

Analytical, Nutritional and Clinical Methods

# Determination of phosphorus in food samples by X-ray fluorescence spectrometry and standard spectrophotometric method

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## Abstract

The Wavelength Dispersive X-ray fluorescence (WD XRF) determination of phosphorus in GMO and non GMO food samples is proposed. The tested materials included commercially available transgenic, unmodified soya-foods and popular dairy products. The WD XRF method was compared with the standard molybdenum blue method. Matrix effects were minimised by using standard reference material. Obtained results were discussed in respect of the accuracy, statistical parameters and values recommended by the Recommended Dietary Allowance (RDA). The results indicate the validity of the proposed methods to routine food analysis.

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## 1. Introduction

Phosphorus(V) oxo acids and their salts (i.e., inorganic phosphates(V) and polyphosphates) are compounds of biological, agricultural and food industry interest (Stover, 1999). The Recommended Dietary Allowance (RDA) of phosphorus is: for adults 19–70 years old 700 mg/day and 1.250 mg/day for children. The excessive amount of phosphorus compounds (for example as sodium and potassium phosphates) in food causes disturbance of the homeostasis. Therefore the level of this element in food should be monitored with a precise, fast and simple method.

Different analytical methods were developed for phosphorus determination in food samples among others: spectrophotometry (Galhardo & Masini, 2000; Roig, Gonzalez, & Thomas, 1999; Pettersson & Karlberg, 1999), flow injection analysis (Gervasio, Miranda, Luca, Tumang, Campos, & Reis, 2001; Pérez-Ruiz, Martínez-Lozano, Tomás, & Martín, 2001), capillary electrophoresis (Stover, 1999), high resolution <sup>31</sup>P NMR (Stanislawski & Van Wazer, 1980) or gradient elution ion chromatography (Stover, Bulmahn, & Gard, 1994).

WD-XRF analysis is universal and widely used with respect to different types of environmental and geochemical samples, such as soils, plants, atmospheric aerosol, water and alloys (Belikov, Blank, Shevtson, Nadzhafova, & Tananaiko, 1999; Guohui & Shouzhong, 1995; Hettipathirana, 2001; Hoffmann et al., 1997; Krishna, Ravindra, Gopalan, & Syamsundar, 1994; Omote, Kohno, & Toda, 1995; Parus, Raab, & Donohue, 2001; Pereira & Brandao, 2001; Reimann et al., 2000). Majewska et al. (1999) determined the traces of elements in biological samples (urine and women placenta) using XRF and total reflection X-ray fluorescence. XRF analyses of heavy metals in natural water and pure salts after samples preconcentration was studied by Eksperiandova, Makarovska and Blank (1998).

However, this method is seldom used in food analysis. In recent years the WD-XRF were used for iron determination in olives (Aragero et al., 2001), total phosphorus and sulfur content in edible oils (Van Dalen, 1998) and heavy metals in Madeira wine (Carvalho, Barreiros, Costa, Ramos, & Marques, 1996). Selected microelements were analysed in fruit juice (Sheng-Xiang Bao, Zhi-Hong Wang, & Jing-Song Liu, 1999). Mahgoub, Ahmed, Ahmed, and Agib (1999) determined total content of Ca, Fe, Cu, Zn, Pb, Sr and Mo in three types of flour and spices by X-ray fluorescence method.

Different decomposition techniques were tested on specific samples, in order to find the best procedure,

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which results in the lowest scattering background, and therefore the optimal detection limits for trace element determination (Kump, Nečemer, & Šnajder, 1996). There is no standard acceptable temperature, for example: 40 °C for 24 h (Herzig, 1993), 90 °C for 24 h (Fuchs & Garty, 1983), 105 °C for 12 h (Kocman, Peel, & Tomlinson, 1991) were used. Sheng-Xiang et al. (1999) proposed drying fruit juice samples for 2–3 h in 150–200 °C and carbonized for 30 min at 300 °C in a muffle furnace. Even though accurate results can be obtained with this method, the possible loss of the volatile elements during ashing in high temperature makes it not useful.

The attractive features of XRF, compared to other analytical methods, are the rapidity of the determination, non-destructive and easily automated procedure. Therefore we have found it interesting to study the XRF method for total phosphorus determination in food samples. This technique has usually been accomplished with the dry digestion of the food samples. In our studies we have decided to use the temperature 200 °C in sample preparation. In order to compare our data, phosphorus in samples was determined with standard molybdenum blue method using hydrazine sulphate as a reductor [Polish Standard, PN-88/A-86930, eatable vegetable fats], for accuracy, sensitivity and precision. Additionally standard reference material SRM (non-fat milk powder) was used for verification of both methods and minimised matrix effects. Obtained data were compared with the respective values recommended by the Recommended Dietary Allowance (RDA) of phosphorus.

## 2. Materials and methods

### 2.1. Materials

Phosphorus rich food were chosen as a studied samples. The tested materials included commercially available transgenic and unmodified soya-products (soya steak, soya meat, soya pudding, soya milk) and popular dairy products (melted and cottage cheese). Samples of soya food and dairy products were purchased at a local market, Poland. The dairy products were manufactured by a local producer (marked as A). Transgenic and unmodified soya dry semi-products (soya *Clear Hilium*, Canada), such as: steak, meat, milk and pudding manufactured in Poland (producer B) were analysed. Moreover, two type of transgenic soya food produced in the Czech Republic and packaged in Poland (producer C, *White Hilium*, Canada) were studied.

### 2.2. Reagents

All reagents were of analytical grade and used without purification for solutions preparations in deionised water. Standard: stock solution of phosphate(V) (1000

mgdm<sup>-3</sup> PO<sub>4</sub><sup>3-</sup>) was obtained from Merck. Certified reference material NIST—non-fat milk powder was from Promochem, Poland. Standard ashes: BCR176 (Commission of the European Communities, Community Bureau of Reference), ASCRM 010 (Standards Association of Australia) and SRM2690 (National Institute of Standards & Technology, USA) were used for the validation of XRF method. Ammonium molybdate(VI) (0.0404 M), hydrazine sulphate (0.0804 M) and nitric(V) acid (10%) were purchased from POCh, Gliwice, Poland.

### 2.3. Apparatus

Spectroscan-V X-ray fluorescence spectrometer (Spectron-Optel Inc. Russia) equipped with a PET analytical crystal was employed at voltage 40 kV and current of 4.0 mA. The method of Wavelength Dispersive X-ray fluorescence (WD-XRF) was used. UV-VIS spectrophotometer (Helios  $\alpha$ —UNICAM, in 1-cm cell) was applied for spectrophotometric measurements.

## 3. Analytical methods

### 3.1. Food samples preparation

The analytical samples were prepared according to Polish Standard (PN-ISO 13690, 2000). Prior to analyses, the samples of food were dried in a thermostated dryer at 105 °C to constant weight. Samples of soya food were ground in a grinder to a particle size <0.4 mm. In the case of dairy products, materials were crushed in porcelain mortar.

For XRF measurements the food samples were dried at 200 °C for 2 h. For standard method (PN-88/A-86930, 1988) samples were homogenized and digested with dry ashing methods as follows: 5 g of sample in a crucible were placed in a furnace and ashed at 450 °C for 12 h. After cooling, 5 cm<sup>3</sup> of 10% nitric acid was added and the solution heated 15 min. The resulting solution was placed into a 50 cm<sup>3</sup> volumetric flask, made up to the mark and analysed by spectrophotometric method. Blank digestions were also carried out in the same way.

### 3.2. Standard spectrophotometric method [PN-88/A-86930, 1988]

A sample (4 cm<sup>3</sup>) was transferred to 25 cm<sup>3</sup> volumetric flask followed by the addition of 2 cm<sup>3</sup> of the ammonium molybdate reagent (0.0404 M) and 2 cm<sup>3</sup> of hydrazine sulphate and made up to the mark. Obtained solutions were left for 30 min to ensure colour development and absorbance measured at 730 nm. The same procedures were used for blanks.

The calibration curves of the spectrophotometric measurements were performed using a standard solution of  $\text{KH}_2\text{PO}_4$  in  $\text{H}_2\text{O}$  (traceable to SRM from NIST) in the identical way as described above. The working standards of 1, 2, 3, 4, 6 and 10 ppm of P were used. The correlation coefficients were as follows:  $R^2 = 0.9979$  for dairy products and  $R^2 = 0.9989$  for soya food, values indicate the acceptable precision of linear calibration curves. The molar absorptivity ( $\epsilon$ ) was  $4.4610^3 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$  for dairy products and  $6.5010^3 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$  for soya food. The above results suggested that the sensitivity of the standard spectrophotometric method was acceptable.

### 3.3. XRF measurements

Total phosphorus was determined at analytical line  $\text{P-K}_\alpha$  0.58 nm and the counting time 60 s. The calibration of the X-ray measurements was carried out with the external standard method using BCR176, SRM2690 and ASCRM-010 standard ashes. The matrix effects were minimised by using the mentioned standards.

## 4. Results and discussion

### 4.1. Food sample analyses

The obtained results of the total phosphorus content in food samples for XRF and standard method are listed in Table 1.

The highest concentration of phosphorus in dairy product was observed in a melted cheese for both methods ( $758 \pm 32$  and  $807 \pm 28 \text{ mg}/100 \text{ g}$ ). The determined level of this element in the examined samples can be related to the presence of the added compounds such as: sodium phosphate E-339, sodium diphosphate E-450a, potassium diphosphate E-450c, potassium orthophosphate E-451b, sodium and potassium triphosphate E-451a,b. The lowest concentration was detected in the cottage cheese ( $113 \pm 12$  and  $121 \pm 8 \text{ mg}/100 \text{ g}$ ), which is in accordance with the manufacturer's declaration about the lack of preservatives. The noted concentrations of phosphorus in the dairy products were in the range of RDA values.

The obtained results indicate phosphorus content in the studied soya products ranged  $132 \pm 8$ – $700 \pm 27 \text{ mg}/100 \text{ g}$  of dry mass for XRF and  $92 \pm 3$ – $565 \pm 13$  for standard method, respectively. The highest concentration of phosphorus was observed in the unmodified genetically soya milk ( $700 \pm 27$  and  $565 \pm 13 \text{ mg}/100 \text{ g}$ ), whereas the lowest was in the modified soya meat ( $132 \pm 8$  and  $92 \pm 3 \text{ mg}/100 \text{ g}$ ). In the remaining products obtained values of phosphorus content were similar.

The total phosphorus content in transgenic soya food (steak and meat) manufactured by two producers was studied (Table 1). The manufacturers do not specify phosphorus content in products (B) and (C). In the case of soya steak, the obtained values of phosphorus content were similar. The higher level of this element was recorded in genetically modified soya meat (B) than soya meat (C) and ranged from 294 to  $422 \text{ mg}/100 \text{ g}$ .

Table 1  
Concentrations of total phosphorus in food samples ( $n = 5$ ) determined with XRF and standard method (calculated as  $\text{mg P}/100 \text{ g dry mass}$ )<sup>a</sup>

Food sample	X (mg P/100 g dry mass)	S.D.	RSD [%]
<i>XRF method</i>			
Melted cheese (A)	$758 \pm 32$	20.01	2.64
Cottage cheese (A)	$113 \pm 12$	7.85	6.95
Genetically modified soya steak (B)	$407 \pm 16$	15.01	3.69
Genetically modified soya steak (C)	$383 \pm 16$	15.15	3.96
Unmodified soya steak (B)	$278 \pm 16$	15.19	5.47
Unmodified soya milk (B)	$700 \pm 27$	25.56	3.65
Genetically modified soya meat (B)	$422 \pm 16$	15.51	3.68
Genetically modified soya meat (C)	$132 \pm 8$	7.22	5.46
Soya pudding contain unmodified soya protein (B)	$142 \pm 8$	8.10	5.71
<i>Standard method</i>			
Melted cheese (A)	$807 \pm 28$	27.72	3.43
Cottage cheese (A)	$121 \pm 8$	2.46	2.03
Genetically modified soya steak (B)	$287 \pm 16$	7.95	2.77
Genetically modified soya steak (C)	$299 \pm 17$	7.51	2.51
Unmodified soya steak (B)	$195 \pm 9$	4.24	2.17
Unmodified soya milk (B)	$565 \pm 13$	5.84	1.03
Genetically modified soya meat (B)	$294 \pm 9$	4.03	1.37
Genetically modified soya meat (C)	$92 \pm 3$	1.49	1.62
Soya pudding contain unmodified soya protein (B)	$111 \pm 9$	2.74	2.47

<sup>a</sup> Where: A, producer of dairy products; B, C, producer of soya food. X, mean value of the obtained results with confidence limit calculated using u-Lord test (probability level,  $P = 95\%$ ), S.D., standard deviation, RSD, relative standard deviation.

The differences in phosphorus content can be explained by the different variety of soya beans and various technological processes, probably.

The level of the examined element was within the standards for products containing soya protein and sufficient to cover 80% of daily recommended intake (RDA).

Furthermore, there are significant differences in determined phosphorus content for food containing transgenic soya in comparison with its non-modified equivalent (manufactured by producer B). The level of this element was lower in modified food. The observed difference between the results was about 100 mg/100 g dry mass for the discussed methods.

In order to assess the precision of the proposed procedures, the statistical evaluation of the sample solution was made. Repeatability (calculated using the relative standard deviation, RSD,  $n=5$ ) for phosphorus determination by spectrophotometric methods was below 4 and 7% for XRF methods, respectively (Table 1). The values of S.D., RSD and confidence limit were comparable to those obtained by standard and tested methods. Statistical parameters obtained for the tested methods are similar and satisfactory (Miller & Miller, 2000). Sheng-Xiang Bao et al. (1999) reported XRF and ICP-AED methods applied for phosphorus and other elements' determination in fruit juice and obtained precision, calculated as RSD, in the range 1.4–9.5%.

The obtained results for XRF were compared to the standard method and are presented in Fig. 1.

The correlation coefficient was 0.9285 and it indicates an acceptable linearity of the curve for compared methods. Somogyi, Braun, and Posta (1997) applied XRF and ICP-AES in sediment analysis and reported regression coefficients were in the range 0.980–0.993. In our study there was a satisfactory correlation (irrespective of the analytical technique) between the concentration of the total phosphorus in tested dairy and soya products. The presented results suggested that XRF procedure could be applied for total phosphorus determination in food samples.

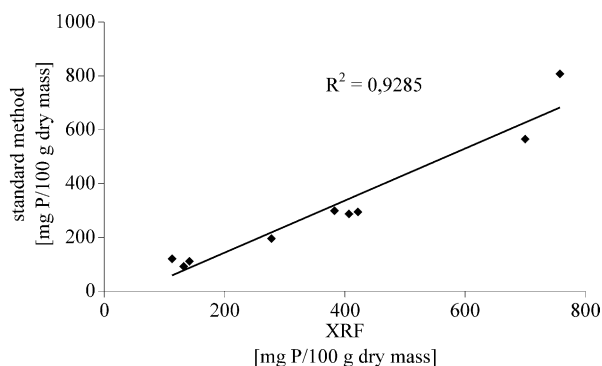


Fig. 1. Phosphorus concentration of different food samples measured by XRF and standard method.

#### 4.2. Validation of tested procedures

The accuracy of the XRF method was examined with standard reference material (van Zoonen, Hoogerbrugge, Gork, van de Wiel, & van't Klooster, 1999). The total phosphorus concentration in non-fat milk powder (certified reference material NIST) was  $1.06 \pm 0.02$  weight%. The achieved data were as follows:  $0.94 \pm 0.04$  for standard method and  $0.96 \pm 0.03$  weight% for XRF. Results comparable to the CRM values were obtained in XRF (91% confidence interval) with standard method (88% confidence interval). However, the determined phosphorus content of standard reference material indicated a good agreement with the certified value for proposed techniques.

The obtained results varied depending on the method used. It might be due to different ways of preparing samples, the homogeneity in the samples, and systematic errors. F-Snedecor test (the relative standard deviation ratio  $F$ -test) was employed to evaluate the precision of the used methods (Miller et al., 2000). The statistical analysis of the results presented above using  $F$ -test revealed no significant differences between the performed determinations at the probability level 95%, because obtained  $F_{\text{calculated}}$  value were smaller than  $F_{\text{theoretical}}$  (19). This fact and the results presented above indicated that the reported XRF method appeared to be more accurate and precise than standard method.

#### 5. Conclusions

The results obtained by the XRF were compared with those obtained by the official method (PN-88/A-86930). The phosphorus determination with the tested method is accurate and has repeatability for samples in a wide range of concentrations ( $92 \pm 3$ – $807 \pm 28$  mg/100 g dry mass). The differences of the examined element contents for GMO and non-modified food were noticed.

The XRF spectrometry analysis, compared to standard method, is less time consuming and sample preparation is simple. Therefore XRF can be applied as a suitable technique for food inspection authorities or factory quality control laboratories.

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