Sodium chloride, nitrate and/or nitrite and polyphosphate ions are widely used as preservatives in meat production [1]. From technological point of view, the most important reason for adding nitrites and/or nitrates is the formation of red colour, which is a major determinant in consumer purchasing attitude [1, 2]. The use of these ions in the manufacturing of meat products is commonly expressed as “curing processes”, and the norms are different in various European regions [3, 4].

However, an obvious benefit of nitrites use is the increased safety of products. Nitrates and nitrites used in combination with sodium chloride serve as important antimicrobial agents in meat to inhibit the growth of bacterial spores, which cause botulism, a deadly food-borne illness. In many countries the use of both substances, usually added as potassium or sodium salts, is limited [5], because high intake of nitrites presents a risk to human health [3, 5–7]. Due to these toxic effects many papers described the presence and determination of both ions in meat samples, whereas less attention was paid to nitrites. It can be noted that nitrites in food can be reduced to nitrites and both can be hazardous to humans if ingested in large amounts [6, 8]. On the other hand, this process is slow, mainly through reduction by microbial enzymes.

From both technological and health standpoints, it is essential to develop analytical methods for determination of these ions in meats, in order to ensure sufficiently accurate results. Spectrophotometric methods based on the variations of the Griess reaction [9] and flow-injection analysis (FIA) systems are most widely used for nitrates and nitrites determination [10–13]. However, these methods have such disadvantages as employment of large volumes of toxic reagents and time-consuming procedures. Other methods based on new spectrophotometric reagents [8, 14, 15] and alternative detection techniques, such as potentiometry [16, 17] or chemiluminescence spectroscopy [18, 19] have been also reported. Most of them involve prior reduction of nitrates to nitrites [10, 20], usually by flowing through a copperized cadmium column [8, 21–23]. Moorcroft et al. [24] reviewed methods of nitrates and nitrites determination in various matrices. Parolari et al. [25] studied the spectral and extraction properties of nitrate-free dried hams as a function of maturing time and muscle type. Ion chromatography [26–28] or HPLC [29, 30] are other common methods of determination of these ions, however they employ expensive columns and have relatively long run times. Furthermore, in the case of biological or food samples, HPLC requires complicated sample preparation (e.g., clean-up). Capillary electrophoresis (CZE) is an alternative separation technique that has the potential for the determination of nitrate and nitrite ions [31–34]. The advantage of the CZE methods is the considerable reduction of sample preparation and analysis times, as well as reagent consumption. Simultaneous determination of nitrates and nitrites in meat products by capillary electrophoresis was examined by Özték et al. [6]. This method was applied by Marshall and Treherry [33] for determination of nitrates and nitrites in cheese, cabbage puree, fruit juice, water and a variety of meat products.

Application of Capillary Isotachophoretic Method to the Determination of Nitrate and Nitrite Ions in Meat Products1

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Abstract—The application of capillary isotachophoretic method to the simultaneous determination of nitrates and nitrites in meat products was studied. Simple electrolyte system allowed separation of ions in standard solution with within-day and between-days coefficient of variation (CV) of relative step height in the ranges 2.3–2.5% and 4.1–5.5%, respectively. The levels of nitrates and nitrites ranged from 3 to 65 mg/kg NaNO₃ and from 36 to 111 mg/kg NaNO₂. The coefficients of variation for nitrites were 0.7–3.5% and for nitrates 2.3–5.9%, that indicated satisfactory precision of the proposed method. The recoveries for meat samples spiked with nitrites and nitrates varied between 95–99% and 91–102%, respectively.

Key words: cITP, cITP–cITP, meat products, nitrates, nitrites

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1 The article is published in the original.
Table 1. cITP results for relative step height, RSH (n = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>nitrates</th>
<th>nitrites</th>
<th>nitrates</th>
<th>nitrites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard solution</td>
<td>Meat extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-day RSH</td>
<td>0.0398</td>
<td>0.172</td>
<td>0.0403</td>
<td>0.179</td>
</tr>
<tr>
<td>Within-day CV_{RSH}, %</td>
<td>2.3</td>
<td>2.5</td>
<td>3.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Between-days RSH</td>
<td>0.0401</td>
<td>0.177</td>
<td>0.0427</td>
<td>0.182</td>
</tr>
<tr>
<td>Between-days CV_{RSH}, %</td>
<td>4.1</td>
<td>5.5</td>
<td>7.3</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Concluding, many methods for determination of nitrate and nitrite ions already exist but they are often complicated and difficult to use in-line. In this paper capillary isotachophoresis (cITP) was proposed for determination of nitrates and nitrites in meat samples after simple sample pretreatment. In contrast to HPLC or CZE, this technique does not require purification and preconcentration of the sample before analysis. In this study the extraction with redistilled water was applied for evaluation of nitrites and nitrates level. In literature, there are no reports on nitrates and nitrites determination in meat samples after simple extraction by cITP method. It should be noted that this technique permits on separation and determination of both ions during one measurement.

In some previous papers the cITP method was proposed for determination of phosphate ions in meat samples [35, 36]. The main goal of the present work is an application of one-dimensional and two-dimensional capillary isotachophoresis to simultaneous determination of nitrate and nitrite ions in meat products. The proposed procedure was validated by obtaining statistical parameters (linearity, limit of detection (DL) and quantification (QL), precision and accuracy) and using certified reference materials.

**EXPERIMENTAL**

**Reagents.** Analytical grade: glutamic acid, hydroxyethylcellulose (HEC) and β-alanine (BALA) were purchased from Sigma Aldrich (Poznań, Poland), whereas HCl, KNO₃, NaNO₂ were from POCH (Gliwice, Poland). Redistilled water was used in all solution preparations (specific conductivity < 10 μS).

**Apparatus.** Isotachophoretic separations were performed using a Villa Labeco EA 100/101 analyzer equipped with a conductivity detector. The isotachophorograms were evaluated with the PC software package supplied with the analyser (KasComp, Bratislava Slovakia). Samples of fixed volume 30 μL were injected via a sample valve by internal sample loop. Meat products samples were centrifuged by laboratory centrifuge MPW-350 (MPW, Warszaw, Poland). For pH measurements a CX-742 (Elmetron, Gliwice, Poland) pH-meter with a combined glass electrode (ERH-11, Hydromet, Gliwice, Poland) was used.

**RESULTS AND DISCUSSION**

The nitrate and nitrite anions are identified by cITP using the relative step height (RSH) parameter [35, 36]. Precision was evaluated as the within-day and between-days coefficient of variation (CV) [37]. Within-day analyses were determined by injection of the nitrates and nitrites standard solutions five times per day. Intralaboratory reproducibility was determined by analysis of the standard solutions during 5 consecutive days. Meat product samples were analysed during two days. The obtained results are presented in Table 1. Data in Table 1 exhibited that nitrate and nitrite ions were separated and could be quantified. The levels of RSH were stable for standard solutions. Between-days RSH were 0.0401 0.177 0.0427 0.182 and 4.1 5.5 7.3 8.9, respectively, that indicated reasonable repeatability and

**Sample preparation.** Ten meat products samples (corned pork) were purchased from local markets. The products acquired nitrites and nitrates in composition (declaration of producer). Prior to analyses, samples were minced and homogenized with a plate of 3 mm diameter holes. The meat products samples (5 ± 0.0001 g purchased products—p.p.) were extracted with 30.0 mL of redistilled water using an orbital shaker for 30 min. The extracts were separated using centrifuge at 9000 rpm for 30 min, followed by double filtration. All extracts were transferred into a 50 mL volumetric flask, made up to the mark and analysed with cITP method. In the case of several samples, dilution with redistilled water was applied.

**Conditions of cITP analysis.** The electrolyte system described in the literature [36] was used. Determination of nitrate and nitrite ions were performed with leading electrolyte LE1 : 5 mM HCl with β-alanine (BALA) to pH 4.5; LE2: 10 mM HCl + 0.1% hydroxyethylcellulose (HEC) + β-alanine (BALA) to pH 4.5, whereas 5 mM glutamic acid, pH = 5.0 was applied as terminating electrolyte (TE). The driving current of the pre-separation column was 200 μA, which decreased to 150 μA during detection. In the case of analytical column 25 μA was applied.