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The determination of nitrite and nitrate in foods by capillary ion electrophoresis

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Capillary ion electrophoresis (CIE) has been used to determine the nitrite and nitrate content of a range of foods. The samples tested were cheese, cabbage puree, fruit juice, water and a variety of meat products. A 75 cm×75 μm i.d. fused silica capillary column was used, with a simple OFM Anion–BT/sodium chloride electrolyte. The operating voltage was −20kV, the temperature 28°C and the detection wavelength 210 nm. Significant amounts of nitrate were found in some of the meat products, its source being the chemicals used in the curing process. Initial work with iodide and tungstate as internal standard was discontinued in favour of thiocyanate, owing to poor resolution of chloride and iodide, and relatively poor peak shape and inconsistent peak areas for tungstate. Good recoveries of nitrite and nitrate were obtained with thiocyanate as internal standard. The data gathered indicate that the method is suitable for determining nitrite and nitrate in a variety of foods. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Nitrate occurs in the environment through the natural process of bacterial nitrogen fixation, and also from man's use of nitrogenous fertilizers (Meah *et al.*, 1994). Relatively high levels can occur in vegetables, which are the main source of nitrate in the human diet (Beljaars *et al.*, 1994).

Nitrate salts are also added to certain foods (meats, some fish and dairy products) as preservatives, but do not become active until converted to nitrite by processing or microbial activity, which is not controllable (Lueck, 1980). In the meat curing process, nitrite is added (particularly to ground meat products) to speed curing and the formation of the required colours and flavours.

Nitrite can interfere with the oxygen transport system in the body and may result in the condition known as methaemoglobinaemia, in which the ability of haemoglobin to exchange oxygen is seriously reduced (Cotton & Wilkinson, 1988). Infants under 3 months are thought to be more susceptible than adults (Meah *et al.*, 1994). The other major concern is formation of carcinogenic *N*-nitroso compounds by reaction of nitrite with various amines and amides in foods *in vivo* (Mirvish, 1975). Because of the microbial conversion of nitrate to nitrite which takes place *in vivo* (Meah *et al.*, 1994) it is prudent to analyse for both ions simultaneously.

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Capillary ion electrophoresis (CIE) is a relatively new technique for the analysis of nitrite and nitrate in foods. Jimidar *et al.* (1995) used CIE with indirect detection to determine these ions in vegetables, while Swallow & Low (1994) used an electrolyte consisting of 10 mM NaCl/0.4 mM OFM–OH for determining nitrate in orange juice and orange pulpwash. The classical method of determining nitrites and nitrates is by colourimetry, after cadmium column reduction of nitrate and azo dye formation, but this is a lengthy procedure (Lippsmeyer *et al.*, 1990). Another common method of determining these ions is by ion chromatography (Lippsmeyer *et al.*, 1990; Murcia *et al.*, 1995), however this employs expensive columns and has relatively long run times. The main advantages of CIE are simplicity and speed. In addition, it has excellent resolving power and relatively low operating costs (Swallow & Low, 1994), however, poor sensitivity is a problem in some applications. Direct UV absorption has been used in the present work to achieve adequate sensitivity.

The present work describes the analysis of a variety of locally available foods for nitrite and nitrate. The cabbage puree and fruit juice were analysed as part of international proficiency studies.

MATERIALS AND METHODS

Reagents

Sodium nitrite and sodium tungstate were obtained from BDH Ltd, Poole, England. Potassium nitrate was

obtained from May and Baker Australia Pty. Ltd, West Footscray, Victoria. Potassium thiocyanate was obtained from Ajax Chemicals Ltd. OFM Anion–BT reagent was obtained from Waters Chromatography, Milford, MA 01757, USA. All other chemicals were A.R. grade.

Methods

Samples

Samples were either received directly at our laboratory, or purchased at a local supermarket. Meat and cheese samples were thoroughly blended in a commercial food processor, and the fruit juice and cabbage puree were thoroughly mixed. All samples were stored at 4°C until analysed.

For solid samples (meat, cheese, cabbage puree) 5 g was weighed accurately into a beaker. To generate recovery data, known volumes of standard solutions of nitrite and/or nitrate were added to a second portion of the sample when required. 50 mL of water was added, and the mixture blended for 2 min with a hand-held blender. The volume was adjusted to 200 mL with water, and then filtered through a Whatman No. 1 or 4 filter paper. The filtrate was passed through either an IC–RP cartridge (Alltech Australia) or C18 Sep–Pak cartridge (Waters Chromatography, Milford, MA). The two types of cartridge were found to perform the same function in this application. 4.5 mL was collected in a volumetric flask containing 0.5 mL of internal standard stock solution, the resulting solution being mixed thoroughly.

The fruit juice sample was diluted with water, internal standard added, and the resulting solution passed through an activated C18 Sep–Pak cartridge, while the water samples were simply diluted with internal standard.

All final solutions were filtered through a 0.8 μm cellulose acetate filter disc before analysis.

Standards

A stock solution of approximately 100 μg/mL nitrite and 100 μg/mL nitrate was prepared by accurately weighing appropriate amounts of sodium nitrite and potassium nitrate into a 100 mL volumetric flask then dissolving and making to volume with water. Internal standard stock solution was prepared by weighing approximately 5 mg of potassium thiocyanate into a 50 mL volumetric flask then dissolving and making to volume with water, producing a solution concentration of about 100 μg/mL. The working standard was prepared by diluting the nitrite/nitrate and internal standard stock solutions 25-fold and 10-fold respectively in the same volumetric flask.

Apparatus

An ISCO Model 3140 electropherograph (Isco Inc., Lincoln, NE, USA) was used for the analyses. The operating voltage was −20 kV and the temperature 28°C. The extracts were loaded under vacuum (level 2, 20 kPa.s) onto a 75 cm×75 μm i.d. fused silica capillary column purchased from Polymicro Technologies (Phoenix, AZ, USA). The effective length to the detector was 50 cm, and the electrolyte consisted of 0.5 mM OFM Anion–BT, 1000 ppm chloride (1540 ppm sodium chloride). The current produced under these conditions was typically 50–60 μA. The detection wavelength was 210 nm, and the sensitivity 0.005. The detector response to both nitrite and nitrate was shown to be linear to 25 μg/mL, and the precision obtained from seven injections of each of three standards at different concentrations was satisfactory (0.2 μg/mL, %cv nitrite 10.7%, %cv nitrate 6.6%; 2.5 μg/mL, %cv nitrite 2.2%, %cv nitrate 1.5%; 25 μg/mL, %cv nitrite 1.6%, %cv nitrate 1.2%). The capillary was cleaned after each sample with a sequential water/0.1 M hydroxide/water wash for 2 min/3 min/2 min, respectively, followed by a 3 min flush with running electrolyte. Electropherograms were recorded with the ICE Data Management and Control Software supplied with the Model 3140 Electrophrerograph.

RESULTS AND DISCUSSION

Apart from vegetables, smallgoods are one of the main sources of nitrite and nitrate in the diet. Initial work focused on a range of meat products. The electrolyte used in this work was 0.5 mM OFM Anion–BT/5 mM sodium chromate (Romano *et al.*, 1991). Indirect detection at 254 nm was used, and the internal standard was iodide. Internal standards are desirable in capillary electrophoresis because they reduce the uncertainties created by drifting migration times and very small injection volumes. By using ratios of migration times of analyte and internal standard, more reliable peak assignments are obtained. Variation in injection volumes is compensated for by taking internal standard peak areas into account in calculations. Initial work with iodide as the internal standard gave satisfactory results (Table 1). However, the high concentration of salt used in the curing process resulted in a substantial chloride peak appearing immediately before the iodide internal standard. In addition, iodide was occasionally migrating very close to nitrite. These migration time characteristics were deemed undesirable, and an alternative internal standard was sought.

Three different anions were assessed as potential internal standards, in an attempt to find one which had

Table 1. Results of duplicate analyses and recovery data for three different processed meats, using iodide as internal standard

Sample	mg kg^{-1}		% Recovery	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-
Processed meat 1	72, 76	44, 48	118	106
Processed meat 2	4, 6	31, 35	109	100
Processed meat 3	15, 16	31, 42	88	73

a significantly different migration time to chloride. Molybdate ($Mo_7O_{24}^{6-}$), tungstate (WO_4^{2-}), and fluoride were tested, and all had suitable migration times (slightly longer than nitrite and nitrate). Molybdate and fluoride peaks showed undesirable tailing, and tungstate was finally adopted for further work on the basis of its sharper peak profile. A variety of other electrolytes were also prepared in an attempt to find one better suited to the separation. In the first of these, chloride (1000 ppm) was substituted for chromate in an attempt to reduce the effect of chloride in the sample. Since chloride and chromate have similar mobilities (Jandik & Jones, 1991), there was no significant change in migration behaviour of the analyte ions. However, substituting chloride for chromate destroyed the UV absorbance of the electrolyte, and the change to direct detection at 210 nm was made, giving increased sensitivity compared to indirect detection. Slight tailing of the tungstate peak was evident with this electrolyte. Replacing the OFM Anion–BT with 0.5 mM CTAC (cetyltrimethylammonium chloride) electrolyte with 1000 ppm chloride resulted in too broad a peak for tungstate, with a longer migration time of about 4 min. A 0.5 mM OFM Anion–BT/1000 ppm fluoride electrolyte produced sharp peaks ($\sim 10^5$ theoretical plates/metre) but was not a significant improvement over the OFM Anion–BT/chloride system already tried.

Eight cured meat products were purchased, to be used in further method validation. The products were salami, cooked silverside, leg ham, bacon, saveloys, liverwurst, garlic metwurst and chicken/ham meat spread. All had sodium nitrite listed as an ingredient, and one (garlic metwurst) also contained sodium nitrate. The eight products were analysed using the OFM Anion–BT/chloride system described, with tungstate as internal standard. Electropherograms were clean with no obvious interferences and little migration time drift, but the peak areas of tungstate for the silverside and leg ham samples were only about half those for the salami and bacon. Figure 1 shows the electropherogram of a sample of saveloy. Most of the samples contained predominantly nitrate and only small amounts of nitrite, suggesting added nitrite has undergone reaction during or after processing. To confirm the presence of nitrite in the samples, a sample (bacon) was spiked with nitrite. Consistent peak areas were obtained for the saveloys, liverwurst and meat spread, with significant nitrite being detected in the saveloys and liverwurst. To test if there was some component in the uncooked meat which was reacting rapidly with nitrite, a sample of uncooked beef mince spiked with nitrite was also included. For this sample, the peak area of the internal standard (tungstate) was significantly less than for the other samples, resulting in a nitrite recovery approximately two and a half times the actual spiking level. Recalculating without the internal standard gave a satisfactory recovery (105%), indicating no rapid nitrite conversion. For the metwurst, no definite tungstate peak was observed. These were further instances of interference with tungstate, and raised a question about its suitability as an internal standard for meat products. Such variation of an internal standard is not analytically desirable, and casts doubt on the validity of the results. A possible reason for the problem is binding of a large biomolecule (e.g. protein) and tungstate, reducing the availability of the latter.

An attempt was made to remove large biomolecules from the unpurified metwurst sample extract using

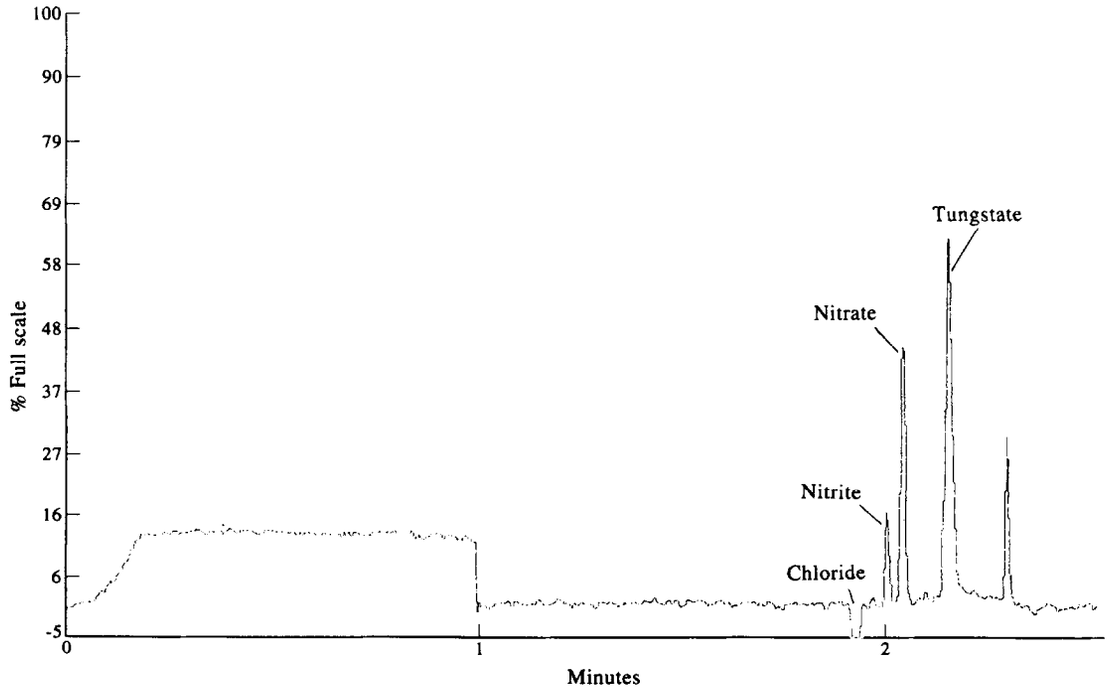

Fig. 1. Electropherogram of saveloy extract showing the separation of chloride, nitrite, nitrate and tungstate (internal standard) using an electrolyte consisting of 0.5 mM OFM Anion–BT, 1000 ppm chloride. The *x*-axis gives the migration time in minutes.

Table 2. Results of analysis for eight cured meat products, with recovery data for nitrite. Thiocyanate was the internal standard

Sample	mg kg^{-1}		% Recovery
	NO_2^-	NO_3^-	NO_2^-
Salami	< 10	137, 146	92
Leg ham	< 10, < 10	126, 139	
Bacon	< 10, < 10	39, 57	
Silverside	< 10	46, 55	95
Chicken/ham spread	< 10, < 10	144, 170	
Saveloys	< 10, < 10	99, 111	
Liverwurst	< 10	140, 153	99
Metwurst	< 10	230, 241	91

Table 3. Results of analysis for a variety of meat products, with recovery data. Thiocyanate was the internal standard

Sample	mg kg^{-1}	
	NO_2^-	NO_3^-
Corned beef	< 10	15
Camp pie	< 10	25
Corned beef	< 10	25
Corned beef	< 10	16
Smoked ham	< 10, < 10	< 10, 10
Cooked leg ham	< 10	15
Frankfurts	< 10	14
Corned beef	< 10	15
Corned beef	< 10	15
Leg ham	< 10	11
Lamb tongues	< 10, < 10	< 10, 10
Luncheon meat	< 10	10
Camp pie	< 10	45
Ham	< 10, 104% recovery	13, 97% recovery

molecular weight cut-off filters (10 000 and 5000 M.W.). Tungstate was added to the filtrate. For the 10 000 M.W. filtrate the tungstate peak was small, and for the 5000 M.W. was slightly larger, but in both cases the peak remained small in comparison to the standard.

After consideration, iodide was again tried as internal standard, attempts being made to overcome the problems described earlier by changing operating voltage and electrolyte composition. Changing the operating voltage from −20 kV to −10 kV and −30 kV had very little effect on the separation of the chloride, iodide, nitite and nitrate peaks. Doubling the concentration of OFM Anion–BT to 1 mM resulted in poor resolution of iodide, nitrite and nitrate.

Further work was done to identify an alternative internal standard, the choice limited to ions with significant UV absorbance and similar migration time to nitrite and nitrate. Ions tested were thiosulphate ($S_2O_3^{--}$), bromate (BrO_3^-), iodate (IO_3^-), and thiocyanate (NCS^-). Thiosulphate had a similar migration time to iodide and was therefore unsuitable, while iodate migrated too close to other sample components, risking interference. Bromate and thiocyanate had similar migration times, and thiocyanate was adopted based on its superior UV absorptivity and good separation from nitrite and nitrate.

All cured meat samples were re-analysed using the same conditions, with thiocyanate as internal standard (Table 2). Very consistent peak areas were observed, with good nitrite recoveries (91–99%) and reasonable duplicates for all samples. An electropherogram of a salami extract is presented in Fig. 2. Significant amounts of nitrate were again detected, but they do not correlate well with the results obtained using tungstate as internal standard. The nitrite previously found in the saveloys

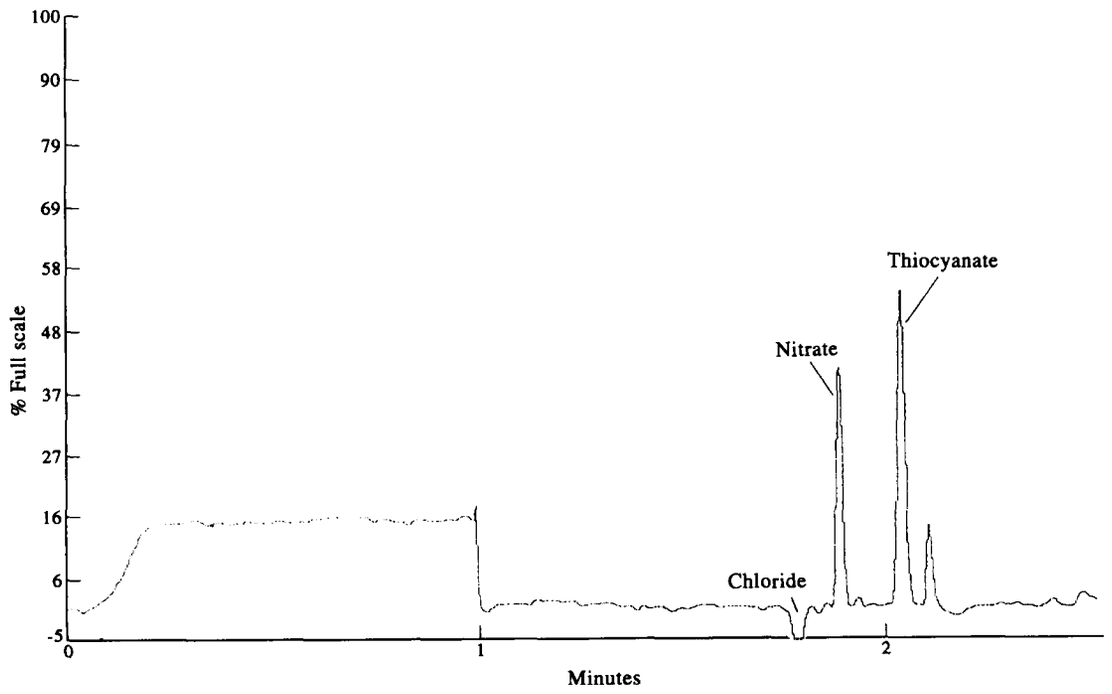

Fig. 2. Electropherogram of salami extract showing the separation of chloride, nitrate and thiocyanate (internal standard) using an electrolyte consisting of 0.5 mM OFM Anion–BT, 1000 ppm chloride. The *x*-axis gives the migration time in minutes.

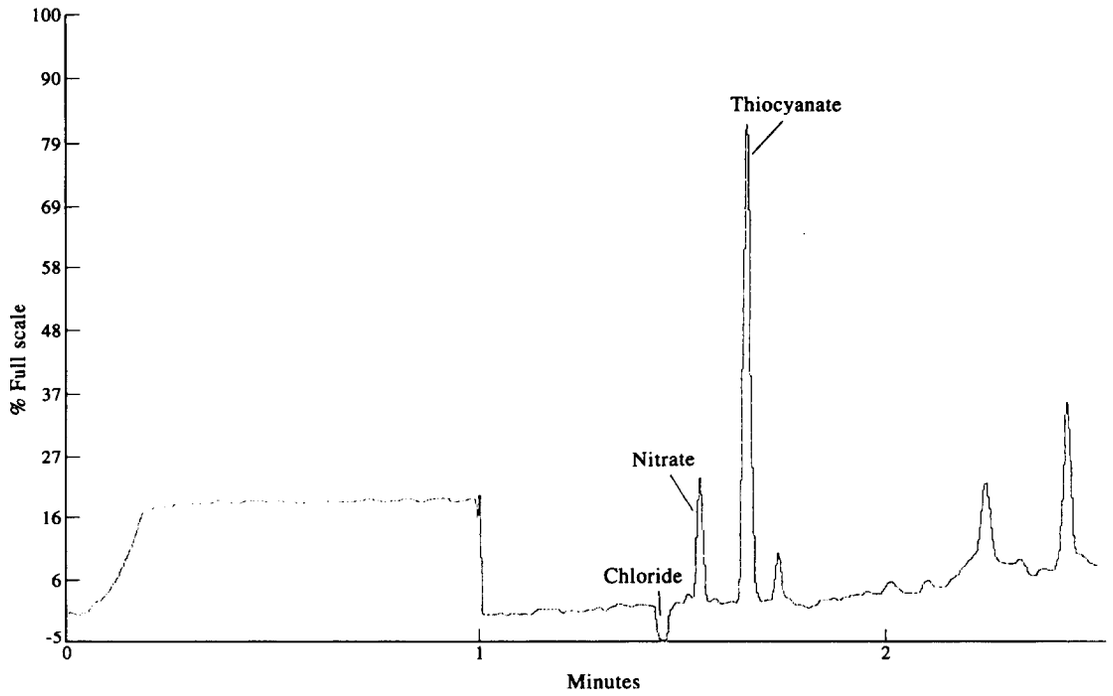

Fig. 3. Electropherogram of camp pie extract showing the separation of chloride, nitrate and thiocyanate (internal standard) using an electrolyte consisting of 0.5 mM OFM Anion–BT, 1000 ppm chloride. The *x*-axis gives the migration time in minutes.

and liverwurst was no longer significant. This indicates the amount of nitrite in the sample diminishes with storage, since extracts were analysed soon after preparation. Meah *et al.* (1994) found measurable nitrite in many of the cured meats they analysed, however the levels were generally less than nitrate. The limit of reporting was set at 10 ppm, based on the lowest standard run (0.2 μg/mL) corresponding to approximately 8 ppm in the sample. The estimated limit of detection was 5 ppm, based on a signal level three times the height of the baseline noise.

The method was applied to a fruit juice sample (unknown type) in an ANAQUAL proficiency study for nitrate (October, 1994) with excellent results (submitted value 225 ppm, official/median value 225 ppm). No nitrite was detected.

Fourteen different canned meat products were also analysed (Table 3). Small amounts of nitrite (less than 10 mg kg^{-1}) were detected, while nitrate levels were slightly higher (mostly 10–25 mg kg^{-1}). Again good recoveries were obtained (104% for nitrite and 97% for nitrate in a ham sample). The electropherograms were clean and uncomplicated. Figure 3 shows the electropherogram of a sample of camp pie. The run time was 2.5 min. It was necessary to flush the capillary with water/0.1 M hydroxide/water (as described earlier) after each run to clean the capillary surface and maintain satisfactory performance. Without this cleaning procedure, sample components (e.g. proteins) built up on the capillary surface, affecting the electroosmotic flow and increasing the migration times.

Sixteen cheese samples were analysed using the same procedure (Table 4). No nitrite and only small amounts of nitrate were found. Electropherograms of spiked and unspiked cheese sample extracts are presented in Fig. 4. 96% recovery of nitrate was obtained from the spiked sample. An unidentified peak migrated close to the internal standard, however this did not affect results for any of the samples.

Several samples of ice were also analysed. Some electropherograms had a significant unidentified peak migrating between the negative chloride peak and where nitrite was expected, while others showed a slight negative dip between the small nitrite and nitrate peaks. Concentrations found in the samples were all less than the allowable levels listed in the Australian Food Standards Code (10 mg L^{-1} nitrate and 1 mg L^{-1} nitrite, calculated as N).

Table 4. Results of analysis for a variety of cheeses, with recovery data for nitrate. Thiocyanate was the internal standard

Sample	mg kg^{-1}	
	NO_2^-	NO_3^-
Cheese	< 10	< 10
Cheese	< 10	< 10
Cheese	< 10	< 10
Cheddar	< 10	< 10
Cheddar	< 10	< 10
Brie	< 10, < 10	12, < 10
Brie	< 10	< 10
Brie	< 10	< 10
Edam	< 10	< 10
Havarti	< 10	< 10
Cheddar	< 10	< 10
Gouda	< 10	< 10
Feta	< 10	< 10
Cheddar	< 10, < 10	< 10, 11
Cheddar	< 10, < 10	< 10, 96% recovery

Cabbage puree, as a FAPAS proficiency study (Nitrate analysis, Series XV, Round 1, August 1995) was also analysed. Nitrate was determined at a level of 550 mg kg^{-1} (Fig. 5). This compared favourably with the assigned value of 573 mg kg^{-1} resulting in a satisfactory z-score of -0.7. This suggests the method may also be applicable to vegetable products.

CONCLUSION

A rapid, simple and reliable CIE method for determining nitrite and nitrate in a variety of foods has been developed, and validated by recovery data and satisfactory proficiency test results. Thiocyanate was found to be the best internal standard for the system and

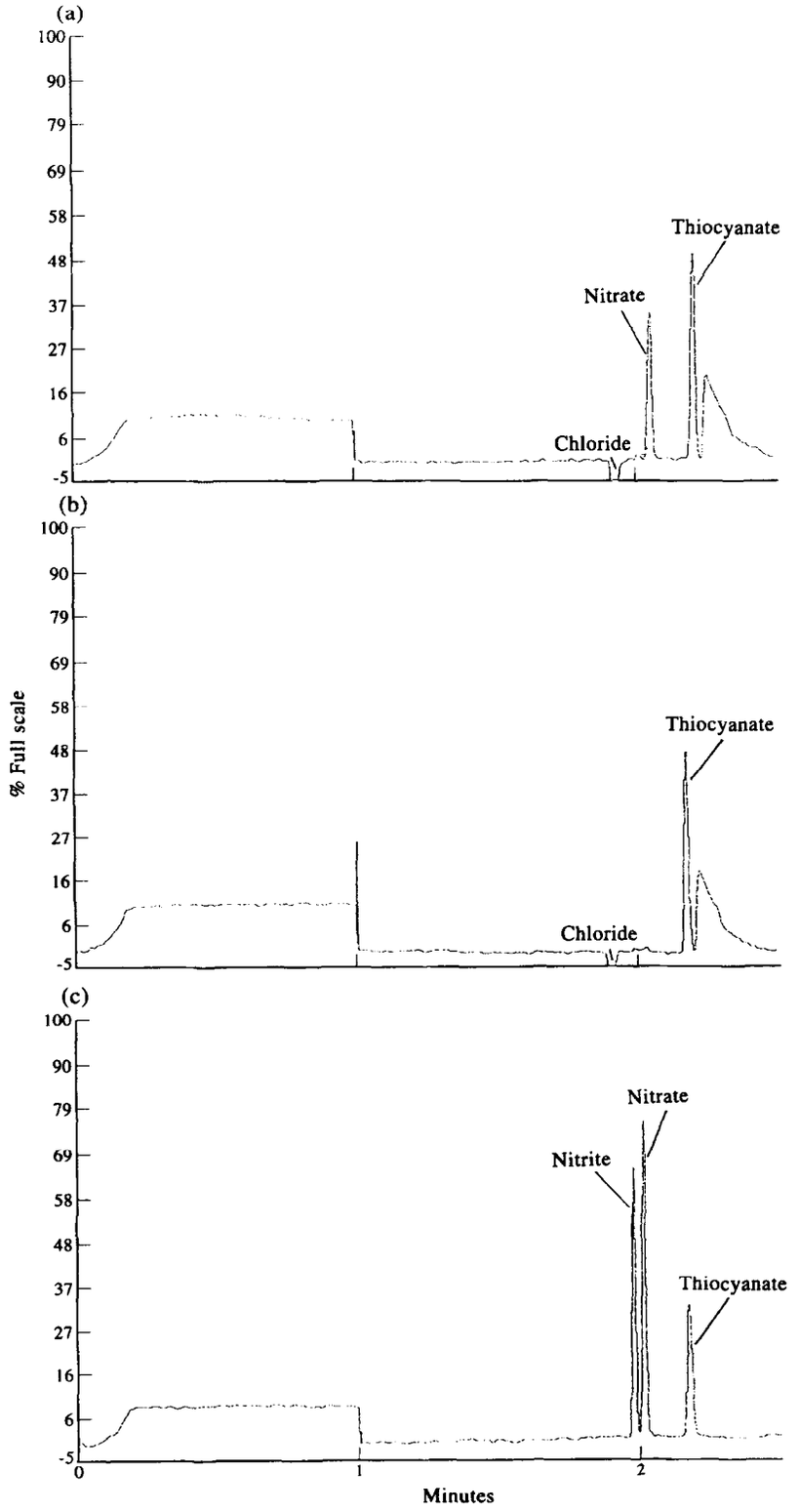

Fig. 4. Electropherograms of cheddar cheese extracts. (A) Sample spiked with 104 mg kg^{-1} nitrate, (B) sample extract and (C) standard solution. The electrolyte consisted of 0.5mM OFM Anion–BT, 1000ppm chloride. Thiocyanate was the internal standard. The x-axis gives the migration time in minutes.

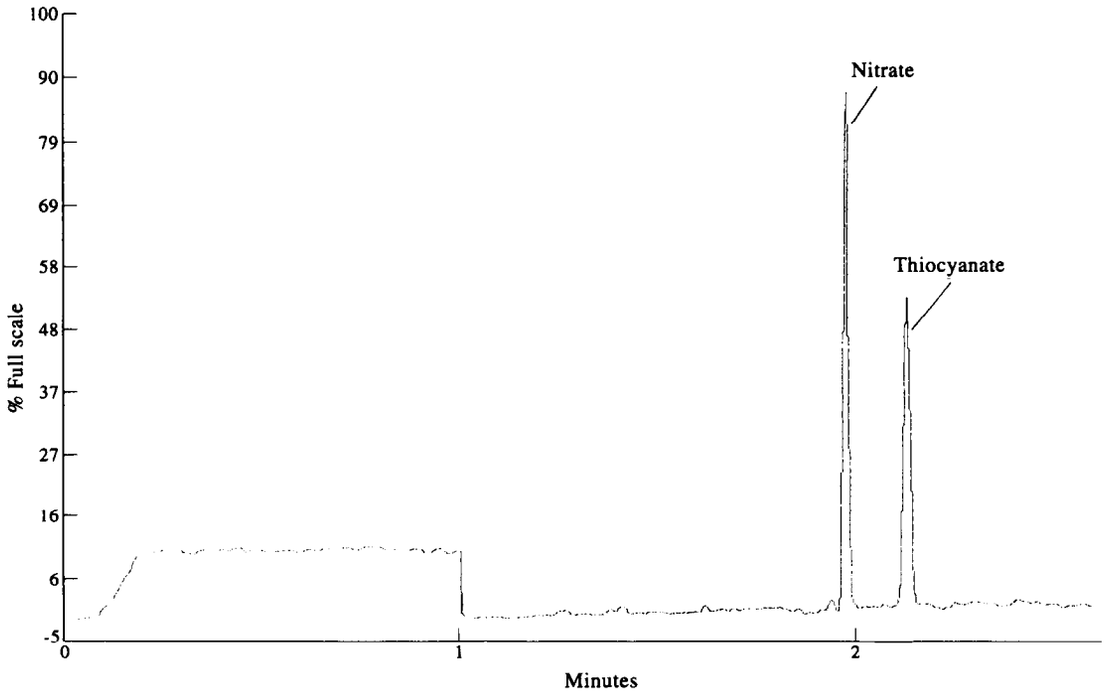

Fig. 5. Electropherogram of cabbage extract, using an electrolyte consisting of 0.5 mM OFM Anion–BT, 1000 ppm chloride. Thiocyanate was the internal standard. The *x*-axis gives the migration time in minutes.

conditions used. The method is a useful alternative to colorimetric and ion chromatographic methods.

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