Trace element determination in different milk slurries

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Summary. We have studied the contents of trace elements of nutritional or toxicological interest in 90 samples of whole, low-fat, skim, condensed, evaporated and powdered milks. Slurries of the samples were prepared with Triton X-100 and analysed using electrothermal atomic absorption spectrometry. The temperature–time programme of the graphite oven was optimized for each element, and the accuracy, precision, selectivity and sensitivity of the method were verified. Concentrations of the trace elements we investigated were: Pb 0–0·211 μg/g, Cd 0–28·985 ng/g, Al 0·528–4·025 μg/g, Cu 0·041–0·370 μg/g, Cr 0–0·177 μg/g, Mn 0·024–0·145 μg/g, Se 0–23·333 ng/g, Zn 0·297–0·827 μg/g and Ni 0·058–1·750 μg/g. (A value of zero indicates that the element was undetectable by our methods.) Concentrations of the pairs of elements Cu–Cd, Mn–Cd, Mn–Cu, Zn–Mn, Ni–Cu, Ni–Mn and Ni–Zn were significantly correlated ($P < 0·001$). Linear discriminant analysis confirmed the separation between the six types of milk analysed.

A variety of minerals are essential for health, especially during growth and old age, and deficiencies and/or excesses may result in metabolic irregularities and pathological changes. Food processing can modify trace element content (Reilly, 1980; Concon, 1988) and external factors such as the environment, eating habits and stress can alter absorption. In addition, bioavailability may depend on interactions with other dietary components, including other minerals, and both competitive and cooperative interdependence has been reported (Klaassen *et al.* 1986).

Milk contains most of the nutrients necessary for a healthy diet, and in some groups, such as children and the elderly, it may constitute the main or even the only food. For this reason, toxic elements need to be detected and measured, and adequate concentrations of essential elements ensured. The composition of the mineral fractions of milk and dairy products has been extensively investigated (Favretto *et al.* 1987, 1994; Coni *et al.* 1990, 1995; Fresno *et al.* 1995).

We have measured the contents of nine trace elements of nutritional or toxicological importance (Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni) in samples of whole, low-fat, skim, condensed, evaporated and powdered cows' milk. All were determined directly by electrothermal atomic absorption spectrometry in slurries of samples. The accuracy, precision and specificity of the analytical method were verified. We

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also investigated possible correlations between concentrations that might influence the availability or physiological effects.

MATERIAL AND METHODS

Apparatus

A Perkin–Elmer model 1100B spectrometer (Perkin–Elmer, Norwalk, CT 06859, USA) equipped with a deuterium background corrector was used. Atomization was performed under the conditions shown in Table 1, using a Perkin–Elmer HGA-700 graphite furnace and heat-resistant tubes with a L'Vov platform. The samples were injected manually and Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni hollow cathode lamps made by the same manufacturer were used. Argon (99·999% pure) at 300 ml/min was used as the internal gas. Signals (peak-height or peak-area mode) were recorded on a Perkin–Elmer 024 potentiometric recorder.

Slurries of the samples were prepared with a Rotaterm Selecta 2000 W rotary shaker (J. P. Selecta, E-08630 Barcelona, Spain). Samples were ashed with a Parr 4782 microwave acid digestion bomb (Parr Instruments, Moline, IL 61265, USA) and a Moulinex FM-460 microwave oven (F-75008 Paris, France).

Reagents

All solutions were prepared with water of specific resistivity 1800 MΩ m obtained by filtering distilled water through a Milli-Q purifier (Millipore, F-91191 Gif-sur-Yvette, France) immediately before use. Standard solutions of Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni (Titrisol, $1{\cdot}000 \pm 0{\cdot}002$), Triton X-100 (analytical grade), silicone antifoaming emulsion (analytical grade), nitric acid (suprapure) and vanadium pentoxide (analytical reagent) were from Merck (D-64293 Darmstadt 1, Germany). Blown zirconium spheres were from Glen Creston (Stanmore IM8 1RF, UK). Magnesium nitrate, nickel(II) nitrate hexahydrate, ammonium nitrate, ammonium dihydrogenphosphate and ammonium molybdate (analytical grade, Merck) were used as chemical modifiers.

Materials

To eliminate background due to detergents and samples, all glassware and polythene sample containers were washed with tap water after each use, soaked in 6 M-nitric acid solution (at least overnight) and rinsed several times in ultrapure water.

Milk samples

We analysed 90 samples of whole, low-fat, skim, condensed, evaporated and powdered milk, representing all the types of milk widely available in Spain. Three items (each from a different batch) of each type and brand of milk were obtained. Preliminary assays established the appropriate amount of sample for analysis to ensure homogeneity between samples and ensure they were representative (Pomeranz & Meloan, 1994; Cabrera *et al.* 1995, 1996).

Procedure

All samples were homogenized and portions (1·0 g) weighed into small polythene bottles, and then 10 g blown zirconium spheres and 10·0 ml Triton X-100 solution (10 ml/l) were added. The bottles were shaken for ~ 30 min with a flask shaker until a slurry had formed. The slurries were separated from the zirconium spheres using a Büchner funnel and transferred into a calibrated flask. A few drops of silicone

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Table 1. *Instrument conditions for determinations of Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni in milk slurries by electrothermal atomic absorption spectrometry*

	Pb	Cd	Al	Cu	Cr	Mn	Se	Zn	Ni
Wavelength, nm	283·3	228·8	309·3	324·8	357·9	279·5	196·0	213·9	232·0
Slit width, nm	0·7	0·7	0·7	0·7	0·7	0·2	2·0	0·7	0·2
Atomization system	Platform	Platform	Platform	Wall tube	Platform	Platform	Wall tube	Wall tube	Wall tube
Ashing temperature, °C	800	700	1700	1200	1650	1400	1000	600	1400
Atomization temperature, °C	1800	2000	2500	2300	2500	1200	2200	1800	2500
Injection volume, μl	10	20	10	10	20	10	20	10	10
Matrix modifier	$NH_4H_2PO_4$	$NH_4H_2PO_4$	$Mg(NO_3)_2$	—	$Mg(NO_3)_2$	$Mg(NO_3)_2$	$Ni(NO_3)_2$	$Mg(NO_3)_2$	HNO_3
Signal mode	Peak area	Peak area	Peak area	Peak height	Peak area	Peak area	Peak height	Peak height	Peak height

antifoaming emulsion in water (300 ml/l) were added before the slurry was diluted with deionized Milli-Q water. All samples were prepared in triplicate to a final volume of 25 ml. The final dilutions of each element are given in Table 2.

Method validation

For validation of the method, 2·5 ml pure nitric acid and a few micrograms of vanadium pentoxide catalyst were added to 1·0 g portions of the homogeneized samples, and the mixture was digested in the microwave acid digestion bomb. Ashing was complete in 90 s with the oven at its highest setting. The digestion bomb was cooled by freezing at −18 °C for 45 min and the solution then diluted to a total volume of 25 ml in ultrapure water. This treatment was used for ten different randomly chosen samples. All subsequent determinations were performed in triplicate. The reference material was skim milk powder no. 063 R-BCR (Community Bureau of Reference, Commission of the European Communities).

Normality of the values from both procedures (slurry and microwave acid digestion) was confirmed with Kolmogorov-Smirnov's test, and the results were subjected to Snedecor's test. No significant differences were found at the 95% confidence level.

Sample analysis

Pb, Cd, Al, Cu, Cd, Mn, Se, Zn and Ni were determined directly with electrothermal atomic absorption spectrometry under the conditions given in Table 1. For all elements, we tested different temperatures for the drying, matrix mineralization and analyte atomization stages to reach an acceptable compromise between ramp time and atomization period. The most suitable integration time and reading mode were also verified. The instrumental and analytical parameters were adjusted to control for interference, improve the sensitivity and detection limit, and enhance the precision and accuracy of the determinations.

The most critical parameters were the type of tube (pyrolytic or normal; with or without a L'Vov platform) and the use of matrix modifiers (see Table 1). A deuterium background corrector was used for all determinations. We pretreated the graphite tubes with saturated ammonium molybdate and heated them several times in accordance with the temperature programme. All determinations were performed in triplicate, and the same procedure was used to run the blanks. The blank solution was prepared fresh daily.

Analytical characteristics

Detection limits were calculated according to IUPAC rules (Long & Winefordner, 1983), corresponding to three times the SD of the blank for an injection volume of 10 or 20 μl depending on the element (see Table 1). Sensitivity and precision were evaluated for each element. Accuracy was checked with recovery assays by adding known amounts of analyte to five different randomly chosen samples and processing the mixtures as described for experimental samples. These results are summarized in Table 2, which also gives the concentration of each element in the final sample volume.

To evaluate method selectivity, standard addition graphs were prepared for blanks and samples and used to calculate the blank-to-sample slope ratio for each element in the different samples. No matrix effect was observed, as shown by slope ratio approaching 1 (Table 2). The use of standard additions was avoided.

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Table 2. *Analytical characteristics for determinations of Pb, Cd, Al, Cu, Cr, Mn, Se, Zn, Ni in milk slurries*

(Values are means of the results for all six types of milk)

	Detection limit, pg	Characteristic mass, pg	Precision, residual SD, %	Final concn in 25 ml, μg/g	Recovery, Mean ± SD	Blank : sample slope
Lead	4·0	10·0	4·5	0·034	98·6 ± 0·7	1·080
Cadmium	0·5	0·4	6·1	0·060	97·4 ± 1·0	1·083
Aluminum	4·0	10·0	5·2	0·980	98·6 ± 1·2	1·050
Copper	4·0	4·5	3·5	0·100	99·5 ± 0·5	0·999
Chromium	1·0	3·0	6·5	0·040	98·5 ± 0·7	1·060
Manganese	2·0	2·5	4·5	0·042	97·9 ± 1·0	0·997
Selenium	30·0	25·0	6·0	0·030	97·6 ± 1·2	0·996
Zinc	0·5	0·5	3·6	0·610	99·1 ± 0·6	0·998
Nickel	10·0	13·0	5·0	0·070	98·5 ± 0·5	0·995

Table 3. *Levels of Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni in different milks*

(Values are means ± SD for *n* samples; a value of zero indicates that levels were below our detection limits)

n ...	Whole 10	Skim 7	Low fat 4	Powdered 3	Condensed 3	Evaporated 3
Pb, μg/g	0·034 ± 0·023	0·052 ± 0·038	0·079 ± 0·070	0·126 ± 0·056	0·066 ± 0·040	0·183 ± 0·032
Cd, ng/g	6·086 ± 4·796	3·519 ± 2·957	3·170 ± 3·000	22·585 ± 10·352	13·180 ± 10·016	16·062 ± 11·436
Al, μg/g	1·095 ± 0·378	1·761 ± 0·571	1·465 ± 0·352	2·914 ± 1·046	0·851 ± 0·589	2·310 ± 1·130
Cu, μg/g	0·110 ± 0·039	0·103 ± 0·043	0·118 ± 0·057	0·413 ± 0·051	0·185 ± 0·148	0·214 ± 0·121
Cr, μg/g	0·043 ± 0·040	0·057 ± 0·054	0·039 ± 0·031	0·021 ± 0·020	0·043 ± 0·040	0·023 ± 0·020
Mn, μg/g	0·042 ± 0·015	0·036 ± 0·009	0·029 ± 0·008	0·121 ± 0·034	0·050 ± 0·020	0·100 ± 0·046
Se, ng/g	2·874 ± 2·676	0·625 ± 0·600	2·031 ± 2·000	7·154 ± 0·432	2·893 ± 0·474	9·791 ± 9·600
Zn, μg/g	0·614 ± 0·214	0·777 ± 0·273	0·525 ± 0·193	1·629 ± 0·247	0·505 ± 0·111	1·125 ± 0·362
Ni, μg/g	0·073 ± 0·034	0·046 ± 0·033	0·050 ± 0·031	1·629 ± 0·247	0·100 ± 0·011	0·133 ± 0·004

RESULTS

Applicability of the proposed method

The method proved highly suitable for determining minerals in milk and milk products, because the advantages of electrothermal atomic absorption spectrometry as an analytical technique were enhanced by the slurry procedure. The use of slurries reduced sample manipulation to a minimum, avoided the loss of analytes through volatization, reduced the risk of contamination and made it possible to analyse several elements simultaneously.

Table 3 shows the concentrations of Pb, Cd, Al, Cu, Cr, Mn, Se, Zn and Ni found in milk samples.

Statistical procedures

Correlation analysis. Statistical analyses were performed with the Excel v. 7.0 software package. We first sought correlations between trace elements that might affect their availability and subsequent physiological effects.

Table 4 shows the results of the initial correlation analyses of all samples. We found a direct correlation between Cu and Cd in whole milk, and an inverse correlation between Se and Pb in condensed milk. In skim milk there was an inverse correlation between Ni and Mn, and a direct correlation between Zn and Mn in condensed milk. In evaporated milk, there were direct correlations for the pairs Se–Cr, Zn–Pb and Zn–Cd, and an inverse correlation between Se and Cu. It should be noted that Mn and Ni were directly correlated in whole milk, but inversely correlated in skim milk, a difference possibly associated with fat removal.

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Table 4. *Correlation matrix between concentrations for pairs of trace elements in 90 different milk samples*

	Pb	Cd	Al	Cu	Cr	Mn	Se	Zn	Ni
Pb	1·000***								
Cd	0·373*	1·000***							
Al	0·311	0·229	1·000***						
Cu	0·394*	0·734***	0·411*	1·000***					
Cr	−0·205	−0·174	−0·024	−0·197	1·000***				
Mn	0·518**	0·622***	0·341	0·777***	−0·207	1·000***			
Se	0·514**	−0·029	0·350	0·012	0·009	0·089	1·000***		
Zn	0·422*	0·400*	0·500**	0·661***	−0·236	0·730***	−0·011	1·000***	
Ni	0·345	0·507**	0·532**	0·760***	−0·190	0·662***	−0·095	0·750***	1·000***

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

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Table 5. *Linear discriminant analysis of trace element contents of different types of milk*

Discriminant function	Eigenvalues	Relative percentage	Canonical correlation
1	66·942	86·05	0·992
2	9·247	11·891	0·949
3	1·001	1·29	0·707
4	0·519	0·67	0·584
5	0·087	0·91	0·284

Derived function	Wilks' λ	χ^2	df	Significance, $P < \alpha$
0	0·00043	166·4636	45	0·00000
1	0·02948	75·76232	32	0·00002
2	0·30215	25·73191	21	0·21686
3	0·60488	10·80834	12	0·54541
4	0·91919	1·81161	5	0·87455

Table 6. *Classification according to the results of linear discriminant analysis of trace element levels of different types of milk*

	Predicted group						
Actual group	Whole	Low-fat	Skim	Condensed	Evaporated	Powdered	Total
Whole	7	1	1	0	1	0	10
Low-fat	0	6	1	0	0	0	7
Skim	0	0	4	0	0	0	4
Condensed	0	0	0	3	0	0	3
Evaporated	1	0	0	0	2	0	3
Powdered	0	0	0	0	0	3	3
Total	8	7	6	3	3	3	30

In a similar study, Cabrera *et al*. (1995) found a significant correlation between Zn and Cd in dairy products ($P < 0·001$). Favretto *et al*. (1994) reported direct correlations for the pairs Mo–Mn (0·59), Cu–Ni (0·48), Cu–Mo (0·47) and Zn–Cu (0·42) in milk, with the highest inverse correlation being between Zn and Fe (– 0·49).

One way analysis of variance. The results were subjected to one way analysis of variance (ANOVA) using element and equality of the means as the variables. The different types of milk were classified with the least significant differences method of Asher. Mean levels of all trace elements except Cr differed significantly between types of milk.

Linear discriminant analysis. The multivariate classification technique known as linear discriminant analysis is used to assign individuals to a group (cluster) on the basis of similarities in defined characteristics. A series of groups were defined a priori (types of milk) and observations for a set of relevant variables were recorded for each individual (nine variables corresponding to each of the trace elements studied here). This information was used to calculate a discriminant function that was used to make predictions about similarities between types of milk.

The results of linear discriminant analysis are shown in Tables 5 and 6. The six types of milk were distributed in three well defined groups: evaporated, powdered and all others. In the last group skim and condensed milks appeared to form a subgroup but whole and low-fat milks were not clearly distinguishable on the basis of the two most significant discriminant functions. The use of all discriminant

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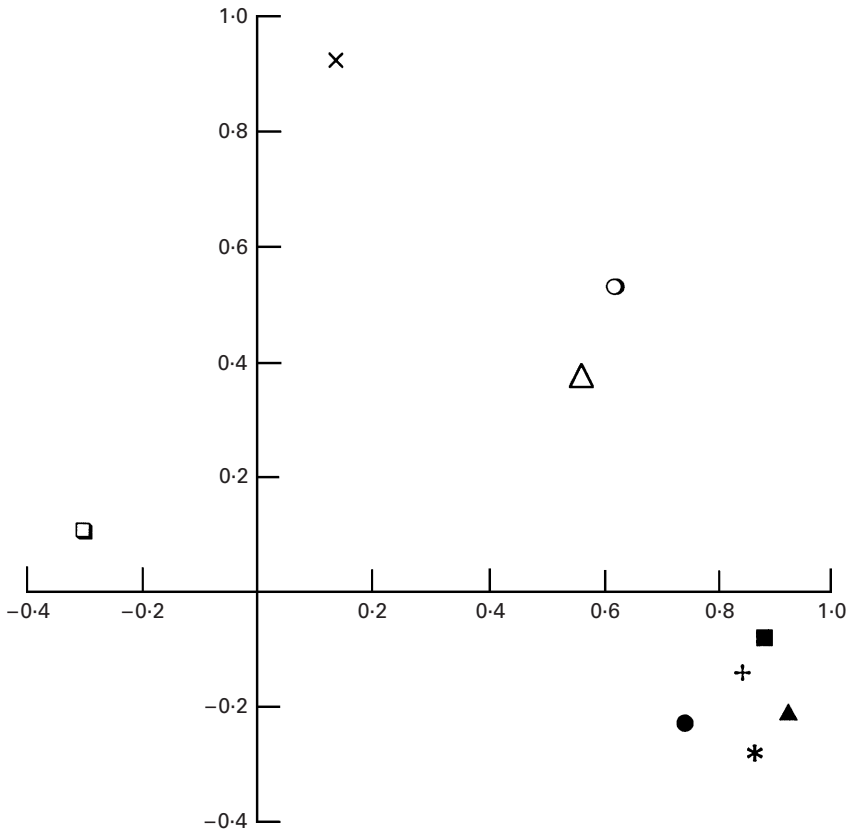


Fig. 1. Clusters of trace elements in cows' milk as determined by factor analysis: ○, Pb; ●, Cd; △, Al; ▲, Cu; □, Cr; ■, Mn; ×, Se; +, Zn; ∗, Ni.

functions allowed us to distinguish powdered (discriminant function 1) and evaporated (discriminant function 2) milk, but the predictions obtained for other types of milk were uninformative.

Factorial analysis. The results of factorial analysis showed that 64·5% of the variance was explained by two factors. The amounts of Cd, Cu, Zn, Ni and Mn all correlated directly (see pairs of elements in Table 4), a finding suggesting that these five trace elements can be considered a single variable in milk. Thus the information provided by nine variables can be reduced to two: Cd, Cu, Zn, Ni and Mn on the one hand, and Cr on the other. In addition, the nine elements could be grouped into four clusters (Fig. 1): Se, Cd + Cu + Ni + Mn + Zn, Pb + Al and Cr.

DISCUSSION

The greatest differences in Pb concentration were found among different brands of low-fat milk. Significant differences were detected between different types of milk, although differences between samples of a given product were small. These differences may be due to contamination during factory processing, or to the quality of the packaging, although the precise cause is difficult to identify because of the variety of factors involved. Canning leads to a considerable increase in the Pb content of evaporated milk (Reilly, 1980; Concon, 1988), and elevated Pb concentrations in forage and pastures are associated with high Pb levels in the viscera and milk. Reilly

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(1980) showed that the Pb content of milk was three to four times normal values when cows consumed grass with 50–100-fold the usual Pb levels.

The Cd levels we found (0–28·985 ng/g) were similar to those found in a previous study (1–20 ng/g; Concon, 1988). The largest variations were in samples of powdered milk. Boudene (1990) found that Cd concentrations were low except in milk from animals fed with contaminated feeds or forage. However, processing or packaging may raise concentrations. In canned evaporated milk levels approaching 40 ng/kg have been found, and clay, ceramic or plastic containers used for dairy products can also release metals (Cabrera *et al.* 1995).

As in previous studies, we found some variation in Al levels among samples of a given type of milk. Significant differences have been noted in relation to environmental, geochemical and technological factors (Reilly, 1980; Klaassen *et al.* 1986; Concon, 1988). Arruda *et al.* (1995) found higher Al levels in dairy desserts than in the raw milk used, probably as a result of transfer from fruit, egg, chocolate or additives.

Cu levels varied for different brands of milk, with somewhat higher values for condensed milk, possibly because of the added sugar. Although cows' milk is a poor source of Cu, it is a good vehicle for this element and it is sometimes added to avoid deficiencies (Levy *et al.* 1985; Rincón *et al.* 1994).

Concentrations of Cr in powdered and evaporated milk were somewhat lower than in whole, low-fat and skim milk, perhaps owing to losses during processing, as Cr compounds are relatively volatile (Pomeranz & Meloan, 1995). Variations among samples may have arisen because Cr is widely used in the manufacture of stainless steel and other materials used in packaging systems (Reilly, 1980).

The levels of Mn were slightly higher in condensed milk, again possibly because of the added sugar. Rincón *et al.* (1994) found significant differences in milk from different species.

Se concentrations in whole, skim and low-fat milk were lower than in the other types of milk tested. They were lower than the levels of other essential trace elements such as Cu or Zn. The Se content of milk depends largely on the animals' feed (Hatano *et al.* 1985; Klaassen *et al.* 1986; Rincón *et al.* 1994) and supplementation can increase levels by $\sim 80\,\%$. There are seasonal variations, with higher levels in winter (Olson & Palmer, 1984; Frøslie *et al.* 1985; Benemariya *et al.* 1993). Diseases such as mastitis can also alter Se concentrations in milk (Alaejos & Romero, 1995). Van Dael *et al.* (1991) found that skim milk contained only 93 % of the Se present in whole milk. After centrifugation, 1–3 % of the total Se was present in the lipid fraction (Debsky *et al.* 1987). However, we found slightly lower Se concentrations in whole milk than in low-fat milk, with even lower levels in skim milk. A direct correlation has been reported between Se and the protein content in milk (Shen *et al.* 1995).

Zn concentrations were similar to those found in earlier studies (Rincón *et al.* 1994; Shen *et al.* 1995). The levels of Ni were also similar to earlier findings (Favretto *et al.* 1994). Despite the current interest in this trace element, few studies have investigated Ni in milk.

The Cd–Cu and Cd–Mn correlations were noteworthy for the association of a highly toxic element (Cd) with nutritionally important elements (Cu and Mn). The associations of Mn, Cu, Zn and Ni may reflect the presence of these elements in machinery used in dairy processing.

Our results show that milk can contribute a considerable proportion of the supply of trace elements in the human diet.

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