JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Simple and Fast Method for Recognition of Reducing and Nonreducing Neutral Carbohydrates by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

MARKÉTA LAŠTOVIČKOVÁ* AND JOSEF CHMELÍK

Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Veveri 97, 611 42 Brno, Czech Republic

Negative-ion mode matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) was used for the characterization of storage, neutral oligosaccharides extracted from Jerusalem artichoke, red onion, and wheat. The oligosaccharides from the real samples were analyzed with 2,4,6-trihydroxyacetophenone as the most convenient matrix that was selected in advance with the standard carbohydrate samples (inulin and maltooligosaccharides). The oligosaccharides from Jerusalem artichoke and red onion (similarly as inulin) produced $[M - H]^-$ peaks as the main distribution, which reflects their nonreducing composition. On the contrary, the cross-ring fragmentations $[M - H - 120]^-$ formed the main distribution in the mass spectra of hydrolyzed wheat starch similarly to reducing maltooligosaccharides and dextrans. The negative-ion mode MALDI-TOF MS is capable of recognizing reducing and nonreducing oligosaccharides. Such a simple differentiation of malto or inulin type of oligosaccharides is not possible in the positive-ion mode.

KEYWORDS: MALDI-TOF/TOF MS; inulin; malto-oligosaccharides; negative-ion mode; vegetable; reducing and nonreducing carbohydrates

INTRODUCTION

Carbohydrate properties are related to their composition and molecular masses. Therefore, the oligosaccharide molecular mass distributions are significant in this regard. The linkage, degree of branching, and stereochemistry of monosaccharide units must be determined for full characterization of oligo- and polysaccharides, and also, recognition of reducing and nonreducing composition is very important. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) has already shown its potential for the characterization of carbohydrates (*1*, *2*).

Inulin belongs to the fructan group. It is a nonreducing polysaccharide containing D-fructofuranosyl units linked by $\beta 1 \rightarrow 2$ glycosidic bonds and ended with one glucose unit (see **Figure 1A**) (*3*). Inulin is found in the roots and tubers of plants from the *Compositae* (aster, dandelion, dahlia, chicory lettuce, Jerusalem artichoke, etc.), *Liliacae* families (lily bulbs, onion, tulips, etc.), and it is produced by some algae (*4*). Glucose syrups are concentrated, aqueous solutions of reducing, low molecular mass oligosaccharides (containing $1 \rightarrow 4$ and $1 \rightarrow 6$ glycosidic bonds) obtained by hydrolysis of starch. These starch hydrolysates can be trespassed as the cheap sweeteners at the adulteration of food, e.g., fruit juices (*5*−*7*).

MS was used for analysis of fructans and starch oligosaccharides from various plants [agave (*8*), different cultivars of garlic (*9*−*11*), onion (*10*−*12*), shallots (*11*), Dahlia variabilis (*12*), barley (*6*, *7*, *10*, *13*), rice (*14*), etc.]. However, the authors used only the positive-ion mode MS for these measurements. In this way, the oligosaccharides are detected usually as the adducts with sodium or potassium ions ($[M + Na]^+$, $[M + K]^+$), which allows one to determine only some basic characterizations of oligosaccharide distribution, but it is not possible to find out more details about oligosaccharide structure without MS/MS experiments (*10*).

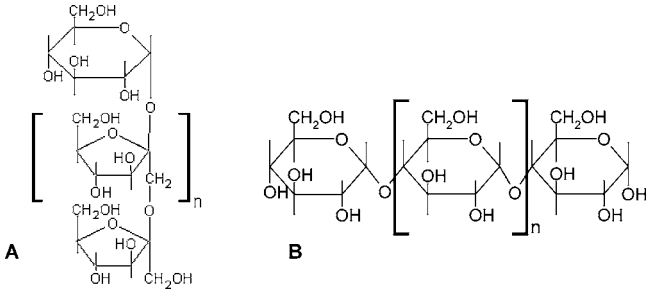

Figure 1. Structure of standard samples used in this study: (**A**) inulin and (**B**) maltooligosaccharides; *n*-DP.

The lack of acidic sites in neutral oligosaccharides reducing the ability of forming the deprotonated molecules ($[M - H]^-$) is probably the reason for the small number of the papers dealing with the negative-ion MALDI Time of Flight (TOF) MS analyses of neutral oligosaccharides (*15*, *16*). Alternative ways to charge the neutral oligosaccharides are either attachment of a small inorganic anion A^- to form the anionic adduct [M +

* To whom correspondence should be addressed. Tel: +420532290109. E-mail: lastovickova@iach.cz.

10.1021/jf052988s CCC: $33.50
Published on Web 06/15/2006

A]$^-$ (*17*, *18*) or covalent binding of negatively charged labels (*19*−*21*). There is no general anion dopant for oligosaccharide analysis by MALDI-TOF MS; by now, mainly hydrogen sulfate and chloride are used (*17*, *18*).

Although the negative-ion mode is often neglected, it can provide structurally informative fragments that are required for the determination of the oligosaccharide linkage (*15*, *22*). In general, fragmentation patterns in MALDI are similar to those obtained with other ionization methods [fast atom bombardment (FAB), electrospray ionization, and FTMS) (*2*, *23*, *24*). The nomenclature for fragment ions of carbohydrates was introduced by Domon and Costello (*25*). Generally, B (= M − 162 − 18) and Y (= M − 162) ions are usually the most abundant in mass spectra of most carbohydrates. The relative proportions of the different types of fragment ions vary with the type of parent ions (*1*, *24*, *26*, *27*). An overview on the negative-ion mode FAB mass spectra of different underivatized oligosaccharides published Garozzo et al., who found that the fragmentation process occurs stepwise, going from the reducing to the nonreducing end (*24*).

MALDI-TOF/TOF MS is able to provide high-energy fragmentation information (*26*). Cross-ring fragmentation patterns of oligosaccharides and *N*-glycans derived from glycoproteins in the positive-ion mode of MALDI-TOF/TOF-MS were investigated by Mechref and Novotny (*28*). They studied three isobaric oligosaccharides with different linkages. They found that the cross-ring fragment ions were more intense than the most commonly observed Y and B ions, in some cases. A type fragment ions allowed the distinction between α1→6- and α1→4-linked isomers. The difference was based on the type of ions formed but also on their intensities. Both isomers formed ions resulting from the loss of 120 *m*/*z* units but with different intensities. This is due to the fact that they correspond to the different ions (^{0,4}A and ^{2,4}A) (*28*). Spina et al. (*29*) presented MALDI-TOF/TOF MS/MS spectra of complex carbohydrates from human milk, where they found the peaks originating from glycosidic cleavages as well as from carbohydrate ring fragmentations. Stephens et al. described the fragmentation characteristics of native and permethylated oligosaccharides (*30*). The 2,5-dihydroxybenzoic acid (2,5-DHB) matrix adducts of type [M + DHB − H]$^-$ were found in mass spectra of glucose derivates using both ion modes of MALDI-TOF MS by Mele and Malpezzi (*31*). New extensive studies about fragmentation of the negative ions of high-mannose, hybrid, and complex *N*-linked glycans have been recently published by Harvey (*32*−*34*).

We demonstrated here a potential of the negative-ion mode of MALDI-TOF MS for the characterization of underivatized neutral oligosaccharides extracted from real samples. Partially hydrolyzed wheat starch and oligosaccharides from Jerusalem artichoke and onion have already been studied in the positive-ion mode (*10*), but contrary to the negative-ion mode, these experiments have not revealed any difference between the reducing and the nonreducing oligosaccharides. The influence of the matrix selection on the negative-ion mode measurements was studied with inulin and malto-oligosaccharides (MOSs) (G4−G10) as the standards. The experiments were carried out in both linear (LIN) and reflectron (REF) negative-ion mode.

MATERIALS AND METHODS

Standard Sample Preparation. Inulin from Dahlia tubers M_w 5000 (Fluka, Buchs, Switzerland) and MOSs G4−G10 (Sigma, St. Louis, MO) were prepared at concentrations of 1 or 4 mg/mL in deionized water.

Extraction of Carbohydrates from Real Samples. Red onion and Jerusalem artichokes were acquired from a private producer from the Czech Republic. A procedure for the carbohydrate extraction from fresh samples was described previously (*10*, *11*). Low glucose syrup (LGS) from 80% wheat starch (w/v), obtained from Amylon Co. (Havlickuv Brod, Czech Republic), was used at a concentration of 4 mg/mL in deionized water.

MALDI MS. The linear negative-ion mode mass spectra were acquired with a Kompact MALDI IV mass spectrometer (Shimadzu-Kratos, Manchester, GB) equipped with a nitrogen laser (337 nm). The optimal laser power was selected from the relative scale 0−180. The reflectron negative-ion mode experiments were performed with an Applied Biosystems 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA) utilizing a Nd:YAG laser (355 nm). The optimal laser power was selected from the relative scale 0−8800.

Matrix Selection. Five matrices were compared in order to get the highest sensitivity and resolution of oligosaccharides in the negative-ion mode: 2,5-DHB (Aldrich), 2,4,6-trihydroxyacetophenone (THAP; Fluka), α-cyano-4-hydroxycinnamic acid (HCCA; Aldrich), 2-4-hydroxyphenylazobenzoic acid (HABA; Aldrich), and 3-aminoquinoline (3-AQ; Fluka). The experiments were performed with two standard oligosaccharides: inulin and MOSs. Two techniques of slide preparation were used, thin layer (TL) and dried droplet (DD). The optimal experimental conditions (concentration of the samples and matrices, convenient slide preparation, and laser power) were based on those determined for the positive-ion mode previously (*10*). The particular experimental conditions are described in the figure legends.

RESULTS AND DISCUSSION

Selection of the Proper Matrices in LIN and REF Modes. To determine the storage oligosaccharides from real samples in the negative-ion modes MALDI-TOF MS, it is necessary to select a proper matrix. This task was carried out with inulin standard sample. It was analyzed with five matrices under optimal conditions that had been determined for the positive-ion mode (*10*). The obtained spectra were evaluated for several aspects—the range between the shortest and the highest detected oligomers = the range of the degree of polymerization (DP), the total number of detected oligomers (n_p), number-average molecular mass (M_n), weight-average molecular mass (M_w), and polydispersity (δ). In **Table 1**, there is also information about the most abundant peak (M_r, intensity, and DP).

The preferences of different matrices for particular oligosaccharides observed in the positive-ion mode were confirmed in the negative-ion mode. The results of measurements with various matrices are also presented as LIN MALDI-TOF mass spectra of inulin in **Figure 2**. The spectra with all five matrices were found in LIN mode, but HCCA, HABA, and 3-AQ showed the spectra with low signal:noise ratios under the conditions used, and the repeability of measurements was low. THAP was selected as the most convenient matrix for LIN experiments with inulin, because both the total number of oligomers detected (three times) and the sensitivity (22 times) was higher than in the case of 2,5-DHB.

The advantage of LIN mode in comparison to the positive-ion mode is the relatively higher sensitivity for oligomers with higher molecular masses, which was demonstrated by higher or comparable values of M_n (only if the proper matrix is used); on the contrary, LIN mode gave the peaks with lower resolution and intensity (based on the comparison to the data published in ref *10*). Because the resolution was relatively low in LIN mode, inulin was also analyzed in the negative reflectron mode (REF) for confirmation of the peak origin using an Applied Biosystems 4700 instrument with THAP as the matrix (**Figure 3A**). The same experimental conditions (optimal concentrations of samples and matrices and slide preparation) as in LIN mode were also

Table 1. Evaluation of Mass Spectra of Inulin (LIN mode) for Five Different Matrices

matrix	2,5-DHB	THAP	HCCA	HABA	3-AQ
n_p	17	49	16	11	24
DP	14–30	9–57	10–25	15–25	8–31
M_n	2988	3475	2779	3283	1128
M_w	3032	3683	2840	3338	1369
δ	1.01	1.06	1.02	1.02	1.21
peak types	$[M - H]^-$	$[M - H]^-$, $[M - H - H_2O]^-$	$[M - H]^-$, $[M - H - H_2O]^-$	$[M - H]^-$	$[M - H]^-$, $[M + K - 2H]^-$, $[M + K + Na - 3H]^-$
		the most abundant peak			
M_r (average)	2935.57	3097.71	2935.95	3259.85	2611.28
intensity (mV)	1.5	33	1.3	0.4	2.7
DP	18	19	18	21	16

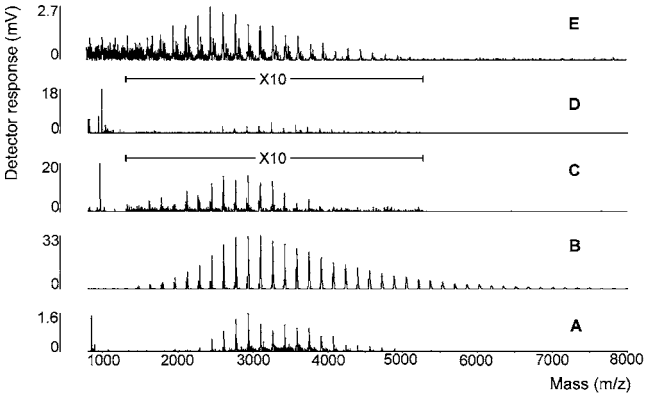

Figure 2. LIN MALDI-TOF mass spectra of inulin obtained with various matrices: (**A**) 2,5-DHB (1 mg/mL of inulin, 15 mg/mL 2,5-DHB in deionized water, DD technique); (**B**) THAP (4 mg/mL of inulin, 100 mg/mL THAP in acetone, TL technique); (**C**) HCCA (1 mg/mL of inulin, HCCA saturated in acetone, TL technique); (**D**) HABA (1 mg/mL of inulin, HABA saturated in acetone, DD technique); and (**E**) 3-AQ (4 mg/mL of inulin, 20 mg/mL 3-AQ in ethanol, DD technique). A Kratos instrument was used.

used for the REF measurements, and the instrument settings (laser intensity, number of laser shots, and mass range) were optimized. Higher resolution was achieved with the proper experimental setting; isotopic resolution and oligomer masses with better mass accuracy were obtained. The values of n_p, n_{s-h}, M_n, M_w, and δ were comparable to those obtained in LIN mode. MOSs were measured under the same conditions (**Figure 3B**,**C**). A fundamental difference was observed between the REF mass spectra of inulin and MOS. The mass spectra of inulin showed the expected deprotonated molecules $[M_x - H]^-$ as the main distribution, whereas the main observed distribution of MOS (the linear oligomer containing only 1→4 linkages; for structure, see **Figure 1B**) corresponded to the fragment ions $[M_x - H - 120]^-$. This in-source fragmentation has already been described for negative-ion mode MALDI-TOF mass spectra of dextrans (the polymer containing the main chain with 1 → 6 glycosidic bonds and different degree of branching) (*16*). These MS experiments showed that 1 → 4 glycosidic bond containing oligosaccharides are also subjected to the fragmentation of the carbohydrate ring in the negative-ion mode.

Determination of Storage Oligosaccharides Using Negative-Ion Mode and Evaluation of the Peak Origin. After finding the optimal experimental conditions and selection of the proper matrix, the negative-ion mode MALDI-TOF mass spectra of oligosaccharides from real samples were also obtained. Both LGS and oligosaccharides isolated from Jerusalem artichoke and red onion were analyzed with THAP. Generally, no multicharged peaks were observed in the negative-ion mass spectra of these oligosaccharides, and as you can see in **Figure 4** (REF mass spectra), the oligomers showed the mass difference of 162 Da (hexose residues) for all samples. The distribution of oligosaccharides showed DP in the range from 6 to 22 for Jerusalem artichoke, from 6 to 11 for red onion, and from 7 to 25 for LGS. Other characteristics of these mass spectra are summarized in **Tables 2** and **3**.

Because the REF mode was found as more convenient for measurement of oligosaccharide samples (better resolution and signal:noise ratio), the repeability of measurements was studied in this mode. M_n and M_w are the most convenient values for the comparison. Therefore, M_n and M_w were determined for minimally five different measurements, and then, the average values and the standard deviations (%) were calculated. The distribution of oligosaccharides from Jerusalem artichoke showed $M_n = 1744 \pm 60$ Da (3.5%) and $M_w = 1868 \pm 95$ Da (5.1%); from red onion, $M_n = 1359 \pm 33$ Da (2.5%) and $M_w = 1398 \pm 38$ Da (2.7%); and from LGS, $M_n = 1668 \pm 103$ Da (6%) and $M_w = 1817 \pm 127$ Da (7%). The ranges of oligosaccharide distribution observed in the negative-ion mode were comparable to those in the positive-ion mode (*10*), but the values of M_n and M_w were higher in the negative-ion mode.

An interesting point is the origin of the peaks and the differences between the positive- and negative-ion modes. The positive-ion mode mass spectra of all studied oligosaccharides showed the same type of distribution containing $[M + Na]^+$, $[M + Na - H_2O]^+$, and $[M + K]^+$ peaks (*10*). However, their negative-ion mode mass spectra were different. $[M - H]^-$ ions formed the dominant distribution for oligosaccharides from both Jerusalem artichoke and red onion. The mass spectra differed in the quantity of the adducts, which was dependent on the amount of ions in the samples (see **Tables 2** and **3** and **Figure 4A**,**B**). The main distribution of LGS was formed by the fragment ions $[M - H - 120]^-$ (see **Figure 4C**). LGS showed this fragmentation because of its structure that contains the main chain formed by 1→4 glycosidic bonds and branches with 1→6 glycosidic bonds and a reducing end group. It is supposed that fragmentation of LGS takes place at the ring with the reducing end group similarly to dextrans (*16*); however, the structure of $[M - H - 120]^-$ ions is not yet known.

There are some important conclusions on the mass spectrometric behavior of the neutral oligosaccharides. In the positive-ion mode, the main distribution of the oligosaccharides is formed by the alkali-ion adducts. The negative-ion mode MALDI-TOF mass spectra showed a potential for the determination of some features of the neutral oligosaccharides. Generally, in the negative-ion mode, reducing and nonreducing oligosaccharides can be differentiated, because of easy fragmentation of reducing end ring, which is not evident in the positive-ion mode of

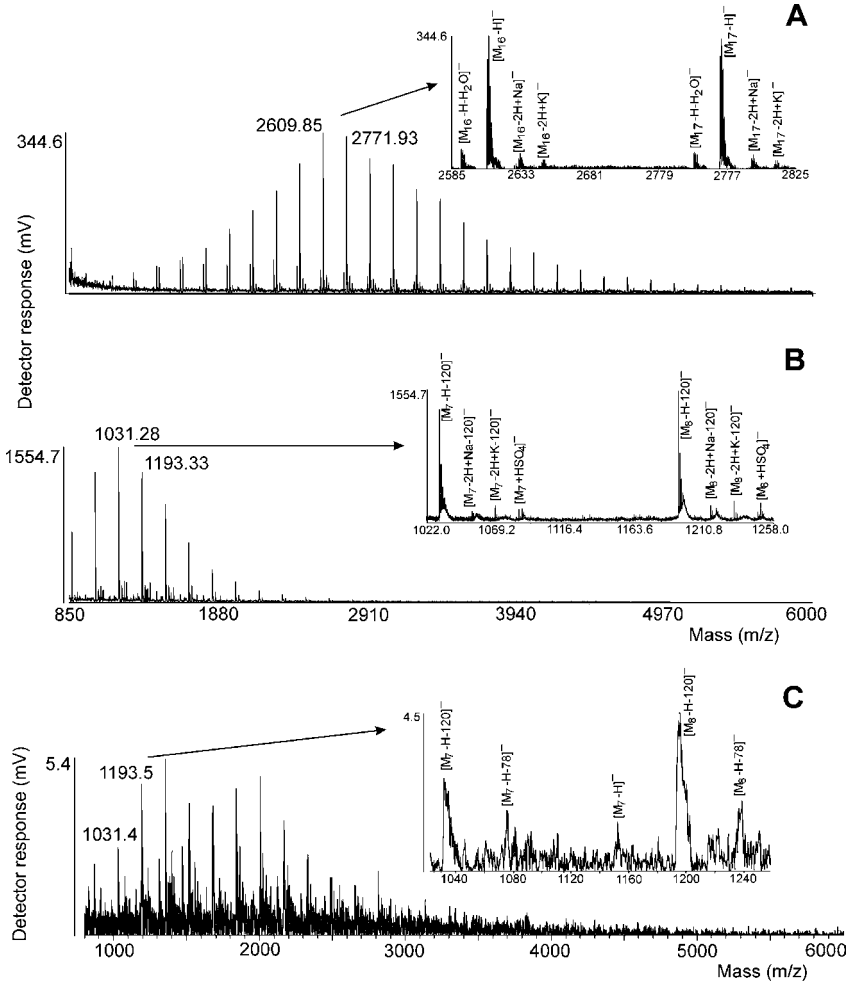

Figure 3. REF MALDI-TOF mass spectra of standard oligosaccharides: inulin (**A**) and MOS (**B**); LIN MALDI-TOF mass spectra of MOS (**C**). Conditions: sample concentration, 1 mg/mL deionized water; matrix, THAP (100 mg/mL acetone); TL technique.

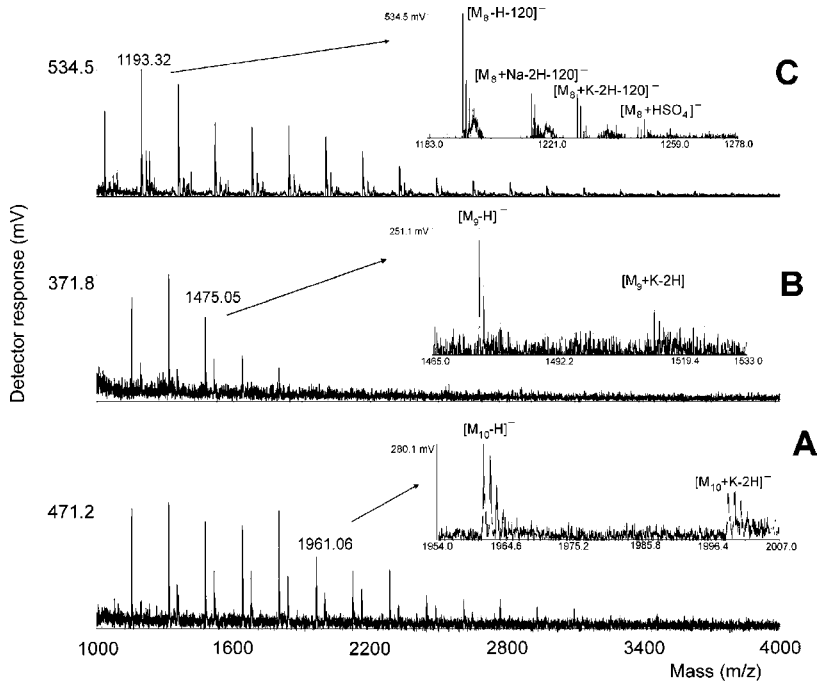

Figure 4. REF MALDI-TOF mass spectra of oligosaccharides from the real samples: Jerusalem artichoke (**A**); red onion (**B**); and LGS (4 mg/mL deionized water; **C**). Conditions: matrix, THAP (100 mg/mL acetone); TL technique. An Applied Biosystems instrument was used.

MALDI-TOF MS where both types of oligosaccharides form the alkali-ion adducts.

Because of these in-source fragment ions that were found in the negative-ion mode MALDI-TOF mass spectra of LGS,

Table 2. Evaluation of LIN Mode Mass Spectra of LGS and Oligosaccharides Isolated from Red Onion and Jerusalem Artichoke

	LIN mode		
	Jerusalem artichoke	red onion	LGS
n_p	15	6	12
DP	6–20	6–11	7–18
M_n	1874	1212	1681
M_w	2064	1250	1778
δ	1.10	1.03	1.06
peak types	$[M-H]^-$, $[M+K-2H]$	$[M-H]^-$, $[M+K-2H]^-$	$[M-H]^-$, $[M-H-120]^-$, $[M+Na-2H-120]^-$, $[M-H-78]^-$
	the most abundant peak		
M_r (average)	1638.43	1314.15	1356.29
intensity (mV)	15	6.5	4
DP	10	8	9

Table 3. Evaluation of REF Mode Mass Spectra of LGS and Oligosaccharides Isolated from Red Onion and Jerusalem Artichoke

	REF mode		
	Jerusalem artichoke	red onion	LGS
n_p	17	6	19
DP	6–22	6–11	7–25
M_n	1805	1392	1763
M_w	1962	1426	1966
δ	1.09	1.02	1.11
peak types	$[M-H]^-$, $[M+K-2H]^-$	$[M-H]^-$, $[M+K-2H]^-$	$[M-H-120]^-$, $[M+Na-2H-120]^-$, $[M+K-2H-120]^-$, $[M+HSO_4]^-$
	the most abundant peak		
M_r (mon.)	1313.06	1313.06	1193.31
intensity (mV)	471.2	371.8	534.5
DP	8	8	8

MOS, and dextran on one side and $[M-H]^-$ ions forming the main distribution of inulin and oligosaccharides from Jerusalem artichoke and onion on the other side, it is possible to differentiate reducing maltooligosaccharides and nonreducing fructooligosaccharides. This is very useful for the identification of storage carbohydrates isolated from plants.

LITERATURE CITED

(1) Harvey, D. J. Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates and glycoconjugates. *Int. J. Mass Specrom.* **2003**, *226*, 1−35.

(2) Zaia, J. Mass spectrometry of oligosaccharides. *Mass Spectrom. Rev.* **2004**, *23*, 161−227.

(3) French, A. D.; Waterhouse, A. L. Chemical structure and characteristics. In *Science and Technology of Fructans*; Suzuki, M., Chatterton, J. K., Eds.; CRS Press Inc.: Boca Raton, Florida, 1993; pp 41−82.

(4) Hendry, G. A. F.; Wallace, R. K. The origin, distribution, and evolutionary significance of fructans. In Science *and Technology of Fructans*; Suzuki, M., Chatterton, J. K., Eds.; CRS Press Inc.: Boca Raton, Florida, 1993; pp 119−140.

(5) Origin Agrostar Limited. Bulk Products. Glucose Syrup. [Online]. Last updated: 2nd January 2002. Accessible from http://www.originagrostar.com/glucose.html.

(6) Žídková, J.; Chmelík, J. Determination of saccharides in fruit juices by capillary electrophoresis and MALDI-TOF mass spectrometry. *J. Mass Spectrom.* **2001**, *36*, 417−421.

(7) Cabálková, J., Žídková, J., Přibyla, L., Chmelík, J. Determination of carbohydrates in juices by using capillary electrophoresis, high performance liquid chromatography and matrix assisted desorption/ionization time-of-flight mass spectrometry. *Electrophoresis* **2004**, *25*, 487−493.

(8) Lopez, M. G.; Mancilla-Margalli, N. A.; Mendoza-Diaz, G. Molecular structures of fructans from Agave tequilana Weber var. azul. *J. Agric. Food. Chem.* **2003**, *51*, 7835−7840.

(9) Losso, D. N.; Nakai, S. Molecular size of garlic fructooligosaccharides and fructopolysaccharides by matrix-assisted laser desorption ionization mass spectrometry. *J. Agric. Food Chem.* **1997**, *45*, 4342−4346.

(10) Štikarovská, M.; Chmelík, J. Determination of neutral oligosaccharides in vegetables by matrix assisted laser desorption/ionization mass spectrometry. *Anal. Chim. Acta* **2004**, *520*, 47−55.

(11) Wang, J.; Sporn, P.; Low, N. H. Analysis of food oligosaccharides using MALDI-MS: Quantification of fructooligosaccharides. *J. Agric. Food Chem.* **1999**, *47*, 1549−1557.

(12) Stahl, B.; Karas, M.; Hillenkamp, F.; Steup, M. Analysis of fructans from higher plants by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Biochem.* **1997**, *246*, 195−204.

(13) Chmelik, J.; Krumlova, A.; Caslavsky, J. Characterization of starch materials from barley by gravitational field-flow fractionation and matrix-assisted laser desorption/ionization mass spectrometry. *Chem. Pap. Chemicke Zvesti* **1998**, *52*, 360−361.

(14) Grimm, C. C.; Grimm, D. A.; Bergman, C. J. Oligosaccharides in food and agriculture. *ACS Symp. Ser.* **2003**, *849*, 32−42.

(15) Hao, Ch.; Ma, X.; Fang, S.; Liu, Z.; Liu, S.; Song, F.; Liu, J. Positive- and negative-ion matrix-assisted laser desorption/ionization mass spectrometry of saccharides. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 345−348.

(16) Čmelík, R.; Štikarovská, M.; Chmelík, J. Different behavior of dextrans in positive-ion and negative-ion mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 1467−1473.

(17) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. An anion dopant for oligosaccharides in matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **1999**, *71*, 205−211.

(18) Cai, Y.; Jiang, Y.; Cole, R. B. Anionic adducts of oligosaccharides by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Anal. Chem.* **2003**, *75*, 1638−1643.

(19) Macasek, F.; Bruder, P.; Patakyova, A.; Buriova, E. Atmospheric pressure electrospray ionization mass spectra of glucose and 2-fluorodeoxyglucose for quantitative analysis of 2-fluorodeoxyglucose by high-performance liquid chromatography. *Eur. J. Mass Spectrom.* **2003**, *9*, 129−137.

(20) Sajid, B. A.; Giannakopulos, A. E.; Derrick, P. J.; Critchley, P.; Bottrill, A.; Padley, H. D. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. A comparison of fragmentation patterns of linear dextran obtained by in-source decay, post-source decay and collision-induced dissociation and the stability of linear and cyclic. *Eur. J. Mass Spectrom.* **2004**, *10*, 109−120.

(21) Lattova, E.; Perreault, H. N.; Krokhin, O. Matrix-assisted laser desorption/ionization tandem mass spectrometry and post-source decay fragmentation study of phenylhydrazones of N-linked oligosaccharides from ovalbumin. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 725−735.

(22) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. An anion dopant for oligosaccharides in matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **1999**, *71*, 205−211.

(23) Cai, Y.; Jiang, Y.; Cole, R. B. Anionic adducts of oligosaccharides by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Anal. Chem.* **2003**, *75*, 1638−1644.

(24) Que, A. H.; Novotny, M. V. Structural characterization of neutral oligosaccharide mixtures through combination of capillary electrochromatography/ion trap tandem mass spectrometry. *Anal. Bioanal. Chem.* **2003**, *375*, 599−608.

(25) Reinhold, V. N.; Reinhold, B. B.; Costello, C. E. Carbohydrate molecular weight profiling, sequence, linkage and branching data: ES-MS and CID. *Anal. Chem*. **1995**, *67*, 1772−1784.
(26) Garrozzo, D.; Giuffrida, M.; Impallomeni, G.; Ballistreri, A.; Montaudo, G. Determination of linkage position and identification of the reducing end in linear oligosaccharides by negative ion fast atom bombardment mass spectrometry. *Anal. Chem.* **1990**, *62*, 279−286.
(27) Domon, B.; Costello, C. E. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate J.* **1988**, *5*, 397−409.
(28) Carroll, A.; Willard, D.; Lebrilla, C. B. Energetics of cross-ring cleavages and their relevance to the linkage determination of oligosaccharides. *Anal. Chim. Acta* **1995**, *307*, 431−447.
(29) Reis, A.; Coimbra, M. A.; Domingues, P.; Ferrer-Correia, A. J. Fragmentation pattern of underivatised xylo-oligosaccharides and their alditol derivatives by electrospray tandem mass spectrometry. *Carbohydr. Polym.* **2004**, *55*, 401−409.
(30) Mechref, Y.; Novotny, M. V. Structural characterization of oligosaccharides using MALDI-TOF/TOF tandem mass spectrometry. *Anal. Chem.* **2003**, *75*, 4895−4903.
(31) Spina, E.; Sturiale, L.; Romeo, D.; Impollomeni, G.; Garozzo, D. New fragmentation mechanisms in matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry of carbohydrates. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 392−398.
(32) Stephans, E.; Maslen, S. L.; Green, L. G.; Williams, D. H. Fragmentation characteristics of neutral N-linked glycans using a MALDI-TOF/TOF tandem mass spectrometer. *Anal. Chem.* **2004**, *76*, 2343−2354.
(33) Mele, A.; Malpezzi, L. Noncovalent association phenomena of 2,5-dihydroxybenzoic acid with cyclic and linear oligosaccharides. A matrix-assisted laser desorption/ionization time-of-flight mass spectrometric and X-ray crystallographic study. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 228−236.
(34) Harvey, D. J. Fragmentation of negative ions from carbohydrates: Part 1. Use of nitrate and other anionic adducts for the production of negative ion electrospray spectra from N-linked carbohydrate. *J. Am. Soc. Mass. Spectrom.* **2005**, *16*, 622−630.
(35) Harvey, D. J. Fragmentation of negative ions from carbohydrates: Part 2. Fragmentation of high-mannose N-linked glycans. *J. Am. Soc. Mass. Spectrom.* **2005**, *16*, 631−646.
(36) Harvey, D. J. Fragmentation of negative ions from carbohydrates: Part 3. Fragmentation of hybrid and complex *N*-linked glycans. *J. Am. Soc. Mass. Spectrom.* **2005**, *16*, 647−659.

Received for review November 30, 2005. Revised manuscript received April 14, 2006. Accepted May 1, 2006. We thank the Grant Agency of the Czech Republic (Project 526/03/1182) and a research plan Z40310501 for financial support.

JF052988S