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Essential and non-essential elements in milks and plant-based drinks: a comparison study

TREBALL DE FI DE GRAU

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L'avaluació del treball pràctic tindrà en compte la nota referida pel tutor respecte a la memòria impresa i el seguiment del treball. El resultat de l'avaluació del tutor ha de ser favorable per tal que l'alumne pugui presentar i defensar el treball i representa el 25 % de la nota total del treball escrit (salvo excepcionalitat per la pandèmia).

ENSENYAMENT: Nutrició Humana i Dietètica

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TÍTOL DEL TREBALL: Essential and non-essential elements in milks and plant-based drinks: a comparison study

| SEGUIMENT I AVALUACIÓ DEL TREBALL PER PART DEL TUTOR DEL TREBALL PRÀCTIC | | |
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| (0-10) | | |
| Ha mostrat capacitats d'anàlisi, síntesi i raonament al llarg del treball | | |
| El seu grau d'implicació durant el desenvolupament del treball ha estat elevat | | |
| El procés d'elaboració del treball ha estat continuat | | |
| Ha mostrat habilitat de cerca i gestió de la informació | | |
| Ha mostrat capacitat d'organització i planificació | | |
| Ha seguit la normativa pròpia del Centre en quan a la presentació escrita del treball | | |
| El treball és ordenat i redactat amb cura, expressant-se correctament amb la llengua escollida | | |
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| El treball presentat supera les expectatives del tutor | | |
| <u>Comentaris del tutor</u> | | |
| MITJANA DE LA NOTA DEL TUTOR (0-10) | 9.56 | |
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NO FAVORABLE

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MARQUÉS BUENO, MONTSER MONTSERRAT MONTSERRAT (AUTENTICACIÓN) sn=MARQUES, givenName=MONTSERRAT, cn=MARQUES BUENO, MONTSERRAT (AUTENTICACIÓN) Fecha: 2021.05.27 12:08:06 +02'00'

Reus, a 27 de maig de 2021

*Lliurar una còpia al tutor i adjuntar una còpia amb la signatura original al Treball escrit. La suplantació de la signatura original està tipificada com a falta greu i serà objecte d'expedient.

Abstract

Milk and plant-based drinks are widely consumed foodstuffs with high nutritional value. However, their consumption might represent the intake of non-essential elements becoming a risk for human health. The present study was aimed at determining the concentrations of essential (Ca, Co, Cu, K, Mg, Mn, Mo, Na, Ni, P and Zn) and non-essential (As, Hg, Pb, U and V) elements in milks (cow and goat), plant-based drinks (soy, almond, rice and oat) and infant formulas from organic and conventional production systems. Lactose-free, fresh and Ultra High Temperature (UHT) milks were also included. The chemical analysis was performed by means of ICP-MS. No significant differences were found when comparing the two production systems and lactose to lactose-free milks. On the contrary, significant differences were found in the concentration of multiple elements when comparing sterilization methods (fresh vs. UHT, being K and Na higher in UHT than fresh milks, and Mg higher in fresh than UHT milks), source (animal vs. plant-based, being Ca, K, Mg, Na, P higher in milks than plant-based drinks, while Mn was higher in plant-based drinks than in milks) and animal species (cow vs. goat, being Cu, Mg and P higher in goat than cow). Non-essential elements were not detected in milks and plant-based drinks, except Pb which was detected in 1 sample of conventional skimmed cow milk, 1 sample of organic semi-skimmed fresh cow milk and 1 sample of organic soy-based drink. Whilst the consumption of goat milk is recommended considering the intake of essential elements and the nodetection of non-essential elements in any sample, more extensive studies should be carried out to confirm the absence of our target - and non-target - toxic elements at very low trace levels. On the other hand, the best plant-based drink option is the soy-based drink, although the point detection of Pb in one of the samples should be more deeply explored to confirm its safety. Finally, recommendations derived from this research should be publicly available to help the population to balance the benefits and risks from milk and plant-based drink consumption and make appropriate decisions in their dietary habits.

1. Introduction

Milk is a nutritious, white liquid food excreted by the mammary glands of mammals. Milk is composed mainly of water, carbohydrates (lactose), proteins (casein), fatty acids (triacylglycerides, diacylglycerides, saturated and polyunsaturated fatty acids, and phospholipids), vitamins (mainly retinol, thiamine, riboflavin and niacin) and other trace elements [1, 2, 3]. Milk contains many biologically essential and non-essential trace elements [4, 2].

Essential elements play a vital role in the maintenance of biochemical and physiological functions in living organisms. Essential elements are as follows: Calcium (Ca), Chrome (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), Selenium (Se) and Zinc (Zn). Their role depends on the element. For example, Ca is involved in vascular, endocrine and neuromuscular function [5]; Cu and Fe are involved in physiological balance (i.e.: homeostasis) [6, 7, 8]; K is involved in vascular function (i.e.: blood pressure regulation) [9]; Mg in endocrine function (i.e.: blood glucose control) and biochemical reactions [10]; Mn is involved in immune function and in physiological balance (i.e.: homeostasis, coagulation) [7, 11]; Na is involved in vascular and neuromuscular function (i.e.: transmission of nerve impulses) [9]; P is involved in multiple physiological functions [12]; and Zn is involved in many biochemical and enzymatic reactions [7]. However, if certain concentration thresholds are exceeded, these elements can bioaccumulate and biomagnify in the body, leading to the formation of free radicals, oxidative stress disorders, and consequently becoming harmful for human health [13, 14].

Non-essential elements do not have any known function in the human body and they might be toxic even at low concentrations [15, 16]. Non-essential trace elements are the group of heavy metals and metalloids (semimetals). This group of chemicals entails: Aluminium (Al), Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb), Antimony (Sb), Tin (Sn), Uranium (U) and Vanadium (V). Their toxicity is related to their capability to damage vital organs such as the brain, kidney and liver [17]. The long-term exposure to non-essential elements might lead to physical (i.e.: chronic pain, blood pressure alteration, blood composition change, etc.) and psychological (i.e.: anxiety, passivity, etc.) disorders, neurodegenerative diseases (i.e.: Alzheimer, Parkinson's, etc.), and cancer [17, 18]. Regarding the latter, inorganic As and Cd are classified as "Carcinogenic to humans (Group 1) by the IARC [19].

Heavy metals and metalloids are natural components of the Earth's crust [17]. However, the industrialization, the high-energy demand and the exploitation of natural resources increased their occurrence in the environment [20]. After their emission from the source, they can be transported and deposited on the soil [20, 21]. Hence, livestock is exposed to these chemicals, which enter into

the trophic chain through the consumption of their meat or dairy products, such as milk [22], becoming a potential cause of several illnesses [23, 24].

The presence of trace elements in milk and the composition of milk can vary due to different factors, such as: climate, season of production, the origin country and breed of the producing animal, among others [25, 26, 27]. Most of these studies assess the influence of the aforementioned parameters on the content of a few essential and non-essential elements. However, these studies do not analyse the wide range of milks available for consumption in the supermarkets. Furthermore, in a desire for a healthy lifestyle, an aversion to animal cruelty and an increasing environmental awareness, consumers in recent years have been opting to substitute dairy milks by plant-based driks [28]. The occurrence of essential and non-essential elements in plant-based drinks is less explored, while to the best of our knowledge, there is a gap in the comparison between the content of these elements in animal and plant-based drinks [29, 30, 31].

This study was aimed at assessing the concentrations of essential elements (Ca, Co, Cu, K, Mg, Mn, Mo, Na, Ni, P and Zn) and non-essential elements (As, Hg, Pb, U and V) in milks (cow and goat), plantbased drinks (soy, almond, rice and oat) and infant formulas from organic and conventional production systems. Lactose-free, fresh and Ultra High Temperature (UHT) milks were also included when available in the Spanish markets. Present results will allow to: i) determine if the content of essential and non-essential elements depends on the production system (conventional or organic), origin (animal or plant-based), the animal source (cow or goat), the sterilization method (fresh or UHT), and the presence (or absence) of lactose; and ii) identify the best type of milk in terms of benefits (intake of essential elements) and risks (intake of non-elements).

2. Materials and methods

2.1. Sampling

From January 25 to February 1, 2021, milks and plant-based drinks were bought in several supermarkets located in Reus (Spain). The most consumed types of animal and plant-based drinks were selected and purchased according to the results of ENALIA surveys (National Survey of Nutrition in the Child and Adolescent Population of Spain) [32]. Triplicates of each dairy and plant-based drink were purchased when up to three different brands were available (Table 1).

2.2. Sample treatment

Composites of each milk and plant-based drink were done with 5 mL of each individual sample. In the case of infant formula powder, 15 g were mixed with 30 mL of purified water, and subsequently, the composite was made up with 5 mL of each sample as previously described. Immediately, composites were placed into vials and frozen at -20 °C until further analysis

Up to 5 μ L of each sample was mixed with 5 mL of HNO₃ (10 %) in hermetic Teflon vessels during 8h at room temperature (pre-digestion) and 8h at 80 °C (digestion). Once the digestion was completed, and after samples were cooled to room temperature, extracts were filtered and made up to 25 mL with purified water [13, 33]. Samples were analysed in duplicate to achieve an acceptable accuracy in the results.

2.3. Analytical determination

The concentrations of Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sn, U, V and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS). The analytical method has already been described elsewhere [33, 34].

2.4. Quality control

Spinach leaves (SRM 1570a) and whole milk powder (SRM 1549a) certified by the National Institute of Standards and Technology were used as standard reference materials. Spinach leaves were used to determine the recoveries of the following elements: Cu, Zn, As, Mn, Hg, Pb, Ni, V, Co and U. In turn, the whole milk powder was used for: Cu, Zn, Mo, K, Ca, Mg, Na and P. Recoveries were between the range 75 % and 110 %. Only Mo showed a slightly low recovery (56 %).

The limit of detection (LOD) was set at 250 μ g/g for K, Na and P; at 50 μ g/g for Ca; at 25 μ g/g for Mg; at 0.5 μ g/g for Zn; at 0.1 μ g/g for Cu, As and V; at 0.05 μ g/g for Mn, Hg, Pb, Ni, Mo, Co; and at 0.025 μ g/g for U.

2.5. Statistical analysis

Data are presented as medians and 25th and 75th percentiles for continuous variables with a nonnormal distribution or as the means and standard deviations (SDs) for variables with a normal distribution. Categorical variables are reported as percentages. Continuous variables were tested for normality using the Shapiro–Wilk test. Differences between groups were analysed using the nonparametric Mann–Whitney U test or Welch's parametric t test for continuous variables and the chisquare test or Fisher's exact test for categorical variables. In the significant associations, we have measured the strength of the observed effect (i.e., the effect size) with Cohen's d method.

An unsupervised clustering procedure was used to understand the similarities and differences among the different types and procedences of the studied milks. We used a k-means clustering method and chose the number of clusters (3) using the elbow method. A Principal Component Analysis (PCA) was performed to visualize the relationship between such clusters and each of the studied elements with a significant detection rate.

Confidence intervals were given with a 95% confidence and type I error was set to 5%. Metals that did not show enough observations above the detection limit were not included in the analysis.

All statistical analyses were performed using the R software package version 4.0. [35] along with R Commander [36].

3. Results and discussion

3.1. Concentrations of essential and non-essential elements in animal and plant-based drinks

Most of the milks and plant-based drinks contained Ca, Cu, K, Mg, Mn, Na, P and Zn (Figure 1A-1H). In turn, Mo (Figure 1J) and Ni (Figure 1K) were detected but in less samples (3 and 10, respectively). Finally, Co was not detected in any of the milk and plant-based drinks samples (Supplementary Material, Figure S1). The highest levels of these elements were found in conventional soy-based drink (Cu, Mn, Mg and Ni), organic soy-based drink (Zn and Mo), organic whole goat's milk (K and Na), organic whole fresh goat milk (Ca) and conventional semi-skimmed goat's milk (P).

Regarding the non-essential elements, Pb was detected in conventional skimmed cow's milk, semiskimmed fresh cow's milk and organic soy-based drink (Figure 1L). Even though the concentration of the Pb was below the theoretical LOD in the two latter, Pb exceeded the maximum limit established by the Codex Alimentarius (CODEX STAN 193-1995) in these 3 samples. On the contrary, As, Hg, U and V were not detected in any of milks and plant-based drinks hereby analysed (Supplementary Material, Figure S2-S5).

3.2. Comparison between groups

Those elements detected with a high frequency (>50%) (Ca, Cu, K, Mg, Mn, Na, P, Zn) in milks and plant-based drinks were compared between production systems (conventional vs. organic),

sterilization methods (fresh vs. UHT), source (animal vs. plant-based), animal species (cow vs. goat), presence of lactose (lactose vs. lactose-free) and production system of infant formula (conventional vs. organic),

Production system

No significant differences (p > 0.05) were found between the occurrence of Ca, Cu, K, Mg, Mn, Na, P and Zn (Supplementary Material, Figure S6-13) in conventional and organic milk and plant-based drink. These results are in agreement with other studies [37, 38], and with the fact that the organic certification does not include the occurrence of essential and non-essential elements in food. However, a study conducted in Northwestern Spain showed that the concentrations of some trace elements (Cu, I, Se, Zn) were lower in organic milks than in conventional milks, nevertheless the authors stated that these differences could be related to animal supplementation [39].

Sterilization method

This comparison group includes milks, being plant-based drinks excluded from the analysis because only UHT plant-based drinks samples were included in the study. The occurrence of K (Figure 2A) and Na (Figure 2C) were significantly higher (p < 0.05) in UHT than in fresh milks (effect size measured by Cohen's d, 95% CI of -1.03 (-2.05, -0.02) and -0.80 (-1.80, 0.22), respectively). In contrast, the content of Mg (Figure 2B) was higher (p < 0.05) in fresh than UHT milks and the effect size measured by Cohen's d, 95% CI of 1.05 (0.00, 2.07). Differences between the concentrations of the remaining essential elements (Ca, Cu, Mn, P, Zn) (Supplementary Material, Figure S14-S18) were not significant (p > 0.05).

Our findings are in agreement with Guney et al. [40] who reported that heat treatment does not change the content of Ca and P in milk. However, these authors concluded that the application of heat does not affect the concentration of K, Mg, Na, which is in disagreement with our results. Finally, Singh et al. [41] demonstrated that the content of Ca and P increased with the application of a heat-treatment, which was not found in the present study.

Origin (animal or plant-based)

The levels of Ca, K, Mg, Na and P (Figure 2D-2F and Figure 2H-2J) were significantly higher (p < 0.05) in milks than in plant-based drinks (effect size measured by Cohen's d, 95% Cl of 3.23, (1.96; 4.46), 3.69 (2.32; 5.02), 1.05 (0.15; 1.94), 0.85 (-0.03; 1.72) and 4.22 (2.73; 5.68), respectively). On the contrary, the occurrence of Mn (Figure 2G) was significantly higher (p < 0.05) in plant-based drinks

than in animal ones. We did not find significant differences between the concentrations of Cu and Zn (Supplementary Material, Figure S19-S20) in milks and plant-based drinks.

Dairy products have already been identified as significant sources of Ca, K, Mg, Na and P [5, 29]. In addition, Astolfi et al. [29] stated that Ca, K and P were the elements occurring in the highest concentrations in milks, while Mn was the most abundant element in plant-based drinks, especially in soy beverages, being in agreement with our findings. On the contrary, Davila de Campagnaro [30] stated that almond vegetable drink is rich in Mg i P and has a higher concentration of Ca than cow's milk, which is in disagreement with the present study and Astolfi et al. [29]. Finally, Davila de Campagnaro [30] highlighted that rice and oat drinks have a low content of Ca.

Animal species

The concentrations of Cu, Mg and P (Figure 2K-2M) were statistically higher (p < 0.05) in goat than in cow milk (effect size measured by Cohen's d 95% Cl of -0.87 (-2.02; 0.30), -4.14 (-5.97; -2.27) and -2.52 (-3.93; -1.06), respectively). In contrast, no significant differences were detected between the concentrations of the rest of the elements (Ca, K, Mn, Na, Zn) in goat and cow milks (Supplementary Material, Figure S21-S25).

Whilst some authors [42] have detected Cu and Mg in different kinds of milk, others [43] have not been able to find Cu above the LOD (< $0.50 \ \mu g/g$) [43]. In turn, Lopez et al. [44] reported a significant (p < 0.05) higher content of Cu, Mg and P in goat's milk than in cow's milk, which is in agreement with our study. Beyond these findings, cow milk has already been reported as a good source of Cu, Mg and P taking into account the estimated recommended daily allowances [44].

Presence of lactose

Only milks were included in this group. Vegetable drinks excluded from this analysis because of their plant-based source. Present results did not show significant differences (p > 0.05) in the concentration of the Ca, Cu, K, Mg, Mn, Na, P and Zn between regular milk and lactose-free milk (Supplementary Material, Figure S34-S41).

These results are in agreement with Dekker et al. [45], who neither found that the content of essential and non-essential elements depends on the presence of lactose. However, it must be taken into account that lactose might increase the bioavailability of Ca, although further studies are needed to confirm this mechanism [45, 46].

Production system of infant formula

No significant differences (p > 0.05) were found between the concentration of essential and nonessential elements (Supplementary Material, Figure S26-S33) in terms of the production system (conventional or organic) in infant formulas. However, a previous study reported higher levels of Ca in organic than in conventional infant formula [47].

Multivariate Analysis of results

The PCA allowed to verify the significant differences observed through the K-means clustering, in which distinctions can be visually appreciated in three clusters, depending on the amount of several essential elements (Ca, Cu, K, Mg, Mn, Na, P, Zn). Those elements below the LOD (As, Hg, U and V) or with a detection rate below 50 % (Mo, Ni and Pb) were discarded. Due to the large difference in the concentration of elements between the soy-based drinks and the other plant-based drinks, these two were not included in the same group, being separated in 2 clusters. The three clusters are: 1) milks; 2) soy-based drinks; and 3) the rest of plant-based drinks (Figure 3). The PCA also demonstrates that milks have a higher content of essential elements, mainly Na, Ca, P and K, than plant-based drinks, which is in accordance with other studies [27, 42]. However, it should be highlighted the relevant intake of a few essential elements (Cu, Mn and Zn) that soy-based drinks represent compared to milks and other plant-based drinks. Soy-based drinks are the best choice among all the non-animal drinks hereby assessed, and even in case of a desire to increase these essential elements.

Finally, these results are particularly relevant in light of the tendency to perceive plant-based food as healthier than animal foodstuffs, mainly based on different reasons: i) the recommendations to increase the consumption of fruits, vegetables, whole grains and nuts; ii) environmental and ecological issues related to the higher carbon footprint of animal than plant-based products; iii) the widespread intolerance to lactose or casein; and iv) taste reasons.

4. Conclusions

Milk and plant-based drinks are essential foodstuffs in the diet of a large part of the population. To the best of our knowledge, there are very few studies comparing the content of essential and nonessential elements in different types of milks, plant-based drinks, infant formula products. Likewise, the impact of organic and conventional production systems, sterilization methods and the presence of lactose on their concentrations has been scarcely assessed. Our study provides evidence on which type of milk or plant-based drink contains the highest concentration of each essential element. In this sense, goat milk is rich in Ca, K, Na and P, while soy drink is rich in Cu, Mg, Mn, Mo, Ni and Zn. The presence of non-essential elements (such as As, Hg, Pb, U and V) is hardly appreciated in dairy milks and plant-based drinks, being the potential risks derived from their consumption minimized. However, the detection of Pb in point samples of cow milk and soy-based drink exceeded the maximum limit established by the Codex Alimentarius (CODEX STAN 193-1995), which should be more deeply assessed.

Overall, milk is the best choice among all the types of milks and plant-based drinks hereby assessed, being goat's milk the best option balancing the benefits and the potential risks. Soy drinks are recommended in a desire to avoid consuming animal products, or for those who have allergies or intolerances to milks, or even in a desire to increase the intake of Cu, Mn and Zn. The rest of plant-based milks do not represent a significant intake of essential either non-essential elements. Anyhow, individuals who would rather consume almond, oat or rice drinks have the chance to enhance the intake of these essential elements through other food sources.

Present findings allow us to recommend both conventional and organic production systems, as well as regular or lactose-free milks. In turn, more extensive studies are needed to confirm the potential role of the sterilization methods on the content of essential elements, while the absence of Pb and other toxic elements should be further assessed.

Finally, recommendations derived from this research should be publicly available because of their crucial value to help the population to balance the benefits and risks from milk and plant-based drink consumption through making appropriate decisions in their dietary habits.

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6. References

[1] Ballard O, Morrow AL. "Human milk composition: nutrients and bioactive factors". Pediatr Clin North Am. 2013;60(1):49-74.

[2] German JB, Dillard CJ. "Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules". Crit Rev Food Sci Nutr. 2006;46(1):57-92.

[3] Food and Agriculture Organization of the United Nations (FAO). "Milk and Dairy products in human nutrition". Rome, 2013. Available: www.fao.org/publications

[4] Mehri A. "Trace Elements in Human Nutrition (II)" - An Update. Int J Prev Med. 2020 Jan 3;11:2.

[5] Reid IR, Bristow SM, Bolland MJ. "Calcium supplements: benefits and risks". J Intern Med. 2015 Oct;278(4):354-68.

[6] Royer A, Sharman T. "Copper Toxicity". 2020 Oct 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 32491388.

[7] Filippini T, Cilloni S, Malavolti M, Violi F, Malagoli C, Tesauro M, Bottecchi I, Ferrari A, Vescovi L, Vinceti M. "Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community". J Trace Elem Med Biol. 2018 Dec;50:508-517.

[8] Dev S, Babitt JL. "Overview of iron metabolism in health and disease". Hemodial Int. 2017 Jun;21Suppl 1(Suppl 1):S6-S20.

[9] Newberry SJ, Chung M, Anderson CAM, Chen C, Fu Z, Tang A, Zhao N, Booth M, Marks J, Hollands S, Motala A, Larkin J, Shanman R, Hempel S. "Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks" [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2018 Jun. Report No.: 18-EHC009-EF

 [10] Rude RK. Magnesium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. "Modern Nutrition in Health and Disease". 11th ed. Baltimore, Mass: Lippincott Williams & Wilkins; 2012:159-75.

[11] Evans GR, Masullo LN. "Manganese Toxicity". 2020 Jul 15. In: StatPearls [Internet]. Treasure Island(FL): StatPearls Publishing; 2021 Jan–2020 Jul 15.

[12] Chang AR, Anderson C. "Dietary Phosphorus Intake and the Kidney". Annu Rev Nutr. 2017 Aug 21;37:321-346.

[13] Binder GA, Metcalf R, Atlas Z, Daniel KG. "Evaluation of digestion methods for analysis of trace metals in mammalian tissues and NIST 1577c". Anal Biochem. 2018 Feb 15;543:37-42.

[14] Gupta, S., Satpati, S., Nayek, S. et al. "Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes". Environ Monit Assess 165, 169–177 (2010).

[15] Rawn DFK, Sadler AR, Casey VA, Breton F, Sun WF, Arbuckle TE, Fraser WD. "Dioxins/furans and PCBs in Canadian human milk: 2008-2011". Sci Total Environ. 2017 Oct 1;595:269-278.

[16] AESAN (Spanish Agency for Food Safety and Nutrition). "Heavy metals". [Internet]. Publicationdate:Oct.2,2015.Availablefrom:http://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/subdetalle/metales_pesados.htm

[17] Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. "Toxicity, mechanism and health effects of some heavy metals". Interdiscip Toxicol. 2014;7(2):60-72. doi:10.2478/intox-2014-0009

[18] Al Osman M, Yang F, Massey IY. "Exposure routes and health effects of heavy metals on children".Biometals. 2019;32(4):563–73.

[19] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. "Some drinking-water disinfectants and contaminants, including arsenic". IARC Monogr Eval Carcinog Risks Hum. 2004;84:1-477.

[20] P.K. Gautam, R.K. Gautam, M.C. Chattopadhyaya, S. Banerjee, M.C. Chattopadhyaya, J.D. Pandey.
"Heavy metals in the environment: fate, transport, toxicity and remediation technologies". Editor:
Deepak Pathania. Heavy metals: sources, toxicity and remediation techniques. Nova Science
Publishers. 2016 (pp.101/130)

[21] Briffa J, Sinagra E, Blundell R. "Heavy metal pollution in the environment and their toxicological effects on humans". Heliyon. 2020 Sep 8;6(9):e04691.

[22] Numa Pompilio CG, Francisco CS, Marco Tulio FM, Sergio Samuel SM, Fernanda Eliza GJ. "Heavy metals in blood, milk and cow's urine reared in irrigated areas with wastewater". Heliyon. 2021 Apr 15;7(4):e06693.

[23] Beikzadeh S, Ebrahimi B, Mohammadi R et al. "Heavy Metal Contamination of Milk and Milk Products Consumed in Tabriz". Current Nutrition & Food Science. 2019; 15: 484-492.

[24] Mahmoudi R, Kazeminia M, Kaboudari A et al. "A review of importance, detection and controlling of heavy metal in milk and dairy products". Malaysian Journal of Science. 2017. 36 (1): 1– 16.

[25] Ministry for Ecological Transition and Demographic Challenge (Government of Spain). "Heavy metals" [Internet]. Available from: https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/temas/atmosfera-y-calidad-del-aire/emisiones/prob-amb/metales_pesados

[26] Schwendel BH, Wester TJ, Morel PC, Tavendale MH, Deadman C, Shadbolt NM, Otter DE. "Invited review: organic and conventionally produced milk-an evaluation of factors influencing milk composition". J Dairy Sci. 2015 Feb;98(2):721-46.

[27] GONZALEZ-MONTANA, José R. "Heavy metals in meat and milk and certification for the European Union (EU)". Rev Colom Cienc Pecua [online]. 2009, vol.22, n.3 [cited 2021-05-05], pp.305-310.

[28] Battisti I, Ebinezer LB, Lomolino G, Masi A, Arrigoni G. "Protein profile of commercial soybean milks analyzed by label-free quantitative proteomics". Food Chem. 2021;352(129299):129299.

[29] Astolfi ML, Marconi E, Protano C, Canepari S. "Comparative elemental analysis of dairy milk and plant-based milk alternatives". Food Control. 2020;116:107327.

[30] Dávila de Campagnaro E. "Plant-based drinks and milks of other mammals". Arch Venez Pueric Pediatr. 2017;80(3):96–101.

[31] Llorent-Martínez EJ, de Córdova MLF, Ruiz-Medina A, Ortega-Barrales P. "Analysis of 20 trace and minor elements in soy and dairy yogurts by ICP-MS". Microchem J. 2012;102:23–7.

[32] Cuadrado-Soto E, López-Sobaler AM, Jiménez-Ortega AI, Bermejo LM, Aparicio A, Ortega RM. "Breakfast Habits of a Representative Sample of the Spanish Child and Adolescent Population (The ENALIA Study): Association with Diet Quality". Nutrients. 2020 Dec 8;12(12):3772.

[33] Perelló G, Vicente E, Castell V, Llobet JM, Nadal M, Domingo JL. "Dietary intake of trace elements by the population of Catalonia (Spain): results from a total diet study". Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2015;32(5):748-55. [34] Marquès M, Sierra J, Drotikova T, Mari M, Nadal M, Domingo JL. "Concentrations of polycyclic aromatic hydrocarbons and trace elements in Arctic soils: A case-study in Svalbard". Environ Res. 2017 Nov;159:202-211.

[35] R Core Team. R: "A language and environment for statistical computing". R Foundation for Statistical Computing. 2020. Vienna, Austria. URL: https://www.R-project.org/

[36] Fox J, Bouchet-Valat M (2020). "*Rcmdr: R Commander*". R package version 2.7-1, https://socialsciences.mcmaster.ca/jfox/Misc/Rcmdr/.)

[37] G. Anacker. "Differences between composition of organic milk and conventional milk Lebensmitt. Milchwirtsch., 128 (2007), pp. 17-25

[38] Schwendel BH, Wester TJ, Morel PC, Tavendale MH, Deadman C, Shadbolt NM, Otter DE. "Invited review: organic and conventionally produced milk-an evaluation of factors influencing milk composition". J Dairy Sci. 2015 Feb;98(2):721-46.

[39] Rey-Crespo F, Miranda M, López-Alonso M. "Essential trace and toxic element concentrations in organic and conventional milk in NW Spain". Food Chem Toxicol. 2013 May;55:513-8.

[40] Guney B, Gokmen S. "Effects of different heat treatment and radiation (microwave and infrared) sources on minerals and heavy metals contents of cow's milk". J Food Process Preserv. 2021;45:e15084.

[41] Singh R, Gautam N, Mishra A, Gupta R. "Heavy metals and living systems: An overview". Indian J Pharmacol 2011;43:246-53

[42] LANTE, A. – LOMOLINO, G. – CAGNIN, M. – SPETTOLI, P. 2004. "Content and characterization of minerals in milk and in crescenza and squacquerone Italian fresh cheese by ICP-OES". Food Control, vol. 17, 2004, p. 229–233.

[43] M. Tunegová, R. Toman, V. Tančin. "Heavy metals - environmental contaminants and their occurrence in different types of milk". Slovak J. Anim. Sci., 49, 2016 (3): 122–131

[44] Lopez A, Collins WF, Williams HL. "Essential elements, cadmium, and lead in raw and pasteurized cow and goat milk". J Dairy Sci. 1985;68(8):1878–86.

[45] Dekker PJT, Koenders D, Bruins MJ. "Lactose-Free Dairy Products: Market Developments, Production, Nutrition and Health Benefits". Nutrients. 2019 Mar 5;11(3):551.

[46] Kwak H.-S., Lee W.-J., Lee M.-R. "Reviewing lactose as an enhancer of calcium absorption". En t. Dairy J. 2012; 22 : 147-151.

[47] Ljung K, Palm B, Grandér M, Vahter M. "High concentrations of essential and toxic elements in infant formula and infant foods - A matter of concern". Food Chem. 2011 Aug 1;127(3):943-51.

[48] Scholz-Ahrens KE, Ahrens F, Barth CA. "Nutritional and health attributes of milk and milk imitations". Eur J Nutr. 2020 Feb;59(1):19-34.

Tables and Figures

Table 1. List of milks and plant-based drinks, abbreviations and number of samples analysed.

| Kind of milk/plant-based drink | Abbreviation | Number of samples |
|--|--------------|-------------------|
| Whole cow's milk conventional | CON-WCM | 3 |
| Whole cow's milk organic | ORG-WCM | 3 |
| Semi-skimmed cow's milk conventional | CONV-SSCM | 3 |
| Semi-skimmed cow's milk organic | ORG-SSCM | 3 |
| Skimmed cow's milk conventional | CONV-SCM | 3 |
| Skimmed cow's milk organic | ORG-SCM | 3 |
| Lactose-free cow's milk conventional | CONV-LFCM | 3 |
| Lactose-free cow's milk organic | ORG-LFCM | 3 |
| Whole goat's milk organic | ORG-WGM | 1 |
| Semi-skimmed goat's milk conventional | CONV-SSGM | 3 |
| Whole fresh cow's milk conventional | CONV-WFCM | 3 |
| Whole fresh cow's milk organic | ORG-WFCM | 2 |
| Semi-skimmed fresh cow's milk conventional | CONV-SSFCM | 3 |
| Semi-skimmed fresh cow's milk organic | ORG-SSFCM | 3 |
| Skimmed fresh cow's milk conventional | CONV-SFCM | 2 |
| Whole fresh goat's milk conventional | CONV-WFGM | 1 |
| Whole fresh goat's milk organic | ORG-WFGM | 1 |
| Almond-based drink conventional | CONV-ABD | 3 |
| Almond-based drink organic | ORG-ABD | 3 |
| Oat-based drink conventional | CONV-OBD | 3 |
| Oat-based drink organic | ORG-OBD | 3 |
| Soy-based drink conventional | CONV-SBD | 3 |
| Soy-based drink organic | ORG-SBD | 3 |
| Rice-based drink conventional | CONV-RBD | 3 |
| Rice-based drink organic | ORG-RBD | 3 |
| Follow-on formula conventional | CONV-IF | 3 |
| Follow-on formula organic | ORG-IF | 3 |
| Follow-on formula 2 conventional | CONV-CIFF2 | 3 |
| Follow-on formula 2 organic | ORG-CIFF2 | 3 |
| Follow-on formula 3 conventional | CONV-CIFF3 | 3 |
| Follow-on formula 3 organic | ORG-CIFF3 | 2 |
| Follow-on formula 4 organic | ORG-CIFF4 | 3 |

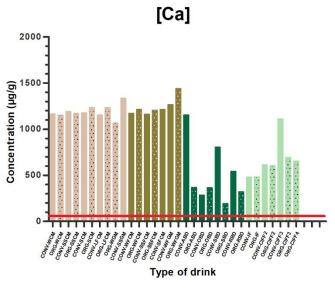
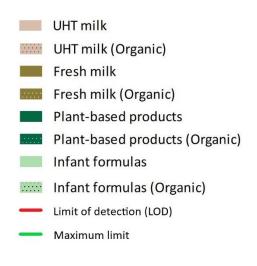


Figure 1a. Concentration of calcium (Ca)



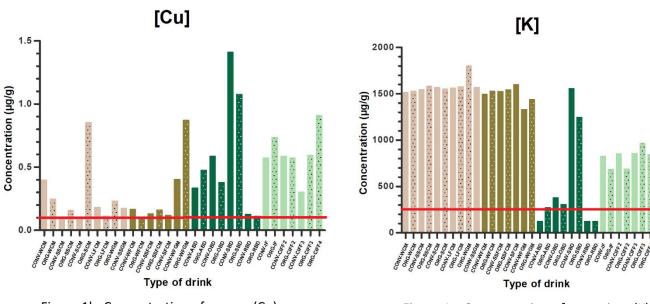
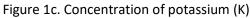
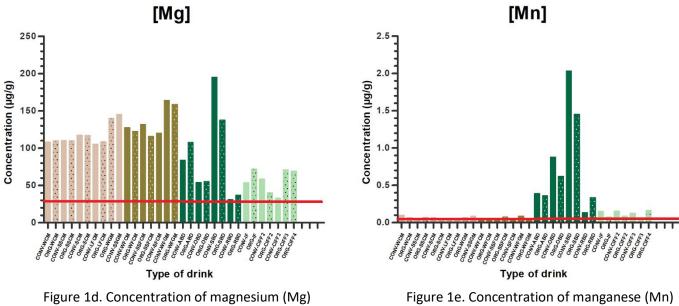
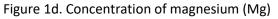
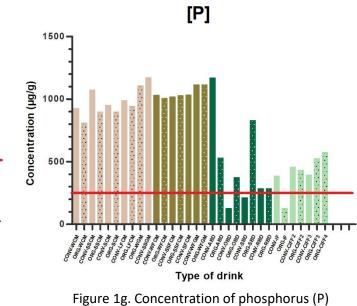


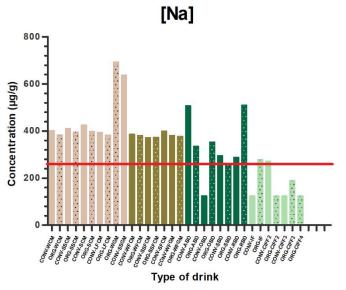
Figure 1b. Concentration of copper (Cu)

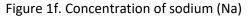


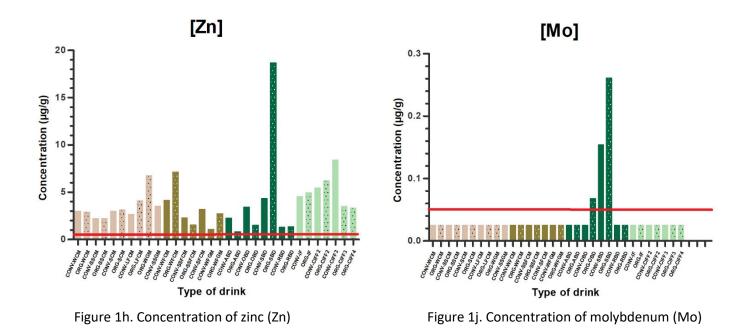


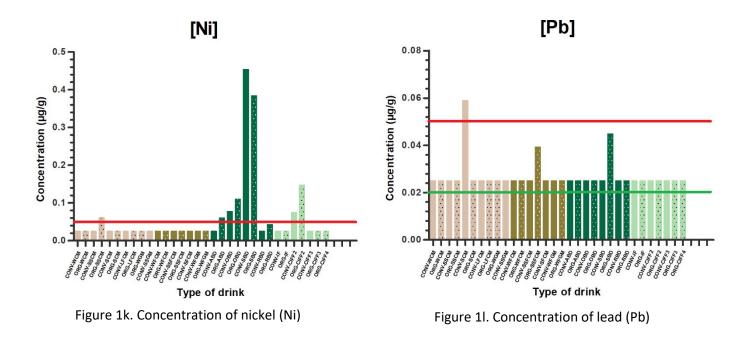












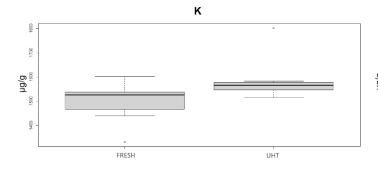


Figure 2a. Box plot of K concentration in milk according to sterilization method (fresh or UHT).

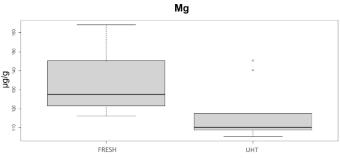


Figure 2b. Box plot of Mg concentration in milk according to sterilization method (fresh or UHT).

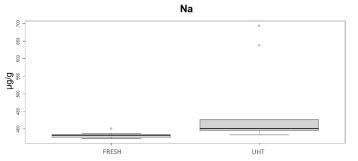


Figure 2c. Box plot of Na concentration in milk according to sterilization method (fresh or UHT).

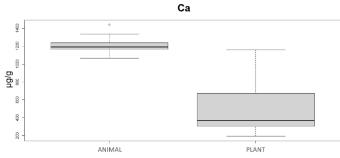


Figure 2d. Box plot of Ca concentration in milk and plant-based drinks according to the origin (animal or plant-based).

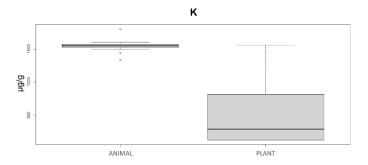


Figure 2e. Box plot of K concentration in milk and plant-based drinks according to the origin (animal or plant-based).

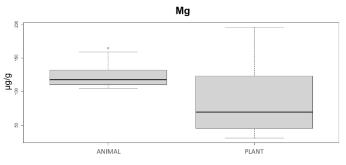


Figure 2f. Box plot of Mg concentration in milk and plant-based drinks according to the origin (animal or plant-based).

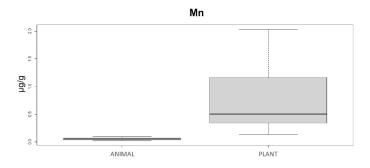


Figure 2g. Box plot of Mn concentration in milk and plant-based drinks according to the origin (animal or plant-based).

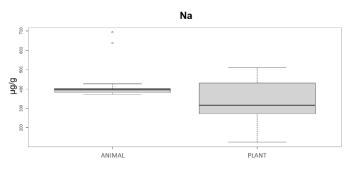


Figure 2h. Box plot of Na concentration in milk and plant-based drinks according to the origin (animal or plant-based).

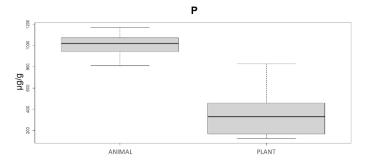


Figure 2j. Box plot of P concentration in milk and plant-based drinks according to the origin (animal or plant-based).

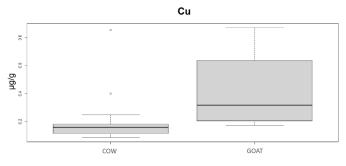


Figure 2k. Box plot of Cu concentration in milk according to the animal species (cow or goat).

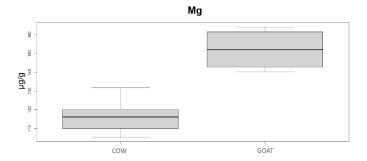


Figure 2l. Box plot of Mg concentration in milk according to the animal species (cow or goat).

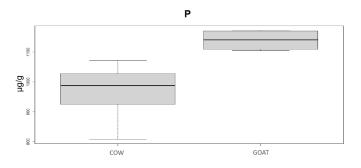


Figure 2m. Box plot of P concentration in milk according to the animal species (cow or goat).

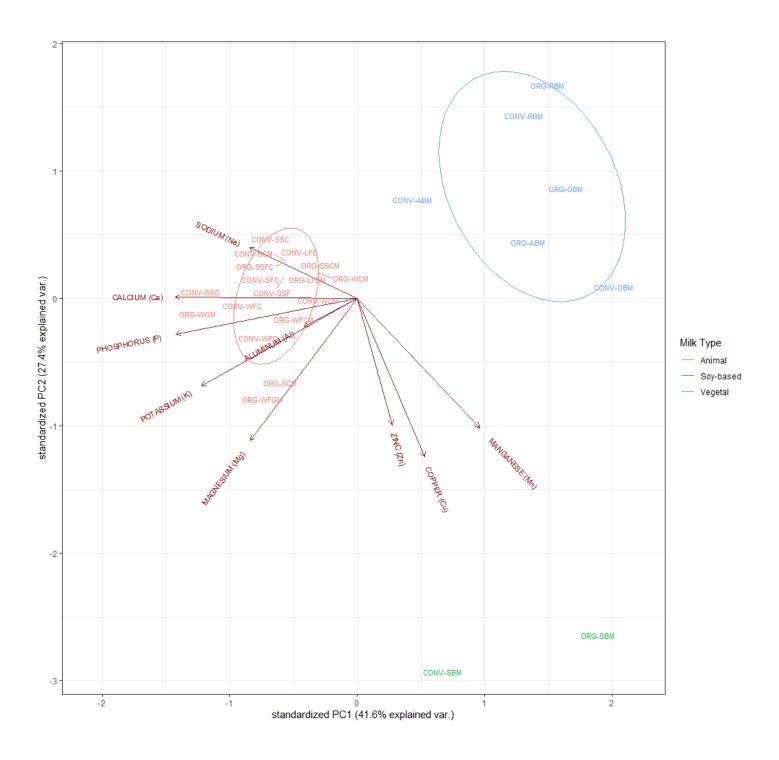


Figure 3. Principal component analysis of essential elements (Ca, Cu, K, Mg, Mn, Na, P, Zn).

Supplementary material

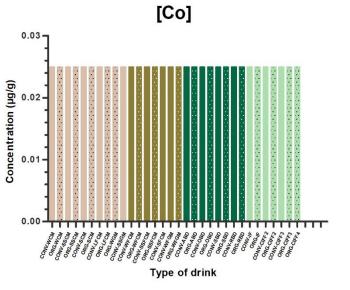
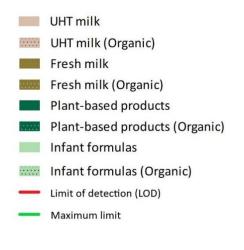


Figure s1. Concentration of cobalt (Co)



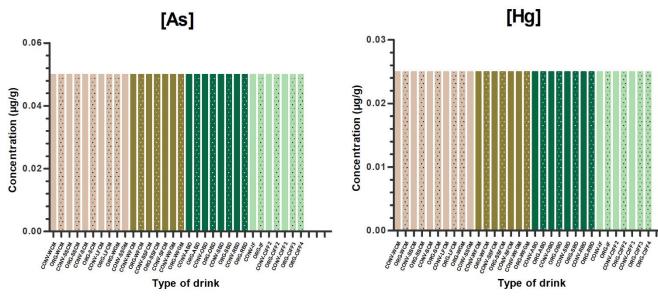
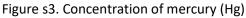
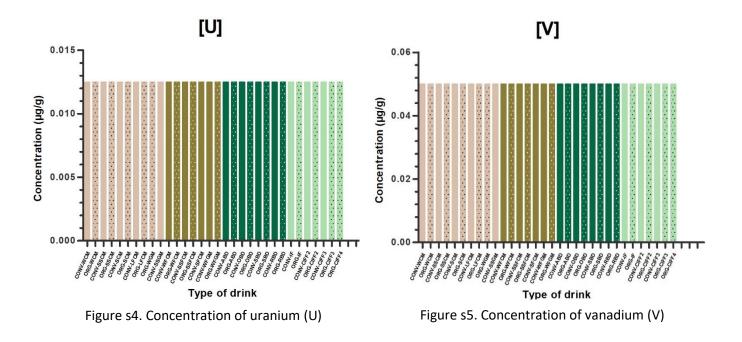


Figure s2. Concentration of arsenic (As)





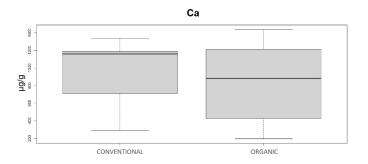


Figure s6. Box plot of Ca concentration in milks and plant-based drinks from conventional and organic production systems.

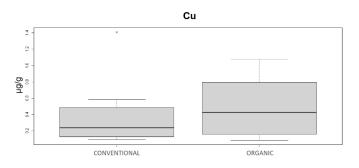


Figure s7. Box plot of Cu concentration in milks and plant-based drinks from conventional and organic production systems.

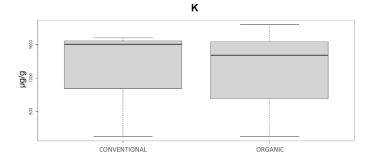


Figure s8. Box plot of K concentration in milks and plant-based drinks from conventional and organic production systems.

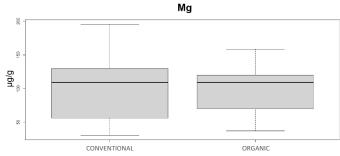


Figure s9. Box plot of Mg concentration in milks and plant-based drinks from conventional and organic production systems.

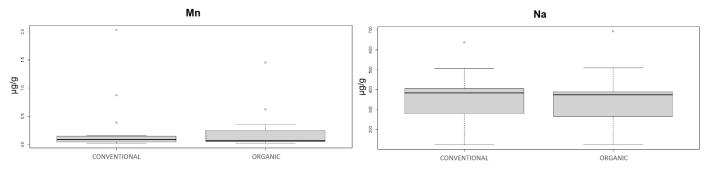


Figure s10. Box plot of Mn concentration in milks and plant-based drinks from conventional and organic production systems.

Figure s11. Box plot of Na concentration in milks and plant-based drinks from conventional and organic production systems.

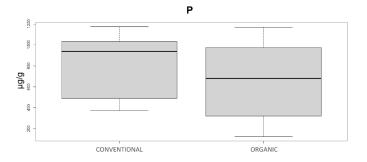


Figure s12. Box plot of P concentration in milks and plant-based drinks from conventional and organic production systems.

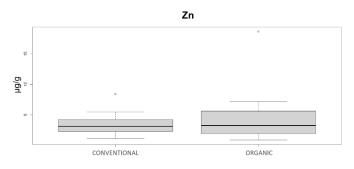


Figure s13. Box plot of Zn concentration in milks and plant-based drinks from conventional and organic production systems.

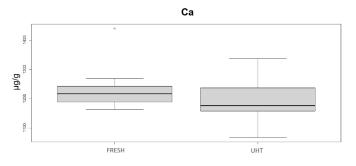


Figure s14. Box plot of Ca concentration in milk according to sterilization method (fresh or UHT).

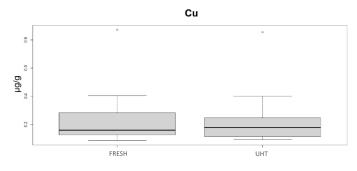
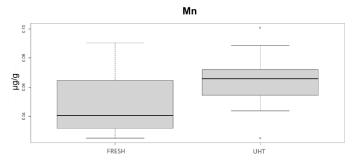
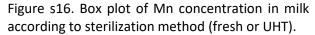


Figure s15. Box plot of Cu concentration in milk according to sterilization method (fresh or UHT).





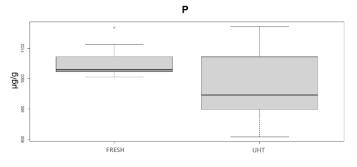


Figure s17. Box plot of P concentration in milk according to sterilization method (fresh or UHT).

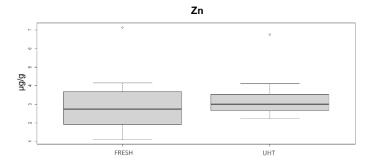


Figure s18. Box plot of Zn concentration in milk according to sterilization method (fresh or UHT).

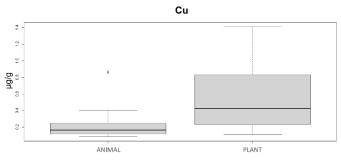


Figure s19. Box plot of Cu concentration in milk and plant-based drinks according to the origin (animal or plant-based).

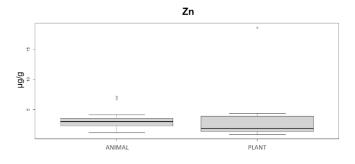


Figure s20. Box plot of Zn concentration in milk and plant-based drinks according to the origin (animal or plant-based).

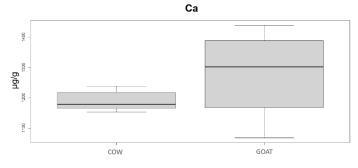


Figure s21. Box plot of Ca concentration in milk according to the animal species (cow or goat).

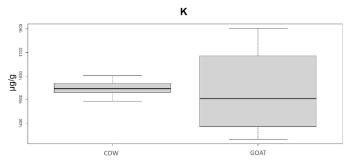


Figure s22. Box plot of K concentration in milk according to the animal species (cow or goat).

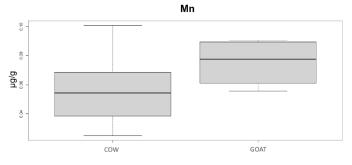


Figure s23. Box plot of Mn concentration in milk according to the animal species (cow or goat).

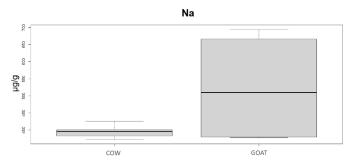


Figure s24. Box plot of Na concentration in milk according to the animal species (cow or goat).

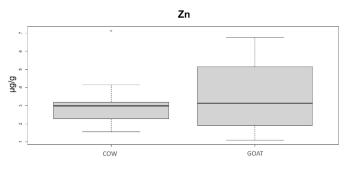


Figure s25. Box plot of Zn concentration in milk according to the animal species (cow or goat).

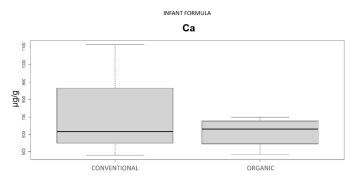


Figure s26. Box plot of Ca concentration in milk from conventional and organic production systems in infant formula.

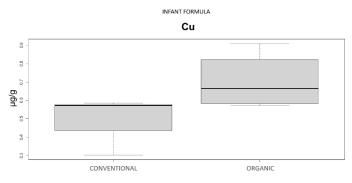


Figure s27. Box plot of Cu concentration in milk from conventional and organic production systems in infant formula.

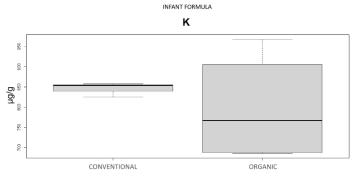


Figure s28. Box plot of K concentration in milk from conventional and organic production systems in infant formula.

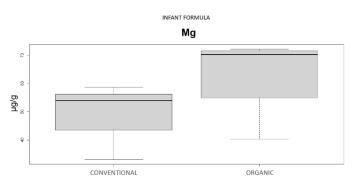


Figure s29. Box plot of Mg concentration in milk from conventional and organic production systems in infant formula.

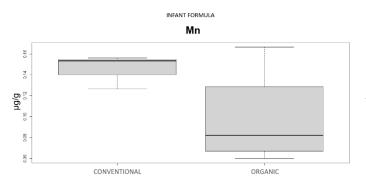


Figure s30. Box plot of Mn concentration in milk from conventional and organic production systems in infant formula.

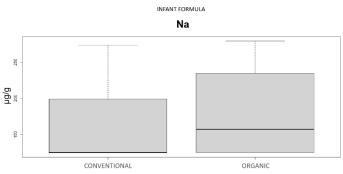


Figure s31. Box plot of Na concentration in milk from conventional and organic production systems in infant formula.

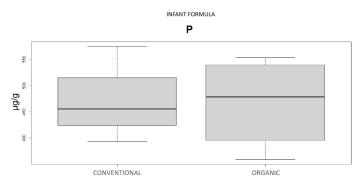


Figure s32. Box plot of P concentration in milk from conventional and organic production systems in infant formula.

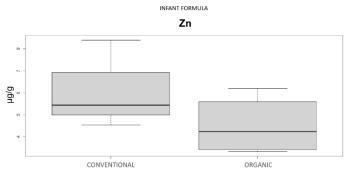


Figure s33. Box plot of Zn concentration in milk from conventional and organic production systems in infant formula.

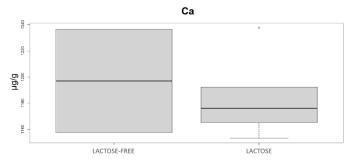


Figure s34. Box plot of Ca concentration in milk according to the presence or absence of lactose.

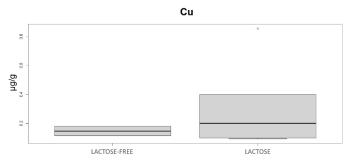


Figure s35. Box plot of Cu concentration in milk according to the presence or absence of lactose.

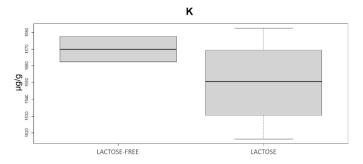


Figure s36. Box plot of K concentration in milk according to the presence or absence of lactose.



Figure s37. Box plot of Mg concentration in milk according to the presence or absence of lactose.



Figure s38. Box plot of Mn concentration in milk according to the presence or absence of lactose.

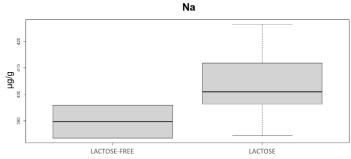


Figure s39. Box plot of Na concentration in milk according to the presence or absence of lactose.

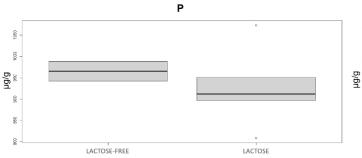


Figure s40. Box plot of P concentration in milk according to the presence or absence of lactose.

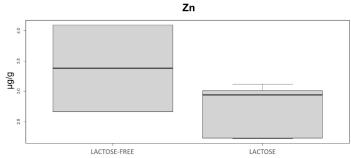


Figure s41. Box plot of Zn concentration in milk according to the presence or absence of lactose.