ACTA IMEKO
ISSN: 2221-870X
April 2016, Volume 5, Number 1, 15-21

Multi-elemental composition of Slovenian milk: analytical approach and geographical origin determination

Doris Potočnik[1,3], Marijan Nečemer[2], Darja Mazej[1], Radojko Jaćimović[1] and Nives Ogrinc[1,3]

[1] *Department of Environmental Science, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia*
[2] *Department of Low and Medium Energy Physics, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia*
[3] *Jožef Stefan International Postgraduate School, Jamova 39, 1000 Ljubljana, Slovenia*

ABSTRACT
The main objective in multi-elemental analysis in food has traditionally been and still is, to ensure food quality and safety. Three different methods were investigated in the present study to obtain the elemental content in milk samples: energy dispersive X-ray fluorescence spectrometry (EDXRF), k_0-instrumental nuclear activation analysis (k_0-INAA) and the inductively coupled plasma mass spectrometry (ICP-MS). Results indicate that the obtained data with different methods are in good agreement with certified reference materials. Precision was found to be satisfactory with relative standard deviations always between 1 and 10 %, except for Se, As, Cd and Pb. The concentrations of these elements were close to the detection limit and thus, precision higher than 10 %. An intercomparison exercise between EDXRF and k_0-INAA showed satisfactory agreement and only two samples exceeded 95 % confidence interval for Rb and Zn with lower data obtained by k_0-INAA. It was found that EDXRF was the cheapest, simplest and most environmental friendly method for analysis of multi-elemental composition (P, S, Cl, K, Ca, Zn, Br, Rb, Sr) in milk samples, while for the determination of Mn, Fe, Cu, Se content and possible identification of pollutants such as As, Cd and Pb ICP-MS it was a method of choice due to its excellent sensitivity and accuracy. These two methods were also used to determine the multi-elemental composition in Slovenian raw cow milk from different geographical regions: Alpine, Mediterranean, Dinaric and Pannonian in December 2013. Linear discriminant analysis (LDA) was used to explore multi-elemental analysis of milk samples to obtain classification according to geographical regions. Regional discrimination was most successful taking into account Ca, S, P, K, and Cl with prediction ability of 66.7 %.

Section: RESEARCH PAPER

Keywords: milk; EDXRF; k_0-INAA; ICP-MS; LDA; geographical origin

Citation: Doris Potočnik, Marijan Nečemer, Darja Mazej, Radojko Jaćimović and Nives Ogrinc, Multi-elemental composition of Slovenian milk: analytical approach and geographical origin determination, Acta IMEKO, vol. 5, no. 1, article 5, April 2016, identifier: IMEKO-ACTA-05 (2016)-01-05

Section Editor: Claudia Zoani, Italian National Agency for New Technologies, Energy and Sustainable Economic Development affiliation, Rome, Italy

Received September 6, 2015; **In final form** December 20, 2015, year; **Published** April 2016

Funding: Project funding by IAEA under Contract No. 17897

Corresponding author: Nives Ogrinc, e-mail: nives.ogrinc@ijs.si

1. INTRODUCTION

Milk is an important everyday nutrition, since it is a source for many key nutrients including proteins, energy, many essential minerals and vitamins, factors that all contributed to an increased consumption in recent years. Milk contains more than 20 different micro and macro elements. Among them are elements such as Cu, Co, Se, Zn, Mn, Mo, I and Fe that are essential for human health in certain concentrations. These elements have a beneficial effect on animals and humans, since they are important for normal functioning of metabolism, growth and development and any concentration discrepancies of these elements may cause disturbance in organisms [1], [2].

Concentration of micro and macro elements in milk is not constant, but is influenced by genetics of individual animal species, stage of lactation, farm management (nutritional regime) and environment factors such as season, locality of the farm, nature of soil, fertilizer application and industrial activities [3]. Industrial and other anthropogenic activities could also influence the distribution and levels of elements, mainly toxic elements such as Cd, Ni, Co, Pb, Cr, Hg in milk, since they are accumulated through the food chain [1], [4], [5]. They can easily accumulate in plants grown on polluted soils and thus cause toxic effects in cattle and consequently also in humans who consume "contaminated" milk [4], [6]. For this reason, it is necessary to control the concentration of micro and macro elements/toxic elements in consumed food.

Further, multi-element analysis has been successfully used for the determination of the geographical origin of different foodstuff such as wine [7], [8] onion [9]-[11], tea [12] and tomatoes [13]. In relation to milk and dairy products, most of the studies have been performed on cheese for descriptive purposes [14] and for origin authentication purposes [15]. Sacco et al. [16] used multi-elemental analysis in combination with stable isotope analysis for differentiation between Southern Italy and foreign milk. Trace elements in milk and dairy product have been used to investigate and to provide information about correlation between animals fodder, time of year, elemental conditions. It was found that season has more influence on mineral concentration than regions [15].

Several different techniques are available for simultaneous multi-element chemical analysis including: energy dispersion X-ray fluorescence spectrometry (EDXRF), neutron activation (k_0-instrumental nuclear activation analysis; k_0-INAA), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) [1]. Each of these techniques has the capacity to provide a chemical profile or chemical fingerprinting, which can be used to characterize the material in question and to compare it with other samples or reference materials. Among inductively coupled plasma systems ICP-AES and ICP-MS have been widely used for the analysis of minor and trace elements in food, since they provide satisfactory result and allow performing simultaneous-sequential determination of multiple elements. The advantages of these techniques are short time of analysis and low limit of detection [4], [17]. On the other hand, EDXRF as a highly sensitive, fast and cheap technique offers the possibility of performing direct multi-element analysis of solid samples over a wide dynamic range. This technique is especially useful in food authentication and traceability studies where there is a requirement for a database of genuine samples to which the sample can be compared to establish its authenticity. For effective data processing, the large number of independently measured parameters is needed including multi-elemental composition [18]-[20]. Another nondestructive multi-element technique is k_0-INAA. The main disadvantages of this method are long turn-around time, is labour intensive and a nuclear reactor or other source of activating particles is required. Nevertheless, providing a different approach, this method is often an important asset in studies regarding the analysis of standard reference materials [21]-[23] and as an additional technique in evaluation of the accuracy of analytical results [24].

Thus, the main objectives of this paper are: (1) to verify the methods for elemental composition in milk samples including EDXRF, k_0-INAA and ICP-MS; and (2) to differentiate the milk according to geographical region in Slovenia based on elemental composition. Sample preparation and the analysis procedure for each of the above mentioned analytical techniques are described and a comparison of analytical and other parameters such as uncertainty, accuracy, limits of detection (LOD), cost of analysis per sample, instrumental cost etc. is critically evaluated.

2. MATERIALS AND METHODS

2.1. Sampling

Samples of the whole milk were provided by four Slovenian diary producers: Ljubljanske mlekarne d.d., Pomurske mlekarne d.d., Mlekarna Planika d.o.o. and Mlekarna Celeia d.o.o. in December 2013 covering different geographical regions (Mediterranean, Pannonia, Dinaric and Alpine) in Slovenia. The samples were stored at -20°C before analysis. All together 40 samples were obtained for multi-elemental analysis.

2.2. Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF)

Multielement determination of macro (P, S, Cl, K, Ca) and micro elemental (Zn, Br, Rb, Sr) content was nondestructively performed by Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF). The analysis was performed on freeze-dried samples. The pellets were prepared from 0.5 to 1.0 g of powder sample material by a pellet die and hydraulic press. For excitation, the disc radioisotope excitation source of Fe-55 (25 mCi) and Cd-109 (20 mCi) from Eckert and Ziegler were used. The emitted fluorescence radiation was measured by en energy dispersive X-ray spectrometer constituted of a Si(Li) detector (Canaberra), a spectroscopy amplifier (Canberra M2024), ADC (Canberra M8075) and PC based MCA (S-100 Canberra). The spectrometer was equipped with a vacuum chamber (Fe-55) for the measurement of light elements P-Cl, while the energy resolution of the spectrometer was 175 eV at 5.9 keV.

The analysis of complex X-ray spectra was performed by the AXIL spectral analysis program [24], [25]. For the evaluated uncertainty of this procedure, it is required to include the statistical uncertainty of measured intensities and the uncertainty of the mathematical fitting procedure. For this purpose, quantification was then performed utilizing QAES (Quantitative Analysis of Environmental Samples) software, developed in our laboratory [24], [25]. The estimated uncertainty of the analysis was around 5 % to 10 %. Rather high total estimated uncertainty is mainly due to contributions of matrix correction and geometry calibration procedures, which include errors of tabulated fundamental parameters, and contributions of spectrum acquisition and analysis. In the case of EDXRF, the limits of detection calculated from the signal to background ratio [26] on the realistic sample NIST 1549 for P, S, Cl, K, Ca, Zn, Br, Rb and Sr were 400, 200, 100, 20, 15, 4, 2, 1, 1 μg/g of dry milk sample, respectively.

2.3. k_0-INAA measurements

For k_0-INAA measurements aliquot of the freeze-dry sample (0.10 to 0.20 g) was weighed into a polyethylene ampoule (SPRONK System, The Netherlands). For the determination of long-lived radionuclides the sample was irradiated together with the standard Al-Au (0.1 %) for 12 hours. Irradiation was carried out in a reactor TRIGA Mark II. After irradiation the sample was kept in a polyethylene ampoule and gamma activity of radionuclides induced in the sample after 4, 9-15, and 24-33 days of cooling was measured. All measurements were performed on HPGe detector (40 and 45 % yield). For the evaluation of gamma spectra we used the program HyperLab 2002, and element content was calculated with the program Kayzero for Windows. Limits of detection for Br, Ca, K, Rb, Zn calculated as three times the standard deviations of the blank sample, are 1, 500, 500, 5, 5 μg/g milk sample, respectively.

The uncertainty level for elemental determination by k_0-INAA was 6–10 %, in the case of lower elemental content such as Se and Mn the uncertainty is higher, around 20 %.

2.4. ICP-MS measurements

For multi-elemental analysis of milk samples with ICP-MS two procedures of sample preparation were used:

a) Microwave digestion: about 1 mL of milk sample (0.15 g of lyophilized sample) was weighed into quartz tubes. 1 mL 65 % HNO3, (Merck, Germany, suprapur) and 1 mL 30 % H_2O_2, (Merck, Germany, suprapur) were added and samples were subjected to closed vessel microwave digestion (Microwave system ETHOS 1, MILESTONE SN 130471) at max. power of 1500 W: ramp to 130°C 10 min, ramp to 200 °C 10 min, hold 20 min, cooling 20 min. Then the samples were equilibrated to room temperature. The solution was quantitatively transferred into 10 mL polyethylene graduated tubes and filled to the mark with Mili-Q water. Before determination by ICP-MS, samples were diluted five times. External calibration was made with ICP Multi Element Standard solution XVI CertiPUR (Merck).

b) Dilution (adapted from Barany [27]): An aliquot of 1 mL of milk sample (0.15 g of lyophilized sample) was diluted five times with alkaline (Merck, suprapur) solution containing Triton X-100 (Sigma Aldrich, sigmaultra) and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Fisher Scientific, analytical reagent grade). For calibration the standard addition procedure was utilised.

Measurements of prepared solutions were made by an Octapole Reaction System Inductively Coupled Plasma Mass Spectrometer (7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). Instrumental conditions: Babington nebulizer, Scott-type spray chamber, spray chamber temperature 5 °C, plasma gas flow rate 15 L/min, carrier gas flow rate 0.8 L/min, make-up gas flow rate 0.1 L/min, sample solution uptake flow rate 1 mL/min, RF power 1500 W, reaction cell gas helium 4 mL/min, isotopes monitored ^{55}Mn, ^{56}Fe, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{111}Cd, ^{114}Cd, ^{206}Pb, ^{207}Pb, ^{208}Pb, isotopes monitored as internal standard added to all solutions: ^{45}Sc, ^{69}Ga, ^{89}Y, ^{157}Gd. Tuning of the instrument was made daily using a solution containing Li, Mg, Y, Ce, Tl and Co.

Concentrations of Cd, Pb and As in milk samples are usually very low <0.5 ng/g and thus, insufficient detection limits were obtained with microwave digestion. Therefore preparation with simple dilution of milk samples was introduced. Limits of detection for Cd, Pb, As, Se, Mn, Cu, Zn and Fe, calculated as three times the standard deviations of the blank sample, were 0.1, 0.2, 0.05, 2, 1, 6, 35 and 30 ng/g milk sample, respectively. Estimated combined uncertainty of results was 5 % for Cu, Zn, Fe, 10 % for Se, Mn and 15 % for As, Cd, Pb.

2.5. Quality control

The accuracy of results was checked as follows:

a) analysis of the certified reference materials: Whole milk powder NIST 8435, Non-fat Milk Powder NIST 1549 (both National Institute of Standard and Technology) and Skim Milk Powder BCR 150 (EC-JRC-IRMM), ERM-BD150 and ERM-BD151 (EC-JRC-IRMM);

b) comparison with independent methods: EDXRF and k_0-INAA;

c) for As there are no reference materials, therefore, comparison was made by an independent method radiochemical neutron activation analysis [28], [29]: result for internal quality control milk sample by k_0-RNAA 0.28 ± 0.02 ng/g and by ICP-MS 0.36 ± 0.05 ng/g;

d) participation in interlaboratory comparison scheme FAPAS (Food and Environmental Research Agency, Sand Hutton,York, VB).

2.6. Statistical evaluation

Statistical calculations and multivariate analysis were carried out using XLSTAT software package (Addinsoft, New York, USA) Basic statistics included mean values (median and arithmetic mean), standard deviation (S.D.), minimum and maximum. Multivariate analysis involved linear discriminant analysis (LDA).

3. RESULTS AND DISCUSSION

3.1. Results on certified reference materials

Results of the validation of the accuracy of multi-elemental analysis performed by ICP-MS with certified reference materials (NIST 1549, NIST 843) are collected in Table 1, while results of the validation of the accuracy with certified reference material ERM-BD 150 and ERM-BD 151 with all three methods are collected in Table 2.

Results indicate that the obtained data with different methods are in good agreement with certified reference materials. Precision was also found to be satisfactory with relative standard deviations (RSDs) always between 1 and 10 %. Only in four cases (of Se, As, Cd and Pb), for which the

Table 1. Validation of the accuracy of ICP-MS multi-elemental procedures proposed (NIST 1549, NIST 8435 and BCR 150).

	NIST 1549		NIST 8435		BCR 150	
Element	**Certified value**	**Found Value**	**Certified value**	**Found value**	**Certified value**	**Found value**
			dry weight mg/kg			
Mn	0.26 ± 0.06	0.25 ± 0.03	0.17 ± 0.05	0.19 ± 0.02	-	-
Fe	1.78 ± 0.10	1.71 ± 0.09	1.80 ± 1.10	2.0 ± 0.1	11.8 ± 0.6	9.79 ± 0.48
Cu	0.70 ± 0.10	0.67 ± 0.03	0.46 ± 0.10	0.40 ± 0.02	2.23 ± 0.08	1.95 ± 0.1
Zn	46.1 ± 2.2	39.3 ± 2.0	28.0 ± 3.1	23.8 ± 1.2	-	-
Se	0.11 ± 0.1	0.11 ± 0.1	0.131 ± 0.14	0.128 ± 0.01	-	-
Cd	0.0005 ± 0.0002	0.0005 ± 00001	-	-	0.0218 ± 0.0014	0.0203 ± 0.0031
Pb	0.019 ± 0.003	0.023 ± 0.004	-	-	1.00 ± 0.04	0.84 ± 0.13

Table 2. Determination of element concentrations in mg/kg in reference materials by ICP-MS, EDXRF and k$_0$-INAA (ERM-BD 150 and ERM-BD 151).

	ERM-BD 150				**ERM-BD 151**			
Element	**Certified value**	**Found value by EDXRF**	**Found value by k$_0$-INAA**	**Found value by ICP-MS**	**Certified value**	**Found value by EDXRF**	**Found value by k$_0$-INAA**	**Found value by ICP-MS**
Ca	13900 ± 800	13000 ± 1000	13487 ± 1013	-	13900 ± 700	13200 ± 1100	13309 ± 1018	-
Mg	1260 ± 100	-	1266 ± 117	-	1260 ± 70	-	1268 ± 112	-
Mn	0.289 ± 0.018	-	0.313 ± 0.101	-	0.29 ± 0.03	-	0.306 ± 0.072	-
P	11000 ± 600	9100 ± 900	-	-	11000 ± 600	9600 ± 900	-	-
K	17000 ± 700	16500 ± 1300	17665 ± 1263	-	17000 ± 800	16200 ± 1300	17447 ± 1246	-
Se	0.188 ± 0.014	-	0.201 ± 0.053	0.227 ± 0.022	0.19 ± 0.04	-	0.188 ± 0.046	0.224± 0.022
Na	4180 ± 190	-	4397 ± 313	-	4190 ± 230	-	4348 ± 308	-
Sr	-	3.71 ± 0.3	-	-	-	4.10 ± 0.3	-	-
Zn	44.8 ± 2.0	44.3 ± 3.5	46.7 ± 3.7	42.2 ± 2.1	44.9 ± 2.3	43.0 ± 3.4	46.1 ± 4.0	44.3 ± 3.2
Cu	1.08 ± 0.06	-	-	1.05 ± 0.05	5.00 ± 0.23	-	-	5.24 ±0.33
Cl	9700 ± 2000	9600 ± 900	10143 ± 743	-	9800 ± 1200	9000 ± 900	10077 ± 737	-
Cd	0.0114 ± 0.0029	-	-	0.0122 ± 0.002	0.106 ± 0.013	-	-	0.106 ±0.008
Pb	0.019 ± 0.004	-	-	0.019 ± 0.003*	0.207 ± 0.014	-	-	0.206 ± 0.02
S	-	3300 ± 300	-	-	-	3300 ± 300	-	-
Br	-	13.2 ± 1.0	-	-	-	13.6 ± 1.0	-	-
Rb	-	17.7 ± 1.4	-	-	-	17.0 ± 1.4	-	-

measured concentrations were very close to the detection limit, precision was worse and higher than 10 %.

3.2. Comparison of elemental composition between EDXRF and k$_0$-INAA methods

Comparison of results of milk samples obtained by EDXRF and k$_0$-INAA is presented in Table 3. It was found that only two samples exceeded 95 % confidence interval for Rb and Zn with lower data obtained by k$_0$-INAA (bold values in Table 3).

The possible reason for this discrepancy could be in the inhomogeneity of distribution of these two elements in the two aliquotes of the same sample analysed by EDXRF and k$_0$-INAA.

3.3. Results of the interlaboratory comparison scheme FAPAS

We participated in the interlaboratory comparison scheme FAPAS in 2012, 2013 and 2014 for milk samples in powdered form with all analytes present at low natural levels. Results are collected in Table 4. The results indicated good agreement between measured and assigned values. With these independent assessment competence of our laboratory to analyse low levels of As, Cd and Pb was demonstrated.

Common characteristic of all three analytical techniques applied EDXRF, k$_0$-INAA and ICP-MS is their multi-element capability. Preparation of samples was simple and nondestructive in the case of EDXRF and k$_0$-INAA, meanwhile ICP-MS required decomposition of samples. It is obvious that the most sensitive method applied in this study was ICP-MS with LODs in the range of few tens of ng/g.

The sensitivity of EDXRF and k$_0$-INAA is comparable to each other, according to obtained estimated uncertainty (5-10 %) and LODs for the analysed elements in the range from hundred to few μg/g. This means that LODs of ICP-MS were approximately two orders of magnitude lower compared to EDXRF and k$_0$-INAA. On the other hand, the determination of elements such as P, S, Cl by ICP-MS was impossible due to the fact that the ions formed by the ICP discharge are typically positive ions, M+ or M+^{2}, therefore, elements that prefer to form negative ions (such as Cl, I, F, etc.) are very difficult or impossible to determine by ICP-MS.

On the other hand, EDXRF enables analysis of very important macro elements (P, S, and Cl) in milk samples, meanwhile k$_0$-INAA as an alternative analytical tool allows the determination of only Cl. Determination of P was impossible due to the production of pure negatron emitter 32P after activation. In the case of S, poor activation of ^{37}S (short half life t$_{1/2}$ = 5.05 min) resulted in high LODs (3000-5000 μg/g). Since the S content found in milk was in the concentration range of 2000-3000 μg/g, it was evident that determination of S by k$_0$-INAA was omitted due to its insufficient sensitivity. The advantage of k0-INAA in relation to EDXRF is the determination of other important macronutrients Mg and Na. The determination of Mg and Na by EDXRF was impossible due to insufficient instrumental sensitivity in concentration range from one to few thousand μg/g found in real milk samples. Another disadvantage of the k$_0$-INAA and EDXRF techniques was their inability to perform the analysis of toxic elements such as As, Pb and Cd in the concentration range found in milk (Table 2).

Further, considering the cost per sample, its multi-elemental capability, simple non-destructive sample preparation, the minimum number of steps in the measurement and quantification procedure, EDXRF was undoubtedly the cheapest, simplest and most environmental friendly method among the applied analytical techniques and the most suitable for analysis of multi-elemental composition (P, S, Cl, K, Ca, Zn, Br, Rb, Sr) in milk samples. However, for the analysis of elemental content of Mn, Fe, Cu, Se and possible identification of pollutants such as As, Cd and Pb the ICP-MS method was found as a method of choice due to its excellent sensitivity and accuracy. These two methods were also used for the determination of multi-elemental content in authentic Slovenian raw cow milk.

3.4. Multi-elemental composition of Slovenian milk

The elemental content of Slovenian raw cow milk from different geographical regions collected in December 2013 is presented in Table 5. The elemental composition is as follows K > Ca > P, Cl > S > Zn > Rb > Br > Fe > Sr > Cu> Mn >

Table 3. Comparison between k_0-INAA and EDXRF methods for selected macro- and micro-elements in milk samples. Samples exceeding 95 % Confidence interval of comparison are marked bold.

	k_0-INAA					**EDXRF**				
Sample no.	Br	Ca	K	Rb	Zn	Br	Ca	K	Rb	Zn
	dry weight mg/kg									
1	14.0	7970	10969	12.1	28.8	13.9	8710	11200	13.2	30.4
2	11.4	8210	10595	22.1	18.9	11.7	9530	10600	23.3	21.5
3	9.04	7520	8706	6.85	23.3	8.87	7430	8210	7.98	25.2
4	7.71	8680	11800	12.6	31.7	7.85	9210	11600	14.0	33.3
5	7.77	8200	11110	10.3	29.2	8.38	8720	11600	8.85	30.9
6	8.32	8350	11300	12.9	28.5	6.97	8760	11200	14.2	29.9
7	8.10	7670	10390	11.9	27.3	8.38	8740	10600	12.5	28.7
8	7.59	8610	10880	11.5	26.7	8.27	10000	12100	12.9	29.8
9	8.34	8590	11140	16.6	26.6	7.74	9000	11300	19.3	29.6
10	11.4	8120	10980	9.33	29.2	11.4	8810	11300	8.3	30.6
11	8.22	8550	11332	14.0	29.0	9.74	8950	11900	15.0	31.8
12	14.6	8410	11170	12.5	28.6	13.2	9680	12600	11.9	29.6
13	9.12	8420	11230	20.3	27.7	7.93	9660	12400	20.3	29.8
14	16.0	6040	8595	11.1	23.0	15.9	7150	8610	11.4	23.0
15	10.9	7020	9898	**22.3**	**23.6**	14.3	6150	8070	**28.1**	**30.9**
16	14.1	9470	12251	14.1	29.8	14.0	9930	12600	13.3	30.1
17	16.6	9000	12635	22.8	31.2	17.4	9630	12900	23.6	29.6
18	9.44	6070	8113	**9.72**	**20.7**	12.5	6360	8320	**15.4**	**26.0**
19	13.7	7340	10140	15.7	25.6	13.8	8700	10700	16.5	26.1
20	27.5	7660	10332	14.8	23.2	28.2	8510	10900	14.9	26.1

Se > Pb > As > Cd and is consistent with literature data. The content of the elements, such as Cd, Cu, Fe, Mn, Ni, Pb, Sr and Zn is influenced by feed and environmental conditions [30]. The concentrations of toxic elements such as As, Cd and Pb, are low and do not represent a threat to human health.

Multi-elemental composition of milk was used for possible discrimination among four Slovenian geographical regions. Results of statistical evaluation by LDA are presented in Figure 1. Partial separation between groups was obtained, where Alpine and Pannonian groups were well separated, while Dinaric and Mediterranean groups were overlapping. The later two groups are close to each other and thus insufficiently separation was obtained; however their discrimination tendency is promising. The most important factors affecting the geographical origin were Ca, S, P, K, and Cl. In a cross validation test 66.7 % of the samples are classified correctly. The highest rate of classification was for the Alpine and Dinaric samples (78.6 % and 71.4 %, respectively). It is expected that more precise and efficient separation between four geographical regions could be obtained by the involvement of more analysed parameters such as stable isotope values of milk samples.

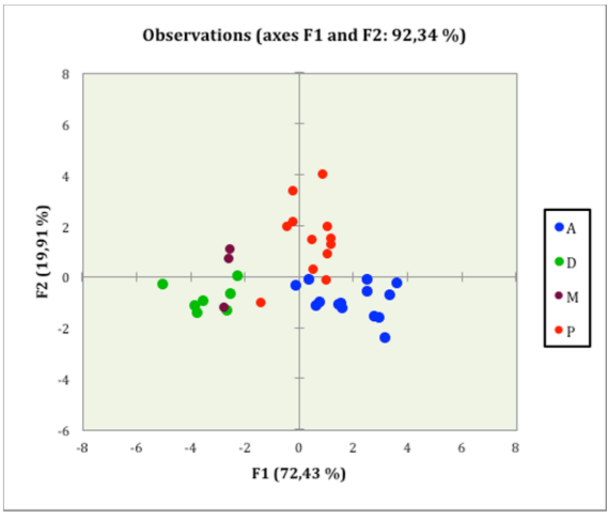

Figure 1. Discrimination between geographical regions using significant elemental parameters. Function 1 represents 72.43 % of variability, while function 2 represents 19.91 % of variability.
Label: A-Alpine, D-Dinaric, M- Mediterranean, P – Pannonian.

Table 4. Results of the metallic contaminants in milk powder in interlaboratory schemes FAPAS in 2012, 2013 and 2014.

	2012: FAPAS 07172		**2013: FAPAS 07190**		**2014: FAPAS**	
Element	Assigned value	Found value	Assigned value	Found value	Assigned value	Found value
	μg/kg					
As	56.4 ± 12.4	45.2 ± 6.1	49.1 ± 10.8	53.5 ± 6.0	58.1 ± 12.8	53.8 ± 1.6
Cd	18.59 ± 4.09	19.9 ± 5.8	16.2 ± 3.56	15.8 ± 1.0	12.2 ± 2.69	12.5 ± 0.8
Pb	66.17 ± 14.6	51.8 ± 3.3	50.8 ± 11.2	47.8 ± 3.4	47.5 ± 10.5	49.3 ± 3.0

Table 5. The summary of elemental data in Slovenian raw cow milk collected in December 2013.

Elements	Mediterranean			Pannonia			Alpine			Dinaric		
Ca (mg/100 g)	110	±	14	113	±	11	113	±	15	111	±	14
K (mg/100 g)	128	±	20	145	±	14	146	±	20	144	±	18
Cl (mg/100 g)	74	±	17	84	±	9	89	±	10	91	±	13
S (mg/100 g)	24	±	3	27	±	4	26	±	5	28	±	5
P (mg/100 g)	62	±	12	77	±	10	78	±	13	76	±	12
Na (mg/100 g)	33	±	4	35	±	2	35	±	3	33	±	7
Zn (mg/100 g)	329	±	57	392	±	28	374	±	34	368	±	34
Br (mg/100 g)	147	±	32	106	±	14	139	±	40	197	±	71
Rb (mg/100 g)	190	±	100	196	±	67	210	±	63	215	±	40
Sr (mg/100 g)	27	±	6	29	±	5	30	±	15	20	±	7
Fe (mg/100 g)	33	±	3	25	±	6	28	±	5	29	±	5
Ni (mg/100 g)	4.5	±	0.7	4.6	±	1.2	4.5	±	1.0	5.6	±	1.1
Mo (mg/100 g)	7.4	±	1.2	7.5	±	2.5	8.7	±	1.8	8.8	±	2.2
Mn (mg/100 g)	3.9	±	0.9	2.8	±	1.1	2.4	±	0.8	2.9	±	0.8
Cu (mg/100 g)	7.0	±	3.4	4.6	±	1.2	4.4	±	1.4	5.3	±	1.5
Se (mg/100 g)	2.0	±	0.2	1.6	±	0.4	1.6	±	0.5	2.0	±	0.8
As (mg/100 g)	0.043	±	0.004	0.042	±	0.017	0.043	±	0.015	0.057	±	23
Cd (mg/100 g)	0.006	±	0.004	0.005	±	0.003	0.004	±	0.002	0.007	±	0.004
Pb mg/100 g)	0.076	±	0.051	0.055	±	0.035	0.076	±	0.057	0.064	±	0.027

4. CONCLUSIONS

There is a need for reliable milk element concentration data to provide information about nutritional uptake and at the same time to provide information about the excess amounts of trace and toxic elements. In addition, multi-elemental composition provides important information on the authenticity and geographical origin of food including milk and dairy products. This paper provides some interesting comparisons between three different techniques (EDXRF, k_0-INAA and ICP-MS) in determination of multi-elemental composition in milk samples. Quality assurance proved entirely satisfactory for all involved measurements and an intercomparison exercise between EDXRF and k_0-INAA showed satisfactory agreement.

The simple, fast, and inexpensive EDXRF method in combination with ICP-MS, which was found the most appropriate technique for the analysis of elemental content of Mn, Fe, Cu, Se and toxic elements such as As, Cd and Pb, were further used for multi-elemental analysis of Slovenian raw cow milk samples. Multi-elemental content in milk samples was combined further in a linear discriminant model to discriminate milk according to geographical origin. Only partial separation was possible using elemental content with overall predicting ability of 66.7 %. It is expected that if elemental data are used in conjunction with other characteristic chemical indices such as isotope analysis, a more holistic and accurate picture in relation to geographical region separation could be created. These data represent a basis for a database of authentic samples of milk in Slovenia, which could be incorporated into a traceability system.

ACKNOWLEDGEMENT

The research represents part of the ERA Chair ISO-FOOD project and was partially supported by the IAEA Project under Contract No. 17897 entitled "The use of stable isotopes and elemental composition for determination of authenticity and geographical origin of milk and dairy products" as part of CRP D5.20.38 "Accessible technologies for the verification of origin of dairy products as an example control system to enhance global trade and food safety".

REFERENCES

[1] N. Khan, I. S. Jeong, I. M. Hwang, J. S. Kim, S. H. Choi, E. Y. Nho, J. Y. Choi, K. S. Park, K. S. Kim, Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS), Food Chem. 147 (2014) pp. 220 – 224.

[2] B. H. Schwendel, T. J. Wester, P. C. H. Morel, M. H. Tavendale, C. Deadman, N. M. Shadbolt, D. E. Otter, Invited review: Organic and conventionally produced milk – An evaluation of factors influencing, milk composition, J. Dairy Sci. 98 (2015) pp. 721 – 746.

[3] C. Sola-Larrañaga, I. Navarro-Blasco, Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain, Food Chem. 112 (2009) pp. 189 – 196.

[4] S. Birghila, S. Dobrinas, G. Stanciu, A. Soceanu, Determination of major and minor elements in milk through ICP-AES, Environ. Eng. Manag. J. 7 (2008) pp. 805 – 808.

[5] N. Bilandžić, M. Đokić, M. Sedak, B. Solomun, I. Varenina, Z. Knežević, M. Benić, Trace element levels in raw milk from

northern and southern regions of Croatia, Food Chem. 127 (2011) pp. 63 – 66.

[6] R. Pilarezyk, J. Wojick, P. Czerniak, P. Sablik, B. Pilarezyk, A. Tomza-Mirciniak, Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environ. Monit. Assess. 185 (2013) pp. 8383 – 8392.

[7] V. F. Taylor, H. P. Longerich, J. D. Greenough, Multielement analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. J. Agr. Food Chem. 51 (2003) pp. 856 – 860.

[8] P. P. Coetzee, F. E. Steffens, R. J. Eiselen, O. P. Augustyn, L. Balcaen, F. Vanhaecke, Multi-element analysis of South African wines by ICP-MS and their classification according to geographical origin, J. Agr. Food Chem. 53 (2005) pp. 5060 – 5066.

[9] K. Ariyama, Y. Aoyama, A. Mochizuki, Y. Homura, M. Kadokura, A. Yasui, Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition, J. Agr. Food Chem. 55 (2007) pp. 347 – 354.

[10] G. A. Chope, L. A. Terry, Use of canonical variate analysis to differentiate onion cultivars by mineral content as measured by ICPAES, Food Chem. 115 (2009) pp. 1108 – 1113.

[11] E. Furia, A. Naccarato, G. Sindona, G. Stabile, A. Tagarelli, Multielement fingerprinting as a tool in origin authentication of PGI food products: Tropea red onion. J. Agr. Food Chem 59 (2011) pp. 8450–8457.

[12] A. Moreda-Piñeiro, A. Fisher, S. J. Hill, The classification of tea according to region of origin using pattern recognition techniques and trace metal data. J. Food Compos. Anal. 16 (2003) pp. 195 – 211.

[13] G. Lo Feudo, A. Naccarato, G. Sindona, A. Tagarelli, Investigating the origin of tomatoes and triple concentrated tomato pastes through multielement determination by inductively coupled plasma mass spectrometry and statistical analysis. J. Agr. Food Chem. 58 (2010) pp. 3801 – 3807.

[14] L. Pillonel, R. Badertscher, P. Froidevaux, G. Haberhauer, S. Hölzl, P. Horn, A. Jakob, E. Pfammatter, U. Piantini, A. Rossmann, R. Tabacchi, J. O. Bosset, Stable isotope ratios, trace and radioactive elements in emmental cheese of different origin. Food Sci. Technol. 26 (2003) pp. 615 – 623.

[15] M. T. Osorio, A. Koidis, P. Papademas, Major and trace elements in milk and Halloumi cheese as markers for authentication of goat feeding regimes and geographical origin, Int. J. Dairy Technol. 67 (2015) pp. 1 – 10.

[16] D. Sacco, M. A. Brescia, A. Sgaramella, G. Casiell, A. Buccolieri, N. Ogrinc, A. Sacco, Discrimination between Southern Italy and foreign milk samples using spectroscopic and analytical data, Food Chem. 114 (2009) pp. 1559 – 1563.

[17] J. Nobrega, Y.Gelinas, A. Krushevska, R. M. Barnes, Direct Cetermination of Major and Trace Elements in Milk by Inductively Coupled Plasma Atomic Emission and Mass Spectrometry, J. Anal. Atom. Spectrom. 12 (1997) pp. 1243 – 1246.

[18] S. Kelly, K. Heaton, J. Hoogewerff, Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis, Trends Food Sci. Tech. 16 (2005) pp. 555 – 567.

[19] U. Kropf, M. Korošec, J. Bertoncelj, N. Ogrinc, M. Nečemer, P. Kump, T. Golob, Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. Food Chem. 121 (2010) pp 839 – 846.

[20] S. A. Drivelos, C. A. Georgiou, Multi-element and multi-isotope-ratio analysi to determine the geographical origin of foods in the European Union. Trac-Trend. Anal. Chem 40 (21012) pp. 38 – 51.

[21] R. Jaćimović, P. Makreski, V. Stibilj, T. Stafilov, Determination of major and trace elements in iron reference materials using k_0-NAA, J. Radioanal Nucl. Ch 278 (2008) pp. 795 – 799.

[22] M. A. Menezes, R. Jaćimović, k_0-INAA quality assessment by analysis of soil reference material GBW07401 using the comparator and neutron flux monitor approaches, Appl. Radiat. Isotopes. 69 (2011) pp. 1057 – 1063.

[23] R. Jaćimović, Comparison of relative INAA and k_0-INAA using soil and sediment reference materials. J. Radiosnsl. Nucl. Ch. 300 (2014) pp. 663 – 672.

[24] M. Nečemer, P. Kump, J. Ščančar, R. Jaćimović, J. Simčič, P. Pelicon, M. Budnar, Z. Jeran, P. Pongrac, M. Regvar, K. Vogel-Mikuš, Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies, Spectrochim. Acta B, 63 (2008) pp 1240 – 1244.

[25] M. Nečemer, P. Kump, K. Vogel-Mikuš, “Use of X-ray fluorescence-based analytical techniques in phytoremediation”, in: Handbook of phytoremediation, (Environmental science, engineering and technology), I.A. Golubev, New York, 2011, ISBN 978-1-61728-753-4, 1-61728-753-9, pp. 331 – 358.

[26] P. Kump, Some considerations on the definition of the limit of detection in X-ray fluorescence spectrometry, Spectrochim. Acta Part B 52 (1997) 405 – 408.

[27] E. Barany, I. A. Bergdal., A. Schutz, S. Skerfving, A. Oskarsson, Inductively Coupled Plasma Mass Spectrometry for Direct Multi-element Analysis of Diluted Human Blood and Serum, J. Anal. Atom. Spectrom. 12 (1997) pp. 1005 – 1009.

[28] A. R. Byrne, Low level simultaneous determination of As and Sb in standard reference materials using radiochemical neutron activation analysis with isotopic 77As and 125Sb tracers. Fresenius Z. Anal. Chem. 326 (1987) pp. 733 – 735.

[29] A.R. Byrne, A. Vakselj, Rapid neutron activation analysis of arsenic in a wide range of samples by solvent extraction of the iodide, Croat. Chem. Acta 46 (1974) pp. 225 – 235.

[30] Y. J. Tu, X. Y. Han, Z. R. Xu, Y.Z. Wang, W. F. Li, Effect of cadmium in feed on organs and meat colour of growing pigs, Vet. Res. Commun. 31 (2007) pp. 621 – 630.