Journal of Food Composition and Analysis 24 (2011) 1049–1056

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Article

Capillary isotachophoresis as rapid method for determination of orthophosphates, pyrophosphates, tripolyphosphates and nitrites in food samples

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ARTICLE INFO

Article history:
Received 8 March 2010
Received in revised form 4 November 2010
Accepted 5 December 2010
Available online 18 January 2011

Keywords:
Capillary isotachophoresis
Nitrites
Tripolyphosphates
Pyrophosphates
Orthophosphates
Seafood
Meat products
Food analysis
Food composition

ABSTRACT

Environmentally friendly, simple and sensitive isotachophoretic method to identify and quantify orthophosphates, pyrophosphates, tripolyphosphates, nitrites and nitrates in various food products was developed. Two electrolyte systems (A) and (B) were tested and applied for determination of the above ions in meat (different canned products, smoked, cooked and long-matured pork ham, headcheese) and seafood (raw and cocktail prawns, squids and different mixes of seafood) products. For nitrogen compounds, both systems fulfilled the criteria for separation, precision and recovery. The better separation of pyrophosphates (pyroP) and tripolyphosphates (tripolyP) was obtained from system (B) with the following electrolytes: leading – 10 mM hydrochloric acid + 0.02% hydroxyethylcellulose and glycine to pH = 3.0 and terminating – 10 mM phosphoric acid. This system was characterised by linearity (R^2 = 0.999 for all ions), accuracy (recoveries ranged from 97 to 98% for pyroP and 95 to 97% for tripolyP), detection: 0.64 (pyroP) and 0.27 mgP L^{-1} (tripolyP) and quantification: 2.12 (pyroP) and 0.91 mgP L^{-1} (tripolyP) limits and intra-assay of relative step height RSH (1.27–10.73%) and inter-assay of RSH (3.95–11.17%). Additionally, the obtained results of phosphate additives determination were compared at the level of added phosphorus calculating as the difference between the total phosphorus and protein bound phosphate.

1. Introduction

Meat, poultry and fish are highly perishable when fresh. Curing is a conservation technique widely used to prolong the shelf life of food products. These techniques vary from one region of Europe to another. However, sodium chloride, nitrate and/or nitrite and polyphosphate ions are the most popular preservatives in the curing processes irrespective of the country (Marco et al., 2006). On the other hand, the usage of nitrite ions in muscle food may lead to the formation of carcinogenic nitrosamines (Dineen et al., 2000). Besides nitrites, sodium chloride is one of the unaccented additives in food products. It is an important compound not only from the technological point of view, but also for its role in sensory characteristic of the final products. Because NaCl contributes to water and fat binding in meat products, reduction of its concentration has an adverse effect on these parameters increasing cooking loss and weakening the texture (Fernández-Ginés et al., 2005). Partial replacement of NaCl by other compounds (phosphate ions, calcium ascorbate) is proposed as a viable way of decreasing sodium level in food. During the last decade the change of the image of meat products was achieved by elimination or reduction of different additives (Fernández-Ginés et al., 2005). The problem of additives has been widely recognized, and, as a consequence, statutory frameworks, aimed at controlling their level within food products, have been imposed in the most industrialized countries (Moorcroft et al., 2001). It can be concluded that the requirements to monitor these ions in food samples are unquestionable. The evolution of food production technologies has resulted in an increased need of new analytical methods that can quantitatively determine some food additives, in particular nitrite, nitrate and phosphate ions.

Many techniques possess sufficient applicability to enable their detection among the potential interferences that can be encountered within food, environmental or industrial samples. Various analytical techniques have been developed to determine nitrite, nitrate and phosphate additives but only few allow for the simultaneous determination of ions in a single measurement. Usually, chromatographic (Helaleh and Korenaga, 2000; Stalikas et al., 2003; Stefanović et al., 2001; Jobgen et al., 2007; Gapper et al., 2004; Hsu et al., 2009) and capillary electrophoresis (Mikuška and Večeřa, 2003; Fukushia et al., 2003; Alonso and Prego, 2000) methods are proposed for this purpose. The expensive equipment, operating costs and complicated sample preparation (in the case of biological or food samples) are the main disadvantages of the chromatographic methods. Capillary

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0889-1575/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2010.12.007

isotachophoresis (cITP) has been studied recently as a new approach for the analysis of food additives. The use of cITP method has grown rapidly during the last years. The reason for the increased acceptance of this method is a short analytical time, uncomplicated and economical procedure with the minimal sample preparation and small sample requirements (Sádecká and Polonský, 2003; Deck et al., 2003; Ježek and Suhaj, 2001). The main advantage of this technique is the possibility of fast simultaneous detection of a wide variety of anions. Recently this method has been applied to the determination of selected ions, food preservatives and functional additives (Kvasnička et al., 2005; Sádecká and Polonský, 2001; Sádecká et al., 2001; Jastrzębska, 2006, 2010; Jastrzębska et al., 2008, 2009).

In this paper a new electrolyte system of cITP method for the selective and sensitive determination of some food additives was described. The proposed method allowed for simultaneous determination of pyro- and tripolyphosphates, nitrites and nitrates in food samples after simple sample preparation. The obtained results were compared with electrolyte systems discussed earlier for determination of sodium tripolyphosphate (Jastrzębska, 2006) and nitrogen additives (Jastrzębska, 2010) in meat samples. Additionally, the results of phosphate additives (pyro- and tripolyphosphates) obtained from the proposed electrolyte system were compared at the level of added phosphorus calculating as the difference between the total phosphorus and protein bound phosphate.

The phosphorus and nitrogen additives are usually applied in the presence of sodium chloride, for this reason the level of NaCl was determined as well.

2. Experimental

2.1. Reagents

Analytical grade: β-alanine (BALA), bis–tris-propane (BTP), hydroxyethylcellulose (HEC), glycine, $Na_5P_3O_{10}$, $Na_3P_3O_9$, $Na_2H_2P_2O_7$, were purchased from Sigma–Aldrich (Poznań, Poland). NH_4VO_3, $(NH_4)_6Mo_7O_{24}$, HCl, HNO_3, H_2O_2, KH_2PO_4, $NaNO_2$, NH_4NO_3, $(NH_4)_2SO_4$, H_3PO_4, H_2SO_4, CuO, NaOH, $AgNO_3$, and K_2CrO_4 were obtained from POCH (Gliwice, Poland). Redistilled water was used in all solutions preparation (specific conductivity <10 μS).

2.2. Apparatus

Isotachophoretic separations were performed using a Villa Labeco EA 100/101 analyzer equipped with a conductivity detector. The isotachopherograms were evaluated with the PC software package supplied with analyser (KasComp, Bratislava Slovakia). Samples of 30 μL fixed volume were injected via a sample valve by internal sample loop. Food product samples were homogenized in household food grinder (Braun Triumph G 3000) with a plate of 3 mm diameter holes and centrifuged by laboratory centrifuge MPW-350 (max speed 9000 rpm, RFC 8693 × *g*, angle 30°, falcon tubes 50 mL, MPW, Warsaw, Poland). Microwave mineralization of certified reference material products was performed by microwave digestion system (ERTEC MAGNUM II, Ertec-Poland, Wrocław) working in the closed system. The maximum power was 600 W and the maximum temperature and pressure in the Teflon vessel (Hostaflen TFM, Berghof) were 300 °C and 50 bar, respectively. Absorption spectra (total phosphorus determination) were recorded with a Helios α-UNICAM spectrophotometer in a 1 cm quartz cell, against reagent blank.

2.3. Sample preparation for isotachophoretic (cITP) method

A total of 9 meat product samples and 6 seafood samples were purchased from local markets. The analyzed samples comprised: different canned meat products (marked as 2–5m and 8m), pork ham: smoked (6m), cooked (1m) and long-matured (7m), headcheese (9m), raw (1s) and cocktail prawns (2s), squids (3s) and three different mix of seafoods (4–6s). All seafoods were bought as deep-frozen food. According to the producer declaration, all meat products contained the sodium chloride, phosphorus(V) compounds (orthophosphates, pyrophosphates and tripolyphosphates) and nitrites. Additionally, three samples: smoked ham (6m), long-matured ham (7m) and canned meat product (8m) contained also sodium nitrate. In the case of seafood samples all of them contained sodium chloride, polyphosphates and/or orthophosphates.

Prior to analysis, samples were minced and homogenized in household food grinder with a plate of 3 mm diameter holes. The food product samples (5 ± 0.0001 g purchased products – p.p.) were extracted with 30 mL of redistilled water using an orbital shaker for 30 min. The extracts were separated using a centrifuge at 9000 rpm for 30 min, followed by double filtration. All extracts were transferred into 50 mL volumetric flasks, made up to the mark and analyzed with cITP method. In the case of several samples, dilution with redistilled water was applied. Isotachophoretic analysis for all tested food samples were carried out in sixfold repetition.

2.4. Microwave mineralization procedure for total phosphorus determination

0.7–0.8 (±0.0001 g) of samples were poured with 3 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) into the reaction vessel (Teflon Hostaflen TFM, Berghof) and digested in a closed microwave system. Program of mineralization included 3 stages: (I) 5 min at 180 W (temp. 295–300 °C, pressure 16–19 MPa), (II) 10 min at 240 W (temp. 295–300 °C, pressure 32–35 MPa) and (III) 10 min at 300 W (temp. 295–300 °C, pressure 42–45 MPa). Obtained clear solution was transferred into 50 mL volumetric flasks and made up to the mark with redistilled water.

The total phosphorus determination was carried out by the previously reported UV–vis method (Jastrzębska and Szłyk, 2009) according to Polish Standard Method (PN-ISO 13730, 1999). Five replicate analyses of all food samples were carried out.

2.5. Conditions of isotachophoretic analysis

Two electrolyte systems (A) and (B) for selected additives determination in food samples were used; the conditions of the analyses are listed in Table 1.

The tested anions were identified by one-dimensional cITP using the relative step height (RSH) (Jastrzębska, 2006). Precision was evaluated as the within-day and between-days coefficient of variation (CV) (Miller and Miller, 2000). Within-day analyses were determined by injection of the tested ions in the solutions five times per day. The intralaboratory reproducibility was determined by analysis of the standard solutions during 5 consecutive days.

Calibration curves were constructed using six calibration solutions of tested ions in the range: 10–100 mg L^{-1}. Results were calculated as an average of five replicates. Calibration points were established by measuring the zone length (*L*) versus standard concentration (*C*).

The accuracy of cITP method (recovery studies) was evaluated by standard addition method using all ions as spiking standards to meat sample extracts. The solution of orthophosphates, pyrophosphates, tripolyphosphates, nitrites and nitrates in concentration of 15 and 30 mg L^{-1} were added to meat sample extracts. For the