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Determination of some Ionic Additives in Meat Products by Ion Chromatography

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Introduction

Meat products like sausages, cold cuts etc. are made of meat, connective tissues, and fat. Additives like salt, nitrate and nitrite are used to suppress the growth of harmful micro-organisms as well as to achieve pleasant sensorial properties. Furthermore, different condensed phosphates are used because of technological reasons. Polyphosphates like di- and tri-phosphate improve certain rheological properties and increase the water binding ability. Most countries have set up regulations concerning the maximum amount of the stated additives.

There are several analytical approaches to measure *nitrite* and *nitrate* in meat products: The coupling of nitrite with sulfanilamide and N-1-naphthylethylenediamine to a reddish-purple azo dye is successfully used for spectroscopic determinations (1). Nitrate ions can be reduced to nitrite by metallic cadmium (2) or nitrate reductase (3) to make them accessible for the coupling reaction. There are also purely enzymatic methods which reduce nitrite to ammonia by nitrite reductase. The resulting NADH/NAD⁺ ratio is measured photospectrometrically (4). Several separation techniques utilise the ionic character of nitrite and nitrate: Ion-interaction HPLC (ion-pair chromatography) can be used to determine nitrite and nitrate in one run (5, 6). The detection is based on UV or UV and electrochemical signals. Encouraging results were reported by the use of capillary ion electrophoresis (7). Ion chromatography (IC) has been used by several authors (8–10).

There are very few reports concerning the determination of *polyphosphates* in meat products. Thin layer chromatography (TLC) was the only reported technique (11, 12). However, this method is neither quantitative nor does it provide the required sensitivity. The most commonly used P-number (P₂O₅) is of limited value, because it is a composite value combining the different inorganic phosphates and

phospholipides as well. HPLC or IC methods were reported for polyphosphates in the detergent and chemical industry (13–15). Flame photometry (13) or dual post column derivatization (14), as well as suppressor based conductivity detection (15, 16) were reported.

Chloride is commonly determined by argentometric titration. Meat products are homogenised, acidified and titrated with silver nitrate (17).

The ever increasing demand to analyse more parameters and more samples with less manpower has created a considerable interest to determine several parameters in one analytical run. This demand has been partially met by the use of continuous flow auto-analysers (2).

It is the aim of this work to present a fast, simple and affordable IC method for the quantification of all the relevant anions in meat and sea food products.

Method

Instruments and columns

IC was performed with a stainless steel LC: quaternary pump HP1100 (Agilent Technology) GINA, UV-detector UVD 170S and column oven STH 585 (all Gynkotech). The IC 753 suppressor module and IC 732 IC detector (both Metrohm) served as electrolytic conductivity detector. As analytical columns Metrosep Anion Dual 1 (3,0×150 mm) with precolumn Anion Dual 1 (3,0×35 mm) and a Hypercarb (4.6×10 mm, 5 µm) post column were used (all Metrohm, Herisau). For sample preparation a Polytron PT 3000 (Kinematica, Littau) mixer and a centrifuge Sorvall RC 5C (Kendro Laboratry, Zurich) were employed.

Chemicals, materials

Sulphuric acid 98 % p.a, tetra-sodium pyrophosphate decahydrate, penta sodium triphosphate (all Fluka). Di-sodium hydrogen phosphate dodecahydrate, sodium hydrogencarbonate, sodium carbonate (waterfree), sodium chloride, nitrate standard (1 g/l), nitrit standard (1 g/l) (all Merck, Darmstadt). Bidest. water. Micro filter: Chromafil PES-20/25.

Alkaline phosphatase from calf intestine mucous membrane (Fluka).

Solutions standards

Mobile phase A: water

Mobile phase B: 50 mmol sodium hydrogencarbonate and 50 mmol sodium carbonate/l water

Suppressor regenerent: sulfuric acid 80 mmol/l

Extraction solution: 60 ml mobile phase B/l water

Standard stock solutions

Chlorid and phosphate: 3 g NaCl; 2.019 g $\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$; 0.314 g $\text{Na}_4\text{P}_2\text{O}_7 \times 10 \text{ H}_2\text{O}$; 0.173 g $\text{Na}_5\text{P}_3\text{O}_{10}$ dissolved in 100 ml bidest water

Nitrite nitrite standard 1 g/l

Nitrate nitrate standard 1 g/l

Working standard solution

Working standard A: 10 ml of chlorid and phosphate stock solution, 1 ml of nitrite and 2 ml of nitrate stock solution is added into a 100 ml graduated flask and filled with water to volume.

One liter contains:

3000 mg NaCl

400 mg P_2O_5 (as NaH_2PO_4)

100 mg P_2O_5 (as $\text{Na}_4\text{P}_2\text{O}_7$)

100 mg P_2O_5 (as $\text{Na}_5\text{P}_3\text{O}_{10}$)

10 mg NO_2

20 mg NO_3

Working standard B: 50 ml working standard A, filled with water to 100 ml

Working standard C: 25 ml working standard A, filled with water to 100 ml

Working standard D: 10 ml working standard A, filled with water to 100 ml

Working standard E: 5 ml working standard A, filled with water to 100 ml

Procedure

- Weigh 5.00 g sample into a plastic centrifugation vessel.
- If desired spiking solutions might be added: 5 ml chloride and phosphate, 0.5 ml nitrite and 0.2 ml nitrate stock solution.
- Add 150 ml extraction-solution.
- Close the screw cover of the vessel and put it immediately into a boiling water bath for 10 minutes.
- Use the polytron to homogenise the still hot sample solution.
- Put the closed vessel again into the boiling waterbath for 10 min.
- Let the vessel cool in an ice bath for 1 hour.
- Centrifuge at 14 500 rotations per minute (equivalent of 15 000 g) for 5 minutes.
- Filtrate the supernant liquid (below the fat film) through a microfilter directly into a HPLC vial.

All fermented sausage samples or sea products should be quickly processed because of the present enzymatic activities.

HPLC conditions

Flow: 0.5 ml/min

Gradient: 0 min: 6 % B; 5 min: 6 % B; 14 min: 20 % B; 20 min: 100 % B; 20.9 min: 100 % B; 21 min: 6 % B; 29 min: 6 % B

UV detector: 215 nm (nitrite and nitrate)
Conductivity: chloride, mono-, di- and triphosphate
Suppressor switch time: 0.2 min
Injection volume: 10 μ l

Positive results can be confirmed by adding a drop of phosphatase solution to the final HPLC vial and by incubating it for one hour at 30°C. di- and triphosphate peaks disappear selectively after this treatment.

Calibration

Chromatograms can be more easily integrated if a blank (extraction solution) is electronically subtracted. Peak areas are used for quantification. A nonlinear quadratic calibration (cubic, if available) should be applied. If spiking solutions are added, resulting dilutions have to be considered in the calculation.

Validation

Meat products present a number of different matrixes, hence the validation did not focus on a single type of meat product. Hence, the described method was validated thrice. Two important Swiss meat products and fish were chosen (a cooked sausage "Servelat", a fermented sausage "Landjäger" and a plaice fillet). The samples were repeatedly spiked with different volumes of stock solutions (see procedure). The highest spike represented a fortification of 60 g NaCl, 8 g PO_4^{3-} (calculated as P_2O_5), 2 g $\text{P}_2\text{O}_7^{4-}$ (calculated as P_2O_5), 2 g $\text{P}_3\text{O}_{10}^{5-}$ (calculated as P_2O_5), 0.2 g NO_2^{1-} and 0.5 g NO_3^{1-} to 1 kg matrix sample. The linearity was tested by reducing the volumes of added stock solutions. Five different spiking levels were produced. In terms of chloride, this represents fortifications of 60 g; 30 g; 12 g; 6 g; 3 g; 0 g NaCl/1 kg calibration matrix. Three repetitions (independent sample processing and analysing) were performed for each matrix and each level, resulting in a total of 54 HPLC injections.

Linear regression

Figure 1 represents the calibration curves (peak areas of the various analytes versus spike concentration). Table 1 shows the statistical data (spiked concentrations explained by calculated concentrations $y = a + b \cdot x$). The squared coefficient of correlation " r^2 " was always higher than 0.994. The intercept of the linear regression " a " is in many cases clearly above zero. This can be explained by the presence of analyte in the unspiked matrix (e.g. NaCl). There are instances where the slope of the calibration curve " b " lies outside $1 \pm$ the confidence limit. This can be best understood by the non-linear character of the calibration curve, as discussed later. Hence the quadratic calibration function used by the HPLC integration system is not anymore sufficient to describe the uppermost part of the calibration curve. This is clearly visible when comparing the slope for chloride produced by spiking the different matrixes. The slope " b " measured in fish is close to one. On the other hand, a sig-

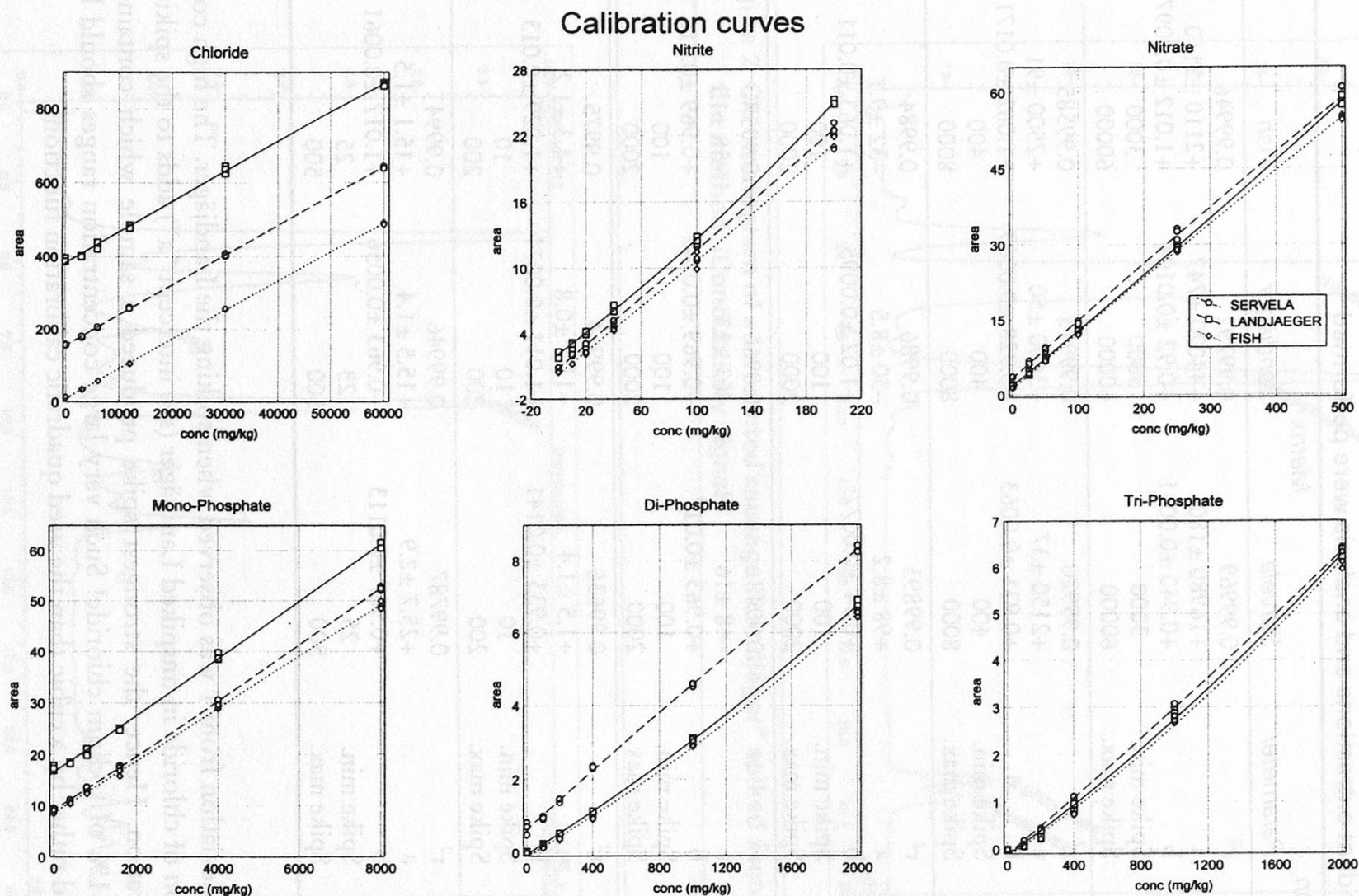


Figure 1 Calibration curves for all analytes in the three matrices (peak area versus spike concentration)

Table 1

Linear regression (spike concentration predicted by the measured concentrations.
Each of the three matrices was spiked with five levels. For each spike level three independent extractions and analysis were performed

| Validation | | Matrix | | |
|--|------------|---------------------|---------------------|---------------------|
| Analyt | Parameter | Servela | Landjäger | Fish |
| NaCl | r^2 | 0.99969 | 0.99792 | 0.99946 |
| | a | +16980 \pm 180 | +44020 \pm 747 | +2110 \pm 2110 |
| | b | +0.940 \pm 0.0041 | +0.92 \pm 0.0104 | +1.012 \pm 0.0097 |
| | Spike min. | 3000 | 3000 | 3000 |
| | Spike max. | 60000 | 60000 | 60000 |
| PO ₄ (P ₂ O ₅) | r^2 | 0.99926 | 0.99913 | 0.99585 |
| | a | +2150 \pm 37 | +3600 \pm 50 | +2500 \pm 91 |
| | b | +0.931 \pm 0.0063 | +0.928 \pm 0.0069 | +1.062 \pm 0.0171 |
| | Spike min. | 400 | 400 | 400 |
| | Spike max. | 8000 | 8000 | 8000 |
| P ₂ O ₇ (P ₂ O ₅) | r^2 | 0.99893 | 0.9986 | 0.9984 |
| | a | +98 \pm 8.2 | -30 \pm 8.5 | -32 \pm 9.1 |
| | b | +0.94 \pm 0.0077 | +1.02 \pm 0.0095 | +1.063 \pm 0.011 |
| | Spike min. | 100 | 100 | 100 |
| | Spike max. | 2000 | 2000 | 2000 |
| P ₃ O ₁₀ (P ₂ O ₅) | r^2 | 0.9948 | 0.9966 | 0.9957 |
| | a | -48 \pm 16 | -63 \pm 13 | -58 \pm 15 |
| | b | +0.963 \pm 0.017 | +0.969 \pm 0.0142 | +0.969 \pm 0.016 |
| | Spike min. | 100 | 100 | 100 |
| | Spike max. | 2000 | 2000 | 2000 |
| NO ₂ | r^2 | 0.99636 | 0.99914 | 0.9975 |
| | a | +1.5 \pm 1.4 | +13.2 \pm 0.8 | +4.3 \pm 1.2 |
| | b | +0.933 \pm 0.0141 | +1.011 \pm 0.0074 | +1.029 \pm 0.013 |
| | Spike min. | 10 | 10 | 10 |
| | Spike max. | 200 | 200 | 200 |
| NO ₃ | r^2 | 0.99787 | 0.99946 | 0.9994 |
| | a | +25.7 \pm 2.9 | +15.5 \pm 1.4 | +15.1 \pm 1.5 |
| | b | +0.967 \pm 0.0113 | +0.963 \pm 0.0056 | +1.017 \pm 0.0061 |
| | Spike min. | 25 | 25 | 25 |
| | Spike max. | 500 | 500 | 500 |

nificant deviation from 1 was observed when spiking the Landjäger. The high concentration of chloride in unspiked Landjäger (see intercept "a") adds to the spiking concentration. Hence, the strongest spike produced a sample which contained almost 11% of sodium chloride! Such very large concentration ranges should be processed rather by a cubic than the used quadratic calibration function.

Limits of detection

Nitrite: 2.5 mg/kg, nitrate 2 mg/kg, di- and triphosphate: 20 mg/kg each. Figure 2 shows a chromatogram (conductivity signal) of a "Landjäger" spiked with

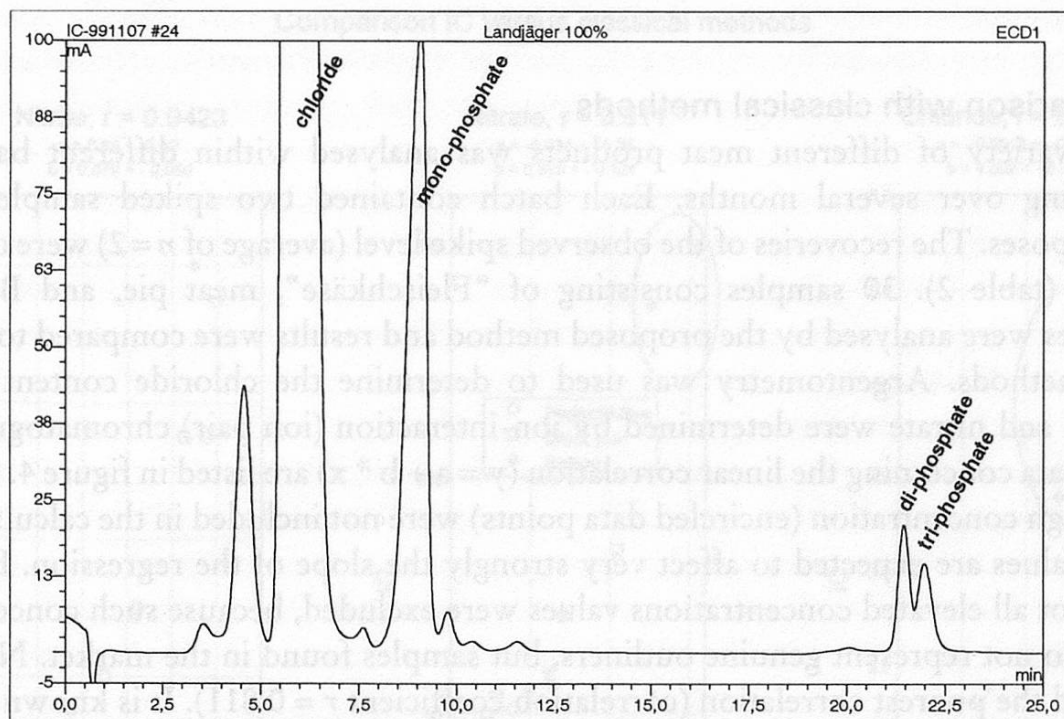


Figure 2 **Chromatogram of a fermented sausage "Landjäger" spiked according to the method. (Conductivity signal)**

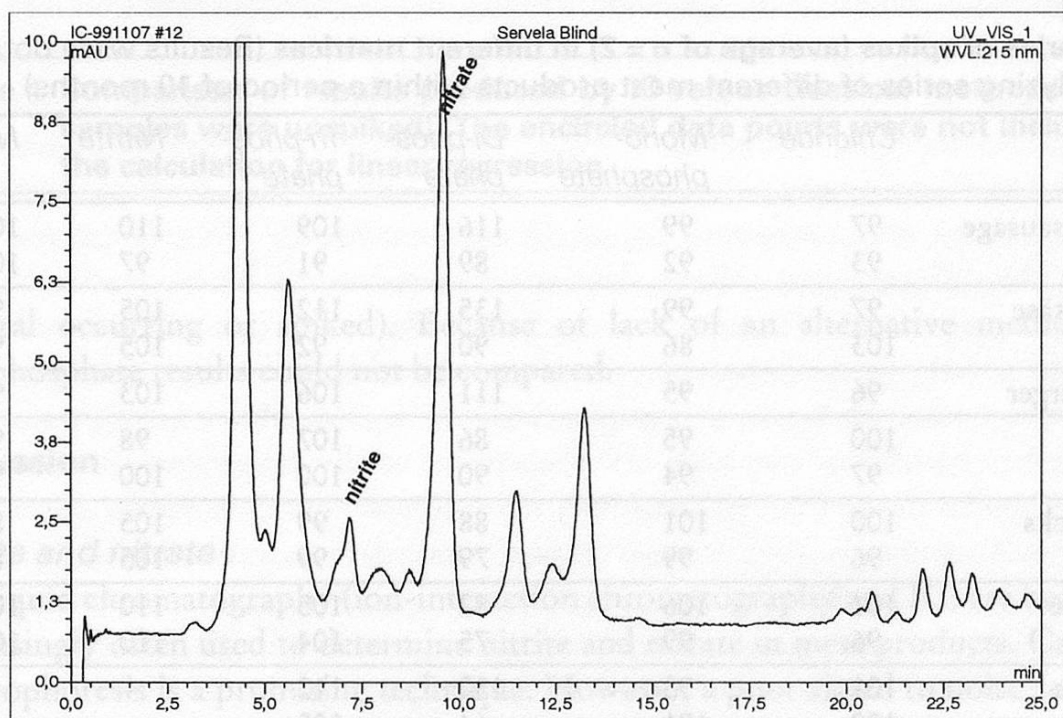


Figure 3 **Chromatogram of a cooked sausage "Servela" unspiked (UV signal)**

stock solution. Figure 3 shows a chromatogram (UV signal) of a "Servela" without spike.

Comparison with classical methods

A variety of different meat products was analysed within different batches spreading over several months. Each batch contained two spiked samples for Q-purposes. The recoveries of the observed spike level (average of $n=2$) were determined (table 2). 30 samples consisting of "Fleischkäse", meat pie, and Balkan sausages were analysed by the proposed method and results were compared to classical methods. Argentometry was used to determine the chloride content (17). Nitrite and nitrate were determined by ion-interaction (ion pair) chromatography (18). Data concerning the linear correlation ($y = a + b \cdot x$) are listed in figure 4. Some very high concentration (encircled data points) were not included in the calculation. Such values are expected to affect very strongly the slope of the regression. However, not all elevated concentrations values were excluded, because such concentrations do not represent genuine outliers, but samples found in the market. Nitrate showed the poorest correlation (correlation coefficient $r = 0.811$). It is known from literature that the determination of low nitrate concentrations presents certain difficulties. A comparison (6) between the cadmium reduction and an HPLC method reported (for a similar nitrate concentration range) a correlation coefficient of 0.67. A significant improvement of the correlation is observed at higher concentrations

Table 2

Recoveries of spikes (average of $n = 2$) in different matrices (Results were obtained by analyzing series of different meat products within a period of 10 months)

| | <i>Chloride</i> | <i>Mono-phosphate</i> | <i>Di-phosphate</i> | <i>Tri-phosphate</i> | <i>Nitrite</i> | <i>Nitrate</i> |
|----------------|-----------------|-----------------------|---------------------|----------------------|----------------|----------------|
| Balkan sausage | 97 93 | 99 92 | 116 89 | 109 91 | 110 97 | 102 104 |
| Fleischkäse | 97 103 | 99 86 | 135 90 | 112 92 | 105 105 | 97 93 |
| Hamburger | 96 | 95 | 111 | 106 | 103 | 93 |
| Ham | 100 97 | 95 94 | 86 90 | 107 100 | 98 100 | 94 96 |
| Fish sticks | 100 96 | 101 99 | 88 79 | 99 99 | 105 105 | 85 83 |
| Meat pie | 101 96 | 106 99 | 82 75 | 105 104 | 110 110 | 105 102 |
| Shrimps | 101 102 | 93 101 | 130 64 | 112 105 | — — | — — |
| Prawns | 103 | 86 | 90 | 92 | 65 | 100 |

Recoveries in % (Spiking level according to procedure)

Comparison IC versus classical methods

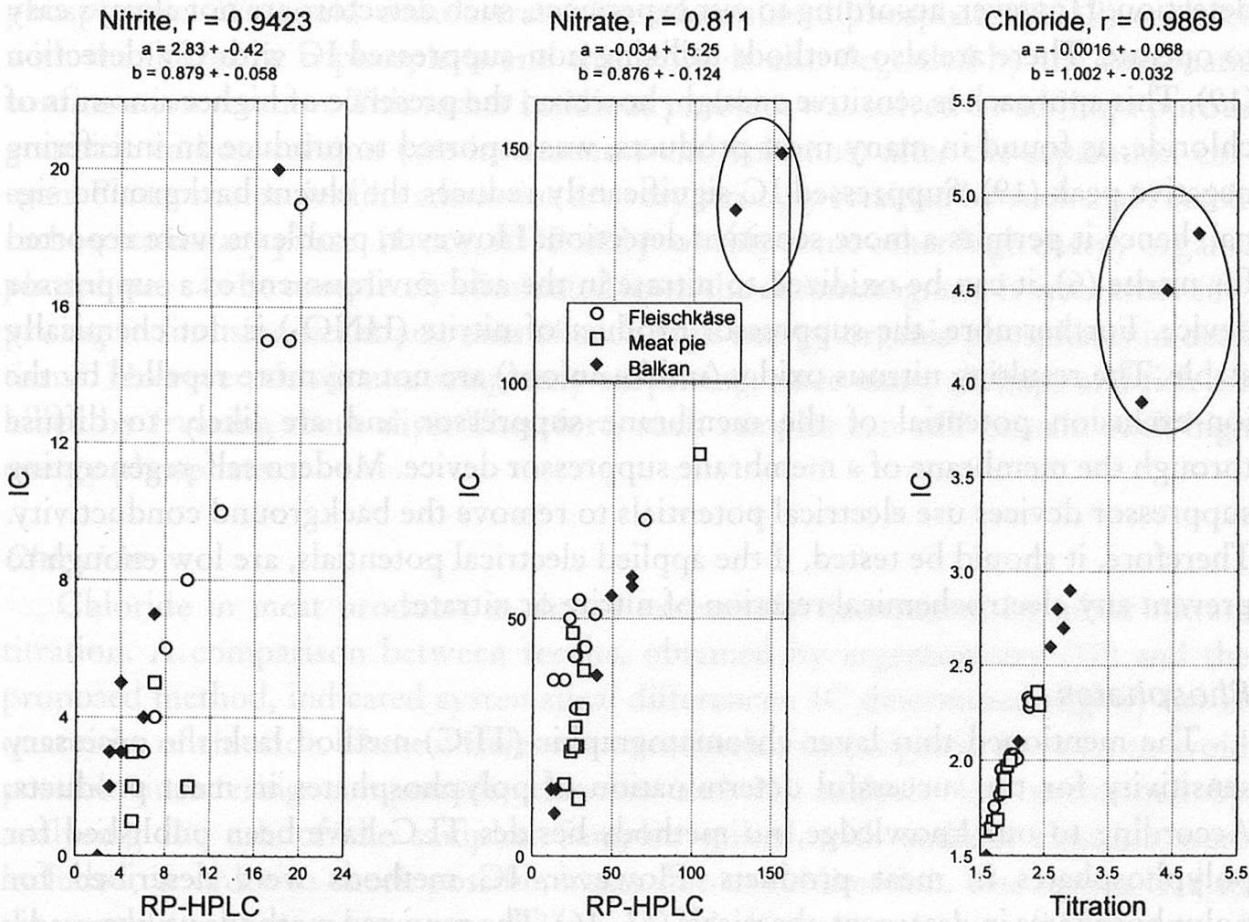


Figure 4 Comparison of results produced by IC versus classical methods (all 30 samples were unspiked). The encircled data points were not included in the calculation for linear regression

(natural occurring or spiked). Because of lack of an alternative method, the polyphosphate results could not be compared.

Discussion

Nitrite and nitrate

Liquid chromatography (ion-interaction chromatography and IC) are becoming increasingly often used to determine nitrite and nitrate in meat products. Capillary electrophoresis is a promising technique. However, a poor signal to noise ratio and a high sensitivity towards changing matrices presents major problems. Ion-interaction chromatography (C-18 column and mobile phase containing a cationic tensid) employs low wavelength UV detection (18). Most meat products produce rather

clean chromatograms without interferences. Still we observed in our laboratory some samples containing a number of interfering matrix peaks. The detection of nitrite can be enhanced by utilizing the more selective and sensitive electrochemical detection. However, according to our experience, such detectors are not always easy to operate. There are also methods utilizing non-suppressed IC with UV detection (19). This approach is sensitive enough, however, the presence of higher amounts of chloride, as found in many meat products, was reported to produce an interfering negative peak (19). Suppressed IC significantly reduces the eluent background signal, hence it permits a more sensitive detection. However, problems were reported for nitrite (6), it can be oxidized to nitrate in the acid environment of a suppressor device. Furthermore, the suppressor product of nitrite (HNO_2) is not chemically stable. The resulting nitrous oxides (unlike anions) are not anymore repelled by the ion-exclusion potential of the membrane suppressor and are likely to diffuse through the membrane of a membrane suppressor device. Modern self-regenerating suppressor devices use electrical potentials to remove the background conductivity. Therefore, it should be tested, if the applied electrical potentials, are low enough to prevent any electrochemical reaction of nitrite or nitrate.

Phosphates

The mentioned thin layer chromatographic (TLC) method lack the necessary sensitivity for the successful determination of polyphosphates in meat products. According to our knowledge, no methods besides TLC have been published for polyphosphates in meat products. However, IC methods were described for polyphosphates in detergent chemicals (13–16). The reported methods utilize gradient IC, because an isocratic elution of mono-, di- and triphosphate is not feasible. The k' -values of the various phosphates are too different to elute them by an isocratic chromatography. There are basically two approaches to separate polyphosphates on an IC column.

Initial work was performed by using acid eluents (HNO_3). Detection was achieved by different post column derivatization reactions. We have utilized iron-nitrate as reagent and UV as detection mode (14). A major problem observed by this approach was the periodic baseline ripples produced by the reagent pump. Chromatograms with such baselines can also be seen in (16). Minor pump pulses can cause spikes because of the high UV absorption of the iron-nitrate reagent. The observed spikes prevent a high sensitivity detection of polyphosphates.

Recently, alkaline eluents, membrane suppressor devices and conductivity detection (15, 16) has been suggested for the quality control of pure polyphosphate chemicals. Reported were high resolution separations from P_1 to P_{35} phosphate units. However, there are no reports how such high performance separations are affected by heavy matrixes like extract of food products.

The proposed method has been used to analyze a great variety of meat products without any interference. However, shrimps, some fish and ham samples produced

an interfering peak in the region of the di- and triphosphate. This peak was visible as a conductivity and a strong UV signal as well. Furthermore, this substance still produced false positives when employing the enzymatic confirmation reaction with phosphatase. We could demonstrate that adenosine-diphosphate (ADP) coelutes with the inorganic triphosphate and that ADP is also degraded by the enzymatic confirmation reaction. This rather insidious problem was solved by adding a porous graphitic carbon column (see instruments and columns) after the separation column. Planar molecules like adenosine are very strongly retained by such a pH stable carbon stationary phase. Hence, ADP and probably some other high energy organic phosphates can be completely eliminated from the chromatogram. It was rather surprising to find significant amounts of such high energy organic phosphates in dead tissue. However, this is not completely surprising, since many shrimps and fish are killed by freezing them alive. Therefore, such samples can still contain such high energy phosphates.

Chloride

Chloride in meat products is almost exclusively determined by silver nitrate titration. A comparison between results, obtained by argentometry (17) and the proposed method, indicated systematical differences. IC determined slightly lower quantities of chloride. These differences were further investigated. In order to avoid possible interfering compounds, titration and IC analysis was performed by analysing the ash of the samples. Samples spiked with sodium chloride were included, in order to check possible losses due to sublimation. The results lead to the conclusion that argentometry delivers slightly higher results, possible because of some organic interference (probably proteins). This bias disappeared when the ash of the sample was analysed.

It is an important feature of this IC method that trace analytes like nitrite are analyzed together with chloride as major component. In order to quantify trace levels of nitrite, a rather concentrated sample has to be injected. On the other hand, high chloride levels tend to overload the column, which results in a non linear peak area response. Yet, the discussed validation showed satisfactory linearities for the three different matrices. However, non-linear calibration is required if lower or higher sodium chloride levels have to be analyzed.

Extraction, clean up

Our results demonstrated that all the mentioned analytes can be easily quantitatively extracted by homogenizing the sample in cold water. Hence we successfully performed initial experiments with cold water extraction. Upon validating the matrix of fermented sausages (e.g. salami), we observed very low recoveries of di- and triphosphates. The reason for this behaviour was attributed to the activity of *phosphatase enzymes* in raw meat products. Surprisingly, decomposition of tri- and di- to monophosphates still proceeded in the HPLC vial. Therefore a hot water

extraction was reintroduced. The high extraction temperature is not important in terms of extraction efficiency, but in terms of enzyme denaturation. Hence the extraction conditions are similar to the ones specified by Charrière (18).

A centrifugation step separates the extraction solution from the insoluble protein and fat. Still, a rather significant portion of protein does not precipitate and remains dissolved in solution. These charged molecules are prone to *contaminate the IC column* (10) and possibly membrane suppressor. However, the standard method for protein precipitation, Carrez solution fails. Because it precipitates mono-, di- and triphosphates almost quantitatively. Perchloric and trichloric acetic acid produced huge, broad IC peaks which occupies large tracts of the chromatogram. But there are other methods to remove proteins, however, such procedures are rather laborious. The risk of shortening the lifetime of expensive latex-type IC columns and membrane suppressors by such "dirty" samples has to be considered. Therefore, ways were sought to cope with the heavy matrix. A reasonable priced spherical hydroxyethyl methacrylate column was used with a precolumn consisting of the same material. This column does not provide the same resolution for polyphosphates as latex based columns. However, hydroxyethyl methacrylate columns are sold at much lower prices than latex based columns. The Anion Dual 1 column separates monophosphate clearly from di- and triphosphate, yet, di- and triphosphate are not completely baseline separated. Longer polyphosphates were not considered because di- and triphosphate are more useful than longer polyphosphates in the field of meat processing technology (20). Another important step was the use of a modern *solid phase suppressor* instead of a membrane suppressor device. The used solid phase suppressor shows low dead volume and is capable of suppressing eluent concentrations which enable the elution of triphosphate. A solid phase suppressor device consists of three mini-cation exchanger cartridges. The first is used to suppress the eluent stream, while the second column is being regenerated by sulfuric acid and the third column is rinsed with water. The three suppressor columns are located in a revolver which rotates from one position to the next. The first commercial introduced solid phase suppressors were known to affect the retention time of some analytes. Weakly dissociated acids were retained by ion-exclusion. The observed shift in retention time depended on the remaining capacity of the suppressor column. No such effects could be observed when employing the Metrohm device, probably because of the reproducible switching time. However, there was a problem concerning the reproducibility of peak areas when using gradient IC. It was observed that one of the three suppressor columns produced significant lower peak areas. This phenomena occurred only, when the suppressor was used until break-through. If a suppressor column is not completely regenerated after a break-through, some sodium ions will consequently remain in the regenerated suppressor column. Such an incompletely regenerated suppressor column permits some analyte anions to elute together with sodium, instead with hydrogen counter-ions. Since sodium has a much lower equivalent

conductivity than hydrogen, the detector registers a lower overall conductivity response. This problem was easily solved by the use of a more concentrated regenerate solution (sulfuric acid). With this solution, all residual sodium ions were removed from the suppressor column. This adjustment resulted in highly reproducible peak areas. Solid phase suppressors were found to be clearly less sensitive to contamination than membrane based devices. Until now (22 months), no replacement of the suppressor cartridge was needed. Furthermore, the replacement of a solid phase suppressor is much cheaper than of a membrane suppressor. The switching of the suppressor is done automatically by the LC data system and has been successfully used for routine analysis. However, because of the mechanics involved, solid phase suppressors require more attention than membrane suppressors. Besides, the present generation of solid phase suppressors show lower exchange capacities than membrane suppressors.

Probably one of the most significant advantages of modern solid phase suppressors is the capability to use *hydrogencarbonate/carbonate gradients*. Carbon dioxide is produced during the suppression of such eluents. Carbon dioxide ultimately degases and makes detection impossible. The application of a back pressure after the detector prevents successfully the formation of carbon dioxide gas bubbles. Unfortunately membrane suppressors cannot be used in this mode, because they are pressure sensitive devices. The needed back pressure would quickly disrupt the suppressor membrane. A hydrogencarbonate/carbonate gradient has major advantages over a classic (membrane suppressor friendly) sodium hydroxide gradient. Sodium hydroxide has no buffer capacity at all. Hence the dissociation of the eluting analytes is not controlled. Uncontrolled dissociation results in analyte concentration dependent retention time drifts. Multiple charged ions like phosphate are especially affected by these phenomena. These negative effects are clearly less observed when using the described method because of the large buffer capacity of the eluent. Furthermore, it is possible to analyze in one chromatogram ppm concentrations of nitrite and chloride in the ranges of up to ten percents. Otherwise a large dynamic range still presents a formidable challenge for the present day IC technology. There are additional benefits of sodium-carbonate as eluent. Sodium-carbonate is less caustic towards human and UV detector cells than sodium-hydroxide. Sodium-hydroxide eluents are known to adsorb carbon-dioxide from the air, which affects strongly the eluting strength of the eluent. This does not happen with the used carbonate/hydrogencarbonate eluent.

A disadvantage, related to the carbonat/hydrogencarbonat eluent, is the clearly higher background conductivity which is caused by carbonic acid. The presence of a dissociated analyte pushes the dissociation equilibrium of carbonic acid towards the undissociated form. Hence the conductivity of the eluent is locally decreased. This baseline dip is not visible since the analyte responsible for this effect produces a much stronger positive conductivity signal response. As a result, small analyte concentrations show peak areas which are lower than expected. Higher analyte con-

centrations are equally affected, however, the small loss in peak area is hardly noticeable, because of the large signal. An argument in favour of this theory is the observation that nitrite and nitrate produce non linear conductivity but linear UV calibration curves. However, all conductivity IC methods (carbonate and hydroxide eluents) produce slightly non-linear calibration curves. This is due to the dissociation of polyvalent anions and the ion activity coefficient. Both effects are affected by the concentration of the analyte ion.

Injection of "dirty" samples affect the performance of the separation system. We consider the replacement of the disposable pre-column as an efficient and affordable way to restore deteriorating separation efficiencies. Once we observed a situation, where phosphate peaks showed some tailing. This behaviour could be linked to a contaminated suppressor. The performance of the system was fully restored by rinsing it with nitric acid. All separations were performed with standard stainless steel HPLC instruments. No negative effects related to the metal environment were observed, hence in our opinion there is no need to use metal-free IC equipment.

Summary

A gradient ion chromatography method was described and validated which permits the determination of chloride, nitrate, nitrite, mono-, di- and triphosphate in meat products. *Detection*: UV and electrolytic conductivity. *Column type*: Metrosep Anion Dual 1. The sample clean up is very simple, as a result, somewhat "dirty" extracts are obtained. In order to minimize the costs for columns and suppressor replacements, affordable IC columns and robust solid-phase suppressors were employed. In addition, the use of a solid phase suppressor permitted the use of well buffered hydrogencarbonate/carbonate eluents. The stability of retention times, linearity of the detector response and extend of the dynamic range were found to be superior to classical latex based columns and unbuffered sodium hydroxide eluents.

Zusammenfassung

Eine Gradienten Ionenchromatographie-Methode für Chlorid, Nitrat, Nitrit, Mono-, Di- und Triphosphat in Fleischerzeugnissen wurde beschrieben und validiert. *Detektion*: UV- und elektrolytische Leitfähigkeit. *Säulentyp*: Metrosep Anion Dual 1. Die Probenaufarbeitung ist sehr einfach, was auf Kosten der Reinheit der Extrakte geht. Aus diesem Grund wurden preisgünstige IC-Säulen und robuste Festphasen-Suppressoren eingesetzt. Der Einsatz eines solchen Suppressors erlaubt die Verwendung von Hydrogencarbonat/Carbonat als Eluent. Die Puffereigenschaft dieses Mediums verbessert die Stabilität der Retentionszeiten. Der dadurch erweiterte dynamische Bereich stellt den Hauptvorteil gegenüber klassischen Latex-IC-Säulen mit ungepufferten Natronlauge Eluenten dar.

Résumé

Une méthode par chromatographie ionique (IC) à gradient est décrite et validée pour la détermination des chlorures, nitrates, nitrites, mono-, di- et tri-phosphates dans les produits carnés. *Détection*: UV et conductibilité électrolytique; *type de colonne*: Metrosep Anion Dual 1. La préparation d'échantillon est très simple et conduit à des extraits impurs. Pour cette raison, des colonnes peu coûteuses et des «solid phase suppressors» durables ont été utilisés. Cette technique permet l'utilisation d'un éluant hydrogénocarbonate/carbonate ce qui améliore la stabilité des temps de rétention. Comparé aux colonnes-IC classiques sur base latex et aux éluents de soude caustique non tamponés, ce système offre l'avantage d'un domaine de quantification plus étendu.

Key words

Meat products, Gradient IC, Polyphosphates, Nitrite, Nitrate, Chloride

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