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Short communication

Ion chromatographic determination of nitrate and nitrite in meat products

Daniel C. Siu*, Alan Henshall

Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94086, USA

Abstract

Nitrate and nitrite are usually added to processed meat products to provide protection against microorganisms that cause food poisoning. Nitrite may react with secondary amines to form nitrosoamines, a class of carcinogens. Nitrate, although it is more stable than nitrite, can act as a reservoir for nitrite. Thus, both nitrate and nitrite need to be monitored to ensure the quality and safety of meat products. In this paper, an accurate and sensitive method is described by which nitrate and nitrite are extracted from food samples, then analyzed by ion chromatography (IC). Commercial samples of ham and salami were analyzed by IC with UV absorbance detection. UV absorbance was specific for nitrate and nitrite, eliminating interference from other ions present at much higher concentrations. Recoveries of nitrate and nitrite were greater than 90%. The method was linear (r^2>0.999) over the working range, and detection limits for nitrate and nitrite were 50 μg/l and 30 μg/l, respectively. © 1998 Elsevier Science B.V.

Keywords: Food analysis; Nitrate; Nitrite; Inorganic anions

1. Introduction

Nitrate and nitrite are routinely added to meat products to serve as a preservative against microorganisms, such as *Clostridium botulinum* [1], that can cause food poisoning. Both nitrate and nitrite are also monitored regularly because of their toxicity. Nitrite can be converted to carcinogenic nitrosoamines in food products and within the human digestive systems [2]. Nitrate, although more stable and less toxic than nitrite, is also a concern because it can readily be converted to nitrite by microbial reduction in food products [3,4].

Spectrophotometric methods are traditionally used to determine nitrate and nitrite in foods. These methods, however, lack the high sensitivity for detection of trace levels of the analytes, and can be unreliable due to sample matrix interferences. During the last 20 years, numerous IC and HPLC methods have been developed, and generally they are faster, more accurate and more sensitive than the spectophotometric methods [5–16]. In addition, several HPLC methods have also been reported for determination of nitrate and nitrite in meat. However, these chromatographic methods frequently involve time-consuming protein precipitation procedures, or sample processing steps using solid-phase extraction (SPE) cartridges [17–22].

This paper describes an analytical method in which nitrate and nitrite are extracted from processed meat samples, and then analyzed directly using anion-exchange chromatography with UV detection. Commercially available ham and salami were used as model samples. The meat analysis method described in this paper does not require protein precipitation or the use of a SPE cartridge. A water

*Corresponding author.

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extraction at 70–80°C is used to quantitatively extract nitrate and nitrite. This step also removes a substantial amount of the matrix components present in the meat sample. Nitrate and nitrite are determined by ion chromatography (IC) using an IonPac AS11 column, a low capacity, hydrophilic anion-exchange column. Bound proteins and residual matrix components were removed from the column using a 5 min, 100 m*M* sodium hydroxide wash step following each run. Using UV detection, the meat method allows simultaneous determination of nitrate and nitrite down to concentrations of low μg/l levels for the extracts, and high ng/g levels for the food samples.

2. Experimental

2.1. Instrumentation

A Dionex (Sunnyvale, CA, USA) DX500 chromatography system consisting of a GP40 gradient pump, an AD20 UV-Vis detector, a LC20 enclosure, was used. Chromatographic data were analyzed using a Dionex PeakNet chromatography workstation. Meat samples were homogenized using a Scovill Hamilton Beach blender. A Beckman Spinchron R centrifuge (Palo Alto, CA, USA) was used for all centrifugation procedures. The IonPac AS 11 (250×4 mm), AG11 guard (50×4 mm) columns were obtained from Dionex.

2.2. Materials

Light salami and cooked low fat ham were obtained from a local supermarket. Sodium hydroxide, 50% (w/w), sodium nitrate and sodium nitrite were obtained from Fisher Scientific, Pittsburgh, PA, USA. Sterile Acrodisc were obtained from Gelman Sciences, Ann Arbor, MI, USA. Filter papers were obtained from Whatman LabSales, Hillsboro, OR, USA.

2.3. Methods

2.3.1. Extraction

Ten grams of ham or salami were removed from commercial packages. Deionized water was added to the meat sample to make up to a final volume of 100 ml. The mixture of meat sample and water was then homogenized in a blender for 1 min. The homogenized sample was heated and the temperature was maintained between 70°C and 80°C for 15 min. After cooling to room temperature, the sample was centrifuged at 4960 *g* (6000 rpm in a Beckman GA-10 rotor) for 10 min. Following centrifugation, the supernatant was removed, and filtered successively through the following filters—Whatman No. 2 and GF/A filters, and then through the 1.2 μm and 0.2 μm Acrodisc filters. The filtrate was then collected for IC analysis.

2.3.2. Ion chromatography

Nitrate and nitrite were separated using isocratic conditions with an IonPac AG11guard column and AS11 analytical column—5 m*M* sodium hydroxide for 10 min, followed by a column wash with 100 m*M* sodium hydroxide for 5 min, and equilibration with 5 m*M* sodium hydroxide for 10 min. The injection volume was 25 μl and eluent flow-rate was 1 ml/min. Analytes were detected using UV detection at 225 nm.

2.3.3. Calibration curves

Calibration curves for nitrate and nitrite were generated by plotting the peak areas against the concentrations of the standards injected. For nitrate, triplicate injections at nine levels between 50 μg/l and 375 mg/l (equivalent to 500 μg/kg and 3.75 g/kg for the meat sample) were made. For nitrite, triplicate injections at nine levels between 30 μg/l and 300 mg/l (equivalent to 300 μg/kg and 3.00 g/kg for the meat sample) were made. At least two peak area data points were collected per order of magnitude.

3. Results and discussions

Fig. 1 shows a chromatogram of nitrate and nitrite of a ham extract using an IonPac AS11 column with low UV detection. The negative peak eluting before the nitrite peak is due to chloride (approximately 400 mg/l). The presence of this chloride peak was confirmed by suppressed conductivity detection in-line with UV detection. The ratio of the negative

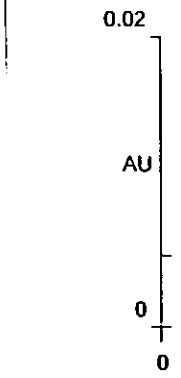

Fig. 1. IC
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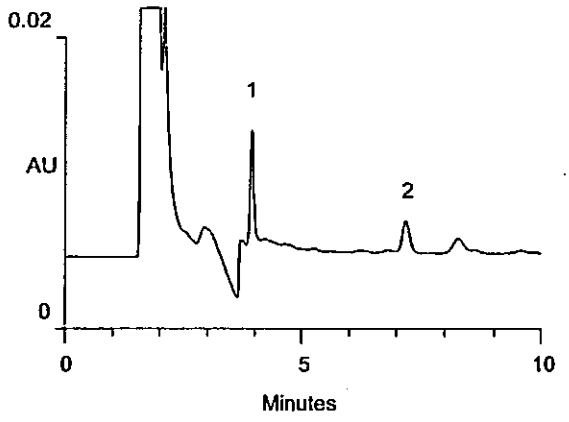

Fig. 1. IC determination of nitrate and nitrite in ham with UV detection. Peaks: 1: nitrite, 1.16 mg/l. 2: nitrate, 0.54 mg/l. Columns: IonPac AS11 and AG11. Eluent: 5 m*M* sodium hydroxide. Flow-rate: 1 ml/min. Detection: UV, 225 nm. Injection volume: 25 μl.

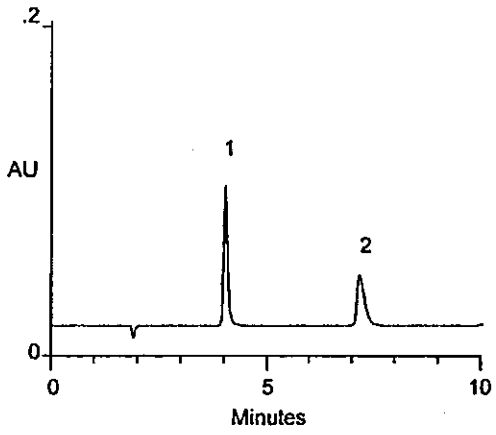

Fig. 2. Chromatogram of nitrate and nitrite standards. Peaks: 1: nitrite, 10 mg/l. 2: nitrate, 12 mg/l. Columns: IonPac AS11 and AG11. Eluent: 5 m*M* sodium hydroxide. Flow-rate: 1 ml/min. Detection: UV, 225 nm. Injection volume: 25 μl.

chloride peak height to baseline noise is the lowest with detection at 225 nm, as compared with detection at 210 nm or 214 nm. Thus, 225 nm was selected to minimize the magnitude of the chloride dip. It was found that the IonPac AS11, which is a hydrophilic, low capacity column, gives the best separation of nitrite from the chloride dip, as compared to the IonPac AS 10 and AS14 columns (data not shown). The extraction process apparently removes the bulk of the fat and protein-based interferences so that a low capacity column is sufficient to provide good efficiencies for the nitrite and nitrate peaks. A chromatogram of nitrate and nitrite standards is shown in Fig. 2. The amounts of nitrate and nitrite, as shown in Table 1, were determined to be 5.4 mg/kg and 11.6 mg/kg (0.54 mg/l and 1.16 mg/l for the extract), respectively. Fig. 3 shows a chromatogram of a salami extract. The chromatogram is similar to the one obtained from ham. As shown in Table 1, the amounts of nitrate and nitrite in salami were determined to be 98.5 mg/kg and 108 mg/kg (9.85 mg/l and 10.8 mg/l for the extract), respectively. As was the case for the ham sample, a negative chloride peak eluting before the nitrite peak is also observed for the salami sample.

Spike recovery experiments were performed to determine the percent recovery of added standards. Known amounts of nitrate or nitrite standards were added to each of the meat samples and allowed to be absorbed into the meat samples for 10 min. The amounts of nitrate and nitrite were then determined following the same extraction and separation processes. Recoveries were between 90% and 100% for nitrate, and between 90% and 105% for nitrite.

The degree of agreement among individual test results was determined and was expressed as R.S.D.s (relative standard deviations). Table 1 shows the R.S.D.s of retention time and peak areas of nitrate and nitrite. For both ham and salami, peak area R.S.D.s were below 3% and retention time R.S.D.s were less than 0.5%. No detectable changes in retention time were noticed after 117 injections of either meat samples.

Detection limits and linearity data are also shown in Table 1. The coefficients of determination for nitrate and nitrite were 0.9991 and 0.9995, respectively. These values were calculated over three orders of magnitude. Detection limits for nitrate and nitrite from the extracts, determined at three times the noise, are 50 μg/l and 30 μg/l (500 μg/kg and 300 μg/kg for the meat sample), respectively.

4. Summary

A simple and accurate analytical method for determining nitrate and nitrite in meat samples is described. Extraction procedures were developed

Table 1
Concentration of nitrate and nitrite, R.S.D.s (%, n=5) and linearity data

	Amount of nitrite (mg/kg)	Amount of nitrate (mg/kg)	Nitrite peak area R.S.D. (%)	Nitrate peak area R.S.D. (%)	Nitrite retention Time R.S.D. (%)	Nitrate retention Time R.S.D. (%)	Nitrite concentration range and r^2	Nitrate concentration range and r^2
Salami	108	98.5	2.7	2.9	0.2	0.3	300 μg/kg–3.00 g/kg, r^2=0.9995	500 μg/kg–3.75 g/kg, r^2=0.9991
Ham	11.6	5.37	2.3	1.0	0.2	0.2	300 μg/kg–3.00 g/kg, r^2=0.9995	500 μg/kg–3.75 g/kg, r^2=0.9991

which remove the bulk of the potentially interfering matrix components such as protein and fat-based substances. Nitrate and nitrite were determined by ion chromatography using pellicular anion-exchange columns in conjunction with low-UV detection. The pellicular anion-exchange column was chosen to provide the appropriate selectivity, not only for the separation of nitrate and nitrite, but also for the separation of the analytes from the residual matrix components. The meat method allows for determination of nitrate and nitrite at the sub-mg/l levels.

5. Precautions

Detectable changes (more than 10%) of nitrate and nitrite concentrations from the meat extracts were observed after the extracts were kept at room temperature for more than 12 h. Analysis should be completed within 12 h after extraction.

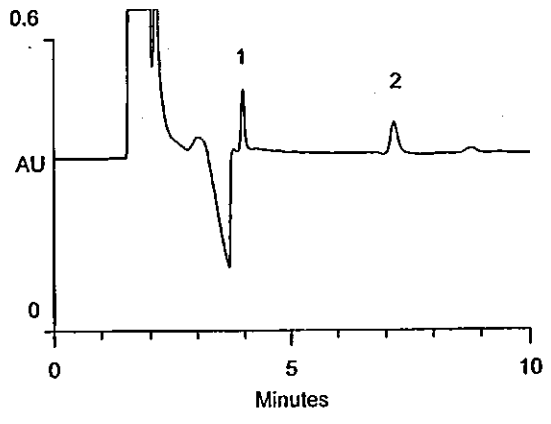

Fig. 3. IC Determination of nitrate and nitrite in salami with UV detection. Peaks: 1: nitrite, 10.8 mg/l. 2: nitrate, 9.85 mg/l. Columns: IonPac AS11 and AG11. Eluent: 5 mM sodium hydroxide. Flow-rate: 1 ml/min. Detection: UV, 225 nm. Injection volume: 25 μl.

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