Journal of Environmental Science and Health, Part B

Essential and xenobiotic elements in cottage cheese from the Slovak market with a consumer risk assessment

--Manuscript Draft--

Full Title:	Essential and xenobiotic elements in cottage cheese from the Slovak market with a consumer risk assessment
Manuscript Number:	JESHB-2019-0442
Article Type:	Original Research
Keywords:	cottage cheese, elements, correlations, consumer exposure, MOE, PTWI
Manuscript Classifications:	Food contaminants; Organic matter; Risk assessment; Toxicology; Xenobiotics
Abstract:	The examination of various elements in milk products is very important in the food sector in respect of food quality and safety. The aim of this study was to determine the concentrations of calcium (Ca), cobalt (Co), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), mercury (Hg), potassium (K), magnesium (Mg), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb) and zinc (Zn) in white cottage cheese or supplemented with various additives (white, lacto-free, chive, tzatziki, mustard+onion, chilli, active protein) available on the market of Slovakia. All essential elements were within the reference range. Cottage cheese enriched with tzatziki showed higher amount of Cu, Fe, K, and Zn. Mustard+onion cheese contained high values of Ca, Co, Mg, and Ni. In white cottage cheese high amount of Cr, Mn, and P was measured. The content of xenobiotic metals was below permitted limit. The contribution to PTWI (Provisional tolerable weekly intake) suggested very low dietary exposure to heavy metals as Cd, Hg, and Pb as well as other metals (Cu, Ni, and Zn) in cottage cheese. Numerous correlations between concentrations were observed. MOE (Margin of Exposure) evaluation denoted that average consumption of cottage cheese does not pose any high cardiovascular and nephrotoxicity threat.
Order of Authors:	Marcela Capcarova, Ph.D. Lubos Harangozo Julius Arvay Tomas Toth Lucia Gabriny Lukasz Binkowski Lucia Palsova Magdalena Skalicka Maria de la Luz Garcia Pardo Robert Stawarz Peter Massanyi

Manuscript

Essential and xenobiotic elements in cottage cheese from the Slovak market with a consumer risk assessment

Marcela Capcarova[1], Lubos Harangozo[2], Julius Arvay[2], Tomas Toth[2], Lucia Gabriny[3], Lukasz J. Binkowski[4], Lucia Palsova[5], Magdalena Skalicka[6], Maria de la Luz Garcia Pardo[7], Robert Stawarz[4], Peter Massanyi[1]

[1]*Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic*

[2]*Department of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic*

[3]*AgroBioTech, Slovak University of Agriculture in Nitra, Slovak Republic*

[4]*Institute of Biology, Pedagogical University of Krakow, Krakow, Poland*

[5]*Department of Law, Faculty of European Studies and Regional Development, Slovak University of Agriculture in Nitra, Slovak Republic*

[6]*University of Veterinary Medicine and Pharmacy in Kosice, Institute of Nutrition, Dietetics and Feed Production, Kosice, Slovak Republic*

[7]*Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, Orihuela, Spain*

Abstract

The examination of various elements in milk products is very important in the food sector in respect of food quality and safety. The aim of this study was to determine the concentrations of calcium (Ca), cobalt (Co), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), mercury

(Hg), potassium (K), magnesium (Mg), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb) and zinc (Zn) in white cottage cheese or supplemented with various additives (white, lacto-free, chive, tzatziki, mustard+onion, chilli, active protein) available on the market of Slovakia. All essential elements were within the reference range. Cottage cheese enriched with tzatziki showed higher amount of Cu, Fe, K, and Zn. Mustard+onion cheese contained high values of Ca, Co, Mg, and Ni. In white cottage cheese high amount of Cr, Mn, and P was measured. The content of xenobiotic metals was below permitted limit. The contribution to PTWI (Provisional tolerable weekly intake) suggested very low dietary exposure to heavy metals as Cd, Hg, and Pb as well as other metals (Cu, Ni, and Zn) in cottage cheese. Numerous correlations between concentrations were observed. MOE (Margin of Exposure) evaluation denoted that average consumption of cottage cheese does not pose any high cardiovascular and nephrotoxicity threat.

---

*Address correspondence to Marcela Capcarova, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, Phone: +421376414343, E-mail: marcela.capcarova@uniag.sk

KEY WORDS: cottage cheese, elements, correlations, consumer exposure, MOE, PTWI

Introduction

The minimum consumption of milk and milk products per person given by World Health Organisation (WHO) should be at least 220 kg per year. In Slovak Republic this consumption is evaluated on the level of 160 kg/person/year. [1] The industrial processing of milk has long tradition in Slovakia, however, for the last 17 years, the consumption of drinking milk decreased in average by 0.998 kg per person per year, excluding consumption of cheese, cottage cheese, sour milk products and butter. [2] The milk products are considered as the most important source of calcium in the diet, [3] as well as more than 20 different minor and trace elements. [4]

Cottage cheese belongs to a fresh cultured dairy product, fresh cheese curd that has been drain, [5, 6] commonly consumed on its own but also applicable in other products. [6] Quality cottage cheese should have a mild, diacetyl flavour, curds with uniform shape and size, meaty texture without being too firm, rubbery or tough. [7] A 125 g provides an important source of vitamin B12, calcium, phosphorus, zinc, folate, riboflavin and vitamin B6. [8] Cottage cheese with various additives like herbs, spices, or vegetables is very popular. [9] Herb and spices contain essential, aromatic oils with antimicrobial activity, includes phenolic compounds revealing antioxidant activity, and contribute to the taste of food and its aroma. [10] Moreover, current modern trends in food industry as well as in cheese manufacture concentrate on the production of on-coming functional products with health benefits. The chesses with pepper, chive, herbs are flavoured products with acceptable sensory properties, nutritional value microbial quality and sought after by consumers. [9] Generally, dairy products including cottage cheese are important source of minerals and proteins essential for development and growth and health in human. Nevertheless, they can contain some contaminants as the results of increasing environmental pollution. [11, 12] The presence of various trace elements in dairy products is an indicator of qualitative parameters of the food and can image both, utility and beneficially aspects (calcium, phosphorus, iron, sodium and others), but also hazardous condition concerning except for environmental pollution as well sanitation, hygiene conditions during the manufacturer process, processing conditions, used tolls, machines and packaging lines. [12-14] Toxic elements may be harmful also at low concentration when ingested during the long time. On the other hand, the essential elements indispensable for human organism can cause toxic effects in higher doses received for long time. [15] At any rate, the determination of elements in the milk products is very useful in the assessment of the quality of food. [16]

Thus, the present study aim was to measure the concentrations of calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), zinc (Zn) and toxic metals as cadmium (Cd), mercury (Hg), and lead (Pb) in various types of cottage cheeses commercially available in Slovakia using atomic absorption spectrophotometry (AAS) techniques. The evaluation of consumer risk assessment was carried out based the contributions to provisional tolerable weekly intake (PTWI) and the margin of exposure (MOEs). Additionally, the relationships between concentrations of elements were inquired.

Material and Methods

The samples of commercially available cottage cheeses (n = 42) were purchased from different markets in Nitra city, Slovak Republic, in 2017. Brand of cottage cheese represents the most frequently consumed in the Slovak Republic. According to the flavour and additives, six types of cheese were studied: lacto-free, chive, tzatziki, mustard+onion, chilli and active protein. The cottage cheese without any additives - white served as the control sample.

Sampling

The samples were left in their original packages and transferred to the lab in an ice box. The material was carefully handled with the use of glassware reduced to a minimum to minimalize the risk of sample contamination. The samples were placed in the marked tubes and stored in a freezer (-20ºC) until a further analysis.

Laboratory Analysis

All the analyses were carried out in the laboratory of Department of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.

Analysis of mercury

A cold-vapor AAS analyser (AMA 254, Altec, Czech Republic) was applied for mercury (Hg) determination. The samples (200 mg) were subjected to an *in situ* dry decomposition in a stream of oxygen and passed through the combustion gases to the catalytic column, [17] followed by trapping Hg on the gold amalgamator. Further heating the amalgamator quickly evaporates Hg preconcentrated. Mercury was then moved into the measuring cells system, and its atomic absorption was noted at 254 nm. The working range of the method was 0.05 to 500 ng Hg per sample with the recoveries 100%. The detection limit was 1.5 ng/kg dm.

Analysis of phosphorus

The extract of a sample (1 cm^3) was diluted in the volumetric flask of volume 50 cm^3 by adding 8 cm^3 of a specific solution (concentrated H_2SO_4, NH_4MoO_4, tartrate antimony potassium, ascorbic acid). The flask was complemented with distilled water to 2 000 cm^3. After two hours the absorbance were measured by a spectrophotometer at 666 nm (Shimadzu, Japan).

Analysis of other elements

Cottage cheese samples (3 g from each) were put into teflon cups with 5 mL of deionized water (0.054 µS/cm) from Simplicity 185 (Millipore, UK) and 5 mL of concentrated HNO_3 (Suprapur, Merck, Germany). The teflon cups were shaken to mix the volume. The closed cups were located inside a microwave mineralisation appliance (microwave digestion, MARS X-press, CEM Corp., USA). The samples rotted in three phases. A blank sample was processed in the same manner. The mineralised samples were filtered through filter paper (Munktell grade 390.84 g/m^2) into 50 cm^3 volumetric flasks and filled up with deionized water.

Such prepared samples were analysed for Cd and Pb levels with electrothermal AAS technique (AA240 Z, Varian, Australia) at defined wavelengths and conditions (Table 1). A flame AAS spectrometer (AA240FS, Varian, Australia) was used to measure concentrations of other elements.

Risk Assessment

The results were compared regard to the safety thresholds establish by law and risk assessments were evaluated basing of EDI, PTWI and MOEs.

The EDI (expected dietary exposure) for elements studied in the cottage cheese was estimated per consumer (70 kg of body weight) according to the formula:

$$EDI\ \mu g/kg\ bw/day = \frac{M_{Me}\ \mathrm{x}\ 0.16}{70}$$

M_{Me} - mean concentration of a given metal in micrograms per gram, 0.16 kg of cottage cheese per day/per capita (approximately 1 crucible per day): according to the average consumption of milk in Slovak Republic given by the Ministry of Agriculture and Rural Development of the Slovak Republic is 0.46 kg of milk and milk products per capita/per day, the consumption of milk is 0.13 L per capita/per day

We calculated also contribution to Provisional Tolerable Weekly Intake (PTWI) for Cd, Pb, Hg and also for Cu, Ni, and Zn as these elements are grouped into essential elements, but only at low doses. The contribution was calculated based on values available in the literature according to the formula:

$$contribution\ \% = \frac{\mathrm{EDI\ x\ 7}}{\mathrm{PTWI}} x100$$

According to EFSA [18] the risk to human health related to the presence of cadmium in milk by applying the Margin of Exposure (MOE) was calculated. A summary of the estimated

MOE parameters for the different endpoints was prepared. Systolic blood pressure (SBP) and chronic kidney disease (CKD) were considered to be the most sensitive endpoints used in MOE approach. MOE value of 10 or more brings no significant risk of clinical effects on SBP and change in the CKD. The risk of MOE higher than 1.0 is very low. [18] We determined MOEs for normal consumption (160 mg/per capita/per day) and higher consumption (220 mg/per capita/per day) of cottage cheese in Slovakia.

Statistical analysis

The analysis of variance (one-way ANOVA) was used to determine significant differences in concentrations between groups studied. The relationships among elements were examined by Pearson´s correlation coefficients. Significance level was set at 0.05. Statistical software SAS Release 9.1 (SAS Institute Inc. Cara, USA, 2002-2003) was used for all the calculations.

Results

The element contents observed are expressed as the mean ± SD (standard deviation). The elements were divided into two groups, essential elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, and Zn) and xenobiotic elements (Cd, Hg, and Pb).

Concentrations of essential elements

The essential elements concentrations are shown in Table 2. The significant differences among the groups were found for Co, K, Mg, Na, Ni, and P. Significantly ($P<0.05$) lower content of Co was noted in the white cottage cheese when compared to the chive, mustard+onion and chilli. The K concentration was the lowest in the white cottage cheese. Statistically higher contents ($P<0.05$) were found in the tzatziki and mustard+onion. In the mustard+onion the highest concentration ($P<0.05$) of Mg was measured in comparison to the all other groups of cottage cheese. In the case of Na the lowest concentration ($P<0.05$) was observed in the white cottage against chive, tzatziki, mustard+onion, and chilli. The Ni concentration was the lowest also in the white cottage cheese ("control") in comparison to mustard+onion and chilli. The highest content of P ($P<0.05$) was measured in the white cottage when compared to chive, tzatziki, and chilli. The contents of Ca, Cr, Cu, Fe, Mn, and Zn were in all groups of cottage cheese similar and the differences were not significant ($P>0.05$).

Concentrations of xenobiotic elements

The highest concentration ($P<0.05$) of Cd was found in the group of mustard+onion when compared to the tzatziki and active protein (Table 3). In the white cottage cheese the level of Cd was very low, below LoD. In the case of Hg and Pb the contents of these element were in all groups very low and differences among them were not significant ($P>0.05$).

Correlations

The correlation analysis showed some strong positive/negative correlations among the elements in each group of cottage cheeses (Tables 4-10). In white cottage cheese (Table 4) strong positive correlations between P and Cu (r=0.68), Cr and Mn (r=0.85), Cr and Fe (r=0.86), Ca and Mn (r=0.74), Ca and Fe (r=0.74), Mg and Mn (r=0.72), Mg and Fe (r=0.71), as well as strong negative between Ca and K (*r*=-0.81), Mg and K (*r*=-0.68) and P and Ni (*r*=-0.98).

In the case of toxic elements a strong positive correlations were observed between Hg and Mn (*r*=0.91), Hg and Fe (*r*=0.87), Hg and Cr (*r*=0.74) and strong negative correlations between Na and Pb (*r*=-0.72), Na and Cd (*r*=-0.72).

In the case of lacto-free samples (Table 5) the strong positive correlations were found between Cr and Cu (*r*=0.89), K and Zn (*r*=0.84), Cr and Mn (*r*=0.77), Cr and Fe (*r*=0.79), Co and Ni (*r*=0.67), Ca and Co (*r*=0.71), Mg and Ca (*r*=0.91). Strong negative correlations were noticed between Na and Zn (*r*=-0.95), Ca and Mn (*r*=-0.71), Ca and Fe (*r*=-0.72), Co and Cr (*r*=-0.72), Ca and Cr (*r*=-0.67), Na and K (*r*=-0.68), P and Ca (*r*=-0.81), and P and Mg (*r*=-0.97). Correlations between toxic elements were as follows: strong positive between Cd and Ni (*r*=0.81), Na and Pb (*r*=0.96), Ca and Cd (*r*=0.82) and strong negative between Pb and Cu (*r*=-0.88), Pb and Zn (*r*=-0.92), Cd and Cr (*r*=-0.69), K and Pb (*r*=-0.72).

In chive cottage (Table 6) strong positive correlations was noted between Zn and Cu (*r*=0,95), Ni and Cu (*r*=0.88), Co and Cu (*r*=0.93), K and Cu (*r*=0.91), Na and Cu (*r*=0.72), Ca and Cu (*r*=0.83), Ni and Zn (*r*=0.71), Co and Zn (*r*=0.78), K and Zn (*r*=0.84), Na and Zn (*r*=0.86), Ca and Zn (*r*=0.88), Fe and Mn (*r*=0.83), P and Mn (*r*=0.76), P and Fe (*r*=0.96), Co and Ni (*r*=0.94), K and Ni (*r*=0.81), Ca and Ni (*r*=0.71), K and Co (*r*=0.85), Ca and Co (*r*=0.68), Na and K (*r*=0.72), Ca and K (*r*=0.88), Ca and Na (*r*=0.78), Mg and Na (*r*=0.67), and Mg and Ca (*r*=0.84). Strong negative correlations were found between Cr and Cu (*r*=-0.81), K and Mn (*r*=-0.69), Ca and Mn (*r*=-0.72), Ni and Fe (*r*=-0.81), K and Fe (*r*=-0.81), Ca and Fe (r=-0.67), Ni and Cr (*r*=-0.82), Co and Cr (*r*=-0.94), K and Cr (*r*=-0.70), P and Ni (*r*=-0.75). In this cottage cheese correlated toxic elements in this way: strong positive between Cd and Cr (*r*=0.80), Hg and Pb (*r*=0.82), Hg and Cd (*r*=0.86) and strong negative correlations between

Cd and Cu (*r*=-0.91), Hg and Cu (*r*=-0.67), Cd and Zn (*r*=-0.88), Hg and Zn (*r*=-0.77), Cd and Co (*r*=-0.88), K and Cd (*r*=-0.86), Na and Cd (*r*=-0.68), Ca and Cd (*r*=-0.73) was found.

In cottage cheese enriched with tzatziki (Table 7) followed strong positive correlations were found: between Zn and Cu (*r*=0.90), K and Cu (*r*=0.97), K and Zn (*r*=0.92), Na and Zn (*r*=0.83), Fe and Mn (*r*=0.97), Cr and Fe (*r*=0.68), P and Cr (*r*=0.75), Na and Ni (*r*=0.87), Ca and Co (*r*=0.85), Na and K (*r*=0.73). Strong negative correlations were between Co and Cu (*r*=-0.89), Ca and Cu (*r*=-0.84), Co and Zn (*r*=-0.79), Ca and Zn (*r*=-0.82), Ni and Cr (*r*=-0.92), P and Ni (*r*=-0.73), K and Co (*r*=-0.80), and Ca and K (*r*=-0.84). In the group of toxic elements were strong positive correlations found between Pb and Cu (*r*=0.99), Pb and Zn (*r*=0.94), Cd and Co (*r*=0.91), K and Pb (*r*=0.95), Ca and Cd (*r*=0.76). Also strong negative correlations were found between Cd and Cu (*r*=-0.82), Cd and Mn (*r*=-0.76), Cd and Fe (*r*=-0.85), Pb and Co (*r*=-0.90), Cd and Pb (*r*=-0.79), Ca and Pb (*r*=-0.86), K and Cd (*r*=-0.69), Na and Hg (*r*=-0.67).

Cottage cheese mustard+onion (Table 8) showed strong positive correlations between Zn and Cu (*r*=0.89), Fe and Mn (*r*=0.78), Ni and Cu (*r*=0.67), Ni and Zn (*r*=0.71), K and Cu (*r*=0.97), K and Zn (*r*=0.84), P and Fe (*r*=0.91). Strong negative correlations were found between Ni and Cr (*r*=-0.92), Ca and Mn (*r*=-0.79), Ca and K (*r*=-0.67), Mg and Ca (*r*=-0.89). Strong positive correlations in the case of toxic elements were found between Pb and Cu (*r*=0.93), Pb and Zn (*r*=0.78), Pb and K (*r*=0.96), and strong negative correlations between Cd and Cu (*r*=-0.96), Cd and Zn (*r*=-0.87), Cd and Pb (*r*=-0.98), Cd and K (*r*=-0.98), Hg and Na (*r*=-0.89).

In the case of cottage cheese chilli (Table 9) we found strong positive correlations between Zn and Cu (*r*=0.98), Co and Ni (*r*=0.85), K and Cu (*r*=0.88), K and Zn (*r*=0.78), Ca and Mn (*r*=0.67), Mg and Cu (r=0.70), Mg and Mn (*r*=0.87), and strong negative correlations between Cr and Cu (*r*=-0.91), Cr and Zn (*r*=-0.84), Ni and Fe (*r*=-0.76), Na and Mn (*r*=-0.78), Ca and

Ni (*r*=-0.74), Mg and Cr (*r*=-0.66), Mg and Na (*r*=-0.83), P and Ni (*r*=-0.85). Strong positive correlations were determined between Pb and Cu (*r*=0.92), Pb and Zn (*r*=0.92), Pb and K (*r*=0.82), Cd and Mn (*r*=0.75), Cd and Ca (*r*=0.98), and strong negative between Cd and Ni (*r*=-0.78), Cd and Co (*r*=-0.69), Hg and Cu (*r*=-0.79), Hg and Zn (*r*=-0.87), Hg and Pb (*r*=-0.82), Hg and K (*r*=-0.67).

Cottage cheese enriched with active proteins (Table 10) showed strong positive correlations between P and Zn (*r*=0.86), Fe and Mn (*r*=0.88), Co and Cr (*r*=0.73), Na and Cr (*r*=0.85), Ca and Cr (*r*=0.82), Ca and Na (*r*=0.89), Mg and Ca (*r*=0.88), P and Mg (*r*=0.73). Strong negative correlations were found between K and Cr (*r*=-0.97), K and Co (*r*=-0.83), Na and K (*r*=-0.76), Ca and K (*r*=-0.83), Mg and K (*r*=-0.69). In the case of toxic elements strong positive correlations between Pb and Mn (*r*=0.75), Hg and Ni (*r*=0.76), K and Pb (*r*=0.79), Na and Hg (*r*=0.76), P and Hg (*r*=0.68), and strong negative correlations between Na and Pb (*r*=-0.76), Pb and Cr (*r*=-0.89) were found.

Discussion

Food safety and its nutritional values is a fundamental concern for both consumers and food producers. [9, 19] Dairy products as cottage cheese are good sources of minerals. Minerals are considered essential elements. Calcium, selenium, cooper, for instance, are essential for human body functions and are involved in many physiological processes. [20-22] Nevertheless, heavy metals such as cadmium, lead, mercury may also be detected in the dairy products. [23, 24]

In our study the content of Ca was balanced in all samples and ranged from 1152.33±81.93 µmg/mL in tzatziki to 1375.62±90.62 µmg/mL in mustard+onion. The consumption of dairy products is important in preventing osteoporosis, proper development of bones and dental health. [25] It is more efficient than Ca supplementation due to the presence of lactose which improves calcium assimilation. [26-28] In this study all essential and major elements were within the reference range and they contribute to the high quality of cottage cheese and valuable source of necessary element for human body. There were some differences among the observed samples. Data revealed that the cottage cheese enriched with tzatziki obtained higher amount of Cu, Fe, K, and Zn. In cottage cheese with mustard+onion a higher amount of Ca, Co, Mg, Na, and Ni was measured. White cottage cheese has higher content of Cr, Mn, and P.

Usually, the content of toxic metals (Cd, Hg, Pb) in milk is very low. The content may be increased in particular region with higher industrial activity. The quantification of toxic metals in milk and milk products has significance in respect of food safety and consumer risk assessment. [14, 16, 29] In the milk available on the Slovak market very low values of Cd, Hg and Pb were found. [14] In compliance with the Ministry of Agriculture of Slovak Republic, the maximum tolerable limits of Cd, Hg and Pb in milk products are 0.05 mg/L, 0.02 mg/L, and 0.3 mg/L. In our study the values of Cd ranged from 0.008±0.002 to 0.017±0.001 mg/L. In the white cottage cheese samples the concentration of Cd was below LoD. The values of Hg were in the range between 0.0002±0.0001 and 0.0008±0.0003 mg/L. In the case of Pb the values ranged from 0.01±0.002 to 0.07±0.002 mg/L. All values of the observed toxic metals in samples of cottage cheese were below maximum tolerable limits. Nevertheless, Pb and Cd are known for their cumulative properties, what can result to the various metabolic disorders. [30-33] Thus, the measurement and monitoring of these elements in food is required, as they are nonbiodegradable and their presence in the environment raises agricultural and public health apprehensions. [34]

Correlations

The correlations between the elements in cottage cheese were rarely analysed. The miscellaneous correlations among the observed elements in all groups of cottage cheeses in our study are the results of possible influences among the elements as antagonism, synergism or summation. [35] Ca in cow milk positively correlated with Cu, Zn, and Mg and negatively with Fe. [36] But, the correlations between various elements in the cottage cheese are not reported yet. In our previous study we found high positive correlations in the milk coming from local producer in Nitra (Slovak Republic) between Na and K (r= 0.67) and between Na and Ca (r=0.71). [14] In this study also strong, but negative, correlations between Na and K were found in the white cottage cheese (r=-0.81), active protein (r=-0.76), and lacto-free (r=-0.68). Strong positive correlation between Na and K was observed in the cottage cheese enriched with chive (r=0.72) and tzatziki (r=0.73). A strong positive correlation between Ca and Mg (r=0.873) in the milk of cows found. [37] In our study the same strong positive correlations were in the cottage cheese from the groups lacto-free (r=0.91), chive (r=0.84) and active proteins (r=0.88). Strong positive as well as negative correlation between Na and Mg we found in this study in the cottage cheese enriched with chive (r=0.67) and chilli (r=-0.83), and in our previous study in milk coming from producer from Czech Republic (r=0.67). [14]

The interesting point of this study is the correlations (positive or negative) between heavy metals (Cd, Hg, Pb) and other major or trace elements (Mn, Fe, Cr, Na, Ni, Zn, Cu, K, P) in cottage cheese as the milk and milk products are important for health. The interactions between trace elements or heavy metals (Cd and Mg, Cd and Mn, Zn and Cu, Zn and P, Ca and P, Mg and P, Ca and Mg) or between heavy metals (Pb and Cd) in milk reported Pilarczyk et al. [38] Positive correlations between Cd and Ca (r=0.22) and negative correlation between Pb and Ca (r=-0.295) found Stawarz et al. [39] in breast milk in Poland. The same correlations, strong positive (*r*=0.82) we found in the lacto-free, tzatziki and chilli cottage cheese and strong negative (*r*=-0.86) in tzatziki sample. Pilarczyk et al. [38] found statistically significant correlations found between Cd and Mn (*r*=0.61). Similarly, in our study we also observed strong positive correlation between Cd and Mn in the case of chilli cottage cheese (*r*=0.75). In the milk of Simmental and Holstein-Friesian cows from organic farm Pb concentration was highly correlated with Cd (*r*=0.85 and *r*=0.87), [38] whereas in our study we found strong negative correlation between these two metals in tzatziki (*r*=-0.79) and mustard+onion cottage cheese (*r*=-0.98). It was published that interactions between Cd or Pb and Zn in the organism result in a high degree from an affinity of both metals to metallothionein, a small, cysteine-rich metal-binding protein, and their ability to induce its synthesis. [40] In our study strong positive correlations between Pb and Zn (*r*=0.94), in mustard+onion (*r*=0.78) and in chilli (*r*=0.92) as well as strong negative correlation between Cd and Zn in chive cottage cheese (*r*=-0.88) and mustard+onion (*r*=-0.87) were found. The interaction between toxic metals and trace elements (Ca, Mg, P, Cu, Fe, Mn, Zn) has not been understood clearly, particularly in milk. [41] Correlations between toxic metals (mainly Pb and Cd) and major and trace elements were observed in humans, in milk of nursing mothers [39, 42] and blood and urine. [43] Pilarczyk et al. [38] considered the correlations between Pb and Zn and between Cd and Zn as noteworthy. Pb negatively correlated with Cu (*r*= -0.67) in our study with milk coming from Slovakia. [14] Similarly, in this study strong negative correlation between Pb and Cu was found in the lacto-free cottage cheese (*r*=-0.88). On the contrary, in this study we found strong positive correlation between Pb and Cu in the case of chilli sample (*r*= 0.92), mustard+onion (*r*=0.93) and tzatziki (*r*=0.99). At this moment, we have no biological explanations to some of the reported correlations in cottage cheese and more studies and assessments are needed in this area.

Risk Assessment for Consumers

European Food Safety Agency [18] officially regulated and settled the PTWI for particular elements which is an acceptable level of toxic metals that can be ingested on a weekly basis. It was specified for the purpose of estimating the potential risk to human health. [44] EDI means an estimate of expected dietary exposure of elements consumed through the cottage cheese (Table 11). The results revealed that the normal consumption of cottage cheese (1 crucible per day, 160 mg) is beneficial in term of content of various minerals essential for human body. Additionally, the shifting various cheese (white, lacto-free, chive, tzatziki, mustard+onion, chilli, active protein) supply the human organism with necessary spectrum of minerals. The highest value of EDI for Ca and Co was found in cottage cheese enriched with mustard+onion, for Cr it was white cottage cheese, for Cu tzatziki, for Fe tzatziki as well as mustard+onion, for K mustard+onion, for Mg mustard+onion, for Mn white cheese, for Na mustard+onion, for Ni mustard+onion and chilli, for P white cottage cheese, and for Zn lacto-free, tzatziki, mustard+onion, chilli and active proteins. It seems that additional component given to the white cottage cheese has meaning for regular consumption of cottage cheeses.

We determined the Cd, Hg, and Pb contribution to PTWI (Table 12) in the cottage cheeses available at the market in Slovak Republic. The estimated contribution did not exceed the values settled by the European Commission. Furthermore, the contribution to PTWI suggested only very low dietary exposure to heavy metals as Cd, Hg, and Pb as well as other trace elements (Cu, Ni, and Zn) in cottage cheese. Similarly, Starska et al. [45] concluded that intake of metals noxious by milk and milk products regarding PTWI do not pose any threat to human health in Poland. Thus, the consumption of cottage cheese may contribute to the daily intake of minerals and nutrients to the human organism.

In compliance with the report of EFSA [18] and JECFA, [46] risk assessment based on the MOE value was determined in this paper (Table 13). MOE is defined as the ratio between a defined point on the dose-response curve for the adverse effect and the estimated intake of food. [47] We found that the consumption of cottage cheese available on the market of Slovak Republic poses no risk of CKD and SBP. Similar results we determined in our previous study with milk and wine. [14, 48]

Conclusion

The consumption of cottage cheeses enriched by various additives as chive, tzatziki, mustard+onion, chilli, active proteins, lacto-free as well as pure white cottage cheese available on the market of Slovak Republic may contribute to the daily dietary intake of important essential elements. The content of heavy metals present in these products is lower than legal limits. The contribution to PTWI calculation denoted the exposure to heavy metals (Cd, Hg, and Pb) from cottage cheese is very low. MOE evaluation revealed that the average consumption of cottage cheese poses no high cardiovascular and nephrotoxicity threat. Food safety is one of the most important priorities in Europe. Therefore, constant monitoring and control is needed.

Acknowledgments

This work was financially supported by the grants APVV-16-0289, KEGA 024SPU-4/2018, and KEGA 010/SPU-4/2018. The research has been conducted with the support of the

Erasmus+ Programme of the European Union, Jean Monnet Centre of Excellence project CEEAG No. 611446-EPP-1-2019-1-SK-EPPJMO-CoE.

References

[1]. Petriľák, M.; Horská, E.; Šumichrast, J.; Palkovič, J. Comparison of Slovak dairy products with and without added value sold by commercial chains. Proceedings of the 8th International Scientific Conference Rural Development 2017, 1238-1243. eISBN 978-609-449-128-3.

[2]. Kubicová, Ľ.; Kádeková, Z.; Dobák, D. Trends in consumption of milk and dairy products in Slovakia after accession. Polityki Europejskie, Finanse i Marketing **2014**, *12*(61), 90-95.

[3]. Gonnelli, S.; Rossi, S.; Montomoli, M.; Caffarelli, C.; Cuda, C.; Lazzeri, G.; Giacchi, M.; Nuti, R. Accuracy of different reduced versions of a validated foor-frequency questionnaire in Italian men and women. Calcif. Tissue Int. **2009**, *85*, 221-227.

[4]. Kurajdova, K.; Taborecka-Petrovicova, J.; Kascakova, A. Factors influencing milk consumption and purchase behaviour-evidence from Slovakia. Procedia Econ. Financ. **2015**, *34*, 573-580.

[5]. Hubbard, E.M.; Jervis, S.M.; Drake, M.A. The effect of extrinsic attributes on linking of cottage cheese. J. Dairy Sci. **2016**, *99*, 183-193.

[6]. Yasin, N.M.N.; Shalaby, S.M. Physiochemical and sensory properties of functional low fat cheesecake manufactured using cottage cheese. Ann. Agric. Sci. **2013**, *58*(1), 61-67.

[7]. Bodyfelt, F.W.; Potter, D. Sensory evaluation of creamed cottage cheese. In *Sensory evaluation of dairy products*; Clark, S.; Costello, M.; Drake, M.A.; Bodyfelt, F., Eds.; Van Nostrands Reinhold: New York, NY, 2009; 167-190.

[8]. Hill, A.R. Cheese making technology book. Dairy Education Series at the University of Guelph, Canada. 2012, https://www.uoguelph.ca/foodscience/book-page/cheese-making-technology-ebook

[9]. Josipovic, R.; Kneževic Z.M.; Frece, J.; Markov, K.; Kazazic, S.; Mrvcic, J. Improved properties and microbiological safety of novel cottage cheese containing spices. Food Technol. Biotechnol. **2015**, 53(4), 454-462.

[10]. Shan, B.; Cai, Y.Z.; Brooks, J.D.; Corke, H. Potential application of spice and herb extracts as natural preservatives in cheese. J. Med. Food. **2011**, *14*, 284-290.

[11]. Kazi, T.G.; Jalbani, N.; Baig, J.A.; Kandhro, G.A.; Afridi, H.I.; Arain, M.B.; Jamali, M.K.; Shah, A.Q. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. Food Chem. Toxicol. **2009**, *47*, 2163-2169.

[12]. Capcarova, M.; Harangozo, L.; Toth, T.; Schwarczova, L.; Bobkova, A.; Stawarz, R.; Guidi, A.; Massanyi, P. Detection of selected trace elements in yogurt components. J. Environ. Sci.Health part B **2017**, *52*(12), 858-863.

[13]. Ayar, A.; Sert, D.; Akin, N. The trace metal levels in milk and dairy products consumed in middle Anatolia – Turkey. Environ. Monit. Assess. **2009**, *152*, 1-12.

[14]. Capcarova, M.; Binkowski, L.J.; Stawarz, R.; Schwarczova, L.; Massanyi, P. Levels of essential and xenobiotic elements and their relationships in milk available on the Slovak market with the estimation of consumer exposure. Biol. Trace Elem. Res. **2019**, *188*(2), 404-411.

[15]. Uluozlu, O.D.; Tuzen, M.; Mendil, D.; Soylak, M. Assessment of trace element contents of chicken products from Turkey. J. Hazard Mater. **2009**, *163*, 982-987.

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

[17]. Arvay, J.; Tomas, J.; Hauptvogl, M.; Kopernicka, M.; Kovacik, A.; Bajcan, D.; Massanyi, P. Contamination of wild grown edible mushrooms by heavy metals in former mercury-mining area. J. Environ. Sci. Health part B **2014**, *49*(11), 815-827.

[18]. EFSA Scientific opinion on lead in food. EFSA panel on contaminants in the food chain. European Food Safety Authority, Parma. EFSA J. **2010**, *8*(4), 1570.

[19]. Gyawali, R.; Ibrahim, S.A. Impact of plant derivates on the growth of foodborne pathogens and the functionality of probiotics. Appl. Microbiol. Biotechnol. **2012**, *95*, 29-45.

[20]. Licata, P.; Di Bella, G.; Potorti, A.G.; Lo Turco, V.; Salvo, A.; Dugo, G.M. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. Food Addit. Contam. B **2012**, *5*(4), 268-271.

[21]. Krajcovicova, V.; Omelka, R.; Durisova, J.; Martiniakova, M.; Galbavy, D.; Bauerova, M. The effect of A163G polymorphism in the osteoprotegerin gene on osteoporosis related traits in Slovak postmenopausal women. Anthropol. Anz. **2015**, *72*(3), 311-319.

[22]. Mondockova, V.; Adamkovicova, M.; Lukacova, M.; Grosskopf, B.; Galbavy, D.; Martiniakova, M.; Omelka, R. The estrogen receptor 1 gene affects bone mineral density and osteoporosis treatment efficiency in Slovak postmenopausal women. BMC Med. Genet. **2018**, *19*, article number 174, DOI: 10.1186/s12881-018-0684-8.

[23]. Southage, D.A.T. Milk, milk products, fat and oils. In *Human nutrition and dietetics*; Garrow, J.S.; James, W.P.T.; Ralph, A. 10 th ed. London: Churchill Livingstone, UK, 2000, 375-383.

[24]. Swarup, D.; Patra, R.C.; Naresh, R.; Kumar, P.; Shekhar, P. Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Sci. Total Environ. **2005**, *347*(1-3), 106-110.

[25]. Weaver, C.M. Milk consumption and bone health. JAMA Pediatr. **2014**, *168*(1), 12-13.

[26]. Wlodarek, D.; Glabska, D.; Lange, E. The effect of dairy products choice on calcium dietary intake in female university students of nutritional faculty. Rocz. Panstw. Zakl. Hig. **2014**, *65*(1), 35-39.

[27]. Uenishi, K.; Ishida, H.; Nakamura, K. Development of a simple food frequency questionnaire to estimate intakes of calcium and other nutrients for the prevention and management of osteoporosis. J. Nutr. Sci. Vitaminol. **2008**, *54*, 25-29.

[28]. Heaney, R.P. Calcium, dairy products and osteoporosis. J. Am. Coll. Nutr. **2000**, *19*, 83-99.

[29]. Tunegova, M.; Toman, R.; Tančin, V. Heavy metals-environmental contaminants and their occurrence in different types of milk. Slovak J. Anim. Sci. **2016**, *49*(3), 122-131.

[30]. Morais, S.; Garcia e Costa, F.; de Lourdes Pereira, M. Heavy metals and human health. In *Environmental health – emerging issues and practice*; Jacques Oosthuizen, IntechOpen, DOI: 10.5772/29869. https://www.intechopen.com/books/environmental-health-emerging-issues-and-practice/heavy-metals-and-human-health, 2012; 227-246.

[31]. Soylak, M.; Turkoglu, O. Trace metal accumulation caused by traffic in agricultural soil near a motorway in Kayseri-Turkey. J. Trace Microprobe Tech. **1999**, *17*, 209-217.

[32]. Soylak, M.; Cihan, Z.; Yilmaz, E. Heavy metal contents of organically produced, harvested, and dried fruit samples from Kayseri, Turkey. Environ. Monit. Assess. **2013**, *185*, 2577-2583.

[33]. Binkowski, L.J.; Sawicka-Kapusta, K. Lead poisoning and its *in vivo* biomarkers in Mallard and Coot from two hunting activity areas in Poland. Chemosphere **2015**, *127*, 101-108.

[34]. De Vries, W.; Römkens, P.F.; Schütze, G. Critical soil concentrations of cadmium, lead, and mercury in view of helath effects on humans and animals. Rev. Environ. Contam. Toxicol. **2007**, *191*, 91-130.

[35]. Backhaus, T.; Faust, M. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. **2012**, *46*, 2564-2573.

[36]. Proskura, N.; Podlasinkska, J.; Proskura, W.S.; Frost-Rutkowska, A.; Dybus, A.; Szydlowski, K. Concentration of macroelements and trace elements in milk of Jersey cows. Indian J. Anim. Res. B **2016**, *369*, 1-4.

[37]. Rodriguez Rodriguez, E.M.; Sanz Alaejos, M.; Diaz Romero, C. Chemometric studies of several minerals in milks. J. Agric. Food Chem. **1999**, *47*(4), 1520-1524.

[38]. Pilarczyk, R.; Wojcik, J.; Czerniak, P.; Sablik, P.; Pilarczyk, B.; Tomza-Marciniak, A. Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environ. Monit. Assess. **2013**, *185*(10), 8383-8392.

[39]. Stawarz, R.; Formicki, G.; Massanyi, P. Daily fluctuation and distribution of xenobiotics, nutritional and biogenic elements in human milk in Southern Poland. J. Environ. Sci. Health part A **2007**, *42*(8), 1169-1175.

[40]. Cai, Q.; Long. M.L.; Zhu, M.; Zhou, Q.Z.; Zhang, L.; Liu, J. Food chain transfer of cadmium and lead to cattle in a lead-zinc smelter in Giuzhou, China. Environ. Pollut. **2009**, *157*(11), 3078-3082.

[41]. Isaak, C.P.; Sivakumar, A.; Kumar, C.R. Lead levels in breast milk, blood plasma and intelligence quotient: a health hazard for women and infants. Bull. Environ. Contam. Toxicol. **2012**, *88*(2), 145-149.

[42]. Perrone, L.; Di Palma, L.; Di Toro, R.; Gialanella, G.; Moro, R. Interaction of trace elements in a longitudinal study of human milk from full-term and preterm mothers. Biol. Trace Elem. Res. **1994**, *41*(3), 321-330.

[43]. Wang, Y.; Ou, Y.L.; Liu, Y.Q.; Xie, Q.; Liu, Q.F.; Wu, Q.; Fan, T.Q.; Yan, L.L.; Wang, J.Y. Correlations of trace element levels in the diet, blood, urine, and feces in the Chinese male. Biological Trace Element Research **2012**, *145*(2), 127-135.

[44]. Bilandzic, N.; Sedak, M.; Calopek, B.; Luburic, D.B.; Kolanovic, B.S.; Varenina, I.; Dokic, M.; Kmetic, I.; Murati, T. Lead concentrations in raw cow and goat milk collected in rural areas of Croatia from 2010 to 2014. Bull. Environ. Contam. Toxicol. **2016**, *96*, 645-649.

[45]. Starska, K.; Wojciechowska-Mazurek, M.; Mania, M.; Brulinska-Ostrowska, E.; Biernat, U.; Karlowski, K. Noxious elements in milk and milk products in Poland. Polish J. Environ. Stud. **2011**, *20*(4), 1043-1051.

[46]. JECFA Safety evaluation of certain food additive and contaminants. Prepared by the Seventy-third meeting of the Joint FAO/WHO Expert Committee on Food

Additives. Cadmium. Mercury. World Health Organization (WHO), 2011, Geneva, pp. 305-684.

[47]. Gasparik, J.; Binkowski, .LJ.; Jahnatek, A.; Smehyl, P.; Dobias, M.; Lukac, N.; Blasczyk, M.; Semla, M.; Massanyi, P. Levels of metal in kidney, liver, and muscle tissue and their influence on the fitness for the consumption of wild boar from western Slovakia. Biol. Trace. Elem. Res. **2017**, *177*(2), 258-266.

[48]. Semla, M.; Schwarz, P; Mezey, J.; Binkowski, L.J.; Blaszczyk, M.; Formicki, G.; Gren, A.; Stawarz, R.; Massanyi, P. Biogenic and risk elements in wines from the Slovak market with the estimation of consumer exposure. Biol. Trace Elem. Res. **2018**, *184*, 33-41.

[49]. Shahbazi, Y.; Ahmadi, F.; Fakhari, F. Voltametric determination of Pb, Cd, Zn, Cu and Se in milk and dairy products collected from Iran: An emphasis on permissible limits and risk assessment of exposure to heavy metals. Food Chem. **2016**, *192*, 1060-1067.

Table 1. Parameters of the analytical procedures. Limits calculated for the analytical solution. Recovery and RSD calculated for quality check solutions of known concentrations

Element	Wavelength (nm)	LoD (mg/L)	LoQ (mg/L)	Recovery (%)	RSD (%)
Ca	422.7	0.0642	0.1237	108.2	6.6
Co	240.7	0.0150	0.0300	95.2	3.1
Cr	359.7	0.0300	0.0500	92.2	2.4
Cd	228.8	0.0621	0.1200	91.6	4.3
Cu	324.8	0.0876	0.0911	99.6	2.7
Fe	248.3	0.2980	0.4705	101.6	6.2
Hg*	253.7	0.02*	0.04*	100.0	2.6
K	766.5	0.1788	0.2823	92.2	1.7
Mg	285.2	0.0089	0.0141	90.3	5.5
Mn	279.5	0.0250	0.0300	102.4	4.8
Na	589.0	0.0179	0.0282	94.1	6.9
Ni	232.0	0.3576	0.5646	104.9	4.1
Pb	217.0	0.0894	0.1411	93.7	6.2
Zn	213.9	0.0870	0.1740	106.2	5.3

LoD – limit of detection, LoQ – limit of quantification,
*LoD and LoQ values for Hg expressed as nanograms per sample.

Table 2. Mean concentrations (μg/g) followed by standard deviation (SD) of trace elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Zn) in the cottage cheese studied

element	Cottage cheese						
	white	lacto-free	chive	tzatziki	mustard+onion	chilli	active protein
Ca	1189.97±28.98	1206.43±50.26	1371.67±81.93	1152.33±33.04	1375.62±90.62	1177.62±25.92	1310.50±61.21
Co	0.03±0.01[a]	0.05±0.02	0.10±0.01[b]	0.05±0.01	0.12±0.02[b]	0.11±0.01[b]	0.03±0.01
Cr	0.17±0.04	0.13±0.04	0.08±0.01	0.12±0.03	0.10±0.01	0.08±0.00	0.08±0.02
Cu	0.11±0.03	0.11±0.04	0.08±0.03	0.22±0.03	0.15±0.03	0.08±0.02	0.09±0.02
Fe	1.75±0.34	1.54±0.90	1.21±0.34	2.67±0.59	2.61±0.65	1.16±0.14	1.25±0.54
K	540.57±.86.00[a]	577.72±33.96	622.15±43.72	747.25±28.64[b]	693.98±46.25[b]	637.68±34.60	631.43±11.87
Mg	62.47±1.13[b]	57.68±1.43[b]	64.85±2.15[b]	68.58±1.26[b]	84.42±3.52[a]	66.38±1.77[b]	66.75±2.34[b]
Mn	0.68±0.37	0.16±0.11	0.15±0.03	0.31±0.06	0.41±0.04	0.25±0.01	0.08±0.02
Na	2038.13±60.60[a]	2144.32±34.46	2329.93±25.69[b]	2419.95±45.38[b]	2473.02±72.97[b]	2285.02±51.53[b]	2065.57±30.89
Ni	0.09±0.02[a]	0.13±0.04	0.19±0.04	0.20±0.03	0.27±0.04[b]	0.26±0.04[b]	0.20±0.03
P	1099.02±55.09[a]	969.35±33.11	905.97±24.72[b]	898.03±20.59[b]	966.25±36.92	916.98±38.18[b]	1026.52±24.44
Zn	1.80±0.08	2.20±0.16	1.86±0.15	2.33±0.18	2.08±0.19	2.08±0.21	2.28±0.13

[a,b] different letters in rows indicate statistically significant differences.

Table 3. Mean concentrations (μg/g) followed by standard deviation (SD) of xenobiotic trace elements (Cd, Pb and Hg) in the cottage cheese studied

element	Cottage cheese						
	white	lacto-free	chive	tzatziki	mustard+onion	chilli	active protein
Cd	< LoD	0.014±0.002	0.013±0.002	0.008±0.002[b]	0.017±0.001[a]	0.013±0.001	0.010±0.001[b]
Hg	0.0007±0.0001	0.0006±0.0004	0.0002±0.0001	0.0005±0.0001	0.0004±0.0002	0.0008±0.0003	0.0027±0.0017
Pb	0.01±0.002	0.07±0.002	0.05±0.004	0.01±0.002	0.06±0.001	0.04±0.001	0.03±0.001

[a,b] different letters in rows indicate statistically significant differences.

Table 4. Correlations among concentrations of different elements in white cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	0.60	0.20	0.25	0.03	-0.57	-0.23	0.25	0.25	-0.13	0.25	-0.09	-0.10	0.32	**0.68**
Zn		1	0.12	0.14	0.34	0.04	-0.23	0.62	0.62	-0.07	0.64	-0.61	-0.52	0.08	0.02
Mn			1	0.14	**0.85**	0.29	0.36	0.32	0.32	**0.91**	-0.36	0.19	**0.74**	0.72	-0.24
Fe				1	**0.86**	0.28	0.39	0.30	0.30	**0.87**	-0.33	0.19	**0.74**	0.71	-0.22
Cr					1	0.66	0.57	0.41	0.41	0.74	-0.13	-0.18	0.50	0.52	-0.60
Ni						1	0.60	-0.03	-0.03	0.32	0.10	-0.16	0.14	-0.17	**-0.98**
Co							1	-0.14	-0.14	0.20	-0.45	-0.13	0.59	0.26	-0.48
Pb								1	0.36	0.36	-0.07	-0.72	-0.10	0.63	0.02
Cd									1	0.36	-0.07	-0.72	-0.10	0.63	0.02
Hg										1	-0.44	0.24	**0.71**	0.65	-0.35
K											1	-0.14	**-0.81**	**-0.68**	-0.11
Na												1	0.43	-0.18	0.12
Ca													1	0.65	-0.10
Mg														1	0.22
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 5. Correlations among concentrations of different elements in lacto-free cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	**0.70**	**0.70**	**0.72**	**0.89**	-0.19	-0.35	**-0.88**	-0.41	0.52	0.43	**-0.80**	-0.48	-0.41	0.42
Zn		1	0.46	0.47	0.36	0.44	0.36	**-0.92**	0.35	0.28	**0.84**	**-0.95**	0.10	0.03	0.02
Mn			1	0.47	**0.77**	-0.02	-0.46	-0.46	-0.37	-0.18	0.11	-0.49	**-0.71**	-0.59	0.48
Fe				1	**0.79**	-0.06	-0.47	-0.48	-0.39	-0.12	0.12	-0.50	**-0.72**	-0.59	0.46
Cr					1	-0.45	**-0.72**	-0.59	**-0.69**	0.37	0.14	-0.46	**-0.67**	-0.48	0.41
Ni						1	0.67	-0.17	**0.81**	-0.55	0.40	-0.36	0.45	0.16	-0.08
Co							1	-0.09	**0.90**	-0.07	0.45	-0.24	**0.71**	0.49	-0.39
Pb								1	-0.03	-0.53	**-0.72**	**-0.96**	0.08	0.13	-0.21
Cd									1	-0.30	0.56	-0.16	**0.82**	0.65	-0.58
Hg										1	0.34	-0.34	0.06	0.15	-0.07
K											1	**-0.68**	0.54	0.55	-0.49
Na												1	0.09	0.21	-0.29
Ca													1	**0.91**	**-0.81**
Mg														1	**-0.97**
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 6. Correlations among concentrations of different elements in chive cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	**0.95**	-0.49	-0.62	**-0.81**	**0.88**	**0.93**	-0.40	**-0.91**	**-0.67**	**0.91**	**0.72**	**0.83**	0.56	-0.46
Zn		1	-0.41	-0.43	-0.61	**0.71**	**0.78**	-0.51	**-0.88**	**-0.77**	**0.84**	**0.86**	**0.88**	0.64	-0.24
Mn			1	**0.83**	0.35	-0.64	-0.58	0.21	0.52	0.35	**-0.69**	-0.20	**-0.72**	-0.46	**0.76**
Fe				1	0.55	**-0.81**	**-0.70**	-0.16	0.49	0.07	**-0.81**	-0.25	**-0.67**	-0.55	**0.96**
Cr					1	**-0.82**	**-0.94**	0.30	**0.80**	0.50	**-0.70**	-0.26	-0.40	-0.40	0.44
Ni						1	**0.94**	-0.27	**-0.70**	-0.37	**0.81**	0.34	**0.71**	0.51	**-0.75**
Co							1	-0.40	**-0.88**	-0.60	**0.85**	0.43	**0.68**	0.34	-0.57
Pb								1	0.52	**0.82**	-0.15	-0.21	-0.34	0.01	-0.27
Cd									1	**0.86**	**-0.86**	**-0.68**	**-0.73**	-0.29	0.26
Hg										1	-0.54	-0.61	-0.58	-0.13	-0.16
K											1	**0.72**	**0.88**	0.63	-0.64
Na												1	**0.78**	**0.67**	-0.04
Ca													1	**0.84**	-0.53
Mg														1	-0.51
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 7. Correlations among concentrations of different elements in tzatziki cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	**0.90**	0.37	0.47	0.00	0.34	**-0.89**	**0.99**	**-0.82**	-0.27	**0.97**	0.58	**-0.84**	0.44	-0.36
Zn		1	-0.05	0.08	-0.26	0.61	**-0.79**	**0.94**	-0.56	-0.61	**0.92**	**0.83**	**-0.82**	0.34	-0.33
Mn			1	**0.97**	0.61	-0.56	-0.45	0.28	**-0.76**	0.63	0.19	-0.52	-0.20	0.12	-0.03
Fe				1	**0.68**	-0.57	-0.61	0.39	**-0.85**	0.45	0.28	-0.44	-0.31	0.19	**0.80**
Cr					1	**-0.92**	-0.25	-0.07	-0.35	0.15	-0.15	-0.63	0.23	0.41	**0.75**
Ni						1	-0.08	0.41	0.10	-0.39	0.49	**0.87**	-0.50	-0.17	**-0.73**
Co							1	**-0.90**	**0.91**	0.31	**-0.80**	-0.35	**0.85**	-0.34	0.06
Pb								1	**-0.79**	-0.36	**0.95**	0.63	**-0.86**	0.35	-0.36
Cd									1	-0.12	**-0.69**	-0.07	**0.76**	-0.26	0.19
Hg										1	-0.33	**-0.67**	0.25	-0.24	-0.31
K											1	**0.73**	**-0.84**	0.52	-0.44
Na												1	-0.60	0.28	-0.45
Ca													1	-0.15	0.47
Mg														1	0.25
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 8. Correlations among concentrations of different elements in mustard+onion cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	**0.89**	0.22	-0.22	-0.54	**0.67**	0.16	**0.93**	**-0.96**	-0.28	**0.97**	0.38	**-0.70**	0.63	-0.45
Zn		1	0.21	-0.04	-0.49	**0.71**	0.46	**0.78**	**-0.87**	-0.09	**0.84**	0.31	-0.59	0.57	-0.14
Mn			1	**0.78**	0.13	0.05	-0.55	-0.11	-0.01	0.26	0.13	-0.50	**-0.79**	0.59	0.48
Fe				1	0.38	-0.26	-0.39	-0.47	0.30	0.20	-0.26	-0.38	-0.33	0.09	**0.91**
Cr					1	**-0.92**	-0.52	-0.45	0.47	0.55	-0.35	-0.49	0.04	0.04	0.38
Ni						1	0.59	0.51	-0.56	-0.26	0.48	0.29	-0.27	0.26	-0.30
Co							1	0.22	-0.25	-0.05	0.10	0.37	0.40	-0.22	-0.10
Pb								1	**-0.98**	-0.37	**0.96**	0.57	-0.47	0.45	-0.62
Cd									1	0.38	**-0.98**	-0.57	0.54	-0.47	0.45
Hg										1	-0.23	**-0.89**	-0.18	0.50	0.19
K											1	0.40	**-0.67**	0.63	-0.48
Na												1	0.26	-0.45	-0.26
Ca													1	**-0.89**	0.05
Mg														1	-0.22
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 9. Correlations among concentrations of different elements in chilli cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	**0.98**	0.52	-0.37	**-0.91**	0.35	0.36	**0.92**	-0.13	**-0.79**	**0.88**	-0.65	-0.26	**0.70**	-0.17
Zn		1	0.51	-0.55	**-0.84**	0.46	0.43	**0.92**	-0.15	**-0.87**	**0.78**	-0.58	-0.27	0.61	-0.30
Mn			1	0.04	-0.33	-0.38	-0.23	0.39	**0.75**	-0.36	0.29	**-0.78**	**0.67**	**0.87**	0.17
Fe				1	0.16	**-0.76**	-0.64	-0.48	0.46	**0.80**	-0.17	-0.09	0.48	0.20	0.59
Cr					1	-0.21	-0.13	**-0.92**	0.21	0.61	**-0.91**	0.45	0.37	-0.66	-0.06
Ni						1	**0.85**	0.33	**-0.78**	-0.50	0.19	0.16	**-0.74**	-0.40	**-0.85**
Co							1	0.19	**-0.69**	-0.54	0.31	-0.21	-0.62	-0.24	**-0.70**
Pb								1	-0.19	**-0.82**	**0.82**	-0.37	-0.36	0.58	-0.06
Cd									1	0.27	-0.29	-0.36	**0.98**	0.54	0.42
Hg										1	**-0.67**	0.42	0.37	-0.39	0.25
K											1	-0.61	-0.43	0.65	0.16
Na												1	-0.31	**-0.83**	-0.11
Ca													1	0.40	0.31
Mg														1	0.39
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 10. Correlations among concentrations of different elements in active proteins cottage cheese from market in Nitra (Slovak Republic)

	Cu	Zn	Mn	Fe	Cr	Ni	Co	Pb	Cd	Hg	K	Na	Ca	Mg	P
Cu	1	-0.02	-0.08	**-0.70**	-0.56	-0.05	**-0.95**	0.24	0.06	-0.24	**0.72**	-0.17	-0.50	-0.61	-0.04
Zn		1	0.17	0.23	0.14	0.40	0.11	0.18	-0.30	0.65	-0.14	0.47	0.64	0.60	**0.86**
Mn			1	**0.88**	-0.59	-0.12	0.00	**0.75**	0.58	-0.45	0.52	-0.64	-0.38	-0.25	-0.23
Fe				1	-0.33	-0.22	0.12	0.49	0.52	-0.42	0.34	-0.33	-0.16	-0.19	0.00
Cr					1	0.35	**0.73**	**-0.89**	-0.25	0.66	**-0.97**	**0.85**	**0.82**	0.57	0.44
Ni						1	0.09	-0.22	0.31	**0.76**	-0.29	0.32	0.31	0.06	0.20
Co							1	-0.44	-0.07	0.32	**-0.83**	0.39	0.65	0.63	0.20
Pb								1	0.16	-0.42	**0.79**	**-0.76**	-0.54	-0.21	-0.22
Cd									1	-0.33	0.32	-0.50	-0.52	**-0.72**	-0.59
Hg										1	-0.65	**0.76**	**0.80**	0.63	**0.68**
K											1	**-0.76**	**-0.83**	**-0.69**	-0.41
Na												1	**0.89**	0.62	**0.80**
Ca													1	**0.88**	**0.84**
Mg														1	**0.73**
P															1

0-0.33 – weak correlation, 0.34-0.66 – medium correlation, **0.67 – 1 – strong correlation**

Table 11. Estimated daily intake (EDI) of elements studied with the consumption of the cottage cheese available on Slovak market (calculated for 70 kg person)

element	EDI (µg/kg bw/day)						
	White	Lacto-free	Chive	Tzatziki	Mustard onion	Chilli	Active protein
Ca	1189.97	1206.43	1371.97	1152.33	1375.62	1177.62	1310.50
Co	0.00007	0.00011	0.00023	0.00011	0.00027	0.00025	0.00007
Cr	0.00039	0.00030	0.00018	0.00027	0.00023	0.00018	0.00018
Cu	0.00025	0.00025	0.00018	0.00050	0.00034	0.00018	0.00021
Fe	0.004	0.004	0.003	0.006	0.006	0.003	0.003
K	1.236	1.321	1.422	1.708	1.586	1.458	1.443
Mg	0.143	0.132	0.148	0.157	0.193	0.152	0.153
Mn	0.0016	0.0004	0.0003	0.0007	0.0009	0.0006	0.0002
Na	4.659	4.901	5.326	5.531	5.653	5.223	4.721
Ni	0.0002	0.0003	0.0004	0.0005	0.0006	0.0006	0.0005
P	2.512	2.216	2.071	2.053	2.209	2.096	2.346
Zn	0.004	0.005	0.004	0.005	0.005	0.005	0.005

EDI values calculated on the basis of 160 g consumption per person (70 kg) per day

Table 12. Contribution to Provisional Tolerable Weekly Intake (PTWI) of elements studied in the cottage cheese available on Slovak market (calculated for 70 kg person)

Element	PTWI (µg/kg bw/week)	Contribution to PTWI (%)						
		White	Lacto-free	Chive	Tzatziki	Mustard onion	Chilli	Active protein
Cd	7 [18]	0.0001	0.0032	0.0029	0.0015	0.0032	0.0024	0.0019
Hg	4 [18]	0.0003	0.0002	0.00001	0.0002	0.0001	0.0003	0.0009
Pb	25 [49]	0.0006	0.0045	0.0032	0.0005	0.0031	0.0021	0.0016
Cu	3500 [18]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Ni	35 [18]	0.0041	0.0059	0.00867	0.0091	0.0123	0.0119	0.0091
Zn	7000 [18]	0.0004	0.0005	0.0004	0.0005	0.0005	0.0005	0.0005

Table 13. Estimated MOEs for different endpoints by the intensity of cottage consumption

Cottage origin	Endpoint	MOE	
		Normal total daily exposure	Higher total daily exposure
White	Cardiovascular effect	1.2 – 4.2	1.2 – 4.1
	Nephrotoxicity	0.5 – 1.8	0.5 – 1.8
Lacto-free	Cardiovascular effect	1.2 – 3.8	1.1 – 3.8
	Nephrotoxicity	0.5 – 1.6	0.5 – 1.5
Chive	Cardiovascular effect	1.2 – 3.8	1.2 – 3.7
	Nephrotoxicity	0.5 – 1.6	0.5 – 1.5
Tzatziki	Cardiovascular effect	1.2 – 4.0	1.2 – 3.9
	Nephrotoxicity	0.5 – 1.7	0.5 – 1.6
Mustard+onion	Cardiovascular effect	1.2 – 3.8	1.2 – 3.6
	Nephrotoxicity	0.5 – 1.6	0.5 – 1.5
Chilli	Cardiovascular effect	1.2 – 3.8	1.2 – 3.7
	Nephrotoxicity	0.5 – 1.5	0.5 – 1.6
Active proteins	Cardiovascular effect	1.2 – 3.9	1.2 – 3.8
	Nephrotoxicity	0.5 – 1.6	0.5 – 1.6

MOE values calculated for normal consumption (160 g/per capita/per day) and higher consumption (220 g/per capita/per day)