

UDC 543.427.4

## Possibilities of the X-ray Fluorescence Analysis of Dairy Products with the Help of the Spectrometer with Total External Reflectance

G. V. PASHKOVA<sup>1</sup>, A. N. SMAGUNOVA<sup>2</sup>, A. L. FINKELSTEIN<sup>3</sup>

<sup>1</sup>*Institute of the Earth's Crust, Siberian Branch of the Russian Academy of Sciences,  
Ul. Lermontova 128, Irkutsk 664033 (Russia)*

*E-mail: pashkova.gv@yandex.ru*

<sup>2</sup>*Irkutsk State University,  
Ul. K. Marksa 1, Irkutsk 664033 (Russia)*

<sup>3</sup>*Vinogradov Institute of Geochemistry, Siberian Branch of the Russian Academy of Sciences,  
Ul. Favorskogo 1a, Irkutsk 664033 (Russia)*

(Received August 6, 2010)

### Abstract

Effect of the surface density of the emitter on the results of X-ray fluorescence analysis of dairy products was studied theoretically and experimentally with the spectrometer having the geometry of total external reflectance (XRD TER). The models of the dependence of the error of milk sample preparation on the level of milk dilution with water and on the volume of aliquot deposited on the substrate were obtained with the help of mathematical planning of experiments. The physical interpretation of these models is taken into account. The models of the dependence of the detection limit of elements in milk on the above-indicated factors were obtained. A procedure of non-destructive determination of P, S, Cl, K, Ca, Fe, Ni, Cu, Zn, Br, Rb, Sr, Pb in milk; and in dairy products with the help of XRD TER was developed. Metrological investigation of the developed procedure was carried out.

**Key words:** X-ray fluorescence analysis with total external reflectance, milk, dairy products

### INTRODUCTION

The nutritional value of milk and dairy products is largely determined by the mineral content and ratio between minerals therein [1]. Such information is important for the studying the human diet [2], the features mammalian species [3], lactation period [4], breeds [5] and the state of animal health [6], as well as biogeochemical studies on the migration of elements in the food chain soil plants-animals-milk [7]. The analysis of papers and abstracts for 1998–2008 (more than 100 papers) demonstrated that the determination of elements in milk and dairy products is most often performed using the following techniques: atomic absorp-

tion analysis (AAA), mass spectrometry with inductively coupled plasma (ICP-MS) and atomic emission spectrometry with inductively coupled plasma (ICP-AES) [8]. These methods are focused on the analysis of solutions after decomposition of the organic matrix of milk. In order to transfer entire milk to the solution, researchers most often use “wet ashing” either in an open vessel or under pressure (in an autoclave, in the units of microwave decomposition), where HNO<sub>3</sub> [5], a mixture of HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> [4] or HNO<sub>3</sub>/HClO<sub>4</sub> [3] are used as oxidizing agents. Matrix of milk is rather difficult for mineralization in the course of acidic decomposition due to a high content of organic substances, in particular milk fat [9]. As the

result, this stage takes about 60 % of time spent for analysis, and its contribution to the total error of the result of analysis is often as high as 30 % [10].

Non-destructive X-ray fluorescence analysis (XFA) is used much less often. [8] Generally, milk XRD is performed using energy-dispersive (ED XFA), less often wave-dispersive spectrometers (WD XFA), and only in rare cases, the spectrometers with the geometry of total external reflection (XFA TER) [8]. In the first two cases, the liquid milk samples are usually dried, and then they are pressed from to obtain emitters. In this case, one of the limitations of ED XFA and WD XFA is presented by the requirement for a calibration sample, adequate with respect to the analyzed samples of the object in chemical composition [8].

In the last decade XFA TER was often used for the analysis of biological liquids [11], but for the analysis of milk, this method has not been widespread, despite its apparent promise. In the case of XFA TER technique, the liquid sample aliquot (5–10  $\mu\text{L}$ ) is applied to the substrate, which, after drying, it is used as an emitter. The excitation of the sample using an X-ray beam with the geometry of total external reflection results in reducing the matrix effects of absorption and the intensity of scattered radiation [12]. The content of the elements is determined, as a rule, basing on an internal standard, whose role is often played by gallium [11]. Among the papers available we now

[13–15] those are devoted to the analysis of milk using X-ray spectrometers with the total external reflection, in two cases [13, 14] the samples under investigation were previously subjected to wet ashing and only in the case of determining selenium, initial breast milk was [15]. No detailed studies concerning the use of XFA TER in determining the major and trace elements in milk were found in the literature.

This work was aimed at the assessment of potentialities for the XFA of liquid samples for dairy products using a spectrometer with the geometry of total external reflection.

#### EQUIPMENT

The measurements were performed using a tabletop S2 PICOFOX spectrometer (Bruker AXS) at the Analytical Center of the Institute of the Earth's Crust (Irkutsk, Russia). The main characteristics of the spectrometer are they: power consumption up to 150 W, a cermet X-ray tube with Mo anode and air cooling that can work in the following mode: voltage 50 kV, current up to 750  $\mu\text{A}$ , power up to 37 W. The spectrometer is equipped with a Ni/C multi-layer monochromator to select  $\text{MoK}_\alpha$  radiation of the X-ray tube and a semiconductor detector (Silicon Drift Detector) with the area of 10  $\text{mm}^2$ , the energy resolution being equal to 145 eV at the  $\text{MnK}_\alpha$  line. Together with the spectrometer, Spectra 6 software is supplied,

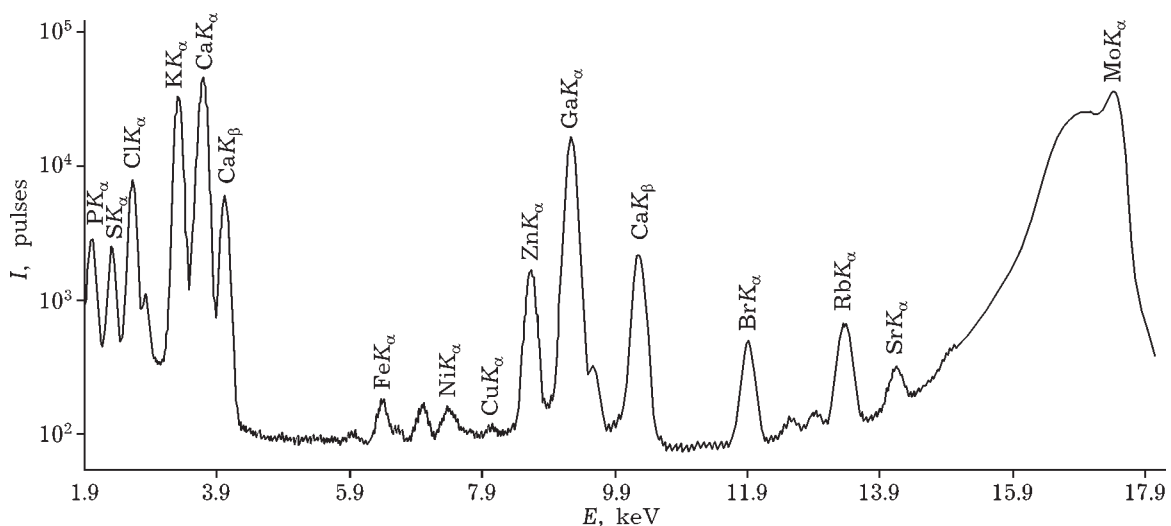


Fig. 1. X-ray spectrum of the sample of milk with added internal standard (Ga).

allowing one to process the spectrum and to calculate the content of the elements.

Figure 1 demonstrates the X-ray spectrum of the milk within the energy range of 1.8–18 keV. The registration time of the spectrum was equal to 1000 s. The spectrometer operates in an open air mode, which does not provide any possibility of measuring the  $K_{\alpha}$  line for the elements with  $Z < 13$ . The spectrum distinctly exhibits the peaks of  $K_{\alpha}$  lines for P, S, Cl, K and Ca, the concentration  $C_i$  for those ranges within 500–1500 mg/L; among trace elements the we observed  $K_{\alpha}$  lines for Fe, Zn, Br, Rb and Sr ( $C_i \sim 0.5$ –5 mg/L), as well as we could measure also weak peaks of  $K_{\alpha}$  lines for Ni, Cu and Pb ( $C_i < 0.5$  mg/L). Within the energy range 16–18 keV, we observed intense peaks inherent in the coherent and incoherent scattering of the primary radiation of X-ray tube.

#### ASSESSMENT OF ACCOUNTING FOR CHEMICAL COMPOSITION AND EMITTER SURFACE DENSITY USING INTERNAL STANDARD TECHNIQUE

For determining the concentration *via* the internal standard method the spectrometer should be preliminary calibrated using synthetic multi-element solutions. The analyte concentration is determined according to the formula

$$C_i = \frac{S_{is}I_i}{S_iI_{is}} C_{is} \quad (1)$$

where  $I$  is the fluorescence intensity of an analytical line;  $S$  is the relative sensitivity, the indices “ $i$ ” and “ $is$ ” are related to the element under determination and reference element (internal standard), respectively. It is assumed that the sample meets the criterion of “thin layer”. The internal standard is introduced in order to reduce the influence of the inhomogeneity of a material under investigation located on the substrate [12]. In the case of the analysis of organic substances, preparing a “thin” sample is rather problematic [11], since its thickness for analytical lines of elements in the energy range 1–20 keV should not exceed 4  $\mu\text{m}$  [16]. When applying biological fluids onto a substrate, the surface emitter density is more often corresponds to the surface density of “intermediate layer” [17]. In this case the results of XFA TER could depend on the matrix effects as well

as the uneven distribution of sample dry matter samples over the surface of the substrate. To reduce these effects, one uses diluting biological fluids with water [11, 17]. However, due to differences between the energy of analytical lines of the element under investigation and the reference element, accounting for the chemical composition and the surface density of the emitter *via* the internal standard method could be incomplete [18]. In order to quantify the dependences of these effects on the energy of the analytical lines of elements in the course of XFA applying to dairy products, theoretical studies were performed.

The intensity of X-ray fluorescence ( $I_i$ ), excited by the monochromatic primary radiation of Mo tube (I0), is determined by [17], as

$$I_i = \text{const } I_0 C_i f_i \quad (2)$$

where const is a constant, independent of the chemical composition;

$$f_i = \frac{\exp[-2\mu(E_0)\varphi^{-1}P_s] - \exp[-\mu_{\Sigma}(E_i)P_s]}{\mu(E_i) - \mu(E_0) / \sin \varphi} + \frac{1 - \exp[-\mu_{\Sigma}(E_i)P_s]}{\mu_{\Sigma}(E_i)} \quad (3)$$

$$\mu_{\Sigma} = (\mu(E_0) / \sin \varphi) + (\mu(E_i) / \sin \psi) \quad (4)$$

Here  $P_s$  is the surface density of the emitter;  $\mu(E_0)$  and  $\mu(E_i)$  are the mass attenuation coefficients for the primary and fluorescent radiation in the sample, respectively;  $\varphi$  and  $\psi$  are the angles of incidence of the primary radiation to the sample selection and fluorescent radiation, respectively. For the geometry used in our spectrometer  $\varphi = 0.1^\circ$ ,  $\psi = 90^\circ$ . Expression (2) is an approximation for a more general formula presented in [19], excluding the term describing the excitation of X-ray standing waves near the surface of the substrate. For biological samples with thickness more than 200 nm, this term could be neglected [19].

The surface density of an emitter meeting the criteria of thin layer (10 % approaching), was estimated by means of expression [20]

$$P_s^t \leq 0.195 / \mu_{\Sigma} \quad (5)$$

Basing on the average content protein, fat and carbohydrates in milk we calculated the composition of the organic matrix, %: H 8, C 51, N 5, and O 36. The calculated value of the attenuation coefficient for the primary radia-

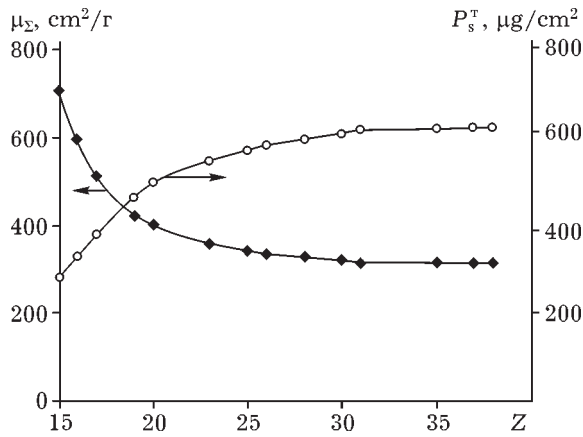


Fig. 2. Mass attenuation coefficient ( $\mu_\Sigma$ ) and the surface density of thin layer ( $P_s^t$ ) for  $K_\alpha$  lines of elements depending on  $Z$ .

tion of  $MoK_\alpha$  line is equal to  $\mu(E_0) = 0.54 \text{ cm}^2/\text{g}$ , then  $\mu(E_0)/\sin \varphi = 310 \text{ cm}^2/\text{g}$ . The values of the mass attenuation coefficient of the fluorescent light  $\mu(E_i)$  ranged from 394 ( $PK_\alpha$ ) to  $2.5 \text{ cm}^2/\text{g}$  ( $SrK_\alpha$ ). Figure 2 demonstrates the values of  $\mu_\Sigma$  and  $P_s^t$ , calculated using formula (5) depending on the atomic number of an element  $Z$ . It is seen that the for  $K_\alpha$  lines inherent in elements with  $Z > 20$ , the  $P_s^t$  value is relatively constant and is  $500\text{--}600 \text{ }\mu\text{g}/\text{cm}^2$ . This fact could be caused by a weak dependence of  $\mu_\Sigma$  value on  $Z$ , since  $\mu(E_0)/\sin \varphi \gg \mu(E_i)/\sin \psi$ . For elements with  $Z < 20$ , the contribution of  $\mu(E_i)$  to the value of  $\mu_\Sigma$  could not be ignored, thus  $P_s^t$  increases with  $\mu(E_i)$  ranging from 280 to  $500 \text{ }\mu\text{g}/\text{cm}^2$ .

The actual surface density of the emitter when using the XFA TER depends on the mass fraction of solid residue ( $\omega_{sol}$ ) in the initial liquid, its dilution level and the volume of aliquots applied onto the substrate. The milk content exhibits most wide varying the content of milk fat ( $C_f$ ), whereas the content of other components is relatively constant [21]. The mass fraction of the solid residue in the milk ranges from 8–9 (with  $C_f \sim 0.1 \%$ ) to 14 % (with  $C_f = 5\text{--}6 \%$ ). When applied onto the substrate  $5 \text{ }\mu\text{L}$  of milk with the mass fraction of solid residue equal to 8–14 %, the values of  $P_s$  range within  $2000\text{--}3600 \text{ }\mu\text{g}/\text{cm}^2$  which significantly exceeds the  $P_s$  value inherent in thin layer. In this connection, formula (1) for the calculation of the required concentration  $C_i$  of element  $i$  in the sample should include factor  $K_i$  (correction factor)  $K_i = f_{Ga}/f_i$  (6) where  $f_i$  and  $f_{Ga}$  are calculated for the element under determination and the reference element (Ga) according to expression (3). For the method of internal standard, the  $K$  factor depends on the surface density of the sample under analysis, on the difference in the radiation energy of compared lines and on the mass attenuation coefficient for the radiation of these lines. In determining the content of P, S, Cl, K, Ca, Zn and Sr in the milk, we studied the dependence of the factor  $K_i$  on the content of solid residue in milk (Fig. 3). The emitter surface density was calculated for the following conditions: the volume of milk aliquot amounted to  $5 \text{ }\mu\text{L}$ , spot diameter equal to 5 mm. One can

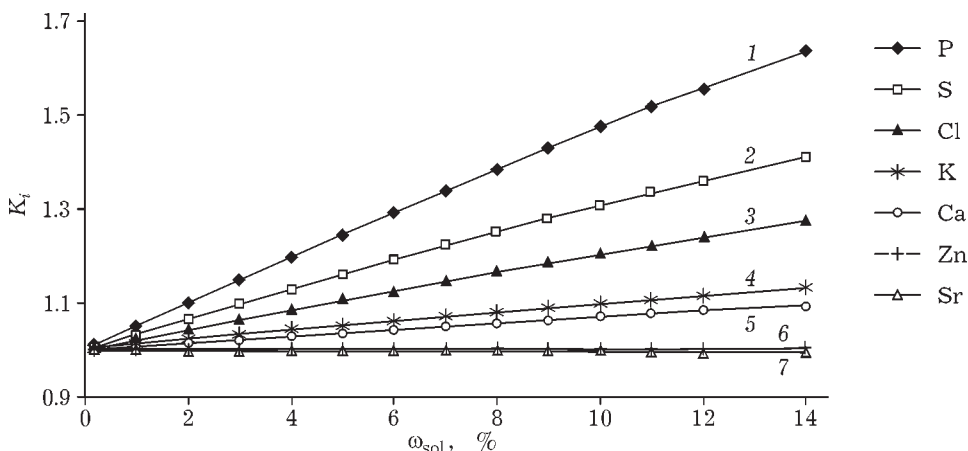


Fig. 3. Value of correction factor ( $K_i$ ) depending on the mass fraction of dry solid residue  $\omega_{sol}$ .

TABLE 1

Theoretical and experimental models ( $Y = I_i/I_{Ga}$ )

| Elements | Theoretical models                            | Experimental models                           |                    |
|----------|---|---|--------------------|
| P        | $Y = 1 - 0.372X_1 - 0.050X_2 + 0.028X_1X_2$   | $Y = 1 - 0.178X_1 - 0.140X_2 + 0.097X_1X_2$ , | $\Delta a = 0.077$ |
| S        | $Y = 1 - 0.417X_1 - 0.035X_2 + 0.022X_1X_2$   | $Y = 1 - 0.263X_1 - 0.113X_2 + 0.075X_1X_2$ , | $\Delta a = 0.069$ |
| Cl       | $Y = 1 - 0.443X_1 - 0.025X_2 + 0.017X_1X_2$   | $Y = 1 - 0.346X_1 - 0.088X_2 + 0.093X_1X_2$ , | $\Delta a = 0.087$ |
| K        | $Y = 1 - 0.471X_1 - 0.013X_2 + 0.009X_1X_2$   | $Y = 1 - 0.445X_1$ ,                          | $\Delta a = 0.071$ |
| Ca       | $Y = 1 - 0.479X_1 - 0.010X_2 + 0.007X_1X_2$   | $Y = 1 - 0.411X_1$ ,                          | $\Delta a = 0.054$ |
| Zn       | $Y = 1 - 0.500X_1 - 0.0002X_2 + 0.0001X_1X_2$ | $Y = 1 - 0.465X_1$ ,                          | $\Delta a = 0.035$ |
| Sr       | $Y = 1 - 0.501X_1$                            | $Y = 1 - 0.457X_1$ ,                          | $\Delta a = 0.036$ |

see that as the difference in the energies of radiation for analytical lines decreases, the correction exhibits a decrease. Thus, the values of  $K_i$  at the maximum content of solid residue ( $\omega_{sol} = 14\%$ ) for different elements are as it follows: P 1.6, S 1.4, Cl 1.3, K 1.13, Ca 1.09, Zn 1.001, Sr 0.996. Thus, for elements with  $Z > 20$  the internal standard method allows taking into account the surface density of emitter, but for elements with  $Z > 20$  it is required to take corrections in order to reduce systematic errors in the results of analysis.

To assess the impact of changing the surface density of emitter on the value of analytical signal  $I_i/I_{Ga}$ , under real experimental conditions we used a mathematical design of experiments when the response  $Y = I_i/I_{Ga}$ . The factors were presented by the dilution level of the initial milk by bidistilled water ( $X_1$ ) and sample volume applied onto the reflector ( $X_2$ ). Natural values for the lower level of the factors amounted to:  $X_1' = 0$  (without dilution) and  $X_2' = 5 \mu\text{L}$ ; those for the upper level were equal to  $X_1'' = 3$  (dilution 1 : 2), and  $X_2'' = 11 \mu\text{L}$ . The dependence of the response on the factors under investigation were approximated by a polynomial expression:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{12}X_1X_2 \quad (7)$$

First we obtained model (7) using the theoretical analysis of line intensities for elements  $i$  calculated using equation (2) for the samples, whose chemical composition and the surface density is set by the matrix of planning the experiment under consideration. The Ga concentration in the samples amounted to 10 mg/L.

The models obtained for elements P, S, Cl, K, Ca, Zn and Sr normalized with respect to the constant term are presented in Table 1. One can see that the dilution of the sample with water (factor  $X_1$ ) causes reducing the analytic parameter  $I_i/I_{Ga}$  for all the elements. This could be connected with an increase in the intensity of the  $K_\alpha$  line of Ga due to increasing its concentration in the solid residue of diluted milk on the substrate. Increasing the milk aliquots applied onto the substrate (factor  $X_2$ ) results in increasing in the thickness of the emitting layer. Under these conditions, the radiation intensity of long-wave analytical  $K_\alpha$  lines (P, S, Cl, K and Ca) increases more slowly than the intensity of the  $GaK_\alpha$  line, for that the sample is to a greater extent corresponding to thin emitter, so coefficient  $a_2$  is negative. When diluting milk with water the emitting layer is thinner, so the effect  $X_2$  would be manifested in a weaker manner, which determines the positive sign of the pair interaction  $a_{12}$ .

The theoretical conclusions concerning the influence of diluting the milk and the volume of aliquots upon the value of  $I_i/I_{Ga}$  were tested experimentally using milk samples for those  $C_f = 1.5\%$ . To the sample (undiluted or three times diluted) of 500  $\mu\text{L}$  in volume was added 5  $\mu\text{L}$  of standard Ga solution ( $C_{Ga} = 1 \text{ g/L}$ ). After mixing them we took an aliquot (5 or 11  $\mu\text{L}$ ) to apply it onto a quartz substrate which, after air drying, was used as an emitter. For each experiment within the framework of the planning matrix we independently prepared 10 emitters, and then for each emitter we twice measured fluorescence intensity and background analytical lines. The experimental models (see



Table 1,  $\Delta a$  is the confidence interval for the coefficients of the models) confirmed the theoretical conclusions within experimental error. The exception is presented by the models obtained for the  $K_\alpha$  lines of the P and S, due to uneven sample distribution over the substrate surface. The variations  $P_s$  values for different parts of the emitter result in the variations of the coefficients  $K_i$  (6), which would affect the values of the coefficients  $a_1$ ,  $a_2$  and  $a_{12}$  in the experimental models. The theoretical models were obtained provided that for each experiment of experimental design matrix the value of  $P_s$  is constant.

#### CHOOSING THE CONDITIONS FOR PREPARING THE EMITTERS

The errors of preparing emitters in the case of XFA TER could be caused by the uneven distribution of material over the surface of the emitter and the random changes in the sample mass involved in the formation of fluorescence [18]. In order to choose optimal preparation conditions for emitters preparing from liquid milk, we performed a two-factor experiment, when the response  $Y$  was presented by the coefficient of variation ( $Y = V_{p/e}$ ), which characterizes preparation error of emitters, whereas the factors and the values of their levels remained the same. The investigations

were performed using three samples of cow's milk with low ( $C_f = 1.5\%$ ), medium ( $C_f = 4\%$ ) and high ( $C_f = 6\%$ ) fat concentration. The confidence interval for the coefficients of the models ( $\Delta a$ ) was assessed from the instrumental error (the coefficient of variation) which varied within the range of 0.4–2.7% depending on the content of the element in the sample.

The models obtained for milk with the fat content of 1.5 and 4% are listed in Table 2. One can see that within the long-wave region of the spectrum ( $K_\alpha$  line P, S, Cl, K and Ca), the dilution with water (factor  $X_1$ ) results in reducing the errors of making emitters. So, for a mediate fat content in the milk ( $C_f = 4\%$ ), the value  $V_{p/e}$  for P decreased from 28 to 4%, for S from 16 to 3%, for Cl from 17 to 4%, for K from 10 to 3%, for Ca from 8 to 2%. This could be caused by a more uniform distribution of the dried diluted sample dried over the substrate surface. As noted above, the method of internal standard does not provide full accounting for the effect of the uneven distribution of milk solid residue over the substrate. As the energy of the analytical line  $E_i$  (in going from P to K) increases, the value of coefficient  $a_1$  decreases. A violation of this dependence for the  $K_\alpha$  of Ca could be associated with an increase in the absorption characteristics of the sample due to a significant number of K atoms.

TABLE 2

Models ( $Y = V_{p/e}$ ) for milk with different fat content

| Elements | Milk fat content, %                         |                    |                    |                    |
|----------|---|--------------------|--------------------|--------------------|
|          | 4   | 1.5                |                    |                    |
| P        | $Y = 16.3 - 12.1X_1 + 2.3X_2 - 2.3X_1X_2$   | $Y = 6.3 - 3.5X_1$ | $\Delta a = 0.59$  | $\Delta a = 1.1$   |
|          | $Y = 1 - 0.741X_1 + 0.137X_2 - 0.138X_1X_2$ | $Y = 1 - 0.516X_1$ | $\Delta a = 0.036$ | $\Delta a = 0.198$ |
| S        | $Y = 9.2 - 6.7X_1 + 1.1X_2 - 0.93X_1X_2$    | $Y = 4.8 - 2.4X_1$ | $\Delta a = 0.77$  | $\Delta a = 1.1$   |
|          | $Y = 1 - 0.727X_1 + 0.124X_2 - 0.101X_1X_2$ | $Y = 1 - 0.512X_1$ | $\Delta a = 0.081$ | $\Delta a = 0.245$ |
| Cl       | $Y = 10 - 6.5X_1 + 1.7X_2 - 1.2 X_1X_2$     | $Y = 6.0 - 2.9X_1$ | $\Delta a = 0.48$  | $\Delta a = 1.7$   |
|          | $Y = 1 - 0.606X_1 + 0.156X_2 - 0.112X_1X_2$ | $Y = 1 - 0.485X_1$ | $\Delta a = 0.045$ | $\Delta a = 0.183$ |
| K        | $Y = 6.7 - 3.4X_1 + 0.77X_2 - 0.44X_1X_2$   | $Y = 4.6 - 2.2X_1$ | $\Delta a = 0.40$  | $\Delta a = 1.3$   |
|          | $Y = 1 - 0.508X_1 + 0.116X_2 - 0.066X_1X_2$ | $Y = 1 - 0.479X_1$ | $\Delta a = 0.059$ | $\Delta a = 0.259$ |
| Ca       | $Y = 5.3 - 3.5X_1 + 0.85X_2$                | $Y = 3.4 - 1.7X_1$ | $\Delta a = 0.34$  | $\Delta a = 0.69$  |
|          | $Y = 1 - 0.656X_1 + 0.162X_2$               | $Y = 1 - 0.496X_1$ | $\Delta a = 0.065$ | $\Delta a = 0.193$ |
| Zn       | $Y = 3.2$                                   | $Y = 2.5$          | $\Delta a = 0.37$  | $\Delta a = 0.86$  |
|          | $Y = 1.0$                                   | $Y = 1.0$          | $\Delta a = 0.117$ | $\Delta a = 0.134$ |
| Sr       | $Y = 2.0$                                   | $Y = 3.0$          | $\Delta a = 2.3$   | $\Delta a = 2.7$   |
|          | $Y = 1.0$                                   | $Y = 1.0$          | $\Delta a = 0.935$ | $\Delta a = 0.655$ |

The influence of factor  $X_2$  exhibited for elements P, S, Cl, K and Ca only for the milk with the mediate fat content ( $C_f = 4\%$ ). The positive sign at the coefficient  $a_2$  indicates that with increasing the volume of the solution under applying an increase is observed in the surface density of emitter, which results in increasing the uneven distribution of milk solid residue over the substrate. This effect ( $X_2$ ) is reduced by diluting milk with water, which determines the negative sign of the  $a_{12}$ . As the value  $E_i$  increases, the absolute magnitude of coefficient  $a_{12}$  decreases; already for calcium this coefficient becomes insignificant.

In the short-wave region of the spectrum ( $K_\alpha$  lines for Zn, Br and Sr) there was no action of factors  $X_1$  and  $X_2$  observed, since the emitter is close to the thin layer. In addition, the effect of the uneven distribution of the sample on the substrate can be more completely taken into account *via* the internal standard method due to decreasing the differences in the wavelengths of the lines under comparison.

From the comparison of non-normalized models obtained for milk with different fat content (see Table 2), one can see that for the elements P, S, Cl, K and Ca with medium fat content of milk ( $C_f = 4\%$ ) the values of  $V_{p/e}$  are 1.5–3 times higher than for low-fat milk ( $C_f = 1.5\%$ ). However, in the case of diluting milk with water, the  $V_{p/e}$  values become comparable (see Table 2). Basing on the data concerning high sample preparation errors obtained for the milk of medium fat level, it can be concluded that the analysis of undiluted milk with high fat content is inappropriate. In this regard, for the construction of models for milk with fat level equal to 6% we changed the values of  $X_1$  factor levels: the lower level  $X_1' = 2$  (dilution 1 : 1), the upper level  $X_1'' = 5$  (dilution 1 : 4). The values of factor  $X_2$  remained the same. The models are demonstrated in Table 3. As it can be seen, the coefficients of models constructed for dilute fat milk ( $C_f = 6\%$ ), are comparable with the coefficients of models obtained for the low fat milk ( $C_f = 1.5\%$ ). This is, to all appearance, connected with the fact that under the dilution conditions chosen the surface density of fat milk diluted at a ratio of 1 : 1 applied then onto the substrate is close to the

TABLE 3

Models ( $Y = V_{p/e}$ ) for milk with fat content equal to 6%

| Elements | Model               |                    |
|----------|---------------------|--------------------|
| P        | $Y = 5.4 - 3.1X_1$  | $\Delta a = 1.1$   |
|          | $Y = 1 - 0.564X_1$  | $\Delta a = 0.198$ |
| S        | $Y = 3.9 - 2.2X_1$  | $\Delta a = 1.1$   |
|          | $Y = 1 - 0.550X_1$  | $\Delta a = 0.245$ |
| Cl       | $Y = 1.8 - 0.65X_1$ | $\Delta a = 1.7$   |
|          | $Y = 1 - 0.368X_1$  | $\Delta a = 0.183$ |
| K        | $Y = 1.4 - 0.49X_1$ | $\Delta a = 1.3$   |
|          | $Y = 1 - 0.362X_1$  | $\Delta a = 0.259$ |
| Ca       | $Y = 2.0 - 1.2X_1$  | $\Delta a = 0.69$  |
|          | $Y = 1 - 0.596X_1$  | $\Delta a = 0.193$ |
| Zn       | $Y = 1.5$           | $\Delta a = 0.86$  |
|          | $Y = 1.0$           | $\Delta a = 0.134$ |
| Sr       | $Y = 3.9$           | $\Delta a = 2.7$   |
|          | $Y = 1.0$           | $\Delta a = 0.655$ |

surface density of the undiluted low fat milk. Milk with the fat content equal to 6% exhibits the mass fraction of solid residue ( $\omega_{sol}$ ) after 1 : 1 dilution is equal to 7%, whereas when diluted at a ratio of 1 : 4  $\omega_{sol} = 2.8\%$ , for the low fat milk without dilution  $\omega_{sol} = 9.6\%$ , and when diluted at a ratio of 1 : 2  $\omega_{sol} = 3.2\%$ .

Thus, the error of sample preparation for XFA TER of milk depends not only on the content of milk fat, but also on the surface density of emitter, and with increasing the mass fraction of solid residue in the milk this value increases. This is well illustrated by the dependence of the variation coefficient  $V_{p/e}$  which characterizes the error of making the emitters on the dry solid matter content in the milk for elements P, S, Cl, K, Ca, and Zn (Fig. 4). The  $V_{p/e}$  values were obtained for emitters prepared by applying 5  $\mu\text{L}$  of undiluted and diluted milk with different fat content.

Theoretical and experimental studies performed have demonstrated that the determination of elements P, S, Cl, K and Ca in milk the original sample should be diluted with water for reducing the influence of the chemical composition and the uneven distribution of material over the substrate surface. Increasing the amount of the liquid applied from 5 to 11  $\mu\text{L}$  is impractical because this results in increasing the uneven distribution of fat milk solid residue over a substrate.

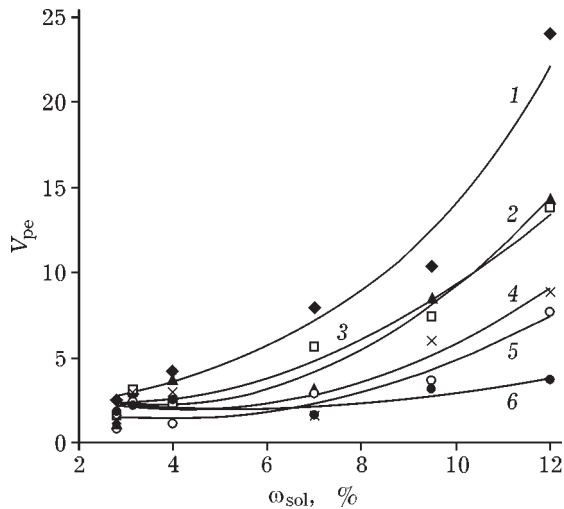


Fig. 4. Coefficient of variation ( $V_{p/e}$ ) characterizing the error of sample preparation depending on the mass fraction of dry solid in milk ( $\omega_{sol}$ ) for P (1), S (2), Cl (3), K (4), Ca (5), Zn (6).

Models obtained with the help of mathematical planning the experiment, when as the response  $Y$  one uses the detection limit value ( $Y = C_{0.997}$ ), support this conclusion:

$$Y_P = 1 - 0.653X_1 + 0.501X_2 - 0.502X_1X_2; \\ \Delta a = 0.135 \quad (8a)$$

$$Y_S = 1 - 0.548X_1 + 0.134X_2 - 0.124X_1X_2; \\ \Delta a = 0.094 \quad (8b)$$

$$Y_{Zn} = 1 - 0.475X_1; \Delta a = 0.048 \quad (8c)$$

$$Y_{Sr} = 1 - 0.472X_1; \Delta a = 0.075 \quad (8d)$$

The values of  $C_{0.997}$  were calculated for the samples whose composition is set by the matrix of experimental design. In order to estimate the standard deviation ( $\sigma_{bl}$ ) characterizing the scatter of measurements from the signals resulted from the blank sample, we used the values of background intensity measured near the analytical line. It has been found that the resulting models are weakly dependent on the fat content in the milk. From the models constructed (8a)–(8d) it follows that increasing the level of dilution causes decreasing the detection limit for all of these elements, whereas for P and S an increase in aliquot volume results in an inverse effect. With increasing  $X_2$  one observes an increase in scatter  $\sigma_{bl}$  of measured background values, which results in increasing the value of  $C_{0.997}$ . The negative sign of the pair interaction coefficient  $a_{12}$  could be connected with the fact that applying a great volume of dilute milk causes increasing the intensity  $I_i$ ,

which reduces the value of  $C_{0.997}$ . For elements Zn and Sr, whose analytical lines are located within a short-wave spectral region, this effect appeared insignificant.

The conclusions drawn were taken into account in the course of the developing the methods for the analysis of milk and dairy product liquid samples with different fat content employing an S2 PICOFOX X-ray spectrometer with the geometry of total external reflection. For preparing the emitters, we diluted the initial sample three-fold with double distilled water, to sample then a 500  $\mu$ L aliquot with adding therein 5 mL of standard gallium solution with concentration equal to 1 g/L. After mixing them, we took a 5  $\mu$ L aliquot from this mixture to apply onto a quartz substrate, which, after air drying, was used as an emitter. The registration time for the spectrum was equal to 1000 s. For processing the spectrum and calculating the required content of components we used Spectra 6 software. In order to reduce systematic errors in the calculation of P, S, Cl, K and Ca concentration we could introduce a correction  $K_i$  for solid residue content in the sample, after determining this value from experiments *via* milk drying at  $(102 \pm 2)^\circ\text{C}$  up to constant mass.

#### METROLOGICAL STUDIES

We evaluated the metrological characteristics of the technique developed. The values of  $C_{0.997}$  were calculated using  $3\sigma$  criterion. The standard deviation was determined as scatter  $\sigma_{bl}$  among 20 measurements of the background intensities near the analytical line of element. Figure 5 demonstrates the detection limit ( $C_{0.997}$ ) for elements in milk with fat content of 1.5% depending on their atomic number  $Z$ . The minimum detection limit values ( $C_{0.997} = 0.008$  mg/L) were obtained for elements Ni, Cu and Zn, for Pb  $C_{0.997} = 0.012$  mg/L.

The coefficients of variation ( $V_{IP}$ ) characterizing the intralaboratory precision of measurement results were equal to, %: P 5, S 3.4, Cl 4.7, K 4.4, Ca 3.1, Fe 17, Ni 19, Cu 18, Zn 3, Br 4, Rb 4, Sr 3, Pb 16. High values of  $V_{i/p}$  for Fe, Ni, Cu and Pb could be related to their low content in dairy products.



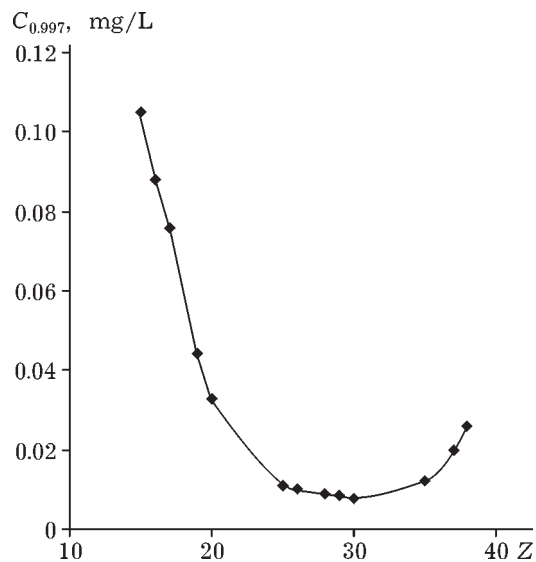


Fig. 5. Detection limit depending on the atomic number Z.

To check the correctness of the analysis of dairy products we used a standard sample of milk powder IAEA-153 [22]. For performing the analysis, a sample (0.03 g) was dissolved in 1 mL of double distilled water. The results of XFA TER ( $C_{XFA\ TER}$ ) and certified content ( $C_{cer}$ ) in the sample IAEA-153 are presented in Table 4. It is seen that for most of elements the value of  $C_{XFA\ TER}$  are within the confidence

TABLE 4

Results of the analysis of a standard sample of milk powder, mg/kg

| Elements | $C_{at}$    | $C_{XFA\ TER}$ |
|----------|-------------|----------------|
| P        | 10100±1015  | 9501±320       |
| K        | 17620±1140  | 15600±516      |
| Ca       | 12870±315   | 12600±193      |
| Fe       | 25±0.9      | 3.0±0.5        |
| Cu       | (0.57±0.2)  | 0.75±0.15      |
| Zn       | 39.6±1.8    | 41.0±0.6       |
| Br       | 12.3±1.3    | 12.2±0.3       |
| Rb       | 14.0±1.9    | 14.6±0.28      |
| Sr       | (4.09±0.62) | 4.1±0.2        |

Note. In parentheses there is information values presented.

interval. An exception is presented by potassium, whose content is underestimated by 10% comparing to the certified value. The reasons for this fact are not understood yet.

In addition, the correctness of the technique was confirmed *via* comparing the results of non-destructive XFA TER with the results of XFA TER for the samples of dairy products after acidic decomposition (Table 5). The samples were decomposed using  $HNO_3/HClO_4$  mixture as described in [3]. Cl and Fe content in

TABLE 5

Results of determining the elements by XFA TER without decomposition and with the decomposition of milk, mg/L

| Elements | Cow's milk            |                         | Goat milk               | Kefir                   | Milk formula*       |
|----------|-----------------------|-------------------------|-------------------------|-------------------------|---------------------|
|          | $C_f = 1.5\%$         | $C_f = 4\%$             | ( $C_f = 2.5\%$ )       | ( $C_f = 0.1\%$ )       |                     |
| P        | 689±27/710±26         | 615±44/654±8            | 722±30/727±19           | 800±25/812±22           | 2999±205/3100±172   |
| S        | 200±6/197±6           | 177±8/168±3             | 213±9/204±5             | 272±5/229±10            | 919±32/878±28       |
| Cl       | 748±30/-              | 750±24/-                | 820±33/-                | 1080±20/-               | 2862±413/-          |
| K        | 1220±60/1190±72       | 1160±30/1020±25         | 958±40/995±34           | 1550±80/1340±150        | 4820±677/4600±487   |
| Ca       | 918±30/1193±6         | 872±29/856±3            | 808±24/882±10           | 1130±22/1080±26         | 4695±245/4430±171   |
| Fe       | 0.68±0.1/-            | 1.2±0.4/-               | 0.45±0.05/-             | 0.14±0.02/-             | 90±8/-              |
| Ni       | 0.04±0.02/0.05±0.02   | 0.10±0.02/0.12±0.03     | 0.08±0.01/0.13±0.01     | 0.08±0.01/0.15±0.01     | 0.27±0.04/0.52±0.02 |
| Cu       | 0.10±0.01/0.07±0.02   | 0.11±0.03/0.09±0.04     | 0.14±0.02/0.11±0.02     | 0.13±0.01/0.08±0.01     | 3.2±0.14/3.4±0.15   |
| Zn       | 3.6±0.2/3.8±0.2       | 4.2±0.2/3.9±0.1         | 3.1±0.1/3.2±0.1         | 4.4±0.1/4.5±0.1         | 55±1/53±0.5         |
| Br       | 2.7±0.03/-            | 2.4±0.04/-              | 1.8±0.08/-              | 5.0±0.04/-              | 13±1/-              |
| Rb       | 1.1±0.04/0.95±0.05    | 1.3±0.04/1.2±0.02       | 1.6±0.05/1.5±0.03       | 1.2±0.03/1.1±0.05       | 4.0±0.5/4.0±0.2     |
| Sr       | 0.78±0.03/0.82±0.02   | 0.89±0.05/0.90±0.02     | 0.75±0.04/0.80±0.03     | 1.1±0.02/1.1±0.03       | 3.9±0.29/4.2±0.06   |
| Pb       | 0.045±0.01/0.049±0.01 | 0.068±0.007/0.053±0.008 | 0.070±0.004/0.059±0.008 | 0.040±0.007/0.028±0.006 | 0.80±0.07/0.61±0.06 |

Note. The first value corresponds to the content in the samples without decomposition; the second value corresponds to the content after the acidic decomposition of milk samples.

\* Calculated for dry solid, mg/kg.

the samples after decomposition could not be quantified due to high signal intensity inherent in the concentration of these elements in the blank sample, the concentration of Br was not determined due to the loss of this element in the course of decomposing the milk in an open container. The results of assessing the correctness of the technique demonstrated that there are no significant systematic errors. The relative standard deviations characterizing the difference between the results under comparison for P, S, K, Ca, Zn, Rb and Sr were less than 10 % for Ni, Cu and Pb these values were less than 20–30 %.

## CONCLUSION

Thus, the use of X-ray spectrometer with a total external reflection allows researchers to determine both macro- and micronutrients in dairy products with no chemical decomposing the samples under investigation. It was demonstrated that the internal standard method with the use of Ga as a reference element allows one to take into account the surface emitter density for the elements with  $Z > 20$ . In the course of determining the elements with  $Z < 20$  (P, S, Cl, K, Ca) in order to reduce the influence of the surface emitter density and the errors of sample preparation upon the results of the analysis, the initial sample should be diluted with water.

## REFERENCES

- 1 Skurikhin I. M. (Ed.), *Khimicheskiy Sostav Rossiyskikh Produktov Pitaniya* (Handbook), De LI Print, Moscow, 2002.
- 2 Waheed S., Zaidi J. H., Ahmad S., Saleem M., *J. Radioanal. Nucl. Chem.*, 254, 3 (2002) 597.
- 3 Rodriguez E. M. R., Alaejos M. S., Romero C. D., *Agric. Food Chem.*, 47, 4 (1999) 1520 .
- 4 Abdulrazzaq Y. M., Osman N., Nagelkerke N., Kosanovic M., Adem A., *J. Environ. Sci. Health. A Tox. Hazard. Subst. Environ. Eng.*, 43 (2008) 329.
- 5 Hermansen J. E., Badsberg J. H., Kristensen T., Gundersen V., *J. Dairy Res.*, 72 (2005) 362 .
- 6 El Zubeir Ibtisam E. M., El Owni O. A. O., Mohamed G. E., *Res. J. Agric. & Biol. Sci.*, 1, 1 (2005) 45.
- 7 Solis C., Isaac-Olive K., Mireles A., Vidal-Hernandez M., *Microchem. J.*, 91 (2009) 9.
- 8 Pashkova G. V., *Analitika i Kontrol*, 14, 1 (2010) 4.
- 9 Greenberg R. R., *Anal. Chem.*, 58, 12 (1986) 2511.
- 10 Handbook of Dairy Food Analysis, in L. Nollet, F. Toldra (Eds.), Boca Raton, CRC Press, 2009.
- 11 Szoboszlai N., Polgari Z., Mihucz V.G., Zaray G., *Anal. Chim. Acta*, 633 (2009) 1.
- 12 Revenko A. G., *Analitika i Kontrol*, 14, 2 (2010) 42.
- 13 Da Costa R. S. S., Do Carmo M., Lopes C., De Jesus E. F., Simabuco S. M., *J. Food Comp. Anal.*, 15 (2002) 27.
- 14 Da Costa R. S. S., Do Carmo M. G. T., Saunders C., De Jesus E. F. O., Lopes R. T., Simabuco S. M., *Int. J. Food Sci. Nutr.*, 54 (2003) 111.
- 15 Gross A., Stosnach H., Lab Report XRF 434 S2 PICOFOX, Trace Elements in Biological Matrices and Their Impact in Clinical Chemistry, Bruker Nano GmbH, Berlin. URL: [http://www.bruker-axs.de/uploads/tx\\_linkselectorforpdfpool/LR\\_XRF\\_434\\_Biological\\_Matrices\\_low\\_res.pdf](http://www.bruker-axs.de/uploads/tx_linkselectorforpdfpool/LR_XRF_434_Biological_Matrices_low_res.pdf)
- 16 Klockenkamper R., von Bohlen A., *Spectrochim. Acta B*, 44, 5 (1989) 461.
- 17 Zarkadas Ch., Karydas A. G., Paradellis T., *Spectrochim. Acta B*, 56 (2001) 2219.
- 18 Pavlinskiy G. V., Smagunova A. N., Karpukova O. M., Bolormaa O., Dorzh, D., *Zh. Anal. Khim.*, 57, 3 (2002) 231.
- 19 de Boer D. K. G., *Spectrochim. Acta B*, 46, 10 (1991) 1433.
- 20 Losev N. F., *Kolichestvenny Rentgenospektralny Fluorestantsnyy Analiz*, Nauka, Moscow, 1969.
- 21 Gorbatova K. K., *Khimiya i Fizika Moloka* (High School Handbook), GIORD, St. Petersburg, 2004.