

ASSESSMENT OF THE OCCURRENCE AND RISK ESTIMATION OF INTAKE OF SELECTED CHEMICAL ELEMENTS IN SLOVAK SHEEP'S CHEESE – BRYNDZA ON ORGANIC FARMS FROM DIFFERENT REGIONS OF SLOVAKIA

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Abstract

This study aimed to assess the concentrations of selected chemical elements in the traditional Slovak sheep product - bryndza, from farms of different regions of Slovakia with organic animal farming systems, and with the assessment of the intake of these elements through the consumption of this product. Slovak bryndza is a natural white, finely spreadable ripened cheese with occasional grains, made in the traditional way - by grinding ripened sheep's lump cheese or a mixture of sheep's and cow's lump cheese. Six chemical elements (Ca, Zn, Mg, Al, Ni, Cd) were monitored in sheep's cheese – bryndza samples (32 samples in total) and determined by the ICP-OES. Comparing the concentration of element between farms, statistically significant differences ($P < 0.05$) were found in the case of Ca, Zn, Mg, Al, with all concentrations of these elements being higher on organic farm from area with undisturbed environment. When evaluating the intake of essential elements, were found that if the categories of Slovak peoples with the highest need for selected essential elements consumed 100 g of bryndza cheese per day, they would meet their Ca intake 33.52%, Zn intake 12.6% and Mg intake 7.23 % through bryndza cheese from an organic farm in a region with an undisturbed environment. When estimating the intake risk of toxic elements, we found that Al and Ni concentrations were significantly higher on a farm from a region with an undisturbed environment compared to farm with a heavily disturbed environment. Consuming 100 g of bryndza cheese per day would represent 7.36% PTWI (permissible tolerably weekly intake) for Al and 8.13% TDI (tolerable daily intake) for Ni for peoples of Slovakia, which, however, means a very low intake. From the results of the work, we can conclude that the consumption of bryndza from the monitored farms is unlikely to pose a health risk to consumers, especially based on the assessment of exposure to toxic elements, and on the contrary, it can be an important part of the diet of the population of Slovakia due to its health benefits which are high in calcium and magnesium, which is also confirmed by the results of this work

Keywords: sheep cheese, essential elements, toxic elements, bryndza, organic farms

1. INTRODUCTION

Sheep milk is recognized for its rich nutritional profile, which includes both essential and potentially toxic elements. The essential minerals found in sheep milk, such as calcium (Ca), magnesium (Mg), and phosphorus (P), are vital contributors to human health. Research indicates that sheep milk contains significantly higher levels of these essential elements compared to cow milk, rendering it an excellent source for dietary intake (Şensoy & Çiftçi, 2022; Burrow et al., 2018; Balthazar et al., 2017). Specifically, the bioavailability of minerals in sheep milk is enhanced by its protein structure, particularly casein, which aids in mineral absorption during digestion (Şensoy & Çiftçi, 2022; Balthazar et al., 2017). The mineral composition of sheep milk is influenced not only by the ruminant's genetics but also by its feeding regime, environmental conditions, and health status (Lawal et al., 2023; Flis & Molik, 2021). Various studies have reported increased concentrations of essential minerals, such as zinc (Zn) and manganese (Mn), in sheep milk compared to cow milk (Burrow et al., 2018; Flis & Molik, 2021). For instance, sheep milk offers approximately double the fat and protein content compared to

cow milk, thus providing a denser nutrient profile beneficial for both lactating ewes and consumers (Hamad & Baiomy, 2010). Research has demonstrated that differences in the grazing environment and feed impact the mineral concentration in sheep milk, emphasizing the importance of sustainable feeding practices (Pšenková et al., 2021; Tunegová et al., 2018). Conversely, the potential for contamination with toxic elements, such as heavy metals, poses significant risks to the safety of sheep milk products. Heavy metals like lead (Pb), cadmium (Cd), and arsenic (As) can accumulate in sheep milk due to environmental exposure from grazing on contaminated lands or consumption of polluted water and feed (Pšenková et al., 2021). These studies emphasize that trace elements in sheep milk—including both essential and non-essential minerals—require rigorous monitoring to ensure food safety (Kiliçaltun, 2020; Şanal et al., 2011). The impact of environmental factors on toxic element accumulation suggests that sheep dairy products should be tested for contaminants, especially in regions where industrial activity may influence soil and water quality (Pšenková et al., 2021). Recent advances in analytical techniques allow for better detection and quantification of both toxic and essential elements in sheep dairy products. Techniques such as inductively coupled plasma mass spectrometry (ICP-MS) have been employed to profile the mineral content, confirming the presence of essential minerals while highlighting the urgency of monitoring toxic elements to protect consumer health (Şanal et al., 2011). Significant variances have been documented in the levels of cadmium and lead in dairy products derived from sheep in different geographical locations, emphasizing that local environmental assessments are vital for the dairy industry (Tunegová et al., 2018). Slovak bryndza is a natural white, finely spreadable ripened cheese with rare grains, made in the traditional way - by grinding matured sheep's lump cheese or a mixture of sheep's and cow's lump cheese. A characteristic feature of the production of Slovak bryndza is the crushing and grinding of matured sheep's or a mixture of sheep's and cow's lump cheese and their mixing with salt or a specially prepared salt solution, which distinguishes this production from the production of other types of sheep's cheese produced outside the territory of Slovakia. Its characteristic sensory properties are given by the natural microflora contained in raw sheep's milk and sheep's lump cheese and the characteristic production method. The basic raw material to produce Slovak bryndza is sheep's lump cheese or a mixture of sheep's lump cheese and cow's lump cheese, or a mixture of barreled sheep's lump cheese and cow's lump cheese, matured under specific conditions (Council Regulation, n. 510/2006). Sheep dairy products are a good source of necessary nutrients but can pose risks from toxic elements. Its mineral profile supports comprehensive environmental assessments and consumer safety protocols. As the appreciation for sheep dairy products grows, so does the importance of understanding their mineral composition and ensuring the safety of these products amidst potential environmental hazards.

Therefore, this study was conducted to quantify the concentrations of essential and toxic elements in slovak sheep cheese- bryndza and to assess the suitability and safety of this product from organic farm from different part of Slovakia when including these products in the diet of children and consumers.

2. MATERIALS AND METHODS

2.1. Materials

Thirty-two samples of Slovak traditional sheep's cheese – bryndza were collected from 2 organic farms, which are situated in different part of Slovakia (Figure 1). Farm 1 is situated on the North of Slovakia. According to the Environmental Regionalization of Slovakia (Klinda et al., 2016), this part has character undisturbed environment. This farm is situated in a region that does not have specific industry that could burden the environment and therefore the character of this region is with an undisturbed environment. The second farm is situated in the southeast of Slovakia. This region has character slightly disturbed environment. The territory of the region in which the farm is located is specific to metal processing, and therefore this territory may show certain values of environmental pollution, on the basis of which the farm is characterized by slightly disturbed environment. Farmers on these farms strive to raise animals and produce feed without the use of synthetic chemicals, pesticides, and artificial fertilizers. They focus on sustainable practices such as crop rotation for soil health, gentle animal husbandry, and organic food production. All samples of bryndza (200 g per sample) were taken twice a month during the six months,

directly on the farms where this type of product was produced. After collecting, all samples were stored in plastic sample containers and stored in freezers at -18°C .

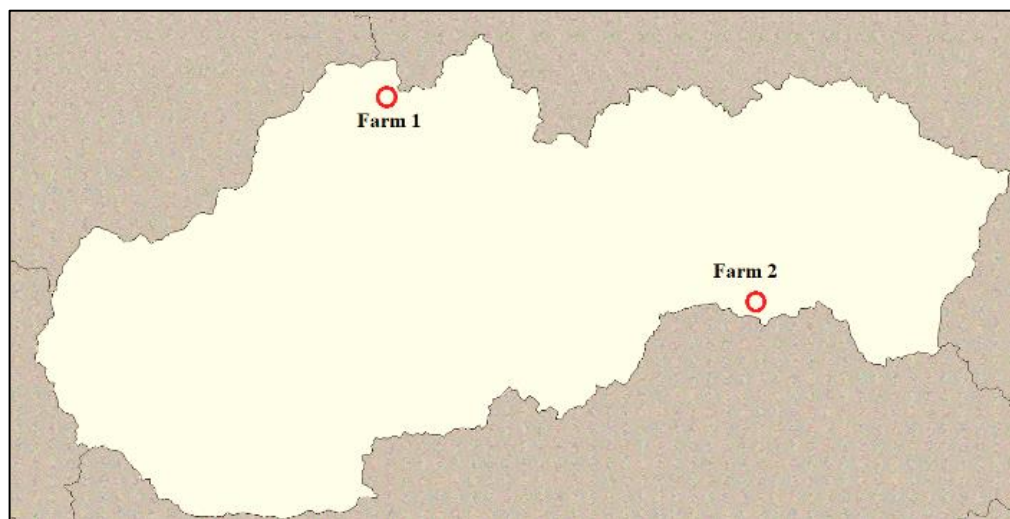


Fig. 1. Location of analyzed samples of Slovak bryndza cheese

2.2. Methods

In all samples of bryndza cheese were analyzed 6 chemical elements (essential element – Ca, Zn, Mg; toxic elements – Cd, Al, Ni). Samples (1 g) were weighed into Teflon containers, to which 8 ml $\geq 69\%$ nitric acid and 2 ml $\geq 30\%$ hydrogen peroxide were added. Each sample during the microwave dissolution process was supplemented with a blank sample containing 8 ml of $\geq 69\%$ nitric acid alone and 2 ml $\geq 30\%$ hydrogen peroxide. The digestion of the samples took place for 45 minutes with the applied temperature increase algorithm, as specified for biological samples, without exceeding 200°C and 1800 W. The microwave process was carried out using the Ethos One microwave digestion system, Milestone. After the completion of the mineralization process, the samples were cooled to room temperature and then filtered through quantitative filter paper and supplemented with ultrapure water ($18.2\text{ M}\Omega\text{ cm}^{-1}$, 25°C , Synergy UV, Merck Millipore) to a volume of 50 ml. The measurements were performed using ICP-OES spectrometer, Agilent, Series 700, axial type. Before the measurements, the method was calibrated using certified Merck and Agilent models, with concentrations of 10 000 ppm for selected microelements. The analytical methods were validated by two independent tests, using a certified reference material (NIST – 1515).

2.3. Statistical Analysis

Statistical analysis of the data was performed using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA). Differences in concentrations of the analyzed chemical elements between farms were compared by the ANOVA and Student's t-test. All data were expressed as mean and standard deviation. The probability level of $p < 0.05$ was considered statistically significant.

2.4. Risk Assessment Analysis

The concentrations of essential elements found in sheep products were compared with the recommended nutritional doses for the population of Slovakia (MZ SR, 2015), in the age categories with the highest need for the given element. For each essential element, the % share of dairy product consumption in their recommended intake was expressed.

For toxic elements, we calculated the value of the estimated daily intake (EDI) based on the average concentrations found in the dairy products samples.

The relationship was used for the calculation:

$$EDI = C_{\text{metal}} \times W_{\text{milk}} / BW$$

where C_{metal} is the concentration of the element in the analyzed sample, W_{cheese} is the daily cheese consumption and BW is the average body weight (70 kg). The average consumption of fresh cheese is 0.02 kg per capita in Slovakia, according to the Statistical Office (2022).

From the determined values, we calculated the Estimated Weekly Intake (EWI) for Al:

$$EWI = EDI \times 7_{(\text{days})}$$

Subsequently, we expressed the proportion (%) of intake of the toxic element on the preliminary tolerable weekly PTWI intake for Al established by JEFCA (2012):

$$\%PTWI = EWI / PTWI \times 100$$

For Ni for which the PTWI value is not determined, we will express the share (%) of element intake on the Tolerable Daily Intake (TDI) determined by WHO (2003, 2010) and EFSA (2020):

$$\%TDI = EDI / TDI \times 100$$

When assessing the intake of essential elements through bryndza, we focused on expressing the intake of an essential element in relation to the recommended nutritional intakes of the Slovak population, based on the age categories of the Slovak population who have the highest need for elements, the concentrations of which we monitored in sheep's bryndza samples. Subsequently, the concentrations found were calculated as a percentage of the recommended nutritional intake for the selected amount of sheep's bryndza.

3. RESULTS

In table 1 and table 2 are listed concentrations of six essential and toxic elements in sheep cheese – bryndza from farms, which are situated in different part of Slovakia with different environmental burden. From all analyzed chemical elements in sheep cheese were detected all elements, except Cd. Concentration of this element was detected below the LOQ limit. The concentrations of essential elements in sheep's cheese was determined in the order $Ca > Mg > Zn$ in both farms and in the case of toxic elements concentrations were determined in order $Al > Ni > Cd$ in both farms as well. In all cases the chemical elements were recorded statistically significant differences between farms ($P < 0.05$), except for cadmium, where concentrations of this element were not detected in the cheese samples. The highest concentrations of essential elements were detected on Farm 1, which is situated in area of Slovakia with undisturbed environment, in cases all essential elements. An interesting result that we noted in our work was that the concentrations of toxic elements were lower on Farm 2, which is in region of Slovakia with slightly disturbed environment, compare to Farm 1, which is in undisturbed area of North Slovakia. Even though both farms have an organic way of raising animals and managing, the higher concentrations of toxic elements in sheep's cheese, especially in a region with an undisturbed environment, could have been caused by some secondary source (e.g. technological processing).

Table 1. Concentration of essential elements in bryndza cheese

Elements	Ca		Zn		Mg	
Farm	Farm 1	Farm 2	Farm 1	Farm 2	Farm 1	Farm 2
Mean	5363.23*	3883.52	18.97*	13.00	303.72*	203.17
SD	1039.51	154.85	4.06	2.07	51.10	2.52

*Statistically significant difference between farms $P < 0.05$; SD – standard deviation

Table 2. Concentrations of toxic elements in bryndza cheese

Elements	Al		Ni		Cd	
Farm	Farm 1	Farm 2	Farm 1	Farm 2	Farm 1	Farm 2
Mean	14.69*	5.69	0.74*	0.21	ND	ND
SD	5.40	2.93	0.02	0.04	-	-

*Statistically significant difference between farms $P < 0.05$; SD- standard deviation

In table 3 it is noted the percentages expression of the intake of essential elements in relation to the recommended nutritional doses. The results are listed in 2 scenarios of consumption of sheep cheese and that in amount of consumption of 20 g of sheep cheese per day, which is the consumption of sheep cheese according to the consumption by Slovak population in 2023. When expressing the fulfilment of recommended nutritional doses through the consumption of sheep's bryndza cheese, in the second scenario we based our chosen amount of cheese or recommended amount of cheese consumption.

The highest percentage coverage of the daily Ca requirement in the case of consuming 20 g of sheep bryndza cheese was found on Farm 1, where consuming this amount would ensure the daily Ca requirement at the level 6,7 %, which is 2% more compared to farm 2. In cases of Zn and Mg, it was found that the daily need for these elements would also be ensured in higher coverage through cheese on farm 1, in the case of Zn at the level of 2,52% and Mg at the level of 1,47%. In both cases, this is approximately 1% higher daily requirement compared to farm 2. From table 3 it is clear if the consumption of sheep's cheese was at the level of the second scenario, i.e. the recommended amount of the daily need for selected essential elements would be ensured.

However, it should be noted that sheep bryndza cheese is a sheep product with excellent probiotic effects, but it contains a significant amount of salt, and therefore the consumption of this sheep product needs to be adjusted accordingly.

Table 3. The percentage expression of the intake of essential elements in relation to the recommended nutritional doses

Farm	Farm 1		Farm 2	
RND*	Consumption			
	20g	100 g	20 g	100 g
Ca (1600 mg)	6.70%	33.52%	4.85%	24.27%
Zn (15 mg)	2.52%	12.60%	1.73%	8.70%
Mg (420 mg)	1.47%	7.23%	0.97%	4.84%

*RND- recommended nutritional doses for different age categories with the highest need: Ca – category of breastfeeding women in 19-51 years, Zn – category of boys 15-18 years with increased physical activity, Mg – category of men 35-62 years with hard work

In table 4 are listed results of estimation of the risk intake of toxic elements in relation to toxicological limits. It is clear from the table that in case of both monitored toxic elements, based on their detected concentrations and calculations in relation to toxicological limits, the Slovak population would receive a higher amount of these toxic elements on farm 1 through sheep bryndza cheese. The highest share was recorded in the case of Ni in the second scenario, where the Slovak people would receive 8.13% TDI through sheep bryndza cheese and the lowest values was recorded in the case of Ni, where they received 0.46% TDI through bryndza when consuming 20g of bryndza on farm 2. The results of the risk analysis

of the consumption of this cheese show that the share of monitored toxic elements in PTWI and TDI, in the case of current consumption of this cheese by the population of Slovakia (scenario 1), was low and the values ranged at 1.63% and less. However, if consumers were to consume this cheese frequently and in larger quantities, despite its very good probiotic properties, this could pose a risk from the point of view of the intake of toxic elements, as the proportion of toxic elements in TDI and PTWI ranged from 2.31 - 7.36% (scenario 2), which is a higher proportion compared to scenario 1.

Table 4. Estimation of the risk of intake of toxic elements in relation to toxicological limits

Farm		Farm 1				Farm 2			
Toxicological limit		EDI	EWI	% TDI	% PTWI	EDI	EWI	% TDI	% PTWI
Consumption 20 g (scenario 1)	Al	4.2 E-03	2.94 E-02	-	1.47 %	1.62 E-03	1.13 E-02	-	0.57%
	Ni	2.1 E-04	-	1.63%	-	6.00 E-05	-	0.46%	-
Consumption 100 g (scenario 2)	Al	2.1 E-02	1.4 E-01	-	7,36 %	8.10 E-03	5.70E-02	-	2.85 %
	Ni	1.06 E-03	-	8.13%	-	3.00 E-04	-	2.31%	-

EDI- estimated daily intake; PTWI (Al) – 2 mg/kg (JECFA, 2012); TDI (Ni) – 13 µg/kg (EFSA, 2020)

4. DISCUSSION

Sheep milk products have garnered increasing attention in the dairy industry due to their unique compositional properties, nutritional benefits, and wide-ranging applications, particularly in cheesemaking. The nutritional profile of sheep milk is notably superior to that of cow's and goat's milk, with higher concentrations of proteins, fats, vitamins, and minerals, making it a highly nutritious option for food products (Barłowska et al., 2011). Sheep milk's rich nutrient content is attributed to its elevated levels of total solids, essential amino acids, calcium, and phosphorus, which significantly contribute to its value in various dairy applications (Hamad & Baiomy, 2010). In the presented study, 3 essential and 3 toxic elements were monitored in traditional Slovak sheep product – bryndza on two organic farms from different regions of Slovakia. The result of the work indicate that the highest concentrations of Ca, Zn and Mg were detected of farm 1, which is in the northern region of Slovakia, which has the character of an undisturbed environment. The highest concentrations were recorded in the case of Ca, where the Ca concentration was at 5363.23 mg/kg on farm 1 and 3883.52mg/kg on farm 2. If we compare these results with our previous study (Pšenková et al., 2022), we find that in commercially available hard cheese from the retail chain, the Ca concentration was found in the range of 5793.35 - 6225.816 mg/kg. Also in this study, we recorded a similar and lower Ca concentration in commercially available bryndza cheese from the retail chain, where the Ca concentration ranged from 3000.816 - 4108.503 mg/kg. Cheeses are concentrates of milk components (De Oliveira Filho et al., 2022), and it is known that the content of elements in cheeses or other products is influenced by biochemical processes during their production. The loss of water content during the different stages of cheese production is one of the reasons for the presumed higher concentration of trace elements compared to fresh cheeses or dairy products that contain more water (Elbaraby and Hamouda, 2013; Barone et al., 2018). As reported by Astolfi et al. (2020) and Vannucii et al. (2018), calcium is one of the most important elements in milk, which provides important functions in the animal and human body, and one of its most important functions is bone mineralization, therefore it is important to ensure sufficient Ca intake through food. If we evaluate the need for calcium in the age category with the highest need for Ca, which are breastfeeding women aged 19-51, we find that in the case of both farms, consuming 100g of bryndza cheese per day would significantly contribute to covering the daily need for Ca in this age category, at the level of 33.52% on farm 1 and 24.27% on farm 2. However, with the current real consumption for 2023, where the consumption of bryndza was at the level of 20g per day, the intake of this element is low compared to the recommended dietary allowance of 1600mg Ca (6,7% farm 1 and 4,85% farm2). Another element analyzed in sheep's cheese - bryndza was Zn. Zn is considered a heavy metal if it exceeds the values of 5 g/cm³ (Duffus, 2002). Nevertheless, zinc plays an important role in physiological

processes and the functional performance of the human immune system. It participates in the structure and activity of approximately 300 enzymes in the body. These enzymes are responsible for the replication and secretion of insulin and cell differentiation, synthesis of proteins and nucleic acids. On the other hand, if there is an excessive intake of Zn, its chronic exposure in high concentrations has negative effects on the human body, leading to anemia, leukopenia and can cause gastrointestinal diseases, e.g. diarrhea (Meshref et al., 2014; Vahčić et al., 2010). Similar to the case of Ca, higher concentration of Zn was recorded in sheep's cheese on farm 1 compared to farm 2, which is in a region with a slightly disturbed environment, and the difference in these concentrations was statistically significant, similar to Ca. Authors Manzi et al. (2021) reported in their study the Zn concentrations in traditional Italian cheeses. They found higher Zn concentrations in cow's milk cheeses (1.83 – 7.75 mg.100g⁻¹) compared to sheep's milk cheeses.

In samples of sheep cheese – bryndza was analyzed Mg as well. Astolfi et al. (2020) report that milk and milk products are the primary source of Ca, Mg, K, Na. For example, the role of Mg in muscles is to control ATP content and its utilization for contraction and reticular Ca²⁺ uptake and release (Romani, 2013). In soft tissues, Mg acts as a cofactor for many enzymes involved in energy metabolism, protein synthesis, and RNA and DNA synthesis. It also plays a major role in maintaining the electrical potential of nervous tissue and cell membranes (Cowan, 2002). In our work we recorded higher Mg concentrations on farm 1, i.e. in a region with an undisturbed environment. The recommended nutritional dose of Mg in Slovakia, for the category with the highest Mg requirement, is 420 mg, for hard-working men aged 35-62. When consuming sheep's cheese, which we analyzed, the current consumption (20 g) of the Slovak population will receive approximately 1.47% and 0.97% from farms 1 and 2. If they consumed 100 grams of this cheese every day, the cheese from farm 1 would represent a higher percentage of fulfilling the OVD, at the level of 7.23% compared to farm 2.

The regulation of chemical elements is not balanced and for some known toxic elements there are no limits set, such as PTWI. In the case of cadmium, the original PTWI value was abolished in 2010 because it did not meet the requirements of consumer protection in the long term and was replaced by the PTMI (preliminary tolerable monthly intake) value (JECFA, 2010). However, in our work, we found concentrations of cadmium in sheep's cheese samples below the detection limit and therefore we could not determine an intake risk estimate for this element. In general, however, milk and dairy products contain low levels of cadmium. However, if Cd is present in these products, it is a consequence of the animals being fed contaminated feed or the drinking water for the animals containing this element. Acute Cd toxicity in humans mainly leads to damage to the skeletal and cardiovascular systems (Elboudy et al., 2016; Özcan a Juhaimi, 2012).

The results of the risk analyses of human consumption of these products show that the proportion of most elements in PTWI and TDI is low, and in the case of both elements Al and Ni, we recorded values below 1%, which is low exposure on both farms. Milk and dairy products can be a source of toxic elements, especially in areas with industrial contamination. In our analysis, we found the presence of Al and Ni out of the three monitored elements, and an interesting result was that we recorded higher concentrations on the first farm, which is in an undisturbed environment, compared to farm 2 in a slightly disturbed environment, where the concentrations of these elements were significantly lower. In the case of Al, the concentration was at the level of 14.69 and 5.69 mg/kg on farm 1 and farm 2. Significantly lower concentrations compared to our results were recorded by Danezis et al. (2019) in the analysis of Greek cheeses, in which the Al concentration ranged from 0.-1.8 mg/kg. However, Meshref et al. (2015) found an average Al concentration in milk of up to 19.92 mg/kg and in cheeses at a concentration of 52.36 mg/kg. Dietary intake of Al has been associated with the risk of anemia and neurological syndrome known as dialysis encephalopathy, and the development of serious brain disorders such as Alzheimer's disease.

Significantly lower Ni concentrations compared to our results in Slovak and Czech producers of cow's milk compared to our sheep's cheese are reported by Capcárová et al. (2019), who found Ni concentrations at the level of 0.84 - 1.01 µg/l. On the other hand, higher Ni concentrations, compared to the presented results of Ni concentrations, were recorded in our previous study, where we found Ni

concentrations in cheeses from the retail chain at the level of 0.356 mg/kg and 0.347 mg/kg in Eidam cheese and in parenica at the level of 0.123 and 0.477 mg/kg (Pšenková et al., 2022).

In the case of both of farms, we recorded an excess compared to the value declared by the regulation of the Ministry of Health of the Slovak Republic, where the highest permissible limit of Al in milk and dairy products is 1 mg/kg. Similarly, in the case of Ni, we recorded an exceedance of the maximum permissible limit (0.1 mg/kg), but the concentrations were only slightly exceeded (0.74 mg/kg of Ni farm 1; 0.21 mg/kg of Ni farm 2). If there is long-term exposure to nickel, high concentrations would lead to its neurotoxicity in the body as well as damage to the male reproductive system and oxidative stress (Beckett et al., 2007; Das et al., 2008). Therefore, it will be necessary to monitor the long-term development of these concentrations and possibly identify the sources of these elements.

5. CONCLUSIONS

From the results of the presented work, we can conclude that sheep's cheese - bryndza from the monitored farms - is unlikely to pose a health risk to consumers, especially based on the assessment of exposure to the toxic elements Al and Ni, which were found in the work. This result also confirms the risk estimate, where the values of % PTWI and % TDI for the toxic elements Al and Ni were low. However, with frequent consumption and increased consumption of this cheese, the risk of ingesting toxic elements may increase. The essential element - Ca would cover the daily need in the age category of lactating women with the highest need for this element at a significant level and both farms in the case of consumption of the amount of analyzed sheep's cheese we selected. However, it is important to deal with the sources of toxic elements that were monitored in the work and monitor their long-term intake in further research, since the detected concentrations of these elements exceeded the highest permissible limit set by Slovak legislation.

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REFERENCES

1. Astolfi, M.L., Marconi, E., Protano, C. & Canepari, S., 2020. Comparative elemental analysis of dairy milk and plant-based milk alternatives. *Food Control*, 116, p.107327.
2. Balthazar, C. et al., 2017. Sheep milk: physicochemical characteristics and relevance for functional food development. *Comprehensive Reviews in Food Science and Food Safety*, 16(2), pp.247–262.
3. Barłowska, J., Sz wajkowska, M., Litwińczuk, Z. & Król, J., 2011. Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive Reviews in Food Science and Food Safety*, 10(6), pp.291–302.
4. Barone, G. et al., 2018. Traditional Italian cheeses: Trace element levels and estimation of dietary intake. *Journal of Food Composition and Analysis*, 66, pp.205–211.
5. Beckett, W.S., Nordberg, G.F. & Clarkson, T.W., 2007. Routes of exposure, dose and metabolism of metals. In *Handbook on the Toxicology of Metals*, 3rd ed. Amsterdam: Elsevier B.V.
6. Burrow, K. et al., 2018. The distribution of essential, trace, and nonessential minerals in weanling male rats fed sheep or cow milk. *Molecular Nutrition & Food Research*, 62(21).
7. Capcarova, M. et al., 2019. Levels of essential and xenobiotic elements and their relationships in milk available on the Slovak market with the estimation of consumer exposure. *Biological Trace Element Research*, 188, pp.404–411.

8. Cowan, J.A., 2002. Structural and catalytic chemistry of magnesium-dependent enzymes. *Biometals*, 15(3), p.225.
9. Danezis, G. et al., 2019. Greek graviera cheese assessment through elemental metabolomics—Implications for authentication, safety and nutrition. *Molecules*, 24(4), p.670.
10. Das, K.K., Das, S.N. & Dhundasi, S.A., 2008. Nickel, its adverse health effects oxidative stress. *Indian Journal of Medical Research*, 128, p.412.
11. de Oliveira Filho, E.F. et al., 2022. Concentrations of essential trace and toxic elements associated with production and manufacturing processes in Galician cheese. *Molecules*, 27(15), p.4938.
12. EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2020. Update of the risk assessment of nickel in food and drinking water. *EFSA Journal*, 18(11), e06268.
13. Elbarbary, H.A. & Hamouda, A.F., 2013. Variations in some heavy metals' level during processing of soft cheese. *Journal of Food Measurement and Characterization*, 7, pp.194–198.
14. Eleboudy, A.A. et al., 2016. Heavy metals residues in some dairy products. *Alexandria Journal of Veterinary Sciences*, 51, pp.334–346.
15. Flis, Z. & Molik, E., 2021. Importance of bioactive substances in sheep's milk in human health. *International Journal of Molecular Sciences*, 22(9), p.4364.
16. Hamad, M. & Baiomy, A., 2010. The physical and chemical properties of camel's and sheep's milk in Qena Governorate. *Journal of Food and Dairy Sciences*, 1(7), pp.405–412.
17. JECFA, 2012. *Safety evaluation of certain food additives and contaminants*, Vol. 68. Geneva: World Health Organization.
18. Kiliçaltun, S., 2020. Essential elements and heavy metal levels in sheep milk and its dairy products. *Middle East Journal of Science*, 6(2), pp.94–103.
19. Klinda et al. 2016. Ministry of the Environment of the Slovak Republic. Environmental regionalization of the Slovak Republic. 4th, updated and expanded edition. Bratislava: Ministry of the Environment of the Slovak Republic and Slovak Environmental Agency. ISBN 978-80-89503-48-3.
20. Lawal, A. et al., 2023. Fatty acids and mineral content in milk of locally reared ruminant in Sokoto. *Nigerian Journal of Basic and Applied Sciences*, 30(2), pp.92–99..
21. Manzi, P., Di Costanzo, M.G. & Ritota, M., 2021. Content and nutritional evaluation of zinc in PDO and traditional Italian cheeses. *Molecules*, 26(20), p.6300.
22. Meshref, A.M., Moselhy, W.A. & Hassan, N.E.H.Y., 2014. Heavy metals and trace elements levels in milk and milk products. *Journal of Food Measurement and Characterization*, 8, pp.381–388.
23. Özcan, M.M. & Juhaimi, F.Y.A., 2012. Determination of heavy metals in bee honey with connected and not connected metal wires using inductively coupled plasma atomic emission spectrometry (ICP–AES). *Environmental Monitoring and Assessment*, 184, pp.2373–2375.
24. Pšenková, M. et al., 2021. Changes in the concentrations of selected toxic and essential elements in ewe milk from area with a potentially undisturbed environment. *Science Technology and Innovation*, 13(2), pp.28–34.
25. Romani, A.M., 2013. Magnesium in health and disease. In *Metal Ions in Life Sciences*, 13, pp.49–79.
26. Şanal, H., Güler, Z. & Park, Y., 2011. Profiles of non-essential trace elements in ewe and goat milk and their yoghurt, torba yoghurt and whey. *Food Additives and Contaminants Part B*, 4(4), pp.275–281.

27. Şensoy, T. & Çiftçi, G., 2022. Determination of alpha s1-casein, beta-lactoglobulin, some biochemical and mineral levels in milk of different animals. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, 11(1), pp.28–36.
28. Vahčić, N. et al., 2010. Essential minerals in milk and their daily intake through milk consumption. *Mljekarstvo/Dairy*, 60(2).
29. Vannucci, L. et al., 2018. Calcium intake in bone health: a focus on calcium-rich mineral waters. *Nutrients*, 10(12), p.1930.