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Determination of sodium tripolyphosphate in meat samples by capillary zone electrophoresis with on-line isotachophoretic sample pre-treatment

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Abstract

The usefulness of zone capillary electrophoresis (CZE) in combination with isotachophoresis (cITP) as on-line preconcentration technique was examined for analysis of tripolyphosphate (STPP) in meat and meat products. The mean concentrations of STPP in different types of meat products varied from 39 mg P_2O_5/100 g to 219 mg P_2O_5/100 g, these values are below the legal requirements. The detection (LOD) and quantification (LOQ) limits for STPP in extracted solutions were 0.80 mg P_2O_5/dm^3 and 2.69 mg P_2O_5/dm^3, respectively. Obtained results were compared with the Kjeldahl method. Accuracy (97.4–98.3%) was determined using recovery assay based on standard additions method. Precision was evaluated by within-day R.S.D. (1.40–2.19%), between-days R.S.D. (3.00–3.82%) and demonstrates the benefit of using this procedure for the routine analysis of STPP in meat and their products. The *F*-Snedecor test was employed to compare the precision of the used methods and calculated *F*-test values (4.00, 6.13) were less than the theoretical (6.39).

Keywords: Sodium tripolyphosphate; Meat sample; cITP; cITP–CZE

1. Introduction

The combination of capillary zone electrophoresis (CZE) with capillary isotachophoresis (cITP) was used as an effective method to increase the separation capability and sensitivity of capillary electrophoresis [1]. Successful application of this technique depends on different separation mechanisms of the analyte in cITP and CZE electrolyte systems and the minimum amount of the sample fraction transferred into the CZE column [2]. In cITP–CZE separation, reported by Kaniansky and Maràk [3], combination of the preseparation capillary, filled with leading electrolyte (L) and the analytical column, with terminating electrolyte (T) or some other background electrolyte (BGE) was applied. Other electrolytes systems combinations (L–S–L, T–S–T and BGE–S–BGE) in cITP–CZE were reported as well [4,5]. Kwasnička et al. [5] proposed new combination of the electrolyte system such as T–S–BGE, which was verified by cations (creatinine and 3-methylhistidine in meat samples) and anions determination (fumaric and malic acids in apple juice). Other applications and instrumentation development of cITP–CZE technique were reviewed extensively [2,6–12].

Blatný et al. [13] described determination of iron in drinking and table water (limit of detection (LOD) = 10 μg Fe/dm^3) by on-line coupled cITP–CZE. Anionic EDTA was determined in mayonnaise by on-line cITP–CZE, after extraction of the sample with water [14]. The same technique was used for chlorite determination in drinking water [15]. Lysozyme in selected food [16] and 14 natural constituents (flavonoids and phenolic acids in red wines samples) were separated and determined by the on-line coupled cITP–CZE [17] as well. Therefore, the discussed method can be considered as an alternative way, to standard methods, for the anionic additives determination in food.

Various phosphates are widely used as functional additives in meat, poultry and seafood industry [18]. Among the different poly- and pyrophosphates used in food industry, sodium tripolyphosphate (STPP) is widely applied by meat industry [19].

STPP found numerous applications in meat industry. It improves the water holding capacity of meat and their texture, prevents denaturation of proteins and stabilises the colour of the products. In Poland, the amount of added phosphates is limited to 5 g P_2O_5/kg meat products [20].

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Because the protein-rich food, such as meat, contain natural phosphorus compounds (nucleotides, phospholipids, etc.) along with naturally occurring orthophosphates, hence the direct determination of polyphosphates in meat products is difficult. Therefore, different analytical techniques, such as ion chromatography (IC) [21–23], capillary electrophoresis (CE) [24–26], spectrophotometry (UV–vis) [27], combination of enzymatic and colorimetric methods [28], were used for mono-, di- and polyphosphates determination in solution. Sekiguchi et al. [23] applied IC with an on-line hydroxide eluent generator for determination of the condensed phosphates in ham, fish paste and cheese samples. The CE was used for determination of polyphosphates in a commercial detergent in water solution [29] and LOD for tripolyphosphate, pyrophosphate and orthophosphate were in the range 2.0×10^{-5} to 5.0×10^{-5} M. Separation of inorganic phosphorus-containing anions by isotachophoresis, capillary zone electrophoresis and capillary gel electrophoresis (CGE) was reviewed by Wang and Li [30].

Unfortunately, methods of polyphosphates determination in food sample were not explained clearly and most of these techniques were too complex to apply in a standard laboratory conditions. Therefore, it would be beneficial for quality control laboratories to elaborate simple and accurate technique to determine the amount of the added phosphates. There are only few reports on phosphorus ions determination in meat samples by cITP in the literature [31,32]. Hence, in this study isotachophoretic method for analyses of phosphate species in meat products was proposed. Furthermore, capillary zone electrophoresis (CZE) with capillary isotachophoresis (cITP) on-line combination, which could be simple and sensitive method of tripolyphosphate determination in meat samples, was applied.

In the presented work, three separation systems: cITP (one-dimensional), cITP–cITP (two-dimensional) and cITP–CZE (T–S–T electrolyte system) will be compared using isotachophoretic analyser with coupled column system and conductometric detection. Sodium tripolyphosphate (STPP), the major phosphate additive in meat industry, was chosen for anionic separation and determination tests (cITP–CZE and cITP–cITP). Because the official method for determination of STPP in meat products does not exist, hence the obtained results will be related to standard method of added phosphorus determination (Kjeldahl) [33,34]. Sample preparation is an important stage, in food analysis by means of capillary zone electrophoresis, particularly when the analyte concentration is low. Therefore, the extraction procedure [35] and dry ashing method [36,37] were used for samples preparation in phosphorus determination.

2. Experimental

2.1. Reagents

Sodium tripolyphosphate STPP (anhydrous pentasodium tripolyphosphate), bis–tris-propane (BTP), β-alanine (BALA), hydroxyethylcellulose (HEC), succinic acid for cITP and CZE were purchased from Sigma–Aldrich, whereas HCl, NH_4VO_3, NaH_2PO_4, $(NH_4)_6Mo_7O_{24}$, NaOH, CuO, H_2SO_4, K_2SO_4, $Na_2B_4O_7$ and HNO_3 were purchased from POCH (Gliwice, Poland). All reagents were of analytical grade. Redistilled water (specific conductivity below 1 μS/cm) was used in all solutions preparation.

2.2. Apparatus

Isotachophoretic separations were performed using a Villa Labeco EA 100/101 isotachophoretic analyser equipped with a conductivity detector. The PTFE pre-separation capillary (160 mm; 0.8 mm i.d.) was connected with PTFE analytical capillary (160 mm; 0.3 mm i.d.). Samples of 30 μl fixed volume were injected via a sample valve by internal sample loop. The isotachopherograms were evaluated with the software supplied with analyser (KasComp Ltd., Slovakia).

Absorption spectra (total phosphorus analysis) were recorded with a Helios α-UNICAM spectrophotometer in a 1-cm quartz cell against reagents blank.

2.3. Conditions of analysis

The electrolyte system described in the literature [31] for STPP determination in meat samples was used (Table 1). Furthermore, two-dimensional cITP and the on-line combination of capillary zone electrophoresis with capillary isotachophoresis in anionic separation of tripolyphosphate with coductometric detection were elaborated (analyses conditions listed in Table 1).

2.4. Analytical procedure

2.4.1. Samples preparation

Raw pork meat was purchased from local slaughterhouse and two meat products (cooked and smoked ham) from local market in Toruń, Poland. The analytical samples of meat and their products were prepared according to Polish Standard [36] as follows: meat products were analysed within 24 h. Prior to analyses, samples were minced and homogenised with a plate of 3 mm diameter holes. According to ref. [35] meat sample 5 ± 0.01 g was extracted with 15 cm^3 of NaOH on an orbital shaker for 30 min. Blatný et al. proposed 0.1 M NaOH but in this paper the concentration of NaOH was 1 mM. The extracts were separated using centrifuge at 15,000 rpm for 20 min and filtrated. The pH was slightly above 8 stabilised by buffer solution (20.5 cm^3, 1 M HCl + 4.77 g $Na_2B_4O_7$), since the polyphosphates are not stable in aqueous solutions and lower pH would accelerate their hydrolysis [18,21,31]. All extracts were transferred into a 50 cm^3 volumetric flask and made up to the mark. Solutions were analysed within 2 h after preparation. The reproducibility of the methods was checked by five replicate determinations of STPP in the same meat samples over the period of 5 days.

For total phosphorus analysis samples were digested with a dry ashing method as follows: 5 ± 0.01 g of sample in a quartz crucible was placed in a furnace and ashed at 450 ± 25 °C, cooled, 10 cm^3 of 22% nitric acid was added and heated up to 150–160 °C on a hotplate. The resulting solution was filtrated, placed into a 50 cm^3 volumetric flask and made up to the mark. Blank samples were also carried out in the same way.

Table 1
Composition of the electrolyte systems and conditions of analyses

Electrolyte system		Conditions of analysis	Detection
1	One-dimensional cITP	LE: 8 mM HCl + 3 mM bis–tris-propane (BTP) + 0.2% hydroxyethylcellulose (HEC) + 31.2 mg β-alanine (BALA) TE: 5 mM citric acid I_1: 250 μA	CON
2	Two-dimensional cITP	LE 1: 8 mM HCl + 3 mM BTP + v0.2% HEC + 31.2 mg BALA LE 2: −1 mM HCl + 0.1% HEC + 22.3 mg BALA TE: −5 mM citric acid I_1: 250 μA	CON
3	cITP–CZE	LE: 8 mM HCl + 3 mM BTP + 1.5 M BALA + 0.1% HEC TE: 5 mM succinic acid BGE: 10 mM succinic acid + 15 mM BALA + 0.1% HEC I_1: 250 μA	CON–CON

2.4.2. Determination of total phosphorus by standard spectrophotometric method [37]

Mineralised sample (2.5 cm^3) was transferred into the 50 cm^3 volumetric flask followed by the addition of ammonium vanadate(V), ammonium molybdate(VI), nitric(V) acid (15 cm^3) and made up to the mark. Obtained solutions were left for 15 min to ensure colour development and absorbance was measured at 430 nm against reagents blank.

The calibration curve was measured using five working standards prepared from standard solution of KH_2PO_4 (0.0070 M) in the same way as above and calculated regression equation was $y = (0.3521 \pm 0.0041) \times x + (0.0040 \pm 0.0007)$. The calibration plots were linear in the concentration range from 1.0 mg P_2O_5/dm^3 to 6.0 mg P_2O_5/dm^3 with the correlation coefficient $R^2 = 0.9978$, whereas molar absorptivity, $\varepsilon = 1.11 \times 10^4$ dm^3 mol^{-1} cm^{-1}. According to Polish Standard [37] phosphorus content was expressed as phosphorus pentoxide.

2.4.3. Determination of net phosphorus by Kjeldahl method [33]

The amount of added phosphorus was obtained by calculating the difference between the total phosphorus and protein bound phosphate, which were determined from the total nitrogen by the Kjeldahl method. Samples (3 g) were digested with a mixture of sulphuric acid, K_2SO_4 and CuO. After cooling, the solution of NaOH was added to pH 8.3 and the sample was distilled after addition of 10 cm^3 33% NaOH in Parnas–Wagner analyser. The obtained solution was used for the titrimetric determination of nitrogen (N × 6.25 = net protein).

Added phosphorus as phosphorus pentoxide in the meat samples was calculated as follows: mg $P_2O_5/100$ g = 2.29 × 0.01 mg net protein/100 g (where 2.29, factor of P_2O_5; 0.01, factor of net phosphorus in pork meat).

2.5. Validation of electrophoretic method

For LOD calculation the value $(y + 3 \times s_{y/x})/b$ was used, where the intercept of the calibration curve estimates y, $s_{y/x}$ the standard deviation in the y-direction of the calibration line and b is the slope of the calibration line. The value of $10 \times s_{y/x}/b$ was used for estimation of LOQ [38].

The precision of the presented method was expressed in terms of the relative standard deviation (R.S.D.). Five injections of standards were performed sequentially. Within-day and between-days precision validation of polyphosphates determination was calculated. The analyses were repeated after 5 days. The linearity of calibration curves was determined from repeated injection at six different concentrations of STPP. Accuracy was calculated using recovery test based on standard additions method. The content of tripolyphosphate in the real samples determined by the studied methods were compared with the Kjeldahl method [33].

3. Results and discussion

The electrophoretic methods used for the analysis of meat samples were applied for identification and determination of tripolyphosphates.

Figs. 1–3 demonstrate the typical isotachopherograms and electropherogram of real samples for all tested systems.

The STPP in cITP techniques was identified with the relative step height (RSH) parameter, which was calculated from the relation: RSHX = (HX − HL)/(HT − HL), where HX is the zone height of STPP, HL and HP is step height of leading and

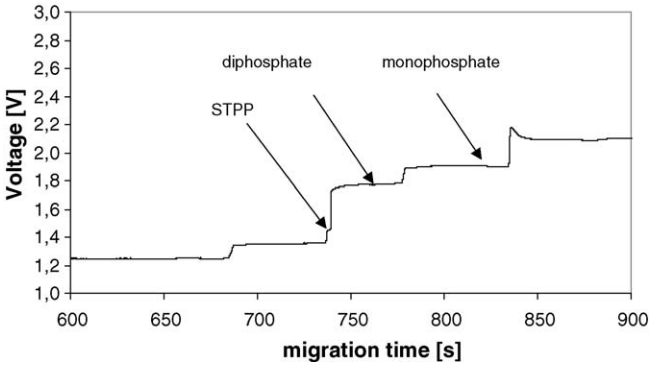

Fig. 1. Determination of smoked ham samples by one-dimensional cITP (analytical capillary). Electrolyte system 1 (Table 1).

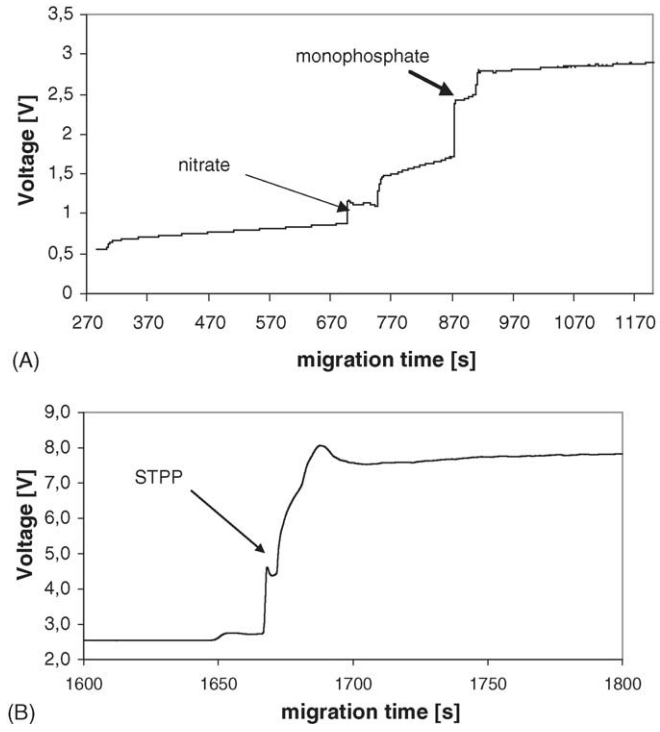

Fig. 2. Determination of smoked ham samples by two-dimensional cITP. Electrolyte system 2 (Table 1): (A) preseparation capillary and (B) analytical capillary.

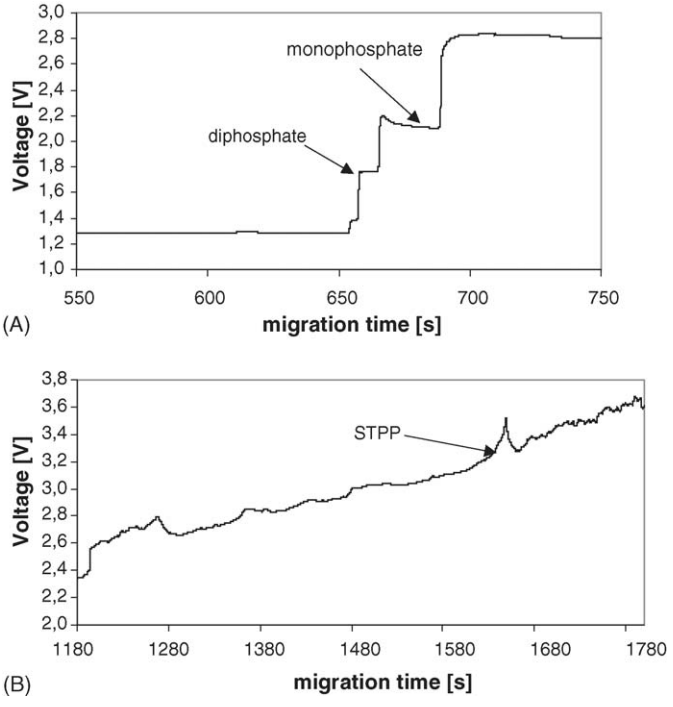

Fig. 3. Determination of smoked ham samples by cITP–CZE combination. Electrolyte system 3 (Table 1): (A) preseparation capillary and (B) analytical capillary.

Table 2
Analytical parameters for cITP, cITP–cITP and cITP–CZE determination of sodium tripolyphosphate (STPP) ($n = 5$)

Parameter	Electrolyte system		
	1	2	3
Relative step height, RSH	0.481	0.416	0.425
Within-day R.S.D.$_{RSH}$ (%)	3.66	2.59	1.68
Between-days R.S.D.$_{RSH}$ (%)	3.53	4.02	2.05
Slope of regression line (b)	0.4339	1.8948	2.932
Intercept of regression line (a)	0.4808	1.0231	1.9962
Coefficient of determination (R^2)	0.9987	0.9991	0.9993
Detection limits, LOD (mg/dm^3)	0.89	0.79	0.80
Quantification limit, LOQ (mg/dm^3)	2.96	2.64	2.69

terminating ion, respectively [39]. To verify these results the isotachopherograms of real samples were compared with the standard solutions. Quantitative analyses of STTP in cITP system were based on the calibration curve expressed as $y = b \times x + a$, where x is the STTP concentration (mg P_2O_5/dm^3) and y is the zone length (s).

Quantitative analyses of STTP in cITP system were based on the calibration curve expressed as $y = b \times x + a$, where x is the STTP concentration (mg P_2O_5/dm^3) and y is the zone length (s). Applied instrument collected the data from cITP–CZE analyses as isotachopherograms or electropherograms. Any significant differences between qualitative analyses applying the relative step height (RSH) and the migration time were not observed. In this paper, the comparison of one- and two-dimensional isotachophoretic techniques with cITP–CZE method was discussed. For this reason the RSH value was used.

cITP–CZE combination allows on quantitative analysis both in the function of peak area (PA) or peak height (PH) versus concentration. In this paper, the quantitative analysis was based on the calibration curve expressed as $y = b \times x + a$, where x is the STTP concentration (mg P_2O_5/dm^3) and y is the peak height (mV).

Three calibration curves for STPP solutions (0.1 M $Na_5P_3O_{10}$) were plotted and the regression parameters are listed in Table 2.

For the studied samples, the relative standard deviations values of the zone heights were below 3.7% ($n = 5$), what demonstrate high reliability. The values of between-days R.S.D.$_{RSH}$ were less than 4.1% indicating reasonable inter-day precision of tested methods for determination of tripolyphosphate. Considering R.S.D. presented in this paper one can suggest the proposed isotachophoretic methods of STPP determination are enough repeatable and reproducible. It is noteworthy, that intra- and inter-day precisions of migration time in cITP–CZE mode were fairly high, the R.S.D. value does not exceed 2.1%.

The lowest LOD (0.79 mg P_2O_5/dm^3) and LOQ (2.64 mg P_2O_5/dm^3) were obtained by means of two-dimensional cITP system. The correlation coefficients values (0.9987, 0.9991 and 0.9993) indicate good precision of linear calibration curves for STPP in all modes. In comparison, Cui et al. found linear calibration curve up to 100 mg/dm^3 with the correlation coefficient $R^2 = 0.9991$ and LOD = 5 mg STPP/kg [21].

Table 3
The average zone length of STPP ($n = 5$) measured during day and after 5 days

Electrolyte system	Length of the STPP zone (s)	Length of the STPP zone after 5 days (s)
1	21.16	12.72
2	20.18	10.34

Hydrolysis of polyphosphates is an important factor, which must be considered in determination of these anions [40]. Linear polyphosphates are reasonably stable in neutral or alkaline solution in room temperature, but their hydrolysis is strongly acid-catalysed and they can be eventually converted to orthophosphates. Dušek et al. [32] discussed isotachophoretic determinations of mono- and polyphosphates anions directly in water extracts. Motooka et al. [41] examined the effect of hydrolysis in the leading solution. He has reported the lack of hydrolysis influence within 24 h of the polyphosphate samples preparation. Also there was no impact on the zone length. Because for the studied system the length of STPP zone (STPP was dissolved in water) decreased with time (Table 3), hence in this study STPP was analysed after extraction with 1 mM NaOH [35,40].

Tested systems (one-, two-dimensional cITP and cITP–CZE) were applied for tripolyphosphate determination in fresh pork and meat products and results are listed in Table 4. Since no certified reference material for STPP was available, the accuracy of the methods was evaluated through recovery assay based on standard additions method. Precision was evaluated as the within-day and between-days R.S.D.

Raw meat was not analysed because the tripolyphosphate zone was not observed on the isotachopherograms, what indicated the lack of tripolyphosphate in fresh meat. Determined STPP contents in cooked ham were between 39–40 mg P_2O_5/100 g purchased product (p.p.) and 177–239 mg P_2O_5/100 g p.p. for smoked ham. The results in Table 4 indicate the significant differences between the mean concentrations of STPP in smoked ham samples, depending on the tested electrolytes systems. In the present study, bis–tris-propane was applied as the counter-ion with complexing ability in anionic mode (Table 1, system 1). The application of the same electrolyte system for determination of total phosphorus in soya food was described [42]. This buffering system was successfully used for analysis of tri- and diphosphate in cooked sausage [31]. Results in Table 4 suggest, that the proposed system can be applied for determination of tripolyphosphate only in smoked ham. In the case of cooked ham, polyphosphate and monophosphate formed mixed zone. The recovery experiments were performed by adding a fixed amount of standard solutions of STPP to the meat samples and were between 85.5% and 89.7% (Table 4). Analysis of data in Table 4 suggest that usage of electrolyte system in one-dimensional cITP is limited due to its low separation capacity for STPP, hence the isotachophoretic experiments were carried out with two-dimensional cITP (Table 1, system 2).

In this system, the composition of the leading electrolyte in preseparation column allowed to apply a suitable pH. Moreover, the complexation properties of BTP for the phosphate analysis permit for quality improvement. In the second capillary, the use of divalent organic cation as a co-counter ion was not necessary.

Mean concentrations of STPP determined by two-dimensional cITP were higher in comparison to the other electrolyte systems. It is noteworthy that the values of R.S.D. were below 5.6% for within-day and between-days precisions. The same parameters in the case of smoked ham for one-dimensional system (mode 1) were significantly higher and ranged between 5.19% and 8.13%. On the other hand, the accuracy, expressed as recovery, was between 105.1% for cooked ham and 110.6% for smoked ham (Table 4). Blatný et al. [35] reported recoveries between 94% and 98% for inorganic acids, including phosphoric, in feed additives after extraction with NaOH, using two-dimensional capillary isotachophoresis. Considering recoveries presented in this paper one can concluded that in this electrolyte system results are to high.

Therefore, samples were analysed by the cITP–CZE using T–S–T electrolyte system with succinic acid as a background anion. The zone of STPP was transferred quantitatively into the analytical capillary filled with BGE. Comparison of studied systems revealed that mean levels of STPP determined by cITP–CZE were slightly lower than by two-dimensional method. On the other hand, proposed method revealed the better statistical parameter, because repeatability and reproducibility (calculated using the R.S.D., $n = 5$) for STPP determination with cITP–CZE method were below 2.20 and 3.90%, respectively.

Table 4
Results of STPP determination (calculated as mg P_2O_5/100g purchased product, p.p.) in pork meat products ($n = 5$)

Electrolyte system parameter	One-dimensional cITP	Two-dimensional cITP	cITP–CZE
Cooked ham			
$X \pm \mu$	–*	41 ± 2	39 ± 1
Within-day R.S.D. (%)	–	3.72	2.19
Between-days R.S.D. (%)	–	4.94	3.82
Recovery (%)	85.5	105.1	97.4
Smoked ham			
$X \pm \mu$	177 ± 11	239 ± 5	219 ± 4
Within-day R.S.D. (%)	5.19	4.43	1.40
Between-days R.S.D. (%)	8.13	5.53	3.00
Recovery (%)	89.7	110.6	98.3

Where X: mean value (mg P_2O_5/100 g purchased product), μ: confidence limit (probability level, $P = 0.05$), R.S.D.: relative standard deviation; *: anions formed the mixed zone.

Table 5
Results of total and net phosphorus in meat samples (mg P_2O_5/100 g p.p.) ($n = 5$)

Parameter	Sample		
	Pork meat	Cooked ham	Smoked ham
Total phosphorus			
$X \pm \mu$	417 ± 15	508 ± 20	656 ± 26
R.S.D. (%)	3.54	4.26	5.51
Net phosphorus			
$X \pm \mu$	398 ± 36	438 ± 7	289 ± 18
R.S.D. (%)	8.57	13.90	10.13

Where X: mean value (mg P_2O_5/100 g purchased product), μ: confidence limit (probability level, $P = 0.05$), R.S.D.: relative standard deviation.

In comparison, the R.S.D. values obtained by Hamoudova et al. [17] ranged between 0.1% and 11%. Similar to system 2, the STPP concentration decreased significantly during 5 days.

Separation and determination of polyphosphates were studied by IC [21–23] or capillary electrophoresis [26]. The obtained recovery and reproducibility for tripolyphosphate were 91.8 and 3.2% (as a peak area), respectively [29]. Determination of mono- and diphosphate was discussed by Stover and Keffer [43]. The CZE results were reported and compared with the IC results. Obtained precision for diphosphate was lower than 3%, but R.S.D. for monophosphate of low concentration was very poor, i.e., about 16%.

To the best of the Author's knowledge the same combination of on-line cITP–CZE was not applied in polyphosphate determination in meat products. Kvasnička and Mikova [14] used on-line cITP–CZE for EDTA determination in mayonnaise and repeatability was better than 5% and recovery higher than 95%. In the case of chlorite in drinking water recoveries were 96–106% for the analysed range of 0.02–0.20 mg/dm^3 [15]. When applied for determination of lysozyme in selected food, the following parameters were obtained: linearity (0–50 μg/cm^3), recovery 96 ± 5%, intra-assay (3.8%), LOQ (1 μg/cm^3) and LOD (0.25 μg/cm^3) [16]. Masár et al. [44] discussed the using of zone electrophoresis with on-line isotachophoresis sample pretreatment on a column-coupling chip with conductivity detection for sulphite determination in wine and obtained recoveries were between 88% and 90%.

In this paper, the accuracy and precision were slightly better than reported [14,15,43,44]. Presented results proved that the on-line cITP–CZE method could be useful for isolation and identification of polyphosphates.

The content of STPP in the meat samples determined by the studied method was compared with the official method of added phosphates determination [33]. The total and net phosphorus content in tested meat products are listed in Table 5.

The total and net phosphorus contents in cooked and smoked ham obtained by standard method were higher than in raw pork meat, due to usage of phosphates as stabilisers in meat products. Obtained concentrations of phosphorus in pork meat (Table 5) are lower in comparison to the reported by Dušek et al. [32] (493.7 ± 12.4 mg/100 g P_2O_5 in a pork meat). Nevertheless, the obtained values for pork meat and ham cooked are in good agreement with the data reported in *Food Composition Table* [45].

According to the reference method [33] the added phosphorus content was calculated by subtraction of the net phosphorus from the total content of this element. The average values for pork meat, cooked and smoked ham were as follows: 19 mg P_2O_5/100 g p.p., 70 mg P_2O_5/100 g p.p. and 367 mg P_2O_5/100 g p.p., respectively.

In the case of raw pork, the difference between the net phosphorus content and total amount can be caused by sample treatment in the Kjeldahl method. It should be noted, that the standard method is indirect and complicated. In comparison, Ünal et al. [18] proposed modified spectrophotometric method of added phosphates determination in meat sample. In this technique all the phosphates were hydrolysed to orthophosphates using combined reagent. However, the ratio of free soluble and total phosphate is more variable than the total phosphorus levels in meat due to post-mortem changes as phosphate is released from compounds such as proteins, nucleotides, phospholipides and phosphocreatine [32]. Comparing studied electrolyte systems with the standard method, it is evident that mean values of STPP determined by cITP methods were lower (Table 4). The latter one can be related to the fact that the electrophoretic methods allow on separation and quantification of the exact form of phosphates, whereas by the standard method all added phosphorus forms were determined. Moreover, the Kjeldahl method revealed worse statistical parameters, because repeatability (calculated using the R.S.D., $n = 5$) for total and net phosphorus were between 3.54–5.51% and 8.57–13.90%, respectively.

The *F*-Snedecor test was employed to compare the precision of the used methods [38]. The calculated *F*-test values were less than the theoretical values, indicating that there was not significant difference between the proposed cITP–CZE and standard methods with regard to precision.

Obtained results suggest usefulness of cITP–CZE in determination of phosphorus ions, because the accuracy and precision of the elaborated method is better than the isotachophoretic methods. cITP eliminates the influence of sample matrix and provides a selective preconcentration of STPP before transfer to the CZE stage. On the other hand, CZE assure final separation simultaneously with tripolyphosphate detection. Sufficient sensitivity and precision, low running cost and low consuming of organic solvents are in favour of the tested configuration as an alternative to the commonly used separation methods (IC, CE). Considering the above facts one can propose cITP–CZE method as convenient way of minor components analysis in the presence of large amounts of macro-components extracted from meat samples. Moreover, presented results indicate, that developed cITP–CZEcITP–CZE method of tripolyphosphates determination in meat products is reliable and reproducible and can be an alternative to the official method.

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