Journal of Chromatography, 354 (1986) 490–494
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 388

Note

High-performance liquid chromatographic method for the determination of nitrate and nitrite in cured meat

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(Received December 3rd, 1985)

Cured meat is normally meat which has been treated with nitrate and/or nitrite and immersed in brine. The nitrite (either added or produced from nitrate-reducing organisms) reacts with myoglobin to produce the red colour of pickled meats[1]. Nitrate can act as a reservoir for nitrite[2] but as a preservative it is similar in effectiveness to sodium chloride[1], the difference being that nitrate prevents the growth of *Clostridium botulinum*. Nitrate may react with amines to form carcinogenic nitrosamines and consequently it has become increasingly important to monitor nitrate and nitrite levels in meat products. New Zealand regulations[3] do not permit wet cured meats to contain more than 125 mg/kg of nitrite and nitrate (calculated as sodium nitrite) and do not allow the addition of nitrates. The usual methods for nitrate determination involve cadmium reduction to nitrite, followed by diazotisation, coupling and spectrophotometric estimation. These methods are time consuming and suffer from interferences which become critical at 10–20 mg/kg levels of nitrate and nitrite[4].

High-performance liquid chromatographic (HPLC) techniques have been examined as alternative procedures for the determination of nitrate and nitrite. Suppressed ion chromatography is perhaps an obvious alternative, but complications arise with the oxidation of nitrite and nitrate in the acidic conditions used on the suppressor column[5]. Jackson *et al.*[6] have assessed many of the HPLC methods and have proposed a method using a low-capacity silica-based anion-exchange column with 11 m*M* chloromethanesulphonic acid as eluent and UV detection. Excellent resolution of nitrate and nitrite was obtained and no interference from the high concentrations of chloride found in cured meats was observed. Two recent methods have made use of ion-interaction chromatography. Wootton *et al.*[7] used a silica-based reversed-phase column (Waters Radial Pak C_{18}) and direct UV detection and found that matrix interference was a problem with meat samples. De Kleijn and Hoven[8] found that a neutral styrene–divinylbenzene copolymer column (Hamilton PRP-1) and UV detection at 240 nm gave a clean chromatogram with low sensitivity. A fourth method for the determination of nitrate and nitrite in cured meats makes use of a high-capacity anion-exchange column, 0.04 *M* sodium perchlorate as eluent and UV detection. This method has a detection limit of about 17 mg/kg nitrate in ham. Improved resolution was obtained with 0.2 *M* methanesulphonate as eluent. At high mobile phase concentrations, however, the column was damaged[9].

0021-9673/86/$03.50

In a laboratory where many samples are analysed it is necessary to have quality assurance checks regularly and to be able to assess the precision of the method at frequent intervals. This can be accomplished easily using an automated HPLC method. We have used a high-capacity polymethacrylate gel anion-exchange (quaternary ammonium) column with dipotassium phosphate as eluent and detection by UV spectrophotometry at 215 nm. As nitrite is susceptible to oxidation at pH values less than 5[4,5] and will not be retained on an ion-exchange column near or below its pK_a of 3.37, dipotassium hydrogen phosphate (5 m*M*) was chosen as eluent for it has sufficient buffer capacity at pH 9 and is an effective anion at displacing nitrate and nitrite. The response of nitrate is considerably enhanced if it is detected at 190 nm[10]. We chose 215 nm as the detection wavelength as it is a suitable compromise between sensitivity and interference from other compounds.

EXPERIMENTAL

Instrumentation

An HPLC system (Waters Assoc., Milford, MA, U.S.A.), a Model 8700 solvent delivery system (Spectra-Physics, San Jose, CA, U.S.A.), a Model PU4020 variable-wavelength spectrophotometer (Pye Unicam, Cambridge, U.K.), and a Model SP4270 integrator (Spectra-Physics) were used. The column was an IC-Pak anion column (Waters Assoc.) with a capacity of 30 mequiv./ml and it was maintained at 40°C using a purpose-built column heater.

Mobile phases

Dipotassium hydrogen phosphate solution (5 m*M*) was prepared from analytical grade reagent (Ajax Chemicals) and sodium methanesulphonate (30 m*M*) was prepared from methanesulphonic acid (Riedel de Haën, Prosynth grade) titrated with sodium hydroxide (0.1 *M*) to pH 6.0. The flow-rate was 0.6 ml/min.

Sample preparation

Sample preparation follows the method of Fudge and Truman[11]. The samples of meat were purchased from a retail outlet. A 250-g sample was homogenised in a food processor (Toshiba, Model TFP-1200), and 10.00 g were slurried thoroughly with hot distilled water (100 ml) at 80°C, saturated borax solution (5 ml) and activated charcoal (0.5 g) and then placed on a boiling water bath for 15 min. After cooling to room temperature, Carrez Reagents I and II were added (2.0 ml of each), followed by saturated borax solution (5 ml) and the solution was made up to 250 ml in a volumetric flask. After standing for 30 min, the solution was filtered through Whatman No. 44 paper (previously washed with 50 ml of 0.1 *M* hydrochloric acid), the first portion of the filtrate being discarded, and then through a 0.45-μm membrane filter: aliquots of 20 μl were injected into the chromatograph.

RESULTS AND DISCUSSION

The chromatogram of a standard solution containing 5 ppm nitrite and nitrate (both as sodium nitrite) recorded at 215 nm using 5 m*M* dipotassium phosphate as eluent is shown in Fig. 1a. The relative standard deviation for replicate injections

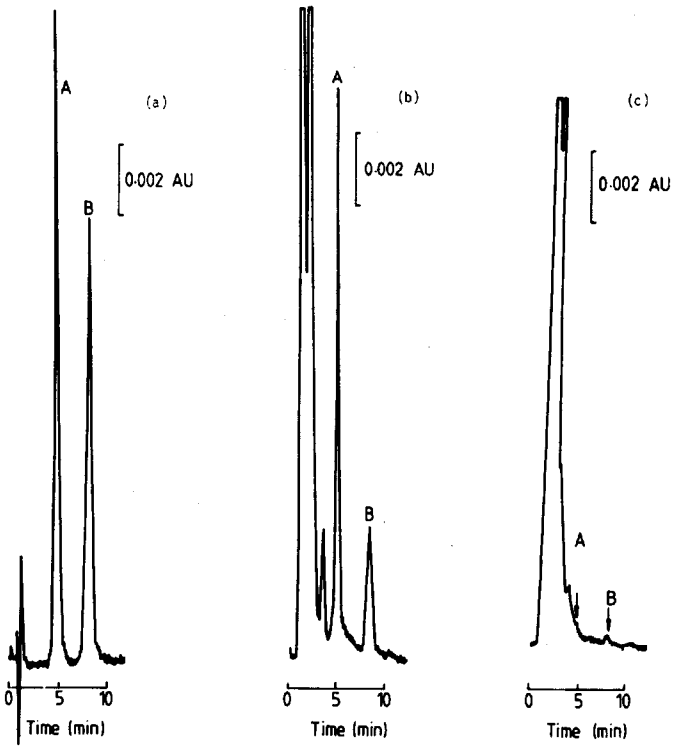

Fig. 1. Separation of nitrite (A) and nitrate (B). (a) 5 ppm of nitrite and nitrate. (b) Corned beef sample. The estimated concentration in the aqueous extract corresponds to 57 mg of nitrite and 32 mg of nitrate per kg corned beef. (c) Beef sample. Conditions: Waters IC-Pak A column with 5 m*M* dipotassium phosphate; flow-rate, 0.8 ml/min; injection volume, 20 μl, detection wavelength, 215 nm.

obtained under repeatability conditions[11] was dependent on the amount of solute injected and varied from 1 to 4%. The regression of peak area *versus* concentration over the range 0.2–100 ppm (as sodium nitrite or sodium nitrate) was linear for nitrite and nitrate. Fig. 1b shows a chromatogram of an aqueous extract of cured meat. The estimated concentration in the extract corresponds to 58 mg of nitrite and 25 mg of nitrate per kg of cured meat. Fig. 1c shows the chromatogram of a sample of similar beef which was not cured. The method has also been used on luncheon sausage, saveloys and frankfurters and shown to be free from interferences.

When 30 m*M* methanesulphonic acid (adjusted to pH 6 with sodium hydroxide) was used as eluent, retention times of nitrite (5.6 min) and nitrate (9.2 min) were similar to those obtained with the above eluent. First proposed by Ivey[13] as an eluent for the chromatography of inorganic anions using both UV absorption and conductivity detection, methanesulphonic acid offers no advantages in this application.

Prior to the development of this method, this laboratory determined nitrate in cured meat samples by reduction with cadmium followed by diazotisation of sulphanilamide and coupling with N-(1-naphthyl)ethylenediamine hydrochloride. The absorbance of the resulting solution was measured at 540 nm[11].

Samples of cured meat were extracted and analysed for nitrite and nitrate by both methods and the results are compared in Table I. The agreement for nitrite is excellent while for some samples the nitrate is quite variable. Usher and Telling[4] in their critical review on the analysis of nitrate and nitrite with special reference to meat products discuss in detail the methods for nitrate which use reduction by spongy cadmium. They find the disadvantages are the variable sample blanks which can range from 0 to 30 mg/kg and occasionally up to 80 mg/kg, and inconsistent reduction efficiencies, particularly if polyphosphate is present in the meat. They conclude by suggesting the method is not ideal and is suspect for low levels of nitrate (10 mg/kg) in food samples.

TABLE I

COMPARISON OF CHROMATOGRAPHIC AND FUDGE AND TRUMAN'S[11] METHODS FOR DETERMINATION OF NITRITE AND NITRATE IN CURED MEATS

Nitrite (mg/kg)		*Nitrate (mg/kg)*	
HPLC	*Fudge and Truman*[11]	*HPLC*	*Fudge and Truman*[11]
8.7	8.5	21.7	27.1
17.5	17.0	27.8	28.8
58.0	57.9	33.0	25.1
38.4	38.7	229	178
17.0	17.2	18.4	34.6
13.7	13.1	29.7	49.0
42.3	41.9	25.2	23.7
11.7	11.6	18.3	18.0
318	330	48.0	48.8
23.8	24.5	174	165
12.6	12.6	19.4	20.2

In contrast the HPLC method, particularly when automated, is considerably more convenient and rapid and the results for nitrate are more precise with a lower detection limit (approximately 1 mg/kg with a 100-μl injection volume of the extract).

We have found that peaks arising from malic and tartaric acids are not fully resolved from that of nitrate and care should be taken if these are suspected additives to cured meats, or if the method is used for the determination of nitrate in plant tissues.

The microbiological stability of aqueous solutions of nitrate is always open to question and the nitrate concentrations in unpreserved samples can be dramatically reduced in a short time. The meat extracts are preserved with borax and standards should also be preserved.

CONCLUSIONS

The use of a polymethacrylate gel ion-exchange column with dipotassium phosphate as eluent and detection by UV spectrophotometry permits the precise measurement of nitrite and nitrate at the lower levels required by present legislation.

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