Dormaar, 1984; Cheshire et al., 1990); however, this method presents the disadvantage of a large number of manual preparation steps, which makes the procedure time consuming and tedious to be performed

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(Zhang and Amelung, 1996). Rumpel and Dignac (2006) have tried to simplify the procedure by eliminating the removing step of borate salts during purification, but the recovery of ribose decreased significantly (only 35% on average) and the coefficient of variation of fucose was too high (up to 45%).

In order to overcome the inherent disadvantage of alditol acetates derivatization, trimethylsilyl (TMS) or *O*-methyl-oxime-TMS derivatization has been developed to quantify neutral sugars in soils by GC (Amelung et al., 1996; Larré-Larrouy and Feller, 1997). Indeed, TMS derivatization is much simpler, but it forms multiple peaks for each sugar, which made the GC separation more difficult and would increase the deviation of the determination (Andrews, 1989). In addition, TMS derivatives are unstable during storage (Larré-Larrouy and Feller, 1997); hence, the quantitative analysis is uncertain if the prepared samples are not determined within short time. In this context, still there a need to find a derivatization procedure for more convenient and reliable determination of soil neutral sugars.

Aldononitrile acetate derivatives have been tested to quantify neutral and amino sugars in biological samples (Guerrant and Moss, 1984) and amino sugars in soil samples by GC technique (Zhang and Amelung, 1996). Because the chemical structure of amino sugar and neutral sugar molecules involved in derivatization (i.e. –CHO and –OH) are similar, it is promising to extend the aldononitrile acetate derivatization to soil neutral sugars. According to Zhang and Amelung (1996), the aldononitrile acetate derivatization is sensitive and less time consuming than that of the alditol acetate derivatization. In addition, each neutral sugar produces single chromatographic peak. Therefore, it is worth evaluating this technique for the determination of soil neutral sugars.

Traditionally, soil neutral sugars are released by H_2SO_4 (Cheshire, 1979), but it has been reported that TFA is more efficient than H_2SO_4 in releasing non-cellulosic saccharides from soils (Amelung et al., 1996). However, it is still not clear which hydrolytic procedure is better suited to the aldononitrile acetate derivatization and which purification procedure can be employed. Our objectives were therefore to evaluate the response and precision of aldononitrile acetate derivatization for GC determination of soil neutral sugars and then to find out a suitable hydrolysis and purification procedure for the derivatization.

2. Soils and methods

Four soil samples with different organic carbon (OC) contents were used for neutral sugar analysis and the soils were classified according to US soil taxonomy (Soil Survey Staff, 2003) (Table 1). The Hapludults sample was collected from the Jiangxi Red Soil Research Station, the Hapludolls from Gongzhuling, the Endoaquepts from Hailun, and the Hapludalfs from Shenyang, China. All soils were sampled from the surface layer (0–20 cm), air-dried and ground to pass 250 μm sieve prior to hydrolysis.

Table 1
Some properties of soils (0–20 cm)

Samples	Organic C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	pH	DTPA-Fe ($mg\,kg^{-1}$)
Endoaquepts	34.00	2.49	6.1	86.65
Hapludolls	12.51	1.52	6.1	48.38
Hapludalfs	9.55	0.86	6.7	67.57
Hapludults	7.68	1.07	5.5	166.91

The weighted soil samples (containing about 4 mg organic C) were hydrolyzed in two ways:

(1) 12 M H_2SO_4 (1 ml) in a closed hydrolysis flask at ambient temperature for 16 h followed by treatment with 1 M H_2SO_4 at 100 °C for 6 h (Hu et al., 1995).
(2) 4 M TFA at 105 °C for 4 h (Guggenberger et al., 1994; Amelung et al., 1996).

After hydrolysis, 100 μl of internal standard (adonitol of $1\,mg\,ml^{-1}$) was added to the hydrolysate, and then the mixture was filtered through a glass fiber filters (GF 6, Schleicher and schuell, Germany). The soil neutral sugars liberated by H_2SO_4 were purified according to the procedure of Cheshire (1979) with slight modification. The key step was that the filtrate was neutralized with $Ba(OH)_2$ to pH 6.6–6.8. For TFA hydrolysis, the slightly modified purification procedure of Zhang and Amelung (1996) was applied. Briefly, the filtrate was dried completely by a rotary evaporator at 45 °C under vacuum. Thereafter, the residue was dissolved with 20 ml of distilled H_2O and the pH was adjusted to 6.6–6.8 with 0.4 M KOH. The precipitates produced during purification either from H_2SO_4 or TFA hydrolysate were removed by centrifugation (3000 rpm for 10 min) and the clear supernatant was dried again by a rotary evaporator at 45 °C. The residue was dissolved with 4 ml distilled H_2O, transferred to a Reacti-Vial™ of 5 ml, and then freeze-dried completely under vacuum. Neutral sugars in the four soil samples after TFA hydrolysis were also determined as TMS derivatives by the method of Amelung et al. (1996), but the detail procedures are not described here because the TMS results were used for comparison only.

Derivatization was conducted according to the procedure of Zhang and Amelung (1996). The second internal standard (myo-inositol) was added before derivatization and the area ratio of myo-inositol to adonitol represented the average recovery of the purification and derivatization. The derivation reagent (0.3 ml), which contained 32 mg hydroxylamine hydrochloride ml^{-1} and 40 mg 4-(dimethylamino) pyridine (DMAP) ml^{-1} in pyridine–methanol (4:1, v/v), was added to the Reacti-Vial™ with a dry sample or a mixture of standards in it. The capped vial was shaken and heated for 30 min at 75–80 °C (during heating, the vial was shaken once more). Then, the vial was cooled to room