Determination of Oligosaccharides in Foods, Diets, and Intestinal Contents by High-Temperature Gas Chromatography and Gas Chromatography/Mass Spectrometry

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A rapid and simple gas chromatographic (GC) method was developed for the quantitative determination of oligosaccharides in foods, diets, and intestinal contents. The method includes extraction of oligosaccharides with water, clean up with chloroform/methanol, reduction with KBH_4, neutralization with HAc, methylation with iodomethane in dimethyl sulfoxide and NaOH powder, and analysis by high-temperature capillary gas chromatography with ultrathin stationary phases. Oligosaccharides with two to seven sugar units were quantified and identified by GC and GC/MS. Fructooligosaccharides with up to 12 sugar units were analyzed by GC/MS. The GC method gave reproducible values when applied to rapeseed meal, mung beans, soybeans, chickpeas, red lentils, soy diet, and intestinal contents.

INTRODUCTION

Food legumes, which include oil seeds, peas, and beans, are of nutritional interest as they are important sources of protein and energy. Because of their relatively high content of the raffinose family of oligosaccharides—including raffinose, stachyose, and verbascose—they are considered to cause flatulence in man and animals (Rackis, 1975; Reddy et al., 1980; Olson et al., 1982). There is no α-galactosidase activity in mammalian intestinal mucosa to cleave the α-(1→6) galactose linkage present in the raffinose oligosaccharides (Gitzelmann and Auricchio, 1965). Therefore, they pass into the large intestine. Bacteria in the colon metabolize them to form large amounts of carbon dioxide and hydrogen. High concentrations of these oligosaccharides in the small intestine increase the osmotic pressure of the luminal content, which in turn may cause osmotic diarrhea (Cummings et al., 1986).

Significant quantities of galactopinitol, galactinol, and manninotriose have been reported among the oligosaccharides extracted from several legume seeds (Schweizer et al., 1978; Aman, 1979). The roles of these α-galactosides in flatus formation have not been elucidated. Fructooligosaccharides, which occur in fruits, vegetables, and grains, are also claimed to be indigestible in the small intestine and to be selectively utilized by beneficial colon bacteria. Recent studies indicate that they have favorable effects on carbohydrate and lipid metabolism similar to those of dietary fiber (Hidaka et al., 1990). Oligosaccharides are also formed when starch is hydrolyzed during food processing or digestion in the human gut. These carbohydrates are usually not included in the dietary fiber concept, and their physiological effects have been investigated very little.

High-performance liquid chromatography (HPLC) is commonly used for the quantitative determination of oligosaccharides in foods due to its ease of sample preparation; derivatization is not needed. Several HPLC methods have been developed using amino-bonded silica or ion-exchange columns (Kuo et al., 1988; Scott and Hatina, 1988; Wang and Zopf, 1989; Peelen et al., 1991; Bach Knudsen and Li, 1991) or reversed-phase C_{18} columns (Herbreteau et al., 1990).

Gas–liquid chromatographic (GLC) methods for determination of oligosaccharides in foods are also employed. The sugars are then converted to trimethylsilyl (TMS) derivatives (ethers or oximes) and chromatographed with packed columns (Aman, 1979; Sosulski et al., 1982; Li et al., 1985) or capillary columns (Traitler et al., 1984; Bach Knudsen and Li, 1991). The time necessary to prepare the required derivatives is considered a drawback with GLC methods. However, sample cleanup necessary for HPLC determinations could be as laborious as sample preparation for GLC (Macrae, 1985). The higher saccharides are a problem when analyses are performed with both GLC and HPLC. The TMS derivatives prepared for GLC or higher saccharides form molecules with high molecular weights which are difficult to chromatograph, and when using HPLC, lower resolution is obtained for higher saccharides as compared to the lower saccharides. This means that they are also difficult to quantitate.

Capillary gas chromatography is a higher resolving and more sensitive and selective method than HPLC, which is of importance as higher oligosaccharides in foods and diets are often present in low concentrations. Methylation of oligosaccharides forms more volatile and stable compounds than TMS derivatives. Wang et al. (1983) performed gas chromatography of methylated oligosaccharide alditols up to and including pentasaccharides, and the methylation procedure was recently simplified (Ciucanu and Kerek, 1984; Karlsson et al., 1989). It has been demonstrated that the use of capillary columns with ultrathin films (<0.05 μm) of thermostable bonded stationary phases at high temperatures makes possible the analysis of methylated oligosaccharides with up to 11 sugar units (Karlsson et al., 1989).

The aim of the present investigation was to apply this technique to develop a rapid and simplified capillary gas chromatography method for the qualitative and quantitative determination of oligosaccharides including the higher saccharides in foods, diets, and intestinal contents.

MATERIALS AND METHODS

Materials. Chickpeas, mung beans, red lentils, and soybeans were bought on the Swedish feedstuff market. Defatted rapeseed meal was obtained from the National Institute of Animal Science,

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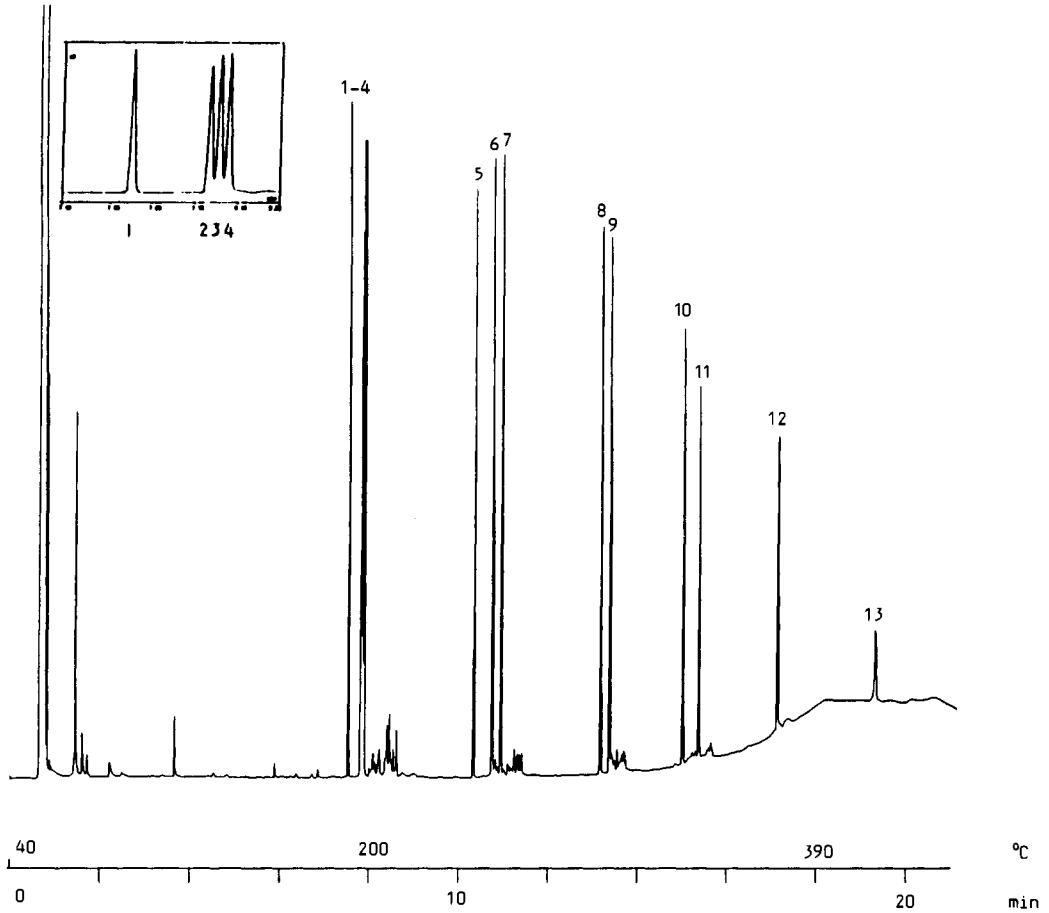

Figure 1. Gas chromatogram of a mixture of reference sugars. (1) Saccharose*; (2) trehalose*; (3) maltose; (4) lactose; (5) melezitose*, IS; (6) raffinose*; (7) maltotriose; (8) stachyose*; (9) maltotetraose; (10) verbascose*; (11) maltopentaose; (12) maltohexaose; (13) maltoheptaose. An asterisk indicates compounds not reduced by KBH_4.

Denmark. Freeze-dried soy diet and intestinal contents were materials from a previous study performed in ileostomy subjects (Sandström et al., 1986).

Pure standards of saccharose were obtained from BDH Chemicals (Poole, England). Trehalose was from Pfanstiehl (Waukegan, IL). Maltose and lactose were from Merck (Darmstadt, Germany), raffinose, stachyose, maltopentaose, maltohexaose, and maltoheptaose were from Serva Finebiochemica (Heidelberg, Germany), and maltotriose and maltotetraose were from Sigma (St. Louis, MO). Verbascose was from MegaZyme (North Rocks, Australia), and melezitose (α-glycosylsaccharose) was from Fluka (Buchs, Switzerland). Other reagents were of analytical grade.

Sample Preparation. Samples of 0.1–0.15 g of dried and ground samples (particle size <0.5 mm) of foods, freeze-dried diets, or intestinal contents with an addition of 1 mg of internal standard (0.1 mL of a solution of 10 mg/mL of melezitose) were extracted with 5 mL of water in a 25-mL glass tube. The extraction was performed for 2 h, the initial 10 min at 100 °C in a boiling water bath and then continued at room temperature. During the extraction, continuous stirring with a magnetic bar was performed.

The extract was centrifuged (5 min at 3000*g*). A 1-mL aliqout of the supernatant was taken out and transferred to a 10-mL tube to which 4 mL of chloroform/methanol (2:1) was added. The mixture was shaken thoroughly to remove compounds containing both hydrophobic and hydrophilic groups and centrifuged (1 min at 1500*g*). The water/methanol phase was transferred to a 10-mL tube. The residual chloroform/methanol phase was then washed with 0.5 mL of H_2O. The combined water/methanol phases were mixed thoroughly. A 1-mL aliquot was taken out, to which 0.5 mL of 0.5 M KBH_4 was added to reduce the oligosaccharides. The samples were allowed to stand for 1.5 h. After neutralization with 0.5 mL of 1 M HAc, approximate pH was checked, and the samples were dried in a stream of air on a water bath (50 °C). The evaporation procedure was repeated four times with 2 mL of methanol to remove the boric acid and obtain a dry residue.

Methylation was performed according to the methods of Ciucanu and Kerek (1984) and Karlsson et al. (1989). The samples were dissolved by sonication in 1 mL of dimethyl sulfoxide (DMSO) for 5 min. For methylation, 0.5 mL of iodomethane was added and a check was made that solubilization was complete before approximately 0.1 g of NaOH powder was added. The samples were stirred with a magnetic bar 15 min, and the reaction was stopped by the addition of 10 mL of water. Four milliliters of chloroform was then added. The water phase was removed after centrifugation, and the chloroform phase was washed four times or until the water phase was neutral. The chloroform phase was evaporated in a stream of air in a water bath at 50 °C. The methylated oligosaccharides were dissolved in 2 mL of ethyl acetate before analysis with GC.

Capillary Gas Chromatography and Gas Chromatography/Mass Spectrometry. Capillary GC was carried out on a Carlo Erba HRGC-5300 HT Mega Series (Milano, Italy) fitted with an on-column injector and an FID detector (temperature 400 °C). An HT polyimide-fused silica column (25 m × 0.32 i.d.) (Quartz and Silice, France) was static coated with cross-linked SE-54 (Alltech Associates, Deerfield, IL) according to the method of Blomberg et al. (1982); the film thickness was 0.05 μm. Helium was used as the carrier gas at a linear gas velocity of 60 cm/s at 60 °C. A high-capacity gas purifier (Oxisorb, Messer Griesheim, Germany) and a 5-Å molecular sieve (Alltech Associates) were used in the carrier gas line. Samples were dissolved in 2 mL of ethyl acetate and (0.6 μL; 3–100 ng/component) injected on-column at 40 °C. A linear temperature program (20 °C/min) was run up to 400 °C. Integration was performed with a PC-pack Softran Kontron Instrument (Milano, Italy). A standard mixture of saccharose, trehalose, maltose, lactose, raffinose, stachyose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, verbascose, and melezitose was used as a reference. The oligosaccharides were identified by comparison with retention

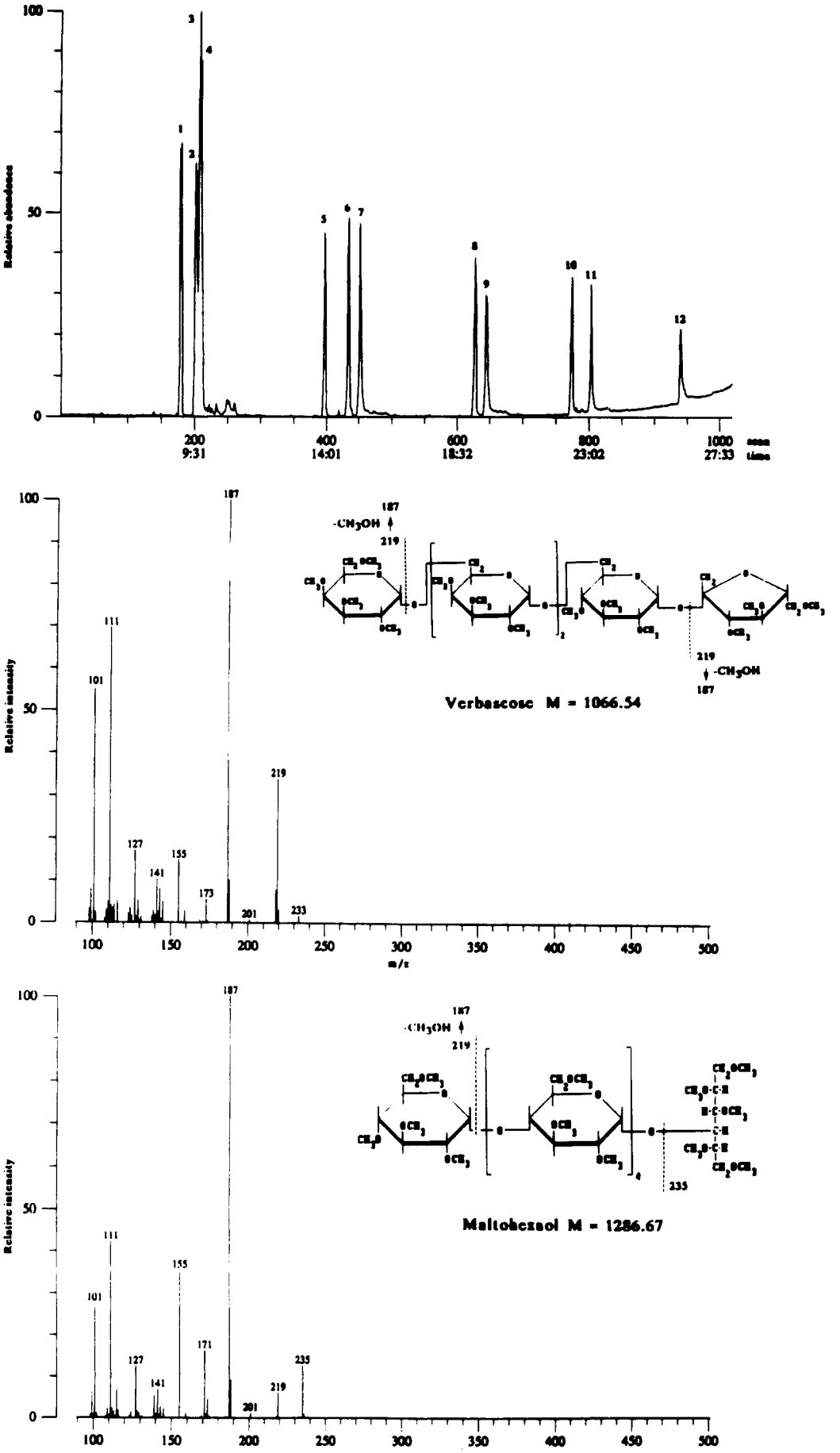

Figure 2. Total ion chromatogram from GC/MS of a mixture of reference methylated oligosaccharides and mass spectra of methylated verbascose and maltohexaol. (1) Saccharose; (2) trehalose; (3) maltose; (4) lactose; (5) melezitose, IS; (6) raffinose; (7) maltotriose; (8) stachyose; (9) maltotetraose; (10) verbascose; (11) maltopentaose; (12) maltohexaose.

Table I. Calculated Correction Factors for Different Sugars[a]

	saccharose	trehalose	maltose	lactose	raffinose	maltotriose	stachyose	maltotetraose	verbascose	maltopentaose	maltohexaose	maltoheptaose
calcd correction factors	0.94 ± 0.004	0.98 ± 0.03	0.94 ± 0.005	1.00 ± 0.03	0.91 ● 0.004	0.91 ± 0.007	0.97 ± 0.004	0.98 ± 0.007	1.09 ± 0.011	0.96 ± 0.02	1.06 ± 0.02	2.00 ± 0.02

[a] Peak areas for individual sugars were compared to that of the internal standard melezitose (mean ± SD of four replicate determinations).

Table II. Investigation of Optimal Extraction Time and Heating Time for Oligosaccharides in Two Soy Diets (Grams of Oligosaccharides per Daily Diet)

heating time at 100 °C, min	saccharose, extraction time of			maltose, extraction time of			raffinose, extraction time of			maltotriose, extraction time of			stachyose, extraction time of			maltotetraose, extraction time of		
	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h
								Total Grams in Soy Diet 1										
0	31.5	30.7	28.7	6.22	6.40	7.10	0.27	0.25	0.26	0.57	0.59	0.60	1.28	1.26	1.26	0.61	0.61	0.52
10	34.7	32.6	33.2	5.70	5.80	5.82	0.21	0.26	0.26	0.49	0.54	0.53	1.21	1.26	1.27	0.62	0.65	0.66
60	32.2	33.7	28.9	5.89	5.99	5.30	0.26	0.27	0.22	0.53	0.56	0.47	1.26	1.33	1.13	0.65	0.68	0.58
								Total Grams in Soy Diet 2										
0	15.9	15.2	14.9	13.2	13.3	13.7	0.26	0.24	0.27	0.56	0.50	0.52	1.35	1.32	1.33	0.51	0.41	0.44
10	15.9	12.6	15.8	11.9	8.37	11.8	0.23	nd[a]	0.23	0.44	nd	0.40	1.33	nd	1.27	0.45	nd	0.48
30	16.1	16.4	15.9	12.1	12.0	11.9	0.21	0.25	0.22	0.45	0.48	0.45	1.28	1.30	1.32	0.46	0.49	0.45

[a] nd, not determined.

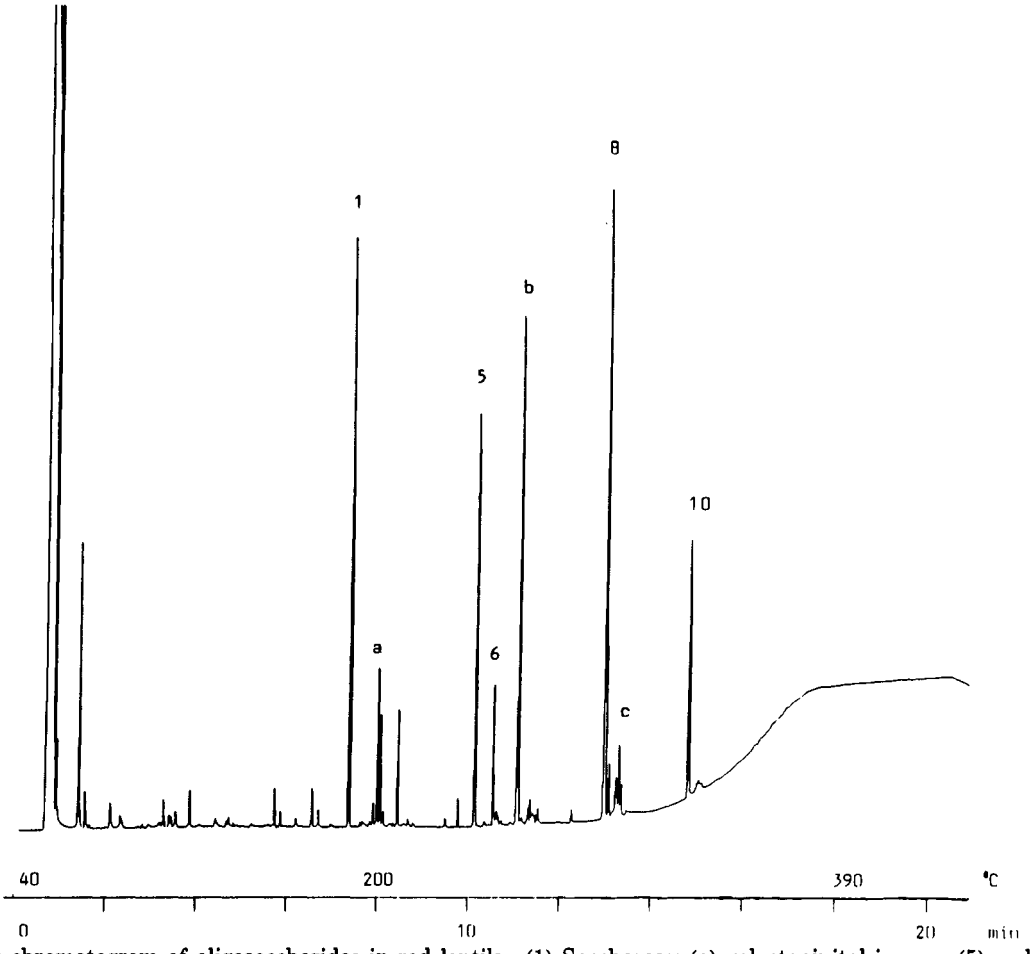

Figure 3. Gas chromatogram of oligosaccharides in red lentils. (1) Saccharose; (a) galactopinitol isomer; (5) melezitose, IS; (b) digalactopinitol isomer; (6) raffinose; (c) trigalactopinitol isomer; (8) stachyose; (10) verbascose.

times of pure standards and with gas chromatography/mass spectrometry.

A standard solution containing 0.2 mg of each sugar was prepared from stock solutions of each sugar containing 2 mg of sugar/mL. Correction factors for differences in response factors between sugars were calculated in relation to the peak area of the internal standard (melezitose). Impurities of the manufactured available sugar standards partly explain that the correction factors deviate from 1.0. Such impurities were also found by Molnár-Perl et al. (1984).

GC/MS was performed on a Carlo Erba 4160 gas chromatograph directly interfaced to a magnetic sector instrument (VG ZAB-HF, data system VG 11-250, VG Analytical, Manchester, U.K.) with a home-built interface. The fused silica column (10 m × 0.25 mm i.d.) was coated with cross-linked PS 264 (Fluka, Buchs, Switzerland); film thickness of 0.02 μm was introduced directly into the ion source, and the tip was positioned 1–2 mm from the electron beam. Helium was used as the carrier gas at 0.2 bar, giving a linear gas velocity of 90 cm/s at 70 °C. A high-capacity gas purifier and an OMI-1-indicating purifier (both

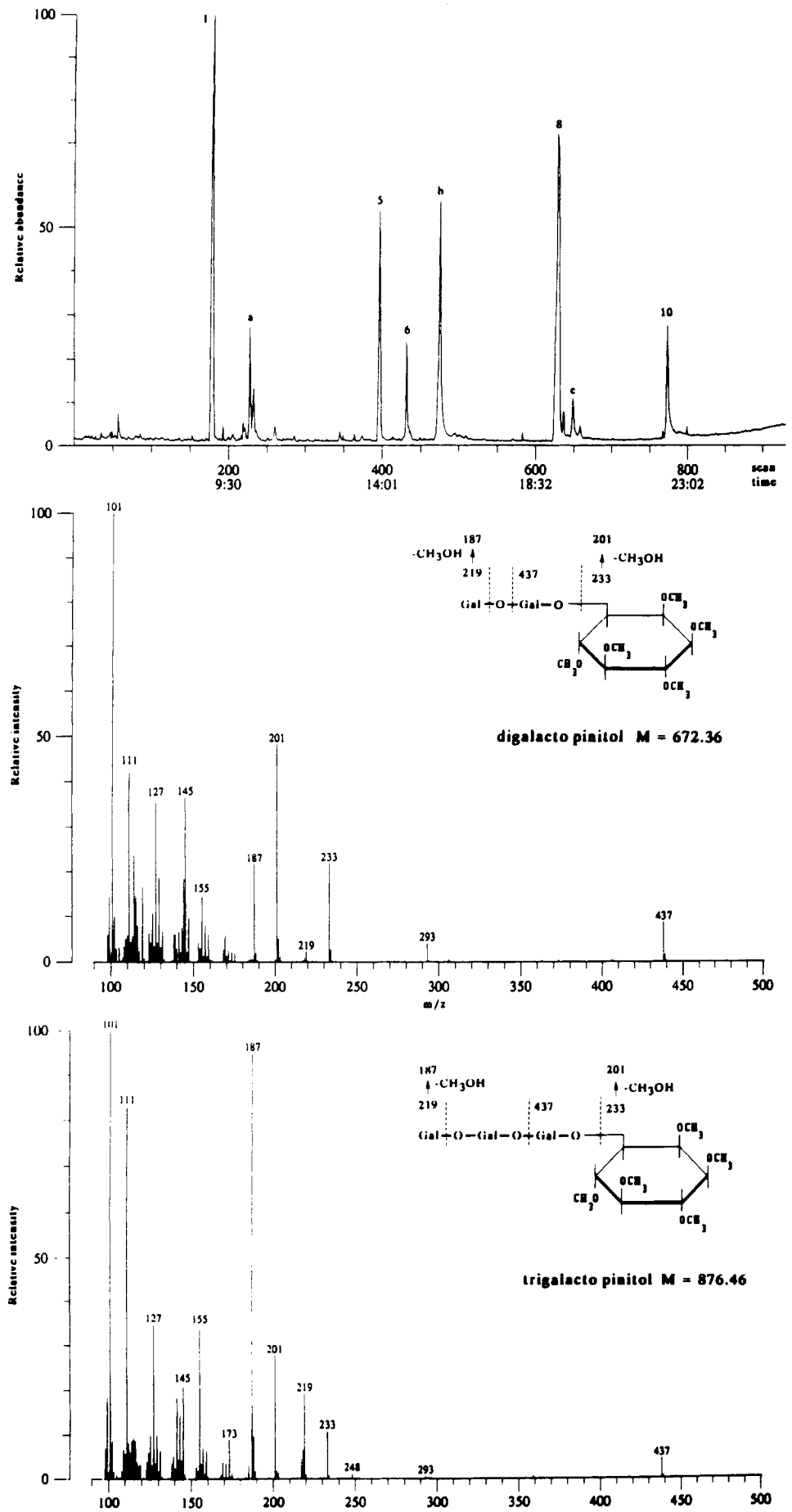

Figure 4. Total ion chromatogram from GC/MS of methylated oligosaccharides in red lentils and mass spectra of methylated digalactopinitol isomer (b) and trigalactopinitol isomer (c). (1) Saccharose; (a) galactopinitol isomer; (5) melezitose; (6) raffinose; (b) digalactopinitol isomer; (8) stachyose; (c) trigalactopinitol isomer; (10) verbascose.

Table III. Recovery (Percent; Mean ± SD of Duplicate Samples) of Standard Additions (0.2–0.8 mg) of Saccharose, Maltotriose, and Stachyose to a Soy Diet and to Ileostomy Contents

mg of sugar added	saccharose	maltotriose	raffinose	stachyose	verbascose	maltohexaose	maltoheptaose
soy diet							
0.2 mg of sugar added	98 ± 0.5	97 ± 0	94 ± 0.7	103 ± 1.2	101 ± 1.9	100 ± 0	95 ± 0
0.4 mg of sugar added	92 ± 1.3	99 ± 0.5	94 ± 0	100 ± 0.4	90 ± 0	90 ± 0	91 ± 0
0.8 mg of sugar added	91 ± 1.7	98 ± 0	95 ± 0	98 ± 0	102 ± 0.6	96 ± 0	91 ± 2.5
ileostomy contents							
0.4 mg of sugar added	90 ± 2.5	96 ± 1.2	92 ± 8.5	89 ± 1.2	nd[a]	nd	nd
0.8 mg of sugar added	90 ± 0.6	98 ± 3	98 ± 1.2	88 ± 1.2	nd	nd	nd

[a] nd, not determined.

Table IV. Oligosaccharide Content of Some Selected Foods, Diets, and Ileostomy Contents (Percent of Dry Weight; Mean ± SD)

sample	no. of sample	saccharose	maltose	raffinose	maltotriose	stachyose	maltotetraose	verbascose	ajugose
rapeseed meal[a] (*Brassica napus*)	8	6.70 ± 0.11	nd[c]	0.44 ± 0.01	nd	1.06 ± 0.02	nd	0.012 ± 0.001	nd
mung beans[a] (*Phaseolus aureus*)	5	1.62 ± 0.06	nd	0.46 ± 0.007	nd	1.85 ± 0.01	nd	3.34 ± 0.04	0.13 ± 0.003
red lentils[a] (*L. esculenta microsperm*)	8	1.35 ± 0.012	nd	0.24 ± 0.004	tr[d]	2.44 ± 0.03	nd	1.30 ± 0.03	nd
chickpeas[a] (*Cicer arietium*)	6	3.69 ± 0.05	nd	0.56 ± 0.01	nd	1.88 ± 0.01	nd	0.054 ± 0.004	nd
soybean[a] (*Glycine max*)	4	6.28 ± 0.2	nd	0.62 ± 0.006	nd	3.23 ± 0.035	nd	0.16 ± 0.005	nd
soybean[b] (*G. max*)	10	6.18 ± 0.16	0.17 ± 0.02	0.61 ± 0.02	nd	3.81 ± 0.11	nd	0.15 ± 0.005	nd
soy diet 1[b] g/24 h	20	32.0 ± 1.9	5.86 ± 0.39	0.25 ± 0.02	0.57 ± 0.04	1.29 ± 0.06	0.63 ± 0.04	nd	nd
intestinal contents[a] from soy period g/24 h	12	nd	nd	0.12 ± 0.01	nd	0.63 ± 0.03	nd	nd	nd

[a] IS melezitose. [b] IS lactose. The soy diets were composed of low-fiber diet with addition of textured soy flour. Intestinal contents were from a subject consuming soy diet. [c] nd, not detected. [d] tr, traces.

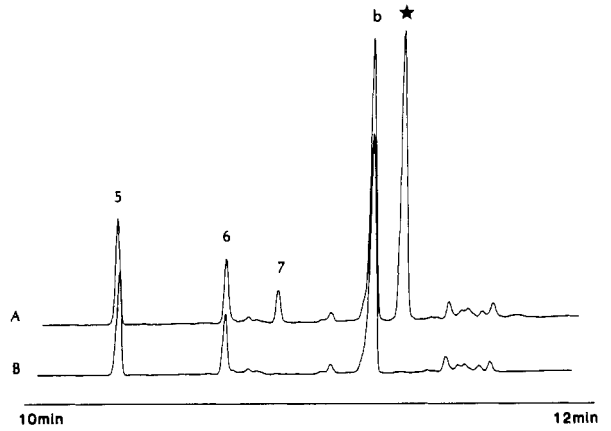

Figure 5. Expanded gas chromatogram of the trisaccharide region of chickpeas. (5) Melezitose, IS; (6) raffinose; (7) maltotriose; (b) digalactopinitol isomer. (A) Chickpeas with addition of manninotriose (★) and maltotriose (7); (B) chickpeas.

Supelco, Bellefonte, PA) were used in the carrier gas line. One microliter of sample (10–100 ng/component) dissolved in ethyl acetate was injected on-column at 70 °C. The column temperature was maintained at 70 °C for 1 min and then programmed to 390 °C at 10 °C/min. MS conditions were as follows: interface, 370 °C; ion source, 360 °C; electron energy, 70 eV; trap current, 500 μA; accelerating voltage, 8 kV; mass range scanned, m/z 900–90; scan speed, 1 s/decade; total cycle time, 1.5 s; resolution, 1000; pressure in the ion source region, 10^{-5} mbar.

RESULTS AND DISCUSSION

We found that oligosaccharides in foods with two to seven sugar units methylated according to the method of Ciucanu and Kerek (1984) were possible to chromatograph and determine quantitatively with the method described in the present paper. The methylation technique provides a simple alternative to the commonly used TMS procedure for the derivatization of sugars in foods. According to Karlsson et al. (1989), this methylation technique gave high yields for oligosaccharides in comparison with earlier methylation techniques (Hakomori, 1964; Finne et al., 1980), which are also more complicated. Figure 1 shows a gas chromatogram of a reference mixture of oligosaccharides containing two to seven sugar units. The sugars were identified by GC/MS. Figure 2 shows the corresponding total ion current chromatogram (TIC) together with the mass spectra of methylated verbascose and maltohexaol. These methylated oligosaccharides easily form a lot of fragment ions under electron impact ionization, but with the exception of a weak B_2 ion at m/z 423, no structurally informative sequence ions (B_i) (Domon and Costello, 1988) are observed. Only terminal fragment ions are present, i.e., at m/z 219 and m/z 187 (219 – CH_3-OH) together with low-mass fragment ions due to cleavage of the pyran rings. For maltohexaol, inductive cleavage of the alditol moiety gives a fragment ion at m/z 235.

The linearity of the sugar concentration vs peak area was investigated in the range 0–500 ng. The area is linearly proportional to the sugars (mixture of sacharose, melezitose, and raffinose) concentration over the entire range. The column is overloaded and the peaks become unsymmetrical at concentrations above 100 ng, and thus concentrations up to 100 ng were used for the calibration.

Melezitose was used as the internal standard as it normally is not present in foods and intestinal contents and it gives a peak that occurs at an appropriate retention time in the chromatogram. In some analyses of foods not containing lactose, this sugar was used as the internal standard. The correction factors for differences in detector response and yield for the different sugars (saccharose, trehalose, maltose, lactose, raffinose, maltotriose, stachyose, maltotetraose, verbascose, maltopentaose, maltohexaose, maltoheptaose) were calculated by comparison of the peak areas with that of the internal standard. Correction factors are given in Table I.

Optimal conditions for the extraction of oligosaccharides in diets were investigated. The soy diet used contained saccharides with up to four sugar units. Higher saccharides are seldom present in detectable concentrations in mixed

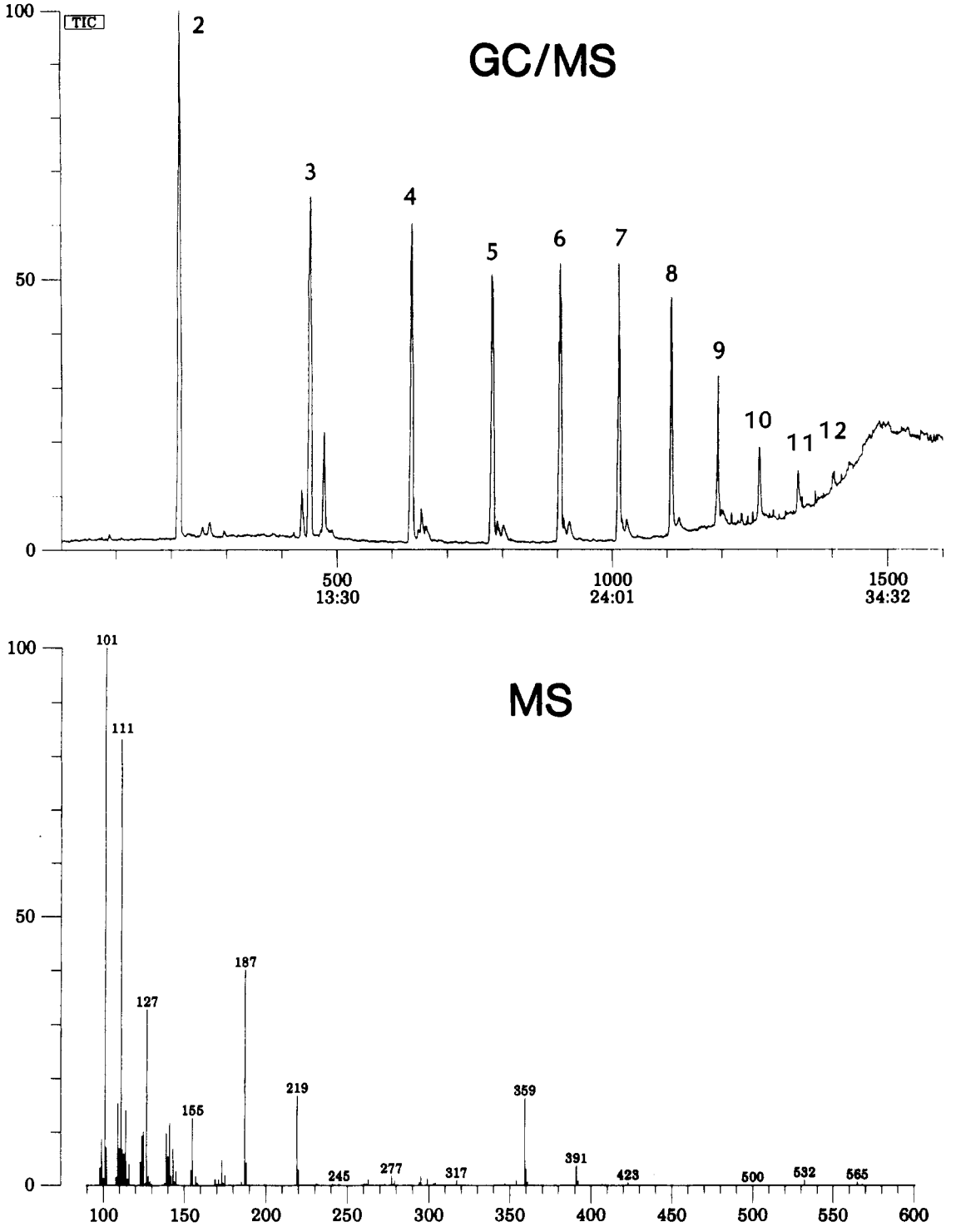

Figure 6. Total ion chromatogram from GC/MS of methylated fructooligosaccharides in Jerusalem artichokes and mass spectrum of a methylated fructooligosaccharide with nine sugar units (peak 9 above; M_r = 1882.9). The figures above the peaks in the GC/MS correspond to the number of sugar residues.

diets or are present in low concentrations. Effects of variations in extraction time and heating time are given in Table II. The differences in analytical values found for different extraction conditions were small. As optimal extraction conditions varied between sugars, a "mean" optimal condition was chosen, i.e., 2 h of extraction and 10 min of heating time (100 °C). The heating reduces the activity of oligosaccharide-degrading enzymes and prevents bacterial growth. When diet samples were spiked with different amounts of saccharose, maltotriose, stachyose, verbascose, maltohexaose, and maltoheptaose, the recoveries of added sugars were approximately 90–105%. The results of these standard additions to diet and intestinal contents are shown in Table III.

Applications of capillary gas chromatography for the analysis of oligosaccarides in foods, diets, and intestinal contents are shown in Table IV and Figures 3–6. The oligosaccharides were identified by GC/MS and comparison with retention times of standards. As demonstrated in Table IV, the method gave reproducible values for the selected materials. The accuracy of the GC method was from these results estimated to be about ±3.5%. Red lentils (Figures 3 and 4), soybeans, rapeseed, mung beans, and chickpeas were found to contain considerable amounts

of the raffinose family of oligosaccharides and of galactopinitol isomers. These pinitols were also found by Traitler et al. (1984) in soybeans. In mung beans detectable levels of a higher sugar, probably ajugose, were also found.

Our results for sugar content of rapeseed meal are in agreement with those of Bach Knudsen and Li (1991), who studied the same kind of meal, except for verbascose, which they could not detect. We found that verbascose was present in low concentrations. These concentrations were probably below the detection limit of their HPLC method. The content of the raffinose family of oligosaccharides in mung beans was similar to that of Kuo et al. (1988), analyzed by HPLC, and that of Aman (1979), determined by GC. However, with their methods it was not possible to determine the highest saccharide, which likely is ajugose. For oligosaccharide content in soybeans it is more difficult to compare with literature values as some studies were done on defatted flours (Schweizer, 1978; Kuo et al., 1988; Bach Knudsen, 1991) and considerable genetic variations occur (Kennedy et al., 1985). The values for raffinose oligosaccharides found in this study are, however, comparable to values found by other groups (Sosulski et al., 1982; Kennedy et al., 1985), which also is the case for chickpeas (Schweizer et al., 1978; Aman, 1979; Sosulski et al., 1982). These authors also found considerable amounts of a saccharide suggested to be manninotriose in chickpeas. Addition of manninotriose, prepared by mild acid hydrolysis [according to Aman (1979)], to the chickpeas and analysis by GC showed a peak separated from the peak suggested to be manninotriose (Figure 5). We believe that this peak occurring before manninotriose in the chromatogram instead constitutes a digalactopinitol isomer as it elutes on the same retention time as the digalactopinitol isomer identified by GC/MS in the red lentils. We have not found values in the literature for the variant of red lentils (*Lens esculenta microsperm*) used in this study.

In the soy diet, mainly composed of rice, meat, fish, and white wheat bread with the addition of textured soy flour (1), considerable amounts of raffinose, maltotriose, stachyose, and maltotetraose were found. Details on this diet have been described previously (Sandström et al., 1986). Raffinose and stachyose were identified in the corresponding intestinal contents of a subject consuming this soy diet. Approximately half of the dietary intake of raffinose and stachyose from the soy diet was recovered in the intestinal contents.

The present method for determination of oligosaccharides also has potential for analysis of fructooligosaccharides in foods. Figure 6 shows a total ion chromatogram from GC/MS of methylated fructooligosaccharides with up to 12 sugar units together with a mass spectrum of a fructooligosaccharide with 9 sugar units (M_r = 1882.9).

CONCLUSIONS

Analyses with high-temperature capillary GC gave consistent and reproducible values for a number of oligosaccharides (with two to seven sugar units) of foods and biological samples. The rapidity of this method as compared with that of previous GC methods and its high sensitivity and selectivity as compared with HPLC make it a useful method for the analysis of oligosaccharides.

The method will be used in further studies of the effect of food processing on hydrolysis of oligosaccharides and their digestion in the human gut.

ACKNOWLEDGMENT

This study was supported by the Swedish Council for Forestry and Agricultural Research (Projects 824/85 L 39 and 599/89L 135:2).

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Received for review June 1, 1992. Accepted August 31, 1992.

Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3; maltotriose, 1109-28-0; maltotetraose, 34612-38-9; maltose, 69-79-4; verbascose, 546-62-3; ajugose, 512-72-1; maltohexaose, 34620-77-4; maltoheptaose, 34620-78-5; trehalose, 99-20-7; lactose, 63-42-3; maltopentaose, 34620-76-3.