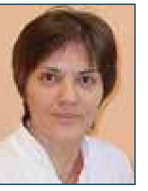
Macro- and microelement contents in sterilized milk of different manufacturers from Croatia and the EU



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Abstract

The aim of this study was to investigate the differences in macro- (Ca, Na, Mg) and microelement (Co, Cr, Cu, Fe, Mn, Mo, Se, Zn) concentrations in processed cow milk samples. Sterilized UHT (ultra-high temperature) milk with 2.8% milk fat produced by five different milk producers in Croatia and one milk producer in the European Union (EU) were randomly purchased from large marketplaces in the Croatian capital, Zagreb. Element concentrations were analysed by inductively coupled plasma mass spectrometry (ICP-MS). Mean element concentrations were in the range (mg/kg): Ca 1111-1285, Na 361.1-453.3, Mg 101.2-113.7, Zn 3.85-4.33; (μg/kg): Fe 180.7-269.1, Cu 36.2-45.1, Mo 33.3–47.7, Mn 22.9-31.1, Se 14.7-26.4, Cr 1.91-5.24, Co 0.19-0.32. Significant differences in the content of Ca, Cr, Na, Mn, Mo, Se and Zn were determined between milk samples of different producers. There were no significant differences in the concentrations of Cu or Co. Milk of Croatian producers showed significant differences in Mg, Fe, Mo, Mn, Se and Cr levels compared to milk from the EU producer. The highest concentrations of Fe, Cr and Co were found in the EU milk. Given the importance and frequency of use of processed cow's milk as a foodstuff, the measurement of macro- and microelement contents plays an important role in the evaluation of its nutritional value.

Key words: *sterilized milk; macroelements; microelements; ICP-MS; Croatia; EU*

Introduction

Cow's milk is an important foodstuff containing many essential amino acids, lipids, vitamins, soluble fibre, and significant quantities of essential elements. Considering the large number of bioactive compounds in milk and dairy products, this food category has a strong positive effect on human health (Król et al., 2017). The main areas of the biological activity of milk involve the proper development of infants, gastrointestinal development, immune

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system development and function, and microbiological activity, including antibacterial and probiotic activity (Park, 2009). Milk contains 20 elements considered to be nutritionally essential for humans and which can be classified as macroelements (calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), and chloride (Cl), phosphorus (P)) or microelements (iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), chromium (Cr), cobalt (Co), molybdenum (Mo), iodine (I), fluoride (F), arsenic (As), nickel (Ni), silicon (Si) and boron (B)) (Cashman, 2003). In a typical serving size of milk (200 mL), the percent contribution of macroelements and selected microelements to the dietary reference value were (%): Ca 22.4, Na 7.1, K 5.8, Mg 5.9, Fe 0.8, Zn 8.2, Cu 2.0, Se 3.6, Cr 1.33, Mo 22.2 (Cashman, 2006).

Among the many macro- and microelements in milk, high levels of calcium (Ca) play an important role in the development of children and give strength and density to bones, which is important in the prevention of osteoporosis in the elderly. High blood pressure and cardiovascular diseases could be prevented by eating a diet high in Ca, Mg and K, and low in Na. Calcium is also beneficial in reducing the absorption of cholesterol and in controlling body weight, and also supporting normal skeletal growth and development and its maintenance during later life (Cashman, 2006). It is considered that cow's milk and dairy products in the human diet provides about 70% of the recommended daily intake of Ca (Park, 2009).

Trace elements such as Cu, Zn, Fe, Se, Cr, Co, Mn and Mo and other micronutrients have varying functions in the maintenance of a wider range of physiological functions, and therefore their presence in milk is also of critical importance for nutrition (Noël et al., 2012). It is important to emphasize that the chemical form of macroelements and microelements in milk influence their intestinal absorption and the process of transport, cellular assimilation and conversion into a biologically active form and therefore bioavailability (Cashman, 2006).

Most studies to date have focused on the content of macro- and microelements in raw cow milk in different countries (Ceballos et al., 2009; Nardi et al., 2009; Sola-Larrańaga and Navarro-Blasco, 2009; Soares et al., 2010; Navarro-Alarcón et al., 2011; Arnich et al., 2012; Chekri et al., 2012; Millour et al., 2012; Noël et al., 2012; Bilandžić et al., 2015). Few studies have examined element contents in processed milk (Soares et al., 2010; Vahčić et al., 2010; Rey-Crespo et al., 2013).

The aim of this study was to measure and compare the content of macro- (Ca, Na, Mg) and microelements (Co, Cr, Cu, Fe, Mn, Mo, Se, Zn) in processed cow's milk from different manufacturers using inductively coupled plasma with mass spectrometry (ICP-MS).

Materials and methods

Sampling

A total of 30 samples of sterilized UHT (ultra-high temperature) milk with 2.8% milk fat content were collected in large market centres in the Croatian capital, Zagreb. Five milk samples were sampled from six milk producers: five producers from Croatia (P1–P5) and one from the European Union (EU). Samples were labelled and stored at + 4 °C until analysis.

Chemicals and Standards

Acid HNO_3 was purchased from Merck (Darmstadt, Germany). For analysis, ultra pure water (18.2 MΩ/cm resistivity) obtained from system Milli-Q Advantage 10V (Millipore Corporation Merck, Darmstadt, Germany) was used. For instrument calibration, certified standards consisting of Ca, Co, Cr, Cu, Fe,

Table 1. ICP-MS operating conditions and measurement parameters.

Torch injector	Quartz
Spray chamber	Peltier Cooled Cyclonic
Sample uptake	0.3 rps (rounds per second)
Nebulizer Type	MicroMist
Interface	Pt-cones
RF power	1550 W
Ar gas flow rate	plasma 15L/min; auxiliary 0.9 L/min
Nebulizer pump	0.1 rps
He gas flow rate	0.03 mL/min
Ion lenses model	x-lens
Lens voltage	10.7 V
Omega bias	- 90 V
Omega lens	10.2 V
Acquisition mode	Spectrum
Peak Pattern	1 point
Integration time	2000 ms
Replicate	3
Sweeps/replicate	100
Tune mode (Stabilization time; Integ. time/mass)	No gas: 0 s; 0.1 s He: 5 s; 0.5 s HEHe: 5 s; 1 s
ICP-MS (standard mode)	No gas: Na^{23}, Mg^{24}, Mo^{95}
	He mode: Ca^{43}, Cr^{52}, Mn^{55}, Co^{59}, Cu^{63}, Zn^{66}
	HEHe: Se^{78}, Fe^{56}
Internal standards	^{209}Bi, ^{115}In, ^{45}Sc

Mg, Mn, Na, Se and Zn (Environmental Calibration Standard, Agilent Technologies, USA) at a concentration of 10 mg/L were used. Stock solutions for ICP-MS analysis were prepared by dissolving the multi-element standard mixture solution with ultra pure water. Working solutions were prepared by serial dilution of stock solutions with 5.0% v/v HNO_3 and kept at room temperature until further use.

The certified standard consisting of Bi, In, Sc, Y and Tb (Inorganic Ventures, Blacksburg, VA, USA) at a concentration of 20 mg/L was used as the internal standard for ICP-MS to correct for sensitivity drift and the matrix effect.

Sample preparation

Samples (1 g) were weighed into a Teflon dish with the addition of 3 mL H_2O, 2.5 ml HNO_3 (65%) and 1 mL H_2O_2. Wet digestion by microwave oven was performed using a digestion programme consisting of three potency steps: first step at 500 W for 2.5 minutes, second step at 1000 W for 20 minutes, and the third step at 1200 W for 30 minutes.

After cooling to room temperature, the digested clear solution was quantitatively transferred to a 50 mL volumetric flask and made up to the mark with ultra-pure water. All solutions were then spiked with the internal standard (Inorganic Ventures, Blacksburg, VA, USA) to the final concentration of 10 μg/L.

Quantitative analysis was performed via the calibration curve method. Calibration curves were built with a minimum of five concentrations of standards per element. The limits of detection (LODs) were calculated as three times the standard deviation of 10 consecutive measurements of the reagent blank, multiplied by the dilution factor used for sample preparation. LOD values determined were (mg/kg): Ca 0.01, Co 0.001, Cr 0.001, Cu 0.001, Fe 0.005, Mg 0.005, Mn 0.001, Mo 0.001, Na 0.01, Se 0.005 and Zn 0.01.

Instruments

Milk samples were digested using a high-pressure microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany). Element concentrations were determined using an inductively coupled plasma instrument with mass detector Agilent ICP-MS system Model 7900 (Agilent, Palo Alto, CA, USA). The sample introduction system consisted of a quartz cyclonic spray chamber and a Meinhard® nebulizer connected to the peristaltic pump of the spectrometer with Tygon® tubes. The ICP-MS was operated with a platinum sampler and skimmer cones. The peristaltic pump of the ICP-MS was set at 20 rpm. High-purity argon (99.999%, White Martins, Brazil) was used throughout. The instrumental working parameters and experimental conditions for ICP-MS are shown in Table 1.

Statistical analysis

Element concentrations were expressed as mean ± standard deviation (SD), and as the minimum and maximum concentration. Statistically significant differences between concentrations of elements in the milk of different producers were determined by the Student *t*-test. Differences at the level of probability $P \leq 0.05$ were considered statistically significant. For statistical analysis, the statistical program Statistica® 10 (StatSoft®, Tulsa, USA) was used.

Results and Discussion

Milk and milk products are important dietary sources of macro- and microelements and thus play an important role in maintaining health. Due to this nutritional relevance for human health, it is therefore necessary to determine the element concentrations in milk. Previous studies on the multi-element analysis of milk have typically been focused on raw milk samples and differentiation of the milk of different animals, or differences due to the sampling period or location. In the present study, elemental analysis was carried out on processed milk types produced by different manufacturers.

The concentrations of macroelements and the most important microelements in processed milk produced by six different manufacturers are shown in Table 2. The element concentrations ranged in the following order from high to low: Ca > Na > Mg > Zn > Fe > Cu > Mo > Mn > Se > Cr > Co. Mean element concentrations were determined in the ranges (mg/kg): Ca 1111–1285, Na 361.1–453.3, Mg 101.2–113.7, Zn 3.85–4.33; (μg/kg): Fe 180.7–269.1, Cu 36.2–45.1, Mo 33.3–47.7, Mn 22.9–31.1, Se 14.7–26.4, Cr 1.91–5.24, Co 0.19–0.32. Significant differences in the concentrations of Ca, Cr, Na, Mn, Mo, Se and Zn were determined between milk samples of different producers. There were no significant differences in the concentrations of Cu or Co. The highest element levels were determined as follows: P1: Mg and Zn; P2: Cu and

Table 2. Concentrations of macroelements and microelements in sterilized cow's milk (2.8% fat) of five producers from Croatia and one from the European Union.

Element	Statistics	P 1	P 2	P 3	P 4	P 5	EU
Ca [a] (mg/kg)	Mean ± SD Min-Max	1281 ± 84.2 1211 - 1374	1130 ± 42.4 1088 - 1173	1111 ± 44.1 1069 - 1157	1114 ± 68.0 1043 - 1178	1285 ± 294.6 1115 - 1625	1116 ± 25.2 1099 - 1134
Na [b] (mg/kg)	Mean ± SD Min-Max	418.2 ± 25.7 399.7 - 447.5	393.1 ± 18.4 373.8 - 410.4	361.1 ± 17.3 342.0 - 375.9	365.5 ± 286.2 342.9 - 397.7	453.3 ± 88.7 390.6 - 554.8	387.0 ± 17.7 374.5 - 399.6
Mg [c] (mg/kg)	Mean ± SD Min-Max	113.7 ± 6.84 109.3 - 121.6	102.1 ± 4.62 96.9 - 105.8	102.7 ± 57.0[a] 96.3 – 107.2	104.9 ± 8.54 97.7 - 114.6	107.5 ± 2.28 91.5 - 133.5	101.2 ± 4.72 97.9 - 104.6
K (mg/kg)	Mean ± SD Min-Max	16.5 ± 1.19 15.7 - 17.9	14.6 ± 0.44 14.2 - 15.1	15.2 ± 0.41 14.8 - 15.6	15.6 ± 1.08 14.8 – 16.8	16.4 ± 3.05 14.6 - 19.9	14.8 ± 043 14.5 - 15.1
Zn [d] (mg/kg)	Mean ± SD Min-Max	4.33 ± 0.16 4.18 - 4.49	3.85 ± 0.052 3.82 - 3.91	3.89 ± 0.14 3.74 – 3.99	3.95 ± 0.11 3.87 – 4.07	3.93 ± 0.85 3.32 - 4.89	4.03 ± 0.21 3.88 - 4.18
Fe [e] (µg/kg)	Mean ± SD Min-Max	233.8 ± 53.7 173.3 - 274.3	243.1 ± 65.6 169.9 - 297.5	180.7 ± 40.5 136.2 – 215.1	205.1 ± 41.4 159.3 - 240.5	222.8 ± 18.6 210.8 - 243.9	269.1 ± 9.3 262.7 - 275.8
Cu (µg/kg)	Mean ± SD Min-Max	42.7 ± 4.02 38.1 - 45.4	45.1 ± 4.86 39.5 - 48.7	44.9 ± 4.22 40.2 – 48.2	36.2 ± 3.92 31.7 - 39.3	43.0 ± 11.3 33.4 – 55.4	41.0 ± 3.91 38.2 - 43.8
Mo [f] (µg/kg)	Mean ± SD Min-Max	37.5 ± 0.88 36.9 - 38.6	33.3 ± 0.59 32.6 - 33.7	36.9 ± 1.16 36.1 - 38.3	33.3 ± 0.74 32.4 - 33.8	47.7 ± 11.3 40.9 - 60.7	36.1 ± 1.16 35.2 - 36.9
Mn [g] (µg/kg)	Mean ± SD Min-Max	27.9 ± 1.5 26.4 - 29.1	31.1 ± 2.14 28.4 – 32.1	23.2 ± 1.71 20.8 – 24.1	22.9 ± 1.41 21.0 – 23.7	27.9 ± 3.42 25.4 – 31.6	31.0 ± 3.7 28.4 – 33.7
Se [h] (µg/kg)	Mean ± SD Min-Max	21.6 ± 0.95 20.5 - 22.3	17.9 ± 2.25 16.3 - 20.5	21.8 ± 1.51 20.4 - 23.4	26.4 ± 2.92 23.2 - 28.9	14.7 ± 5.24 10.7 - 20.6	25.2 ± 0.47 24.9 - 25.6
Cr [i] (µg/kg)	Mean ± SD Min-Max	2.83 ± 1.65 0.93 - 3.85	5.24 ± 4.36 0.20 - 7.89	2.69 ± 1.60 0.84 - 3.67	4.52 ± 1.28 3.04 - 5.28	1.91 ± 1.44 0.25 - 2.81	18.8 ± 1.06 18.1 - 19.6
Co (µg/kg)	Mean ± SD Min-Max	0.19 ± 0.066 0.14 - 0.26	0.23 ± 0.095 0.16 - 0.34	0.14 ± 0.099 0.082 - 0.26	0.32 ± 0.13 0.23 - 0.47	0.25 ± 0.065 0.19 - 0.32	0.35 ± 0.11 0.27 - 0.43

Statistically significant differences in element content between different producers:
[a] P1-P2 $P<0.05$, P1-P3 $P<0.05$
[b] P1-P3 $P<0.05$
[c] P1-EU $P<0.001$, P2-EU $P<0.001$, P3-EU $P<0.001$, P4-EU $P<0.001$, P5-EU $P<0.001$
[d] P1-P2 $P<0.01$, P1-P3 $P<0.01$, P1-P3 $P<0.01$
[e] P5-EU $P<0.05$
[f] P1-P2 $P<0.001$, P1-P4 $P<0.001$, P2-P3 $P<0.01$, P2-EU $P<0.05$, P3-P4 $P<0.01$, P4-EU $P<0.05$
[g] P1-P2 $P<0.01$, P1-P4 $P<0.01$, P2-P3 $P<0.01$, P2-P4 $P<0.001$, P3-EU $P<0.05$, P4-EU $P<0.05$
[h] P1-EU $P<0.01$, P2-P4 $P<0.01$, P2-EU $P<0.01$, P4-EU $P<0.01$
[i] P1-EU $P<0.001$, P2-EU $P<0.05$, P3-EU $P<0.001$, P4-EU $P<0.001$, P5-EU $P<0.001$

Mn; P4: Se; P5: Ca, Na and Mo. The milk of the EU producer contained the highest concentrations of Fe, Cr and Co.

The concentrations of macro- and microelements in raw cow milk and heat-treated milk from previous studies are presented in Table 3. Macroelement concentrations were reported in the ranges (mg/kg): Ca 845.7–1936, Na 372–600, Mg 91.8–150.1 (Ceballos et al., 2009; Nardi et al., 2009; Soares et al., 2010; Sola-Larranaga and Navarro-Blasco, 2010; Vahčić et al., 2010; Navarro-Alarcón et al., 2011; Chekri et al., 2012; Bilandžić et al., 2015). The concentrations of Ca, Na, and Mg determined in this study were within the range specified for raw milk. Studies reporting macroelement concentrations in UHT milk are scarce. In pasteurized milk from Brazil, a Ca content of 888 mg/kg and Mg of 105 mg/kg were reported (Soares et al., 2010). In this study, all milk samples showed significantly higher concentrations of

Table 3. Literature reports on macro- and microelement concentrations in raw and heat treated cow milk.

Element	Croatia [1*, 2]	Brazil [3, 4*]	France [5, 6, 7, 8]	Spain [9, 10, 11, 12*]
Ca (mg/kg)	845.7-927.8 [1*] 1400 [2]	888 [4*]	1026 [5]	970 [9] 1135.8 [10] 1936 [11]
Na (mg/kg)	466.3-492.7 [1*] 600 [2]		432 [5]	372 [9]
Mg (mg/kg)	110.8-122.7 [1*] 100 [2]	101 [3] 105 [4*]	120 [5]	91.8 [9] 94.0 [10] 150.1 [11]
K (mg/kg)	1560.7-1580 [1*] 1900 [2]		1679 [5]	1344 [9]
Zn (mg/kg)	3.22-3.80 [1*] 4.0 [2]	4.8 [3] 4.59 [4*]	3.75 [7]	4.631 [9] 4.63 [10] 4.03 [11] 3.93 [12*]
Fe (µg/kg)	530-990 [1*] 500 [2]	1050 [4*]	399 [6]	290 [9] 900 [10] 351 [12*]
Cu (µg/kg)	140-240 [1*] 13.0 [2]	190 [3] 1730 [4*]	91 [7]	51.8 [9] 140 [10] 68.9 [12*]
Mn (µg/kg)	55 - 68 [1*] 43 [2]	81 [4*]	29 [7]	29.1 [9] 25.3 [12*]
Mo (µg/kg)	20 [2]	45 [3]	46 [7]	41.7 [12]
Se (µg/kg)	25 [2]	43 [3]	40 [7]	9,7 [9] 19.2 [12*]
Cr (µg/kg)	34 [2]	32 [3] 79 [4*]	120 [7]	4 [9] 4.63 [12*]
Co (µg/kg)		10.1 [3]	3.6 [8]	4.95 [12*]

[1*] Vahčić et al. (2010) - pasteurized and sterilised milk
[2] Bilandžić et al. (2015)
[3] Nardi et al. (2009)
[4*] Soares et al. (2010) - pasteurized milk
[5] Chekri et al. (2012)
[6] Millour et al. (2012)
[7] Noël et al. (2012)
[8] Arnich et al. (2012)
[9] Sola-Larrańaga and Navarro-Blasco (2009)
[10] Ceballos et al. (2009)
[11] Navarro-Alarcón et al. (2011)
[12*] Rey-Crespo et al. (2013) - UHT milk

Ca, while the concentrations of Mg were similar. In comparison with a previous study conducted for pasteurized and sterilised milk in Croatia, the Ca levels obtained in this study were lower, while Na concentrations were higher and ranged from 466.3 to 492.7 mg/kg (Vahčić et al., 2010).

The microelement zinc plays an important role in a number of physiological functions and has catalytic, structural and regulatory functions in the organism (King and Cousins, 2014). It is involved in many aspects of cellular metabolism and is necessary for the catalytic activity of over 200 enzymes. It also plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division (Prasad, 1995; Solomons, 1998; Classen et al., 2011). Zinc concentrations in this study were determined in the range 3.85–4.33 mg/kg. Significantly higher Zn levels were measured in the milk of producer P1 than in the milk of producers P2, P3 and P4 (P<0.01, all). Zn contents were similar to previous reports from Croatia and other countries (Vahčić et al., 2010; Soares et al., 2010; Navarro-Alarcón et al., 2011; Noël et al., 2012; Rey-Crespo et al., 2013).

Copper plays an important role in many physiological processes in the organism, such as optimising production and reproduction, the role of cofactors for enzymes responsible for glucose metabolism and the synthesis of haemoglobin, and also connective tissue and phospholipids, in the process of iron utilisation and also in oxidation-reduction reactions (Linder and Hazegh-Azam, 1996; Nardi et al., 2009). Copper metabolism is involved in the synthesis of catecholamines, ATP production and protection against free radicals (Meyer et al., 2001). In this study, no significant differences were found in the Cu concentrations between different UHT milk producers in Croatia. Copper levels were measured in the range 36.2–45.1 µg/kg and were similar to levels obtained in the milk of the EU producer. In previous studies conducted in Croatia, Cu content was significantly lower in raw milk (Bilandžić et al., 2015), but was significantly higher in pasteurized and sterilised milk (Vahčić et al., 2010). Also, very different Cu concentrations have been reported in the literature, in the range 51.8–190 µg/kg (Ceballos et al., 2009; Nardi et al., 2009; Sola-Larranaga and Navarro-Blasco, 2010; Noël et al., 2012; Rey-Crespo et al., 2013). The highest Cu levels of 1730 µg/kg were measured in pasteurized cow milk from Brazil (Soares et al., 2010).

Iron participates in various metabolic processes in the body, and is physiologically important as a component of cytochrome and oxygen-binding molecules, and also in DNA synthesis and electron transfer (Evans and Halliwell, 2001; Kuvibidila et al., 2001). In milk, Fe occurs in combination with several proteins, such as transferrin, lactoferrin and ferrilactin (Park, 2009). In this study, Fe concentrations ranged from 180.7 µg/kg to the highest level of 269.1 µg/kg measured in EU milk. A significantly higher Fe content was found in EU milk than in milk of producer P5 (P<0.05). In previous studies conducted in Croatia, Fe levels were measured in the range 500-990 µg/kg (Vahčić et al., 2010; Bilandžić et al., 2015). A high Fe content, of even 1050 µg/kg has been reported in raw milk from Brazil (Soares et al., 2010).

As an essential element, Se is a component of the enzyme glutathione peroxidase and protects membrane lipids, proteins and nucleic acid against damage by free radicals and oxidants (Liu et al., 2008). In the present study, the mean values of Se in UHT milk of different producers ranged from 14.7 to 26.6 µg/kg. Significant higher Se levels were determined in UHT milk of the EU producer than in milk of producers P1, P2 and P4 (P<0.01, all). Also, milk of producer P2 had a significantly higher Se content than milk of producer P4 (P<0.01). Selenium concentrations determined in this study were significantly lower than those reported in raw milk from Brazil (Nardi et al., 2009) and France (Noël et al., 2012), but similar to previous reports from Croatia (Bilandžić et al., 2015).

Manganese is an essential metal required for many metabolic and cell functions, and is also a structural part of the particular enzyme and cofactor in a series of enzymatic reactions (Liu et al., 2008). In milk, a large proportion of Mn, together with Cu and Zn, is bound to milk casein (Park, 2009). In this study, Mn mean values in UHT milk of Croatian producers ranged from 22.9 to 31.1 µg/kg, and the level measured in EU milk was 31.0 µg/kg. Significant differences in Mn levels were determined between milk of producers P1 and P2, P1 and P4, P2 and P4 ($P<0.01$, all). Also, a significantly higher Mn content was determined in EU milk than in milk of P3 and P4 ($P<0.05$, both). In previous studies, Mn levels in raw milk were measured from 29 to 81 µg/kg (Sola-Larrańaga and Navarro-Blasco, 2009; Soares et al., 2010; Noël et al., 2012). The Mn levels obtained in this study were 1.7 to 3 times lower than previous reports of Mn levels in pasteurized and sterilised milk from Croatia (Vahčić et al., 2010).

Molybdenum plays a role as a cofactor for the enzymes involved in the catabolism of sulphur amino acids and heterocyclic compounds including purines and pyridines (Turnlund et al., 1995). In this study, mean Mo values in UHT milk samples ranged from 33.3 to 47.7 µg/ kg. Significantly higher Mo levels were determined in UHT milk of P1 than in milk of producers P2 and P4 ($P<0.001$, both). The milk of producer P3 contained a significantly higher Mo content than those of P2 and P4 ($P<0.01$, both). Milk of the EU producer had a significantly higher Mo content than the milk of producers P2 and P4 ($P<0.05$, both). In a previous study conducted in Croatia, a lower Mo level of 20 µg/kg was measured in raw milk (Bilandžić et al., 2015). Studies conducted in other countries reported Mn concentrations between 41.7 and 46 µg/kg (Nardi et al., 2009; Noël et al., 2012; Rey-Crespo et al., 2013).

Cobalt is an essential element required for the formation of vitamin B12 (hydroxocobalamin), which is necessary for the synthesis of methionine from homocysteine, and for folate metabolism and purine modification of methylmalonyl-coenzyme A to succinyl-coenzyme A (Barceloux, 1999). In the present study, Co mean values in UHT milk were in the range 0.14–0.35 µg/kg. There were no significant differences in the Co content between the tested milk samples. Concentrations obtained were more than 25–72 times lower than levels obtained in Brazil, France and Spain (Nardi et al., 2009; Noël et al., 2012; Rey-Crespo et al., 2013).

The role of Cr in the organism has not yet been fully clarified. The biological role of Cr and its impact on living organisms and the molecular mechanism is known to be its participation in gene structure and expression (Okada et al., 1989). Furthermore, it has an effect on insulin binding and increasing the number of insulin receptors on the cell surface and the sensitivity of the pancreatic β-cell, together resulting in an overall increase in insulin sensitivity (Anderson, 1997). There is a correlation between Cr and metabolism during increased physiological, pathological and nutritional (food) stress, such as fatigue, trauma, pregnancy, metabolic, physical, emotional and environmental stress (Anderson, 1994). Chromium concentrations in UHT milk of Croatian producers in this study were measured in the range 1.91–5.25 µg/kg. It is important to state that the milk from the EU producer contained 3.7–10 times more Cr than the milk of Croatian producers. Therefore, the statistical analysis showed significantly higher Cr levels in EU milk than in the milk of P1, P3, P4 and P5 ($P<0.001$, all) and P2 ($P<0.05$). Previously measured Cr levels in raw milk in Croatia were substantially higher (34 µg/kg; (Bilandžić et al., 2015). Studies from other countries have reported substantially higher Cr levels, with the highest Cr level of 120 µg/kg measured in France (Noël et al., 2012). On the other hand, in Italy, Cr content showed a mean value of 1.5 μg/kg and Cr was not detectable in 85% of samples (Licata et al., 2004).

It has been previously suggested that the heat treatment of milk may affect its element composition, resulting in milk with lower concentrations than in raw milk (Malbe et al., 2010). Seasonal variations have also been reported to have an influence on microelement concentrations in organic cow milk. In other studies, concentrations were higher during winter for all elements; for example, statistically higher Fe levels were obtained during winter than in summer (Rey-Crespo et al., 2013).

Conclusions

In the present study, significant differences were detected in the contents of Ca, Cr, Na, Mn, Mo, Se and Zn between different Croatian milk producers. The milk of Croatian producers showed significant differences in the concentrations of Mg, Fe, Mo, Mn, Se and Cr compared to milk from the EU producer. Milk from the EU contained the highest concentrations of Fe, Cr and Co, where the Cr content was 3.7-10 times higher than in the milk of Croatian producers. In conclusion, differences in microelement concentrations between this study and reports from other countries may be due to a range of factors, including the important ecological status of geographic locations and differences in soil element contents. Given the importance and frequency of use of processed cow's milk as a foodstuff, the determination of concentrations of macro- and microelements plays an important role in the evaluation of their nutritional value.

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Sadržaj makro- i mikroelemenata u steriliziranom mlijeku različitih proizvođača iz Hrvatske i EU

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Cilj ovog istraživanja utvrđivanje razlika u koncentraciji makro (Ca, Na, Mg) i mikro elemenata (Co, Cr, Cu, Fe, Mn, Mo, Se, Zn) u uzorcima procesiranog kravljeg mlijeka. Sterilizirano UHT (engl. ultra-high temperature) mlijeko s 2,8% mliječne masti pet različitih proizvođača mlijeka iz Hrvatske i jednog iz Europske unije (EU) nasumice su prikupljena u velikim trgovačkim lancima glavnog grada Zagreba. Koncentracije elemenata su određivane primjenom induktivno spregnute plazme s masenom spektrometrijom (ICP-MS). Srednje vrijednosti koncentracija elemenata kretale su se u rasponima (mg/kg): Ca 1111-1285; Na 361,1-453,3; Mg 101,2-113,7; Zn 3,85-4,33; (µg/kg): Fe 180,7-269,1; Cu 36,2-45,1; Mo 33,3-47,7; Mn 22,9-31,1; Se 14,7-26,4; Cr 1,91-5,24; Co 0,19-0,32. Ustvrđene su statistički značajne razlike u sadržaju elemenata Ca, Cr, Mn, Na, Mo, Se i Zn između mlijeka različitih proizvođača. Nije bilo značajnih razlika u koncentracijama Cu i Co. Mlijeko hrvatskih proizvođača pokazalo je značajne razlike u koncentracijama Mg, Fe, Mo, Mn, Se i Cr u odnosu na mlijeko iz EU. U mlijeku iz EU izmjerene su najviše koncentracije Fe, Cr i Co. S obzirom na važnost i učestalost uporabe obrađenog kravljeg mlijeka kao prehrambenog proizvoda, određivanje sadržaja makro i mikroelemenata ima važnu ulogu u procjeni njihove nutritivne vrijednosti.

Ključne riječi: *sterilizirano mlijeko; makroelementi; mikroelementi; ICP-MS; Hrvatska; EU*