JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9931–9935 9931
DOI:10.1021/jf102053h

Fast and Simple Droplet Sampling of Sap from Plant Tissues and Capillary Microextraction of Soluble Saccharides for Picogram-Scale Quantitative Determination with GC-MS

Yupeng Tan,[†] Ke Li,[†,‡] Lei Hu,[§] Shu Chen,[†] Ying Gai,*[,†,‡] and Xiangning Jiang*[,†,‡]

[†]College of Biological Sciences and Biotechnology, Beijing Forestry University, No. 35, Qinghua Donglu, Beijing 100083, People's Republic of China, [‡]The Tree and Ornamental Plant Breeding and Biotechnology Laboratory of State Forestry Administration, Beijing 100083, People's Republic of China, and [§]School of Life Sciences, Tsinghua University, No. 19, Qinghua Donglu, Beijing 100084, People's Republic of China

Soluble saccharides are very important metabolites of the life cycle and synthesis of structural polysaccharide components (cellulose, hemicellulose, pectin, etc.) of cell walls. A new method for droplet sampling of saps from tissues of organisms and manipulation routines in capillaries for extraction, derivation, and partitioning were developed for picogram-scale quantitative determination with gas chromatography−mass spectrometry (GC-MS). Five to ten microliters of sap was sampled with a glass capillary containing ribitol (internal standard). Subsequently, the analytes were acetylated with acetic anhydride and catalyzed by 1-methylimidazole. Finally, the soluble saccharides were qualitatively detected with GC-MS SIM (selective ion monitoring) mode. The linear ranges of the method were up to 1×10^{-6} mol/L and the theoretically lowest limits of detection (LOD, s/n $\geq$ 3) were up to 1×10^{-9} mol/L. The method is suitable and applicable to analysis of soluble monosaccharides in fresh tissues and other aqueous samples in wide fields of agriculture, food science, biological sciences, and even medical studies.

KEYWORDS: Droplet sampling; sap; microextraction; soluble saccharides; gas chromatography and mass spectrometry

INTRODUCTION

Soluble saccharides are the primary products of the photosynthesis in plants. Meanwhile, as the major energy source, soluble saccharides play important roles in the life cycle and material metabolism (*1*). Also, the contents of soluble saccharides have close correlations with drought tolerance, low-temperature stress, and osmosis stresses (*2*−*5*). Concentrations of soluble sugars are associated with the growth and quality of plant (*6*, *7*), so the contribution of endogenous soluble saccharides to the resistance and metabolic activity of plants could be revealed through the research of soluble saccharides. In this research, it is essential that the component and concentration of soluble saccharides in plant tissues be revealed in advance.

For soluble saccharides analysis by traditional method, quite large amounts of samples and organic solvent are required for a single analytical procedure, which is not appreciated by most biological scientists and also not friendly to the environment. Given the unnecessary consumption of original samples and organic solvent, many scientists in the fields of food chemistry, environmental sciences, and agricultural chemistry are dedicated to research using microscale analytical techniques (*8*−*11*). To date, typical microextraction techniques including solid phase microextration (SPME), solvent microextraction (SME), and liquid−liquid microextraction (LLME) are widely used for determination of simple organic compounds in samples (*12*−*14*). It is difficult to carry out pretreatment of target compounds, especially compounds with high polarity, by the above microextraction techniques. Furthermore, special equipment is required for these microextractions, which restricts the wide application of these techniques (*15*, *16*).

To avoid the disadvangtes of traditional microextraction methods for soluble saccharides analysis, we have developed a new method. In this new method, droplet sampling, microextraction, microderivation, micropartitioning, and microanalysis were combined to determine soluble saccharides in plant tissues. Five to ten microliters of sap from plant samples and a small amount of organic solvent are required for an analytical process by this new microextraction method. To validate this new technique, we employed this technique to determine soluble saccharides in tissues of *Populus tomentosa*, *Arabidopsis thaliana*, fresh corn, cucumber, and apple fruit. The results of these analyses demonstrate that this new technique is reliable, fast, convenient, economical, and environmentally friendly.

MATERIAL AND METHODS

Reagents and Solutions. In various plant tissues many soluble saccharides are present according to the literature (*1*, *3*, *5*). However, only about 21 soluble saccharides are major compounds in tissues of a wide range of plant species. Therefore, 21 standards of these saccharides were prepared. Erythritol, 2-deoxy-D-ribose, xylose, rhamnose, fucose, arabinose, ribitol,

*Corresponding authors (phone +861062338063; fax +861062338063; e-mail jiangxn@bjfu.edu.cn and gaiying1981@yahoo.com.cn).

Published on Web 08/31/2010

xylitol, galactose, glucose, fructose, inositol, mannitol, sorbitol, trehalose, and sucrose were purchased from Sigma (USA). The remaining standards, that is, 2-deoxy-D-ribitol, rhamnitol, fucitol, arabinitol, and galactitol, were prepared by the reduction of the corresponding saccharides with $NaBH_4$. $NaBH_4$, methanol, 1-methylimidazole, acetic anhydride, and ethyl acetate were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO).

Apparatus and Main Fragment Ion *m/z* of Each Standard Saccharide. A GC-MS system (Trace-GC Ultra) connected to a Trace-DSQ mass selective detector with EI ionization of full scan and selected ion monitoring (selected ion ± 1.0 mass unit) (Thermo-Finnigan, San Jose, CA) were used in the analysis of acetyl-derivatized analytes. The column used was a 30 m × 0.25 mm i.d., 0.25 μm film thickness, DB-17MS fused silica capillary column (Agilent, USA). The GC oven temperature was programmed as follows: initiation at 100 °C, then gradually ramped to 190 °C (12 °C/min) and held for 6 min, subsequently ramped to 250 °C (30 °C/min) and held for 6 min, finally to ramped 280 °C (40 °C/min) and held for 10 min. The temperature at the injector port was 250 °C and at the detector port, 260 °C. Helium was used as the carrier gas at a constant flow mode at the rate of 1.0 mL/min. Xcalibur 2.0 workstation was used for the data acquisition and quantification.

Authentic standards of 21 soluble saccharides were used to determine the retention times of each component. SIM mode was used to detect target compounds. Main fragment ion *m/z* of each strandard was as follows: erythritol, *m/z* 145, 115, 103; 2-deoxyribose, *m/z* 81, 98, 141; 2-deoxyribitol, *m/z* 159, 103, 117; xylose, *m/z* 128, 170, 157; rhamnose, *m/z* 115, 157, 142; fucose, *m/z* 115, 157, 142; arabinose, *m/z* 128, 170, 115; rhamnitol, *m/z* 128, 170, 115; ribitol, *m/z* 115, 145, 103; fucitol, *m/z* 128, 170, 115; arabinitol, *m/z* 115, 145, 103; xylitol, *m/z* 115, 145, 103; galactose, *m/z* 115, 157, 98; glucose, *m/z* 115, 157, 98; fructose, *m/z* 187, 128, 101; inositol, *m/z* 168, 115, 126; mannitol, *m/z* 115, 145, 139; sorbitol, *m/z* 115, 145, 128; galactitol, *m/z* 115, 187, 127; trehalose, *m/z* 169, 109; sucrose, *m/z* 169, 109, 211.

Droplet Sampling of Sap and Microextraction of Soluble Saccharides from Plant Tissues. A 100 μL glass capillary was used for the manipulation of microextraction and micropartitioning. A droplet of 5 μL of sap from fresh plant tissues was sampled with a 5−20 μL medical capillary. Then the sap was transferred to a 100 μL glass capillary containing internal standard (ribitol) and extracted with the extraction solution (80% methanol). Acetic anhydride was used as acetylation derivation reagent and 1-methylimidazole as the catalyst. Derivation was maintained for 5 min in the glass capillary. Double-distilled H_2O in 2−3-fold volumes was added to the glass capillary to remove the unreacted acetic anhydride. Subsequently, a drop of ethyl acetate was added to the glass capillary to extract the acetylated analytes. Afterward, the glass capillary was centrifuged for 1 min to partition the organic phase. One microliter of supernatant was injected into the GC-MS for analysis.

Derivatization of Authentic Standard Soluble Saccharides. Ten microliters of authentic standard soluble saccharides solution was reacted with 10 μL of derivatization reagent (acetic anhydride and 1-methylimidazole) for 5 min. Then 2−3-fold volumes of double-distilled H_2O with water-soluble components were added to the reaction system. A drop (about 10 μL) of ethyl acetate was used as the extracting solvent, equilibrated between the aqueous and organic phases for 1 min, and then centrifuged for partitioning. Finally, 1 μL of supernatant was injected into the GC-MS.

Calibration Curves, Lowest Limits of Detection (LOD), and Recoveries. With the procedures described above, acetylated authentic standards of soluble saccharides were obtained. Then the stock solution was diluted to a set of seven levels of standard solution of soluble saccharides: 0.5, 1, 2.5, 6.25, 12.5, 25, and 50 ng/μL. One microliter of the prepared acetylated standard solutions was injected into the GC-MS. Three duplicates were made for each contrentration.

To determine the lowest LOD of soluble saccharides, the following concentrations of acetylated standard solutions of soluble saccharides were prepared: 10, 50, 100, 250, and 500 pg/μL.

RESULTS AND DISCUSSION

Determination of Authentic Standards of Soluble Saccharides with GC-MS. All 21 authentic standard soluble saccharides were completely separated on a DB-17 MS capillary column and

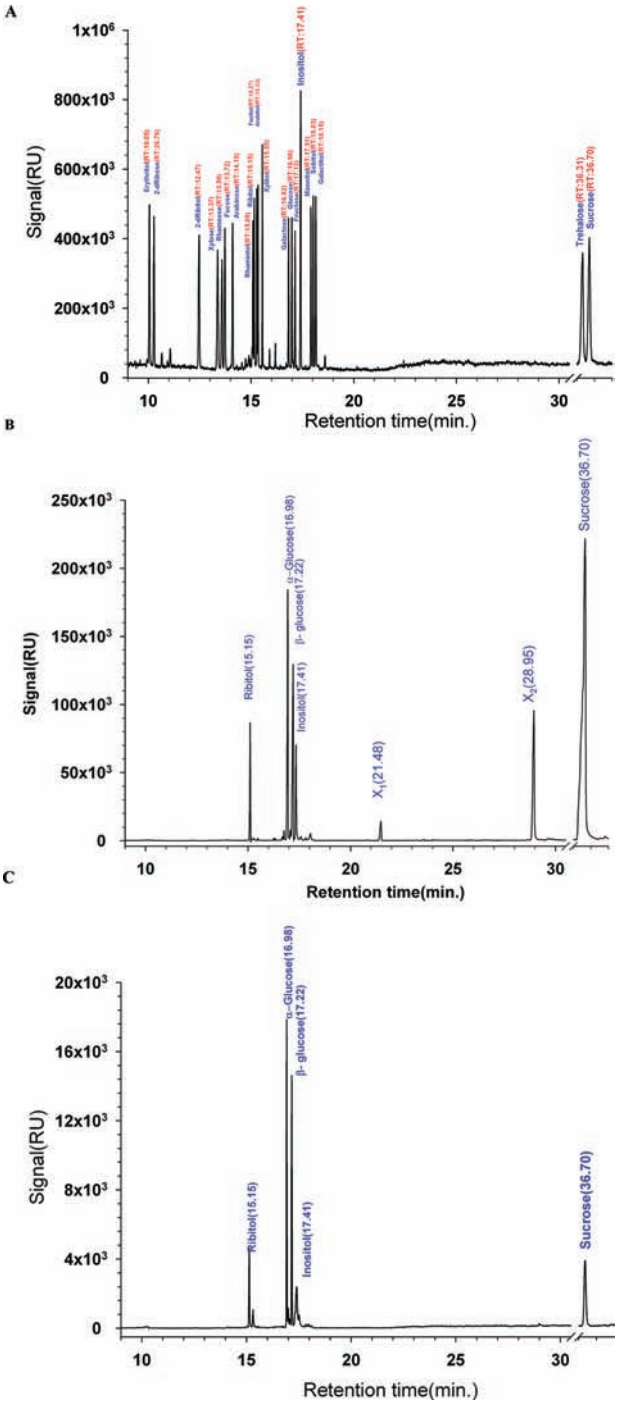

Figure 1. Chromatograms of total ion current (TIC) of acetylated soluble saccharides of standard (**A**), samples of *Populus tomentosa* (**B**), and apple fruit (**C**). (**A**) Soluble saccharides standards [1, erythritol (RT 10.05); 2, 2-deoxy-D-ribose (RT 10.26); 3, 2-deoxy-D-ribitol (RT 12.47); 4, xylose (RT 13.37); 5, rhamnose (RT 13.58); 6, fucose (RT 13.72); 7, arabinose (RT 14.10); 8, rhamnitol (RT 15.09); 9, ribitol (RT 15.15); 10, fucitol (RT 15.27); 11, arabinitol (RT 15.33); 12, xylitol (RT 15.55); 13, galactose (RT 16.82); 14, glucose (RT 16.98); 15, fructose (RT 17.13); 16, inositol (RT 17.41); 17, mannitol (RT 17.91); 18, sorbitol (RT 18.03); 19, galactitol (RT 18.15); 20, trehalose (RT 36.31); 21, sucrose (RT 36.70)]. (**B**) Soluble saccharides from *Populus tomentosa* [1, ribitol (RT 15.15); 2, α-glucose (RT 16.98); 3, β-glucose (RT 17.22); 4, inositol (RT 17.41); 5, unknown 1 (tentative cellobioseocta-acetate, RT 21.48); 6, unknown 2 (tentative β-D-glucopyranoside, 4-nitrophenyl-2,3,4,6-tetraacetate, RT 28.95); 7, sucrose (RT 36.70)]. (**C**) Soluble saccharides from apple fruit [1, ribitol (RT 15.15); 2, α-glucose (RT 16.98); 3, β-glucose (RT 17.22); 4, inositol (RT 17.41); 5, sucrose (RT 36.70)].

Table 1. Detection of Standard Soluble Saccharides

no.	name	retention time (min)	resolution	response factor	R^2	recovery (%)	RSD[a] (%)	theor LOD[b] (mol/L)
1	erythritol	10.05	2.21 ≥ 1.0	695.89	0.9993	97.05	1.23	3.1×10^{-8}
2	2-deo-ribose	10.26	2.21 ≥ 1.0	256.82	0.9988	95.96	2.67	8.4×10^{-8}
3	2-deo-ribitol	12.47	46.53 ≥ 1.0	196.11	0.9975	97.17	3.12	1.1×10^{-7}
4	xylose	13.37	15.65 ≥ 1.0	862.90	0.9990	94.24	1.34	2.5×10^{-8}
5	rhamnose	13.58	4.00 ≥ 1.0	237.06	0.9993	91.11	3.45	9.1×10^{-8}
6	fucose	13.72	3.26 ≥ 1.0	2838.50	0.9992	95.49	1.78	7.6×10^{-9}
7	arabinose	14.10	9.44 ≥ 1.0	196.11	0.9978	97.78	0.97	1.1×10^{-7}
8	rhamnitol	15.09	11.36 ≥ 1.0	468.97	0.9971	94.11	1.28	4.6×10^{-8}
9	ribitol	15.15	2.45 ≥ 1.0	634.49	0.9987	99.04	3.22	3.4×10^{-8}
10	fucitol	15.27	4.90 ≥ 1.0	234.48	0.9986	96.83	3.41	9.2×10^{-8}
11	arabinitol	15.33	2.67 ≥ 1.0	371.94	0.9967	94.77	2.25	5.8×10^{-8}
12	xylitol	15.55	8.35 ≥ 1.0	513.63	0.9980	92.16	1.66	4.2×10^{-8}
13	galactose	16.82	61.95 ≥ 1.0	342.42	0.9979	96.21	2.23	6.3×10^{-8}
14	glucose	16.98	8.00 ≥ 1.0	312.65	0.9988	94.18	3.67	6.9×10^{-8}
15	fructose	17.13	2.00 ≥ 1.0	126.90	0.9978	95.96	2.69	1.7×10^{-7}
16	inositol	17.41	10.77 ≥ 1.0	3852.25	0.9984	96.16	4.28	5.6×10^{-9}
17	mannitol	17.91	20.41 ≥ 1.0	3922.29	0.9990	96.60	1.43	5.5×10^{-9}
18	sorbitol	18.03	4.90 ≥ 1.0	1797.72	0.9985	95.51	2.19	1.2×10^{-8}
19	galactitol	18.15	5.71 ≥ 1.0	1135.40	0.9964	96.89	1.45	1.9×10^{-8}
20	trehalose	36.31	109.25 ≥ 1.0	12.70	0.9988	98.30	3.48	1.7×10^{-6}
21	sucrose	36.70	3.90 ≥ 1.0	12.70	0.9982	103.25	4.84	1.7×10^{-6}

[a] Relative standard deviation. [b] Limit of detection.

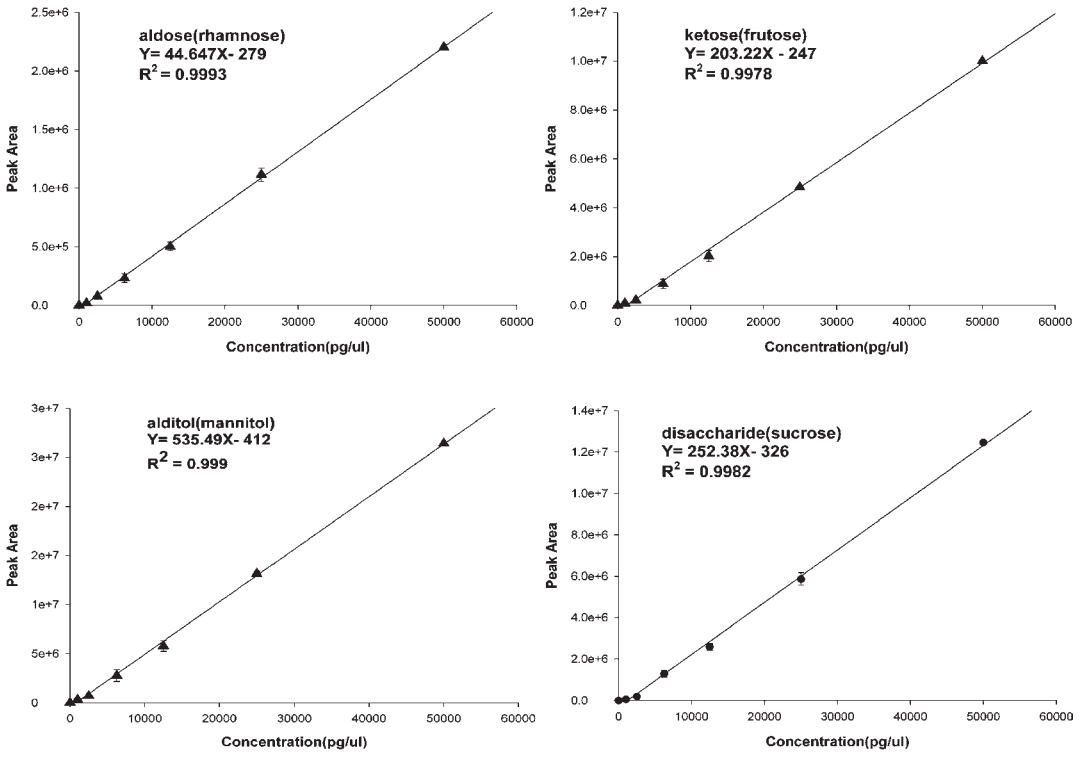

Figure 2. Standard calibration curves of aldose (rhamnitol), ketose (fructose), alditol (mannitol) and disaccharide (sucrose).

successfully detected under the setting conditions (**Figure 1A** and **Table 1**). Qualitative analysis was based on the retention time (RT) and mass spectrometry of the acetylated products.

Quantitative Analysis and Calibration Curve of Standard Soluble Saccharides. The curves of rhamnose, fructose, mannitol, and sucrose, which represent aldose, ketose, alditol, and disaccharide, respectively, are shown in **Figure 2**. Each of the 21 soluble saccharides has its own standard calibration curve. For each standard, the data show a good linearity in the experimental ranges of 0.05−50 mg/L. The correlation coefficients for all of the soluble saccharides were between 0.9978 and 0.9993 ($n = 3$).

Theoretical Lowest LOD of Authentic Standards of 21 Soluble Saccharides. The theoretical lowest LODs, defined as 3 times the signal/noise ratio of the baseline (s/n ≥ 3 signal-to-noise ratio) are presented in **Table 1**. The results indicate that the lowest LODs of these soluble saccharides are up to 10^{-9} mol/L. However, the lowest LODs of soluble disaccharides are up to 10^{-7} mol/L.

The repeatabilities were determined by the analysis of three replicates. A recovery test was carried out by spiking three levels of standard soluble saccharides mixture to the samples. The obtained results were compared with the known amounts of the standard soluble saccharides. The recoveries (%) and the relative

Table 2. Soluble Saccharides Contents in Plant Tissues Comparatively Analyzed by Droplet Sampling Method and Traditional Method

			species (contents (mg/100 g of fw, $n = 3$)									
			Arabidopsis thaliana		fresh corn		fruit (apple)		vegetable (cucumber)		poplar	
no.	name	RT (min)	droplet	traditional	droplet	traditional	droplet	traditional	droplet	traditional	droplet	traditional
1	erythritol	10.05	nd[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	2-deoxyribose	10.26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	2-deoxyribitol	12.47	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	xylose	13.37	0.83 ± 0.03	0.91 ± 0.02	nd	nd	nd	nd	nd	nd	0.05 ± 0.01	0.065 ± 0.01
5	rhamnose	13.58	0.39 ± 0.09	0.82 ± 0.04	0.08 ± 0.04	0.04 ± 0.01	2.02 ± 0.04	1.96 ± 0.06	0.42 ± 0.05	0.31 ± 0.02	0.11 ± 0.03	0.095 ± 0.02
6	fucose	13.72	1.01 ± 0.03	0.43 ± 0.01	nd	nd	0.04 ± 0.02	nd	0.02 ± 0.01	nd	0.19 ± 0.08	0.11 ± 0.02
7	arabinose	14.10	2.00 ± 0.06	1.79 ± 0.10	0.14 ± 0.02	0.11 ± 0.01	15.22 ± 0.61	12.90 ± 0.12	0.70 ± 0.09	0.63 ± 0.05	0.42 ± 0.1	0.24 ± 0.08
8	rhamnitol	15.09	6.02 ± 0.20	5.55 ± 0.21	0.19 ± 0.02	0.13 ± 0.01	nd	nd	0.09 ± 0.03	0.04 ± 0.01	0.39 ± 0.12	0.12 ± 0.01
9	ribitol (IS[b])	15.15										
10	fucitol	15.27	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11	arabitol	15.33	19.20 ± 0.62	20.30 ± 0.33	0.43 ± 0.08	0.24 ± 0.04	6.03 ± 0.58	5.26 ± 0.06	1.03 ± 0.06	0.66 ± 0.04	1.23 ± 0.18	0.93 ± 0.06
12	xylitol	15.55	0.37 ± 0.04	0.58 ± 0.03	0.28 ± 0.03	0.16 ± 0.06	5.99 ± 0.05	6.77 ± 0.03	0.48 ± 0.03	0.36 ± 0.03	0.98 ± 0.13	0.52 ± 0.09
13	galactose	16.82	4.01 ± 0.52	3.80 ± 0.31	0.06 ± 0.04	0.08 ± 0.03	2.40 ± 0.02	2.31 ± 0.02	0.46 ± 0.04	0.35 ± 0.02	2.46 ± 0.4	2.61 ± 0.33
14	α-glucose	16.98	39.31 ± 1.11	34.38 ± 1.52	12.25 ± 0.18	11.01 ± 0.22	561.43 ± 2.23	544.58 ± 2.35	71.47 ± 0.91	77.49 ± 1.03	61.47 ± 4.8	65.89 ± 3.31
15	fructose	17.13	8.21 ± 0.21	8.98 ± 0.31	0.95 ± 0.05	0.80 ± 0.02	6.35 ± 0.25	5.51 ± 0.05	0.35 ± 0.05	0.56 ± 0.07	3.35 ± 0.5	3.88 ± 0.45
16	inositol	17.41	20.04 ± 1.20	18.90 ± 0.93	0.83 ± 0.22	0.66 ± 0.13	36.63 ± 0.22	39.71 ± 0.37	6.23 ± 0.92	5.44 ± 1.02	31.3 ± 3.72	29.85 ± 3.45
17	mannitol	17.91	1.27 ± 0.05	1.42 ± 0.20	0.12 ± 0.04	0.02 ± 0.01	4.15 ± 0.04	3.32 ± 0.08	0.12 ± 0.04	0.15 ± 0.02	2.12 ± 0.34	1.25 ± 0.2
18	sorbitol	18.03	3.02 ± 0.36	2.47 ± 0.61	4.79 ± 0.09	4.11 ± 0.03	121.63 ± 2.04	144.94 ± 3.14	0.39 ± 0.09	0.25 ± 0.01	1.99 ± 0.09	1.19 ± 0.11
19	galactitol	18.15	0.99 ± 0.03	0.83 ± 0.02	1.21 ± 0.07	0.90 ± 0.02	1.35 ± 0.25	1.39 ± 0.15	0.55 ± 0.07	0.76 ± 0.06	1.55 ± 0.27	1.16 ± 0.13
20	trehalose	36.31	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
21	sucrose	36.70	50.26 ± 1.96	53.85 ± 2.02	437.6 ± 2.53	423.59 ± 2.09	12.26 ± 0.05	14.58 ± 0.06	17.6 ± 1.02	12.71 ± 1.11	167.6 ± 5.5	155.17 ± 2.31
22	β-glucose	17.22	39.47 ± 0.99	34.42 ± 1.25	8.09 ± 1.15	6.69 ± 0.18	308.10 ± 0.65	319.09 ± 1.35	38.29 ± 1.25	42.05 ± 1.02	38.29 ± 1.25	42.74 ± 1.52
23	cellobioseoctaacetate	21.48	nd	nd	nd	nd	nd	nd	nd	nd	7.12 ± 0.09	7.83 ± 0.15
24	β-D-glucopyranoside, 4-nitrophenyl, 2,3,4,6-tetraacetate	28.95	nd	nd	nd	nd	nd	nd	nd	nd	36.13 ± 1.11	35.37 ± 1.03
	total saccharides		16	16	14	14	14	13	15	14	18	18
	total amount		196.39 ± 1.96	189.43 ± 2.02	467.02 ± 2.53	448.54 ± 2.09	1082.6 ± 2.23	1097.32 ± 3.14	138.2 ± 1.25	141.76 ± 1.11	356.75 ± 5.5	349.02 ± 3.45

[a] Not detected. [b] Internal standard.

standard deviations (RSDs) are listed in **Table 1**. The recoveries of all measurements are >90%, and the RSDs are <5%. These data indicate that the new method has an excellent feasibility in the analysis of plant samples.

Application in Plant Tissues. The newly developed method was tested in the analysis of soluble saccharides in the leaves of various plants (*P. tomentosa* (Chinese white poplar) and *A. thaliana*), fresh corn, cucumber, and apple. The analysis of soluble saccharides in foliar tissues was performed by using the analytical procedures described as above.

The results showed that the concentrations and components of soluble saccharides in the same plant tissue determined by the microscale method, compared to the traditional extraction method, were almost in the same reliable ranges (**Figure 1B**,**C** and **Table 2**). They also showed that more soluble saccharides could be detected in the developed method compared to those detected by the traditional method. Two unknown components not present in the standards were found in samples of poplar leaves at the retention times of 21.48 and 28.95 min, but these did not appear in apple. The differences were reflected in sucrose, glucose, inositol, fructose, and sorbitol among the different plant tissues (**Table 2**).

The microscale analytical method developed in this study included droplet sampling, microextraction, microderivation, micropartitioning, and microanalysis procedures. All of the pretreatment procedures were completed in a 100 μL glass capillary. No more than 100 μL of organic reagent was consumed for the complete analytical procedure. The amount of plant material used in this analytical process was very small. One drop (ca. 5−10 μL) of sap or milligrams of fresh or dry weight samples were enough for the new method. In contrast, 0.2−1.0 g of fresh or dry weight plant samples was required by the traditional method, which limits its application in tiny tissues in the experiments. In the traditional method, more than 1 mL of organic solvent and relevant reagent is consumed for the pretreatment of the sample, which increases the cost of the research and, much worse, causes more pollution to the environment. Therefore, the microscale analytical technique described here is fast, convenient, economical, and environmentally friendly. Besides fresh plant samples, the microanalytical method is suitable to most other biological samples containing soluble saccharides. This new method can be used in many study fields, such as food science, chemistry, environmental science, agricultural science, biology, and even medical diagnostic tests.

Compared to the reported microanalytical techniques, the present technique also has obvious advantages. It is economical and easy to widely apply because no special equipment or apparatus is required. The container and reactor for sampling, prepurification, derivation, and partitioning are only glass capillaries, which are widely used for melting point tests in chemistry laboratories, and the auxiliary equipment are microscale syringes. They can be found in or affordably purchased by most laboratories. The concominant, but not prerequisite, equipment for the microtechnique is an automatic injector because of its high efficiency and high throughout.

The sensitivity of the method was increased by SIM detection. Therefore, it is beneficial in the precise determination of target compounds at trace amount. In common cases, a GC assistant with a flame ionization detector would be enough to finish the determination, but the resolutions of the components would be disturbed by components that were not of interested. Due to the complexity of the components in plant tissues, it is difficult to separate the derivatized products completely by a chromatographic column. In that case, a detector with high selectivity is necessary.

ACKNOWLEDGMENT

Ke Li and Yupeng Tan contributed equally to this work.

LITERATURE CITED

(1) Ivan, C.; Cecile, S.; Gwenola, S.; Abdelhak, E. A. Involvement of souble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J. Exp. Bot.* **2006**, *57*, 449–461.

(2) Chabrillange, N.; Dussert, S.; Engelmann, F.; Doulbeau, S.; Hamon, S. Desiccation tolerance in relation to soluble sugar contents in seeds of ten coffee (*Coffea* L.) species. *Seed Sci. Res.* **2000**, *10*, 393–396.

(3) Kasuga, J.; Arakawa, K.; Fujikawa, S. High accumulation of soluble sugars in deep supercooling Japanese white birch xylem parenchyma cells. *New Phytol.* **2007**, *174*, 569–579.

(4) Bacchus, S. T.; Hamazaki, T.; Britton, K. O.; Haines, B. L. Soluble sugar composition of pond-cypress: a potential hydroecological indicator of ground water perturbations. *J. Am. Water Resour. Assoc.* **2000**, *36*, 55–65.

(5) Mohammadkhani, N.; Heidari, H. Drought-induced accumulation of soluble sugars and praline in two maize varieties. *World Appl. Sci. J.* **2008**, *3*, 448–453.

(6) Spencer, D. F.; Ryan, F. J.; Aung, L.; Ksander, G. G. Soluble sugar concentrations associated with tuber and winter bud sprouting. *J. Aquat. Plant Manage.* **2001**, *39*, 45–47.

(7) Ali, N. A.; Abdelatif, A. H. Chemical measurement of total soluble sugars as a parameter for cotton lint stickiness grading. *Actes Semin.* **2001**, 112–117.

(8) Altaki, M. S.; Santos, F. J.; Galceran, M. T. Analysis of furan in foods by headspace solid-phase microextraction−gas chromatography−ion trap mass spectrometry. *J. Chromatogr., A* **2007**, *1146*, 103–109.

(9) Paschke, A.; Vrana, B.; Popp, P.; Schüürmann, G. Comparative application of solid-phase microextraction fibre assemblies and semipermeable membrane devices as passive air samplers for semivolatile chlorinated organic compounds. *Environ. Pollut.* **2006**, *144*, 414–422.

(10) Zhao, E. C. Application of a single-drop microextraction for the analysis of organophosphorus pesticides in juice. *J. Chromatogr., A* **2006**, *1114*, 269–273.

(11) Harizanis, P. C.; Alissandrakis, E.; Tarantilis, P. A.; Polissiou, M. Solid-phase microextraction/gas-chromatographic/mass spectrometric analysis of *p*-dichlorobenzene and naphthalene in honey. *Food Addit. Contam.: Part A* **2008**, *25*, 1272–1277.

(12) Desideri, P. G.; Lepri, L.; Heimler, D.; Giannessi, S.; Checchini, L. Concentration, separation and determination of hydrocarbons in sea water. *J. Chromatogr., A* **1984**, *284*, 167–168.

(13) Zalieckeckaite, R.; Adomaviciute, E.; Vickackaite, V. Single-drop microextraction for the determination of phthalate esters. *Chemija* **2007**, *18*, 25–29.

(14) Aubert, C.; Baumann, S.; Arguel, H. Optimization of the analysis of flavor volatile compounds by liquid-liquid microextraction (LLME). Application to the aroma analysis of melons, peaches, grapes, strawberries, and tomatoes. *J. Agric. Food Chem.* **2005**, *53*, 8881–8895.

(15) Kumemura, M.; Yamauchi, T.; Hara, C.; Helaleh, M. I. H.; Korenaga, T. Samping and highly sensitive measurement of formaldehyde based on a droplet method. *Anal. Sci.* **2001**, 17.

(16) Fiamegos, Y. C.; Nanos, C. G.; Stalikas, C. D. Ultrasonic-assisted derivatization reaction of amino acids prior to their determination in urine by using single-drop microextraction in conjunction with gas chromatography. *J. Chromatogr., B* **2004**, *813*, 89–94.

Received for review June 8, 2010. Revised manuscript received August 4, 2010. Accepted August 6, 2010. This research was financially supported by the National Natural Science Foundation (NSF) Project to X.J. (NSF Grant 30630053) and a grant from the China Postdoctoral Science Foundation (20090450015) to Y.G. The work was carried out in The Tree and Ornamental Plant Breeding and Biotechnology Laboratory of State Forestry Administration and National Engineering Laboratory of Tree Breedings, Beijing, People's Republic of China.