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Application Note 112

Determination of Nitrate and Nitrite in Meat Using High-Performance Anion-Exchange Chromatography

INTRODUCTION

Nitrate and nitrite are usually added to processed meat products to protect against microorganisms that can cause food poisoning, such as *Clostridium botulinum*.[1-3] However, nitrite can react with secondary amines to form nitrosoamines, a class of carcinogenic compounds, in food products or in the digestive system. Nitrate, although more stable than nitrite, can act as a reservoir for nitrite. Also, nitrate can readily be converted into nitrite by microbial reduction.[4,5] Thus, both nitrate and nitrite must be monitored to ensure the quality and safety of meat products. This Application Note describes an accurate and sensitive method in which nitrate and nitrite are extracted from meat products and then determined directly using anion exchange chromatography with UV detection. Commercially available ham and salami were used as model samples.

Several HPLC methods have been developed to analyze for nitrate and nitrite in meat. However, these methods require lengthy sample processing or pretreatment steps, such as adding protein precipitation procedures after extraction or using reversed-phase cartridges to remove sample matrix interferences.[6-9] Unlike most other HPLC techniques, the method described in this note does not require the use of protein precipitating reagents. In addition, a reversed-phase or ion-exchange pretreatment cartridge is not needed because a 5-min, 100 mM sodium hydroxide wash step in the chromatographic procedure is sufficient to remove bound proteins and other sample matrix interferences.

EQUIPMENT

Dionex DX-500 chromatography system consisting of:
- GP50 Gradient Pump
- AD25 UV/Visible Absorbance Detector
  - LC20 Enclosure
- AS40 Autosampler
- PeakNet Chromatography Workstation

Scovill Hamilton Beach Blender

Beckman Spinchron R Centrifuge

MATERIALS

Sodium hydroxide, 50% w/w (Fisher Scientific)

Sodium nitrate (Fisher Scientific)

Sodium nitrite (Fisher Scientific)

Whatman Filters (Whatman)

Sterile Acrodisc Syringe Filters, 1.2 and 0.2 mm (Gelman Sciences)

CONDITIONS

Column:	IonPac® AS11 (4 x 250 mm) and AG11 guard (4 x 50 mm)
Injection Volume:	25 µL
Flow Rate:	1 mL/min
Detection:	UV, 225 nm
Eluent A:	50 mM Sodium hydroxide
Eluent B:	Deionized water
Eluent C:	100 mM Sodium hydroxide

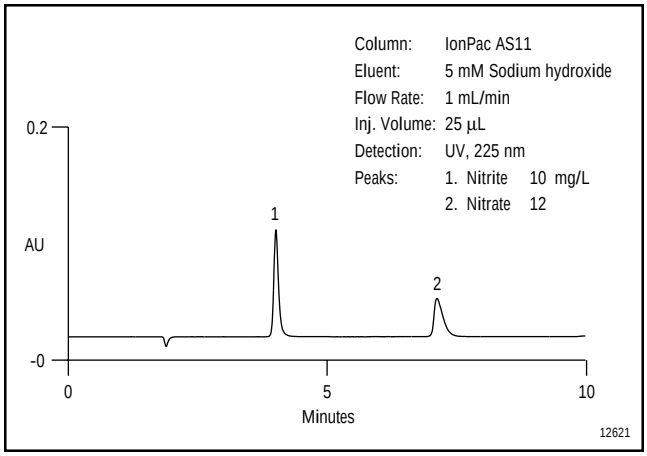

Figure 1 *Separation of nitrate and nitrite standards. Amount injected: 12 mg/L nitrate and 10 mg/L nitrite; injection volume: 25 µL.*

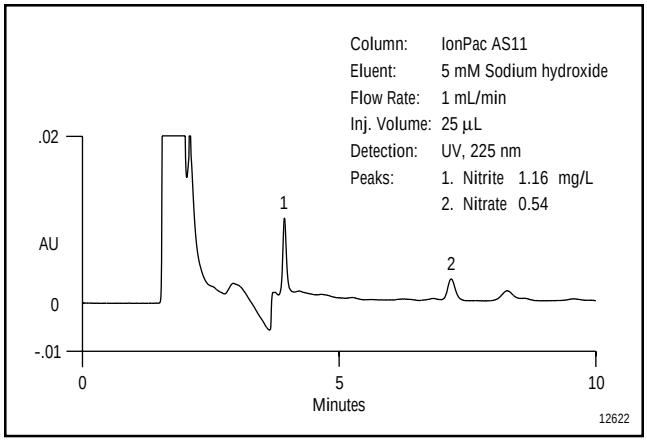

Figure 2 *Separation of nitrate and nitrite from ham. Injection volume: 25 µL.*

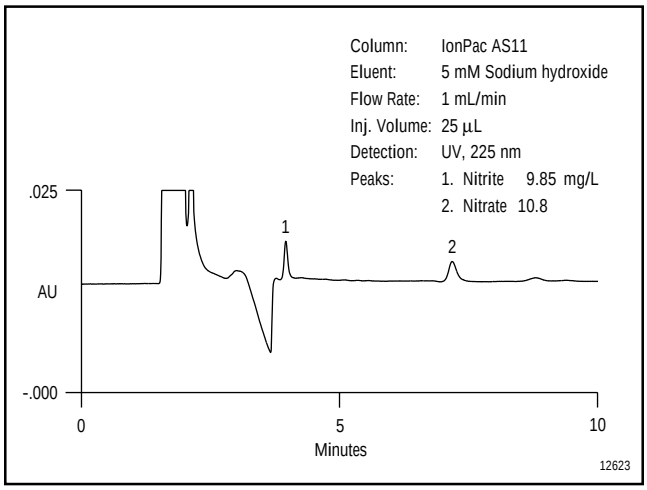

Figure 3 *Separation of nitrate and nitrite from salami. The extract was diluted fourfold before injection; injection volume: 25 µL.*

Method

Time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)
0.0	10	90	0
10.0	10	90	0
10.1	0	0	100
15.0	0	0	100
15.1	10	90	0
25.0	10	90	0

Calibration Curves

Calibration curves for nitrate and nitrite were generated by plotting the peak areas against the concentrations of the standards injected. For nitrate, nine different con-centrations between 50 µg/L (500 µg/kg) and 375 mg/L (3.75 g/kg) were used. For nitrite, nine different concen-trations between 30 µg/L (300 µg/kg) and 300 mg/L (3.00 g/kg) were used. At least two peak area data points were collected per order of magnitude. Each data point was an average of duplicate injections.

PREPARATION OF SAMPLES AND SOLUTIONS

Eluent A: 50 mM Sodium hydroxide

Filter 1.0 L of deionized water through a 0.2 µm filter. Then vacuum degas the deionized water for 5 min. Add 2.5 mL of 50% w/w sodium hydroxide to the 1.0 L of degassed water.

Eluent B: Deionized water

Filter 1.0 L of deionized water through a 0.2 µm filter.

Eluent C: 100 mM Sodium hydroxide

Filter 1.0 L of deionized water through a 0.2 µm filter. Then vacuum degas the deionized water for 5 min. Add 5.0 mL of 50% w/w sodium hydroxide to the 1.0 L of degassed water.

Nitrate Stock Solution

Dissolve 137 mg of sodium nitrate in 100 mL of deionized water to make up a 1-g/L stock solution.

Nitrite Stock Solution

Dissolve 150 mg of sodium nitrite in 100 mL of deionized water to make up a 1-g/L stock solution.

Extraction Procedure

Weigh 10.0 g of ham or salami and transfer to a blender. Add 100 mL of deionized water to the meat sample. Liquify the meat sample in the blender for 1 minute. Heat the liquified sample and maintain the temperature of the sample between 70 °C and 80 °C for 15 min.[6-9] Allow the sample to cool to room temperature. Centrifuge the sample at 4960 x *g* (6000 rpm in a Beckman GA-10 rotor) for 10 min. Remove the supernatant. Successively filter the supernatant through the following filters: Whatman No. 2 and GF/A filters and 1.2 µm and 0.2 µm Acrodisc filters. Collect the filtrate for HPLC analysis.

RESULTS AND DISCUSSION

Figure 1 shows a separation of the nitrate and nitrite standards. Figure 2 shows a separation of nitrate and nitrite from the ham extract. The amounts of nitrate and nitrite, as shown in Table 1, were determined to be 5.37 and 11.6 mg/kg, respectively. The dip before the nitrite peak apparently is due to the elution of chloride (approximately 400 mg/L). The amount of chloride can be determined using a suppressed conductivity detector in series with the UV detector (data not shown).

Figure 3 shows a separation of nitrate and nitrite from the salami extract. As shown in Table 1, the amounts of nitrate and nitrite in salami were determined to be 98.5 and 108 mg/kg, respectively. Similar to the ham sample, there is a dip immediately before the nitrite peak due to the elution of chloride.

Table 1 Concentration of Nitrate and Nitrite in the Ham and Salami Samples

	Amount of Nitrite (mg/kg)	Amount of Nitrate (mg/kg)	Concentration of Nitrite in 100 mL of Extract (mg/L)	Concentration of Nitrate in 100 mL of Extract (mg/L)
Salami	108.0	98.5	10.8	9.85
Ham	11.6	5.37	1.16	0.54

Recovery

As shown in Table 2, predetermined amounts of nitrate or nitrite standards were added to each of the meat samples and allowed to be absorbed by the meat samples for 10 min. The amounts of nitrate and nitrite were then determined following the same extraction and separation processes. Table 2 shows the recovery results. Over 90% recoveries of nitrate and nitrite standards from both the ham and salami samples were obtained.

Table 2 Spike Recovery Data

	Amount Present (mg/kg)	Amount Added (mg/kg)	Total Recovered (mg/kg)	Recovery (%)
Nitrite in Salami	108.0	120.0	218.0	92
Nitrate in Salami	98.5	100.0	195.0	97
Nitrite in Ham	11.6	15.0	27.0	103
Nitrate in Ham	5.37	6.50	11.4	92

Precision

The degree of agreement among individual test results was determined and expressed as Relative Standard Deviations (RSDs). Table 3 shows the RSDs of retention time and peak areas of nitrate and nitrite. For both ham and salami, peak area RSDs were below 3% and retention time RSDs were less than 0.5%. No detectable changes in retention time were noticed after 117 injections of salami and ham samples.

Table 3 Peak Area and Retention Time RSDs

	RSDs of Nitrite Peak Area (%)	RSDs of Nitrate Peak Area (%)	RSDs of Nitrite Retention Time (%)	RSDs of Nitrate Retention Time (%)
Salami, n = 5	2.7	2.9	0.2	0.3
Ham, n = 5	2.3	1.0	0.2	0.2

Linearity and Limit of Detection

Detection limits and linearity data are shown in Table 4. 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, determined at three times the noise, were 50 µg/L (500 µg/kg) and 30 µg/L (300 µg/kg), respectively.

Table 4 Detection Limits and Linearity Data

	Concentration Range	r^2	Detection Limit
Nitrate	500 µg/kg–3.75 g/kg	0.9991	500 µg/kg
Nitrite	300 µg/kg–3.00 g/kg	0.9995	300 µg/kg

CONCLUSION

- The method described in this Note is a simple and accurate analytical method for determining nitrate and nitrite in meat samples. The IonPac AS11 column provides ideal selectivity not only for the separation of
  nitrate and nitrite, but also for the separation of the analytes from matrix components, which are eluted mostly in the void.
- With the 5-min, 100 mM sodium hydroxide wash step, retained ions and organic species are eluted. Thus, this method eliminates the need for time consuming and costly sample pretreatment using reversed-phase or ion-exchange cartridges and protein precipitating reagents.

- The DX-500 system (PEEK system) is designed to be compatible with high-pH eluents such as sodium hydroxide. Combined with the AS40 autosampler, the analytical system provides an ion-free environment for the determination of nitrate and nitrite at the sub-mg/L (sub-ppm) levels.

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 24 h. Analysis should be completed within 24 h after extraction.

Chloride was present at concentrations of approximately 400 mg/L and 1600 mg/L in the ham and salami samples, respectively. It is important to dilute the extracts so that no more that 400 mg/L of chloride is loaded onto the column. If too much chloride is injected, the nitrite peak may elute earlier and be poorly resolved from the chloride dip, causing difficulty in peak area determination.

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LPN 0837-01 4M 7/99