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Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis

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Abstract

A capillary electrophoresis method for the simultaneous analysis of nitrite and nitrate in meat products and vegetables using direct UV detection is reported. The method is based on the separation of two anions in a capillary coated with polyethyleneimine (PEI). Since PEI is a cationic polymer, the electroosmotic flow is reversed over a wide pH range and the fast separation of anions is achieved without the addition of any electroosmotic modifier to the separation buffer. The detection limit of the method is sufficiently low for food products. Good reproducibility and recovery results were obtained using thiocyanate as internal standard.

Keywords: Nitrite; Nitrate; Meat products; Vegetables; Capillary electrophoresis

1. Introduction

Nitrate and nitrite ions are widely used as preservatives in meat products. Furthermore, nitrate abounds in green-leaf vegetables. The nitrates in foods can be reduced to nitrite. It is known that nitrite causes methaemoglobinaemia and, with secondary and tertiary amines, yields the cancerogen nitrosoamines. Due to these toxic effects, it is important to develop new analysis methods for the simultaneous determination of two anions reducing the matrix effect in real samples. The common method to identify nitrites and nitrates in foods is the spectrophotometric method described by the AOAC (1990). However, this is a labor intensive method that is demanding in time and materials.

More recently, the capillary electrophoretic methods have been used in the fast separation of inorganic ions (Buchberger, 1997; Jackson & Haddad, 1993; Jandik & Bonn, 1993). The advantage of the capillary electrophoresis methods is the considerable dimunition in the sample preparation and analysis times, as well as in the reagent consumption Jimidar, Hartmann, Cousment, and Massard (1995) have determined the nitrate amounts in vegetable samples using capillary electrophoresis with the indirect detection method. Marshall and Trenerry (1996) have proposed a direct detection method for the simultaneous determination of nitrites and nitrates in a variety of food stuffs.

The simultaneous detection of nitrites and nitrates by capillary electrophoretic methods depends on the slowing down or reversal of the electroosmotic flow (EOF) by buffer additives. As buffer additives, commercially available reagents of unknown composition or cationic surfactants are commonly used. Recently, we have offered the use of polyethyleneimine (PEI) coated capillaries, instead of the addition of a modifier, to reverse the EOF in the analysis of inorganic anions (Nutku & Erim, 1998). The coating procedure that we have developed is very simple and the resulting coating can be used long term without losing its efficiency (Erim, Cifuentes, Poppe, & Kraak, 1995). By this procedure, especially in studies of real samples with complex structures, the different selectivity effects of the additives in the buffers can be avoided, as the sample can be injected directly to a simple buffer solution. The current work shows the application of PEI coated capillaries in the determination of nitrite and nitrate in food samples.

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2. Materials and methods

2.1. Reagents

PEI (molecular mass range 6.10^5–1.10^6) was purchased from Fluka (Fluka AG, Buchs, Switzerland). Sodium nitrite, potassium nitrate, potassium tiocyanate, and Tris were obtained from Merck (Darmstadt, Germany). Meat products and fresh vegetables were purchased from local markets. All solutions were prepared with distilled water purified in an Elgacan C114 filtration system.

2.2. Apparatus

Separations were carried out with a commercial CE injection system (Prince Technologies BV, Emmen, Netherlands) in combination with an on-column variable wavelength UV Visible detector (Lambda 1000, Bishoff, Leonberg, Germany). The wavelength was set at 210 nm. The fused silica capillaries used for separation experiments were 75 μm I.D. and were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 75 cm, and the length to the detector was 60 cm. Data processing was carried out using commercial CE software (Prince Technologies BV, Emmen, Netherlands). Washing for 2 min with 0.1 mol/l HCl and 2 min with buffer between runs was applied.

2.3. Method

2.3.1. Standards

Stock standard solutions of 1.5 mmol/l of each anion, nitrite, nitrate, and tiocyanate were prepared in deionized water and stored in a refrigerator.

2.3.2. Samples

Meat products (10 g) were weighed in a beaker. Deionized water (150 ml) was added and blended for 2 min in a laboratory blender. The suspension was incubated for 15 min in a warm water bath at 50 °C. After cooling, the volume was diluted to 250 ml and filtered through a Whatman filter paper. An aliquot of this solution was then filtered from a 0.45-μm celluloze acetate filter disc. Three milliliters of solution were collected, 0.267 ml of internal standard stock solution (KSCN) was added and the volume was diluted to 4 ml. The resulting solution was injected directly.

For vegetables, 5 g was weighed, 100 ml deionized water was added and incubated for 30 min in a warm water bath at 50 °C. After homogenizing in blender for 2 min, the volume was diluted to 250 ml. After the same filtration process, 0.5 ml of sample for parsley and dill, 0.1 ml sample for spinach and 0.267 ml of internal standard for all were diluted to 4 ml with water. Internal standard (0.267 ml) was diluted to 4 ml with leak sample solution.

For recovery experiments, the known amount of nitrite and nitrate solutions were added to the second portions of earlier solutions.

2.3.3. Coating procedure

The fused silica capillary was first etched by flushing the capillary with a solution of 1 mol/l sodium hydroxide for 30 min at 1×10^{-1} MPa followed by water for 15 min at the same pressure. The capillary was then flushed with a solution of 10% PEI in water at 1.5×10^{-1} MPa for 10 min and the PEI solution was left in the capillary for 1 h. Next the polymer solution was forced out of the capillary with air at 1.5×10^{-1} MPa. Finally the capillary was rinsed with water for 15 min.

The RSD values for run-to-run, column-to-column and long-term (for 1 month, 70 injections) reproducibilities of the PEI columns used were reported by Erim et al. (1995). The coating was used for successive injections throughout the study without affecting reproducibility.

3. Results and discussion

3.1. Standard samples

The determination of nitrite and nitrate anions were performed by CE using a PEI coated capillary. PEI is a

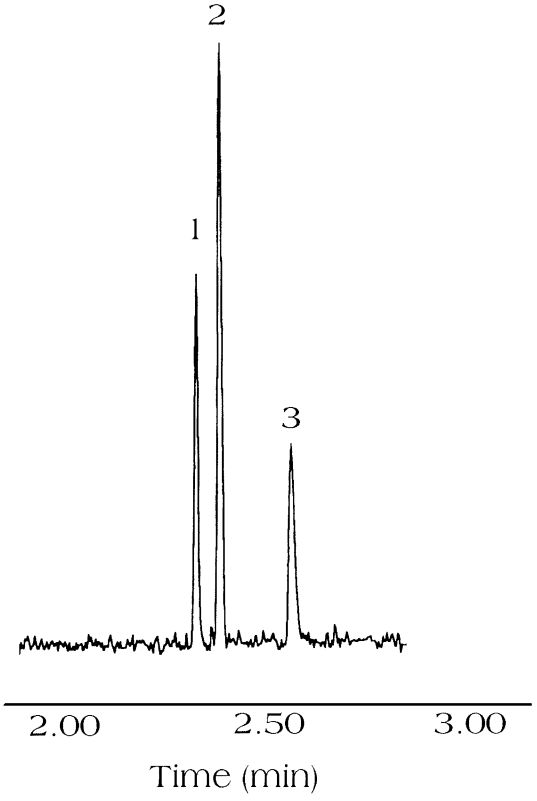

Fig. 1. Electropherogram of a standard mixture. Buffer: 20 mmol/L Tris, pH: 7.5. Voltage: 28 kV, Injection 4.10^{-3} Mpa. PEI coated capillary. Total length 75 cm, effective length 60 cm., i.d. 75 μm. Direct UV detection at 210 nm. Peaks:1 = nitrite, 2 = nitrate, 3 = thiocyanate.

cationic polymer and is adsorbed irreversibly on the capillary wall. The polymer layer has a positive charge over a wide pH range, which results in an EOF towards the anode. Since the anions will move in the same direction with the EOF, the fast separation of inorganic anions can be performed in the PEI-coated capillary without any modifier addition to the buffer. This is one of the advantages of the optimized CE method. Nitrite and nitrate samples are injected directly to the simple Tris buffer and peaks come in 2.5 min. Fig. 1 shows the electropherogram of a standard sample mixture. Direct determination was used for nitrite and nitrate anions at 210 nm.

Only several anions like bromide, thiocyanate, and iodide having UV absorbancy at 210 nm can be determined simultaneously with the proposed method. Since the migration times of these anions are sufficiently different from nitrite and nitrate peaks (Nutku & Erim, 1998), they do not interfere with the analysis. In fact, one of them can be used as internal standard during the work with real samples. As seen from Fig. 1, KSCN was selected here as internal standard.

Calibration curves between the concentrations of anions and normalized peak areas (A/t) were used for quantitative determinations. The calibration curves show linear dynamic ranges from 5×10^{-6} to 1×10^{-4} mol/l with an average correlation coefficient of 0.998. The detection limits of the CE method were sufficiently low for the determination of anions in food samples. LOD for nitrite corresponding to a signal/noise ratio of three is 0.105 μg/ml and for nitrate is 0.099 μg/ml.

3.2. Food samples

Four cured meat products and four fresh vegetables were analyzed using the optimized CE method. Sample solutions were prepared as described earlier and a known amount of tiocyanate solution was added as an internal standard to all injection samples. Recovery data was obtained from the samples prepared exactly in the same way with the addition of the known amount of nitrite and nitrate standards.

Electropherograms of salami extract and spinach extract are presented in Figs. 2 and 3, respectively. As seen from the electropherograms, peaks are seen clearly and there is not any unknown peaks in this region. The presence of high concentrated UV inactive chloride and sulphate anions in foods does not interfere with the determination of nitrite and nitrate in these substances. The maximum concentrations of chlorides and sulphates which do not interfere with migration times and normalized peak areas are determined as 150 times and 50 times more than the nitrite concentration. Protein peaks come very late and do not cause any interference. Thus, there is no need for any deproteinization step before injection. As a result of a washing procedure applied between runs, proteins likely adsorb onto the wall were flushed out of the capillary, so that good results for the precision of real samples were obtained.

Table 1 gives the results of analysis for four cured meat products and four vegetables, with recovery data for nitrite and nitrate. As seen from Table 1, except for

Table 1
Results for the determination of nitrite and nitrate in meat products and vegetables

Sample	NO_2^- (mg/kg)	% Recovery	NO_3^- (mg/kg)	% Recovery
Salami	24.3	101	43.6	106
Ham	<4	103	35.6	106
Turkey sausage	31.9	98	58.1	96
Sausage	31.2	101	43.8	105
Spinach		98	2820	98
Parsley		94	1204	92
Dill		102	2243	92
Leek		96	130	94

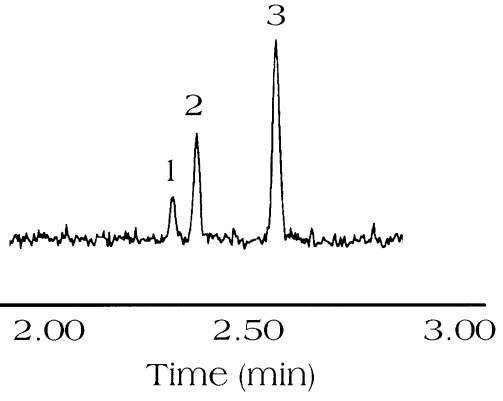

Fig. 2. Electropherogram of salami extract. The symbols and separation conditions are same as Fig. 1.

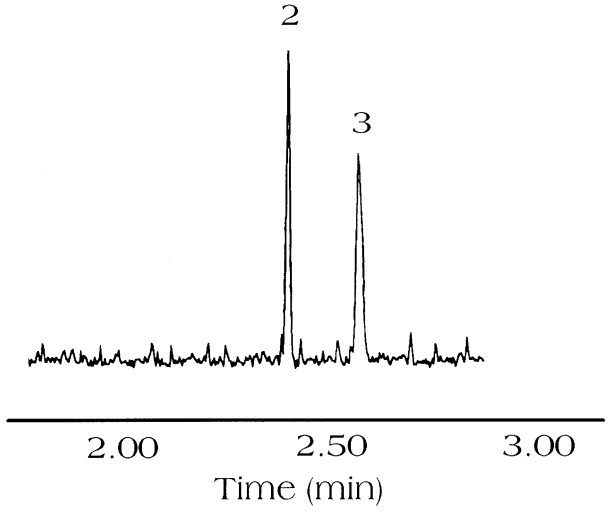

Fig. 3. Electropherogram of spinach extract. The symbols and separation conditions are same as Fig. 1.

Table 2
Reproducibilities of the method for six successive injection of turkey sausage sample

Anion	% RSD migration time	% RSD peak area (A/t)	% RSD (mg anion/kg sample)
NO_2^-	1.43	1.94	4.51
NO_3^-	1.24	1.70	2.54

ham, all meat products contain measurable nitrite. The estimated limit of detection calculated from the standard electropherogram corresponds to 4 mg/kg nitrite in real samples. Nitrite was not detected in the vegetable samples investigated in this study. Nitrite level apparently is below the detection limits.

The repeatability of the CE method was determined by analysing a sample of turkey sausage for six successive injections and is given in Table 2. As seen from Table 2, %RSD values of migration times, peak areas, and the concentrations of nitrite and nitrate in the samples are very good results for capillary electrophoretic separations.

4. Conclusion

A CE method for the simultaneous analysis of nitrite and nitrate ions using PEI coated capillaries was developed. The advantages of the method for food samples are very short analysis time, low electrolyte and sample consumption compared to the classical method of determining nitrites and nitrates by colorimetry. The CE method is also a good alternative to ion chromatography for anion analysis. Fused silica capillaries are less expensive than chromatographic columns, easily washed between runs and free of irreversible contamination of the matrix, unlike the packed columns.

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