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Phosphate additives determination in meat products by 31-phosphorus nuclear magnetic resonance using new internal reference standard: Hexamethylphosphoroamide

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ABSTRACT

New ^{31}P NMR internal reference standard – hexamethylphosphoroamide (HMPA) was applied for determination of added polyphosphates and their ionic forms in raw pork meat and meat products. Phosphate species were determined after extraction with a boric acid buffer (pH = 9) and EDTA solution, using internal standard (HMPA) procedure. Hexamethylphosophoroamide was also used as the NMR reference standard. Linear correlations between phosphates and polyphosphate concentrations and ^{31}P NMR signal areas were found in the range 81–5236 mg P/dm^3, presenting 95–99% recovery and variation coefficient (CV) ≤ 5%. Studied HMPA procedure revealed shorter analysis time and the same recovery (>95%) and precision (CV = 1.3–2.7%) in comparison to MDPA method. Results of phosphate determination by both ^{31}P NMR methods were tested against the molybdenumvanadate yellow spectrophotometric method (standard PN-ISO 13730, 1999) using standard reference material (certified phosphate solution).

1. Introduction

Inorganic phosphates and polyphosphates are used as food additives in raw pork meat and in food products e.g., pork, poultry, fish and sea fruits. The additives improve moisture retention, tenderness, juiciness and protect the flavour of meat products. Besides, phosphate addition reduces the negative effect of lower salt level and improves sensory and technological properties of meat. Jiménez-Colmenero et al. [1] concluded that the antimicrobial and antioxidant activities of phosphates also promote product stability. However, the use of the condensed phosphates (pyrophosphates and tripolyphosphates) in meat products can cause the unnecessary chemical ballast for human organism. In the latest report, Jin et al. [2] correlated the level of phosphates in the animal (rat) diet with the increase of lung cancer risk.

Widely used as food additives are: sodium or potassium:tripolyphosphates, hexametaphosphates, dihydrogenpyrophosphates and orthophosphates [3–5]. Official spectrophotometric method of phosphorus determination with molybdenumphosphonic yellow is usually applied as normalized method in different matrices [3,6–8]. However, preparation of sample by mineralization process (dry or wet) makes impossible determination of other species except orthophosphate ions.

Flow injection analysis (FIA) coupled with spectrophotometry was also applied for phosphate species determination in: milk, beef, brine mixtures and extracts of natural casings [9–12]. The Quantichrom™ phosphate assay kit was used [13], for the orthophosphate determination in water solutions, although better recoveries (90–95%) and CV parameters ≈5% were obtained by ionic chromatography (IC) [14,15]. In the case of chromatographic analysis, identification of phosphate species depended on the retention time and standard analyses, which was related to many parameters and is a time consuming stage. Also electromigration methods [16,17] revealed good analytical parameters, however the speciation and separation of the analyte anions in this procedure depended on the buffer composition and migration time, that were the limiting stages of analysis. Among the above mentioned methods, spectrophotometric methods of phosphate determination in food seems to be the most often used. However, the main drawback of this method was low selectivity, what caused determination of polyphosphate species very complicated or even impossible.

The review on phosphates analysis by NMR technique describes broadly the usage of the method in chemical analysis [18]. ^{31}P NMR spectra of phosphates were presented for the first time by O'Neil and Richards [19] and used for qualitative analysis of polyphosphates. In analysis of phosphate species the hydrolysis process caused changes of ionic forms concentration, hence the EDTA solution was used as the inhibitor of hydrolysis process [19]. In further articles, the hydrolysis process of added pyrophosphates

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and tripolyphosphates in chicken meat was studied [20,21]. As reported, hydrolysis of phosphates depended on NaCl concentration, the period of the meat ageing procedure and ionic formulae of added phosphates. ^{31}P NMR spectroscopy was also applied for quantitative and qualitative analysis of $Na_5P_3O_{10}$ solutions [22], whereas further reports were focused on phosphorus determination in soil, water and other environmental samples [23]. Due to an unambiguous assignment of ^{31}P NMR signals in the spectra of polyphosphates, the anionic species identification became straightforward. Previously, Belloque et al. [24] and Belton et al. [20] identified and quantified diphosphate ions using ^{31}P NMR spectroscopy in samples of commercial UHT milk, by comparing milk spectra with those of several standard polyphosphate compounds. However, the statistical parameters (recovery, LOD, LOQ) of phosphate ions determination were not clearly described, therefore results presented here were not compared in quantitative aspect.

^{31}P NMR signals integration is used for the calibration procedure, because the resonance peak areas can be calculated accurately and due to high molar receptivity (related to $^{1}H = 6.65 \times 10^{-2}$, 100% natural abundance of ^{31}P isotope) by adjusting the quantitative spectrometer parameters (acquisition time, delay time or/and calculated the detector response). ^{31}P NMR resonances used for the determination of phosphates and added phosphates were studied against 80% H_3PO_4 as an internal standard [22,25]. However, overlapping of the orthophosphate anion signal with the standard H_3PO_4 resonance line resulted in the systematic error during calculation of the orthophosphates resonance peak area used in preparation of the calibration curves. Therefore, our research was focused on the application of new ^{31}P NMR standards in phosphate species determination.

Methylenediphosphonic acid (MDPA) was already applied as the external standard because ^{31}P NMR signal did not interfere with resonances of analyzed compounds [26]. First reports on MDPA standard were on structural characteristics of cells, tissues of yeast or bacteria [27,28]. The same standard was used for quantitative analysis of phospholipids in human blood plasma [26,29]. However, authors did not present data on recovery or coefficient of variation (CV). In our previous work on phosphates determination in meat products [30] with MDPA (δ = 18.2 ppm) as standard, reported recovery *ca* 95% and CV *ca* 5% were acceptable in food analysis.

In the presented here work hexamethylphosphoroamide (HMPA) has been used as the new NMR reference standard for determination of the added phosphates, polyphosphates and the products of their hydrolysis in meat products. As reported, HMPA was applied as spectroscopic chemical-shift standard in measurements of ^{31}P NMR [31] or as a solvent in organic synthesis [32].

In this work HMPA was applied as the internal analytical standard, that should provide better accuracy and precision of analysis due to the improved lines resolution and the most important it should reduce time of analysis of all phosphate ions. Another advantage of the HMPA was the lack of physicochemical interferences with the analyzed species and its stability during analytical process. According to our observations HMPA was stable during NMR measurements due to: (a) reduced hydrolysis at pH = 9 applied in analyses, (b) interactions with other phosphates were not observed on NMR spectra (e.g., additional signals or perturbations of resonance width or intensity were not detected). Even if HMPA interacted with naturally occurring in meat phosphates (e.g., ATP and phospholipides) these interactions were not detected in the analyzed matrices, due to the short time of spectra recording (~0.30 h) in comparison to those for natural phosphates detection (more than 18 h) [26]. Correlation between resonance signal area (reference standard and analyzed compound) and concentration of the internal reference standard (HMPA) was tested in order to improve analysis procedure in comparison to reported method with MDPA [30].

2. Materials and methods

2.1. Reagents

Polyphosphates: $Na_2H_2P_2O_7$ (SHP), $K_4P_2O_7$ (PP), $Na_5P_3O_{10}$ (STP) and D_2O (99.9 atom % D), external NMR standard methylenediphosphonic acid (MDPA) and internal NMR standard hexamethylphosphoroamide (HMPA) were purchased from Sigma–Aldrich, whereas KH_2PO_4, H_3BO_3, EDTA, NaOH, KCl, from POCH Gliwice (Poland). All reagents were of analytical grade. Certified solution of orthophosphate ions 1000 ± 0.1 mg P/dm^3 were purchased from Sigma–Aldrich.

2.2. Apparatus

^{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 during acquisition time, number of scans (NS = 196), duration of experiment (AQ = 1 s), sample temperature 298 ± 1 K. Spectra were obtained with an inter-pulse delay 5 s (5 T1 for polyphosphates), pulse: angle 90°, length 8.9 m in 5 mm NMR tubes. Spectra were measured in relation to the HMPA standard (stored under nitrogen atmosphere). Received spectra were compiled by MestReC 4.9.9.1 programme.

Absorption spectra for total phosphorus determination were recorded on a Helios α-UNICAM spectrophotometer in a 1-cm quartz cell against reagent blank. Absorbances were measured at analytical wavelength 430 nm for molybdenumvanadate yellow. Mineralization of meat and meat product samples was performed using a microwave closed system (ERTEC MAGNUM II, Ertec, Poland).

2.3. Samples preparation and ^{31}P NMR procedure

The raw pork ham used in analyses was purchased from the local slaughter house nearby Torun and frozen (−22 °C) for storage. Prior to analyses, meat was minced and homogenized with a plate of 3 mm diameter holes.

Standard meat samples spiked with one of the following salts: SHP, PP, STP (81 mg P × 100 g^{-1} of fresh meat) were prepared and used as laboratory samples. These samples (5 ± 0.0001 g) were extracted with 30 mL of boric acid buffer (pH = 9.00 ± 0.02) and 0.1 M water solution of EDTA (2/1, v/v) using an orbital shaker for 15 min. Extracts were filtrated under reduced pressure on a fritted glass funnels (sieves G0). The filtration procedure was repeated three times. The collected extracts were centrifuged at 9000 rpm for 30 min, transferred into a 50.0 mL volumetric flask and filled to the mark. Clear extracts were placed in NMR tubes (0.3 cm^3 – 4 cm height of 5 mm inner diameter NMR tube). ^{31}P NMR spectra were collected in relation to HMPA standard using NMR procedure described above. Determinations by MDPA method were performed as reported [30]. Internal standard hexamethylphosphoroamide HMPA (^{31}P NMR δ = 29.9 ppm against H_3PO_4, 77.4 mg P/dm^3) was added to the volumetric flask with the extracts prior to spectra recording.

Standardized spectrophotometric method (PN-ISO 13730, 1999), based on molybdenumvanadate yellow compound [33], of phosphorus determination as a second reference method was also used. Meat samples 0.7–0.8 (±0.0001 g) were mineralized with 5.0 mL of HNO_3 (65%) and 1.0 mL of H_2O_2 (30%) in a microwave oven to clear solution, which was diluted to 50.0 ml in the volumetric flask and absorbance measured against the blank solution.

$$\frac{C_A}{C_{HMPA}} = f \frac{I_A}{I_{HMPA}}$$

$$f = \frac{I_{HMPA}}{I_{KH_2PO_4}}$$

Scheme 1.

2.4. Meat products analysis

Elaborated procedure of raw meat sample preparation was applied for meat product samples purchased from the local stores (canned pork: products (1–2); mixed sea food – prawns (3–4) and squid (5)). Samples 5.0 ± 0.1 g were mixed and extracted according to above described procedure for raw meat analysis. Resonance line areas were measured against MDPA as the external NMR standard or HMPA as the internal NMR standard.

Determination of phosphorus based on the integral intensities of ^{31}P NMR resonances were calculated using MestReC program. The following equations were applied (Scheme 1):

The f constant was determined for the same concentrations 0.1 M of KH_2PO_4 solutions and external reference standard HMPA (f = 0.839 ± 0.039 – for 5 repetitions). The use of "f" factor in the presented equation allowed to eliminate the slight differences between consecutive NMR measurements, e.g., receiver gain, tuning, matching or shimming which might cause changes in the instrument response. The relative response of the instrument to the standard was constant over the studied range of concentrations. LD = 32 ± 1 mg P/dm^3 and LQ = 82 ± 3 mg P/dm^3 for HMPA method were calculated in relation to intensity signals of phosphate certified solution and known concentration of HMPA.

3. Results and discussion

In the first stage standard water solutions of polyphosphates were analyzed by ^{31}P NMR using HMPA as NMR standard and were related to the reference methods with MDPA as the external standard [30] and the spectrophotometric normalized method (PN-ISO 13730, 1999) [33]. The sole signal of ^{31}P NMR reference standard caused unambiguous interpretation of spectrum and reduces uncertainity of the qualitative analysis.

Precision and accuracy of new NMR method was tested on certified standard solution and compared to two references method. Orthophosphate water solution was used as certified reference solution (CRS), because CRS standard with polyphosphates is not commercially available. Method with HMPA standard was tested on the certified standard solutions (concentrations 163.1 mg P/dm^3 and 326.2 mg P/dm^3) in relation to the reference methods. Working solutions were prepared by dilution of the initial standard solution (1000 ± 0.1 mg P/dm^3) and used as solutions with "known value" for t-test. Results and statistical parameters calculated for spectrophotometric and NMR methods (with MDPA and HMPA) are listed in Table 1.

For HMPA method t-parameter was below t_{crit} = 2.78, what suggested the lack of statistical differences in reproducibility for both concentrations in relation to MDPA method [30]. In the case of UV–vis method, t_{crit} was above 2.78, for both concentrations, hence there were statistically important differences between the "known value" and obtained by UV–vis method.

Calculated F-parameters were below critical F value (Table 1) for HMPA method, what suggested the similar precision of both reference methods (UV–vis, NMR). The accuracy of HMPA procedure was statistically similar to MDPA method in the case of more concentrated solutions [30]. However, for less concentrated solutions better accuracy was noted for HMPA method (with internal quantitative resonance standard). Tests performed on certified standard solutions confirmed the analytical precision and accuracy of new NMR method with HMPA. It was noted, that at lower concentration of phosphorus, the HMPA method revealed better analytical parameter than other two reference methods.

In the next stage raw meat samples with added in laboratory polyphosphates $K_4P_2O_7$, $Na_2H_2P_2O_7$, $Na_3P_3O_9$, $Na_5P_3O_{10}$ (concentration – 81 mg P × 100 g^{-1} of fresh meat) were analyzed by two ^{31}P NMR methods. Laboratory meat samples were spiked with the one phosphate salt and extracted according to above described procedure. In a similar way phosphates were extracted and determined using the spectrophotometric standard method PN-ISO 13730, 1999 [33] (molybdenumvanadate yellow procedure). The recovery and CV were calculated for total phosphorus determination in the case of UV–vis method – as orthophosphates, whereas for NMR methods as the sum of all phosphate ions (Table 2).

The recoveries obtained by procedure with HMPA were between 96.5 and 98.0%, that was in the same range as for MDPA method [30]. However, CV level for all phosphates was lower than in MDPA method, what confirmed better accuracy of the presented method in the case of laboratory samples. Obtained high recoveries were comparable to reported [24,34] and could be related to the stability of polyphosphates in the extraction solution (borate buffer and EDTA) [19,30]. Recoveries for laboratory meat samples were in the range 96.0–97.0%, which was better than for MDPA method in the case of pyrophosphates determination. Additionally CV (1.2–2.7%) was lower than for spectrophotometric and MDPA methods.

Comparison of HMPA method in relation to MDPA [30] and spectrophotometric method [33] revealed better recoveries and precision of the new HMPA method. Therefore, the HMPA method was used for the phosphates determination in meat products (corned-pork, mixed sea food – prawns and squid). The results of added phosphates analysis were calculated in milligrams of phosphorus per kilogram of sample in the purchased meat products and are listed in Table 3.

Spectra of meat samples (Fig. 1) exhibited the lack of tripolyphosphate signals despite the manufacturer declaration. Moreover, on the spectra of three samples (1–3) additional signals

Table 1
Phosphorus determination in certified solutions by ^{31}P NMR and UV–vis method (n = 5) and statistical parameters (t and F test).

Declared amount (mg P/dm^3)	Determined amount (mg P/dm^3)			Test t and F for UV–vis		Test t and F for NMR methods
	UV–vis	MDPA	HMPA	MDPA	HMPA	MDPA/HMPA
163.1	154.7 ± 6.0; t = 3.88	168.0 ± 7.9; t = 1.71	162.5 ± 2.9; t = 0.62	t – 3.34; F – 1.74	t – 3.09; F – 4.32	t – 2.30; F – 2.89
326.2	315.7 ± 2.8; t = 10.41	324.8 ± 3.2; t = 1.23	321.8 ± 5.6; t = 2.17	t – 11.02; F – 1.30	t – 4.05; F – 4.04	t – 1.76; F – 3.22

F_{crit} = 6.35, t_{crit} = 2.78, n = 5, and α = 95%.

Table 2
Polyphosphates determination in laboratory meat samples by ^{31}P NMR method against MDPA (calibration curve) and HMPA (inner standard) standards (recovery ± CV) for 5 repetitions.

	$Na_5P_3O_{10}$%	$Na_3P_3O_9$%	$K_4P_2O_7$%	$Na_2H_2P_2O_7$%
UV–vis method	93.9 ± (2.7)	93.1 ± (2.8)	88.5 ± (2.3)	93.5 ± (3.3)
MDPA method	98.8 ± (1.2)	99.5 ± (1.2)	95.0 ± (3.8)	96.4 ± (2.3)
HMPA method	98.0 ± (1.3)	97.7 ± (2.7)	96.5 ± (1.8)	97.3 ± (1.6)

Table 3
Determination of added phosphates ions (mg P/kg) in meat products by NMR methods – MDPA (M) and HMPA (H).

	Pork product 1		Pork product 2		Sea food product 3		Sea food product 4		Sea food product 5	
	Methods ^{31}P NMR									
	M	H	M	H	M	H	M	H	M	H
Orthophosphate										
$X \pm \mu$	1297 ± 42	1271 ± 58	1023 ± 26	1055 ± 24	260 ± 16	247 ± 8	569 ± 36	576 ± 34	653 ± 37	657 ± 38
CV [%]	2.92	3.87	1.73	1.35	3.86	2.46	3.60	3.34	3.61	3.66
Pyrophosphate										
$X \pm \mu$	188 ± 2	171 ± 4	107 ± 1	121 ± 3	34 ± 1	33 ± 1	Nd	Nd	Nd	Nd
CV [%]	2.65	3.31	1.53	1.03	3.72	2.23	–	–	–	–
Sum of phosphate ions										
$X \pm \mu$	1478 ± 44	1442 ± 62	1130 ± 27	1176 ± 27	294 ± 17	280 ± 9	569 ± 36	576 ± 34	653 ± 37	657 ± 38

H – method with HMPA, M – method with MDPA, Nd – not detected, $n = 5$, and $\alpha = 95$.

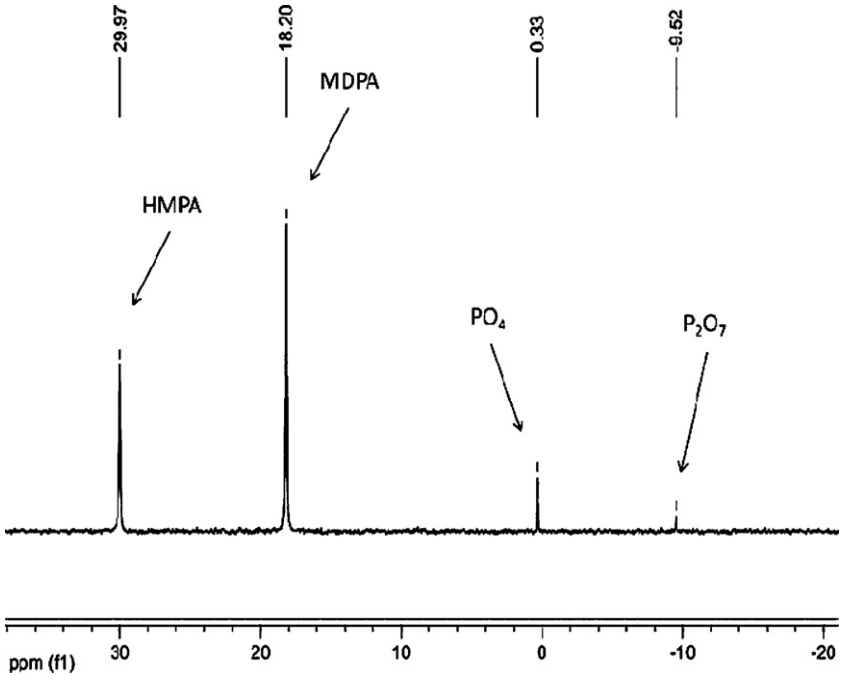

Fig. 1. ^{31}P NMR spectrum of meat product extracts.

from pyrophosphates were noted, what suggested the decomposition of tripolyphosphates in meat products during storage. Calculated CV < 5% was the same as for the laboratory meat samples. Amounts of orthophosphate and pyrophosphate ions found in meat products for both NMR methods revealed similar level. Results of *F* and *t*-test for the comparison of two NMR methods are presented in Table 4.

Calculated *F*-parameter for HMPA method was below F_{crit} for all meat products samples, what suggested the lack of statistical differences of precision in meat products. Therefore, results obtained for pork products and sea food could be applied for phosphates determination in different matrices. The proposed procedure was applied for meat product samples but it could be also used for other matrices.

Table 4
Calculated *t* and *F*-parameters by two NMR methods for meat products.

Test	Pork product 1	Pork product 2	Sea food product 1	Sea food product 2	Sea food product 3
Value *t*	1.34	2.64	0.33	0.31	0.26
Value *F*	2.52	1.41	1.64	1.06	1.04

$F_{crit} = 6.39$, $t_{crit} = 2.78$, $n = 5$, and $\alpha = 95\%$.

4. Conclusions

The use of new NMR reference standard HMPA in phosphate analyses caused the reduction of procedure time with high analytical parameters. The application of this method for the raw meat samples and meat products confirmed the relation between intensity of signal and concentration of phosphorus. High recovery level (in range 95–99%) for NMR method and the possibility of different phosphate ions determination in one procedure suggest application of this method in analysis of meat products. Described NMR method can be used for control of the added phosphates level during production process in quality of food assessment.

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References

[1] F. Jiménez-Colmenero, J. Carballo, S. Cofrades, Meat Sci. 59 (2001) 5–13.
[2] H. Jin, C.-X. Xu, H.-T. Lim, S.-J. Park, J.-Y. Shin, Y.-S. Chung, S.-C. Park, S.-H. Chang, H.-J. Youn, K.-H. Lee, Y.-S. Lee, Y.-C. Ha, C.-H. Chae, G.R. Beck Jr., M.-H. Cho, Am. J. Respir. Cri. Care Med. 179 (2009) 59–68.
[3] S.B. Ünal, F. Erdoğdu, H.I. Ekiz, J. Food Eng. 76 (2006) 119–127.
[4] P.R. Sheard, A. Tali, Meat Sci. 68 (2004) 305–311.
[5] R.T. Baublits, F.W. Pohlman, A.H. Brown, Z.B. Johnson, Meat Sci. 72 (2006) 404–414.
[6] B.J. Lee, D.G. Hendricks, D.P. Cornforth, Meat Sci. 50 (1998) 273–283.
[7] A. Jastrzębska, Chem. Pap. 63 (2009) 47–54.
[8] M. Hermida, M. Gonzalez, M. Miranda, J.L. Rodríguez-Otero, Meat Sci. 73 (2006) 635–639.
[9] J.M. Estela, V. Cerdá, Talanta 66 (2005) 307–331.
[10] L.J. Gimbert, P.M. Haygarth, P.J. Worsfold, Talanta 71 (2007) 1624–1628.
[11] C. Pons, I.V. Tóth, A.O.S.S. Rangel, R. Forteza, V. Cerdà, Anal. Chim. Acta 572 (2006) 148–154.
[12] I.P.A. Morais, I.V. Tóth, A.O.S.S. Rangel, Talanta 66 (2005) 341–347.
[13] J.J. Wijnker, J.L.M. Tjeerdsma-van Bokhoven, E.J.A. Veldhuizen, Meat Sci. 81 (2009) 245–248.
[14] V. Ruiz-Calero, M.T. Galceran, Talanta 66 (2005) 376–410.
[15] Y. Sekiguchi, A. Matsunaga, A. Yamamoto, Y. Inoue, J. Chromatogr. A 881 (2000) 639–644.
[16] A. Jastrzębska, A. Hol, E. Szłyk, LWT Food Sci. Technol. 41 (2008) 2097–2103.
[17] S.Y. Chang, W.L. Tseng, S. Mallipattu, H.T. Chang, Talanta (2005) 411–421.
[18] A. Spyros, P. Dais, Prog. Nucl. Magn. Reson. Spectrosc. 54 (2009) 195–207.
[19] I.K. O'Neil, C.P. Richards, Chem. Ind. 2 (1978) 65.
[20] P.S. Belton, K.J. Packer, T.E. Southon, J. Sci. Food Agric. 40 (1987) 283–291.
[21] R. Li, W.L. Kerr, R.T. Toledo, Q. Teng, J. Sci. Food Agric. 81 (2001) 576–582.
[22] J.G. Colson, D.H. Marr, Anal. Chem. 45 (1973) 370–371.
[23] B.J. Cade-Menun, Talanta 66 (2005) 359–371.
[24] J. Belloque, M.A. De La Fuente, M. Ramos, J. Dairy Res. 67 (2000) 529–539.
[25] A. Jastrzębska, E. Szłyk, Chem. Pap. 63 (2009) 414–419.
[26] S. Bradamante, E. Barchiesi, L. Barenghi, F. Zoppi, Anal. Biochem. 185 (1990) 299–303.
[27] G. Vidal, J.L. Gallis, S. Dufur, P. Canioni, Arch. Biochem. Biophys. 337 (1997) 317–325.
[28] B. Beauvoit, M. Rigoulet, B. Guerin, P. Canioni, FEBS Lett. 252 (1989) 17–21.
[29] M. Kuliszkiewicz-Janus, M.A. Tuz, S. Baczyński, I. Prajs, B. Jaźwiec, Cell Mol. Biol. Lett. 10 (2005) 373–382.
[30] P. Hrynczyszyn, A. Jastrzębska, E. Szłyk, Anal. Chim. Acta 673 (2010) 73–78.
[31] O. Segev, I. Columbus, Y. Ashani, Y. Cohen, J. Org. Chem. 70 (2005) 309–314.
[32] R.R. Dykstra, Hexamethylphosphoric triamide, in: Encyclopedia of Reagents for Organic Synthesis, John Wiley & Sons, 2001, doi:10.1002/047084289X.rh020.
[33] PN-ISO 13730, Meat and meat products – determination of total phosphorus content, Spectrophotometric Method (in Polish) (1999).
[34] C.C Oliveira, E.A.G. Zagatto, A.N. Araùdjo, J.L.F.C. Lima, Anal. Chim. Acta 371 (1998) 57–62.