Sugars in environmental samples and sedimentary rocks – effectiveness in derivatization for GC-MS analysis

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Saccharides (sugars), common constituents of the bio- and geosphere, are useful in determining the source of organic matter (OM) in aerosols and early diagenetic phases. Due to the chemical properties and great diversity of sugars in nature, a wide range of techniques is used for the isolation and quantification of saccharides. Here, gas chromatography-mass spectrometry analysis, preceded by derivatization with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), was undertaken. The effectiveness of this derivatization was demonstrated for soils, aerosols, fungi, and individual sugar standards. The efficacy of BSTFA derivatization for individual sugar standards and, similarly, for samples of sugars in winter and summer aerosols was adequate. In fungi and soil samples, the MSTFA reagent was much more effective. Different mono- and disaccharides were identified in biological samples and sedimentary rocks, showing not only the great diversity of these compounds but also the specificity of their origin in recent and ancient OM. Greater variation in the occurrence of sugars in soils compared to rocks may suggest differences in the stability of some saccharides at an early stage of diagenetic transformation. Comparison of the occurrence of certain sugars in biological samples and sedimentary rocks sheds new light on the origin and preservation of these biomolecules.

Key words: BSTFA, MSTFA, saccharides, particulate matter, fungi, lignites.

INTRODUCTION

Saccharides (sugars) represent the primary form of photosynthetically assimilated carbon in the biosphere and can serve as a tool for clarifying the sources and processes of organic materials in natural environments (Cowie and Hedges, 1984). Mono- and disaccharides are common biomolecules in plants and animals, occurring widely both in marine and terrestrial environments (e.g., BeMiller, 1989; Moers et al., 1989; Jia et al., 2008). Sugars are the main constituents of soils (e.g., Gleixner et al., 2002) formed in various climate conditions, i.e. tropical (Simoneit et al., 2004) or temperate (Marynowski et al., 2020); however, their origin and role in pedogenic processes are usually diverse and complex (Marynowski et al., 2020). Moreover, they are used as molecular tracers in atmospheric aerosols (Simoneit et al., 2004). Anhydrosugars (e.g., levoglucosan, mannosan, and galactosan) are indicators of burning of biomass and lignite (Fabbri et al., 2009); sugar alcohols (arabitol and mannitol) are fungal spore tracers; mono- and disaccharides (fructose, glucose and sucrose) are indicators of pollen grains (Simoneit et al., 2004; Bauer et al., 2008). Given favourable depositional and diagenetic conditions, saccharides may be preserved in Cenozoic and even Mesozoic rocks (Marynowski et al., 2018, 2019; Rybicki et al., 2020). Sugars are typical constituents of various types of lichens and fungi (e.g., Bidochka et al., 1990; Nehls et al., 2010), and their occurrence in geological samples may indicate a plant-based or fungal origin for organic matter (Marynowski et al., 2018, 2019).

The difficulty in evaluating and comparing sugars is due to the variety of techniques used for their isolation and quantification (cf. methods in Simoneit et al., 2004). The chemical properties of saccharides such as high polarity, hydrophilicity, and low volatility necessitate their conversion into volatilisable and stable derivatives prior to gas chromatography-mass spectrometry (GC-MS) analyses (Ruiz-Matute et al., 2011). Derivatization is a chemical process that modifies primary compounds and generates new products with superior chromatographic properties (Schummer et al., 2009). Briefly, the mechanism of the most common derivatization method, i.e. silylation, is based on the introduction of a silyl group in place of a labile hydrogen atom. This group can be readily removed with the reintroduction of active hydrogen (e.g., *via* hydrolysis). Schummer et al. (2009) enumerated the reagents most commonly used in silylation: trimethylchlorosilane (TMCS), trimethylsilylimidazole (TMSI), N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), N-methyl-N-trimethylsilyl-tri-

fluoroacetamide (MSTFA), and N,O-bis(trimethylsilyl)trifluoro-acetamide (BSTFA); for additional reagents, see Poole (2013).

In this study, we show the diversity of saccharides in the atmosphere, biosphere, pedosphere, and lithosphere, as well as the effectiveness of BSTFA and MSTFA in their silylation. Both reagents are known for successful derivatization in environmental and geological studies (Simoneit et al., 2004; Burshtein et al., 2010; Rushdi et al., 2016; Marynowski et al., 2019, 2020), but there is still little information on the performance of these reagents when derivatizing sugar-rich samples in biological and early diagenetic settings such as fungi or soils. In this work, we test which reagent is most appropriate for derivatization of samples with a rich and diverse saccharide distribution. Comparing the occurrence of certain sugars in biological material and sedimentary rocks sheds new light on the origin and preservation of these compounds.

MATERIALS AND METHODS

MATERIALS

Five types of sample were investigated:
1 – standards of individual sugars (Table 1), purchased from Sigma-Aldrich, AMRESCO, Acros Organics, Fluka, Alfa Aesar, and Merc;
2 – examples of soil: arenosol from the Błędowska Desert (S Poland) and soil developed on gypsum from Busko-Zdrój (S Poland);

Table 1

The percentage number of fully derivatized compounds of internal sugar standards (BSTFA *versus* MSTFA) after silylation

Internal standard	BSTFA	MSTFA
	[% fully derivatized]	
Anhydrosaccharides		
Levoglucosan	100	100
Galactosan	89	95
Sugar alcohols		
Threitol	98	99
Arabitol	100	100
Adonitol	98	100
Pinitol	100	100
Mannitol	95	100
Sorbitol	91	94
myo-inositol	100	100
Monosaccharides		
α- + β-arabinose	87	91
α- + β-xylose	92	98
α- + β-fructose	80	97
Mannose	93	96
Galactose	81	91
α-glucose	92	98
Disaccharides		
Sucrose	100	100
Trehalose (mycose)	100	100

3 – two PM10 samples collected during winter (January) and summer (August) in Sosnowiec (S Poland);
4 – wood-decay fungi (*Lenzites betulina, Inonotus obliquus, Fomitiporia robusta,* and *Phellinus tremulae)*;
5 – sedimentary rocks: two fossil wood samples (Doubrava sandpit, Czech Republic, Pleistocene; Jaroszów mine, SW Poland, Miocene), a detrital lignite and an overlying clay (Jaroszów mine, Miocene).

METHODS

EXTRACTION AND DERIVATIZATION

Powdered samples ~15 g (arenosols, fungi, sedimentary rocks) or quartz fibre filters – *Whatman QMA*, 47 mm (PM10 samples) were extracted using a dichloromethane:methanol mixture (1:1 v:v) with an ultrasonic bath (30 minutes at 30°C). The extracted aliquots were filtered using *Munksjö* filters (grade 388, *Ahlstrom-Munksjö*) for the removal of insoluble particles. The excess reagent was removed under blowdown with dry nitrogen, then the sample mixture was dissolved in an equivalent volume of *n*-hexane. Individual standard solutions were prepared by dissolving 10 mg of each standard in 25 cm^3 of methanol and analysing 100 μL of the solution, which was evaporated, derivatized, and dissolved in *n*-hexane to 1.5 mL. We have used 100 μL of both BSTFA and MSTFA to check their effectiveness of derivatization.

The first series was converted to trimethylsilyl (TMS) derivatives *via* reaction with 100 μL BSTFA and the second with 100 μL MSTFA. Both reagents contained 1% trimethylchlorosilane (TMCS). Following the addition of the silylation reagent and 10 μL pyridine, samples were heated for 3 hours at 70°C. A blank sample (silica gel) was analysed using the same method. Trace amounts of phthalates, *n*-fatty acids (FAs), and *n*-alkanols were found. No saccharides were detected in the blank samples.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Gas chromatography-mass spectrometry (GC-MS) analyses were carried out with an *Agilent Technologies 7890A* gas chromatograph and *Agilent 5975C Network* mass spectrometer with a *Triple-Axis Detecto*r (MSD) at the Faculty of Earth Sciences, Sosnowiec, Poland. Helium (6.0 Grade) was used as a carrier gas at a constant flow of 2.6 ml/min. The separation was obtained using a J&W HP-5ms (60 m × 0.32 mm i.d., 0.25 μm film thickness) fused silica capillary column coated with a chemically bonded phase (5% phenyl, 95% methylsiloxane), for which the GC oven temperature was programmed from 45 (1 min) to 100°C at 20°C/min, then to 300°C (held for 60 min) at 3°C/min, with a solvent delay of 10 min. The GC column outlet was connected directly to the ion source of the MSD. The GC-MS interface was set at 280°C, while the ion source and quadrupole analyzer were set at 230 and 150°C, respectively. Mass spectra were recorded from 45–550 (0–40 min) and 50–700 da (>40 min). The MS was operated in the electron impact mode, with an ionization energy of 70 eV.

QUANTIFICATION AND IDENTIFICATION

For data collection and mass spectra processing, *Agilent Technologies MSD ChemStation E.02.01.1177* and *Wiley Reg-*

istry of Mass Spectral Data (9th edition) software were used. To report the values of the monosaccharides, which commonly occur as two isomers (1α- and 1β-configurations of OH on the pyrano or furano ring), the two GC peaks were added together (Medeiros and Simoneit, 2007). Ethylvanillin ≥98% (Sigma-Aldrich) was used as an internal standard in biological samples.

RESULTS AND DISCUSSION

EFFECTIVENESS OF THE DERIVATIZATION OF SACCHARIDES USING BSTFA *VERSUS* MSTFA

INTERNAL STANDARDS

Based on the sugar concentrations in the biological samples, the dominant compounds were selected for verification of the effectiveness of BSTFA *versus* MSTFA derivatization (Table 1). The identification of saccharides was made based on the comparison of mass spectra and retention times with those of commercial sugar standards. The *m/z* 73, 147, 191, 204, and 217 fragments are the key ions for pentoses, hexoses, and heptoses as TMS ethers; disaccharides have an additional ion

at *m/z* 361; while the key ions for the reduced sugars are at *m/z* 205, 217, 307, and 319 (Simoneit et al., 2004). As in the case of environmental samples examined by Medeiros and Simoneit (2007), most of the monosaccharides in our samples showed two GC peaks (1α- and 1β-configurations of OH on the pyrano or furano ring, Fig. 1). The sum of the values for isomers was presented as a percentage of fully derivatized compounds (e.g., α-glucose in Table 1). To identify sugar compounds, especially monosaccharides such as TMS, two mass fragmentograms were primarily used. The *m/z* 204 fragment ion (e.g., glucopyranose as TMS) characterized the mass spectra of saccharides for pyrano ring 5C, the *m/z* 217 ion for furano ring 4C. Medeiros and Simoneit (2007) noted no isomers in their chromatograms, for either sugar alcohols or anhydrosugars; these were also absent in the case of mannitol, arabitol, and *myo*-inositol in fungi and initial soil from this study (Fig. 1). Most disaccharides, such as trehalose or sucrose, are characterized as TMS derivatives by the *m/z* 361 key ion (conjunctly with *m/z* 204). Fully derivatized compounds were identified and calculated (Table 1) based on mass spectra and retention times. The effectiveness of BSTFA and MSTFA in the derivatization of sugar standards was high, ranging from 80 to 100% and 90 to 100%, respectively.
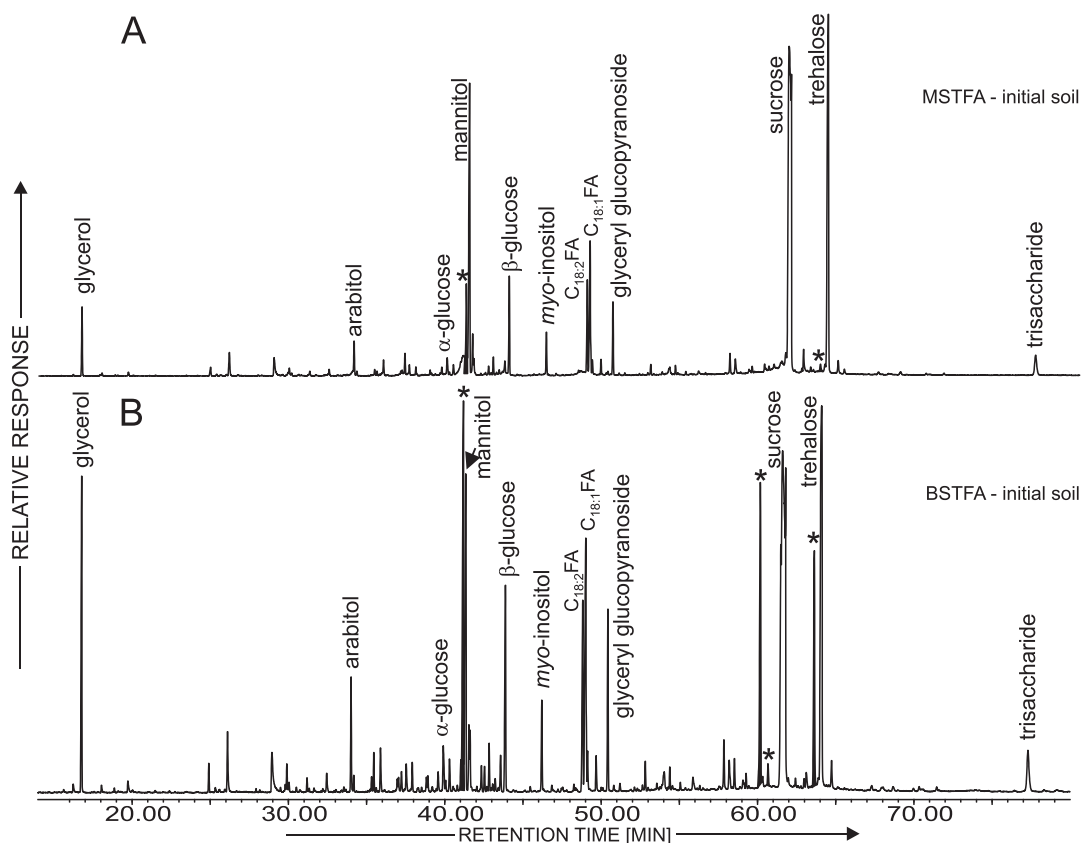

Fig. 1. Total ion current (TIC) chromatogram of arenosols (sandy soils lacking any significant development of a soil profile) from the Błędowska Desert showing the distribution of saccharides as trimethylsilyl derivatives derivatized using: MSTFA – A and BSTFA – B

Asterisks denote compounds not fully derivatized; note a higher number of compounds not fully derivatized in the case of the BSTFA reagent

ARENOSOLS

Saccharides constitute a major fraction of soil organic matter (Gleixner et al., 2002). In temperate climates, glucose, sucrose, and trehalose dominate in soils, with inositols, rhamnose, arabinose, and lyxose present in lesser amounts (Simoneit et al., 2004). The sample of initial soil from the Błędowska Desert represents the third stage of pedogenesis, at which a biological soil crust is formed by fungi, lichens, algae, bacteria and the detritus of higher plants (Marynowski et al., 2020). The dominant sugars in this arenosol were disaccharides – sucrose, trehalose, and sugar alcohol (mannitol); occurring in minor amounts were glycerol, arabitol, β-glucose, *myo-inositol*, and glyceryl glucopyranoside (Fig. 1 and Table 2). The number of non-fully-derivatized compounds was much higher for the samples derivatized with BSTFA compared to MSTFA (Fig. 1 and Table 3). More BSTFA in fact can increase derivatization effectiveness. However, in this research we have studied the usefulness of particular derivatization agents using the same amounts of BSTFA and MSTFA. Even using twice as much BSTFA than MSTFA gives higher concentrations of non-fully derivatized compounds. The generally lower effectiveness of derivatization in arenosols may have been connected with the higher concentration of saccharides in the sample; however, in this case, MSTFA was a superior silylation reagent. All of the samples were dried prior to powdering; hence differences in derivatization efficiency were not due to the susceptibility of the reagents to moisture.

AEROSOLS

In aerosols, depending on the season, the most common saccharides are anhydrosugars (with levoglucosan being dominant), sugar alcohols (arabitol and mannitol), α- and β-glucose, α- and β-fructose, α- and β-arabinose, and trehalose. Other sugars, in lesser amounts, include sucrose, *myo*-inositol, sor-

Table 2

Occurrence and distribution of saccharides in soils, lignites, clays, aerosols and wood-rot fungi

Samples	1	2	3	4	5	6	7	8	9	10	11
	soils		lignites and clays				aerosols		fungi		
Anhydrosaccharides											
Levoglucosan	0.1						100	92			
Mannosan							17	14			
Galactosan							1	4			
Sugar alcohols											
Glycerol	5	15	43	7	11	100	6	13			
Threitol	0.1										
Erythritol				1				9			
Arabitol	3	1		5	5		1	100	2	100	4
Adonitol	0.3	1								1	1
Pinitol	1				100		2	6			
Mannitol	32	10		100	64		2	77	12	42	25
Sorbitol	3	1					1	6			0.1
scyllo-inositol	0.2										0.2
myo-inositol	3							4	1		
Monosaccharides											
a- + β-arabinose	0.7	31	14			2	21	22			
α- + β-xylose			17				2				
α- + β-fructose	0.7	3	100	2	17	8	4	26			
Mannose + galactose	1.4	1	22	5		15	2		0.2		
α- + β-glucose	3	9	24	6	38	48	8	79	2	2	1
Glyceryl glucopyranoside	6								1		
Disaccharides											
Sucrose	100	93	2	4	10	32	7	5			1
Trehalose (mycose)	54	100	9	23	6	27	1	37	100	24	100

1 – arenosole from Błędowska Desert (data from Marynowski et al., 2020); 2 – initial soil from Busko Zdrój; 3 – fossil wood, Doubrava sandpit; 4 – fossil wood sample, Jaroszów mine (JR14K); 5 – detrital lignite, Jaroszów mine (JR7Z); 6 – clay, Jaroszów mine (JRIL1); 7 – aerosol sample, winter (80114); 8 – aerosol sample, summer (80725); 9 – *Lenzites betulina* fungi; 10 – *Inonotus obliquus* fungi; 11 – *Fomitiporia robusta* fungi; relative abundances in %, normalized to major peak – 100

Table 3

**The percentage of fully BSTFA *versus* MSTFA derivatized compounds
of biological samples**

Sample	Fungi species/soil type	L-(-)-arabitol		Mannitol		Trehalose	
		[% fully derivatized]					
		BSTFA	MSTFA	BSTFA	MSTFA	BSTFA	MSTFA
Fungi15	*Lenzites betulina*	85	100	15	90	14	91
Fungi18	*Inonotus obliquus*	68	100	13	79	14	89
Fungi48	*Fomitiporia robusta*	52	84	14	81	2	93
Fungi70	*Phellinus tremulae*	27	100	5	100	5	98
Fungi94	*Ischnoderma resinosum*	80	94	23	91	20	97
OR2	Arenosol	100	100	44	77	72	97

bitol, pinitol, and glycerol (Table 2). Levoglucosan is a widespread anhydrosaccharide and present mostly in winter aerosols as a biomass burning tracer (Simoneit et al., 2004). The efficiency of derivatization with BSTFA compared to MSTFA was similar. Possibly, in the case of lower concentrations of saccharides (concentration of individual compounds not exceeding 2000 ng/m^3 of flowing air, usually within a range of 20–100 ng/m^3), both reagents are effective; derivatized saccharides reached a level near 100%.

FUNGI

Arabitol, mannitol, and trehalose, when they occur together, are characteristic of mycorrhizas and mycorrhizal (e.g., Nehls et al., 2010), entomopathogenic (Bidochka et al., 1990), and wood- and root-rotting species of fungi (Marynowski et al., 2019). The leading role of these compounds in the organisms enumerated is carbohydrate storage (e.g., Asiegbu, 2000). Compared to individual sugar standards, the effectiveness of derivatization with BSTFA was much lower in these samples (Fig. 2 and Table 3). For example, mannitol and trehalose were poorly derivatized by BSTFA (ranging from 13 to 23% and from 2 to 20%, respectively); both show higher values for TMS derivatives with MSTFA: 79–100 and 89–98%, respectively (Table 3 and Fig. 3). BSTFA is not as effective for mixtures of many different organic compounds with different polarities and reactivities. Thus, efficiency in derivatization depends not only on the type of reagent (Schummer et al., 2009) but also on the composition of samples. For example, BSTFA is superior to MSTFA in the derivatization of steroids (Huang et al., 2010). In more complex samples MSTFA is much more effective (compare derivatization of standards *versus* derivatization of fungal samples).

Recent studies suggest that saccharides are useful indicators of organic matter sources and maturity (Medeiros and Simoneit, 2007; Marynowski et al., 2018, 2019). Thus, accurate calculation appears to be crucial both in quantitative research and in palaeoenvironmental reconstruction. Omissions in the integration of peaks and totaling of compounds that are not fully derivatized may underestimate their concentrations. Complete derivatization achieved with the use of MSTFA helps to avoid these problems.

OCCURRENCE OF SACCHARIDES IN BIOLOGICAL MATERIAL AND SEDIMENTARY ROCKS

The distribution of saccharides in soils, rocks, and fungi samples are shown in Table 2 and Figure 4, and in areosols in Table 2. Whereas fungi include only three major and a few minor compounds, the composition of saccharides is the most diverse in soils and aerosols, consisting of various elements of organisms including algae, cyanobacteria, pollen grains, fungi, and lichens and their spores, as well as fragments of higher plants (Bauer et al., 2008; Marynowski et al., 2020), and is less heterogeneous in lignites and clays. Moreover, some saccharide-rich particles in aerosols originate from biomass and coal burning (Fabbri et al., 2009; Rybicki et al., 2020). Although brown coals are also composed of various organisms, some less stable saccharides may disappear during early diagenetic processes, in which case other neutral saccharides, such as hemicellulose and cellulose, are generated from preserved biopolymers (Marynowski et al., 2018, 2019). However, the origin of some sugars appears to be the same in the case of soils, fossil wood, and aerosols. Pinitol and fructose, which are major components of detrital lignites and one fossil wood sample, are associated more closely with pollen grains and primary wood composition (e.g., Roach et al., 2017). Glucose was found in all samples analysed, including extant fungi. This compound appears to be a common constituent of many geological, environmental, and biological materials. Many sugar compounds have been identified in soils (Simoneit et al., 2004; Marynowski et al., 2020), aerosols (Medeiros and Simoneit, 2007; Bauer et al., 2008), and sedimentary OM (Marynowski et al., 2018, 2019), but more studies are required in order to understand their role and origin.

CONCLUSIONS

Saccharides are common and diverse constituents of soils, aerosols and low-rank coals. Based on this study we recommend the application of MSTFA as a derivatization reagent in the case of biological and environmental samples, such as fungi and soils, where saccharides are the dominant compounds. To avoid underestimation of saccharide con-
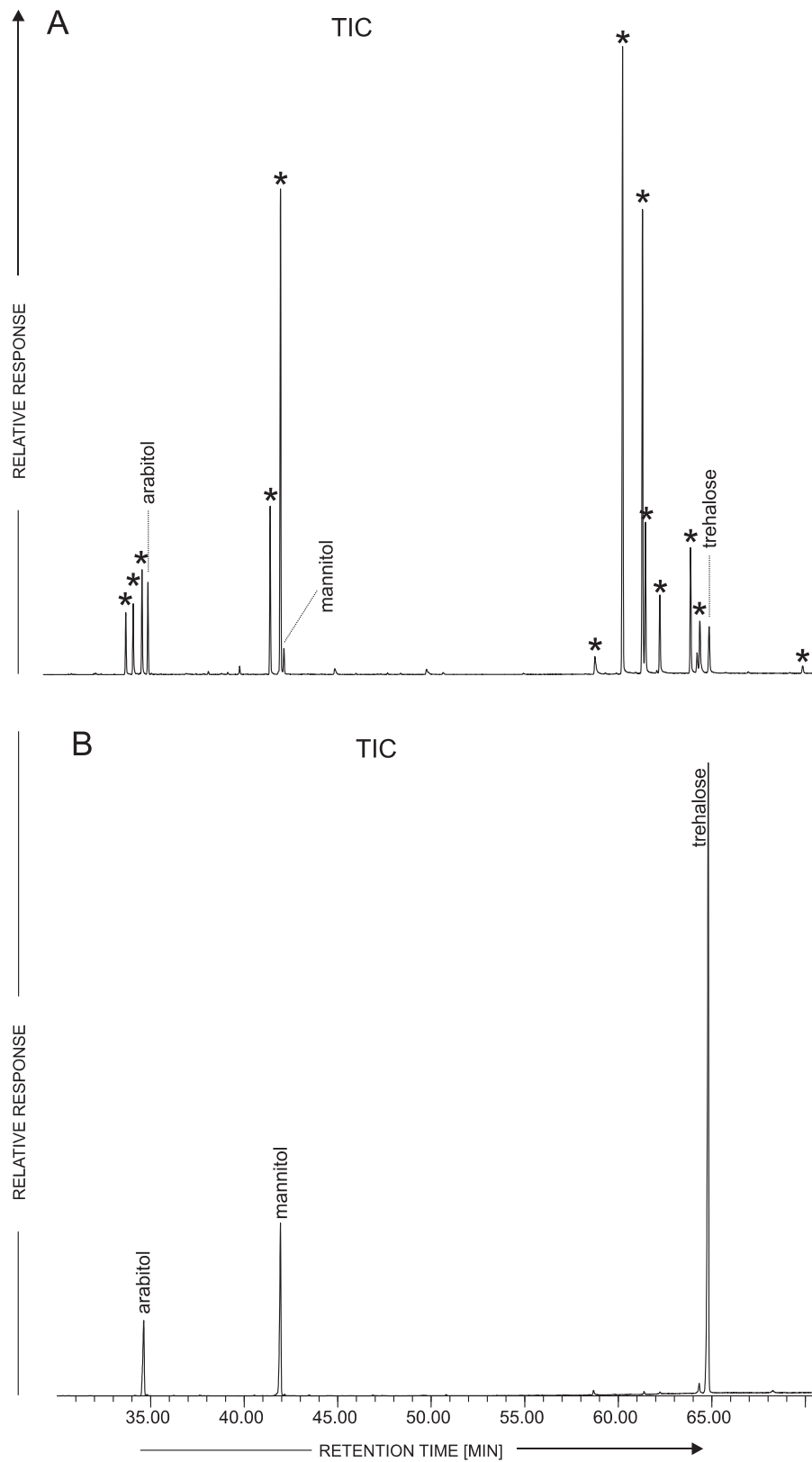
**Fig. 2. TIC chromatogram of *Phellinus tremulae* fungi showing the distribution
of saccharides as trimethylsilyl derivatives derivatized using:
BSTFA – A and MSTFA – B**

Asterisks denote compounds not fully derivatized;
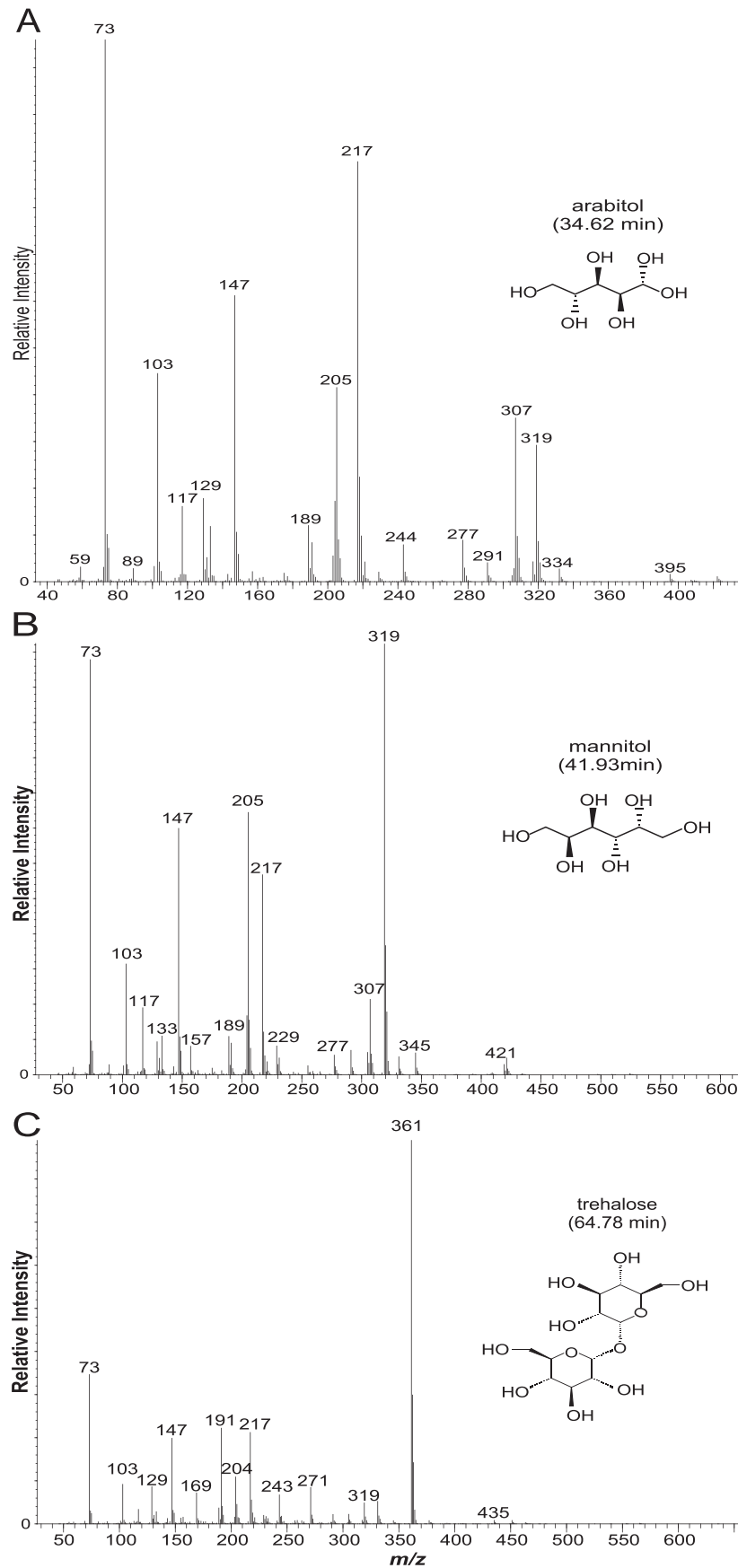note the lower efficiency of BSTFA derivatization

Fig. 3. Mass spectra of TMS derivatives of arabitol (A), mannitol (B) and trehalose (C), the most common fungi sugars detected in wood-rot fungi and sedimentary rocks
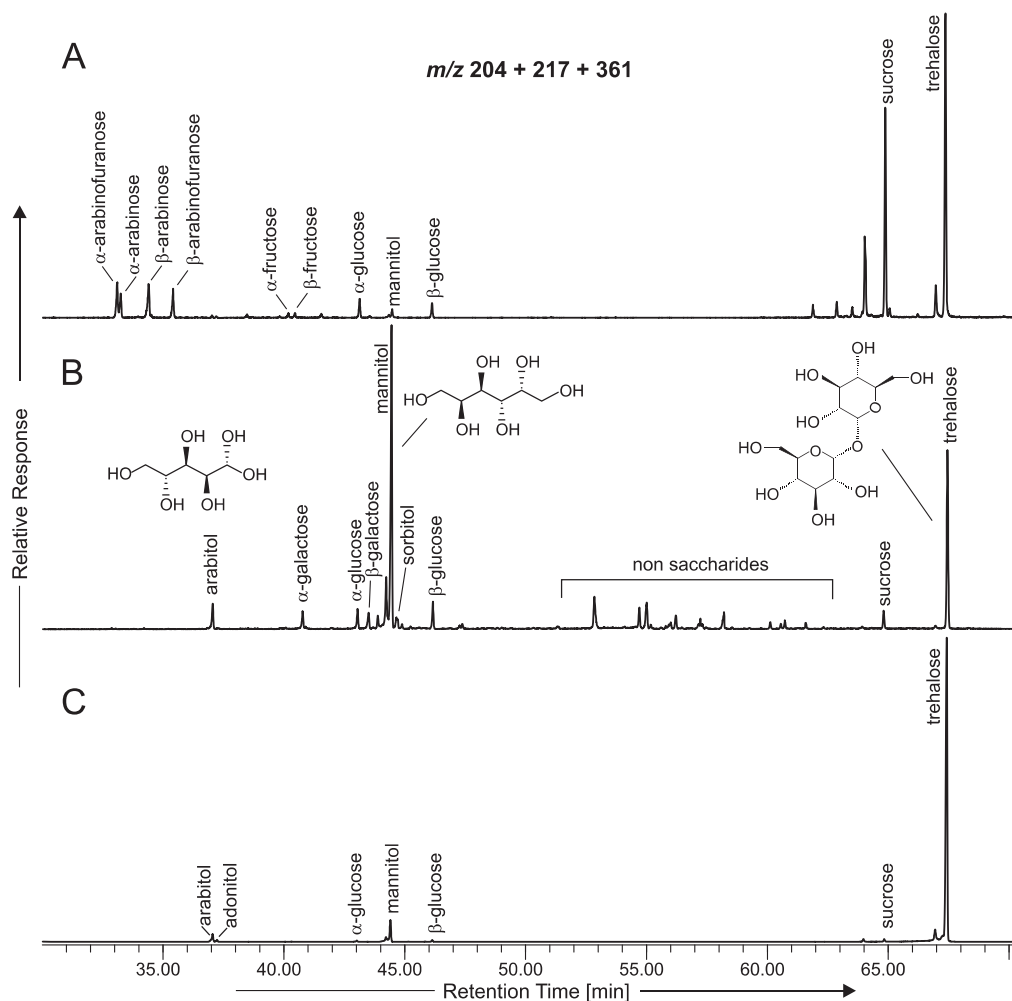
Fig. 4. Summed mass chromatogram (*m/z* 204 + 217 + 361) showing the distribution of mono- and disaccharides of: A – soil developed on gypsum from Busko-Zdrój, derivatized using MSTFA; B – Miocene xylite (fossil wood) from the Jaroszów mine, derivatized using BSTFA; and C – wood rot fungi *Fomitiporia robusta*, derivatized using MSTFA

centrations in sugar-rich natural samples, the use of MSTFA would be crucial. MSTFA enables near-full derivatization of the saccharides. Even though BSTFA is known for successful derivatization in environmental and geological studies, the composition of biological samples may play a role in the effectiveness of derivatization.

Comparing the occurrence of certain sugars in biological material and sedimentary rocks sheds new light on the origin and preservation of these compounds. The origin of arabitol,

mannitol, and trehalose in sedimentary rocks is associated with fungal spores and wood-degrading fungi activity. Fructose and pinitol are more typical of pollen spores as well as of herbaceous plants and trees, whereas glucose origin is heterogeneous.

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