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# Determination of phosphate compounds in meat products by 31-Phosphorus Nuclear Magnetic Resonance spectroscopy with methylenediphosphonic acid after alkaline extraction

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ABSTRACT

Modification of the extraction procedure and application of the $^{31}$P NMR method for the determination of polyphosphates in meat products were studied. In the elaborated procedure threefold water extraction at alkaline pH (borate buffer and 0.1 M EDTA) was applied. Furthermore, the new external standard for $^{31}$P NMR determination of phosphates was proposed. Obtained recoveries were between 95 and 99% and variation coefficients (CV) was $\leq$5%, indicating an increase in accuracy and the precision of the proposed procedure in relation to the spectrophotometric method. The described procedure of sample preparation with $^{31}$P NMR method was applied for the determination of polyphosphate additives in meat products. The satisfactory precision (CV = 0.39–3.40%) shows the benefit of the NMR method in the routine analysis of the phosphate ions in meat products.

## 1. Introduction

In the meat industry, phosphates have many functions including protection against oxidation, water binding, stabilizing, emulsifying and improvement of the meat flavour. Phosphates are approved by legislation and applied as additives for curing of pork, poultry and fish meat. The addition of phosphates to meat products reduces the negative effect of lower salt levels and improves the sensory and the technological properties of meat without the increase of the sodium level [1–4].

Several experimental methods were studied for the phosphate anion determination. The most common spectrophotometric methods [1,5–7] and flow injection analysis (FIA) [8–11] were applied. Better results were obtained with ionic chromatography (IC) [12,13] or electromigration methods [14,15], where ortho-, pyro- and polyphosphates were determined by the one analytical measurement.

Recently $^{31}$P NMR analysis has been applied for phosphorus determination in soil, water and the other environmental samples [16]. $^{31}$P NMR signal area is proportional to the number of $^{31}$P nuclei and the quantitative analysis is based on different calibration procedures [17]. The $^{31}$P NMR method was also applied for the studies of polyphosphate hydrolysis processes and for the determination of phosphate additives in meat samples [18]. In our previous paper [19], we proposed $^{31}$P NMR method for the determination of phosphates in meat samples after water extraction. This method presented the satisfactory accuracy and precision together with the simple procedure of a sample preparation. Then, the application of NMR method for the determination of polyphosphates in meat products became the next stage of our studies. Unfortunately, the simple extraction with redistilled water was insufficient for samples of meat products containing the lowest level of phosphates. The intensity of signals was too low, and phosphate compounds were not determined by low-resolution spectrometer with satisfactory precision and accuracy.

The main purpose of our work was to elaborate an inexpensive method for the routine analysis of phosphate additives in quality assurance laboratories. We proposed the method of sample preparation by threefold extraction with borate buffer solution (pH 8.5) and EDTA 0.1 M (2/1, v/v). This procedure afforded the increase of the concentration of extracted compounds and made possible analysis with low NMR spectrometer resolution. Moreover, we have expected the inhibition of the hydrolysis process of the extracts with proposed pH and prolongation of the sample storage time prior to analysis. Humphrey and Jolley [20] reported that small amounts of $Ca^{2+}$ can decrease the $^{31}$P NMR signal. Therefore, we applied the EDTA solution, which should chelate the $Ca^{2+}$ cation and increase the yield of the extraction process. Additionally, the polyphosphate hydrolysis reactions in water standard solutions at pH 3.5 (acetate buffer) and 8.5 (borate buffer) were studied.

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In our previous paper [19], the chemical shifts were measured against $H_3PO_4$ standard (85%), what caused difficulties in the interpretation of the spectra due to the overlap of sample signals to standard signals. In this paper, we propose $^{31}$P NMR procedure for the determination of polyphosphates using new external reference standard: methylenediphosphonic acid (MDPA). Previously, MDPA was applied in qualitative study of biochemical samples for instance: structure of cells and tissues of yeast, bacterium [21,22] and in quantitative analysis of phospholipids from human blood plasma [23,24].

The new NMR method was compared with the official spectrophotometric method of phosphorus determination (PN-ISO 13730, 1999) after mineralization. Additionally, standard meat samples (laboratory sample) spiked with polyphosphates were used for verification of the proposed method.

## 2. Experimental

### 2.1. Reagents

Polyphosphates: $Na_2H_2P_2O_7$ (SHP), $K_4P_2O_7$ (PP), $Na_5P_3O_{10}$ (STP), $D_2O$ (99.9 at% D) and external NMR standard methylenediphosphonic acid (MDPA) were purchased from Sigma–Aldrich, whereas $KH_2PO_4$, $CH_3COOH$, $CH_3COONa$, $H_3BO_3$, EDTA, NaOH, KCl, $NH_4VO_3$, $(NH_4)_6Mo_7O_{24}$, $HNO_3$, $H_2O_2$ and HCl from POCH Gliwice (Poland). All reagents were of analytical grade.

### 2.2. Instrumentation

Samples of meat products were centrifuged by an MPW-350 laboratory centrifuge (max. speed 9000 rpm, RFC 8693 × g, angle 30°, falcon tubes 50 mL) (MPW, Warsaw, Poland). pH of meat extracts was measured with a CX-742 pH-meter (Elmetron, Gliwice, Poland).

Absorption spectra (total phosphorus determination) were recorded on a Helios α-UNICAM spectrophotometer in a 1-cm quartz cell, in comparison with reagent blank. Mineralization of meat and meat product samples were performed at a microwave mineralizer (ERTEC MAGNUM II, Ertec-Poland) in the closed system.

### 2.3. Sample preparation

#### 2.3.1. Extraction procedure

Seven meat products samples were purchased from local supermarkets, while raw pork ham was purchased from the local slaughter-house. We chose: tinned pork foods – products 2 and 3; luncheon pork meat – products 1 and 4; smoked pork ham – product 5 from among many commercial meat products and mix of sea food – products 6 and 7. Prior to analyses, all samples were minced and homogenized with a plate of 3 mm diameter holes.

$5 \pm 0.0001$ g of meat products was extracted with 30 mL of mixture of borate buffer (pH $8.5 \pm 0.02$) and 0.1 M EDTA (2/1, v/v) using an orbital shaker for 15 min. Extracts were filtrated and the procedure was repeated three times. Then extracts were centrifuged at 9000 rpm for 30 min, transferred into a 100.0 mL volumetric flask and were filled to the mark.

The same procedure was applied for standard meat samples (laboratory samples) spiked with one of the following salts: $Na_2H_2P_2O_7$ (SHP), $K_4P_2O_7$ (PP) and $Na_5P_3O_{10}$ (STP) (81 mgP 100 $g^{-1}$ of fresh meat).

#### 2.3.2. Mineralization procedure

Meat samples of 0.7–0.8 (± 0.0001 g) were poured with 5.0 mL of $HNO_3$ (65%) and 1.0 mL of $H_2O_2$ (30%) and mineralized in a close microwave system. Clear solution was diluted to 50.0 mL in the volumetric flask.

### 2.4. $^{31}$P NMR measurement

$^{31}$P NMR spectra were recorded with a Varian Gemini 200 spectrometer at 80.96 MHz frequency resonance The (dm = "nny" Varian pulse program) was used as the pulse sequence. $^1$H decoupling was used only in acquisition time. The temperature of the samples during spectral acquisition was $298 \pm 1$ K, the number of scans (NS) was 196, and the duration of each experiment (AQ) was 1 s. The spectra were obtained with an interpulse delay of 5 s (5 T1 for polyphosphates) and the angle and length of pulse were 90° and 8.9 ms, respectively.

Spectra were recorded according to the external NMR standard methylenediphosphonic acid (MDPA) in closed micro-tube introduced into 5 mm NMR tubes. The concentration of the external standard MDPA was 0.2 M in $D_2O$ ($^{31}$P NMR $\delta = 18.20$ ppm). Data processing included exponential multiplication, fast Fourier transformation (FFT), manual constant phase correction, and Lorentzian line deconvolution routine and MestReC 4.9.9.1 programme. Obtained solutions were transferred into the NMR tubes by micropipette and 196 scans were collected five times per sample.

Furthermore, the polyphosphate solutions were prepared and stabilized by the acetate buffer (pH 3.5) and borate buffer (pH 8.5). NMR spectra collection parameters for buffered solutions were identical as for meat samples.

### 2.5. $^{31}$P NMR calibration curves

Calibration curves for each phosphate salt ($KH_2PO_4$, $Na_2H_2P_2O_7$, $K_4P_2O_7$, and $Na_5P_3O_{10}$) were constructed using eight standard solutions with the different concentration range. The NMR tubes were filled up with solutions with the constant volume and $^{31}$P NMR spectra were collected according to external reference standard MDPA. The calibration points were calculated by measuring the NMR peaks area (PA) based on constant MDPA signal using the procedure available on MestReC program. The regression parameters of the calibration curve for each phosphate solution are listed in Table 1.

Calibration curves were prepared for water solutions of polyphosphates and orthophosphates over 81–5236 and 50–750 mgP $L^{-1}$, respectively. Calculated $R^2$ (Table 1) was similar to other methods, for example: FIA: 0.9986 [9], ≥0.999 for IC [13,25].

The standard deviations (SD) of the slope and the intercept was $0.15 \times 10^{-5}$–$0.10 \times 10^{-2}$ and $0.16 \times 10^{-6}$–$0.749 \times 10^{-2}$, respectively. The calculated detection (DL) and quantification limits (QL) [26], conformed calibration linearity over studied concentrations ranges for phosphate ion determination. In the case of orthophosphates and tripolyphosphates QL values were higher than the lowest concentration of standard solution.

## 3. Results and discussion

The chemical shifts of phosphate compounds were measured according to MDPA signal as an external standard. Fig. 1 presents $^{31}$P NMR spectra of water extracts of meat samples with previously (before the extraction procedure) added $K_4P_2O_7$, $Na_2H_2P_2O_7$ and $Na_5P_3O_{10}$ and after storage at 4 °C for 96 h. Chemical shifts of $^{31}$P signals for anionic species (relative to an external MDPA standard, $\delta = 18.20$ ppm) were as reported in the literature [14,17,27].

The signal of standard (MDPA) was placed at 18.20 ppm, what simplified the interpretation of $^{31}$P NMR spectra and reduced possible errors during quantification analysis (coinciding $H_3PO_4$ signal with analyzed orthophosphates).