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**Study of the effect of different fermenting microorganisms on the Se, Cu, Cr, and Mn contents in fermented goat and cow milks**

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Running title: Fermenting bacteria do not affect Se, Cu, Cr, and Mn contents in yogurts

ABSTRACT

The aim of this study was to determine the Se, Cu, Cr, and Mn concentrations of different types of goat- and cow-milk fermented products and evaluate the influence of fermenting bacteria (classical fermenting starters and a probiotic strain) on these concentrations. Atomic absorption spectrometry with hydride generation was used to measure Se and electrothermal atomization to measure Cu, Cr and Mn. Analytical parameters determined in the fermented milks demonstrated that the procedures used were adequate for Se, Cu, Cr, and Mn analyses. Se levels were significantly lower in fermented goat milk products than in fermented cow milk products (*p* < 0.05). Se, Cu, Cr, and Mn levels did not differ as a function of the fermenting bacteria used in commercial fermented goat or cow milks or in the lab-produced goat yoghurt. Given the Se, Cu, and Cr intakes for healthy adults, goat and cow yogurts may be important dietary sources.

*Keywords:*

Se, Cu, Cr, and Mn analysis by AAS

Fermented milks

Fermenting microorganisms

**1. Introduction**

Dairy goat farming is a vital part of the national economy in many countries and is particularly well organized in France, Italy, Spain, and Greece (Silanikove, Leitner, Merin, & Prosser, 2010). Goat milk is mainly used for fresh liquid consumption and the manufacture of fermented milks and cheeses (Tamine, Wszolek, Bozanic, & Özer, 2011). In Spain, there has been a recent increase in the consumption of goat milk and fermented products such as yogurt and kefir. However, because of the limited large-scale industrialization of goat milk dairy products (Tamine, Wszolek, Bozanic, & Özer, 2011), a reduced number of commercial brands of goat fermented milk are available in this country (Navarro-Alarcón et al., 2011).

The growing interest in goat milk and its by-products at the expense of cow milk is mainly attributable to their nutritional, health, and therapeutic benefits (Ribeiro & Ribeiro, 2010). Thus, goat milk has a lower allergic potentiality (Silanikove, Leitner, Merin, & Prosser, 2010) and improved fat digestibility/absorption (Haenlein, 2004; Silanikove, Leitner, Merin, & Prosser, 2010) and has been used in the treatment of various clinical disorders (Haenlein, 2004).

It has been reported that the mineral content of goat milk is higher and more available than that of bovine milk. It has been found that fermentation does not affect the mineral content of milk but can modify its bioavailability (Kondyli, Katsiari, & Voutsinas, 2007), as also reported for the Ca, Zn, and P content of milk fermented with classical bacteria starters plus *Lactobacillus fermentum D3* (Bergillos-Meca et al., 2013).

Selenium (Se) is essential for the normal growth and development of humans and animals (Rayman, 2008; Pilarczyk et al., 2010) and exerts antioxidant action as cofactor of glutathione peroxidase, whose activity was reported to be higher in goat *versus* cow milk (Debski, Picciano, & Milner, 1987). It also acts on the regulation of thyroid function against heavy metals, protecting the vascular endothelium as selenoprotein P and behaving as an antineoplastic agent as cofactor of thioredoxin reductase. The range between the recommended (55 μg/day) and toxic daily intake (>400 μg/day) of this element is narrow (Navarro Alarcón & Gil Hernández, 2010).

Copper (Cu) is essential in numerous physiological processes as cofactor of certain enzymes or component of other proteins (lysyl oxidase, tyrosinase, Cu/Zn superoxide dismutase (SOD1), cytochrome C oxidase (COX), ceruloplasmin, and coagulation factors V and VIII, etc) (Wardlaw, 2008; Olivares Grohnert, Castillo Durán, & Dagach-Imbarach, 2010).

Goat milk products have been found to improve the intestinal absorption of Cu (*vs.* cow milk), especially in rats with malabsorption syndrome, due to their elevated cysteine content (Barrionuevo, Alferez, López-Aliaga, Sanz-Sampelayo, & Campos, 2002). It has also been reported (Mrvcic et al., 2013) that *Lactobacillus brevis* L62 strain is highly tolerant to Cu ions, favoring its use in the fermentation of milk with a high concentration Cu2+ ions. Furthermore, the addition of Cu2+ at 0.125 mg/100 g was observed to reduce the postacidification level of fermented cow milk without affecting the fermentation time or counts of viable *Streptococus thermophilus* (Han, Zhang, Du, Yi, Li, & Zhang, 2012); however, the fermenation time lengthened when the concentration was increased to 2 mg/100 g (Han, Zhang, Du, Yi, Li, & Zhang, 2012).

Chromium (Cr) participates in the metabolism of proteins, carbohydrates, and lipids (Navarro-Alarcón, Ruiz-Ojeda, Blanca-Herrera, Kaki, Adem, & Agil 2014), while the most widely studied function is its action on glucose uptake by cells. This element has also been related to reductions in serum total cholesterol, LDL-cholesterol, and triglyceride levels (Wardlaw, 2008; Navarro Alarcón & Gil Hernández, 2010).

Finally, manganese (Mn) is involved as a cofactor of several enzymes (pyruvate carboxylase, arginase, phosphoenolpyruvate carboxykinase, acetyl coenzyme A, and tyrosine carboxylase sulfotransferase) in the metabolism of macronutrients and exerts antioxidant action as cofactor of the mitochondrial SOD (Navarro-Alarcón, Ruiz-Ojeda, Blanca-Herrera, & Agil, 2013). Nevertheless, there has been no report of manganese deficiency in humans, because only a small daily amount is required (Wardlaw, 2008; Navarro Alarcón & Gil Hernández, 2010).

Techniques used for the analysis of trace elements in the quality control of milk and by-products include: inductively coupled plasma emission spectroscopy atomization (ICP-MS; Park, 1994; Ayar, Sert, & Akin, 2009; Guler, 2007; Llorent-Martínez, Fernández de Córdoba, Ruiz-Medina,& Ortega-Barrales, 2012; Khan et al., 2014), atomic absorption spectrometry (AAS) with flame atomization (Mrvcic et al., 2013), high-performance chromatography (HPLC) with ICP-MS (Alzate et al., 2007; Alzate, Fernández-Fernández, Pérez-Conde, Gutiérrez, & Cámara, 2008), and AAS with hydride generation (HG) (Navarro-Alarcón et al., 2011). Although ICP-MS has been used for multiple-element determination in milk and by-products, it does not offer adequate detection limits or sensitivity for all minerals. A useful alternative for mineral measurement in milk and by-products may be to use HG-AAS for Se and electrothermal (ETA)-AAS for Cu, Cr, and Mn.

The production of high-quality fermented goat and cow milk products requires control over several factors, including the chemical composition of the raw milk, type of milk, processing conditions, and starter culture used for the fermentation (Tamine, Wszolek, Bozanic, & Özer, 2011; Bergillos-Meca et al., 2013). Few data are available on the influence of the manufacturing process on the Se, Cu, Cr, and Mn content of fermented goat and cow milk products*.* In the present study, we developed procedures to analyze and investigate the effect of goat and cow milk processing on the mineral (Se, Cu, Cr and Mn) content. The study objectives were: i) to optimize AAS techniques with HG for Se measurement and with ETA for Cu, Cr and Mn measurement; ii) to compare microelement levels between goat and cow yogurts; iii) to study the effect of different fermenting bacteria on the final content of Se, Cu, Cr, and Mn in goat and cow yoghurts; iiii) and given the recommended Se, Cu, Cr and Mn intakes for healthy adults (dietary reference intakes [DRIs] established by the Federación Española de Sociedades de Nutrición, 2010) to evaluate if these dairy products may represent an important dietary source for these minerals.

**2. Materials and Methods**

*2.1. Instrumentation*

A Perkin–Elmer 1100B double beam atomic absorption spectrometer equipped with a MHS-10 hydride generator (Perkin–Elmer, Norwalk CT, USA) was used for the Se determination. Argon of 99.999% purity (Spanish Society of Oxygen, Barcelona, Spain) at 300 ml/min was used as carrier gas for Se determination by HG-AAS. The Perkin-Elmer 5100 Zeeman atomic absorption spectrometer and HGA-5100 graphite furnace (Perkin-Elmer, Germany) with pyrolytically coated graphite tubes and AS-90 autosampler (Perkin-Elmer, Germany) were used in Cu, Cr, and Mn determinations. Measurements were performed at 196.0 nm for Se, 324.8 nm for Cu, 357.9 nm for Cr, and 279.5 nm for Mn, using hollow cathode lamps (Perkin–Elmer). A Selecta multiplace digestion block (Selecta SA, Barcelona, Spain) and Pyrex tubes were used for sample mineralization. A Moulinex blender (Moulinex, Bagnolet, France) was used for sample homogenization. Bidistilled deionized water with a specific resistivity of 18 MΏ/cm was obtained using a Milli-Q system (Millipore, Milford, MA).

*2.2. Reagents*

Standard solutions of Se, Cu, Cr, and Mn (1000 ± 0.002 mg/l) (Tritisol, Merck, Darmstadt, Germany) were used and diluted as necessary to obtain working standards. High-quality concentrated nitric acid (65%) and perchloric acid (65%) were used for sample mineralization. Hydrochloric acid (37%) was used to reduce the sample after mineralization for Se determination. Sodium borohydride and sodium hydroxide were employed for hydride generation. Magnesium nitrate was used to prepare the matrix modifier for Cu and Cr determinations. All solutions were prepared from analytical reagent-grade chemicals (Suprapur, Merck, Germany).

*2.3. Fermented milk samples*

A total of 75 samples were studied. All samples were carefully handled to avoid contamination, and the appropriate quality assurance procedures and precautions were followed to ensure the reliability of results.

The samples were divided between fermented goat (n= 20) and cow milk products (n= 55), and each group was divided into two sub-groups: (a) yogurts fermented with the traditional bacteria *Lactobacillus delbrueckii spss. bulgaricus* (LB) and *Streptococcus thermophilus (*ST); and (b) probiotic yoghurts fermented with other microorganisms used to manufacture probiotic fermented milk products and kefir (LB + ST + different species of *Bifidobacterium* and/or different species of yeasts and/or *L. casei* and/or *L. acidophilus*). The possible influence of the fermentation process (starter culture) on the mineral content was analyzed and compared between the groups. A third subgroup of fermented goat milks was included for a lab-produced yogurt made from local goat milk (Southern Spain) using the traditional fermenting starters plus probiotic bacteria (Bergillos-Meca et al., 2013). The brands in the study represent all eight goat milk fermented products available in this region of Southern Spain. Four samples of each product were purchased for the present study.

*2.4. Material*

The risk of contamination was minimized by using glassware as little as possible and employing plastic (polypropylene) vessels and pipette tips. All material was nitric acid-washed and rinsed several times with bidistilled deionized water.

*2.5. Sample preparation and analysis*

For Se measurement, previous sample mineralization was carried out according to a previously optimized method (Navarro-Alarcón et al., 2011). Briefly, 25 ml of the final analytical solution obtained was transferred to a reaction vessel that was placed in the MHS-10 system. Total Se was then measured by HG-AAS with the addition calibration method. Hydride generation was carried out using a solution of 3% (w/v) NaBH4 in 1% NaOH. Se levels in the final analytical solution were measured by HG-AAS (Navarro-Alarcon et al., 2011).

For Cu, Cr, and Mn measurements, 1 g of homogenized sample was treated with 3 mL of an HNO3-HClO4 (4:1) mixturein Pyrex tubes placed in the digestion block and then heated at 60°C for 45 min, 90°C for 30 min, and 120°C for 45 min. The solutions obtained were cooled to room temperature, transferred to a volumetric flask, and diluted to a final volume of 25 mL with bidistilled deionized water until the analytical solution was obtained. All samples and blanks were mineralized and diluted in triplicate using the same procedure. Finally, the analytical solutions (20, 10 and 20 μl volume, respectively) were injected using a graphite tube without L’Vov platform. Matrix modifiers (20 µl of 0.03% MgNO3 and 10 μl of 0.5% MgNO3) were used for Cu and Cr determination, respectively. Furnace conditions for Cu, Cr, and Mn determination by ETA-AAS were based on previous assays (Velasco-Reynold, Navarro-Alarcón, López-Gª de la Serrana, Perez-Valero, & López-Martínez, 2008a,b,c). The concentration (ppb) in the samples was obtained by linear calibration. Specific time-temperature programs were developed for the determination of Cu, Cr and, Mn in fermented milk samples.

*2.6. Statistical analysis*

SPSS 17.0 for Windows (IBM, Chicago, IL) was used for the data analyses. Results were expressed as arithmetic means ± standard error of the mean (SEM). The normal distribution of variables and the homogeneity of variances were verified by the Kolmogorov-Smirnov and Levene’s tests, respectively. The ANOVA and Kruskall-Wallis tests were used for comparisons of parametric and non-parametric data, respectively. *p* < 0.05 was considered significant.

**3. Results and discussion**

*3.1. Analytical quality assurance*

The analytical characteristics of the methods used to measure Se, Cu, Cr, and Mn were evaluated, establishing the detection limits and sensitivity values (Table 1). The inter-day repeatability, precision, accuracy, and percentage recovery of added Se, Cu, Cr, and Mn were adequate for the measurement of these elements in fermented milk products (Table 1). The accuracy and precision of the Se, Cu, Cr, and Mn measurement procedures were also verified by testing two certified reference standards of the European Union Community Bureau of Reference, CRM 278 mussel tissue and 185R bovine liver. No significant differences were found between the mean Se, Cu, Cr, and Mn concentrations determined in these materials and the certified concentrations (Table 2).

HG-AAS and ETA-AAS methods were developed for measuring total Se and total Cu, Cr and Mn levels in fermented milks, respectively. The results for the certified reference materials support the accuracy and precision of the time-temperature programs developed, and the sensitivity values and detection limits (Table 1) were suitable for the Se, Cu, Cr, and Mn concentrations found. The percentage recovery and reproducibility values of samples pre-treated with Se, Cu, Cr, and Mn endorse the adequacy of these procedures to analyze these elements in fermented milks. Hence, these procedures can be used to measure and assess the magnitude of Se, Cu, Cr, and Mn intakes, evaluating any deficiency or excess/toxicity.

*3.2. Selenium levels*

Se levels were significantly higher in fermented cow milks than in fermented goat milks (*p* < 0.05; Fig. 1). Among the fermented goat milks, no significant difference in Se level was found among our lab-produced yoghurt (n= 8), commercial yoghurts (n= 8), or commercial probiotic yoghurts (n= 4) (Fig. 2). Among the fermented cow milks, no significant difference in Se was found between commercial yoghurts (n= 37) and commercial probiotic yoghurts (n= 18) (*p* > 0.05; Fig. 3).

Dairy products make a major contribution to the daily Se intake, especially in children, due not only to the concentration of this element but also its high bioavailability (around 50%), attributable to its binding to methionine and cysteine (Wardlaw, 2008). Given that the recommended Se intake for healthy adults is 55 μg/day for males and females (Federación Española de Sociedades de Nutrición, 2010), these dairy products may represent an important dietary source (between 6.3 and 10.4% of dietary reference intakes [DRIs]; Table 3). Some other authors have reported that goat milk is a better source of Se than is cow milk (Debski, Picciano, & Milner, 1987; Pappa, Pappas, & Surai, 2006). In the present study, however, a higher concentration of Se was found in the fermented cow milk samples (45.9 ± 3.51 ng/g) than in the fermented goat milk samples (27.7 ± 2.71 ng/g; Fig. 1). Few data are available on Se levels in fermented goat milk, but similar Se levels (28.3 ± 15.8 ng/g) were previously reported by our group (Navarro-Alarcón et al., 2011). Other researchers (Pilarczyk et al., 2013) reported slightly lower mean Se levels (15 ng/ml) in fermented drinks that contained both goat and cow milk. In the present study, no significant differences in Se concentrations were found among the different fermented goat milk products (Fig. 2) or among the different fermented cow milk products analyzed (Fig. 3); hence, Se levels did not change as a function of the fermenting microorganisms utilized.

Higher Se levels were observed in the fermented cow milks in this study (Fig. 1) than previously reported by other researchers in cow milk yogurts [29.9 ± 10.4 ng/g in yogurt with 3.2% fat from Croatia (Klapec, Mnadic, Grgic, Primorac, Perl, & Krstanovic, 2004); 26.9 ± 0.61 ng/g in nonfat yogurt from Greece (Pappa, Pappas, & Surai, 2006); 22.4 ng/g in yogurt from Pakistan (Iqbal, Kazi, Bhanger, Akhtar, & Sarfraz, 2008); 11.0 ng/g in yogurt from Korea (Choi, Kim, Lee, Kim, Hwang, & Park, 2009); and 10.0 ± 9.0 ng/g in yogurt from Poland (Pilarczyc et al., 2010)]. However, markedly higher Se levels were observed by Khan et al. (2014) in commercial cow plain drinking yogurt (PDY; 109 ± 0.64 ng/g), plain paste yogurt (PPY; 98.5 ± 0.56 ng/g), mixed plain yogurt (MPY; 81.4 ± 0.45 ng/g) and mixed drinking yogurt (MDY, 67.9 ± 0.48 ng/g) from South Korea. Murphy and Cashman (2001) described cow milk and by-products as a major source of Se due to their regular consumption and their elevated concentrations of this mineral.

We highlight the significant linear correlation found between Se and the proteins in the studied products (*r*= 0.400), related to its capacity to substitute sulfide-amino acids because of their similar ionic radius (Navarro-Alarcón & Gil Hernández, 2008).

*3.3. Copper levels*

Cu levels did not significantly differ between fermented goat and cow milks (Fig. 1). Among the fermented goat milks, Cu levels did not significantly differ between the lab-produced goat yoghurt and the commercial yoghurts or probiotic yoghurts (Fig. 2). Among the fermented cow milks, Cu levels did not significantly differ between commercial yoghurts and probiotic yoghurts (*p* > 0.05; Fig. 3). Hence, as found for Se, Cu levels did not change as a function of the fermenting microorganisms utilized.

Milks are generally considered poor sources of Cu (Lopez, Collins, & Williams, 1985), but 100 g of milk and yoghurts provide 0.5-1.0% of the daily recommended intake is 900 μg, as we also found in this study (Table 3). The mean concentration of Cu was higher in fermented goat *versus* cow milks, but the difference was not significant (619 ± 100 ng/g *vs.* 488 ± 49.5 ng/g, respectively). Therefore, Cu levels were not influenced by the type of milk used in the manufacture of fermented milk products (Fig. 1).

Cu levels in the present cow milk products ranged from 110 to 1400 ng/g (mean of 400 ng/g), considerably higher than those reported by Suturovic, Kravic, Milanovic, & Williams, 2014 (range 260 to 310 ng/g; mean value= 200 ng/ml) and higher in most cases than the Cu levels reported by Khan et al. (2014) in PDY (140 ng/g), PPY (160 ng/g), MPY (230 ng/g), and MDY (710 ng/g).

Some of the cow milk yoghurts with the highest Cu concentrations were soya yoghurt (1270 ng/g) and yoghurt added with aloe vera (701 ng/g), likely attributable to the plant material included, as previously reported (Sánchez-Segarra, García-Martínez, Gordillo-Otero, Díaz-Valverde, Amaro-López, & Moreno-Rojas, 2000). Likewise, Park (2000) reported a very high Cu concentration (1950 ng/g) in goat yoghurt containing blueberries.

*3.4. Chromium levels*

Although the mean Cr concentration was higher in goat (49.5 ± 11.7 ng/g) than in cow (27.9 ± 10.02 ng/g) fermented milks, the difference was not statistically significant (Fig. 1). There was no significant difference in Cu concentrations of goat (Fig. 2) or cow (Fig. 3) yogurts as a function of the fermenting microorganisms used. Other studies found Cr levels between 6-60 ng/g for cow yoghurts available in Spanish supermarkets (Llorent-Martínez, Fernández de Córdoba, Ruiz-Medina, & Ortega-Barales, 2012). Researchers in Turkey (Güler, 2007) reported higher mean Cr levels in strained goat yogurt (123 ± 30 ng/g) and slightly lower levels in salted goat yogurt (43 ± 7 ng/g) in comparison to the present goat yogurts (49.5 ± 12.3 ng/g).The highest Cr concentration in our study was in goat kefir (106 ± 27.5 ng/g), followed at some distance by skimmed cow yoghurt (45.1 ng/g) and skimmed cow probiotic yoghurt (42.7 ng/g). Given that the recommended Cr intake for healthy adults is 3500 ng/day for males and 2500 ng/day for females (Federación Española de Sociedades de Nutrición, 2010), these dairy products may represent an important dietary source (between 10 and 25% DRIs; Table 3). Markedly higher Cr levels than the present data and previous findings were reported by Khan et al., 2014 in PDY (204 ± 0.32 ng/g), PPY (271 ± 0.38 ng/g), MPY (217 ± 0.23), and MDY (305 ± 0.48 ng/g).

*3.5. Manganese levels*

No difference in Mn levels was found between goat and milk yoghurts (Fig. 1). Mn levels did not change as a function of the fermenting microorganisms utilized in the fermented goat or cow milk products, as observed for the other minerals (Fig. 2 and 3, respectively).

Milks and natural yoghurt are considered to be poor sources of this mineral (Lopez, Collins, & Williams, 1985) as we also reported in the present study (Table 3). The mean Mn concentration was non-significantly lower in fermented goat milks (38.3 ± 9.02 ng/g) than in fermented cow milks (51.1 ± 5.25 ng/g), as also reported by Haenlein (2001). The Mn concentration did not significantly differ among the commercial goat fermented milks, although it was slightly higher in the goat kefir. Similar Mn concentrations have been reported in goat yoghurts by other research groups, although a higher concentration (345 ng/g) was found by Park (2000). Lower Mn concentrations were found by Khan et al. (2014), in PDY (79.3 ± 0.12 ng/g), PPY (73.4 ± 0.12 ng/g), MPY (298 ± 0.35 ng/g) and MDY (237 ± 0.80 ng/g). A markedly higher mean Mn concentration 687 ± 619 ng/g has been reported in fruit yoghurt, but this was attributed to its fruit content (Sánchez-Segarra, García-Martínez, Gordillo-Otero, Díaz-Valverde, Amaro-López, & Moreno-Rojas, 2000).

**4. Conclusions**

The methods developed to measure Se (HG-AAS) and Cu, Cr, and Mn (ETA-AAS) in this study are appropriate for microelement determination in goat and cow yoghurts. Cu, Cr, and Mn levels in fermented milks were not influenced by milk type (goat *versus* cow yoghurt). Se was the only microelement studied that showed lower levels in goat *versus* cow milk yoghurts. The fermenting microorganisms employed in the yoghurt manufacture did not influence Se, Cr, Cu, or Mn levels. Thus, specifically the probiotic strain used to produce our-lab goat yogurt had no influence on mineral levels. Given the recommended Se, Cu, and overall Cr intakes for healthy adults, fermented goat and cow milk products may be important dietary sources for these elements.

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**Figure captions:**

**Fig. 1:** Mean Se, Cu, Cr, and Mn levels in fermented milks according to their animal origin.

**Fig. 2:** Mean Se, Cu, Cr, and Mn levels in fermented goat milks according to the fermenting starters employed in their manufacture.

**Fig. 3:** Mean Se, Cu, Cr, and Mn levels in fermented cow milks according to the fermenting starters employed in their manufacture.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 1**  Analytical parameters of methods used to determine Se, Cu, Cr, and Mn in fermented goat and cow milk products | | | | |
| Element | LODa (ng/g) | LOQb (ng/g) | Recoveryc (%) | Precision, CVd (%) |
| Se | 1.23 | 4.09 | 99.08 ± 2.40 | 7.10 ± 1.48 |
| Cu | 1.61 | 5.36 | 100.06 ± 3.50 | 8.28 ± 1.97 |
| Cr | 2.05 | 6.83 | 99.96 ± 1.77 | 6.36 ± 2.26 |
| Mn | 1.02 | 3.40 | 100.03 ± 1.55 | 6.51 ± 0.26 |
| a Limit of detection  b Limit of quantification.  c Mean recovery obtained by analyte recovery assays in four fractions of the analyte in the same sample.  d Mean coefficient of variation obtained by repeated measurements (*n*= 7) in four goat fermented milks (inter-day variability). | | | | |

**Table 2**

Determination of Se by HG-AAS and Cu, Cr, and Mn by ETA-AAS and in two certified reference materials (*n* = 10; data refer to dry weight)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Element | Mussel Tissue BCR – 278 R | | Bovine Liver BCR 185 R | |
|  | Certified value  (mean ± SD) | Measured value  (mean ± SD) | Certified value  (mean ± SD) | Measured value  (mean ± SD) |
| Se, ng/g | 1660 ± 400 | 1620 ± 120 | 1680 ± 140 | 1760 ± 150 |
| Cu, ng/g | 9600 ± 160 | 9260 ± 260 | 277000 ± 5000 | 290000 ± 15000 |
| Cr, ng/g | 780 ± 60 | 800 ± 80 | - | - |
| Mn, ng/g | 7690 ± 230 | 8050 ± 510 | 11100 ± 290 | 10400 ± 560 |

d Mean ± standard deviation obtained by repeated measurements (*n*= 7) in four goat fermented milks (inter-day variability).

**Table 3**

Mean daily intake and percentage of contribution to dietary reference intakes (DRIs) of healthy adults for minor nutritional elements (Se, Cu, Cr, and Mn) in fermented goat and cow milk products

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Se | | Cr | | Cu | | Mn | |
|  | Goat | Cow | Goat | Cow | Goat | Cow | Goat | Cow |
| Level (ng/g)a | 27.7 | 45.9 | 49.5 | 27.9 | 61.8 | 48.8 | 38.3 | 51.1 |
| Mean daily intake (µg)b | 3.46 | 5.74 | 6.19 | 3.49 | 7.72 | 6.10 | 4.79 | 6.39 |
| DRIs (µg) | 55 |  | 35.0 and 25.0c |  | 900 |  | 2300 and 1800c |  |
| % DRIsb | 6.29 | 10.4 | 17.7 and 24.8c | 9.97 and 14.0c | 0.86 | 0.68 | 0.21 and 0.27c | 0.28 and 0.36c |

a Average level of the determined values (Fig. 1)

b Calculated on the basis of the intake of one yogurt (≅ 125 g)

c For healthy adult men and women, respectively (Federación Española de Sociedades de Nutrición, 2010)