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Selectivity control in the anion-exchange chromatographic determination of saccharides in dairy products using pulsed amperometric detection

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ABSTRACT

The separation of D-glucose, D-galactose, D-fructose, lactose, lactulose, saccharose, maltose, and malto-oligomers on an anion-exchange column in combination with pulsed amperometric detection is described. Resolution was optimized by using separate sodium hydroxide and sodium acetate gradients, generated by a quaternary solvent delivery system. The influence of lithium, potassium, or tetrabutylammonium ions on the separation was found to be negligible. The sample treatment of dairy products consisted of 100-fold dilution with water, followed by ultrafiltration. The recovery of the saccharides in milk was found to be 98.2 to 101.8%, while the repeatability was 0.7% to 5.6%. Determination of saccharides in an infant formula, in a fruit yogurt, and in a candy product are shown. The l.c. anion-exchange chromatographic system described enables the determination of the majority of saccharides present in food products in a single analysis.

INTRODUCTION

The most prominent carbohydrate of milk is lactose. In dairy products lactose may be hydrolyzed to D-glucose and D-galactose by enzymes. In addition to these carbohydrates, other saccharides or saccharide-containing components may be added in order to give the food product more desirable properties. The saccharides encountered most frequently are saccharose, fructose, maltose, and malto-oligomers. Heat treatment (UHT or sterilization) of the food product converts a part of the lactose to lactulose.

High-performance liquid chromatography (h.p.l.c.) has been routinely applied for more than a decade by many laboratories for the determination of saccharides in food products[1–6]. Separations on silica gel-based columns require a high percentage of an organic modifier (e.g., acetonitrile) in the eluent, which severely limits the solubility of the saccharides. Moreover, the response with a refractive index detector is rather poor, due to the unfavorable refractive index of the eluent. Amino- and diol-modified silica gel columns fail to give sufficient resolution between glucose and galactose[7], and the amino-type column shows undesirable reactivity towards reducing sugars[6]. The use

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of cation-exchange resin columns with water as the eluent eliminates the drawbacks of the silica gel-based columns[8]; however the separation capability for di- and higher oligo-saccharides is limited. With a mixed cation-exchange resin column, an acceptable resolution between lactose, maltose and saccharose could be obtained[8], but the combination of maltotriose–saccharose and lactulose–glucose still required an additional analysis.

Anion-exchange chromatography with high-pH eluents in combination with pulsed amperometric detection enabled the separation of mono-, di-, and oligo-saccharides at high sensitivity[10–12]. The present work describes the sample pretreatment and an improved gradient anion-exchange separation of saccharides, in combination with pulsed amperometric detection, for many common food products.

RESULTS

The separation of a standard mixture of saccharides, using a linear gradient of 0–0.25M sodium acetate in 0.1M sodium hydroxide, is shown in Fig. 1. Under these conditions two pairs of saccharides coeluted from the column. Moreover, some minor peaks were present in the chromatogram. These peaks are probably formed by chemical reactions of the saccharides during analysis. The resolution for compounds with a low retention times on an anion-exchange resin column can be improved by lowering the concentration of hydroxide and/or acetate at the outset. Fig. 2a shows the optimized separation of the standard mixture of saccharides. Variations in the first part (0–15 min) of the gradient primarily influence the retention of the malto-oligosaccharides (Fig. 3). The delayed effect of the gradient on the retention of saccharides is caused by the

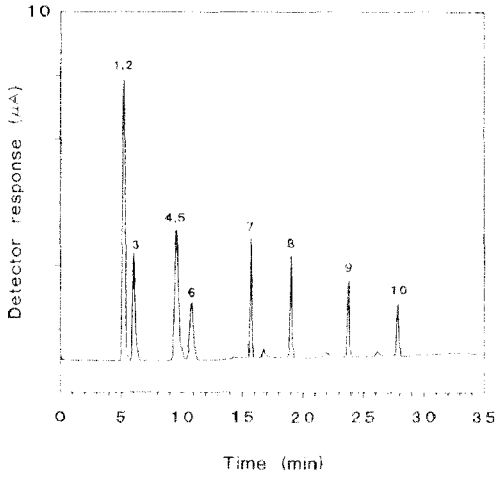


Fig. 1. Separation of a standard mixture of saccharides. Gradient: 100% C linear to 50% C and 50% D in 25 min; Column temperature: 22°. Peak 1, D-galactose; peak 2, D-glucose; peak 3, saccharose; peak 4, D-fructose; peak 5, lactose; peak 6, lactulose; peak 7, maltose; peak 8, maltotriose; peak 9, maltopentaose; and peak 10, maltoheptaose.

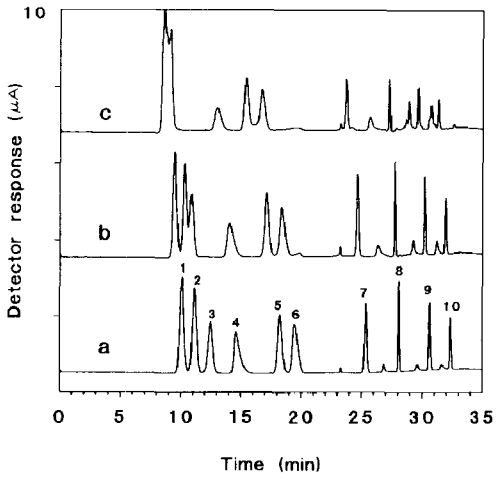


Fig. 2. Influence of the column temperature on the separation of a standard mixture of saccharides. Column temperature: a, 22°; b, 30°, and c, 40°; for gradient, see Table III; for peak identification, see the legend to Fig. 1.

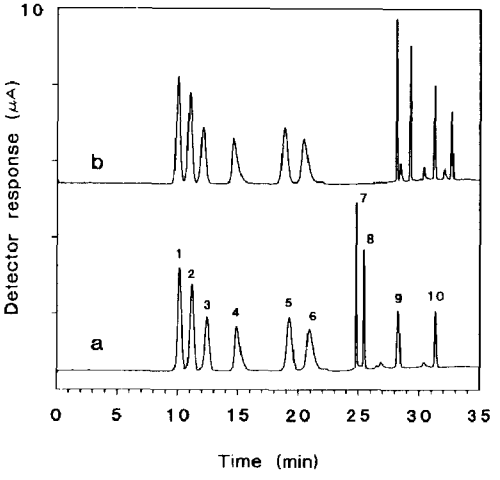


Fig. 3. Separation of a standard mixture of saccharides at a constant hydroxide (a) and a constant acetate (b) concentration during the first 15 min. of the gradient. Gradient: a and b, 70% A and 30% B isocratic during 2 min; a, in 13 min. linear to 26% A, 70% B, and 4% C; b, in 13 min. linear to 41% A, 35% B, and 24% C. Both a and b linear in 10 min. to 50% C and 50% D (see Table III). For peak identification, see the legend to Fig. 1.

considerable ion-exchange capacity of the stationary phase in relation to the ionic strength of the eluent at the initial conditons. The influence of different equilibration delays on the chromatogram of the standard mixture is shown in Fig. 4. The retention of saccharose is especially sensitive to the equilibration delay. Optimum results were obtained with a delay time of 15 min.

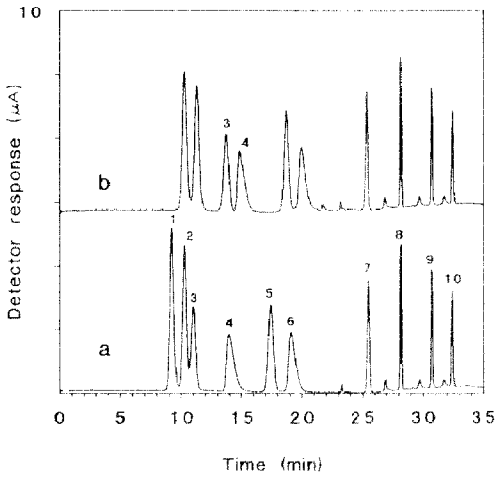


Fig. 4. Influence of the equilibration time of the column on the separation of the standard mixture of saccharides; a, 8 min.; b, 30 min. For peak identification, see the legend to Fig. 1.

If a delay time of 20 min was used, the separation also became very sensitive to the solvent composition at the initial conditions, whereas, at a delay time of 15 min, the separation was shown to the more resistant towards minor changes in solvent composition.

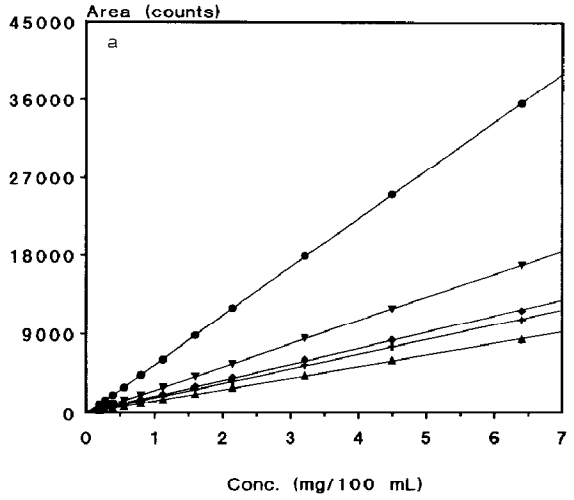
The slow equilibration of the column with low concentrations of hydroxide and acetate is responisble for this phenomenon. Therefore, the column was equilibrated with a high concentration of sodium hydroxide in the presence of a small amount of acetate, in order to rapidly remove the excess of acetate from the column. After this rinse, the column was equilibrated during a period of 15 min with the solvent composition of the initial conditions. While this procedure does not result in a column which is fully equilibrated, when use is made of an automatic sample injector with constant intervals between the injections, repeatable results can be obtained.

The peak shapes of D-fructose and lactulose were sensitive to the age of the dilute sodium hydroxide eluant (A). Excessive peak broadening occured when this eluent was in use for more than 24 h. Exposing the eluent to the ambient air for a few h, in order to induce the uptake of carbon dioxide, did not result in an increase of the peak widths. Possibly, leaching of a component out of the glass eluent bottle could be responsible for the observed phenomenon.

The influence of other monovalent cations on the separation was also investigated. Replacement of sodium by potassium, lithium, or tetrabutylammonium did not markedly change the selectivity between the saccharides. Tetrabutylammonium hydroxide could be used only at low concentrations in combination with sodium hydroxide because of the loss of sensitivity of the detector.

Column temperature influenced the separation. The resolution between D-galactose, D-glucose, and saccharose decreased on increasing column temperatures (Fig. 2).

Morcover, deterioration of saccharides increased at temperatures above 30°, as evidenced by the appearance of an extra peak after the lactulose peak and an increase in the area of unidentified peaks eluting near the malto-oligosaccharides. At 40° a flat valley formed between the peaks of lactose and lactulose, indicating a dynamic equilibrium. To our knowledge, there are no reports in the literature regarding the optimum column temperature for these separations. At room temperature (22°), the unidentified peaks are present near the peaks of the malto-oligosaccharides. As a lower column temperature would thus seem to be desirable, this aspect will be the subject of future research. On the basis of these results, and due to practical limitations, the column temperature



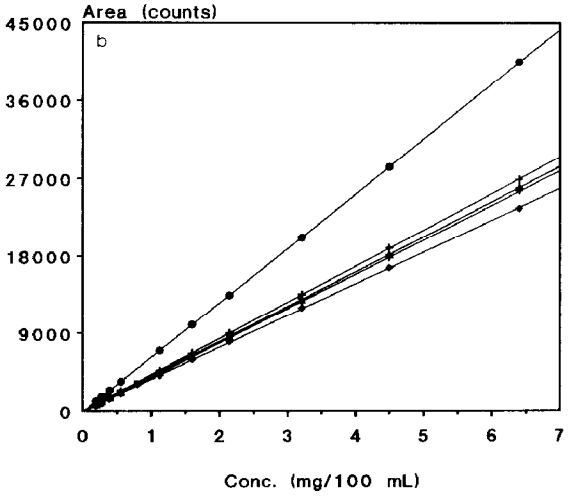


Fig. 5. Calibration curves: a, ●, D-glucose; ▼, maltose; ◆, maltotriose; +, maltopentaose; ▲, maltoheptaose; b, ●, D-galactose; ▼, saccharose; ◆, D-fructose; +, lactose; ▼, lactulose.

TABLE I

Coefficients of linear regression and 95% confidence limits for predicted saccharide content at a mean area count corresponding with a saccharide concentration of 0.2, 3.2, and 6.4 mg/100 mL[a]

Saccharide	*a*[b]	*b*[b]	*95% confidence limit*					
			0.2[c] *μg/100 mL*	*3.2*	*6.4*	*0.2* %	*3.2*	*6.4*
D-Galactose	6340 (± 3)	120 (± 9)	3[d]	3	6	1.7	0.1	0.1
D-Fructose	4018 (± 4)	−167 (±10)	5	5	10	2.7	0.2	0.2
Saccharose	3706 (±17)	−13 (±47)	28	23	48	17	0.7	0.8
Lactose	4221 (± 8)	−25 (±23)	12	10	21	6.0	0.3	0.3
Lactulose	4073 (± 5)	−83 (±14)	7	6	14	3.4	0.2	0.2
D-Glucose	5590 (± 7)	−94 (±21)	8	7	15	3.9	0.2	0.2
Maltose	2635 (± 4)	−6 (±10)	8	7	15	4.4	0.2	0.2
Maltotriose	1818 (±16)	113 (±43)	51	46	94	35	1.4	1.5
Maltopentaose	1653 (±13)	102 (±37)	49	44	89	28	1.3	1.4
Maltoheptaose	1313 (±10)	65 (±27)	45	40	83	26	1.2	1.3

[a] Calculations were performed with the program RRGRAPH (version 4.52, Stichting Reactor Research, 1990, Delft University Press). [b] Area counts = $a \times$ concentration (mg/100 mL) + b. The value in parenthesis gives the standard error. [c] Units: mg/100 mL. [d] At a mean area count, the result of an analysis is 200 ± 3 μg/100 mL with 95% confidence.

was kept at room temperature (22 °). The effect of decomposition of the malto-oligosaccharides can be eliminated to a great extent by appropriate calibration.

The calibration curves for the saccharides of the standard mixture were found to be linear in the range of 0.2 to 6.4 mg/100 mL; using a full-scale setting of 10 μA of the detector (Fig. 5). The malto-oligosaccharides showed slightly less linear behavior, which might be caused by their decomposition during analysis; however, these compounds can be determined with sufficient accuracy, except at the low end of the calibration range (see Table I). The intercepts of the y-axis were, for the majority of the compounds statistically different from zero, but were in all cases small (Table I).

The sample pretreatment according to Biggs and Szijarto[13] was found to be incompatible with the eluent used as a precipitate formed upon mixing. We have, therefore, developed another sample clean-up procedure, which is based on ultrafiltration. As the high sensitivity of pulsed amperometric detection enables a large dilution of the sample solution, and ultrafiltration of dilute solutions proceeds quickly with a minimum of concentration polarization, the technique is a near-ideal technique for sample purification. Table II shows that the recoveries obtained for the saccharides of the standard mixture added to milk vary between 98.2 and 101.8%. The repeatability was determined for the same mixture of saccharides in milk by carrying out the complete determination five times.

An application of this method is shown in Fig. 6 for the determination of saccharides in an infant formula, a fruit yogurt, and a candy.

TABLE II

Recovery of saccharides added to skim milk and repeatability of saccharide determination in skim milk.

Saccharide	*Mean content* *mg/g*	*Recovery*[a] *%*	*Repeatability*[b] *μg/g*	*%*
D-Galactose	5.06	100.9	133	2.6
D-Fructose	5.02	100.5	37	0.7
Saccharose	5.20	101.1	291	5.6
Lactose	46 + 29.8	100.3	119	2.6
Lactulose	5.23	101.8	226	4.3
D-Glucose	4.93	100.2	124	2.5
Maltose	4.89	100.4	181	3.7
Maltotriose	4.80	99.4	255	5.3
Maltopentaose	1.99	98.2	68	3.4

[a] Average of four injections. [b] Repeatability: $r = 2\sqrt{2} \times$ standard deviation; $n = 5$.

TABLE III

Gradient used for chromatography of saccharides

Time min.	*%A*	*%B*	*%C*	*%D*	*curve*
0	70	30	0	0	—
2	70	30	0	0	linear
15	80	0	15	5	linear
25	0	0	50	50	linear
28	0	0	98	2	linear
35	0	0	98	2	—
37	70	30	0	0	linear
52 next injection					

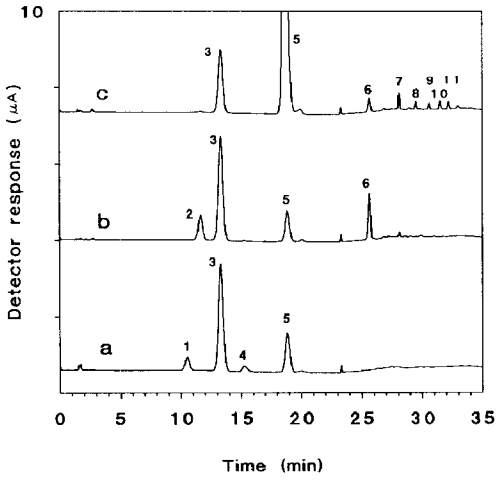


Fig. 6. Determination of saccharides in a, fruit yogurt; b, candy; and c, infant formula. Peak identification: peaks 1–5: see the legend to Fig. 1; peak 6, maltose; peak 7, maltotriose; peak 8, maltotetraose; peak 9, maltopentaose; peak 10, maltohexaose; and peak 11, maltoheptaose.

EXPERIMENTAL

Chromatography. — Separations were carried out on a M600 quaternary gradient pumping system in combination with a WISP 712 automatic sample injector (both from Millipore–Waters) with a Dionex Carbopak PA1 column and matching guard column. The saccharides were detected with a pulsed amperometric detector (EG&G Princeton Applied Research, Model 400 [reference electrode: Ag/AgCl, 3M KCl] or Dionex PED [reference electrode: Ag/AgCl, 3M KCl]) containing a gold electrode to which potentials of E_1 0.1, E_2 0.6, and E_3 −0.8 V were applied for duration times T_1 0.4, T_2 0.3, and T_3 0.3 s. A 50% (w/w) solution of sodium hydroxide (Analar, BDH) and anhydrous sodium acetate (E. Merck) were used to prepare eluents in water, which was filtered and degassed with helium prior to the addition of these compounds. All solvents were stored in closed, pressurized vessels with helium sparging (ESM, Millipore–Waters). Composition of solvents A, B, C, and D were as follows: A, 0.0125M sodium hydroxide (prepared daily!); B, 0.008M sodium acetate; C, 0.125M sodium hydroxide; D, 0.125M sodium hydroxide and 0.5M sodium acetate. The flow-rate was set at 1.0 mL/min, and the gradient used for the chromatography of saccharides is given in Table III.

Sample treatment. — To a weighed amount of dairy product (~1 g, if the individual saccharide content is in the range of 0.5%), 40 mL of water (40°) was added. After swirling the mixture, the volume was made up to 100 mL with water. Of this solution, 2 mL were placed in an ultrafiltration cup (UFP1 LGC, Millipore), the first few drops were discarded, after which 1 mL of ultrafiltrate was collected; 10 µL of the ultrafiltrate was injected into the h.p.l.c. instrument.

Calibration, recovery, and repeatability. — Appropriate amounts (corresponding to the composition of the product to be analyzed) of saccharides were weighed, and water was added to 100 mL. If necessary, dilutions were made. The recovery was determined by weighing about 50 mg of each saccharide (except lactose) of the standard set and a weighed amount (~10 g) of skim milk was added. About 1 g of this solution was used for the sample treatment. The same solution was used to asses the repeatability by carrying out the sample treatment five times.

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