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Trace elements content in cheese, cream and butter

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Nina Bilandžić, Marija Sedak, Maja Đokić, Đurđica Božić, Božica Solomun Kolanović, Ivana Varenina*

Laboratory for Residue Control, Department for Veterinary Public Health, Croatian Veterinary Institute, 10000 Zagreb, Croatia

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Abstract

Trace elements were determined in five types of cheese, cream and butter using inductively coupled plasma-optical emission spectrometry. In cheese samples trace elements were measured as follows (mg/kg): Al 0.01-3.93, Co<0.005, Cr 0.005-1.66, Li 0.008-0.056, Mn 0.068-5.37, Mo 0.003-0.225, Ni 0.01-0.163 and Sr 0.085-3.49. There were significant differences considering the concentrations of Mn, Cr and Al ($p<0.01$, all) among the analysed dairy products. There were no significant differences in Sr, Mo, Ni and Li levels among products. The highest levels were found in following products (mg/kg): 4.23 Mn in semi-hard fat cheese, 2.43 Sr in cream cheese, 0.18 Mo in cream, 0.14 Ni and 0.028 Li in melted cheese, 1.13 Cr and 3.87 Al in butter. The trace element concentrations measured in cheeses and butter varied compared to the literature data. Concentrations of Al, Cr, Mn and Mo found in cheeses and Mn and Ni in butter were in line with contents reported in other countries. These results may demonstrate differences in production processes between countries. The estimated daily intakes (EDIs) calculated for Cr, Mn, Mo and Ni in cheeses showed a low contribution (0.59-3.38 %) to the reference values for the permitted daily exposure (PDE) for these elements. However, the high contribution of Al concentrations (56 and 124 %) to PTWI (provisional maximum tolerable daily intake) calculated in fresh and melted cheese may pose a health risk to consumers.

Key words: cheese, dairy products, trace elements, Al, Co, Cr, Li, Mn, Mo, Ni, Sr, ICP-OES

Introduction

Trace elements such as lithium (Li), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), and molybdenum (Mo) are essential micronutrients that need to be consumed in adequate amounts for proper physiological functioning (Institute of Medicine, 2005). Optimal intakes of essential mineral elements are required to maintain peak health. Mineral deficiencies in humans are widespread throughout the world and may play a negative role on a number of physiological processes, especially in child development, pregnancy and elderly health (Grantham-McGregor and Ani, 2001). All trace elements, including essential elements, may be toxic when taken in excessive amounts, or may lead to deficiencies when taken in insufficient amounts. The presence of trace elements such as aluminium (Al) in food may come from environmental sources, such as the earth's crust and water, or due to food processing and packaging (Arnich et al., 2012).

Milk and dairy products belong to very important components of the human nutrition. They are rich in proteins, carbohydrates, fats, organic acids, enzymes, vitamins and contain more than 20 different minor and trace elements. Microelement content in milk and dairy products is normally very

*Corresponding author/Dopisni autor: Phone/Tel.: +385 1 612 3601, E-mail: bilandzic@veinst.hr

small. The highest levels measured were Zn, Cu, Mg and Cr, which are essential elements and very important for normal metabolism, growth and development (Khan et al., 2014). However, through manufacturing and packaging processes, their contents may be significantly increased. Elements such as Cr, Ni and Co may accumulate in milk and dairy products due to contamination of the environment and thus feed for ruminants (Schuhmacher et al., 1991). Elevated concentrations may cause serious health problems. The extent of toxicity is related to factors such as the route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, age, sex, frequency of intake, absorption rate and efficiency of excretion mechanisms (Khan et al., 2014).

Several Croatian dairies produce traditional and innovative products that are popular as part of the daily consumer diet. Homemade cottage cheese is the most widespread product of traditional Croatian cheese-making practice (Kirin, 2009). To the extent of our knowledge, data on minerals and trace elements in dairy food from Croatia are limited. In recent years, studies related to trace element contents in cheese have been reported in several countries such as Brazil (Nardi et al., 2009), France (Arnich et al., 2012; Millour et al., 2012; Noël et al., 2012), Spain (González-Weller et al., 2013) and Turkey (Mendil, 2006; Merdivan et al., 2004).

The aim of this study was to determine microelement concentrations of Al, Co, Cr, Li, Mn, Mo, Ni and Sr in five types of cheese, butter and cream produced in Croatia and to compare these levels with those reported for other dairy products worldwide.

Materials and methods

Sample collection

In total, 17 dairy products were collected from marketplaces in Croatia: 5 types of cheeses (3 fresh, 2 melted, 3 cream, 3 fat soft, 3 semi-hard fat), 2 samples of cream and 2 samples of butter produced by dairy factories in Croatia. After collection, samples were placed into clean polyethylene bags, labelled and stored at -18 °C until analysis.

Reagents

Analytical reagent grade HNO_3 (65%, v/v) and H_2O_2 (30 %, v/v) were purchased from Kemika (Zagreb, Croatia). Ultra-pure water (18 MŮxcm) generated by the system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia) were used for preparations of solutions.

Standard stock solutions containing 1000 mg/L of Co, Li, Mn, Mo, Ni and Sr were purchased from Inorganic Ventures (Christiansburg, VA, USA). Standard solutions containing 1000 mg/L of Al and Cr were supplied from Sigma-Aldrich (Buchs, Switzerland). The stock solution and working standards were diluted in HNO_3 (0.5 %).

Sample preparation

Dairy product samples (2 g) were weighed into a PFA digestion vessel and 1 mL of H_2O_2 and 6 mL HNO_3 were added. A microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany) was used for acid digestion of samples. The digestion program consisted of step 1 at power 800 W, ramped 15 min, 800 W for 15 min and step II power at 0 W for 15 min. Digested samples were diluted with ultra-pure water to a volume of 50 mL. Analytical batches contained a blank sample and two spiked samples.

Calculation of detection limits were performed according to three times the standard deviation of ten blanks. The limits of detection were (mg/kg): Al 0.01, Co 0.005, Cr 0.02, Li 0.005, Mn 0.01, Mo 0.003, Ni 0.01 and Sr 0.01.

Quality control and recovery for Mn, Mo, Cr, Sr and Li were determined by spiking samples with known element concentrations. Good accuracy was achieved with the following recovery results (%): Al 96.5, Co 95.8, Cr 96.4, Li 96.8, Mn 97.8, Mo 95.5, Ni 97.7 and Sr 99.3.

Trace element analysis

An Optima 8000 (Perkin Elmer, Waltham, Massachusetts, USA) inductively coupled plasma optical emission spectrometer (ICP-OES) was used for element measurement. Instrumental experimental conditions are summarized in Table 1.

Table 1. Instrumental conditions for determination of elements with inductively coupled plasma optical emission spectrometer

Analyte	Al, Cr, Li, Mn, Mo, Sr	Co, Ni
Parameter	Intensity	Intensity
Plasma viewing mode	Axial	Axial
Read time	1-5 s	1-5 s
Measurement replicates	3	3
RF incident power	1300 W	1400 W
Plasma argon flow rate	15 L/min	8 L/min
Nebulizer argon flow rate	0.55 L/min	0.3 L/min
Auxiliary argon flow rate	0.2 L/min	0.2 L/min
Sample uptake rate	1.5 mL/min	1.5 mL/min
Inner diameter of the torch injector	2.0 mm	2.0 mm
Nebulizer type	Concentric glass (Meinhard)	Concentric glass (Meinhard)
Spray chamber type	Glass cyclonic spray chamber	Glass cyclonic spray chamber

Calculation of the estimated daily intake

The estimated daily intake (EDI) was calculated by the equation (Copat et al., 2013):

EDI (μg/day) = [(element concentration; mg/kg or μg/g) per (meal size or daily intake of cheese; g/day)]

In the calculation, the average consumption of cheese in Croatia was set to 20 g/day per adult (Antonić Degač et al., 2007). The values of EDI were used to calculate contributions of each element to the reference toxicological values (PDE, permitted daily exposure; PTWI, provisional maximum tolerable daily intake).

Data analysis

Statistical analysis was conducted using Statistica 6.1 software (StatSoft® Inc., Tulsa, USA). Concentrations were expressed as mean ± SD. One-way ANOVA was used to test statistical differences in element levels between the analysed dairy products. Results were considered significant at $p \leq 0.05$.

Results and discussion

Dietary intakes of trace elements, and therefore in various milk and dairy products, are of great concern for public health, and need to be monitored on a regular basis. The presence of trace elements might indicate qualitative parameters in the production procedures, sanitary conditions, and quality of animal feeding which can affect milk and cheese characteristics, storage or health aspects. Also, some trace elements are correlated to environmental pollution and to releases from packaging and milking equipment in the dairy production (Bakircioglu et al., 2011).

The results of the trace element content (Al, Cr, Li, Mn, Mo, Ni and Sr) in five cheese types, butter and cream, the mean concentrations and the standard deviations (SDs) are presented in Table 2. Statistically significant differences in concentrations of Mn, Cr and Al ($p<0.01$, all) were found among dairy products. There were no significant differences in Sr, Mo, Ni and Li levels among the products. The highest levels determined were in following products (mg/kg): 4.23 Mn in semi-hard fat cheese, 2.43 Sr in cream cheese, 0.18 Mo in cream, 0.14 Ni and 0.028 Li in melted cheese, 1.13 Cr and 3.87 Al in butter.

Cobalt is an essential trace element as a constituent of vitamin B_{12} and some metalloproteins, though its toxicity in many biological systems depends on its concentration range (Antoine et al., 2012). Dietary deficiencies may lead to pernicious anaemia which can be fatal (McDowell, 1992). In the present study, Co levels in dairy products were measured below 0.005 mg/kg. However, the Co contents reported in cheese samples from Turkey were in the range 0.009-1.54 mg/kg (Merdivan et al., 2004; Bakircioglu et al., 2011).

As an essential element, Ni has several possible roles in the maintenance and production of cells, and as an activator of certain enzyme systems (Bakircioglu et al., 2011). However, at higher levels, the most common adverse health effect in humans is an allergic reaction. Nickel contamination in food may be due to environmental contamination or from production or storage processes of food such as drying, cooking and packaging (Bakircioglu et al., 2011). In the present study, variations in Ni content between cheese types were measured (0.041-0.14 mg/kg). The highest Ni content was determined in melted (0.12 mg/kg) and fat soft cheese (0.14 mg/kg). Measured concentrations were 1.5-8 times lower than those reported in Turkey and France (Mendil, 2006; Arnich et al., 2012). Very high levels of Ni of 1.14 mg/kg were measured in white cheese from Turkey (Merdivan et al., 2004). Concentrations found in butter in this study were similar to levels determined in Brazil by Nardi et al. (2009).

Chromium (Cr) is widely found in the environment. However, the main source of the environmental contamination by Cr is its release from stainless steel containers (Bakircioglu et al., 2011). Regarding concentrations of Cr in foods of animal origin, the highest levels are found in oysters and liver, while low contents are reported for meat (Linder, 1988; Aranda and Llopis, 1993). Chromium is an essential element in carbohydrate metabolism as a component of the glucose tolerance factor, and is involved in cardiovascular risk and metabolic syndrome (Hummel et al., 2007). In this study, Cr in cheeses was found in the mean range of 0.11-0.83 mg/kg and the highest content of 1.13 mg/kg was measured in butter samples.

Chromium concentrations measured in cheese samples were similar, though butter samples contained approximately 2-11 times higher concentrations than those reported in Brazil and France (Nardi et al., 2009; Noël et al., 2012). In contrast to that, lower Cr contents were found in two types of Spanish cheese (González-Weller et al., 2013).

Although it is considered an essential element, chronic exposure to lithium (Li) is often associated with toxic effects. Adverse metabolic effects of Li were described for various internal organs and are related to the cardiovascular system, central and peripheral nervous systems, kidneys, gastrointestinal tract and skin, and can also cause changes in

Table 2. Concentrations of trace elements (mean±SD, mg/kg) in cheeses and dairy products

Dairy products	N	Al	Co	Cr	Li	Mn	Mo	Ni	Sr
Fresh cheese	3	0.56±0.52	<0.005	0.12±0.032	0.015±0.006	2.88±0.27	0.115±0.012	0.098±0.016	1.52±1.71
Melted cheese	2	1.24±1.53	<0.005	0.35±0.062	0.028±0.028	2.49±0.62	0.089±0.033	0.14±0.033	1.21±1.24
Cream cheese	2	<0.01	<0.005	0.11±0.0028	0.021±0.004	1.61±0.027	0.062±0.038	0.041±0.027	2.43±0.79
Soft fat cheese	3	<0.01	<0.005	0.195±0.026	0.023±0.028	1.84±0.40	0.021±0.021	0.12±0.008	1.44±1.038
Semi-hard fat cheese	3	<0.01	<0.005	0.66±0.25	0.016±0.002	4.23±1.25	0.047±0.048	0.051±0.013	0.23±0.054
Cream	2	0.47±0.34	<0.005	0.83±1.17	0.006±0.007	0.21±0.087	0.18±0.095	0.092±0.028	0.11±0.028
Butter	2	3.87±0.082	<0.005	1.13±0.018	0.0075±0.003	0.074±0.008	0.005±0.003	0.071±0.0007	0.11±0.014

Table 3. Trace elements concentrations (mg/kg) in dairy samples from other countries

Country	Cheese type	Al	Co	Cr	Li	Mn	Mo	Ni	Sr	Reference
Spain	Fresh cheese			0.010	4.022				3.031	González-Weller et al., 2013
	Hard cheese			0.015	1.535				11.99	
France	Cheese	0.63							0.004	Millour et al., 2012
			0.0153					0.202		Arnich et al., 2012
				0.173	1.89	0.184	0.076			Noël et al., 2012
France	Butter	2.10							0.238	Millour et al., 2012
			0.046					0.046		Arnich et al., 2012
				0.636		0.038	0.440			Noël et al., 2012
Brazil	Cheese	0.09		0.14		0.25	0.075	0.043	1.2	Nardi et al., 2009
Brazil	Butter	0.09	0.0019	0.10		0.09	0.042	0.034	0.068	Nardi et al., 2009
Turkey	White cheese			0.02-0.55		0.28-1.1		0.18-.0.34		Mendil, 2006
	White cheese	2.37	0.200	0.058		0.052	0.34	1.14		Merdivan et al., 2004

calcium levels, interference with glucose metabolism, hypothyroidism, hyperparathyroidism and weight gain (Paquet et al., 2005; González-Weller et al., 2013). In this study, Li levels ranged from 0.015-0.028 mg/kg in cheeses, and were approximately 2.5-3.7 times lower in cream and butter samples than in cheeses. These values are significantly lower than levels reported from the literatures (Noël et al., 2012; González-Weller et al., 2013).

Molybdenum(Mo) is an essential trace nutrient and plays an important role in the regulation of Ca, Mg and Cu metabolism. It is biologically active as a co-factor to several enzymes, as well as the oxidation of sulphite and the formation of uric acid (McDowell, 1992). In this study, the lowest and highest Mo concentrations were found in butter (0.005 mg/kg) and fresh cheese (0.115 mg/kg). Molybdenum contents in cheese samples were within this range, though the content in butter was significantly lower than the literature data presented in Table 3.

Aluminium (Al) has no essential function in human health, and its toxic effects have been established for the central nervous, skeletal and hematopoietic systems (Krewski et al., 2007). Although different food sources contribute variable amounts of Al to the diet, the greatest amount of Al is added through the FDA-approved food additive usage of sodium aluminium phosphates as an emulsifying salt (Lopez et al., 2002; Yokel et al., 2008). This salt is permitted by the FDA up to 3 % in pasteurized processed cheese. It is added during cheese processing to react with the proteins in cheese in order to create a smooth, uniform film around each fat droplet which prevents separation and the bleeding of fat from cheese. In processed cheeses, levels of 320 (Pennington and Schoen, 1995) and 470 mg Al/kg (Saiyed and Yokel, 2005) were reported. The highest levels of Al recently reported were in crustaceans and molluscs (21.1 mg/kg) and in chocolate (15.6 mg/kg), while in other food groups, including dairy products, Al contents were generally lower than 6 mg/kg (Arnich et al., 2012). In this study, the Al content determined in fresh and processed cheese are in agreement with reported data from France (Millour et al., 2012) but lower than those from Turkey (Merdivan et al., 2004). Alvalues measured in butter were 2-43 times higher than those reported previously (Nardi et al., 2009; Millour et al., 2012).

Manganese (Mn) is recognised as an essential trace element for humans, and its metabolic role includes Mn-containing enzyme systems (Reykdal et al., 2011). The Mn contents in cheese samples were between 1.61-4.23 mg/kg and the lowest and highest Mn concentrations were found in cream cheese and semi-hard fat cheese. The estimated Mn values were in agreement with those reported earlier in cheese and butter (Mendil, 2006; Nardi et al., 2009; Noël et al., 2012).

The biological role of strontium has not been clearly established and is generally regarded as non-toxic (Bonjour et al., 2009). As an alkali metal with chemical similarity to calcium, the toxicity of Strontium (Sr) is due to its interference in biological processes involving calcium, primarily sceletogenesis (Oste et al., 2005). In this study, Sr content ranged from 0.074-2.43 mg/kg in cheese products. The content of Sr in cream and butter was similar and was 2-22 times lower than in cheeses. These results were lower than those recorded for cheeses from Spain (González-Weller et al., 2013) and butter from France (Millour et al., 2012).

To evaluate the measured element levels in cheese samples from the risk assessment perspective, the estimated daily intake (EDI) was calculated and compared with reference toxicological values. For the purposes of national and international health authorities and institutes, World Health Organization (WHO) has reported some critical levels for minor and trace elements, such as the provisional maximum tolerable daily intake (PMTDI) level, the provisional tolerable weekly intake level (PTWI), the dietary reference intakes (DRI) and the total dietary intakes (TDI), an upper level (UL) and the acceptable daily intake (ADI). However, to avoid the confusion of differing values for ADI's of the same substance the permitted daily exposure (PDE) was

Table 4. Estimated daily intakes (EDIs) of elements for the two highest mean concentrations among cheeses and contribution to reference toxicological values

Element/Cheese	EDI[a] (μg/day)	PDE[b] or PTWI[c] (μg/day)	Contribution of EDI to PDE or PTWI (%)
		Al	
Fresh cheese	11.2	20	56
Melted cheese	24.8		124
		Cr	
Melted cheese	7.00	250	2.80
Soft fat cheese	3.90		1.56
		Mn	
Semi-hard fat cheese	84.6	2500	3.38
Fresh cheese	57.6		2.30
		Mo	
Fresh cheese	2.30	300	0.77
Melted cheese	1.78		0.59
		Ni	
Melted cheese	2.80	300	0.93
Soft fat cheese	2.40		0.80

[a]EDI was calculated by the equation: [(element concentration; mg/kg or μg/g) per (meal size or daily intake of food; g/day)] (Copat et al., 2013). Meal size of cheese: 20 g/day per adult (Antonić Degač et al., 2007)
[b]PDE (permitted daily exposure)
[c]PTWI (provisional tolerable weekly intake level)

defined as the pharmaceutically maximum acceptable exposure to residual metals on a chronic basis that is unlikely to produce any adverse health effects (EMEA, 2008). The PDE valued defined for Cr, Mn, Mo and Ni were: Cr 250 μg/day (for a 50 kg individual: 0.005 mg/kg/day); Mn 2.5 mg/day; Mo 300 μg/day; Ni 300 μg/day (6 μg Ni/kg/day in a 50 kg person). A Joint FAO (Food and Agriculture Organization of the UN)/WHO Expert Committee on Food Additives established a revised provisional tolerable weekly intake (PTWI) for Al in food, of 1 mg/kg/bw or 20 μg/day for a 50 kg person (FAO/WHO, 2006). However, WHO did not define any nutritional or toxicological references for Co, Li and Sr (Leblanc et al., 2005).

Table 4 contains the estimated daily intakes (EDI) of Al, Cr, Mn, Mo and Ni for the two cheeses with the highest mean concentrations. EDI was not calculated for cream and butter due to the lack of the available literature data for the average consumption in Croatia. The contribution of EDI values to the reference toxicological values (PDE, permitted daily exposure; PTWI, provisional maximum tolerable daily intake) were calculated for each element. Therefore, the EDIs calculated for the highest two mean values showed a contribution to the PDE values for elements: Mo and Ni below 1 %; Mn and Cr between 1.5 and 3.5%. However, EDIs values calculated for contribution of Al for fresh and melted cheese showed a high contribution (56 and 124 %) to PTWI value.

Conclusions

Significant differences in the concentrations of trace elements Mn, Cr and Al were observed between cheeses and butter. There were no significant differences in levels of Sr, Mo, Ni and Li among the tested products. Concentrations of Al, Cr, Mn and Mo found in cheeses were in line with contents reported in other countries. However, Co, Li, Ni and Sr were lower in comparison to the available literature data. Trace elements measured in butter showed higher Al and Cr, lower Co, Mo and Sr, and similar Mn and Ni concentrations in comparison to previous reports. These results may demonstrate differences in production processes between countries.

Regarding the risk assessment results, it is unlikely that the intake of Cr, Mn, Mo and Ni through cheeses and dairy products would involve any risk for the average consumer. However, the high values for Al determined in fresh and melted cheese in comparison to the toxicity reference values may pose a health risk to consumers.

The results demonstrate the trace element distribution in the tested dairy products and may be important for the assessment of the nutritional and toxicological values for each element.

Sadržaj elemenata u tragovima u siru, vrhnju i maslacu

Sažetak

Elementi u tragovima određeni su u pet vrsta sira, vrhnju i maslacu primjenom induktivno spregnute plazme-optičke emisijske spektrometrije. U uzorcima sira određeni su elementi u tragovima u rasponu (mg/kg): Al 0,01-3,93, Co<0,005, Cr 0,005-1,66, Li 0,008-0,056, Mn 0,068-5,37, Mo 0,003-0,225, Ni 0,01-0,163 i Sr 0,085-3,49. Utvrđene su statistički značajne razlike u koncentracijama Mn, Cr i Al (p<0,01, svi) između mliječnih proizvoda. Nije bilo značajnih razlika u koncentracijama Sr, Mo, Ni i Li među proizvodima. Najviše koncentracije izmjerene su u proizvodima (mg/kg): Mn 4,23 za polutvrdi masni sir, Sr 2,43 za krem sir, Mo 0,18 za vrhnje, Ni 0,14 i Li 0,028 za topljeni sir, Cr 1,13 i Al 3,87 za maslac. Koncentracije elementa u tragovima u sirevima i maslacu varirale su u usporedbi s podacima iz literature. Koncentracije Al, Cr, Mn i Mo u sirevima te Mn i Ni u maslacu bile su u skladu sa sadržajem utvrđenim u drugim zemljama. Ovi rezultati pokazuju razlike u proizvodnim procesima između zemalja. Dnevni unosi (eng. EDIs, estimated daily intakes) izračunati za Cr, Mn, Mo i Ni u sirevima pokazuju mali doprinos (0,59-3,38 %) prema vrijednostima dopuštenih dnevnih izloženosti (eng. PDE, permitted daily exposure) određenih za te elemente. Međutim, visoki doprinos Al (56 i 124 %) prema PTWI vrijednosti (eng. provisional maximum tolerable daily intake) u svježem i topljenom siru može predstavljati rizik za zdravlje potrošača.

Ključne riječi: sir, mliječni proizvodi, elementi u tragovima, Al, Co, Cr, Li, Mn, Mo, Ni, Sr, ICP-OES

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