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Determination of sugars and alditols in food samples by HPAEC with integrated pulsed amperometric detection using alkaline eluents containing barium or strontium ions

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The addition, in mobile phase, of alkaline-earth divalent cations such as Sr(II) and Ba(II) is shown to be a versatile means of improving the separation of alditols and carbohydrates, which are conventionally determined by high-performance anion-exchange chromatography (HPAEC) coupled with integrated pulsed amperometry using gold working electrodes. It is believed that Ba(II) or Sr(II) ions are effective both in forming solution complexes with sugar analytes, and ensuring thoroughly carbonate-free alkaline mobile phase flowing through the column. As a result, substantial benefits can be obtained in terms of peak shape, attainable column efficiency, and detection sensitivity. This work describes determination of alditols (*myo*-inositol, D-sorbitol, and D-mannitol), and carbohydrates (glucose, fructose, and sucrose) in some common fruits and fruit products (apricot, plum, watermelon, lemon, mandarin, grape, and dietetic cherry jam) and vegetable foodstuffs (cauliflower, cucumber, fennel, tomato, turnip, and celery). The free sugar contents in fruit juices were determined directly, without pretreatment or derivatization, following a sample extraction. While *myo*-inositol was found in many fruits, fruit products and vegetables examined, no presence of xylitol was detected.

INTRODUCTION

Sorbitol, mannitol, and inositols together with glucose, fructose, and sucrose are naturally occurring and widely distributed in the vegetal kingdom. Their determination is currently of great interest in nutrition, medical cell biology and biotechnology research as these compounds are often used as food additives. A variety of enzymatic and chromatographic methods have been devised for their determination in many real samples (Robards and Whitelaw, 1986; Churms, 1990; Gorton *et al.*, 1994). Carbohydrates and alditols, also known as sugar alcohols, have similar structures, and are difficult to separate by conventional reversed-phase liquid chromatography. The hydroxyl groups of alditols and monosaccharides have pK_a values in the range 12–14 (Rendleman, 1973), allowing ionization at alkaline solutions and potential separation by high performance anion-exchange chromatography (HPAEC).

Pulsed amperometric detection (PAD) is nowadays the most commonly used method for analysis of sugars and alditols (Johnson and LaCourse, 1990; Johnson *et al.*, 1993; Corradini, 1994; Lee, 1996). This detection mode has been used with success to monitor several groups of analytes: sugar alcohols, mono-, di-, and oligosaccharides. Rocklin and Pohl (1983) and Edwards and Haak (1983) first applied HPAEC with PAD to the determination of carbohydrates. Not only does this analytical technique provide more efficient separation and detection than other chromatographic methods, it also minimizes sample preparation. The triple-pulse detector at a gold electrode requires high pH conditions for direct detection of alditols and sugars, so the use of alkaline-tolerant polymeric columns with anion-exchange capabilities seems to be particularly suitable for this aim.

Anion-exchange chromatography is a reliable separation scheme provided that carbonate-free alkaline mobile phases are employed. Moreover, the eluent has to be degassed to prevent absorption of carbon dioxide producing carbonate. A technical note of the column

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manufacture claims 'It is extremely important to minimize contamination of the eluent solutions with carbonate. Carbonate, being a divalent anion at pH 12, binds strongly to the columns and interferes with carbohydrate binding, causing a drastic decrease in column selectivity and a loss of resolution and efficiency' (Dionex, 1994). Recently, we have reported that slightly altering the alkaline mobile phase composition by strontium or barium ions results in enhanced overall column and detection performances (Cataldi *et al.*, 1997). It is believed that the basis of the improved separations involves an effective removal of carbonate ion from the eluent, and the formation in the highly alkaline mobile phase of charged complexes between the analytes and alkaline-earth metal ions. Interestingly, reagent-grade barium or strontium salts are less expensive than sparging the alkaline mobile phase with nitrogen or helium presently used for reducing the uptake of carbon dioxide. The objective of the present study, thus, was to demonstrate the potential of using barium or strontium ions in the alkaline eluent. The analysis of some selected fruits, fruit products and vegetables containing alditols (*myo*-inositol, D-sorbitol, and D-mannitol) and simple carbohydrates (glucose, fructose, and sucrose) in anion-exchange chromatography with integrated amperometry are reported.

EXPERIMENTAL

Reagents and chemicals

Sodium hydroxide, 50% w/w solution in water ($d = 1.515\,g\,ml^{-1}$), $Sr(CH_3COO)_2$ (water ~3%), $Ba(CH_3COO)_2$ 99%, D-fructose, D-ribose were purchased from Aldrich (Milan, Italy), *myo*-inositol, xylitol, D-sorbitol, D-mannitol, D-glucose and sucrose were from Sigma (Milan, Italy), and were used as received. Stock solutions of sugars, and alditols were prepared in pure water containing 0.1% sodium azide to prevent microbial growth. Samples to be injected were prepared, just before use, from the stock solutions by dilution to the desired concentration with pure water. Other chemicals employed were of analytical grade and were used without further purification. Doubly distilled, deionized water was used throughout for preparing solutions. Sodium hydroxide solutions used as the mobile phases were prepared by diluting carbonate-free 50% (w/w) NaOH solution and kept in plastic bottles.

Chromatographic system and detection

All chromatograms were generated using a metal-free isocratic pump (Dionex, Sunnyvale, CA), Model IP20; a Dionex pulsed amperometric detector (Model ED40); a Dionex metal-free rotary injection valve with 10 μl injection loop; a Dionex column CarboPac MA1, 8.5 μm particle dia. (250×4 mm i.d.) coupled with a guard CarboPac MA1column (5×4 mm i.d.). The flow-through detection cell (Dionex) contained a gold working electrode and a Ag/AgCl reference electrode. The counter electrode was provided by the titanium upper half of the detection cell. Acquisition and processing of chromatographic data were done by a personal computer equipped with the Kontron PC Integration Pack software (Kontron Instruments, Milan, Italy). All chromatographic analysis were carried out at ambient temperature with flow-rates of 0.4 or 0.5 ml min^{-1}. Conditions employed here were suitable for the efficient separation and detection of sugars and alditols and did not result in any apparent deterioration of column performance.

As detection mode we adopted a modified version of pulsed amperometry that involves the integration of current, which is called integrated pulsed amperometric detection (IPAD) (LaCourse and Johnson, 1993). The oxidation current integrated with respect to time gives a net charge (q) for the detection cycle, so the response is measured in coulombs. The applied potentials (mV) and pulse durations (ms) were the following: $E_{OX} = +650$, ($t_{OX} = 190$ ms), $E_{DET} = +50$, ($t_{DEL} = 150$ ms, and $t_{INT} = 300$ ms), and $E_{RED} = -150$, ($t_{RED} = 340$ ms). E_{OX} and E_{RED} are used to oxidatively remove adsorbed species and to reduce the gold oxide formed, respectively, in order to minimize fouling of the working electrode. All experiments were carried out at ambient temperature.

Sample preparation

Dietetic cherry jam (Dr Faralli, Arezzo, Italy), a weighed amount (1 g) of dietetic cherry jam, purchased from a local store, was dissolved in water (100 ml), sonicated for a few minutes (10–15 min) and centrifuged; the extract was then filtered, diluted 1:100 and injected. *Celery* (*Apium graveolens*, var. dulce Miller), celery and celery leaves were crushed and weighed amounts of each (5 g) were suspended in water (50 ml), then the mechanical suspensions were sonicated at room temperature for half an hour and two aliquots were diluted 1:25. Then, *ca* 5 ml of the resulting solutions were filtered by passage through 0.45-μm membranes, and the eluate was analyzed. For free sugars, other foodstuff extracts such as *fennel* (*Foeniculum vulgare*, var. dulce Miller), *cauliflower* (*Brassica oleracea*, L. var. Botrytis), and *Italian turnip broccoli* (*Brassica campestris* L.), were treated in a similar way. *Lemon juice* (*Citrus limonum* L.), the juice of a ripe lemon was diluted with pure water 1:100, and 5 ml were filtered and further diluted 1:5. *Mandarin juice* (*Citrus clementei* Hort.), the juice of a mandarin was diluted with pure water 1:100, and 5 ml were filtered and further diluted 1:10. The resulting solution was injected. A similar extraction procedure was accomplished with *watermelon* (*Citrullus lanatus* Thunb.), *Italian grapes* (*Vitis vinifera* L.), and *tomato* (*Lycopérsicon esculentum* Mill.). *Cucumber*

(*Cucumis sativus* L), a cucumber was first peeled, homogenized and upon filtration the extract was diluted 1:100 with pure water. Then, an aliquot was filtered and diluted 2:5 before injection. *Italian plums* (*Prunus domestica*, subspp. *insititia* L.), 1 g of homogenized fresh plums was dissolved in 100 ml of water and sonicated for 15 min. The extract was then filtered, diluted 1:10 and injected. *Apricot juice (Santal, Parmalat, Parma, Italy)*, an apricot juice sample (sugar contents 14.1 g per 100 ml), procured from a local retail shop, was diluted in water (100 ml), sonicated and centrifuged; the extract was then filtered, diluted 1:10 and injected. All samples were passed through 0.45-μm Nylon filters before injection to remove particulate, and 10 μl of the final eluate was injected.

RESULTS AND DISCUSSION

HPAEC with pulsed amperometric detection

Anion-exchange chromatography with integrated pulsed amperometric detection (IPAD) at a gold electrode in strongly alkaline solutions is now a well-established technique for CHOH-bearing compounds. Their combination provides a selective and very sensitive means of analyzing a variety of matrices for sugar and alditol content. The differences in dissociation constants form the basis of a competitive elution mechanism where different retention factors (k') are obtained with solutes of different pK_a values. Because of the weakly acidic properties of alditols (see Table 1), which are even weaker acids than carbohydrates (Rendleman, 1973) there is a need for strongly alkaline eluents in which these compounds are ionized sufficiently to make possible their separation by anion-exchange. Disaccharides are much more strongly retained than monosaccharides. Very recently, we have demonstrated that monosaccharide alditols and simple sugars can be successfully separated by AEC using isocratic conditions with Ba(II)- or Sr(II)-containing sodium hydroxide solutions as the eluent (Cataldi *et al.*, 1997). The action of these alkaline-earth ions seems to be two-fold: very effective removal of carbonate ion content in the mobile phase, and ability to complex cyclic and acyclic polyhydroxy compounds (Angyal, 1989). This last effect leads to a marked shift in the equilibrium of conformers toward the more stable metal–sugar complexes. As a consequence, the peak symmetry and concurrently the column efficiency is greatly improved. A typical chromatogram of a standard solution obtained with 0.58 M NaOH + 2 mM $Ba(CH_3COO)_2$ is shown in Fig. 1. As can be seen, it is possible to perform fast and selective separations under these chromatographic conditions without the need of sparging the alkaline mobile phase with nitrogen or helium gas. Several examples of alditols and sugars profiles of food products are presented below.

Table 1. Dissociation constants of some alditols and saccharides in water at 25°C

Compound	pK_a
myo-Inositol	—
Xylitol	—
D-Sorbitol	13.60[a]
D-Dulcitol	13.43[a]
D-Mannitol	—
D-Galactose	12.39[a]
D-Arabinose	12.43[b]
D-Glucose	12.28[a]
D-Xylose	12.15[a]
D-Mannose	12.15[b]
D-Lyxose	12.11[b]
D-Ribose	12.11[b]
D-Fructose	12.03[a]
Sucrose	12.62[c]
Lactose	11.98[c]
Maltose	11.94[c]

[a]Dean in Lange (1985).
[b]Rendleman (1973).
[c]Degani (1971).

Calibration plots, limits of detection, and precision

The linear response of calibration plots was tested with several model compounds: *myo*-inositol, xylitol, D-sorbitol, D-mannitol, D-glucose, D-fructose, D-ribose, and

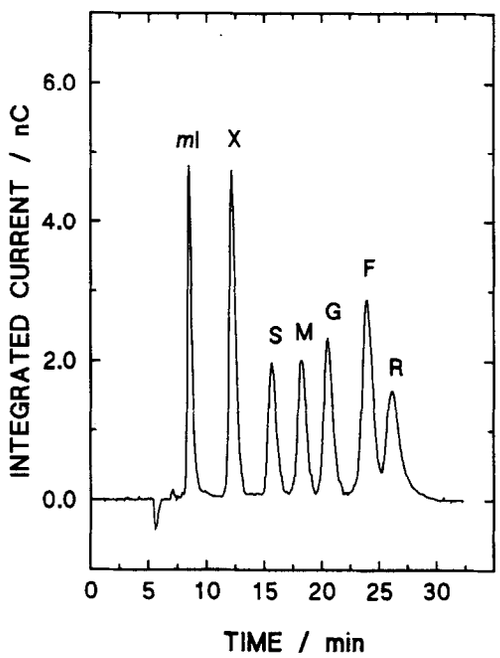

Fig. 1. Separation of alditols and carbohydrates in HPAEC using 2 mM Ba(II) in 0.58 M NaOH as the mobile phase. Peaks and concentrations: (*m*I) *myo*-inositol, 2.5 μM; (X) xylitol, 10 μM; (S) D-sorbitol, 4 μM; (M) D-mannitol, 4 μM; (G) D-glucose, 5 μM; (F) D-fructose, 10 μM; and (R) D-ribose, 5 μM. Flow rate 0.40 ml min^{-1}. Detection at a gold working electrode: $E_{DET} = 0$ mV.

Table 2. Quantitative parameters of common alditols and sugars determined by HPAEC with IPAD[a]

Analyte	k'	Linear range: signal (nC) = $a + b \times C^b$			Repeatability ($n=3$) % RSD; μM
		$a \pm t_{95} \times s_a$ (nC)	$b \pm t_{95} \times s_b$ (nC μM^{-1})	r	
myo-Inositol	0.55	0.6 ± 0.8	1.92 ± 0.01	0.999 98	1.1; 2.5
Xylitol	1.23	−0.1 ± 0.5	0.473 ± 0.001	0.999 98	1.6; 10
D-sorbitol	1.87	−0.1 ± 0.1	0.480 ± 0.001	0.999 98	2.2; 4
D-mannitol	2.34	−0.1 ± 0.1	0.510 ± 0.001	0.999 97	2.3; 4
D-glucose	2.76	−0.1 ± 0.2	0.464 ± 0.001	0.999 98	2.6; 5
D-fructose	3.39	0.1 ± 0.4	0.145 ± 0.002	0.999 96	2.9; 20
D-ribose	3.79	0.1 ± 0.4	0.290 ± 0.001	0.999 97	2.5; 5
Sucrose	5.91	0.1 ± 0.5	0.232 ± 0.003	0.999 91	2.8; 10

[a]Column, CarboPac MA1 plus guard column; flow rate, 0.4 ml min^{-1}; alkaline mobile phase: 0.58 M NaOH + 2 mM $Ba(CH_3COO)_2$; loop, 10 μl.
[b]Regression lines calculated by least-squares analysis; the confidence limits for the slope and intercept were evaluated with t taken at 95% confidence level.

Table 3. Quantitative results of alditol and sugar contents in common food samples

Food sample	Concentration (g $litre^{-1}$)					
	myo-Inositol	D-sorbitol	D-mannitol	D-glucose	D-fructose	Sucrose
Apricot juice[a]	0.21	0.87	—	23.32	9.22	97.8
Cucumber[b] (*Cucumis sativus*)	0.23	0.08	—	3.62	5.43	0.3
Grape juice[a] (*Vitis vinifera*)	—	—	—	6.02	2.45	—
Lemon juice[b] (*Citrus limonum*)	0.34	—	—	17.52	17.44	4.1
Mandarin juice[b] (*Citrus clementei*)	0.60	0.49	—	12.43	14.41	57.6
Watermelon juice[a] (*Citrullus lanatus*)	0.38	—	—	15.64	16.61	29.6
	Concentration (g kg^{-1})					
Cauliflower[b] (*Brassica oleracea*)	0.27	—	0.05	5.80	7.02	1.5
Fennel[a] (*Foeniculum vulgare*)	0.17	0.04	0.24	10.72	10.70	4.0
Tomato[b] (*Lycopérsicon esculentum*)	0.11	0.05	—	8.04	12.32	0.3
Italian turnip broccoli[b] (*Brassica campestris*)	0.39	—	0.17	6.22	4.10	1.0
Celery[b] (*Apium graveolens*)	—	—	4.81	1.64	2.21	0.2
Celery leaves[b] (*Apium graveolens*)	0.13	—	4.43	0.21	—	0.2
Italian Plums[a] (*Prunus domestica*)	0.48	2.43	—	12.13	3.24	45.6
Dietetic cherry-jam[b]	—	641.2	8.5	26.9	19.4	—

[a]Mobile phase: 0.50 M NaOH + 1 mM $Sr(CH_3COO)_2$.
[b]Mobile phase: 0.58 M NaOH + 2 mM $Ba(CH_3COO)_2$.

sucrose. Good chromatographic separation was obtained and data of the peak-height calibration plots are summarized in Table 2. The linearity extended over three orders of magnitude (μM–mM) above the limit of detection (LOD) with correlation coefficients (r) of at least 0.9999. Each data point ($n = 6$–7 concentrations in the linear range) was generated from at least three injections. The standard deviation (SD) of slope and intercept was estimated at the 95% confidence level. The LODs were calculated to be below 500 pmol (S/N = 3) for the eight compounds studied. Precision was evaluated at a concentration level of 10 times the detection limits. In Table 2 the relative standard deviations (RSD) are also reported of peak heights using three replicate injections; good repeatability for each compound was obtained, that is always below 3.0%.

Determination of sugars and alditols in real samples

Carbohydrates and sugar alcohols play a major role as structural (e.g. cellulose and hemicellulose) components of higher plants and fruits. Table 3 summarized the composition of sugars present in the common food samples examined in this study. The compounds of interest were determined, without interferences, in fruits, fruit products and vegetables such as cherry jam, watermelon, lemon, mandarin, grape, plum, apricot, celery, fennel, cauliflower, turnip, and cucumber. The extractions were accomplished as described in the Experimental Section. The analysis of compounds in samples of dietetic cherry jam, celery, and mandarin is illustrated in Figs 2, 3 and 4, respectively. Use of sorbitol as an alternative sweetener is increasing rapidly in

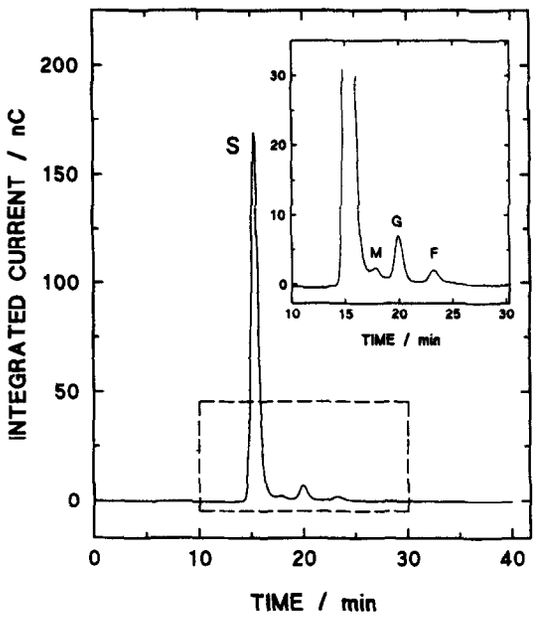

Fig. 2. Chromatographic separation of a dietetic cherry jam. Peak identification: (S) D-sorbitol, (M) D-mannitol, (G) D-glucose, and (F) D-fructose. Other conditions as in Fig. 1.

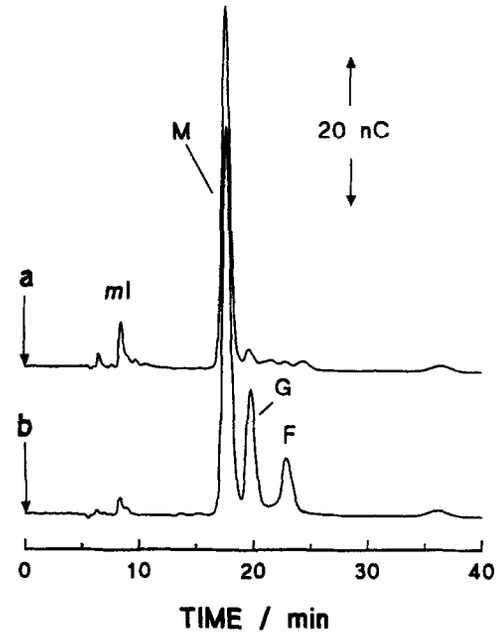

Fig. 3. Chromatographic separations of extracts from celery leaves (a) and celery (b). Peak identification: (*m*I) *myo*-inositol, (M) D-mannitol, (G) D-glucose, and (F) D-fructose. Conditions as in Fig. 1.

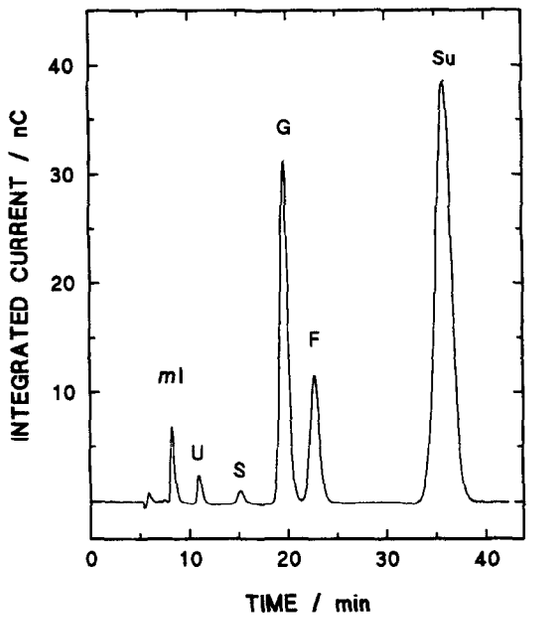

Fig. 4. Chromatogram of a sample of mandarin juice. The dilution factor before injection was 1:1000. Peak identification: (*m*I) *myo*-inositol, (U) unknown, (S) D-sorbitol, (G) D-glucose, (F) D-fructose, and (Su) sucrose. Conditions as in Fig. 1.

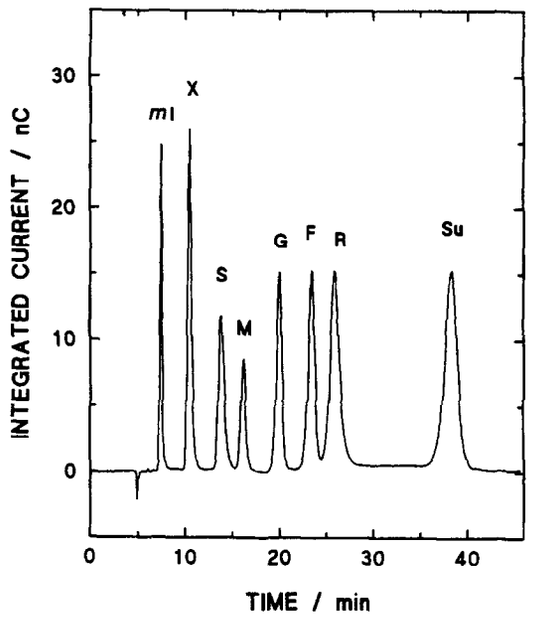

Fig. 5. Separation of a standard solution by HPAEC–IPAD using 1 mM Sr(II) in 0.50 M NaOH as the mobile phase. Peaks and concentrations: (*m*I) *myo*-inositol, 6.2 μM; (X) xylitol, 50 μM; (S) D-sorbitol, 20 μM; (M) D-mannitol, 20 μM; (G) D-glucose, 20 μM; (F) D-fructose, 25 μM; (R) D-ribose, 25 μM; and (Su) sucrose, 50 μM. Flow rate 0.50 ml min^{-1}.

particular as a substitute for glucose in antidiabetic diets. The chromatographic separation of a cherry jam added with sorbitol is shown in Fig. 2. Indeed, a large amount of sorbitol is present (64 %, w/w), in reasonable agreement with the value reported on the label, 56%, but no detectable sucrose. It should be emphasized that when no well-degassed mobile phases are used, D-sorbitol exhibits a pronounced peak distortion due to tailing. We have previously suggested that complex formation between Sr(II) and alditols or sugars is accompanied by a change of conformation that may be responsible of the considerable improvement of peak symmetry (Cataldi *et al.*, 1997).

D-mannitol is widely distributed in higher plants and is abundant in vegetable foodstuffs such as celery (Davis *et al.*, 1988, and Loescher *et al.*, 1992). Direct injection of samples extracted from celery and celery leaves of the

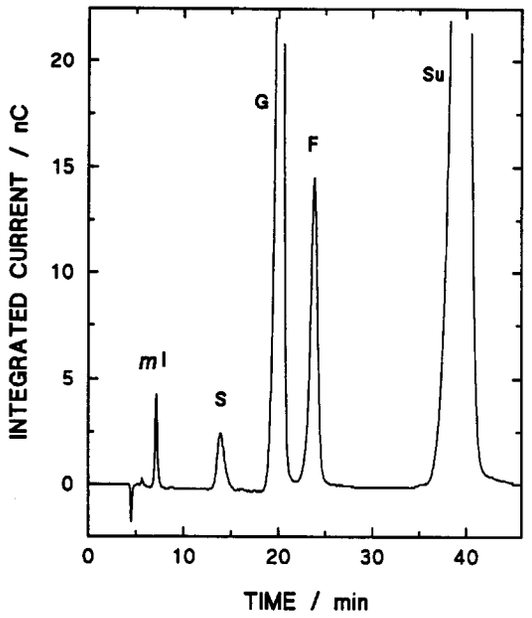

Fig. 6. Chromatographic separation of apricot juice. Peaks: (*m*I) *myo*-inositol, (S) D-sorbitol, (G) D-glucose, (F) D-fructose, and (Su) sucrose. Experimental conditions as in Fig. 5.

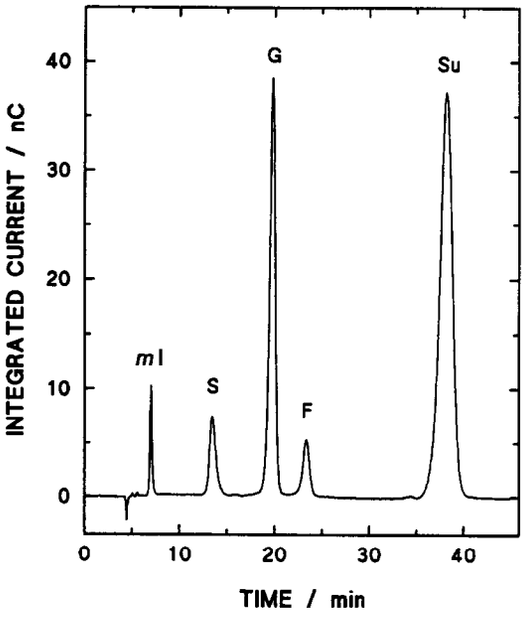

Fig. 7. Chromatographic separation of plum juice. Peaks: (*m*I) *myo*-inositol, (S) D-sorbitol, (G) D-glucose, (F) D-fructose, and (Su) sucrose. Conditions as in Fig. 5.

same plant provides an interesting comparison of the sugar contents (see Fig. 3). The amounts of mannitol were similar in the edible part of celery (4.81 g kg^{-1}) and celery leaves (4.43 g kg^{-1}), and such values are comparable to 10.6 g kg^{-1} reported by Loescher *et al.* (1992). Also, the sugar components of mandarin juice were determined using the present chromatographic method (Fig. 4). The following peaks were identified: (*m*I) *myo*-inositol, (S) D-sorbitol, (G) D-glucose, (F) D-fructose and (Su) sucrose. The unidentified peak (U) may be another naturally occurring sugar or alditol. Although the glucose/fructose ratio was 0.86, a value

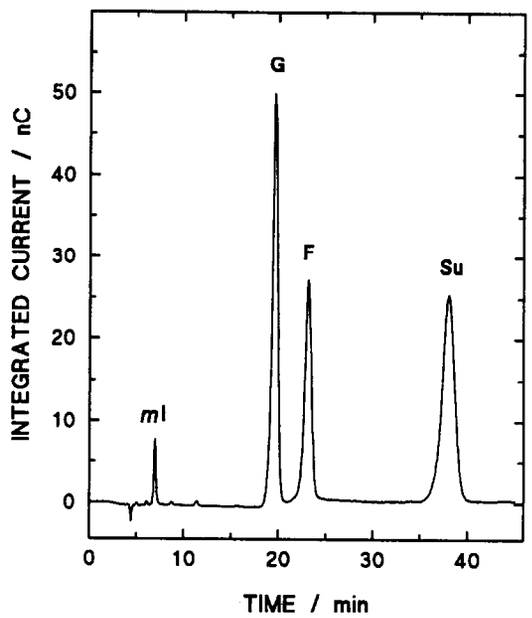

Fig. 8. Chromatographic separation of watermelon juice. The dilution factor before injection was 1:1000. Peaks: (*m*I) *myo*-inositol, (G) D-glucose, (F) D-fructose, and (Su) sucrose. Conditions as in Fig. 5.

very close to that determined in the lemon juice (1.00), the sucrose concentration was found about 14 times greater.

In Fig. 5 is shown a representative chromatogram of a standard solution obtained with 0.50 M NaOH containing 1 mM Sr(II) as the eluent. The mobile phase flow rate was 0.5 ml min^{-1}. Calibration curves were similar to those reported in Table 2. Figures 6 and 7 illustrate the separations of two flavoured fruits, apricot juice and fresh plums, respectively. For each sample the chromatogram is interference-free with few peaks clearly recognizable. Together with *myo*-inositol and D-sorbitol, three major peaks were present as common to most fruit juices. The retention time of standard compounds was used to identify each chromatographic peak. Note that no xylitol was found in any of the samples examined. Figure 8 shows a chromatogram of watermelon juice. In this sample four sugar compounds were identified and quantified: *myo*-inositol, D-glucose, D-fructose, and sucrose. As prevalent to all tested juices three major free sugars were present, albeit their ratios are unique to each juice. Moreover, while *myo*-inositol emerged as common to most fruits and vegetables, the presence of sorbitol and/or mannitol is related to each sample (see Table 3). Grape juice contains only glucose and fructose, but no detectable sucrose or alditols. Less diluted samples of grape juice showed no presence of the sucrose peak.

In summary, we have demonstrated some applications in the analysis of sugars and alditols in fruit juices and vegetable foodstuffs by anion-exchange chromatography with integrated pulsed amperometric detection. In short analysis times (25–35 min) good separations

were achieved with high reliability in retention times, using Ba(II) or Sr(II)-containing alkaline mobile phases. It is anticipated that their use will find increasing application for sugars and alditols determination in real samples.

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REFERENCES

Angyal, S. J. (1989) Complexes of metal cations with carbohydrates in solution. *Advances in Carbohydrate Chemistry Biochemistry* **47**, 1–43.

Cataldi, T. R. I., Centonze, D. and Margiotta, G. (1997) Improved separation and pulsed amperometric detection of alditols and carbohydrates by anion-exchange chromatography using alkaline mobile phases modified with Ba(II), Sr(II), and Ca(II) Ions. *Analytical Chemistry* (in press).

Churms, S. C. (1990) Recent developments in the chromatographic analysis of carbohydrates. *Journal of Chromatography* **500**, 555–583.

Corradini, C. (1994) Recent advances in carbohydrate analysis by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD). *Annalidi Chimica (Rome)*, **84**, 385–396.

Degani, C. (1971) Ionization constants of sugars: a predominant factor in the cyanogen-induced phosphorylation of sugars. *Carbohydrate Research* **18**, 329–332.

Davis, J. M., Fellman, J. K. and Loescher, W. H. (1988) Biosynthesis of sucrose and mannitol as a function of leaf age in celery (*Apium graveolens* L.). *Plant Physiology* **86**, 129–133.

Dionex Corporation (1994) Analysis of carbohydrates by high performance anion exchange chromatography with pulsed amperometric detection (HPAE–PAD). *Technical Note*, **20**.

Edwards, P. and Haak, K. K. (1983) A pulsed amperometric detector for ion chromatography. *International Laboratory* **June**, 38–44.

Gorton, L., Marko-Varga, G., Dominguez, E. and Emnéus, J. (1994) Analysis of mono-, oligo- and polysaccharides by enzyme reaction detection. In *Analytical Applications of Immobilized Enzyme Reactors*, eds S. Lam and G. Malikin, pp. 51–130. Blackie Academic and Professional, Chapman and Hall, London.

LaCourse, W. R. and Johnson, D. C. (1993) Optimization of waveforms for PAD of carbohydrates on pulsed voltammetry. *Analytical Chemistry* **65**, 50–55.

Lange's (1985) *Handbook of Chemistry* ed. J. A. Dean 13th edn. MacGraw-Hill, New York.

Lee, Y. C. (1996) Carbohydrate analysis with high-performance anion-exchange chromatography. *Journal of Chromatography A* **720**, 137–149.

Loescher, W. H., Tyson, R. H., Everard, J. D., Redgwell, R. J. and Bieleski, R. L. (1992) Mannitol synthesis in higher plants. *Plant Physiology* **98**, 1396–1402.

Johnson, D. C. and LaCourse, W. R. (1990) Liquid chromatography with pulsed electrochemical detection at Au and Pt electrodes. *Analytical Chemistry* **62**, 589A–595A.

Johnson, D. C., Dobberpuhl, D. A., Roberts, R. A. and Vandeberg, P. J. (1993) Pulsed amperometric detection of carbohydrates, amines and sulfur species in ion chromatography—the current state of research. *Journal of Chromatography* **640**, 79–96.

Rendleman, J. A. Jr (1973) In *Carbohydrates in Solution, Advances in Chemistry Series*, ed. H. S. Isbell, Vol. 117, pp. 51–68. American Chemical Society, Washington, DC.

Robards, K. and Whitelaw, M. (1986) Chromatography of monosaccharides and disaccharides. *Journal of Chromatography* **373**, 81–89.

Rocklin, R. D. and Pohl, C. A. (1983) Determination of carbohydrates by anion exchange chromatography with pulsed amperometric detection. *Journal of Liquid Chromatography* **6**, 1577–1590.