Application of Fluorimetric Methods for Selected Additives Determination in Food Products

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Abstract: Simple and sensitive spectrofluorimetric method for phosphorus determination in food samples after microwave mineralisation has been described. The proposed method is based on the formation of a fluorescent chelate between morin and aluminium and the fluorescence quenching after addition of phosphates solution. Additionally, the method with quinine sulphate was modified and applied for food samples. The proposed procedures were compared with respect of linearity, precision and accuracy.

Keywords: spectrofluorimetry; quenching the fluorescence; food samples; phosphate

INTRODUCTION

The most popular additives in meat and fish products are nitrates, nitrites and phosphates. From the technological and health standpoints, it is essential to develop analytical methods for determination of these salts in food. Several types of methods exploit the high sensitivity of fluorimetry and the fluorescence quenching with selected compounds were described (Kirkbright *et al*. 1972; Nasu & Minami 1989; Lorenz *et al*. 1997; Jie *et al*. 1999; Taniai *et al*. 2003; Zhang *et al*. 2003). It can be noted that most of described procedures for phosphate determination based on the fluorescence quenching by phosphate ions and did not apply for real samples.

The aim of the presented study was determination of phosphate ions in food samples (meat and fish products) by spectrofluorimetry. In our work we proposed method based on the formation of a fluorescent chelate between morin and aluminium and quenching the fluorescence after addition of phosphate solution. The proposed procedure was applied for determination of phosphate ions in meat and fish samples after microwave mineralisation. Additionally, we modified and applied method of phosphate ions determination described by Kirkbright *et al*. (1972) based on precipitation of quinine molybdophosphate. The discussed methods were validated by statistical parameters (linearity, limit of detection, DL and quantification, QL, precision and accuracy) and using certified reference materials. Furthermore, obtained results were compared with standard spectrophotometric method recommended by Polish Norm (PN-ISO 13730:1999).

MATERIALS AND METHODS

Reagents. Analytical grade: the quinine sulphate, acetone, morin hydrate were purchased from Sigma Aldrich (Poznań, Poland), whereas HNO_3, KH_2PO_4, H_2O_2, $(NH_4)_6Mo_7O_{24}$, H_2SO_4, $AlCl_3$ and C_2H_5OH were from POCH (Gliwice, Poland). Standard reference material: Bovine Muscle Powder, 8414 was supplied by Promochem (Poland), whereas phosphate standard solution, KH_2PO_4 by Merck (Warsaw, Poland).

Apparatus. Fluorescence measurements were performed using a Fluorescence Spectrophotometer F-7000 HITACHI equipped with a Xenon flash lamp. All analysis were performed in 10 mm quartz cells at 20°C. Microwave mineralisation of certified reference materials products was performed by microwave mineraliser (ERTEC MAGNUM II, Poland) working in the closed system.

Samples preparation. The food samples (meat and fish products) were purchased from a local market, minced and homogenised with a plate of 3 mm diameter holes. Next 0.7–0.8 (± 0.0001 g) samples were poured with 5.0 ml of HNO_3 (65%) and 1.0 ml of H_2O_2 (30%) and digested in closed microwave system resulting in clear solution, which was transferred into a 50 ml volumetric flask and made up to the mark.

A. Procedure for phosphates determination with morin-aluminium complex. Aluminium-morin complex was prepared by adding 0.013 g of $AlCl_3$ to 100 ml of morin ethanolic solution (3×10^{-4} M). After 30 min 3 ml of aluminium-morin complex was pipetted into a 10 ml volumetric flask, followed by 1–5 ml of KH_2PO_4 (1×10^{-4} M), 2 ml of HCL (0.2M) and diluted with ethanol up to volume. The fluorescence intensity was measured at 496 nm using an excitation wavelength of 465 nm. The food and CRM samples were prepared simultaneously using 0.5 ml of solution after microwave mineralisation.

B. Procedure for phosphates determination with quinine sulphate solution. A 50 ml of ammonium molybdate (0.04M), 5 ml of H_2SO_4 (2.4M), 50 ml of KH_2PO_4 (25 μgP/ml) and 10 ml of quinine sulphate (0.01M) were added into a 250 ml beaker. The solution was mixed and separated using centrifuge at 9000 rpm for 10 minutes. Obtained precipitate of quinine molybdophosphate was dissolved with acetone-sulphuric acid (0.5M) reagent (9/1 v/v) and diluted to 100 ml. Next 1–6 ml of solution was added into 10 ml volumetric flask and diluted with acetone-sulphuric acid up to volume. The fluorescence intensity was measured at 445 nm using an excitation wavelength of 352 nm. The same procedure was applied for food and CRM samples (10 ml) after microwave mineralisation.

Calibration curves. Calibration curves were constructed using six standard solutions for KH_2PO_4 in the different range. The regression parameters of calibration curves are listed in Table 1.

RESULTS AND DISCUSSION

The proposed methods were tested in order to accuracy and precision by standard addition method using certified solution of KH_2PO_4 (MERCK). Five replicate analyses were carried out with a standard solution using both procedures. Obtained recoveries: 95–98% (for A procedure) and 98–104% (for B procedure) and coefficients of variation, *CV*: 1.26–5.61% and 3.14–7.02%, demonstrate the benefit of spectrofluorimetry application for the routine analysis of phosphorus compounds. For this reason, both methods were applied for determination of total content of phosphorus in meat and fish products. Obtained results are listed in Table 2.

Table 1. Linear regression calibration of phosphates determination (n = 5)

Procedure	Linear range mgP/l	Curve equation	DL	QL	R^2
A	3.10×10^{-2}–1.54×10^{-1}	$y = (-23.678)x + 1362.4$	2.26×10^{-2}	3.54×10^{-2}	0.9983
B	2.50–5.00	$y = (-407.91)x + 3578$	0.44	1.41	0.9998

where: A –procedure with complex of morin-aluminium, B – procedure with quinine sulphate, DL – limit of detection, QL – limit of quantification (Miller & Miller 2000)

Table 2. Results of phosphates determination (g/100 g purchased products – pp) in meat and fish products (n = 6)

Samples	1	2	3	4	5	6	7
Procedure for phosphates determination with complex morin-aluminium (A)							
$\bar{x}$	0.236	0.218	0.165	0.167	0.477	0.253	0.511
CV (%)	4.97	1.44	1.15	4.06	2.38	4.34	3.77
Procedure for phosphates determination with quinine sulphate solution (B)							
$\bar{x}$	0.369	0.323	0.260	0.389	0.630	0.432	0.628
CV (%)	8.36	1.31	3.77	8.0	2.61	9.26	4.01

where: 1–4 – samples of meat products, 5–7 – samples of fish products; $\bar{x}$ – average value (g/100 g); *CV* – coefficient of variation

Table 3. Results of phosphorus determination in Bovine Muscle Powder 8414 by proposed methods (n = 5) (certified value = 0.836 ± 0.045 g P/100 g)

Procedure	A	B
$\bar{x}$ ± μ (g/100 g)	0.778 ± 0.28	1.301 ± 0.33
t (t_{crit} = 2.57)	0.49	3.60

Generally, there are noticeable differences in obtained level of phosphates in food samples analysed by procedure A and B. Procedure based on quenching the fluorescence of morin-aluminium complex demonstrated lower level of phosphorus in analysed samples. The differences ranged from 0.095 to 0.222 g P/100 g of purchased products (p.p.). Comparing the accuracy, expressed as recovery, one can conclude that procedure based on precipitation of quinine molybdophosphate revealed to high values (> 100%). Furthermore, this was not characterised by good precision. It can be noted, that spectrofluorimetry is characterised by many analytical problems, for examples: strong dependence on sample pH, temperature and presence of foreign ions what influence on fluorescence quenching. For the proposed method with morin-aluminium complex, *CV* value below 5% was obtained for all samples, what demonstrates high reliability. Moreover, the obtained results are in good agreement with standard method for phosphorus determination (PN-ISO 13730:1999).

In regard of noticeable difference between tested procedures we used of certified material samples (CRM) to measure and control accuracy. CRM samples were mineralised and determined by tested methods and obtained results were collected in Table 3. The *t*-test was used for comparison of an experimental mean with a certified value of phosphorus (Miller & Miller 2000).

Comparing two proposed procedures, we observed that null hypothesis is retained for quenching the fluorescence of morin-aluminium complex and there are not significant differences between the experimentally determined mean compared with the reference result. On the contrary, recovery obtained by procedure B was 155% and the *t*-test value (t_{calc} = 3.60) exceeds the critical one. It is clearly indicated that determination of phosphates by complex morin-aluminium was found as a better accurate procedure.

In conclusion, the proposed procedure based on quenching the fluorescence of morin-aluminium complex can be used as a simple, accurate, easy and sensitive method for phosphates determination in food samples. It has been found that the phosphorus content in food and certified material samples are measured with a satisfactory accuracy and precision. For this reason, we recommend this method for application in a food laboratory.

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Comparison of cITP and 31 P NMR Methods for Determination of Polyphosphates in Meat Product and Sea Fruits

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Abstract: Phosphates are widely used as additives in different kinds of food, including meat, poultry and seafood. These ions work synergistically with salt and allow meat processors to achieve equivalent water-holding capacity with reduced amounts of salt. Polyphosphates added to meat and seafood products hydrolysed quickly to phosphates and it is difficult to estimation of these compounds (Davis *et al*. 2004; Sheard & Tali 2004; Jastrzębska *et al*. 2008). In our work we compared capillary isotachophoresis (cITP) and nuclear magnetic resonance (31P NMR) methods for phosphate ions determination in food samples. Furthermore, the usefulness of the studied methods to monitoring of poly- and pyrophosphates hydrolysis in meat and sea fruits was discussed. The meat products and sea fruit samples purchased from a local market and acquired polyphosphates in composition were used for analyses. The food were minced, homogenised and extracted with redistilled water using an orbital shaker for 45 minutes. The extracts were separated using centrifuge at 9000 rpm for 30 min, followed by double filtration. All extracts were transferred into a 50 ml volumetric flask, made up to the mark and analysed with one-dimensional cITP and 31 P NMR methods. The obtained results for two proposed methods were discussed in respect of the accuracy, precision and compared by *F*-test and *t*-test. In concluded, cITP method was simpler, lower time consuming, uncomplicated and economical than 31 P NMR technique. Moreover, permitted on expiration date control by qualitative determination of polyphosphates.

Keywords: polyphosphates; cITP; 31 P NMR; food samples

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Chemically Modified Potato Starch as a Source of Nutritional and Non-nutritional Components

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Abstract: Chemical modification of starch for food purposes is strictly limited in terms of the type of chemical reactions, the kind of modifying agents, the degree of substitution as well as the level of contaminants. Commonly applied chemical reactions include only three types, i.e. oxidation, esterification and etherification. Influence of chemically-modified starches on a biological processes occurring in a human body seems to be interesting, especially as a source of the substances indispensable for the course of various metabolic processes. The study was aimed at answering a question: whether or to what extent the commercial preparations of potato starch, obtained upon chemical modification, would constitute a source of macro- and microelements and starch resistant to α-amylase hydrolysis, as components affecting physiological properties in a human body. Industrially obtained native potato starch as well as it food grade modified starches, were studied: oxidised starch (E 1404), acetylated starch (E 1420), and acetylated distarch adipate (E 1422). Protein content was determined with the Kjeldahl method. Resistant starch (RS) content was estimated according to the method described by Champ *et al*. (1999) and amylose content according to Morrison and Laignelet method (1983). The assay of individual elements content in the native and chemically-modified potato starch was carried out using the atomic absorption spectroscopy (AAS) method. Native and chemically-modified potato starch were characterised by different contents of amylose (from 20 to 31% d.m.) and a RS fraction (from 74 to 77% d.m.). All type of the investigated chemically-modified potato starch appeared to be a good source of elements, however the highest contents of phosphorus and potassium were found in native potato starch. Oxidised starch (E 1404) was the best source of macroelements (calcium and magnesium) and microelements (copper, manganese and zinc), as compared to the other investigated starches. Therefore, the application of the investigated chemically-modified potato starch may prove favourable not only due to their functional properties but also a source of RS and minerals.

Keywords: chemically modified potato starch; resistant starch; elements

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