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A sub-minute CZE method to determine nitrate and nitrite in meat products: An alternative for routine analysis

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ABSTRACT

A sub-minute capillary zone electrophoresis (CZE) method was optimized and a simple sample preparation procedure based on the extraction of the analytes with water and sodium tetraborate was developed for the simultaneous determination of nitrate and nitrite levels in meat products. The background electrolyte (BGE) was composed of 20 mmol L^{-1} perchloric acid and 65 mmol L^{-1} β-alanine at pH 3.83. Thiocyanate was used as the internal standard. The proposed method was validated and the uncertainty estimated according to Eurachem guidelines. The run time was only 30 s, allowing analyzing more than 25 samples/h, the good analytical performance confirms the suitability of the method for the analysis of meat products. One sample presented residual nitrite levels above the limit established by MERCOSUL legislation (150 mg kg^{-1}). The use of a fast method in association with a simple sample preparation step means that this procedure represents a possible alternative to fulfill the demand for high throughput in routine laboratory analysis.

1. Introduction

Nitrate and nitrite have been used for centuries in meat curing where they play important roles as food additives (Cammack et al., 1999; Weiss, Gibis, Schuh, & Salminen, 2010), and its effectiveness is well documented; however, the safety of their use has been contested because of toxicological aspects. Nitrate is relatively non-toxic (IDA 3.7 mg kg^{-1} bw^{-1}) but it can be reduced to nitrite, which in high levels can be toxic and even lethal (IDA 0.06 mg kg^{-1} bw^{-1}) (Santamaria, 2006). The principal toxic effect of nitrite is the oxidation of hemoglobin to methemoglobin, a compound incapable of transporting oxygen in the blood, leading to a condition known as methemoglobinemia, which is particularly dangerous in newborn infants (Cammack et al., 1999; Santamaria, 2006). Another aspect of concern related to nitrite is the potential to form N-nitrosamines, compounds formed by the nitrosation of primary and secondary amines, which can be carcinogenic (Ferguson, 2010; Hsu, Arcot, & Alice Lee, 2009; Sindelar & Milkowski, 2012).

Recently, WHO's International Agency for Research on Cancer (IARC) classified the consumption of processed meat as carcinogenic to humans (Group 1), based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer. The experts concluded that each 50 g portion of processed meat eaten daily increases the risk of colorectal cancer by 18% (IARC, 2015). The above-cited N-nitrosamines are one of the reasons of the carcinogenicity of these processed meats.

The European Commission (EC) establishes nitrate levels in non-heated products as 150 mg kg^{-1}, but in some products higher values are allowed (e.g., bacon and Jamón 250 mg kg^{-1}), and nitrite is limited to 150 mg kg^{-1} with some exceptions (e.g., dry cured ham 100 mg kg^{-1}, Wiltshire bacon 175 mg kg^{-1}) (Comission, 2006). The Brazilian Ministry of Agriculture and Livestock (Ministério da Agricultura, Pecuária e Abastecimento, Portuguese acronym MAPA) determines that the maximum allowable levels of nitrate and nitrite in Brazilian meat products are 300 mg kg^{-1} and 150 mg kg^{-1}, respectively (Brasil, 2006). Based on MERCOSUL legislation, MAPA has also established that when nitrate and nitrite are used simultaneously in meat curing, the results should be expressed as residual nitrite and the maximum allowable value is 150 mg kg^{-1} (Brasil, 2009).

The determination of nitrate and nitrite in meat products is part of the routine analysis of meat products for quality control, and the most common procedures performed are the AOAC and ISO official methods. Both of these methods are based on the Griess reaction, but in the ISO method, Carrez I (potassium ferrocyanide) and Carrez II (zinc acetate) are used for the deproteinization (AOAC, 2005; ISO, 1975a, 1975b).

The above-mentioned AOAC and ISO official methods have many characteristics, which are undesirable considering the large numbers of samples associated with routine analysis, and especially with regard to the sample preparation step. This step is laborious and time-consuming (with only one analyte being determined at a time),

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cadmium is used in the nitrate reduction and a large amount of reagents and laboratory supplies are required (generating large amounts of residue) (Öztekin, Nutku, & Erim, 2002; Rincón, Martínez, & Delgado, 2003; Rincón, Martínez, Pérez-Olmos, & Berzosa, 2008; Ruiz-Capillas, Aller-Guiote, & Jimenez-Colmenero, 2007).

Although spectroscopic methods are by far the most widely used for nitrate and nitrite determination in food products, other methods based on analytical techniques have been reported in the literature (Chetty & Prasad, 2009; Öztekin et al., 2002; Santos, Lima, Tanaka, Tanaka, & Kubota, 2009). In the last five years, just a few methods have been published, including ion chromatography (Lopez-Moreno, Perez, & Urbano, 2016) and capillary electrophoresis (CE) (Della Betta, Vitali, Fett, & Costa, 2014; Kalaycıoğlu & Erim, 2016; Pereira, Petruci, & Cardoso, 2012). However, most of these analytical methods are also associated with some of the undesirable characteristics of the official methods mentioned above.

Della Betta et al. (2014) developed and validated a sub-minute capillary zone electrophoresis method for the determination of nitrate and nitrite. With the proposed method, it was possible to separate the analytes in less than 30 s with a simple sample preparation step. When applied to baby food samples, this method provided a high analysis throughput, a characteristic required for routine laboratory analysis, verifying the potential of the method for application to other sample matrices.

Given the importance of determining nitrate and nitrite levels in meat products, the aim of this paper was to optimize and validate a CZE method to determine nitrate and nitrite in meat products. Additionally to develop a sample preparation procedure of low cost which requires a minimal amount of materials, generates low amounts of residue and offers a high throughput.

2. Material and methods

2.1. Reagents and solutions

All solutions were prepared using analytical grade reagents and deionized water (Milli-Q, Millipore, Bedford, MA, USA). Perchloric acid (70%), β-alanine, sodium nitrate, sodium nitrite, potassium thiocyanate, sodium oxalate, potassium bromate, potassium bromide and sodium tetraborate decahydrate (purity > 99%) were purchased from Sigma-Aldrich (St. Louis, CO, USA) and sodium hydroxide from Vetec (Rio de Janeiro, Brazil). Standard solutions (1000 mg L^{-1}) of nitrate, nitrite and thiocyanate and stock solutions (100 mmol L^{-1}) of perchloric acid and β-alanine were prepared and stored at 4 °C until analysis when they were diluted to obtain the working concentration levels.

2.2. Instrumental

The analysis was performed in a capillary electrophoresis system (Agilent Technologies, model 7100, Palo Alto, CA, USA) equipped with a diode array detector, a temperature-control device (maintained at 25 °C) and data acquisition and treatment software supplied by the manufacturer (HP ChemStation®).

Before the first run, the capillary was sequentially rinsed with 1.0 mol L^{-1} NaOH (30 min) and water (30 min). Between runs, the capillary was flushed for 1.0 min with BGE. At the beginning of each day the capillary was conditioned by flushing with 1 mol L^{-1} NaOH (15 min) followed by a 15-min flush with deionized water and an electrolyte solution (15 min). Between runs, the capillary was flushed for 1.0 min with BGE. At the end of each working day, the capillary was rinsed with 1 mol L^{-1} NaOH (10 min) and deionized water (10 min).

CZE method was adapted from Della Betta et al. (2014) and separations were conducted in an uncoated fused-silica capillary of 48.5 cm (8.5 cm effective length × 75 μm I.D. × 375 μm O.D.) purchased from Polymicro Technologies (Phoenix, AZ, EUA.). Direct UV detection set at 210 nm was used and the temperature was maintained at 25 °C. The standards and samples were introduced into the capillary using the short-end injection procedure with a hydrodynamic pressure of 50 mbar for 4 s. The separation voltage applied was −30 kV, with negative polarity on the injection side. The optimized background electrolyte (BGE) used in the proposed method was comprised of 20 mmol L^{-1} perchloric acid and 65 mmol L^{-1} β-alanine at pH 3.83, thiocyanate was used as the internal standard (I.S.) and diluted to obtain a final concentration of 6 mg L^{-1}.

2.3. Samples and sample preparation

Thirteen samples of different meat products were obtained from a local store and kept under refrigeration at 5 ± 2 °C until the analysis. These samples were separated into five categories: fresh meat products (fresh sausage); dry-cured meat products (salami, coppa); heat processed meat products (hot dog sausage, mortadella, ham); raw-salted meat products (salted pork); and cooked-salted meat products (pork ribs, pork loin).

The meat product samples were previously minced and homogenized and 2 ± 0.1 g were weighed into a polyethylene tube to which 18 mL of deionized water and 2 mL of sodium tetraborate decahydrate (STB) 5% were added. The tube was sealed and stirred in a vortex for 1 min and the extraction was performed in a water bath for 20 min at 65 ± 2 °C (stirring occasionally). After cooling to room temperature, the volume was filtered through a Whatman filter paper (no. 1). An aliquot of each sample was collected, appropriately diluted with deionized water, diluted in a proportion of 9:1 (v/v) with I.S. to give a final concentration of 6 mg L^{-1} and then injected into the CE system. All samples were prepared in three independent replicates as soon as possible after their acquirement and before the expiration date.

2.4. Validation for meat products

The method was validated following Eurachem guidelines (EURACHEM, 1998) and the following parameters were assessed: system suitability, linearity, matrix effect, selectivity, precision, accuracy, limit of detection, limit of quantification, robustness and uncertainty measurement.

2.4.1. System suitability

The system suitability was verified considering the relative standard deviation (RSD) of the mean obtained from 10 consecutive injections of the standard solution, for the following parameters: corrected peak area ($area_{(analyte)}$ / $area_{(I.S.)}$) and corrected migration time ($time_{(analyte)}$ / $time_{(I.S.)}$).

2.4.2. Linearity and matrix effect

The linear ranges of the calibration curves (1–12 mg L^{-1} for nitrate and 0.5–6 mg L^{-1} for nitrite) were established considering the limits for the analytes established by the Brazilian regulatory agency. The calibration curves were built from standards at seven equally spaced concentration levels, prepared on each day of analysis in three independent replicates and run randomly. Since blank samples were not available, matrix calibration curves were obtained using standard additions. The ordinary least squares method (OLSM) was chosen to assess the linearity of both calibration curves (standard solution and matrix) and after an exploratory fit by linear regression, residual plots were examined for clear patterns and the presence of discrepant points (outliers). After a visual identification, the outliers were assessed by applying the Grubbs test (Grubbs, 1969) until no further outliers were detected or until there was a drop of 22.2% in the original number of results. The regressions were evaluated for possible violation of the assumptions: normality (Shapiro & Cochran, 1965); homoscedasticity (Cochran, 1941); independence (Durbin & Watson, 1951); and lack of fit in F-test (Snedecor & Cochran, 1989).