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Content of Metals in Cow, Sheep and Goat Milk Samples

Korać, (R) S., Huremović, J.*, Žero, S., Ljubijankić, N.

University of Sarajevo, Faculty of Science, Zmaja od Bosne 33-35, 71000 Sarajevo, B&H

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***Corresponding author:**
Huremović Jasna
E-mail: jasnahuremovic@yahoo.com
Phone: 00-387-33-279-881

Abstract: Milk is an excellent source of many essential nutrients, including Ca, proteins and vitamin D. Nine metals (Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni and Pb) in raw and pasteurized milk of cow, sheep and goat were determined by atomic absorption spectrometry, flame technique. The levels of Cd, Fe, Mn, Ni and Pb were below the detection limit of the used method. The concentration ranges for Ca, Mg, Zn and Cu, in all milk samples, were as follows: 459.8-992.8, 59.5-206.8, <LOD-22.31 and <LOD-3.20 mg/L, respectively. The order of the metal levels in regards to concentration was Ca>Mg>Zn>Cu. The highest content of Ca and Mg was found in goat milk. In the case of cow's milk, the content of Ca, Mg and Zn increased with increasing amount of milk fat in processed milk. Matrix correlation analysis showed that there is a significant correlation for the following pairs: Ca-Mg (r = 0.830), Zn-Cu (r = 0.799) and Ca-Zn (r = 0.624). Also, a strong correlation was found between milk fat and Ca, Mg and Zn (Pearson factor, r > 0.600). Based on daily consumption of 200 mL of milk, the milk consumption does not meet the daily requirements for determined elements. Most significant intake was in case of Ca.

INTRODUCTION

Milk and milk products are basic dietary products and constitute an important source of nutrients in the humans' daily diet required for normal human development and metabolic processes. As far as the basic composition of milk is known, elemental composition is generally unknown (Dobrzañski, Koacz, Górecka, et al., 2005; Khan, Choi, Nho, et al., 2014). Various minerals are essential for the health, especially during the growth and in old age. The efficacy and/or excess of minerals can result in pathological changes and metabolic irregularities (Garcia, Lorenzo, Cabrera, et al., 1999). Consumption of milk is recommended by many nutritionists to meet daily needs for calcium, vitamins B12 and D, and animal proteins. Heavy metals and metallic compounds, as a result of water, air, and soil pollution, pass to animals and people through the food chain and can have a negative impact on health (Karasakal, 2020).
The milk analysis is important because milk is a significant diet source that can contain toxic metal ingested by humans. Milk is also a source of essential nutrients and an indicator of environmental contamination. Animals reduce human trace metals exposure; for example, the levels present in different environmental matrices are higher than those found in food (Pérez-Carrera, Arellano and Fernández-Cirelli, 2016). Trace elements in cow milk, in recent years, have been considered to be good bio-indicators of pollution in the agriculture. The concentration of minerals in raw cow milk vary according to different factors, such as animal species and health status, the season, contamination of environment, climate, lactation period and the dietary composition of animal feed (Vahčić, Hruškar, Marković, et al., 2010).
According to research by Vahčić et al. (2010), the children of 8 to 9 years of age consume 0.46 L of milk per day and average daily milk consumption of an adolescent (15-18 years old) is similar with value of 0.47 L/d when compared to children consumption, but not sufficient. In the United States, the national guidelines for dietary recommendations note that adults should drink three cups or 732 mL/d of milk (Dietary Guidelines for Americans, 2010). However, such level of consumption is rarely observed. According to the Canadian dairy information center (Dairy Facts and Figures) from 2016 year, he mean per capita milk consumption in the United States was 196 mL/d in 2014 and in Europe 171 mL/d, with great heterogeneity in consumption depending on the country (Mullie, Pizot and Autier, 2016). The contents of nine trace elements of toxicological or nutritional importance (Ca, Mg, Mn, Fe, Cu, Zn, Cd, Ni and Pb) in samples of raw and pasteurized cow, sheep and goat milk were measured by atomic absorption spectrometry, flame

technique (FAAS). There are not large numbers of published papers on the elementary analysis of goat's milk compared to cow's milk. In addition, matrix correlations between elements were done and estimated daily intakes of metals from milk samples were calculated.

EXPERIMENTAL

Sample collection, preparation and heavy metal determination

Milk samples were collected from local farms in Sarajevo, Bosnia and Herzegovina and Sarajevo markets, during the summer of 2020. Milk samples were placed into plastic bottles that were rinsed with 10 % HNO_3 in Milli-Q water prior to collection.

One mL of the sample was annealed in a porcelain crucible at 550 ^{0}C for 1 h. The resulting white ash was then moistened with Milli-Q water (10 drops), dissolved in 10 mL of 3 mol/L HCl, filtered through Whatman No. 1 paper into a 100 mL volumetric flask, and diluted to original volume with 3 mol/L HCl.

All reagents used were of analytical reagent grade (Merck, Darmstadt, Germany). Milli-Q water was used during the complete analysis process. The content of metals in milk samples was determined by FAAS (Atomic absorption spectrometer, model Varian AA240FS, Mulgrave, Australia).

The lipid content in raw milk from farms was not determined, while the data for milk fat (table 1) in the milk samples from the markets were taken from the milk packaging.

Analytical quality control

All milk samples were analyzed in triplicate, and standard deviation was calculated. Reagent blanks were also analyzed after each batch of 10 samples. The mean concentration for each milk sample was reported. The limit of detection (LOD, three times the standard deviation of the blank absorbance signal, n = 10) for each metal was calculated. LODs were: 0.035 μg/mL for Ca; 0.021 μg/mL for Cd; 0.071 μg/mL for Cu; 0.941 μg/mL for Fe; 0.014 μg/mL for Mg; 0.210 μg/mL for Mn; 0.163 μg/mL for Ni; 1.091 μg/mL for Pb; 0.660 μg/mL for Zn.

The assessment of accuracy was performed by spiking four already analyzed milk samples with varying analyzed elements concentrations. Samples were spiked at three different known concentrations of standards, high, medium and low, covering the working range. Satisfactory recovery factor values, from 84 % to 109 %, were obtained for all metals.

Estimated daily intake (EDI) of metals through the milk consumption

EDI (mg/kg/day) from milk ingestion was calculated by combining the data on the consumption of milk with the determined levels (c, mg/L) of Ca, Cu, Mg and Zn.

EDI was calculated based on the assumptions that:
1) body weight is 60 kg, and
2) daily intake of milk is 200 mL.

EDI (mg/kg/day) = metal concentrations in milk × 200/1000/60 (Vahčić et al., 2010; Bašić, Beganović, Huremović, et al., 2020)

Statistical analysis

For the data analysis for metals concentrations in milk samples, descriptive statistical parameters such as mean values, standard deviation and Pearson's matrix correlations were used.

RESULTS AND DISCUSSION

The concentrations of Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni and Pb in raw and pasteurized cow, sheep and goat milks were investigated. The content of Cd, Fe, Mn, Ni and Pb was below the detection limit of the method used. The results of the elemental milk analysis are given in Table 1.

Table 1: Content of Ca, Mg, Zn and Cu (mg/L) ± standard deviation in different milk samples, n = 3.

Sample	Milk fat (%)	Ca (mg/L)	Mg (mg/L)	Zn (mg/L)	Cu (mg/L)
Cow's milk 1 (short-lived, sterilized)	0.9	507.5±99.12	68.3±7.92	*<LOD	*<LOD
Cow's milk 2 (short-lived, sterilized)	2.8	459.8±100.4	59.5±14.3	*<LOD	*<LOD
Cow's milk 3 (pasteurized)	2.8	731.9±134.5	181.5±22.1	7.00±0.000	* <LOD
Cow's milk 4 (permanent, pasteurized)	3.2	637.6±56.70	186.5±8.76	10.11±0.61	*<LOD
Cow's milk 5 (permanent, pasteurized)	3.8	663.8±155.4	172.4±5.54	13.00±2.09	*<LOD
Lactose-free cow's milk	1.5	700.2±146.4	175.4±33.3	22.31±0.01	*<LOD
Chocolate cow's milk	2.3	469.2±64.08	85.8±14.2	*<LOD	0.50±0.11
Cow's milk (with addition of Ca and D vitamin)	3.2	757.7±111.9	160.6±19.8	1.40±0.7	*<LOD
Raw cow's milk 1	-	740.4±98.11	183.3±7.00	8.41±0.61	3.20±0.9
Raw cow's milk 2	-	725.7±121.8	182.8±23.1	4.40±0.08	0.51±0.00
Raw sheep milk	-	992.8±223.2	186.7±54.0	5.31±0.90	0.30±0.14
Raw goat milk 1	-	988.7±242.1	206.8±30.1	4.40±0.76	0.90±0.52
Raw goat milk 2	-	931.3±199.9	204.0±9.98	2.80±1.15	0.21±0.13
Goat milk (pasteurized)	2.8	732.1±198.7	180.4±25.7	1.21±0.09	*<LOD
Range	-	459.8-992.8	59.5-206.8	*<LOD-22.31	*<LOD -3.20

*<LOD - below the detection limit of the method used

Concentrations of Cd, Fe, Mn, Ni and Pb in all milk samples were below the detection limit of the method used. The highest content of Ca and Mg was found in raw goat milk and sheep milk. The content of Ca, Mg and Zn mainly increased with the increase of milk fat in processed milk. The concentration of Ca in cow milk with 3.8 % fat was very similar to the concentration of Ca in cow milk with the addition of Ca. The highest Ca, Mg, Zn and Cu content in cow milk samples was found in raw, unprocessed milk. By concentrations, the metals were arranged as the following diminishing series Ca>Mg>Zn>Cu.

Ca was the major metal of interest in milk. It is the most common mineral and needed for the bone formation and bodily functions. It also plays a vital role in activation of different enzymes (Karasakal, 2020). Mg is a cofactor of many important enzymes, including peptidases, arginase, cholinesterase, pyruvate carboxylase, phosphoglucomutase, mitochondrial superoxide dismutase and several glycosyltransferases and phosphates (Vahčić et al., 2010). The Table 2. provides an overview of previous research on the metal content in milk samples in the world.

Table 2: Results of mineral content in different milk samples in the world.

Metal / Sample (Origin)	Ca	Mg	Zn	Cu	Reference
	Concentration of metals in milk (mg/L)				
Cow's milk-processed and raw milk samples (Sarajevo, Bosnia and Herzegovina	459.8-757.7	59.5-186.5	<LOD-22.31	<LOD-3.20	Present study
Goat's milk-pasteurized and raw milk samples (Sarajevo, Bosnia and Herzegovina)	732.1-988.7	180.4-206.8	1.21-4.40	0.2 -0.90	Present study
Raw sheep's milk (Sarajevo, Bosnia and Herzegovina)	992.8±223.2	186.7±54.0	5.31±0.90	0.30±0.14	Present study
Cow's pasteurized milk (Zagreb, Croatia)	901.2±80.2	110.8±17.4	3.22±0.23	0.19±0.10	Vahčić, *et al*., 2010
Raw cow's milk (Virginia, USA)	1164±56	101.4±7.7	3.522±0.526	0.020±0.008	Lopez, Collins, and Williams, 1985
Cow's pasteurized milk (Virginia, USA)	1169±63	99.82 ±8.08	3.488± 0.427	0.043±0.019	Lopez *et al*., 1985
Raw goat's milk (Virginia, USA)	988.9±50.4	120.7±10.0	3.426±0.507	0.084±0.050	Lopez, *et al*., 1985
Pasteurized goat's milk (Virginia, USA)	992.1± 56.4	120.2 ±12.9	3.286±0.570	0.138±0.085	Lopez, *et al*., 1985
Milk powder (Brazil)	731±14	155±5	2.93±0.12	-	Hamid, Abd El-Samad, Soliman, *et al*., 2017
Plain cow's milk (Seoul, Korea)	1085±0.29	104.8±0.03	-	-	Khan *et al*., 2014
Skimmed cow's milk (Seoul, Korea)	1184±0.26	115.2±0.04	-	-	Khan *et al*., 2014
Raw cow's milk (Wroclaw, Poland)	-	-	3.124±0.650	0.077±0.105	Dobrzañski, Koacz, Górecka, *et al*., 2005
Plain cow's milk (California, USA)	-	-	3.700±0.023	-	Voth, 1963
Domestic cow milk (Dhaka, Bangladesh)	-	-	-	0.127±0.029	Muhib, Chowdhury, Easha, *et al*., 2016
Fresh cow's milk (Constanta, Romania)	-	214.00	0.98	0.17	Birghila, Dobrinas, Stanciu, *et al*., 2008
Pasteurized cow's milk (Constanta, Romania)	-	212.82	0.85	0.11	Birghila, *et al*., 2008
Powder milk (Constanta, Romania)	-	919.80	3.24	0.54	Birghila, *et al*., 2008
Cow's milk (Butajira and Meskan districts, south central Ethiopia)	380.1-532.4	159.6-397.9	0.0-8.2	0.03-1.1	Teklu, *et al*., 2022
Cow's milk (Erode District, Tamil Nadu, India)	-	-	1.22-20.94	BDL*-0.35	Yasotha, *et al.*, 2021
Cow's milk (Nuer Zone and Gambella town, Ethiopia)	755.15-812.75	1675.87-2050.65	-	-	Damtew and Gebre, 2020

***** indicates below detection limit

From the data given in Table 2 it can be seen that the metal concentrations obtained through this research were similar to the research conducted in other countries. In this study, slightly lower values for Ca content were obtained compared to previously published papers. Lower values for Mg in milk samples from this study compared to samples from Ethiopia (Damtew and Gebre, 2020) have been obtained.

Matrix correlation analysis

The strength of the correlation between analyzed elements in raw and pasteurized cow, sheep and goat milks is expressed using the guide where Fantu Garcia (1996) described the absolute value of Pearson correlation factor, r: 0.00–0.19, very weak; 0.20–0.39, weak; 0.40–0.59, moderate; 0.60–0.79, strong and 0.80–1.0, very strong. Results of matrix correlation analysis are shown in Table 3.

Matrix correlation analysis showed that there is a very strong correlation for Ca-Mg and strong correlation for Cu-Zn and Ca-Zn pairs. The Cu content was only significantly correlated with Zn, and similar results, for these two metals, were obtained by Garcia et al. (1999). Positive association between Ca and Mg was also found in study by Steck, Omofuma, Su, et al. (2018). Fantuz, Maglieri, Lebboroni, et al., (2009) investigated ass's milk and they found positive and significant correlation between Ca and Mg (r=0.63), Zn and Cu (r=0.50) and Zn and Mg (r=0.45). Also, in this study, a strong correlation was found between milk fat and Ca, Mg and Zn.

Table 3: Correlation matrix between concentrations of trace metals and milk fat in cow, sheep and goat milk samples.

	Milk fat	Ca	Mg	Zn	Cu
Milk fat	1	**0.640**	**0.706**	**0.784**	0.000
Ca		1	**0.830**	**0.624**	0.158
Mg			1	0.571	0.192
Zn				1	**0.799**
Cu					1

For easier visual analysis, correlation values > 0.6 are bold highlighted

EDI values for metals from milk

People need a certain daily intake of minerals. EDI of metals (Ca, Mg, Zn and Cu) depended on the metal concentration level and the amount of milk consumption. Calculated EDI values for metals from raw and pasteurized cow, sheep and goat milk samples are presented in Table 4.

Table 4: EDI of metals from milk samples.

	EDI							
	mg/kg/d	mg/d	mg/kg/d	mg/d	mg/kg/d	mg/d	mg/kg/d	mg/d
Sample /metals	Ca		Mg		Zn		Cu	
Cow's milk 1 (short-lived, sterilized)	1.691	101.5	0.228	13.66	-	-	-	-
Cow's milk 2 (short-lived, sterilized)	1.53	146.4	0.198	11.90	-	-	-	-
Cow's milk 3 (pasteurized)	2.44	146.4	0.605	36.3	0.023	1.38	-	-
Cow's milk 4 (permanent, pasteurized)	2.13	127.5	0.622	37.3	0.034	2.04	-	-
Cow's milk 5 (permanent, pasteurized)	2.21	132.8	0.575	34.48	0.043	2.60	-	-
Lactose-free cow's milk	2.33	140.0	0.585	35.08	0.074	4.46	-	-
Chocolate cow's milk	1.56	93.8	0.286	17.16	-	-	0.002	0.10
Cow's milk (addition of Ca and D vitamin)	2.52	151.5	0.535	32.12	0.005	0.28	-	-
Raw cow's milk 1	2.47	148.1	0.611	36.67	0.028	1.68	0.011	0.64
Raw cow's milk 2	2.42	145.1	0.609	36.56	0.015	0.88	0.002	0.10
Raw sheep milk	3.31	198.6	0.622	37.34	0.018	1.06	0.001	0.06
Raw goat milk 1	3.29	197.7	0.689	41.36	0.015	0.88	0.003	0.18
Raw goat milk 2	3.10	186.3	0.680	40.80	0.009	0.56	0.001	0.042
Goat milk (pasteurized)	2.44	146.4	0.601	36.08	0.004	0.24	-	-

Most significant intake was in case of Ca. The Food and nutrition board has defined the recommended daily intake (RDI) values for a normal adult population, and for Ca, Mg, Zn and Cu is 1000–1200 ppm, 320–420 ppm, 4-25 ppm, 1.0-1.6 ppm, respectively. The results showed that daily milk consumption of 200 mL does not meet the daily requirements for the determined metals. In accordance with the obtained EDI values, it would be necessary for adults to consume about 2 L of milk to meet the needs for Ca, Mg, Zn and Cu, while neglecting the intake of these minerals through other food products. The content of these metals is not necessarily the amount that human body will absorb.

CONCLUSION

Minerals are found in all foods, including milk and milk products. Although the topic is very important, there have not been many published papers dealing with elements in cow, sheep and goat milk. The highest concentration of Ca and Mg were found in raw goat and sheep milk. The content of Ca, Mg and Zn mainly increased with the increase of milk fat. Based on daily consumption of 200 mL of milk, it was shown that the milk consumption does not meet the daily Ca, Mg, Cu and Zn requirements. Most significant intake was in case of Ca. Matrix correlation analysis showed that there is a very strong correlation between Ca and Mg and strong correlation for pairs: Cu-Zn and Ca-Zn. Strong correlation was also found between milk fat and Ca, Mg and Zn.

The content of Ca, Mg, Zn and Cu is the measure of these elements in different milks but not necessarily the amount that human body will absorb. Therefore, the bioavailability of certain minerals should be investigated in future research.

Conflict of interest

The authors declare no conflict of interest.

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Summary/Sažetak

Mlijeko je odličan izvor mnogih esencijalnih nutrijenata, uključujući Ca, proteine i vitamin D. Devet metala (Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni i Pb) u sirovom i pasteriziranom kravljem, ovčijem i kozjem mlijeku je određeno atomskom apsorpcionom spektrometrijom, plamenom tehnikom. Sadržaj Cd, Fe, Mn, Ni i Pb bio je ispod granice detekcije korištene metode. Rasponi koncentracija za Ca, Mg, Zn i Cu, u svim uzorcima mlijeka, bili su sljedeći: 459.8-98.83, 59.5-206.8, <LOD-8.41 i <LOD-3.20 mg/L, respektivno. Redoslijed metala je bio Ca>Mg>Zn>Cu. Najveći sadržaj Ca i Mg nađen je u kozjem mlijeku. U slučaju kravljeg mlijeka sadržaj Ca, Mg i Zn se povećavao sa povećanjem mliječne masti. Korelaciona analiza je pokazala da postoji značajna korelacija za sljedeće parove: Ca-Mg (r = 0.830), Zn-Cu (r = 0.799) i Ca-Zn (r = 0.624). Takođe, nađena je jaka korelacija između mliječne masti i Ca, Mg i Zn (Pearsonov faktor, r > 0.600). Konzumacijom 200 mL mlijeka dnevno, potrošnja mlijeka ne zadovoljava dnevne potrebe za utvrđenim elementima. Najznačajniji unos bio je u slučaju Ca.