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A Rapid Routine Methodology Based on Chemometrics to Evaluate the Toxicity of Commercial Infant Milks Due to Hazardous Elements

Ainara Gredilla[1] · Silvia Fdez-Ortiz de Vallejuelo[2] · Gorka Arana[2] · Alberto de Diego[2] · Marcos L. S. Oliveira[3,4] · Katia da Boit[3] · Juan Manuel Madariaga[2] · Luis F. O. Silva[3]

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Abstract

The toxicity and the health risk assessment associated to the presence of some hazardous elements (HEs) in dried (infant formula and powdered) milks due to manufacturing and packaging process, raw materials used, environmental conditions, etc. need to be determined. With this aim, a new methodology based on the combination of health risk quotients and non-supervised (as cluster analysis (CA) and principal component analysis (PCA)) chemometric techniques is proposed in this study. The methodology was exemplified using the concentration of 27 elements, some of them HEs, measured in 12 powdered milk samples produced for children and adults in Brazil and Colombia. The concentration values were obtained by inductively coupled plasma-mass spectrometry (ICP-MS) after acid microwave digestion. Elemental concentrations vary depending upon the type of milk (initiation, growing-up, follow-on milks and adult milks). However, hazard quotients (HQ) and carcinogenic risk (CR) values showed no risk associated to the presence of HEs on milks. The methodology designed made possible to conclude that adults' milks are more characteristic of elements naturally present in milk. Children milks present major presence of trace and minor elements. Between infant milks, sample H, designed for babies between 12 and 36 months, was identified as of poor quality. Moreover, it was possible to deduce that while the fortification process applied to children powdered milks is a probable metal and metalloid source, together with the manufacturing, the skimming process is not a contamination source for milks.

Keywords Infant milk · Powdered milk · Hazardous elements · Toxicity · Chemometrics

Introduction

Milk, considered a nearly complete food, is a good source of proteins, enzymes, minerals, fats, organic acids, carbohydrates, minerals, nutrients, and vitamins, all of which are necessary for best developmental and psychosocial outcomes for new-borns and good growth of children (Khan et al., 2014; Muhib et al., 2016). There are more than 35 micro and trace elements as inorganic ions reported to be found in raw milk (Muhib et al., 2016). Most of the components of the milk, metals or metalloids such as Ca, Cu, Zn, Mn, Mo and Fe, are considered essential elements for many physiological human functions, but they become toxic when their concentration level exceeds 40- to 200-fold their respective recommended threshold value (Koh and Judson, 1986; Rao, 2005). Some other metals, such as As, Cd, Pb and Hg, however, can be considered hazardous or toxic elements at trace level and may be accumulated along the food chain having toxic effects on human health

✉ Ainara Gredilla
ainara.gredi@ehu.eus

1 Department of Applied Chemistry, Faculty of Chemistry, University of the Basque Country UPV/EHU, P.C. 2018 San Sebastian, Basque Country, Spain

2 Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country UPV/EHU, P.O. Box 644, 48080 Bilbao, Basque Country, Spain

3 Department of Civil and Environmental, Universidad de la Costa, Calle 58 #55-66, 080002 Barranquilla, Atlántico, Colombia

4 Departamento de Ingeniería Civil y Arquitectura, Universidad de Lima, Avenida Javier Prado Este 4600, 1503 Santiago de Surco, Peru

(Zheng et al., 2007). The level of toxicity and accumulative effects from these metals is related to age, route and time of exposure, metal oxidation state, retention percentage, etc. (Mertz, 1986). Metal elimination from milk is not easily achieved, because lipophilic constituents can find their way to bound into the persistent fat compounds from where they cannot be removed (Girma et al., 2014; Muhib et al., 2016). The amount of hazardous elements in uncontaminated milk is usually as minor or trace level, but their contents may be altered through production treatment, manufacturing and packaging processes and environmental conditions causing; in some cases, serious problems. Therefore, the control and quality monitoring of milk products during their production and distribution by the analysis of major, minor and trace elements is necessary (Farid and Baloch, 2012; Khan et al., 2014).

In the case of infant milk, there are synthetic formulas designed to mimic the composition of breast milk. These formulas usually include several raw materials (such as skimmed milk, concentrated milk, lactose, vegetable oils, micronutrient supplies, etc.) and undergo aggressive treatments, such as for example protein hydrolysis. Moreover, infant milks are fortified with essential elements considered nutrients, such as Fe, Zn and Cu, mixed during the manufacturing process at high temperatures and pressures in tanks (Cancela and Yebra, 2006; Hozyasz and Ruszczynska, 2004; Khan, 2008; Landigran et al., 2002; Leotsinidis et al., 2005; Lima de Paiva et al., 2019). Fe is present in all human cells, participates as a catalyst in several metabolic reactions and its deficiency causes anemia, developmental delays and behavioural disturbances. An excess of iron intake, on the other side, may interfere in the absorption of Cu and Zn, favouring oxidative stress and infections (Fernández-Sánchez et al., 2012). Zn is required for the metabolic activity of more than 400 body proteins and has a role in cell-mediated immune and other physiological functions (Fernández-Menéndez et al., 2016). Cu is necessary for the adequate growth, integrity of the cardiovascular system and iron metabolism. Considering that cow milk has low copper content, infants fed solely with milk could develop Cu deficiency and, consequently, anaemia. For these motives, the infant milk formula is usually reinforced with Cu (Campillo et al., 1998). After the fortification of milks during the manufacturing process, the product as powered milk is automatically packaged into each container and inert gas is injected to ensure the quality of the milk during its shelf life.

There are three types of infant milk that can present different elemental compositions: (i) started formulas (0–6 months), used to satisfy by itself the nutritional requirements during the first months of life; (ii) standard follow-up formulas (6–12 months) used as a liquid part of diet for the infant; and finally, (iii) follow-on formulas (12–36 months) (CAC, 2016; CAC, 2017).

Metal input through powdered milk can be considered a health concern in most regions of the world. In fact, it is calculated that infants and young children absorb 50% of dietary toxic metals (compared to 10% in the case of adults) by milk consumption. Clearly, the intake of toxic metals by infants is even more dangerous than in adults, due to their high intestinal absorption capacity of toxic substances, which may cause growth problems and the risk of bad mental development. Therefore, the monitoring of these hazardous elements in the milk is essential to ensure the benefits of consuming commercial synthetic formulas (Krachler et al., 2000; Rebelo and Dutra Caldas, 2016; Saracoglu et al., 2007).

Several studies of milk samples can be found in the references (Abdulkhaliq et al., 2012; Ahmad et al., 2017; Cruz et al., 2009; Cava-Montesinos et al., 2004; Enb et al., 2009; Franco-Uría et al., 2009; Kazi et al., 2009; Kondyli et al., 2007; Krachler et al., 2000; Lutfullah et al., 2014; Martínez et al., 2019; Muñoz and Palmero, 2004; Pereira et al., 2013; Pilarczyk et al., 2013; Sager et al., 2018). Metal inputs in infant milks have also been reported, for example, by Lima de Paiva et al. (2019) for Al in milks from Brazil and by Gardener et al. (2019), for Cd and Pb in baby food from the USA, among others. However, all these studies are based on elemental concentration data recompilation, and none of them proposes an acceptable methodological tool to evaluate infant milk quality and associated risks.

For all these reasons, the objective of this work was to propose a fast, easy and conclusive analytical methodology useful for laboratories and companies working on infant milk manufacturing. The methodology proposed is based in the combination of quality guidelines, the *Standardized Coefficients* (SCs, proposed firstly in this study), and chemometrics applied to the elemental concentration data set. To evaluate and validate the proposed methodology, the elemental composition of some powered milk samples (for infants and adults) collected in Brazil and Colombia was used as a case study.

Materials and Methods

Sampling Collection

Thirty-six commercially available and most consumed milks (3 boxes of each of the 12 milk formulas labelled from A to L, see Table 1) were purchased from different local supermarkets in representative cities of Brazil and Colombia. All the samples were kept in their original packages and transferred to the laboratory, properly labelled and stored at room temperature. Then, samples were shipped to the IBeA laboratory located at the University of Basque Country UPV/EHU (Bilbao, Spain) for chemical analysis. All the samples were stored at room temperature until analysis, which was

Table 1 Code name and type of the analysed milk power samples

Code	Age group	Type of milk
A	Initiation milk: 0 to 6 months	Whole
B	Initiation milk: 0 to 6 months	Whole
C	Initiation milk: 0 to 6 months	Whole
D	Initiation milk: 0 to 6 months	Whole
E	Follow-up milk: 6 to 12 months	Whole
F	Follow-up milk: 6 to 12 months	Whole
G	Follow-up milk: 6 to 12 months	Whole
H	Follow-on milk: 12 to 36 months	Whole
I	Follow-on milk: 12 to 36 months	Whole
J	Adult	Whole
K	Adult	Skimmed
L	Adult	Whole

carried out within 4 months of their purchase and before the expiry date.

Sample Preparation and Analysis

After the reception at the IBeA laboratory, individual units, corresponding to the triplicate milk boxes of each commercial milk, were combined, homogenized and stored in glass tubes of 50 mL.

A total of 0.5 g of milk powder was exactly weighted directly into precleaned Teflon vessels and 6 mL HNO_3/1 mL of H_2O_2 mixture was added (Muhib et al., 2016). In an analytical microwave (Multiwave 3000 Microwave Oven, Perkin-Elmer), a first ramp of 600 W was applied for 5 min, then 10 min of hold, to reach the temperature established of 220 °C, and finally, it was maintained at 800 W during 5 min of ramp and 10 min of hold. After cooling, the digests were filtered (0.45 µm), transferred and diluted in polyethylene bottles to 50 mL with MilliQ water (Millipore, Bedford, MA, USA). For quality assurance, two samples of milk powder were spiked with a known amount of a multi-element standard. All samples were prepared in triplicate. Vessels containing the same acid mixture as used for the samples were also prepared and utilized as the analytical blank. The digests were stored at 4 °C until analysis.

Calibration standards were gravimetrically prepared using a Mettler-Toledo XS205 balance (0.00001 g) by dilution in water of commercial solutions of each individual element of interest at a concentration of 1000 mg L^{-1}. All the standards were acidified (1%) with sub-boiling 69% HNO_3. The argon used for the plasma was supplied by Praxair (99.99 %, Danbury, CT, USA). ^{9}Be, ^{45}Sc, ^{115}In and ^{209}Bi (Specpure, Alfa Aesar, USA) were used as internal standards (10 ng g^{-1}) to correct any instrument drift during analysis. All calibration curves had regression coefficients that were greater than 0.999, showing excellent linearity over the range.

After appropriate dilution (in 1% of HNO_3), the concentrations of the 27 elements (^{7}Li, ^{27}Al, ^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{88}Sr, ^{98}Mo, ^{111}Cd,^{120}Sn, ^{121}Sb, ^{137}Ba, ^{184}W, ^{201}Tl, ^{208}Pb and ^{202}Hg) were measured by ICP-MS (NexION 300, PerkinElmer, Ontario, Canada) inside a clean room (class 100).

The analytical method was validated by detection limits, precision, accuracy and recovery experiments obtaining satisfactory results in all cases. Since no certified reference material was available for all the analytes, the recoveries of spiked samples were considered to assess the accuracy of the method with satisfactory results (89–105%).

The limits of detection (LOD) for each element were calculated following the IUPAC rules, defined as blank signal plus 3 SD, where SD is the standard deviation of 10 measurements of a blank. The LODs (Li: 0.013; Al: 1.4; Sr: 0.010; Mo: 0.075; Sn: 0.090; Sb: 0.00060; Ba: 0.026; W: 0.021; Hg: 0.014; Tl: 0.0036; Pb: 0.053; Na: 5.90; Mg: 0.52; K: 2.64; Ca: 0.25; Se: 0.042; Ti: 1.4; Co: 0.011; Cu: 1.6; Zn: 8.8; As: 0.66; Cd: 0.013; V: 1.5; Cr: 0.21; Mn: 0.13; Fe: 1.6 and Ni: 1.5 mg kg^{-1}) allowed the determination of minor and trace elements at the required levels in the milk samples.

The reproducibility of the method, as relative standard deviation (RSD%), was calculated after triplicate analysis of one of the powdered milk samples. The values obtained were as follow: Li: 1.3%; Al: 3.2%; Sr: 6.1%; Mo: 10%; Sn: 4.5%; Ba: 1.7%; Tl: 4.4%; Na: 6.2%; Mg: 4.1%; K: 3.8%; Ca: 2.8%; Se: 10%; Ti: 5.2%; Co: 6.7%; Cu: 0.85%; Zn: 3.7%; V: 4.5%; Cr: 13%; Mn: 6.0% and Fe: 3.6%.

Statistical Analysis

The data set obtained after the analysis of the samples consisted of a matrix with the concentration of all elements considered in columns (27) and the milk samples in rows (12). Concentrations below the detection limit were substituted by the half of the detection limit. Data treatment was performed by means of different chemometric tools. The cluster analysis (CA) was carried out by SPSS Statistics Software Version 20 (IBM, New York, USA). Principal component analysis (PCA) of the index data was performed by PLS-Toolbox v.7.0.2 (Eigenvector Research, USA) implemented in MATLAB 2010 software (The Mathworks, MA, USA).

Results and Discussion

Basic Statistics on Metals and Metalloids in Milk Samples

The concentrations of metal and metalloids in all the milk samples were of the same order of magnitude as reported in the literature. For example, the results for Al are in concordance with the results found by Lima de Pavia et al. (2019) in infant formulas commercialized in the city of Campinas (Brazil), and with the Zn concentrations found by Fernández-Menendez et al. (2016) in commercial formula milks of local drugstores.

In Table 2 and Fig. 1, standard deviation, variance and the box plot representation with the maximum, minimum and media value of each element are shown as basic statistics. The concentrations of major and minor elements were in the following order Ca>Na>K>Mg>Fe>Zn>Sr>Ti>Al>Cu>Mn>Ba>Pb. Elements present in trace levels were Sn, Li, Mo, W, Tl, Co, V, Cr, Ni, Se, Sb, Hg, As and Cd. Some of these heavy metals, identified as trace elements, are important inorganic contaminants with considerable risk for human health if they enter the food chain from the environment. Some others are essential elements with important nutritional value. The level of Fe, Zn, Cu, Mn, Sn and Li was higher in milks designed for infants between 0 and 6 months due to a greater variation in their composition and additional ingredients present in them. The concentration of the rest of the elements was closely similar in all varieties of infant milk studied. For adult milk samples, higher values were found for Ca, Na, K, Mg, Sr, Ti and Ba with respect to infant milks.

Table 2 Statistical descriptors of the elemental concentrations: range, standard deviation (SD) and variance

	Range (mg/Kg)	SD	Variance
Ca	6871	2414	5,826,365
Na	3298	957	914,947
K	10,094	3003	9,018,511
Mg	748	210	44,227
Fe	124	34	1139
Zn	61	17	302
Sr	22	6	39
Ti	7	2	5.6
Al	4	1	1.36
Cu	3	1	1.83
Mn	1.0	0.4	0.1470
Sn	0.21	0.06	0.0037
Li	0.13	0.04	0.0019
Mo	0.24	0.08	0.0057
W	0.0168	0.005	0.0000
Tl	0.0023	0.0008	0.0000
Co	0.04	0.01	0.0001
V	0.079	0.03	0.0008
Cr	0.17	0.05	0.0023
Ni	0.13	0.04	0.0015
Se	0.11	0.04	0.0014
Sb	0.004	0.001	0.0000
Hg	0.0017	0.0005	0.0000
As	0.09	0.03	0.0007
Cd	0.003	0.001	0.0000
Ba	2.4	0.8	0.5770
Pb	0.9	0.2	0.0613

In order to detect if these differences were statistically significant, a Kruskal-Wallis test was carried out. This test is a nonparametric test, which is used for determining whether three or more independent samples originate from the same population. When this test leads to significant results, at least one sample differs from the others. As Fig. 2 shows, the results obtained in this case reveal that milk samples are significantly different, in a 95% of confidence level, regarding the content of Na, K, Fe, Ti, Ba and Mo. The p value was in all the cases < 0.05, which confirms that the samples are significantly different regarding these elements.

Pattern Recognition by Unsupervised Chemometric Techniques

Firstly, to obtain more information about the samples and find possible connections and groupings between the analysed milks, cluster analysis (CA) was applied. The Euclidean distance between the samples and variables fluctuated between 3 and 25 (Fig. 3a and b). The dendrograms were obtained by applying Ward's algorithm and as it is shown in Fig. 3b, three clusters can be observed for a cut-off value of 17: one includes J, the second K and L and the third includes the rest of the milks. CA also differentiate Ca, Na and K from the rest of the elements. Therefore, CA separates infant milks from adult milks taking into account the concentration of the major elements.

Non-Carcinogenic and Carcinogenic Risks

The non-cancer risk of the investigated heavy metals through the consumption of milk was investigated by means of hazardous quotients (HQ).

Risk from metals intake through digestion was characterized using HQ as the ratio of the estimated metal dose (EDI, mg kg^{-1} of body weight per day) and the reference dose (Rf, mg kg^{-1}) values developed by USEPA and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2005; USEPA, 2011). The EDI for each metal was firstly calculated following Eq. (1):

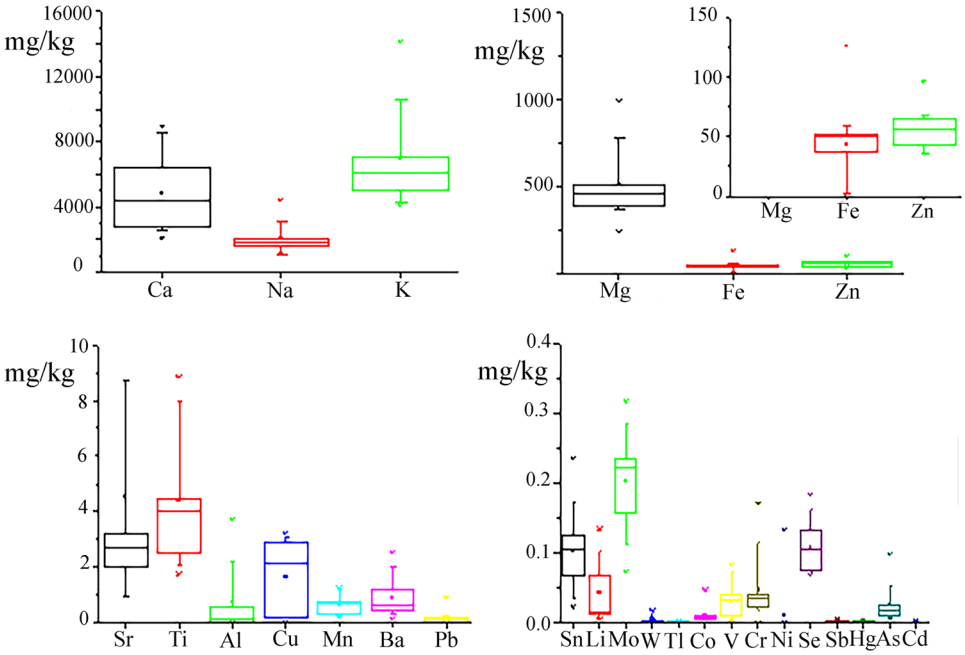


Fig. 1 Box plots of metal and metalloid concentrations measured in milk samples. The three horizontal lines going from bottom to top in the box represent quartile of 25%, 50% and 75% respectively. Square dots represent the mean value and star points represent the outside whisker

$$EDI = C_i \times V_{DIR}/W \quad (1)$$

Here, V_{DIR} = daily milk consumption rate (for infants 500 mg/day and for adults 200 mg/day); W = is the average body weight (8 kg for infants and 60 kg for adults); and C_i = is the experimental metal concentration in milk (mg L^{-1}).

Based on the EDI values, HQ values were calculated (see Eq. 2). If HQ exceeds 1.0, it indicates that there is a potential risk associated with that metal, because the EDI exceeds the Rf. As there is no a Rf value for all the elements analysed, the HQ was estimated in this case for Fe, Sr, Al, Mn, Ba, Pb, Li, Hg, Tl, Co, As, Cd, V, Cr, Ni and Se.

$$HQ = EDI/Rf \quad (2)$$

Despite the follow-on, H milk showed HQ values close to one for some of the elements (Zn, Sr, Pb, As and Co) studied, all the milks were classified as safe. The lowest HQ values were detected in adults' milks (J, K and L). Obviously, the risk associated with the presence of metals is less harmful to the body of an adult, because of its weight, principally. Moreover, the adults' milks are not fortified with extra nutrients; therefore, the contamination of samples through this step is usually avoided.

The possibility of cancer risk in the studied milks through intake of carcinogenic heavy metals was estimated using the carcinogenic risk (CR) (Eq. 3):

$$CR = (EFr \times ED \times EDI \times CSFo)/AT \quad (3)$$

where EFr is the exposure frequency (365 days/year), ED is the exposure duration (2 years in the case of infants and 30 for adults), AT is the averaging time for carcinogens, the lifetime in this case (365 days/year × 70 years for adults and 365 days/year × 6 years for children) and the slope factor (CSFo) is used to estimate an upper-bound probability of an individual developing cancer as a result of a lifetime of exposure to a particular level of a

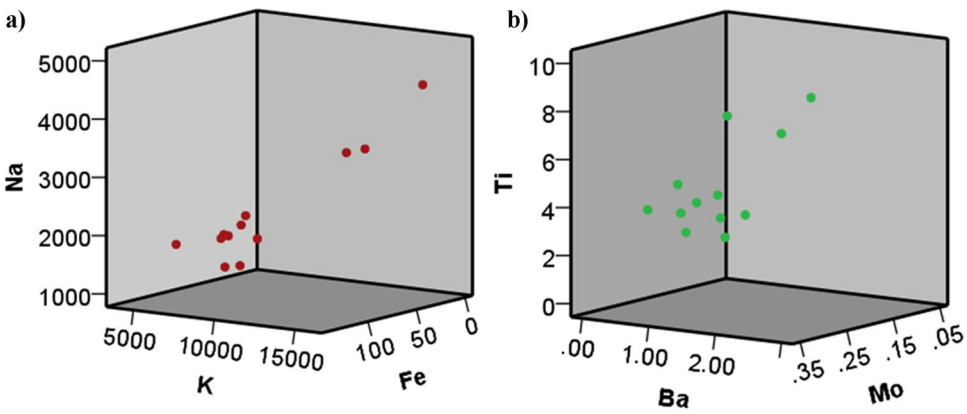


Fig. 2 Differences between samples observed after the Kruskal-Wallis test: **a** sample classification regarding Na, K and Fe; and **b** sample classification regarding Ti, Ba and Mo. All in mg/Kg

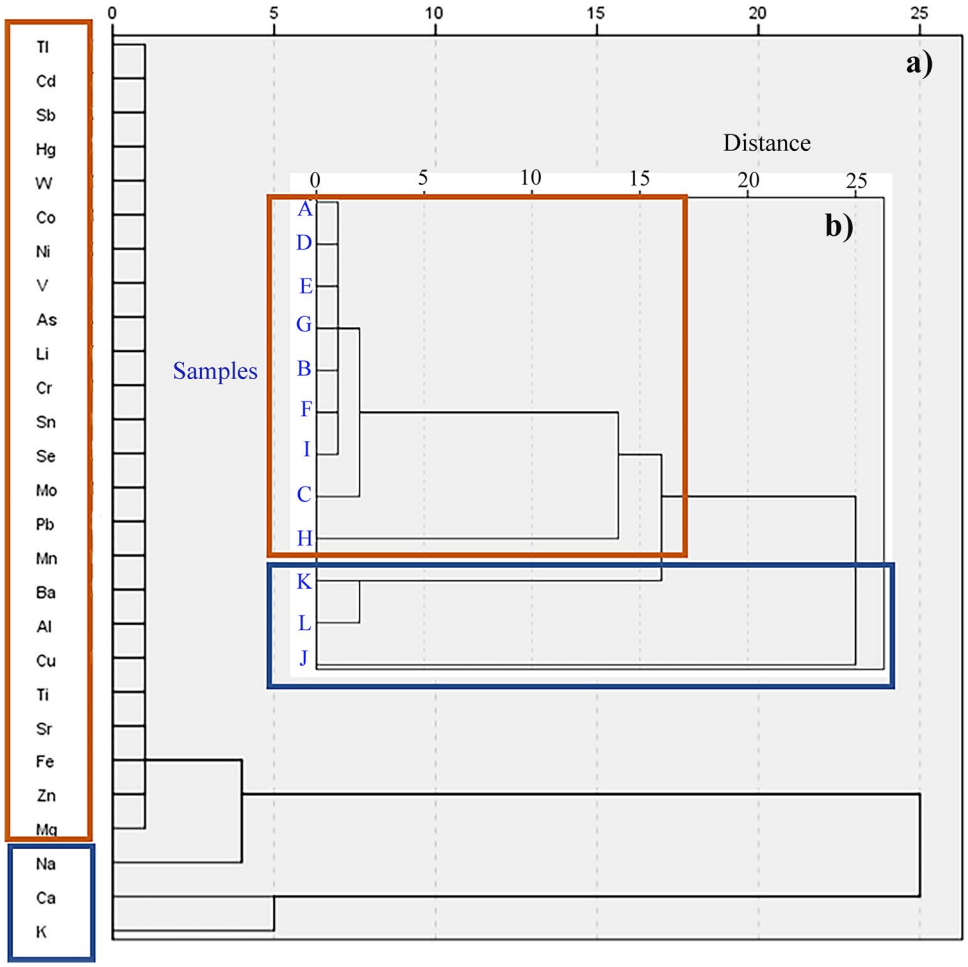


Fig. 3 Dendrogram showing the cluster analysis of **a** variables and **b** milk samples using the Wizard method

potential carcinogen (USEPA 2010; Zeng et al., 2015). In this case, only CR associated with the presence of As, Cd and Cr were calculated (see Table 3). USEPA considers that the CR values lower than 10^{-6} are negligible, and CR above 10^{-4} are considered unacceptable (Luo et al., 2012). The CR values obtained in this study were in all the cases below 10^{-8}, which indicates a probability of 1 chance in 100,000,000 of an individual in developing cancer, which is considered negligible. The metal with higher CR values was As, and the sample showing the highest values was H, with a CR value for As of 1.78E-05. However, the carcinogenic health risk due to consumption of the powdered milks analysed in this study should be neglected.

Statistical Indexes for Nutritional Quality Characterization of Milks

New statistical cumulative indexes called "*Standardized Coefficients*" (SC) were calculated according to metal and metalloid content in the milk samples as a useful tool to predict the nutritional quality of the studied milk samples.

To define the SCs, first, all the concentrations were normalised by linear scaling transformation. Then, a weight was calculated for each element according to the dispersion of the concentrations of that element within the milk samples.

Table 3 Carcinogenic human health risk (CR) calculated for As. Cd and Cr due to milk consumption

Milk	CR (As)	CR (Cd)	CR (Cr)
A	4.64E-06	3.72E-07	9.19E-06
B	1.17E-06	-	7.29E-06
C	3.07E-06	-	4.21E-06
D	7.63E-07	-	6.15E-06
E	5.63E-06	3.44E-08	3.12E-05
F	3.53E-06	6.97E-08	5.39E-06
G	1.35E-06	2.69E-07	6.13E-06
H	1.79E-05	9.85E-09	3.04E-06
I	9.52E-06	3.30E-08	5.03E-06
J	8.72E-09	2.37E-09	9.55E-08
K	1.44E-08	4.00E-10	-
L	1.44E-08	-	1.61E-08

When the concentrations were spread out over a large range of values a high value of weight was defined for that element, and vice versa. To do that, the relative standard deviations calculated for each element, within milk samples, were normalised by linear scaling transformation and the resulting numbers from 0 to 1 were used to weight the normalised concentrations of the elements. Finally, the normalised weighted concentrations were then averaged for each milk and, their values were normalised from 0 to 1 and multiplied by 10 (Gredilla et al., 2014). In this way, different SC values were calculated according to the elements considered for their calculation. SC_{all}s were calculated using the concentration of the 27 elements measured in the milk samples; SC_{major}s were determined based only on the concentration of Ca, Na and K; SC_{minor}s with Mg, Fe and Zn; SC_{toxic}s with Ba, Pb, Hg, As, Cd, Cr and Se; and SC_{trace}s with the rest of elements. In Fig. 4, the values of the SCs obtained for each milk are represented. High values for SC_{all}s, SC_{minor}s and SC_{toxic}s were found in H and J, and also in A for SC_{toxic}s. For SC_{trace}s, higher values were found for H, F and initiation milks. The pattern obtained by SC_{major}s was slightly different, with higher coefficients for adult milks (J, K and L).

The SC values described above include several elements considered essential for humans. For example, SC_{minor}s include Fe and Zn which are considered essential, SC_{toxic}s includes Se and Cr also vital (although at high levels of concentration are considered toxic) and SC_{trace}s comprises Li, Co, Mn, Mo, Ni, Cu, Sn and V, which are also essential. A new index with the mentioned essential elements was calculated called SC_{ess}s (López-García et al., 2007). If all the milks were considered, the higher SC_{ess}s value was found for the J sample (adult milk), but if only infant milk samples were used for the calculation of the SC_{ess}s, samples F and H (see Fig. 5) were the samples with the highest values. Considering that several of these essentials elements at high concentration have toxic effects, a high value of SC_{ess}s could

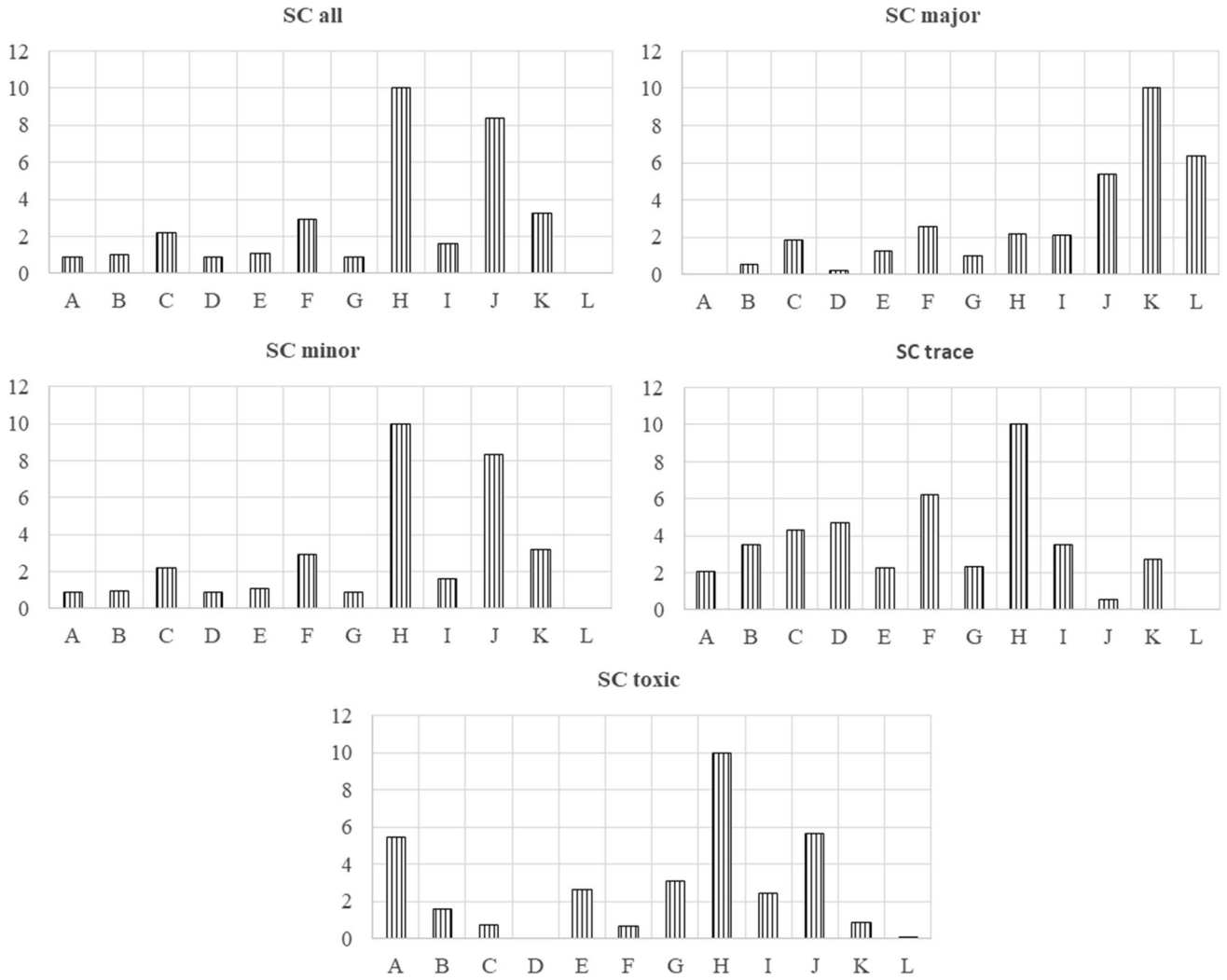
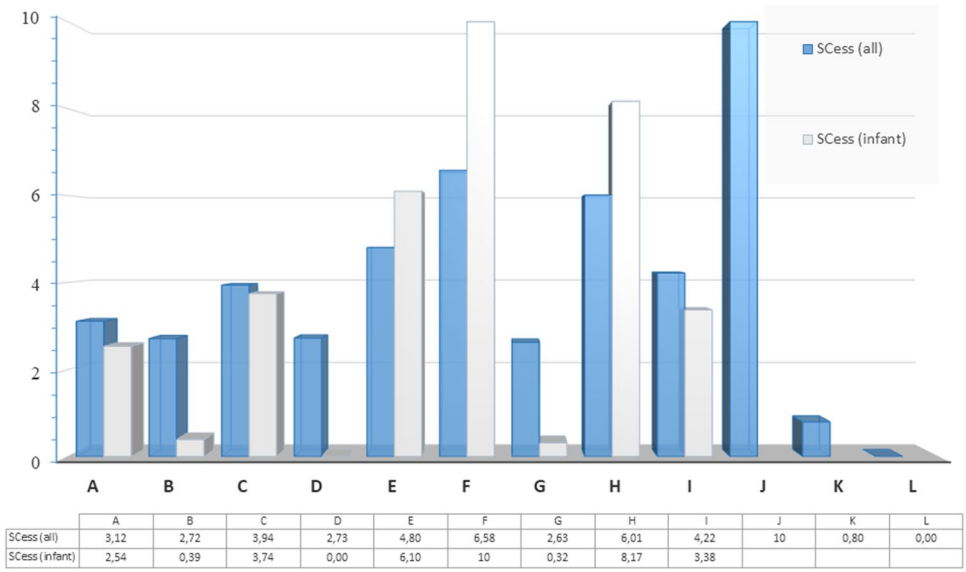


Fig. 4 The SC values obtained for each sample



	A	B	C	D	E	F	G	H	I	J	K	L
SCess (all)	3,12	2,72	3,94	2,73	4,80	6,58	2,63	6,01	4,22	10	0,80	0,00
SCess (infant)	2,54	0,39	3,74	0,00	6,10	10	0,32	8,17	3,38			

Fig. 5 Graphical representation of the values corresponding to SC_{ess}s

be not a good signal of nutritional quality for milk. Given this circumstance, if the objective is to estimate the nutritional quality of the analysed sample, it is better not to focus on this index, and study the concentration values of these elements independently.

In the last step of the proposed methodology, PCA was employed. The objective in this case was to distinguish samples into groups without prior knowledge of group membership. The idea was to evaluate the potential of PCA to differentiate the nutritional level of inorganic constituents of milks by means of the SC indexes. Using the 12 powdered milks as rows and the four SC index as columns in the data matrix (variables as SC_{major}, SC_{minor}, SC_{trace} and SC_{toxic}), a PCA model was extracted whose first three PCs accounted for 94% of variance (PC1: 56%; PC2: 21% and PC3: 17%). The bi-plots (scores and loadings) of the first two PCs and PC1 vs. PC3, shown in Fig. 6, constitute the visual projection of the samples to the PCs. Figure 6a shows the scores and loading plots over the PC1-PC2 and PC1-PC3 spaces. These plots suggest that the first component (56% of variance explained) is strongly related to the average element content of the samples, in such a way that samples placed on its positive part have a higher average concentration of metals than those located on its negative part. The samples with the highest positive loading values on PC1 were H, K and L. H is more related to SC_{toxic}, and K and J to SC_{major}, corroborating their relation with the main constituents of milk (Ca, Na and K). Taking into account the localization of H in both biplots, far away from the other infant milk samples and related to SC_{minor}, SC_{trace} and SC_{toxic}, it could be concluded that it is not adequate for the nutritional

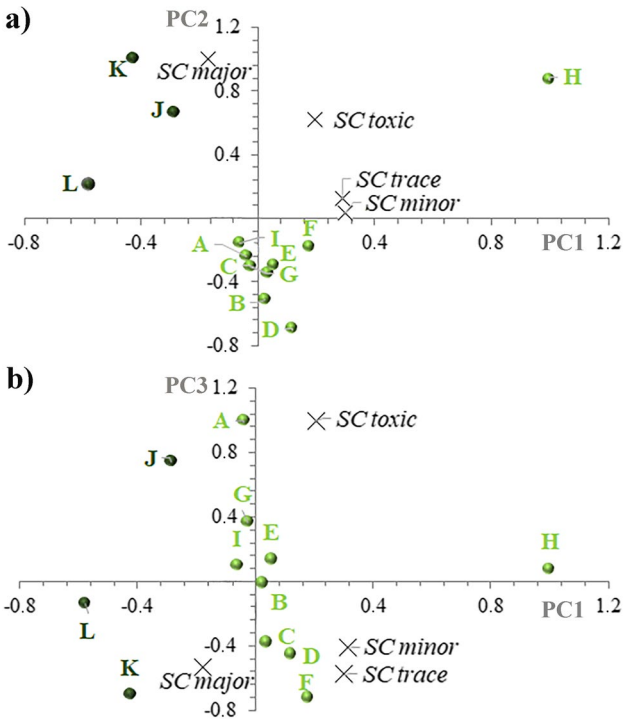


Fig. 6 Principal components result of SC indexes from milk samples: **a** PC1 vs. PC2 and **b** PC1 vs. PC3

necessities of babies. Finally, the rest of the infant samples presented low loading values, generally, on PC1 and PC2. Therefore, the elements added to milks designed for infants between 0 and 6 months due to their nutritional value do not classify them as samples of higher concern with respect to these elements. In fact, initiation milks (A, B, C and D) are placed together and presented negative loading values on PC1 and PC2. Follow-up milks (E, F and G) are also together in Fig. 6a with

positive but low loading values for PC1 and negative values for PC2. This proximity is not observed in milks designed for babies between 12 and 36 months, due to the high loading values of sample H. PC3 divided the samples due to the SC_{toxic}, which confirmed the higher presence of toxic elements in samples A, H and J.

Conclusions

Despite the methodology proposed here being based on the elemental composition of powdered milk samples, the results obtained after its application go far away from the simple conclusions obtained with routine studies. The combination of the elemental composition with chemometric techniques, such as CA and PCA, makes possible the identification of relations between different milks not considering the variables (metals and metalloids in this case) one by one. The use of pattern recognition techniques makes it possible to identify one of the milks made for babies between 12 and 36 months as of poor quality. This was sample H. However, it should be mentioned that this milk has no carcinogenic risk with respect to the elements considered.

This information can be completed with the one provided by the SCs. The SC indexes provide consumers, laboratories, and authorities with a simple and easy-to-understand tool to assess the nutritional quality of the samples under study. Taking into account the samples included in this study, it could be concluded that adults' milks are more characteristic of elements naturally present in milk. The similarities between adult milks revealed that milk skimmed processed process is not a threat to the entry of contaminants into milk. On the other side, children milks present a major presence of trace and minor elements. Therefore, the addition of nutrients and the manufacturing processes should be controlled, because instead of being positive could help in the deterioration of milks designed for children.

Despite milks do not present (non)carcinogenic risks associated with the presence of As, Cd, and Cr, it is important to maintain proper measures for the reduction of metal contamination of milk samples in this area.

Taking into account the results obtained in this study on the quality of the 12 analysed milk samples, we suggest to follow the below-described methodology to apply in future studies over other kinds of foods, especially those directed to infant population.

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Data Availability Data will be made available on reasonable request.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent is not applicable.

Conflict of Interest Ainara Gredilla declares that she has no conflict of interest. Silvia Fdez-Ortiz de Vallejuelo declares that she has no conflict of interest. Gorka Arana declares that he has no conflict of interest. Alberto de Diego declares that he has no conflict of interest. Marcos L.S. Oliveira declares that he has no conflict of interest. Katia da Boit declares that she has no conflict of interest. Juan Manuel Madariaga declares that he has no conflict of interest. Luis F.O. Silva declares that he has no conflict of interest.

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