

GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA



GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 09 | Issue: 02

On 30th June 2023

<http://www.research.lk>

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GARI Publisher | Agriculture | Volume: 09 | Issue: 02

Article ID: IN/GARI/PDL/ICABM/2023/100FEB | Pages: 05-20 (16)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 05.01.2023 | Publish: 30.06.2023

QUALITY OF SHEEP MILK AND MILK PRODUCTS FROM CONVENTIONAL AND ORGANIC FARMING IN SLOVAKIA

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ABSTRACT

Sheep breeding is a traditional and important part of agribusiness in Slovakia. In this work we investigated the occurrence of toxic and essential elements in sheep milk and dairy products depending on different management systems (organic and conventional). By ICP-OES method, Ca, Na, K, Mg, Al, Ba, Cd, Fe, Li, Mn, Ni, Pb, Sr, Zn, Se, As, and Sb in milk, whey, zrnica (acid fermented milk), bryndza cheese, and sheep cheese on farms from the Kysuce (organic farming) and Horné Povázie (conventional farming) regions in Slovakia were analyzed. Concentrations of essential elements (K, Mg, Fe, Mn, Zn, Se) from organic farming were significantly higher compared to conventional farming in most of the dairy products. In milk, Ca, K and Mg were present in significantly higher concentrations in conventional milk. However, significantly higher concentrations of Ba, Li, Sr, and Sb were also found in the bryndza and sheep cheese from the organic farm. Significantly higher concentrations of Al in cheese and Pb in milk were found in conventional cheese. In addition, higher concentrations of Al, Cd, Li, Pb and As were found in milk and milk products from conventional farming. Pb concentrations in milk from the conventional farm composed 30% of the benchmark dose for neurotoxicity. These results indicate that organic farming positively affected the concentrations of essential elements, especially in bryndza

and soft sheep's cheese. These products from organic farming are a good source of essential elements such as Ca, Mg, Mn, Zn, and Se, which are particularly necessary for the healthy development of children and the young generation. However, even under these farming conditions, it is necessary to monitor the occurrence of toxic elements. Toxicological analyses of sheep products can significantly contribute to the development of agribusiness in areas of Slovakia where conditions are suitable for sheep breeding and the production of quality and healthy dairy products.

Keywords: Milk products, Sheep, Chemical elements, Organic, Conventional

INTRODUCTION

The production of healthy and safe raw materials for food production is the main goal of agriculture. High quality food can only be produced in an ecologically undisturbed environment with the lowest possible presence of xenobiotics. However, livestock production often has to occur in conditions that pose a risk in terms of contamination of raw materials and food. Organic farming, unlike conventional farming, uses practices that do not pollute the environment with chemicals, does not use antibiotics, and ensures that animals are reared in a way that protects their health and natural

behavior. These conditions provide high production and quality (Tzamaloukas et al., 2021).

Milk production is one of the basic activities of livestock production. The milk of ruminants, especially cows, is an important component of human nutrition. It is a good source of protein but also of essential elements such as calcium, phosphorus, zinc, and selenium (Turck, 2013; Pereira, 2014). In addition to milk, dairy products such as cheese and fermented dairy products are often consumed. Sheep milk products are an important source of essential elements and fatty acids (Manuelian et al., 2017). Unlike raw milk, processing and production processes play a major role in the distribution of chemical elements in dairy products (Coni et al., 1999). However, due to the farming method, the environment itself and its contamination have a fundamental influence on the quality of the primary raw material, milk. In addition to essential elements, milk and milk products can be a source of toxic elements. These elements pose a health risk, especially for the sensitive child population. As, Cd, Cr, Pb, and Hg are considered systemic toxicants and confirmed or probable human carcinogens (Tchounwou et al., 2012). Disorders of the nervous system, respiratory system, and cardiovascular diseases occur in children (Al Osman et al., 2019). Particular attention should be paid to Pb, Cd, and Co, whose toxicity can be below toxicological reference values (TRVs) and can cause brain development disorders in children even at very low doses (Bocquet et al., 2021). Increases in blood and milk cadmium and lead levels in cows have been reported in relation to environmental exposure of lactating cows to these metals (Patra et al., 2008). Rey-Crespo et al. (2013) found no differences in the concentrations of toxic elements in conventional and organic milk. Moreover, the levels of toxic elements were very low.

However, López-Alonso et al. (2017) point out that in organic farming, it is necessary to pay more attention to the intake of the soil in grazing animals, which may contribute to the intake of toxic elements in contaminated areas. On the other hand, toxic elements (As, Cd, Hg, Pb, and Ni) were not detected in sheep milk in an area with moderate environmental pollution (Pšenková and Toman, 2021) or in a heavily polluted environment (Pšenková et al., 2020). Low levels of toxic elements were found in Galician cheeses corresponding to non-polluted areas. No difference was found between smoked and unsmoked cheeses in the concentration of the 14 elements studied (de Oliveira Filho et al., 2022).

In Slovakia, sheep farming is a traditional branch of agriculture. The production of special sheep products is also typical of Slovak sheep farming areas. For these reasons, the aim of this work was to investigate and compare the occurrence of toxic and essential elements in sheep milk and dairy products depending on organic and conventional farming.

METHODOLOGY

Milk sample collection

Sheep milk and milk products were sampled at two-week intervals during lactation (April-September). A total of 24 pool samples of milk, whey, znicica (acid fermented milk), bryndza cheese (traditional special sheep cheese produced in Slovakia), and sheep cheeses were taken. Samples were taken on two farms with different farming methods (organic and conventional). In addition to milk production, each farm produced its own dairy products, which are listed above. According to the environmental regionalization of Slovakia (Lieskovská and Micuda, 2019), the farm with organic farming is located in the non-polluted area of the Kusyce region with sheep herds of 340 animals (Zoslachtena Valaska breed).

The conventional farm is similarly located in the unpolluted area of the Horne Povazie region with sheep herds of 500 animals (Lacaune breed). The location of the farms where milk sampling took place is marked in Figure 1.

Approximately 500 mL of milk was taken from the tank at each farm and stored in PET bottles at -20°C until processing and analysis.

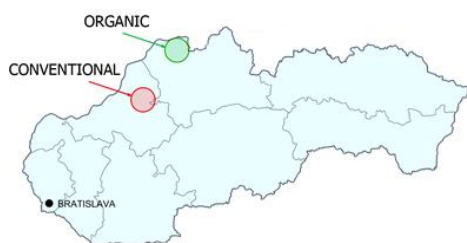


Figure 1 – Map of Slovakia with investigated organic farming (Kysuce) and conventional farming (Horne Povazie) location

Milk sample analysis

The following 17 chemical elements were analyzed in all sheep raw milk samples: Al, As, Ba, Ca, Cd, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sb, Sr, Se and Zn by inductively-coupled plasma optical emission spectrometry (ICP-OES). All chemicals used during sample preparation were ultrapure. Prior to analysis, the samples were thawed and a 2 ml milk sample was collected for analysis. The milk products were homogenized using a DiAx 600 hand homogenizer (Heidolph, Germany). From the homogenate, a 2 g sample was taken for chemical analyses. Samples were mineralized in an Ethos UP high-power microwave system (Milestone Srl, Sorisole, BG, Italy) in a solution of 5 ml HNO₃ ≥69.0% (TraceSELECT®, Honeywell Fluka, Morris Plains, USA), 1 ml H₂O₂ ≥30% (Sigma-Aldrich, Saint-Louis, Missouri, USA) and 2 ml ultrapure (18.2 MΩ cm⁻¹; 25°C, Synergy UV, Merck Millipore, France). Sample digestion was carried out in the heating

and cooling phases. During the heating phase, the samples were heated to 200°C for 15 min and this temperature was maintained for another 15 min. During the cooling phase, the samples were actively cooled for 15 minutes until the temperature reached 50°C. After filtering, the samples were analyzed on an Agilent - ICP OES 720 (Agilent Technologies Australia (M) Pty Ltd.) with an axial plasma configuration and an SPS-3 autosampler (Agilent Technologies, Switzerland). Multielement Standard Solution V for ICP (Sigma-Aldrich Production GmbH, Switzerland) was used for the measurements.

The detection limits - LOD (µg.kg⁻¹) of the measured elements were as follows: Al 0.20; As 1.50; Ba 0.03; Ca 0.01; Cd 0.05; Fe 0.10; K 0.30; Li 0.06; Mg 0.01; Mn 0.03; Na 0.15; Ni 0.30; Pb 0.80; Sb 2.00; Se 2.00; Sr 0.01 and Zn 0.20.

Limit of quantification - LOQ (µg.kg⁻¹) for the analysed elements Al 0.66; As 4.95; Ba 0.10; Ca 0.03; Cd 0.17; Fe 0.33; K 0.99; Li 0.20; Mg 0.03; Mn 0.10; Na 0.50; Ni 0.99; Pb 2.64; Sb 6.60; Se 6.60; Sr 0.03 and Zn 0.66. The accuracy of the method was verified using a certified reference material (CRM-ERM CE278 K, Sigma-Aldrich Production GmbH, Switzerland).

Statistical analysis

Statistical analysis of the data was performed using Statistica CZ ver. 10 (TIBCO Software, Inc., Palo Alto, CA, USA). Differences in the concentrations of the analyzed elements in the products between the different manufacturers were compared using ANOVA and Student's t-test. The data obtained were expressed as mean and standard error. The half-value of the LOD were used for values below the LOD for statistical calculations. A probability level of P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Essential elements

The results of the analyses of essential elements in milk and milk products according to the farming method are shown in Table 1. Milk is one of the important sources of the macronutrients Ca, K, Mg, Na, and P (Astolfi et al., 2020). Calcium has important functions in the animal and human body and one of the most important functions is bone mineralization. For this reason, sufficient dietary Ca intake is necessary (Vannucci et al., 2018). Comparing our results with the analyses of cow's milk published by Toman et al. (2022), we found that sheep milk contained higher Ca levels than cow's milk. However, Li et al. (2022) reported values as high as 2000 mg.kg-1 in sheep milk. Similarly, higher Ca concentrations in sheep milk were found in Tunisia ranging from 1810 to 2020 mg.L-1 (Khaldi et al., 2021). Qin et al. (2021) comparing organic and conventional farming found higher Ca in organic sheep milk (1049.5 mg.kg-1) than in

conventional milk (995.8 mg.kg-1). Sheep's milk is not commonly used for direct consumption but is used for the production of dairy products such as cheese. However, in terms of Ca availability for human intake, Ca bioavailability from sheep milk is not significantly different from human, cow, and goat milk and ranges from 18-23% (Shen et al., 1995). When assessing the calcium requirement at different age classes, the Ca concentrations found in our analyses cover the daily calcium requirement at a consumption of 500 ml of milk per day for lactating women, who have the highest Ca requirement, at 27-45%, and for older preschool children (4-6 years) at 61.7-103%. High Ca concentrations were observed in sheep cheese and in bryndza, which is a traditional Slovak soft sheep cheese similar to Italian Stracchino cheese. These cheeses are good sources of Ca for age groups with high Ca requirements.

Table 1 – Essential elements in milk and milk products of sheeps from different farming systems

	Ca	Na	K	Mg	Fe	Mn	Zn	Se
Organic farming (Mean ± SE)								
Milk	864.99 ±42.05 ^a	234.75 ±10.67	580.43 ±9.34 ^d	89.93 ±3.03 ^c	1.04 ±0.06	0.000015 ±0.000	2.49 ±0.11	0.102 ±0.04
Whey	247.34 ±6.37	406.55 ±16.92	921.84 ±13.80 ^d	83.13 ±1.56	1.02 ±0.05	0.000015 ±0.000	0.11 ±0.03	0.091 ±0.03
Zincica	468.96 ±13.30	399.62 ±20.17	927.06 ±18.05 ^d	93.40 ±2.50	1.10 ±0.15	0.000015 ±0.000	0.19 ±0.03	0.120 ±0.04
Bryndza	5372.07 ±260.30	4769.25 ±149.48 ^d	851.49 ±36.52 ^d	303.85 ±12.75	7.54 ±0.48 ^d	0.460066 ±0.054 ^d	18.97 ±1.02 ^d	0.424 ±0.09 ^d
Cheese	5409.29 ±138.97	285.95 ±27.42	693.38 ±36.20 ^d	330.03 ±13.99 ^c	7.25 ±0.31 ^d	0.487217 ±0.057 ^d	18.53 ±0.62 ^d	0.279 ±0.06 ^b
Conventional farming (Mean ± SE)								
Milk	1439.68 ±16.52 ^a	384.20 ±13.04	852.50 ±13.35 ^d	139.92 ±1.88 ^c	1.47 ±0.10	0.000015 ±0.000	3.67 ±0.15	0.287 ±0.07

Whey	251.86 ±38.79	312.43 ±13.65	637.97 ±40.49 ^d	72.75 ±3.36	1.11 ±0.12	0.000015 ±0.000	0.09 ±0.04	0.033 ±0.02
Zincica	683.59 ±29.82	319.06 ±9.64	644.46 ±21.02 ^d	94.08 ±1.83	1.09 ±0.06	0.000015 ±0.000	0.26 ±0.06	0.001 ±0.000
Bryndza	5301.84 ±198.69	5504.71 ±230.82 ^d	686.82 ±16.58 ^d	298.27 ±12.99	4.79 ±0.23 ^d	0.000015 ±0.000 ^d	16.34 ±0.86 ^d	0.113 ±0.05 ^d
Cheese	5038.20 ±141.62	258.17 ±11.44	513.25 ±18.84 ^d	277.82 ±8.88 ^c	2.56 ±0.14 ^d	0.000015 ±0.000 ^d	14.64 ±0.66 ^d	0.001 ±0.000 ^b

SE – Standard Error; the same index for the same product indicates the statistical differences between the farming systems; ^a P<0.05; ^b P<0.01; ^c P<0.001; ^d P<0.0001

Sodium is mainly found in processed cheeses such as bryndza and sheep's cheese. It is relatively low in milk and the concentrations found in sheep milk are comparable to cow milk (Toman et al., 2022). Qin et al. (2021) report Na concentrations in sheep milk at the same level for both organic and conventional farming. The values found in the authors' analyses are approximately at the level of our results. Similarly, Khaldi et al. (2021) found Na concentrations in the range of 380-400 mg.kg⁻¹, which agrees with our results. However, we found high Na concentrations in bryndza cheese, which is related to the processing method of this cheese. Salt is added at the final stage of its production, which increases the Na concentration in the bryndza. Sodium is a macronutrient that is influenced in sheep by the season (Khan et al., 2006). The authors found an average Na concentration in sheep milk of 358 mg.l⁻¹ in summer and 422 mg.l⁻¹ in winter. Potassium is also a macronutrient and is important for proper cardiovascular and renal function (McLean and Wang, 2021) and sufficient intake is necessary for the prevention of hypertension (Ozemek et al., 2018). When comparing the K content of milk and dairy products, we found significantly higher concentrations in milk from a conventional farm and, conversely, a significantly higher concentration of K in other dairy products from an organic farm.

Similarly, Qin et al. (2021) reported significantly higher K concentrations in conventional milk compared to organic milk in winter. Overall, there is low dietary K intake in the world (McLean and Wang, 2021), so the impact of organic farming is significant in this regard.

The Mg concentration was significantly higher in milk from the conventional farm. Qin et al. (2021) found no difference between organic and conventional milk in Mg levels. The concentrations reported by the authors for organic and conventional milk (95 mg.kg⁻¹ and 95.7 mg.kg⁻¹, respectively), especially for organic milk, were significantly lower than in our experiment. Li et al. (2022) found higher concentrations of Mg in sheep milk (180 mg.kg⁻¹) with higher values at the end of the season. However, organic farming provided higher Mg concentrations in bryndza and sheep cheese. In the case of sheep cheese, the difference with conventional farming was statistically significant. The daily Mg requirement for older preschool children is 120 mg and for boys aged 15-18 years with increased physical activity, who have the highest need for Mg, the recommended daily intake is 430 mg Mg. Sheep's cheese provides 7.7% of the daily Mg requirement for adolescent boys at 100g consumption.

Among other essential elements, there were significantly higher concentrations

of Fe, Mn, Zn, and Se in dairy products from organic farming in bryndza and sheep's cheese compared to conventional farming. Contrasting results were reported by Qin et al. (2021) in milk, where they found lower concentrations of Cu, Fe, Mn, and Zn in organic milk. The authors explain this by the lower intake of concentrates with higher Co, Cu, I, Se, and Zn contents. Similarly, Zwierzchowski and Ametaj (2018) reported significantly lower concentrations of Cu, I, Fe, Mn, Se, and Zn, Rodríguez-Bermúdez et al. (2018) lower concentrations of Cr, Cu, I, Se, and Zn in organic milk compared to conventional milk. Rey-Crespo et al. (2013) also found that significantly lower concentrations of essential elements in organic milk compared to conventional milk occur for Cu, Zn, I, and Se, which are added to feed in conventional farming. In our case, sufficient supplementation with essential elements was also ensured on the organic farm. Our results are significant in terms of providing health benefits from the intake of essential elements such as Fe, Mn, Zn, and especially Se, which occur in Central European geographical areas in low concentrations in the environment and consequently in food. Lower concentrations of Fe in sheep milk (0.8 mg.l^{-1}) were reported by Pietrzak-Fiećko and Kamelska-Sadowska (2020). Even lower concentrations of Fe (0.361 and 0.480 mg.l^{-1}) were found in sheep milk by Khan et al. (2006). Sheep milk and milk products contain higher concentrations of Fe than cow products. Lower values of 0.687 - 0.978 mg.kg^{-1} were found in analyses of cow's milk (Toman et al., 2022). Low concentrations in cow's milk are also reported by Capcarova et al. (2019). The Fe requirement for the human population ranges from 7 to 30 mg with the highest requirement in the pregnant women category. Sheep's cheese and bryndza at 100g consumption only cover this requirement for pregnant women at 2.4 - 2.5%. However, a higher intake of

bryndza is unfavorable, especially during pregnancy, due to the high concentration of Na. Carnicelli et al. (2021) report a positive effect of iodine addition to sheep feed on the Fe concentration in whey. Mn concentrations were below the LOD in most samples except for bryndza and sheep cheese. Magdas et al. (2019) also reported Mn values of 0.729 mg.kg^{-1} (ripened cheese) and 1.678 mg.kg^{-1} (salted cheese) in traditional cheeses from the Transylvania region of Romania. Higher concentrations of Mn are found in cow's milk. Mn concentrations of 0.01 - 0.034 mg.kg^{-1} (Toman et al., 2022), 21.05 - $68.29 \text{ } \mu\text{g.l}^{-1}$ (Saribal, 2020), 28 - $55 \text{ } \mu\text{g.kg}^{-1}$ (Bilandžić et al., 2021) and 24.3 - $31.8 \text{ } \mu\text{g.l}^{-1}$ (Diyabalanage et al., 2021) have been found in milk in Slovakia, Turkey, and Sri Lanka, respectively. Significantly higher values, up to $159 \text{ } \mu\text{g.l}^{-1}$, have been reported in contaminated areas (Diyabalanage et al., 2021). In the case of Mn, it is necessary to monitor its concentrations in milk and dairy products, especially in industrial areas, as excessive intake, especially by children, can have neurotoxic effects (Scher et al., 2021).

Zinc is an essential element whose bioavailability from milk is low, reaching only 1% in the case of sheep milk (Shen et al., 1995). Fe, Zn, and Cu are bound to casein in ruminant milk and to soluble proteins in human milk (Raynal-Ljutovac et al., 2008). Zinc is mainly required for the proper function of many enzymes. It is particularly important for children, adolescents, pregnant and lactating women who are at risk of increased zinc loss (Roohani et al., 2013). This metal is considered to be relatively non-toxic, but in excessive intake, its toxic effects may be manifested, which are related to the induction of copper deficiency (Fosmire, 1990). We observed a demonstrable difference in Zn concentration between organic and conventional farming only in the case of bryndza and sheep cheese in favor of organic farming. Manzi et al.

(2021) analyzed the Zn occurrence in 9 traditional Italian kinds of cheese and 38 protected denomination of origin cheeses. The lowest Zn concentrations were found in traditional Giuncata cheese ($1.83 \text{ mg} \cdot 100\text{g}^{-1}$), which was at the same level as our cheese analysis results. However, in the long-ripened Provola delle Madonie cheese, the authors found up to $7.18 \text{ mg} \cdot 100\text{g}^{-1}$. In milk, we found Zn values that were almost 2-fold lower than in analyses of sheep milk from Poland (Pietrzak-Fiećko and Kamelska-Sadowska, 2020). Raynal-Ljutovac et al. (2008) reported the highest Zn values in sheep milk ($5200 - 7470 \mu\text{g} \cdot \text{kg}^{-1}$) compared to other ruminants and humans. Significantly higher concentrations of $4 - 9 \text{ mg} \cdot \text{l}^{-1}$ are reported by Claye et al. (2014). By comparing the Zn concentration in cheese in our experiment with the recommended daily intake of Zn according to the recommendations of the Ministry of Health of the Slovak Republic (MZ SR, 2015), we found that consumption of 100g of bryndza or sheep's cheese covers the daily requirement for children aged 4 - 6 years at 37% and for men with exhaustive work at 11%. Thus, sheep's cheese contributes quite significantly to the intake of this important metal.

Selenium is an essential element that is a major component of many enzymes and plays a role in antioxidant processes, immune system support, reproduction, thyroid hormone production, cancer prevention, and cardiovascular disease (Mehdi et al., 2013; Avery and Hoffmann, 2018; Mojadadi et al., 2021; Gorini et al., 2021; Vinceti et al., 2018; Shimada et al., 2021). Similar to Zn, Se was found in significantly higher concentrations in our analyses of bryndza and sheep cheese from organic farming compared to conventional farming. Lower concentrations of Se were found in sheep milk in Pakistan at $0.014 \text{ mg} \cdot \text{l}^{-1}$ (Khan et al., 2006). Yanardağ and Orak (1999) also

found significantly lower Se concentrations ($13.5 - 78.92 \text{ ng} \cdot \text{g}^{-1}$) in sheep milk in Turkey than in our analyses. Similarly, in different types of cheese, the authors found lower values ($77.07 - 129.69 \text{ ng} \cdot \text{g}^{-1}$) than in our case. Although Slovakia is located in an area with a lower natural occurrence of Se in the environment, its concentration in milk and especially in dairy products is significant. In terms of Se requirement in the Slovak population, the consumption of 100g of the analyzed cheeses covers the daily Se requirement of 565% for bryndza and 372% for sheep's cheese. Although Se has important biological functions and is essential, its toxicity and the development of chronic degenerative diseases can occur with excessive intake (Spallholz, 1994; Nogueira and Rocha, 2011).

Toxic elements

Milk and dairy products can be a source of toxic elements, especially in areas with industrial contamination. In our analyses of milk and dairy products, we found the presence of 9 toxic elements. The average concentrations of these elements are given in Table 2. Lead, arsenic, cadmium, and barium were present in a smaller number of samples of some of the products analyzed. These elements are considered highly toxic and may be a health risk, especially for children. In the case of Pb and Cd, there are concerns that, despite the toxicological reference values set, their toxicity may be manifested even below these values. These metals can be toxic to brain development even without clearly defined threshold concentrations (Bocquet et al., 2021). In our case, Cd was above the LOD only in organic whey. In contrast, Souza et al. (2019) found Cd concentrations below the LOD in Brazilian whey beverages. The Codex Alimentarius does not specify a maximum permissible level of Cd in whey, but a limit

of 0.01 mg.kg^{-1} is set for milk (MASR, 2006). The concentrations found in whey are at this limit. Rey-Crespo et al. (2013) also reported very low levels of As, Cd, Hg, and Pb in both organic and conventional milk, however, with the exception of Hg, the concentrations of the other elements were higher in organic farming. Zwierchowski and Ametaj (2018) found that Cd, Pb, As, and Ni were present in higher concentrations in milk from conventional farming. In our analyses, Pb was found to be present only in conventional milk at an average concentration of $0.0154 \text{ mg.kg}^{-1}$. This value is below the maximum level set by the European Commission (EC, 2001). Ayar et al. (2009) found higher concentrations in milk (0.11 mg.kg^{-1}) than in our case and the same concentrations were also observed in whey powder. However, they found significantly higher concentrations of Pb in Kaşar cheese (2.5 mg.kg^{-1}). Higher concentrations ranging from 0.14 to 1.2 µg.g^{-1} were also found in different cheeses from Turkey (Mendil, 2006). In Croatia, lower Pb levels of 7.1 and 7.2 µg.kg^{-1} were found in milk by region and, similarly to our case, no sample exceeded the established limit (Bilandžić et al., 2021). Another toxic element, barium, was present only in milk and bryndza from organic farming, and in sheep cheese from both types of farming. For comparison of Ba concentrations in milk, the recommended concentration in drinking water is 0.7 mg.l^{-1} (WHO, 1990). In our samples, the values in organic milk were $0.0258 \text{ mg.kg}^{-1}$ and in conventional milk below the LOD. Concentrations as high as $96\text{-}140 \text{ µg.kg}^{-1}$ are reported by Bilandžić et al. (2021). A toxicological reference value is established for Ba with low reliability for children and its health risks cannot be excluded, even if the tolerable daily intake is not exceeded (Bocquet et al., 2021).

Interesting results were found by analyzing milk and dairy samples for the

presence of Al. In this case, there were higher concentrations in organic bryndza and sheep milk, which was highly significant in the case of sheep cheese. The concentrations of $37.737 \text{ mg.kg}^{-1}$ are quite high and are probably related to the technology of curd processing and the use of aluminium containers. By consuming 100g of sheep's cheese, an adult ingests approximately $0.05 \text{ mgAl.kg}^{-1}$ b.w. The Provisional Tolerable Weekly Intake (PTWI) value set by JECFA (2012) for Al is 2 mg.kg^{-1} , this means that in our case the weekly intake of Al through this cheese will only reach 0.5 mg.kg^{-1} , which is well below the stated PTWI. Therefore, despite the elevated Al concentration, consumption of these products is safe for humans. However, there is a need to monitor this metal in milk especially for young children and infants as they are at higher risk of brain and bone damage, particularly in children with kidney damage (Alasfar and Isaifan, 2021). However, we observed the same trend in milk as reported by Qin et al. (2021), who also found lower concentrations of Al in organic milk than in conventional milk (0.32 vs. 1.14 µg.kg^{-1}). These results are confirmed by Zwierchowski and Ametaj (2018), who reported 6.5-fold higher Al concentrations in conventional milk than in organic milk.

Table 2 – Toxic elements in milk and milk products of sheeps from different farming systems

	Al	Ba	Cd	Li	Ni	Pb	Sr	As	Sb
Organic farming (Mean ± SE)									
Milk	1.327 ±0.23	0.025803 ±0.009	0.000025 ±0.000	0.0035 ±0.0004	0.00457 ±0.003	0.0004 ±0.000 ^d	0.334375 ±0.032	0.00075 ±0.000	0.092 ±0.02
Whey	0.002 ±0.001	0.000015 ±0.000	0.013156 ±0.006 ^b	0.0035 ±0.0007	0.00015 ±0.000	0.0004 ±0.000	0.000005 ±0.000	0.00075 ±0.000	0.125 ±0.03
Zincica	0.104 ±0.03	0.000015 ±0.000	0.000025 ±0.000	0.0034 ±0.0005	0.01314 ±0.009	0.0004 ±0.000	0.035503 ±0.007	0.00075 ±0.000	0.063 ±0.02
Bryndza	14.733 ±1.35	1.331862 ±0.071 ^d	0.000025 ±0.000	0.0261 ±0.0022 ^d	0.18527 ±0.120	0.0004 ±0.000	3.078020 ±0.151 ^d	0.04967 ±0.029	0.286 ±0.05 ^b
Cheese	37.737 ±7.98 ^d	1.387510 ±0.078 ^d	0.000025 ±0.000	0.0062 ±0.0008 ^b	0.30099 ±0.141	0.0004 ±0.000	3.338538 ±0.094 ^d	0.12723 ±0.052	0.295 ±0.07 ^d
Conventional farming (Mean ± SE)									
Milk	8.222 ±3.99	0.000015 ±0.000	0.006159 ±0.004	0.0074 ±0.001	0.12283 ±0.055	0.0154 ±0.007 ^d	0.228475 ±0.010	0.00075 ±0.000	0.153 ±0.03
Whey	6.439 ±1.56	0.000015 ±0.000	0.000025 ±0.000 ^b	0.0078 ±0.001	0.21602 ±0.058	0.0004 ±0.000	0.000005 ±0.000	0.00075 ±0.000	0.022 ±0.01
Zincica	1.370 ±0.84	0.000015 ±0.000	0.000025 ±0.000	0.0062 ±0.0005	0.07891 ±0.054	0.0004 ±0.000	0.000005 ±0.000	0.00075 ±0.000	0.001 ±0.000
Bryndza	8.239 ±2.64	0.000015 ±0.000 ^d	0.000025 ±0.000	0.0013 ±0.0005 ^d	0.22476 ±0.101	0.0004 ±0.000	1.103457 ±0.091 ^d	0.15379 ±0.070	0.071 ±0.03 ^b
Cheese	1.290 ±0.12 ^d	0.023488 ±0.011 ^d	0.000025 ±0.000	0.0009 ±0.0003 ^b	0.00015 ±0.000	0.0004 ±0.000	1.002813 ±0.069 ^d	0.00075 ±0.000	0.001 ±0.000 ^d

SE – Standard Error; the same index for the same product indicates the statistical differences between the farming systems; ^a P<0.05; ^b P<0.01; ^c P<0.001; ^d P<0.0001

Lithium is a metal that is considered hazardous to infants if the mother is treated with this element and should be monitored in the mother's blood serum after childbirth and the dose reduced to prevent exposure to the newborn through breastfeeding (Westin et al., 2017). Low concentrations of Li are found in the milk of ruminants. Toman et al. (2022) report concentrations of 0.001-0.004 mg.kg⁻¹ in different types of processed cow's milk. Nabrzyski and Gajewska (2002) evaluated the occurrence of Li in fermented milk products and found concentrations ranging from 0.01 - 0.50 mg.kg⁻¹. Saribal (2020) reported an average concentration of 9.409 µg.l⁻¹ in milk from Turkey and

Diyabalanage et al. (2021) reported concentrations of 0.53 - 5.21 µg.l⁻¹ in milk from Sri Lanka. In our analyses, significantly higher concentrations occurred in bryndza and in sheep cheese from organic farming compared to conventional farming. However, these values were lower as reported by Nabrzyski and Gajewska (2002) and Magdas et al. (2019).

Comparing the Ni and As concentrations in all products studied between the two farming systems, we found no significant differences. In the case of Ni, the highest concentrations occurred in sheep cheese from organic farming. In organic whey and

conventional sheep's cheese, the Ni values were below the LOD. Similar conclusions were reached by Rey-Crespo et al. (2013), who also found no differences between organic and conventional farming. The authors report milk concentrations of $26.2 \mu\text{g.l}^{-1}$, which are more than 7-fold higher than in our organic milk. Higher Ni concentrations in milk from industrial copper (0.624 mg.l^{-1}) and iron (0.201 mg.l^{-1}) mining areas of India are described by Giri and Singh (2020). Mendil (2006) found values in various types of cheese in Turkey that approximate the Ni concentrations in our analyses. Capcarová et al. (2019) reported low Ni concentrations in cow's conventional milk from Slovak and Czech producers ranging from 0.84 to $1.01 \mu\text{g.l}^{-1}$. Zwierchowski and Ametaj (2018) found significantly higher concentrations of Ni and As in conventional milk compared to organic milk. These results of milk analyses support our findings, where more Ni was present in conventional milk compared to organic milk, although the differences were not significant. In Croatia, Ni concentrations in milk were found to range from 15 to $40 \mu\text{g.kg}^{-1}$ depending on the area. The authors attributed the higher occurrence of Ni in the central region of Croatia to anthropogenic activities (Bilandžić et al., 2021). The tolerable daily intake (TDI) of Ni according to EFSA (2020) is $13 \mu\text{g.kg}^{-1}$. After recalculating the assumed intake of sheep cheese at 100g per day, the exposure to Ni is at 3.3% , i.e. its intake does not pose a significant risk to the average adult consumer.

In the case of As, we found its occurrence above the LOD only in bryndza from both conventional and organic farming and in sheep cheese from organic farming. The highest concentrations were observed in conventional farming bryndza. By consuming 100g of this cheese, a person would ingest 0.015 mg As, which is only

1.42% of the PTWI of $15 \mu\text{g.kg}^{-1}$ set by JECFA (2011). Azar et al. (2009) found As concentrations ranging from 0.010 mg.kg^{-1} (whey powder) - 0.146 mg.kg^{-1} (butter), Magdas et al. (2019) observed As concentrations ranging from 0.113 mg.kg^{-1} (butter) in various dairy products, and 0.113 mg.kg^{-1} (butter) in sheep's milk cheese. Chen et al. (2020) detected relatively high concentrations of As in goat milk in China at $4.27 \mu\text{g.g}^{-1}$. Bilandžić et al. (2021) found As in only 2.3% of milk samples with average concentrations of $5.6\text{-}85 \mu\text{g.kg}^{-1}$ according to the area of Croatia. In the literature, there are very few relevant data comparing organic and conventional farming in terms of the occurrence of toxic metals and metalloids, and data from cow milk production are more frequent (Rey-Crespo et al., 2013). Several authors report the occurrence of As in ruminant milk, especially from contaminated areas. In mining areas in India, As levels of 0.010 and 0.015 mg.kg^{-1} have been found in milk (Giri and Singh, 2019), and in contaminated areas of Italy, 5.3 ng.g^{-1} in sheep milk (Miedico et al., 2016). Rey-Crespo et al. (2013) investigated the occurrence of As in both organic and conventional management and found a non-significantly higher occurrence in organic milk ($1.048 \mu\text{g.l}^{-1}$). They attributed this fact to soil intake during cow grazing. In our analyses, we did not detect As in any milk sample regardless of the farming system.

Strontium may be of importance in terms of food contamination because, together with Li, it can affect the metabolism of essential Ca and may be a cause of osteomalacia (Cohen-Solal, 2002; Ricci et al., 2021). We measured the highest Sr concentrations in samples of bryndza and sheep cheese from both farming systems. A demonstrably higher abundance was found in the organic farming system. Significantly higher concentrations were observed in

Transylvanian cheeses by Magdas et al. (2019). The Sr concentration was 7.683 mg.kg⁻¹ in ripened cheese and 9.361 mg.kg⁻¹ in salted cheese. Concentrations of 620.19 µg.l⁻¹ were found in milk from cows in Turkey (Saribal, 2020), which is about 2-3 times higher than in our analyses. Also, Chen et al. (2020) reported higher Sr concentrations in the milk of different ruminant species. They found the highest concentrations in camel milk (3.05 µg.g⁻¹) and the lowest values were found in buffalo milk (0.695 µg.g⁻¹). Coni et al. (1996) analyzed sheep milk and several types of sheep cheese from different farms. The Sr concentrations in the milk ranged from 4.72 to 6.24 µg.g⁻¹. In curds they found 4.16 - 6.22 µgSr.g⁻¹, in cheeses Sr was present according to processing (after salting, after ripening) in concentrations of 2.89 - 6.56 µg.g⁻¹. These values, especially in milk, were considerably higher than in our case. In our previous experiments, we also found relatively low Sr concentrations (0.252 - 0.637 mg.kg⁻¹) in different types of cow's milk in Slovakia (Toman et al., 2022). The TDI for Sr has been set by WHO at 0.13 mg.kg⁻¹ (WHO, 2010). When consuming 100g of organic sheep's cheese, which contained the highest amount of Sr, an adult would ingest approximately 3.6% of the TDI from this source. Thus, intake of both organic and conventional farm products is safe

Antimony is an industrial contaminant that can occur in industrial areas in elevated concentrations (Bolan et al., 2022). Statistically proven higher concentrations of Sn were found in bryndza and sheep cheese from organic farming. Sanal et al. (2011) reported significantly higher Sb concentrations in milk and dairy products from sheep. The authors found 5.65 mgSb.kg⁻¹ in milk, 3.45 mgSb.kg⁻¹ in whey, and 5.6-7.60 mgSb.kg⁻¹ in yoghurt. Concentrations from LOD to 62 ppb were found in cow's milk (Koyuncu and Alwazeer, 2019),

which are approximately the values found in sheep's milk. However, the authors also found an extreme Sb value in milk, which they attributed to the on-farm processing of milk or long storage of milk in a PET bottle. At high temperatures, Sb can be released from PET bottles and migrate into the contents of the bottle (Al Otoum et al., 2017). Low concentrations of Sb (0.176 µg.l⁻¹) were found in milk from Turkey by Saribal (2020). The TDI for Sn has been set by the WHO at 6 µg.kg⁻¹ (WHO, 2003). Assuming the consumption of 100g of organic sheep cheese, which contained the highest concentrations of Sb, the intake of Sb constitutes about 7% of the TDI. Also in the case of Sb in sheep cheese, we can conclude that its consumption is safe.

CONCLUSION

The results of our work show that the content of essential elements in milk and dairy products of sheep from organic farming in an undisturbed area is higher in most of the studied products compared to conventional farming. Significantly higher concentrations were found in organic farming for K (except of milk), Mg (cheese), Fe (bryndza, cheese), Mn (bryndza, cheese), Zn (bryndza, cheese), Se (bryndza, cheese). However, Ca, K and Mg were present in significantly higher concentrations in conventional milk. The significantly higher concentration of Na in the conventional bryndza is probably due to the higher addition of salt in the processing of this special cheese. On the other hand, organic bryndza and cheese contain significantly higher concentrations of Ba, Li, Sr, and Sb than those in conventional farm. However, significantly higher concentrations of Al in cheese and Pb in milk were found in conventional cheese. In addition, higher concentrations of Al, Cd, Li, Pb and As were found in milk and milk products from conventional farming.

These results indicate that organic farming positively affected the concentrations of essential elements, especially in bryndza and soft sheep's cheese. Their consumption covers a relatively large part of the recommended daily intake in the age categories with the highest need for the elements (young children, pregnant women). In the case of Se, this is as much as 372-565% of the need covered by the consumption of bryndza and sheep's cheese. Pb concentrations in milk from the conventional farm comprised 30% of the

benchmark dose for neurotoxicity. Although we have observed the occurrence of toxic elements in milk and dairy products, their concentrations do not reach dangerous levels for human health. However, even under these farming conditions, it is necessary to monitor the occurrence of toxic elements. Toxicological analyses of sheep products can significantly contribute to the development of agribusiness in areas of Slovakia where conditions are suitable for sheep breeding and the production of quality and healthy dairy products.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-18-0227.

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