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COMPARATIVE EVALUATION OF SOME MINOR- AND MAJOR ELEMENT CONTENTS IN COMMERCIAL YOGURTS

OANA CADAR[a*], MIRELA MICLEAN[a], MARIN SENILA[a], ALEXANDRA HOAGHIA[a], MANFRED SAGER[b]

ABSTRACT. The content of 10 minor and major elements (Cr, Cu, Fe, Mn, Zn, Na, K, Ca, Mg and P) was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) in 15 commercial plain and fruit mixed yogurts, purchased in supermarkets from Romania. Results of both minor and major elements were found mostly similar in all varieties of yogurt studied. The concentrations in mg/kg fresh weight were: Cr (<0.25), Cu (0.30-0.56), Fe (0.53-1.23), Mn (0.42-0.83), Zn (2.56-3.69), Na (325-522), K (999-1356), Ca (932-1206), Mg (82-113) and P (924-1001). Some of the essential elements were found to have good and healthy contribution to daily nutrition of consumers in accordance to Recommended Dietary Allowance (RDA).

Keywords: *yogurt, minor elements, major elements*

INTRODUCTION

Milk and dairy products are important components of human nutrition. Fresh fermented dairy products such as yoghurt are widely consumed foods in many countries due to their potential health benefits for humans and nutritional properties [1-3]. Compared with milk, the mineral concentrations (K, Ca, Mg, P and Zn) are higher in yogurt by nearly 50% [4]. Yogurt is a fermented milk product obtained by fermentation of milk with bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Large numbers of these bacteria remain viable in the product until the time of consumption [5, 6]. Yoghurt is one

[a] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, 400293 Cluj-Napoca, Romania.*
[b] *Competence Centre of Elements, Austrian Agency for Health and Food Safety, 191 Spargelfeldstrasse, A-1220 Vienna, Austria.*
* *Corresponding author: oana.cadar@icia.ro*

of the dairy products whose sales continues to increase due to the diversification of the range available including different flavors (plain, sweetened with fruits or dried fruits, flavored), textures and consistencies (firm, liquid, shakes, smooth, frozen) [7-9].

Yogurt and other fermented dairy products are a good source of nutrients, such as: proteins, fat, important vitamins, major minerals, enzymes and probiotic bacteria [10-12]. The mineral content yogurt is variable due to the factors such as differences between animal species, geographical origin of milk, manufacturing practices and possible contamination from the equipment during the processing. Beside calcium, considered the most important nutrient for bone health, yogurts are a good dietary source of essential and very important elements (like copper, chromium, manganese and zinc) for normal metabolism, growth and development [13].

Although, there are numerous bibliographic references on the major and minor element levels in bovine milk, only several studies have been reported for dairy products, such as yogurt. For the determination of minor and major elements, several analytical techniques have been used: graphite furnace atomic absorption spectrometry, GF-AAS [14], flame atomic absorption spectrometry, FAAS [15-17], inductively coupled plasma optical emission spectrometry, ICP-OES [10, 18], inductively coupled plasma-mass spectrometry, ICP-MS [19, 20], X-ray fluorescence spectrometry, XRF [5] Also, sample preparation is an important step in elemental analysis; microwave digestion has many advantages in comparison with open vessel digestion: short experimental time, low reagent consumption, good recoveries and enhanced operator safety [21].

The objective of this study was the determination of the minor and major element compositions in 15 commercial yogurts (plain and fruit mixed). The study is focused on those essential elements which can be easily determined by ICP-OES, namely Cr, Cu, Fe, Mn, Zn, Na, K, Ca and Mg. The obtained results were used to assess their daily intake and contribution to the recommended dietary allowance (RDA) values.

RESULTS AND DISCUSSION

In order to check the accuracy of the method, CRM (NIST-1549 Non-fat milk powder) was analyzed for the determination of Na, K, Ca, Mg, P, Al, Cu, Fe and Zn (Table 1). The recovery value means of all the investigated elements were found to be in the range of 97.4-103%.

Table 1. Analysis of certified reference material (NIST 1549)

Element	*Certified value[a] (mg/kg)*	*Obtained value[a] (mg/kg)*	*Recovery (%)*
Na	0.497 ± 0.010[b]	0.514 ± 0.043	103
K	1.69 ± 0.03[b]	1.70 ± 0.08	101
Ca	1.30 ± 0.05[b]	1.28 ± 0.03	98.7
Mg	0.120 ± 0.003[b]	0.119 ± 0.006	98.9
P	1.06 ± 0.02[b]	1.08 ± 0.08	102
Cu	0.700 ± 0.100	0.694 ± 0.051	99.1
Fe	1.78 ± 0.10	1.80 ± 0.17	101
Mn	0.26 ± 0.06	0.253 ± 0.031	97.4
Zn	46.1 ± 2.2	46.6± 1.1	101

[a] *Mean±standard deviation*
[b] *Concentration in mass fraction (%)*

The obtained mean concentrations and standard deviations of analyzed elements in both evaluated type of yogurt (plain vs fruit mixed) are presented in Table 2. The elements were categorized into major elements (concentration more than 100 mg/kg) and minor elements (concentration below 100 mg/kg and decreasing order: Zn>Fe>Mn>Cu>Cr). In general terms, the concentrations of most analyzed elements were quite uniform and without relevant differences between brands. Furthermore, there were no significant differences in concentrations between investigated plain and fruit mixed yogurt samples.

Table 2. Concentrations of minor and major elements (mg/kg fresh weight) in yogurt samples

Element / wavelength (nm)	**Plain yogurt[a] (n=10)**	**Fruit mixed yogurt[a] (n=5)**
Minor elements		
Cr / 267.7	<0.25	<0.25
Cu / 327.4	0.38 ± 0.10	0.32 ± 0.10
Fe / 238.2	0.74 ± 0.20	0.92 ± 0.27
Mn / 257.6	0.64 ± 0.12	0.58 ± 0.11
Zn/ 213.9	3.05 ± 0.76	2.78 ± 0.56
Major elements		
Na / 589.6	424 ± 54	401 ± 48.0
K / 769.9	1234 ± 89	1102 ± 101
Ca / 317.9	1045 ± 102	945 ± 76.0
Mg / 279.1	123 ± 24.0	98.0 ± 18.0
P / 213.6	975 ± 10.0	940 ± 11.0

[a] Values are mean ± standard deviations of three (n=3) measurements.

Major elements. In all cases, plain yogurt got the highest major element contents. K was found to be the quantitatively most outstanding of the investigated elements with levels between 1234 mg/kg in plain and 1102 mg/kg in fruit mixed yogurt, respectively. The other major elements follow the descending order: Ca>P>Na>Mg in terms of their content. There were no statistically significant differences between the concentrations of plain and fruit mixed yogurts ($p < 0.05$).

The levels of major elements found in dairy yogurt were comparable with those reported in other studies: Na (356-732), K (946-1630), Ca (796-1405 mg/kg) and Mg (78.5-158) mg/kg fresh weight [3, 10, 22]. The obtained concentrations for P were higher than those found in the most consumed trademarks of yogurt in Spain (627-858 mg/kg fresh weight) [3].

Minor elements. Zn was the most abundant minor element in both types of yogurt (3.05 in plain and 2.78 mg/kg in fruit mixed yogurt) followed by the other minor elements, ranked in descending order, Fe>Mn>Cu>Cr in plain yogurt and Fe>Mn>Cu>Cr in fruit mixed yogurt, respectively. The considerable amount of Fe and Mn may be due to the contamination during manufacturing, packaging processes and transport [14].

The obtained values of minor elements were lower than those reported for Turkish yogurt (1.73 mg/kg Fe, 0.71 mg/kg Cu and 4.51 mg/kg Zn) [18]. In comparison with Korean yogurt determined by Khan et al. 2014 [19], the obtained values for Zn and Cr were lower (Zn - 4.7 mg/kg and Cr - 0.271 mg/kg) while those obtained for Cu and Mn were higher (Cu - 0.158 mg/kg and Mn - 0.080 mg/kg). The higher levels of Fe could be attributed to addition of fruit pieces which cause an increase in the concentration of mineral, but also to the existence of enriched yogurts with minerals (Na, K, Ca, Zn or elements which are deficient in the milk: Fe and Mn) [15]. The minor element contents of the yogurt samples from this work were within the concentration ranges in yogurts consumed in Spain, Cr (0.01-0.06), Cu (0.035-0.46), Fe (0.2-3.6), Mn (0.02-0.04) and Zn (2.09-4.65) mg/kg fresh weight, reported by Luis et al. 2015 [10] and Llorent-Martinez et al., 2012 [20]. Several authors have shown that yogurt and milk (the raw material of this product) have similar mineral composition. Milk composition may vary according to factors such as breed, age, mammary gland health, genetic background, lactation stage, feeding and season) [12, 14, 22].

Table 3 present the obtained results for investigated major and minor nutritional elements (the percentage of contribution to RDA is calculated for each element, considering the intake of one yogurt/day). The RDA data (the levels of intake of essential nutrients considered to be adequate to meet the needs of practically all healthy persons) use in this study, are those provided by the Commission of the European Communities, 2008 and World Health Organization (WHO), 2012 [23, 24]. The concentration of Cr in all yogurt samples was below the limits of quantification (0.25 mg/kg). Thus no conclusion can be drawn regarding the coverage of RDA for Cr. Furthermore, no harmful impacts caused by this element through high consumption of yogurt are to be expected. As a result, the investigated minor and major elements were found to have good nutritional contribution in accordance to RDA. However, it must be considered that yogurts are not the only sources of major and minor elements.

Table 3. Mean daily and percentage of contribution to RDA for investigated nutritional elements in yogurts

Element / yogurt		Level[a] (mg/kg)	Mean daily intake[b] (mg)	RDA[c] (mg)	%RDA
Na	plain	424	53.0	200[d]	26.5
	fruit	401	50.1		25.1
K	plain	1234	154	2000	7.71
	fruit	1102	138		6.89
Ca	plain	1045	131	800	16.3
	fruit	945	118		14.8
Mg	plain	123	15.4	375	4.10
	fruit	98.0	12.3		3.27
P	plain	975	122	700	17.4
	fruit	940	118		16.8
Cu	plain	0.38	0.05	1	4.75
	fruit	0.32	0.04		4.00
Fe	plain	0.74	0.09	14	0.66
	fruit	0.92	0.12		0.82
Mn	plain	0.64	0.08	2	4.00
	fruit	0.58	0.07		3.63
Zn	plain	3.05	0.38	10	3.81
	fruit	2.78	0.35		3.48

[a] Mean concentrations (Table 2);
[b] Calculated on the basis of the intake of one yogurt (approx. 125 g);
[c] EC Commission directive, 2008;
[d] World Health Organization (WHO), 2012.

CONCLUSIONS

In this paper, the determination of macro and trace elements in plain and fruit mixed commercial yogurts has been carried out using microwave digestion with nitric acid and hydrogen peroxide, followed by ICP-OES analysis. Results of both minor and major elements were found mostly similar in all studied yogurt. The levels of investigated essential elements in commercial yogurts were appropriate and thus yogurt having a positive contribution to daily nutrition of consumers in accordance to Recommended Dietary Allowance (RDA). Metal concentrations in yogurt are conditioned by the composition of the initial milk and the technological procedures used in dairy product processing.

EXPERIMENTAL SECTION

Reagents, standard solutions and CRMs

The calibration standards were prepared by appropriate dilution of the ICP multielement standard solution IV (Merck, Darmstadt, Germany) 1000 mg/l of Cr, Cu, Fe, Mg, Zn, Na, K, Ca, Mg and Phosphorous ICP Standard 1000 mg/l P in 2% (v/v) HNO_3. All reagents (HNO_3 65%, H_2O_2 30%) were of analytical grade and were purchased from Merck, Darmstadt, Germany. The certified reference material (NIST-SRM 1549 Whole milk powder) was obtained from LGC Promochem GmbH, Wessel, Germany. For all dilutions, ultrapure water (resistibility 18.2 MΩ/cm) obtained from a Millipore Direct-Q3 UV system (Millipore, France) was used. All PTFE and glass vessels were soaked in 10% HNO_3 for at least 24 h and rinsed extensively with Milli-Q water prior to use.

Sampling

A total of 15 samples of yogurts (10 plain and 5 fruit mixed) from the five major producers were bought from local supermarkets in NW Romania. The samples were purchased in triplicate at different times during September to December, 2014. The selected brands cover more than 75% of the yogurt market in this region, a rate that can also be applied to Romania, where these brands are also distributed. After transport to the laboratory, the yogurt samples were kept in their original packages and stored at 4^oC until analysis.

Sample preparation

0.5 g of each sample was weighted and carefully transferred to a Teflon reaction vessel and 5 ml HNO_3 65% and 2 ml H_2O_2 30% were added. Samples were left on the bench to pre-digest overnight at room temperature. The vessels were closed and the samples were digested in a closed-vessel microwave system Berghof MWS-3+ with temperature control mode (Berghof, Germany), according to Ayar et al. [14]. After cooling down to room temperature, the completely clear and colorless solutions were quantitatively transferred to 25 mL volumetric flasks and diluted to the mark with double deionized water. Certified reference material NIST 1549 and blank, consisting of deionized water and reagents, were prepared in the same way as the sample. All determinations were carried out in triplicate.

Instrumentation

The major and minor element contents were determined by ICP-OES (OPTIMA 5300 DV, Perkin Elmer, USA) equipped with an ultrasonic nebulizer CETAC U-6000AT+ with heater/condenser (CETAC Technologies, USA). The working conditions were: approximate RF power, 1.3 kW; nebulizer gas, 0.8 L/min; plasma gas, 15 L/min; auxiliary gas, 2.0 L/min; sample flow, 1.1 L/min; axial viewing; background correction, 2-point. The analytical emission lines were selected as the most sensitive ones. The calibration range for all evaluated elements was made from 0.005 to 5 mg/l.

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