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Analysis of cationic nutrients from foods by ion chromatography

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ABSTRACT

This paper describes the feasibility of combining two relatively new technologies to generate data on the cationic nutrient content of foods. Single-column ion chromatography was used to monitor several analytes following the use of a microwave digestion scheme aimed at rapid, multiple sample digestion. The result is a more streamline and productive approach to multi-sample preparation and multi-analyte determination when investigating the cation content of foods.

Linearity and limits of detection for the chromatographic procedure were established. Sample size as well as digestion acid type and amount were investigated during the microwave process. The method was applied to a variety of food matrices to evaluate its scope. Results generated with this method compare favorably to those from atomic absorption.

Finally, capillary ion electrophoresis (Waters' trade name: Capillary Ion Analysis), a subset of capillary electrophoresis which has been optimized for ion analysis, was applied to the sample digests to investigate the usefulness of this technology to the analysis of mono-/divalent cations from foods.

INTRODUCTION

Currently, the analysis of cationic nutrients in foods is a routine procedure using atomic absorption spectroscopy (AAS) or inductively coupled plasma spectroscopy (ICP). The former is a tedious process allowing only single analyte determination and the latter represents a significant investment in instrumentation. In both cases, microwave or hot-plate acid digestion as well as combustion oven ashing are used for sample preparation. These techniques are lengthy and require time consuming hands on manipulation of the samples.

The development of new ion chromatography (IC) chemistries has provided the ability to separate mono- and divalent cations in a single analysis. Coordination IC performed on a poly(butadiene–malic acid)-coated silica column with an EDTA–nitric acid isocratic eluent and conductimetric detection allows the separation and detection of nutritionally relevant cations such as sodium, potassium, magnesium and calcium [1,2].

Microwave technology has made a broad entry into the analytical laboratory for a variety of sample preparation procedures. Microwave acid digestion of food matrices has been shown to be useful prior to cationic nutrient determination by AAS for more rapid sample preparation [3]. Complete digestion of multiple samples (10 plus a standard and blank) in less than 1.5 h, as well as the ability to automate the process, positions microwave technology as a desirable sample preparation alternative, especially when used in conjunction with a multiple analyte technique such as IC.

This study incorporates both of these unique technologies. The microwave digestion procedure was optimized relative to sample size and which acids to use in order to provide a digest which was compatible with the ion chromatographic process. Sample concentrations and dilutions were optimized relative to the sensitivity/mass load capabilities of the chromatography. A variety of food samples were chosen to be representative of the diversi-

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ty of matrices which could potentially challenge a food analysis method.

A preliminary evaluation of the application of capillary ion electrophoresis (CIE) (Waters' trade name: Capillary Ion Analysis, CIA) to this analysis was also conducted. All sample digests were introduced into a capillary electrophoresis system in which the chemistry was optimized for cation separation [4]. Good separations of the analytes of interest with no sample induced baseline upsets were observed with this technology and the results were compared to AAS.

EXPERIMENTAL

Instrumentation

The microwave digestion apparatus was a CEM MDS 2000 equipped with lined digestion vessels and the pressure-control option from CEM Corp., Matthews, NC, USA. The liquid chromatograph consisted of an Action Analyzer, a WISP 712 autoinjector, a Model 431 conductivity detector, a Waters IC-Pak C M/D column, and a Model 860 Expert Ease chromatography and data management system, all from Millipore, Waters Chromatography, Milford, MA, USA. A Quanta 4000 capillary electrophoresis unit and AccuPure fused-silica capillaries, also from Millipore, Waters Chromatography, were used for the CIE portion of the study.

Eluents and electrolytes

The eluent used for the IC was a solution of 0.1 m*M* ethylenediaminetetraacetic acid (EDTA free acid) and 3.0 m*M* nitric acid.

The electrolyte used for the CIE separations was a 1.2 m*M* UV-Cat-2 and 3.0 m*M* Tropolone solution.

Reagents

EDTA free acid was analytical-reagent grade from J. T. Baker, Phillipsburg, NJ, USA. Nitric acid was Ultrex grade and hydrogen peroxide (30%) was analytical-reagent grade both from J. T. Baker. Sodium, potassium, magnesium and calcium standards were prepared from sodium chloride, potassium chloride, magnesium nitrate hexahydrate and calcium nitrate tetrahydrate, respectively, and all were Sigma grade, Sigma, St. Louis, MO, USA. Tropolone was reagent grade from Aldrich, Milwaukee, WI, USA. UV-Cat 2 was from Millipore, Waters Chromatography. Water purified (18 MΩ) using a Millipore Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA) was used for all solutions.

RESULTS AND DISCUSSION

All samples were prepared using the procedure outlined in Fig. 1 prior to analysis by IC, AAS and CIE. The amount of nitric acid used for the digestion process was minimized to 3 ml. Through experimentation this proved to be enough acid to complete the digestion without challenging the chromatographic equilibrium during a 100-μl injection.

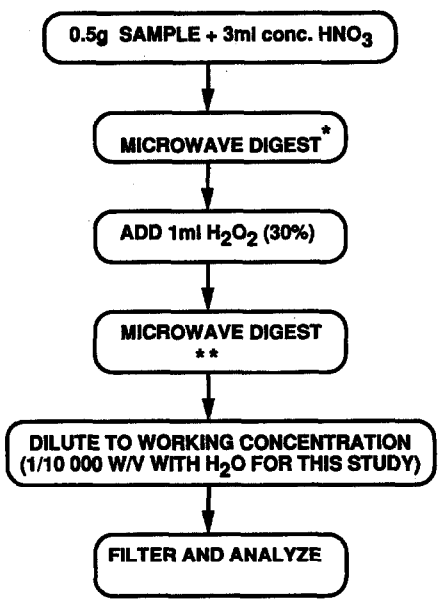

Fig. 1. Schematic of samples preparation used for all three (AAS, IC and CIE) methods of analysis. Notes: * CEM MDS 2000 microwave with lined digestion vessels and pressure control; 1 p.s.i. = 6894.76 Pa.

	Stage				
	1	2	3	4	5
% Power (varies with wattage of system and No. of samples)	30	30	30	30	30
Pressure (p.s.i.)	20	40	85	125	170
Time (min)	15	15	15	15	15
Time at pressure (min)	5	5	5	5	5

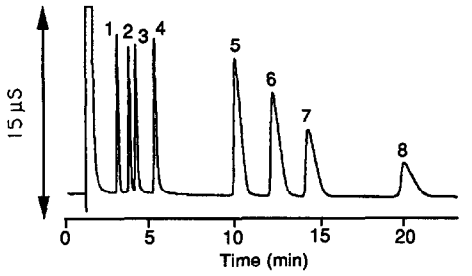

Fig. 2. Standard separation of eight mono- and divalent cations with coordination chromatography. Conditions, column: Waters IC-Pak C M/D; eluent: 0.1 m*M* EDTA–3.0 m*M* nitric acid, flow-rate: 1.0 ml/min; detection: conductivity; injection 100 µl. Peaks: 1 = lithium (0.25 mg/l); 2 = sodium (1.0 mg/l); 3 = ammonium (1.0 mg/l); 4 = potassium (3.0 mg/l); 5 = magnesium (2.0 mg/l); 6 = calcium (3.0 mg/l); 7 = strontium (5.0 mg/l); 8 = barium (5.0 mg/l).

Using more than 3 ml of nitric acid for the digestion procedure produced a large negative response just prior to the sodium peak which restricted integration routines. However, since only 3 ml of nitric acid could be used, the samples had to be submitted to a secondary microwave step involving H_2O_2, and sample size had to be limited to 0.5 g in order for the digestion procedure to produce water clear digests suitable for the subsequent dilutions prior to injection.

The IC used for this experiment had the ability to separate more than just the mono- and divalent cations of nutritional importance. Fig. 2 exemplifies this with an eight-cation separation. Using this chromatography and a series of standard solutions, linearity statements for both the mono- and divalent cations lithium, sodium, potassium, magnesium, calcium, strontium and barium were generated. For these cations the correlation coefficients are better than 0.9996 over a range of 0 to 10 mg/l for monovalent cations and 0 to 20 mg/l for divalent cations. This represents the range of these analytes typically found in the final injection solution of food digests. Table I lists the detection limits of this IC technique as determined with low level standard injections and based on a 3:1 signal-to-noise ratio.

TABLE I

DETECTION LIMITS FOR MONO- AND DIVALENT CATIONS USING COORDINATION CHROMATOGRAPHY WITH CONDUCTIMETRIC DETECTION AS IN FIG. 2

Calculated based on a 3:1 signal-to-noise ratio of standards.

Analyte	Concentration (µg/l)
Lithium	1
Sodium	5
Ammonium	5
Potassium	20
Magnesium	5
Calcium	10
Strontium	50
Barium	100

The samples chosen for this study were intended to represent a wide range of the cations of interest and also a diversity of sample matrices. Pretzels (salted), parsley (dried), bread crumbs, parmesan cheese and peanut butter all present different opportunities for matrix related excipients to potentially interfere with the chromatography. However, as can be supported by the examples of Figs. 3–7, the combination of the acid–peroxide microwave digestion and coordination IC produce an interference free chromatogram.

Pretzels, representing a high sodium (salt added) content sample generated the chromatogram seen in Fig. 3. The sodium peak rises out of the negative response created by the digestion solution residues. This digestion solution response creates a challenge for peak integration. The lift-off point for the sodi-

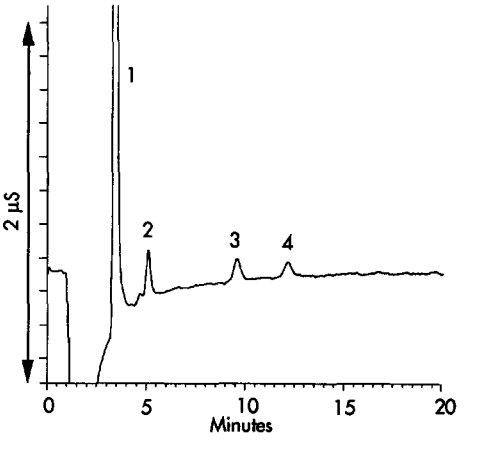

Fig. 3. Cation analysis from pretzels with coordination chromatography following microwave digestion. Conditions as in Fig. 2. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = sodium (1.47%); 2 = potassium (0.106%); 3 = magnesium (0.0224%); 4 = calcium (0.0214%).

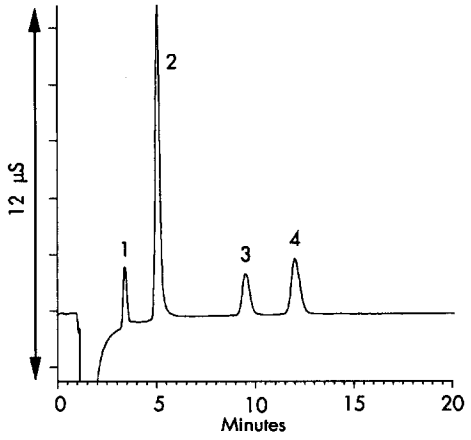

Fig. 4. Cation analysis from parsley with coordination chromatography following microwave digestion. Conditions as in Fig. 2. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = sodium (0.405%); 2 = potassium (4.78%); 3 = magnesium (0.27%); 4 = calcium (0.579%).

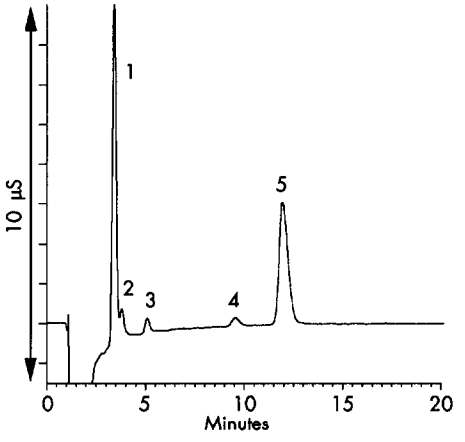

Fig. 6. Cation analysis from parmesan cheese with coordination chromatography following microwave digestion. Conditions as in Fig. 2. Sample diluted 1/10 000. Peaks and amounts in original concentration: 1 = sodium (1.72%); 2 = ammonium (not determined); 3 = potassium (0.15%); 4 = magnesium (0.040%); 5 = calcium (0.944%).

um peak had to be carefully determined with manual integration techniques. Fig. 4 depicts a natural sodium level food example using parsley as the sample. This matrix contained much less sodium which provides a chromatogram where integration of this analyte is much more easily defined because the sodium peak is not rapidly rising out of the negative response of the digestion solution. Also of interest in this example is the high level of magnesium present (0.27%) relative to the other samples. It should be noted that this is a sample high in chlorophyll, a magnesium-containing compound.

Figs. 5–7 demonstrate the presence of ammonium in the chromatogram as well as the other cations

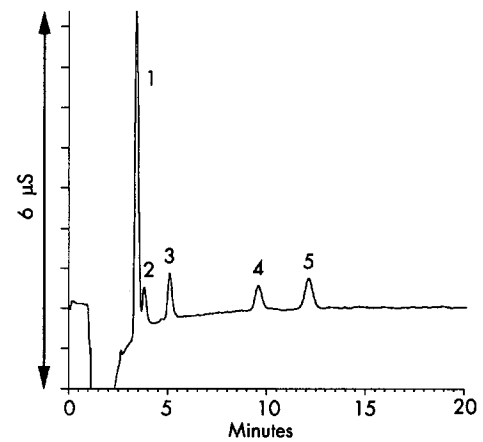

Fig. 5. Cation analysis from bread crumbs with coordination chromatography following microwave digestion. Conditions as in Fig. 2. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = sodium (0.766%); 2 = ammonium (not determined); 3 = potassium (0.219%); 4 = magnesium (0.0485%); 5 = calcium (0.104%).

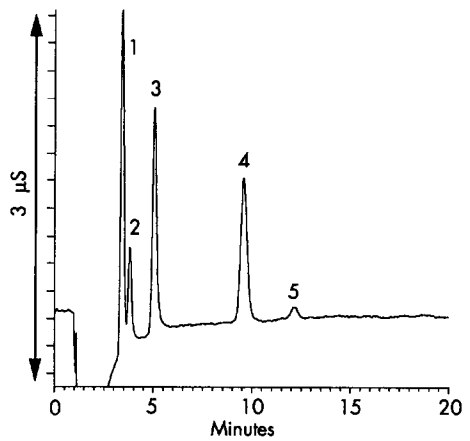

Fig. 7. Cation analysis from peanut butter with coordination chromatography following microwave digestion. Conditions as in Fig. 2. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = sodium (0.456%); 2 = ammonium (not determined); 3 = potassium (0.673%); 4 = magnesium (0.166%); 5 = calcium (0.021%).

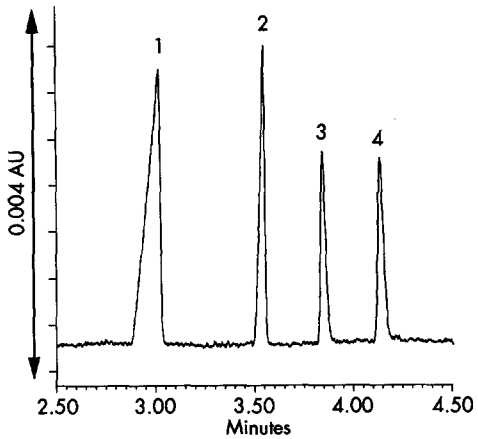

Fig. 8. Cation analysis from parsley with CIE following microwave digestion. Conditions, capillary: 60 cm × 75 μm fused silica; electrolyte: 1.2 m*M* UV Cat-2–3.0 m*M* tropolone; potential: 20 kV positive; detection: indirect UV at 185 nm; injection: hydrostatic, 10 cm for 30 s. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = potassium (4.90%); 2 = calcium (1.23%); 3 = sodium (0.448%); 4 = magnesium (0.39%).

of interest. Since the system had not been calibrated for this analyte, it was not quantified. The latter two of these examples, parmesan cheese and peanut butter, represent this methods ability to deal with relatively high fat matrices.

Figs. 8 and 9 are electropherograms generated by subjecting the microwave digests of parsley and bread crumbs to a CIE system optimized for cations. All four of the cations of interest were readily resolved with a different selectivity than the IC separation and with no intereferences observed. It is known, however, that with the UV Cat-2–tropolone electrolyte used for this feasibility evaluation, there is a comigration of potassium and ammonium. This is reflected in the elevated CIE potassium results of the three samples (Figs. 5–7) where IC showed the presence of ammonium.

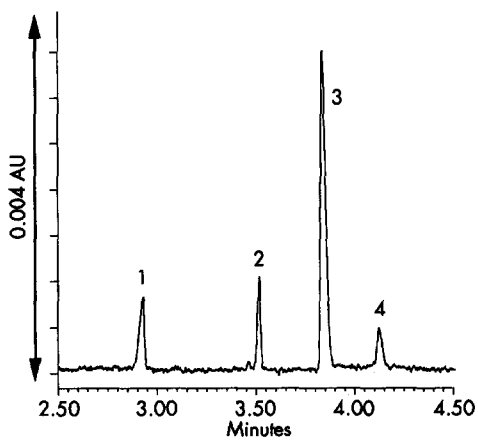

Fig. 9. Cation analysis from bread crumbs with CIE following microwave digestion. Conditions as in Fig. 8. Sample diluted 1/10 000. Peaks and amounts in original sample: 1 = potassium (0.489%); 2 = calcium (0.241%); 3 = sodium (0.752%); 4 = magnesium (0.063%).

TABLE II

MONO- AND DIVALENT CATION RESULTS FOR AAS, IC AND CIE WHEN APPLIED TO THE SAME SAMPLE DIGESTS

	Sodium (%)	Potassium (%)	Magnesium (%)	Calcium (%)
Pretzels				
AAS	1.49	0.25	0.019	0.09
IC	1.47	0.11	0.022	0.02
CIE	1.38	0.26	0.037	0.10
Parsley				
AAS	0.63	5.20	0.22	0.82
IC	0.40	4.79	0.27	0.58
CIE	0.45	4.90	0.39	1.23
Bread crumbs				
AAS	0.82	0.34	0.057	0.20
IC	0.77	0.22	0.049	0.10
CIE	0.75	0.49	0.063	0.24
Parmesan				
AAS	2.00	0.32	0.043	1.28
IC	1.72	0.15	0.040	0.94
CIE	1.63	0.51	0.058	1.96
Peanut butter				
AAS	0.51	0.70	0.140	0.05
IC	0.46	0.67	0.166	0.02
CIE	0.46	1.02	0.246	0.09

Table II presents the data results from the three analytical methods applied in this study. With the exception of calcium for IC and potassium for CIE, the results compare favorably to AAS. In the case of the calcium results by IC, it is known that with coordination chromatography, the calcium is chelated off of the column by the EDTA portion of the eluent. It could be speculated that an increase in this component of the eluent may provide better chelating capabilities for higher IC results for calcium and better correlation to the other data. As previously

mentioned, the elevated potassium results for the CIE data are caused by a comigration of potassium and ammonium. These analytes can be resolved with this technique through the use of complexing agent such as 2.0 m*M* 18-crown-6 ether in the electrolyte [5].

CONCLUSIONS

Microwave digestion and coordination IC can be used in series to provide advantages to current methods of analysis for cation determinations from food. Multi-sample, multi-analyte capabilities offered by this approach can decrease analysis time and increase throughput while minimizing sample handling. Initial studies generated results which are acceptable relative to atomic absorption. Further investigation with respect to low calcium results need to be conducted as do method validation parameters such as precision (reproducibility) and accuracy (spike recoveries). CIE can also be applied to the sample digests as either a substitute for or in addition to IC with good results. This technique offers the advantage of speed with run times of 4.5 min *versus* 20 min for IC. This speed provides a better compliment to the microwave digestion scheme since a batch of 10 samples can now be analyzed in a shorter time than is required to digest another batch of 10 samples. So in this example, an analytical laboratory incorporating microwave digestion followed by CIE determination can process 50 samples times 4 analytes or 200 cation analysis per day.

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