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EVALUATION OF HEAVY METALS AND TOXIC ELEMENTS CONTENT IN TIME IN BIO AND NON-BIO YOGURTS BY ICP-MS METHOD

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This paper presents the analysis of 29 samples of bio and non-bio yogurt (simple and fruits) in order to determine the content of metals, those essential for human health, heavy, and potentially toxic elements, from the content nearby the jar and cap. The metals were determined by ICP-MS, after digestion of the samples. The analysis of 12 toxic elements from yogurt samples revealed that the elements concentration in samples collected from the cap are higher than in those collected from the jar and that the content of elements is higher in bio yogurts. Moreover the variation of element content with time for all yogurts was studied.

Keywords: toxic elements, heavy metals, bio-, non-bio-, simple-, fruits yogurts, ICP-MS, variation

1. Introduction

Dairy products are considered to be a great source of nutrients, such as: proteins, fat, vitamins, minerals and probiotic bacteria; therefore they are included in the human diet and consumed by all age groups [1, 2]. Yogurts are dairy products obtained by fermentation of milk with bacterial cultures, which contributes to colon health, improve the bioavailability of other nutrients, are a rich source of calcium preventing the occurrence of osteoporosis, are an ideal source of macro- and microelements, have cosmetic use in cleaning and hydrating the skin, reducing sun burns, fighting acne and aging process; therefore present a real benefit for human health.

In addition to the macroelements (Ca, P, K) and microelements (Cu, Fe, Zn, Mn, Se), which can be cofactors in enzymatic processes and play an important role in many physiological functions of the body, amounts of heavy metals can occur in yogurts [1, 2]. The presence of heavy metals is caused by different agricultural activities like irrigation with heavy metal-contaminated water, use of pesticides, also by treating the cows with different drugs; all of these resulting in toxic metal contamination of feeds, meat, and milk [3]. Other sources of heavy

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metals are the manufacturing and packaging processes and also the packaging material [1, 2, 4, 5]. The exposure of the organism at levels higher than those admitted by food legislation, even for essential microelements, can cause different adverse effects, including cancer and mutations [1].

For metals determinations several methods are commonly used: flame atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry, atomic fluorescence spectrometry, flow injection spectrometric methods, capillary zone electrophoresis, inductively coupled argon plasma emission spectroscopy, differential pulse anodic stripping voltammetric technique and stripping potentiometry [4, 6].

Due to good sensibility and selectivity inductively coupled plasma-mass spectrometry (ICP-MS) was used to determine the content of metals in a variety of food samples including bio and non-bio yogurts (simple and fruits) [7-9]. The aim of the study was to determine the content of 12 toxic elements – aluminium (Al), cobalt (Co), chromium (Cr), iron (Fe), nickel (Ni), calcium (Ca), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), arsenic (As)- in 29 samples of different types of yogurt, both from the content nearby the jar and cap, in order to evaluate the influence of the packaging on the yogurt inside, especially since some people, mainly from the vulnerable groups, as children or elders, eat the yogurt from the cap, and their health could be affected [10,11]. Also, the variation of toxic elements content with time, for some types of yogurt was evaluated. To our knowledge such investigations have novel character and importance for nutrition education.

2. Experimental

2.1. Sample preparation

29 types of yogurt (8 bio: 4 simple and 4 with fruits, 21 non-bio: 8 simple and 13 with fruits) were obtained from Romanian local markets.

The digestion was carried out in a CEM Mars 5 microwave digestion oven with programmable power control (0 – 1200 W), HP-500 Plus vessel of 100 mL maximum volume with operating pressure and temperature of maximum 350 psi and 210°C, respectively.

For the toxic elements content determination, the digestion was carried out by collecting samples directly in the digestion vessel, for each type of yogurt. 1 g of each sample was collected from the jar of yogurts and from the content nearby the jar and cap. For each sample, 6 mL HNO_3 (69%), from Merck, and 3 mL ultra pure water were added. Then, they were left to react in order to reduce the gases that could accumulate in the vessels, after which they were left to digest for 3 days.

For the variation of elements content with time, the samples preparation was the same but the samples were collected at moment zero (when the jar was opened), and after 24, 48 and 72 hours.

2.2. Samples analysis

Perkin Elmer Sciex ELAN DRC-e inductively coupled plasma-mass spectrophotometer (ICP-MS) was used for the determination of metals content, with the operating conditions described in a previous paper [12] and presented in table 1. Equipment used during experiments was calibrated according to approved calibration procedures. 1 mL from each digested sample was quantitatively transferred into volumetric flasks and diluted to 50 mL with HNO_3 solution 1% in ultra pure water. In order to analyse the solutions, a multi-element calibration standard (10µg/mL) as internal standard was used. A calibration curve was used before reading each sample with a concentration between 1 ppb (part per billion) and 1 ppm (part per million).

Table 1

ICP-MS operating conditions

ICP-MS instrument	**Perkin Elmer SCIEX ELAN DRC-e**
Plasma conditions	
Rf power	1500 W
Plasma gas flow	15 L min^{-1}
Aerosol gas flow	1.2 L min^{-1}
Mass spectrometer settings	
Resolution	Normal
Dweel time	500 ms
Sweeps/reading and Points/spectral peak	5 and 1 respectively
Readings/replicate	400
Sensitivity of DRC-e	
Mg	>50.000 cps/10 ppb
In	>250.000 cps/10 ppb
U	>200.000 cps/10 ppb
Background	220<2 cps
Oxides	Ba^{2+}/Ba<2%
	CeO/Ce<3%

3. Results and discussions

3.1. Toxic elements content

Results of elements content obtained for bio simple yogurts (samples collected from the jar and cap) are presented in table 2.

Table 2

Elements content in bio yogurts from the jar and cap (n=4)

Element	Jar				Cap			
	min (ppb)	max (ppb)	mean (ppb)	σ	min (ppb)	max (ppb)	mean (ppb)	σ
Al	65.01	97.82	83.69	16.18	140.71	242.30	200.02	43.69
Co	1.20	4.86	2.21	1.77	1.55	5.71	3.08	1.93
Cr	12.95	37.49	21.39	11.47	13.31	64.00	33.35	24.42
Fe	1178.30	4886.86	3505.76	1709.63	2831.84	5054.50	4066.60	1079.74
Ni	4.32	25.94	15.98	10.90	7.13	38.51	23.29	16.61
As	0.59	0.85	0.73	0.10	0.63	0.75	0.70	0.056
Ca	5524.23	11188.27	8516.05	2802.20	6014.06	13763.44	9301.41	3601.98
Cd	0.29	11.56	5.93	6.04	0.73	11.63	6.16	6.00
Cu	91.65	226.09	154.59	56.71	113.03	269.76	187.16	66.88
Mn	19.26	201.93	102.71	78.97	36.84	246.65	157.97	87.87
Pb	6.98	14.47	9.88	3.27	7.08	18.84	11.30	5.21
Zn	862.84	2016.75	1376.60	477.18	1066.08	2244.73	1780.44	524.44

It can be observed that the element with the highest content, in both cases, is Ca (8516.05 ppb and 9301.41 ppb), followed by Fe (3505.76 ppb and 4066.60 ppb) and Zn (1376.60 ppb and 1780.44 ppb) and that with the lowest is the As (0.73 ppb and 0.701 ppb) and that the values for the samples collected from the cap are higher than those for the samples collected from the jar. This fact is consistent for all the studied elements except As, were a small decrease is noticed. The most notable difference - 2.4 times - between the content of metal in the yogurt from the jar and the one from the cap is for Al, difference is due to the fact that the caps can be fabricated from aluminium and this element is a contaminant in the present situation.

Results of elements content obtained for bio fruits yogurts (samples collected from the jar and from the cap) are presented in table 3. Like in the case of bio simple yogurts, the metal with the highest concentration is Ca (6281.03 ppb and 6708.35), followed by Fe (3176.02 ppb and 3938.45 ppb) and Zn (1211.37 ppb and 2471.34 ppb) and that with the lowest is Co (1.60 ppb and 2.53 ppb). Also the elements content is increased for the samples collected from the cap, with the exception of As (5.22 ppb and 5.12 ppb). The content of aluminium is smaller than for the bio simple yogurts, but this content in the samples collected from the cap is 6.5 times higher than the one in those collected from the jar.

Table 3

Elements content in bio fruits yogurts from the jar and from the cap (n=4)

Element	Jar				Cap			
	min (ppb)	max (ppb)	mean (ppb)	σ	min (ppb)	max (ppb)	mean (ppb)	σ
Al	17.51	23.31	20.39	3.25	103.40	144.02	125.15	19.10
Co	0.84	3.33	1.60	1.16	1.01	5.68	2.53	2.19
Cr	12.67	14.68	13.91	0.88	13.18	15.76	14.64	1.25
Fe	2427.58	4382.68	3176.02	909.45	3046.48	5605.56	3938.45	1145.98
Ni	8.70	46.84	23.77	16.27	10.24	61.02	32.63	21.16
As	0.56	18.89	5.22	9.11	0.54	18.52	5.12	8.93
Ca	3860.18	8857.27	6281.03	2604.03	4211.74	9814.25	6708.35	2865.33
Cd	0.41	10.50	5.43	5.49	0.44	11.46	5.95	5.84
Cu	96.63	163.32	128.93	35.38	112.08	212.80	158.91	41.42
Mn	70.74	247.99	158.14	83.76	77.71	288.24	182.65	92.179
Pb	6.95	10.89	8.77	1.87	7.065	12.77	9.64	2.63
Zn	591.46	1953.42	1211.37	581.66	810.52	4944.96	2471.34	1950.68

Results of elements content obtained for non-bio simple yogurts (samples collected from the jar and from the cap) are presented in table 4. The results are similar with those reported for bio simple yogurts; with the distinction that the values are smaller, the content of aluminium is relatively low and the difference between the values obtained for the jar and for the cap is minor.

Results of metal content obtained for non-bio fruits yogurts (samples collected from the jar and from the cap) are presented in table 5. The results are in respect with those reported for bio fruits yogurts and the content of Al is similar with the one obtained for non-bio simple yogurts.

Comparing all types of yogurt, it can be observed that the highest content of heavy metals - Pb and Cd - is found in the samples of bio simple yogurts collected from the cap, 11.30 ppb and 6.16 ppb. From the metals with a potential toxic behaviour, chromium has the highest content - 33.35 ppb - in bio simple yogurts, samples collected from the cap. With a few exceptions, the samples collected from the cap have a higher content of metals than those collected from the jar. Also it can be observed the occurrence of similarities between bio and non-bio simple yogurts and bio and non-bio fruits yogurts, respectively. Another aspect that should be mentioned is the large content of Al present in some types of yogurt, especially in the samples collected from the cap, which does not cross the limit imposed by food legislation [13-16] (0.3 – 10 mg/day), but it should be taken into account, because Al brings great damage onto the brain causing Alzheimer disease.

Table 4

Elements content in non-bio yogurts from the jar and cap (n=8)

Element	Jar				Cap			
	min (ppb)	max (ppb)	mean (ppb)	σ	min (ppb)	max (ppb)	mean (ppb)	σ
Al	7.52	42.05	22.57	13.23	12.79	61.69	25.69	16.06
Co	0.18	1.12	0.62	0.43	0.19	1.26	0.67	0.45
Cr	5.32	10.63	7.03	1.82	5.91	11.24	8.47	2.42
Fe	847.89	2984.69	1725.46	954.32	882.53	4335.74	2028.58	1262.97
Ni	2.63	12.75	7.58	3.54	3.81	18.90	10.61	5.46
As	0.26	0.98	0.60	0.23	0.27	0.73	0.54	0.16
Ca	3223.44	8085.17	5334.23	2045.77	3832.60	8381.84	6887.46	2064.28
Cd	0.10	11.05	2.94	4.89	0.13	12.04	3.09	5.12
Cu	43.54	160.12	86.60	86.60	58.06	172.82	100.61	44.93
Mn	13.93	144.65	56.40	56.60	15.59	162.56	64.40	64.23
Pb	0.17	9.82	4.55	3.26	2.97	15.45	6.12	4.34
Zn	560.30	2406.70	1048.42	633.39	595.70	4426.10	1505.95	1236.23

Table 5

Elements content in non-bio fruits yogurts from the jar and cap (n=13)

Element	Jar				Cap			
	min (ppb)	max (ppb)	mean (ppb)	σ	min (ppb)	max (ppb)	mean (ppb)	σ
Al	11.44	53.73	21.76	13.56	12.89	84.07	25.29	19.97
Co	0.15	1.60	0.46	0.47	0.16	3.50	0.64	0.93
Cr	8.17	15.08	10.04	2.32	8.54	20.28	10.90	3.52
Fe	730.95	3324.68	1408.84	915.62	732.69	3342.65	1570.12	1078.56
Ni	2.65	51.27	8.92	13.10	2.93	65.18	12.52	17.54
As	0.21	1.27	0.54	0.29	0.25	0.77	0.52	0.19
Ca	3358.63	7852.06	5587.43	2215.35	3611.65	7992.16	5876.28	2348.51
Cd	0.06	11.48	1.11	3.13	0.09	11.66	1.21	3.16
Cu	49.05	185.55	80.75	44.56	50.79	198.86	90.90	54.80
Mn	19.34	160.98	65.05	55.86	19.60	163.23	73.52	56.81
Pb	1.86	9.34	3.85	2.54	1.98	9.61	4.30	2.68
Zn	456.40	1309.20	710.21	302.27	456.40	2108.50	900.08	515.75

3.2. Variation of elements concentration with time

The variation of elements concentration with time, for all types of yogurt studied is presented in Fig. 1.

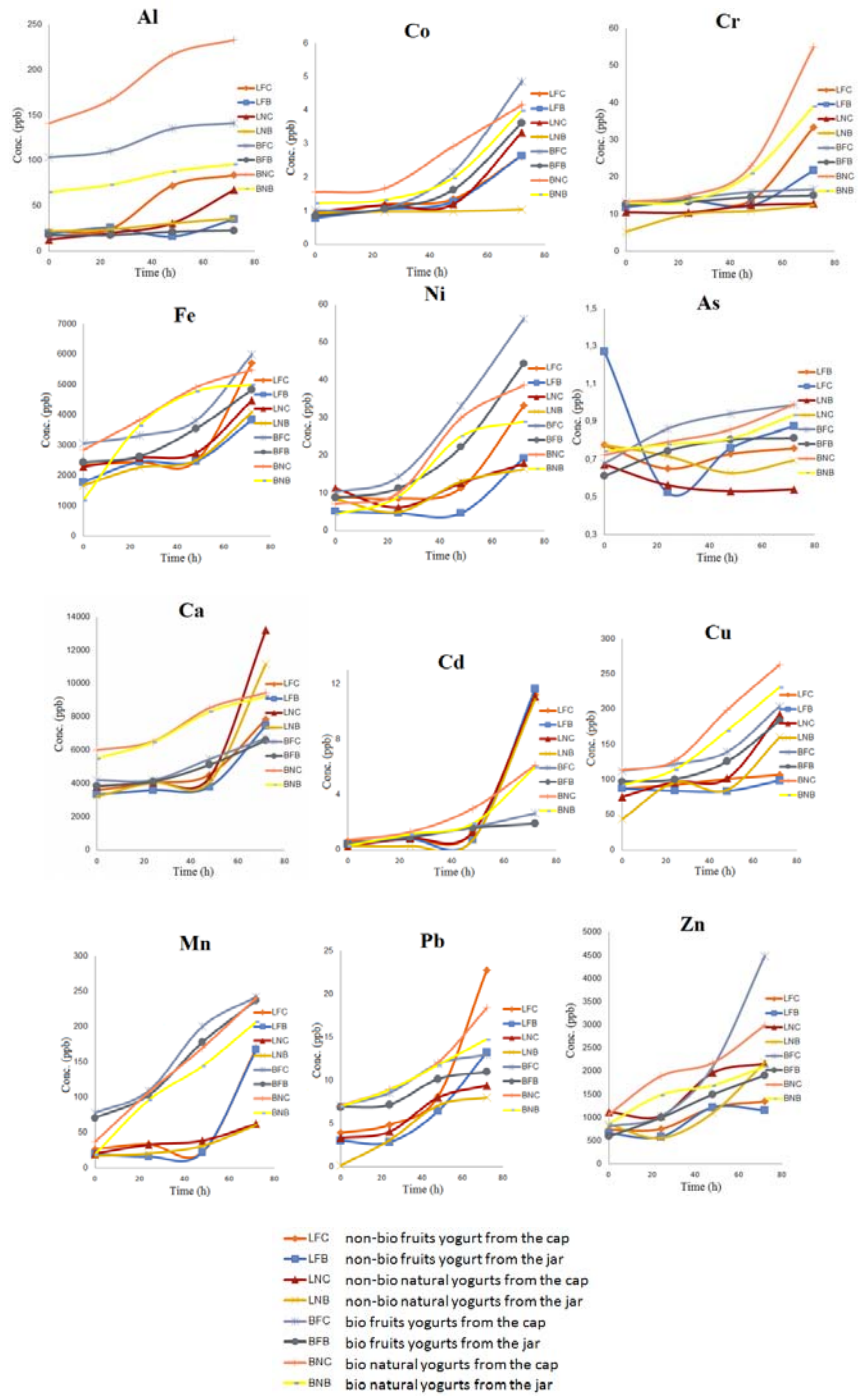

Fig. 1. Variation of elements content with time

It can be observed that in all samples of yogurt the content of all the studied elements, excepting As in some samples, increases with time. The greatest variations of elements content with time, mainly escalations, were recorded for the samples BFC (bio fruits yogurts from the cap) for 4 elements (Co, Ni, As, Zn), followed by non-bio fruits yogurts from the cap (LFC) for 3 metals (Pb, Fe, Cd), and non-bio yogurts from the cap (LNC) for 2 metals (Ca, Cd). It can be also mentioned the fact that for heavy metals the samples with the greatest variation, especially between 48 and 72 hours, are all samples of non-bio yogurts (LFC, LFB, LNC, LNB) for Cd (from 0 to about 12 ppb), and non-bio fruits yogurt from the cap for Pb (from about 7 to about 23 ppb).

4. Conclusions

Based on experimental data, it is to conclude that the micro- and macroelements results show that the highest content of Ca, Fe, Zn and Cu are present in bio simple yogurts and the highest content of Mn is present in bio fruits yogurts. Regarding the heavy metals, the higher content of Cd and Pb are present also in bio simple yogurts. The concentrations of other elements with a certain degree of toxicity are as follows: the highest content of As is found in bio fruits yogurts, the highest content of Ni is found also in bio fruits yogurts, the highest content of Cr is found in bio simple yogurts and the highest content of Co is found also in bio simple yogurts.

As it can be observed, the highest concentration of micro- and macroelements essential for human health are found in bio simple yogurts, but so are the highest concentration of heavy metals and potential toxic ones.

Moreover, with some exception (Ca, Cr, Ni and Mn in non-bio fruits yogurts and As, Ni and Mn in bio fruits yogurts), simple yogurts have a larger content of toxic elements (those considered essential or toxic) than fruits yogurts.

The levels of heavy and potentially toxic elements present in the studied yogurts were below the maximum levels recommended for food, and they present no risk for human health. From literature is it well known that food package can affect the amount of ions release as a function of their type and product degradation. However, it is to notice that various additives from caps and jars, especially from plastic type bring contaminants into the samples of yogurts.

Taking into account that in the concept of safety food "from farm to table", food Safety Knowledge including contaminants and their evolution in time constitute an important part, all such remarks based on experimental data are clearly useful for a safety and healthy nutrition.

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