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Analytical Methods Section

A methodological approach to the assessment of trace elements in milk and dairy products*

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A detailed protocol was applied to the study and quantification of Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn in raw cow's milk and dairy products. The investigation was prompted by the paucity of data available on minor and trace elements in such matrices, despite their importance in nutrition or in food contamination. As a part of an overall monitoring programme, a study was thus undertaken with the following goals: (i) actual measurement of the concentration of the aforesaid elements in milk and related products; (ii) identification of possible correlations between animal feeding, time of year of sample collection, environmental condition and levels of elements in raw cow's milk; and (iii) evaluation of the influence of the manufacturing process on the concentration ranges of certain health-related elements in milk products. Quantitative determinations were carried out by means of inductively-coupled plasma atomic emission spectrometry (ICP-AES). The results obtained show considerable differences among the levels of trace elements in raw milk and those in derived products. This provides evidence of the fact that manufacturing processes play a key role in the distribution of trace elements.

INTRODUCTION

The presence of xenobiotic substances in foodstuffs is today more than ever a cause of concern onto which the interest of consumers, the mass-media and the scientific community is continuously focused. In this connection it is worth recalling that the content in food of toxic metals such as Cd, Cr, Hg and Pb in some cases can reach threshold levels (Nielsen, 1974; Langard & Norseth, 1977; Mills *et al.*, 1985). The commitment of sanitary authorities is to check and guarantee that the concentrations of such elements, as well as of any other which may be recognised to pose a risk in the near future (e.g. Al, Be, Pt and Tl), do not reach toxic levels. Also, improvements in biochemical research have emphasised the importance of maintaining essential element intakes within optimal concentration levels (Passmore *et al.*, 1974; Underwood, 1977).

Sophisticated instrumental techniques are now of fundamental importance in verifying the compliance of food products with some prerequisites for quality, salubriousness and composition of minor and trace elements essential for nutrition (Caroli, 1988; Mumcu & Aras, 1988; Tölg, 1988).

All foodstuffs present the problems of hygienic safety, especially products of animal origin (Stevens, 1991). It is acknowledged that immediate and close contact with the surrounding media makes terrestrial and ichthyc fauna ideal as early indicators of environmental pollution. A study of concentration levels of essential and toxic elements in domestic fauna is therefore of prime importance in obtaining healthy food of animal origin and evaluating of the extent to which the polluting substances interact with the environment. In this context milk is one of the most important foods of animal origin as it has all the nutrients necessary for a healthy diet (even though some of these are found in a somewhat diluted form). Milk is the main food for some consumer groups, such as infants and the elderly.

The composition of the mineral fraction of milk and milk products has been frequently considered, but only a few published papers deal with minor and trace elements, despite their importance in nutrition or in food contamination (Favretto *et al.*, 1987; Gabrielli Favretto *et al.*, 1989; Gabrielli Favretto, 1990; Vojnovic *et al.*, 1991). Investigations on the presence and role of trace elements in milk and dairy products are being promoted at the international level in order to elucidate aspects that are still poorly understood (IDF, 1978; WHO, 1989).

The aim of this work can thus be defined with three distinct yet complementary objectives, namely (i) to assess the concentration ranges to be considered as 'normal' for the elements Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn in raw milk and cheese;

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(ii) to elucidate any possible correlation between environmental conditions potentially affecting livestock in farms, animal feeding, time of year of sample collection and levels of elements in raw milk; and (iii) to evaluate the influence of the manufacturing process on the concentration ranges of certain health-related elements in dairy products.

To approach this problem and to completely fulfil the aims of the study, it was necessary in the preparatory stage to find livestock farms with specific requisites, i.e. (i) adopting a closed cycle; (ii) feeding animals with feed and/or fodder produced locally or of known origin; and (iii) producing cheese in the farm itself. In Italy there are only a few farms that comply with the aforesaid characteristics. Five agriculture farms in the Valsassina area (Lombardy region) were selected, which raise cattle on a closed cycle basis in a well-defined restricted geographical area and produce local cheese such as 'Quartirolo' and 'Semigrasso'. Subsequently, a survey of the environmental situation in which presumably transfer of elements to the animals could occur, was done through a detailed map of the area specifying the following information: (i) extent of geographical area; (ii) geomorphological characteristics of the area; (iii) number and size of surrounding urban sites; (iv) number and type of industrial, zootechnical and agricultural installations; and (v) presence of facilities for waste disposal.

This paper presents the results of the first phase of the investigation and is restricted to the identification and quantification of the fourteen elements already listed in raw bulk milk and cheese samples drawn during a summer period. To adequately carry out this task, it was necessary to resort to an analytical technique which is at the same time multi-elemental and capable of routine applications. These requisites are possessed to a more than satisfactory degree for practical use by inductively coupled plasma atomic emission spectrometry (ICP-AES). Thus, thorough use was made in this study of the above mentioned technique, with the exception of two cases where it was deemed more expedient to use graphite furnace atomic absorption spectrometry (GFAAS).

MATERIALS AND METHODS

Sampling procedure

As reported in previous studies (Coni *et al.*, 1990*a*, *b*; Stacchini *et al.*, 1992), the sampling and storage steps were planned so as to reduce all possible contamination, loss or alteration phenomena in specimens which could affect the reliability of the data. To do this all samples were collected by *ad hoc* trained personnel who used specially prepared and cleaned collection vessels for milk sampling and quartz knives, instead of conventional metal tools, for cheese sampling throughout the campaign to minimise release of contaminating elements during this crucial step of the overall procedure. For transport to the laboratory, samples were then transferred to chemically decontaminated polyethylene flasks. Flasks were marked indelibly with an appropriate code number using a felt-tipped pen, sealed and kept at −20°C until determinations were performed.

The raw bulk milk samples were also collected under normal labour conditions to better evaluate the influence of mechanical milkers and milk metallic containers on concentration levels of the elements under test. Cheese samples were collected in accordance with the procedure reported in the Italian Official Analytical Methods (Ministerial Decree, 21 April 1986). It prescribes, for circular-shaped cheese, the following operations: (i) drawing of a slice of 0·5 kg from a whole cheese of about 5 kg by means of a point-knife; (ii) subdivision of the slice into small pieces and redistribution of these last into five subsamples; and (iii) random selection of two subsamples for weighing the aliquots to be analysed. The same procedure was adopted to collect samples of intermediate products, so as to represent the various phases of cheese production, commonly known as curdling, moulding, salting and seasoning. In this connection, the manufacture of the two types of cheese chosen for this investigation is hereinafter briefly described. The raw milk for cheesemaking is poured into the cheese vats where it is clarified and standardised to the required fat percentage. Thereafter, the partly skimmed milk is conducted into a copper boiler and, after addition of the necessary amounts of starter culture and rennet, it is heated at 35–40°C. The curd so obtained, after having been pressed briefly in the same copper boiler, is cut into blocks and placed in the special cheese moulds and then pressed. After the necessary pressing, during which the curd loses some whey and acquires the desired shape, a 2–4 days immersion in salt brine follows. After this the cheese is cured during a period of 1–4 months, depending on the temperature of the storage room and the type of cheese. Although the manufacture of 'Quartirolo' and 'Semigrasso' cheese in general is similar, there is some difference in the flavour of the cheese as a result of the different ripening and fat content.

In parallel to milk and cheese sampling, samples of water and food to be used in feeding the cattle were collected. In particular, analyses were performed on forage (green hay and vegetables) produced on site and water from wells situated in the geographical area of the farms.

Sample treatment

Before starting determinations, bulk milk underwent lyophilisation in the same flasks in which specimens were stored. This greatly expedited the subsequent destruction of the organic components of the fluid and, at the same time, minimised dilution.

As is well known, elemental analyses by ICP-AES or by GFAAS require preliminary digestion of solid matrices into liquid solutions. To achieve this goal,

Table 1. Instrumentation and working conditions for ICP-AES and GFAAS

	ICP-AES
Spectrometer	Jobin-Yvon 32 + 38 VHR
RF generator	Durr-JY 3848, frequency 56 MHz, nominal output 2·2 kW
Induction coil	5 coils, OD 32 mm, height 30 mm
Torch	INSA, demountable, with plasma argon flow 18 litre/min, coating argon flow 0·9 litre/min and carrier argon flow 0·1 litre/min
Polychromator	HR 1 000 M, focal length 0·5m, Paschen–Runge mounting, equipped with a 3 600 grooves/mm holo graphic concave grating, linear dispersion in the first order 0·55 nm/mm, spectral range 170–410 nm
Monochromator	HR 1 000 M, focal length 1 m, Czerny–Turner mounting, equipped with a 3600 grooves/nm holographic plane grating, linear dispersion in the first order 0·27 nm/mm, spectral range 170–450 nm
Computer	IBM PS/2 55SX with Jobin–Yvon ESS software
Nebuliser	Meinhard-type with Scott-type nebuliser chamber
Slit width	40 μm (entrance and exit for monochromator), 50 μm (entrance and exit for polychromator)
Spectral lines (nm)	Al (I) 237·3 Ba (II) 233·5 Co (II) 238·9 Cr (II) 206·1 Cu (I) 324·8 Fe (II) 259·9 Mg (II) 279·6 Mn (II) 257·4 Ni (II) 231·6 Pt (II) 214·4 Sr (II) 407·8 Zn (I) 213·9
	GFAAS
Spectrometer	Perkin–Elmer 5100 with Zeeman corrector
Monochromator	Czerny–Turner mounting, equipped with a holographic plane grating with 2880 grooves/mm in the UV region and 1440 grooves/mm in the visible region, linear dispersion in the first order 0·65 nm/mm (UV) or 1·30 nm/mm (Vis), spectral range 170–900 nm
Furnace	Perkin–Elmer HGA 600 with autosampler AS-60
Computer	Perkin–Elmer 7300 professional
Thermal programme	Drying 110°C, ashing 800°C (Cd) and 850°C (Pb), atomisation 1 600°C (Cd) and 1 800°C (Pb)
Slit width	0·7 nm
Spectral line (nm)	Cd 228·8, Pb 283·4

various procedures, of both the dry- and wet-ashing type, have been attempted. By comparing their respective performances it was decided that preference should be given to a dry-ashing approach at a relatively moderate temperature. Thus, high combustion efficiency and low contamination risk were achieved. The main steps of this procedure were as follows: (i) transfer, under strictly controlled conditions, of about 5 g of the freeze-dried milk aliquots and of about 2 g for all other samples into quartz cups and careful measurement of the mass; (ii) quantification of humidity content at 120°C for 6 h on separate amounts of samples; (iii) slow charring on a hot-plate with the assistance of the minimum necessary amount of 65% HNO_3 (Suprapur, Merck); (iv) calcining at 420°C in a muffle furnace, the internal walls of which were completely lined with laminar quartz to eliminate any possibility of release of the elements from the refractory material; (v) obtaining white ashes (if this does not occur, steps (iii) and (iv) are repeated); (vi) dissolution of ashes with 1 cm^3 of 65% HNO_3 at 40°C; and (vii) transfer of the final solutions into 25 cm^3 calibrated flasks and final dilution with double distilled water up to the marked volume. The validity of the entire procedure was tested in all possible ways by evaluating the blank contribution of each of the steps to the final concentration values, as discussed below.

Analytical determinations

Extensive use was made in this research of the ICP-AES technique in the simultaneous mode for all elements except Cd, Cr, Pb and V. For Cr and V, the need for a better evaluation of the background around the peaks and the expected levels that are usually close to or below the detection limits made a sequential system more adequate for the purpose given its higher resolution power. For the other two elements, Cd and Pb, the detection power of ICP-AES at the concentrations expected in milk and cheese is not sufficient. This made the use of GFAAS indispensable for these two analytes. Details of the apparatus employed and the working parameters selected are set out in Table 1.

The entire analytical procedure was tested for both measurement accuracy and precision in order to assess the degree of reliability which can be allocated to the data generated by this investigation. The level of accuracy was continuously monitored by adding to each series of unknowns one of the three reference materials suited to the purpose, namely CRM 063 (skimmed-milk powder) and CRM 150 and CRM 151 (both spiked skimmed-milk powder), all supplied by the Measurements and Testing Programme (formerly BCR), Brussels, Belgium. Precision, in turn, was ascertained by replicating the entire analytical cycle, including the pretreatment steps, an adequate number of times (in no case less than 10).

RESULTS AND DISCUSSION

Analytical data for all three CRMs were acceptable, with recovery percentages, calculated as (mean value found/certified value)×100, varying from 90 to 105%

Table 2. Analysis of certified reference materials (CRMs) by proposed method (Results are the mean of 10 independent sample preparations)

Element	CRM 063 Concentration (µg/g)		CRM 150 Concentration (µg/g)		CRM 151 Concentration (µg/g)	
	Certified[a]	Found[b]	Certified[a]	Found[b]	Certified[a]	Found[b]
Co	0·0062	0·006 ± 0·001 (97%, 17%)	0·0064	0·006 ± 0·001 (94%, 17%)	0·0060	0·006 ± 0·001 (100%, 17%)
Cd	0·0029 ± 0·0012	0·003 ± 0·0006 (103%, 20%)	0·0218 ± 0·0014	0·021 ± 0·001 (96%, 5%)	0·101 ± 0·008	0·091 ± 0·005 (90%, 5%)
Cu	0·545 ± 0·030	0·536 ± 0·025 (98%, 5%)	2·23 ± 0·080	2·15 ± 0·10 (96%, 5%)	5·23 ± 0·080	5·09 ± 0·13 (97%, 3%)
Fe	2·06 ± 0·25	2·15 ± 0·11 (104%, 5%)	11·8 ± 0·6	12·0 ± 0·5 (102%, 4%)	50·1 ± 1·3	50·3 ± 1·5 (100%, 3%)
Mg	1 120 ± 30	1098 ± 22 (98%, 2%)	—	—	—	—
Mn	0·226	0·210 ± 0·009 (93%, 4%)	0·236	0·223 ± 0·011 (94%, 5%)	0·223	0·209 ± 0·010 (94%, 5%)
Ni	0·0112	0·011 ± 0·002 (98%, 18%)	0·0615	0·064 ± 0·006 (105%, 9%)	0·056	0·058 ± 0·006 (104%, 10%)
Pb	0·1045 ± 0·003	0·109 ± 0·005 (104%, 5%)	1·000 ± 0·040	1·048 ± 0·052 (105%, 5%)	2·002 ± 0·026	2·075 ± 0·062 (104%, 3%)
Zn	42·0	41·7 ± 0·35 (99%, 1%)	49·5	50·2 ± 0·42 (101%, 1%)	50·4	48·0 ± 0·78 (95%, 2%)

[a] Each mean value is accompanied by its standard deviation. Concentrations without standard deviation are only qualified.

[b] Each mean value is accompanied by its standard deviation.Values in parentheses are in order the recovery percentage and the relative standard deviation (RSD).

Table 3. Concentration values of elements (µg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 1)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	2·44	1·01	0·015	0·025	0·176	3·40	24·6	577	0·195	0·318	0·058	<0·000 1	0·786	19·7
Curds	1·93	2·31	0·035	0·044	0·630	14·0	7·80	347	0·239	0·181	0·070	<0·000 1	0·606	42·0
Cheese after moulding	2·00	2·29	0·044	0·037	0·561	12·8	6·09	294	0·212	0·122	0·075	<0·000 1	0·572	47·5
Cheese after salting	2·18	2·03	0·029	0·036	0·561	11·1	5·91	331	0·202	0·112	0·044	<0·000 1	0·569	51·0
Seasoned cheese	2·24	1·79	0·016	0·031	0·578	11·0	6·75	295	0·242	0·098	0·082	<0·000 1	0·569	52·4

[a] 'Quartirolo' cheese: summer production (July 1992).

Table 4. Concentration values of elements (µg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 1)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	1·15	0·860	0·028	0·008	0·199	3·99	13·0	549	0·258	0·440	0·062	<0·000 1	0·739	26·2
Curds	0·598	1·71	0·029	0·012	0·716	16·2	7·75	346	0·297	0·233	0·082	<0·000 1	0·663	57·7
Cheese after moulding	1·35	1·78	0·028	0·004	0·670	14·8	5·07	242	0·240	0·156	0·090	<0·000 1	0·556	42·4
Cheese after salting	1·37	1·11	0·024	0·017	0·461	10·6	4·17	219	0·188	0·095	0·065	<0·000 1	0·505	35·0
Seasoned cheese	2·01	0·256	0·023	0·006	0·540	11·7	5·79	192	0·229	0·087	0·087	<0·000 1	0·441	23·0

[a] 'Quartirolo' cheese: summer production (September 1992).

according to the element. Precision was also found to be more than satisfactory, with relative standard deviations (RSDs) always between 1 and 5% ($n \geq 10$). Only in the cases of Cd, Co and Ni, where measured concentrations were very close to the detection limit, precision worsened to 15–20%. The corresponding results for both accuracy and precision are summarised in Table 2.

The data obtained thus far refer to eight complete cycles of cheese production (July and September for three farms and July for the other two) and can therefore provide a preliminary and sufficiently reliable indication of the concentration ranges to be considered characteristic for the elements under test. The results of spectrometric analysis of raw bulk milk (collected under normal labour conditions), cheese and intermediate products are shown in Tables 3–10, whereas Table 11 reports the data pertaining to samples of water and feed for cattle breeding. In order to better evaluate the effect of processing on element composition, detailed information on the processes and equipment was gathered by means of a questionnaire.

From an overall point of view the data seem to be supportive of the fact that there are no striking differences in concentration values for the eight bulk milk samples, if these latter were collected under strict precautions to minimise any source of contamination from the outside. Furthermore, these figures compare favourably with those retrievable in the relevant literature for other countries (IDF, 1978; Dabeka & McKenzie, 1987; Krelowska Kulas, 1990; Larsen & Rasmussen, 1991). A more scrupulous examination,

Table 5. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 2)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	2·12	0·693	0·034	0·055	0·208	4·50	16·7	580	0·319	0·438	0·075	<0·000 1	0·643	48·7
Curds	0·792	0·775	0·042	0·093	0·256	5·12	5·22	425	0·345	0·259	0·089	<0·000 1	0·767	54·4
Cheese after moulding	1·39	1·18	0·031	0·125	0·251	5·07	7·45	334	0·330	0·219	0·095	<0·000 1	0·721	55·1
Cheese after salting	1·55	0·434	0·015	0·015	0·265	5·16	5·17	225	0·255	0·144	0·060	<0·000 1	0·806	55·2
Seasoned cheese	1·49	0·164	0·025	0·011	0·356	5·71	5·51	227	0·276	0·117	0·079	<0·000 1	0·517	52·3

[a] 'Quartirolo' cheese: summer production (July 1992).

Table 6. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 2)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	2·60	0·473	0·030	0·035	0·185	3·59	11·8	589	0·244	0·289	0·054	<0·000 1	0·642	25·6
Curds	0·814	1·36	0·040	0·042	0·326	7·17	6·18	320	0·275	0·247	0·079	<0·000 1	0·591	40·1
Cheese after moulding	1·10	1·34	0·040	0·021	0·368	8·22	6·17	327	0·286	0·238	0·080	<0·000 1	0·526	32·1
Cheese after salting	1·06	0·513	0·028	0·018	0·411	9·04	4·22	301	0·249	0·183	0·050	<0·000 1	0·561	33·6
Seasoned cheese	1·09	0·213	0·023	0·019	0·594	9·42	7·49	318	0·365	0·162	0·076	<0·000 1	0·450	35·7

[a] 'Quartirolo' cheese: summer production (September 1992).

Table 7. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 3)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	3·68	0·627	0·025	0·005	0·093	1·49	4·36	532	0·238	0·469	0·049	<0·000 1	0·627	18·2
Curds	1·87	1·30	0·097	0·019	0·493	11·1	8·14	351	0·282	0·190	0·068	<0·000 1	0·706	36·0
Cheese after moulding	2·21	1·01	0·049	0·015	0·283	9·50	8·39	307	0·300	0·185	0·073	<0·000 1	0·712	54·2
Cheese after salting	2·35	0·612	0·026	0·011	0·309	11·5	4·87	255	0·299	0·173	0·045	<0·000 1	0·544	45·0
Seasoned cheeses	2·87	0·324	0·021	0·013	0·347	14·5	5·76	264	0·307	0·142	0·089	<0·000 1	0·458	35·2

[a] 'Quartirolo' cheese: summer production (July 1992).

Table 8. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 3)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	3·65	0·756	0·042	0·015	0·042	0·613	10·1	524	0·226	0·399	0·071	<0·000 1	0·742	33·4
Curds	1·69	0·967	0·044	0·043	0·092	1·58	5·83	276	0·255	0·289	0·098	<0·000 1	0·810	47·9
Cheese after moulding	2·05	0·975	0·039	0·033	0·091	1·46	7·07	273	0·259	0·234	0·102	<0·000 1	0·738	34·3
Cheese after salting	3·71	0·957	0·026	0·013	0·188	1·57	5·79	283	0·284	0·197	0·068	<0·000 1	0·669	36·1
Seasoned cheese	4·22	0·805	0·013	0·017	0·493	1·51	11·0	246	0·357	0·136	0·087	<0·000 1	0·443	36·3

[a] 'Quartirolo' cheese: summer production (September 1992).

however, reveals that for a few elements slight differences occur. It is self-evident in fact that the spread of actual concentrations is much larger in all cases for elements such as Al, Cd, Cr and Pb which have an obvious exogenous origin, this being clearly linked to the rather variable sets of conditions governing their intake with feed.

On the other hand, bulk milk samples collected under normal labour conditions show clearly (see Tables 3–10) that there can be accentuated differences occurring among concentrations of some elements (e.g. Al, Cu, Fe and Ni) depending on the metal release from mechanical milkers and milk metallic containers employed. In particular, the amplitude of concentration intervals increases for the aforesaid elements as follows: 1·42–6·53 versus 1·39–1·96 μg/g for Al, 0·613–4·50 versus 0·589–0·971 μg/g for Cu, 4·3–24·6 versus 3·81–8·84 μg/g for Fe and 0·289–0·609 versus 0·258–0·349 μg/g for Ni. This reflects the fact that the milking equipment of the five farms differs considerably both in typology and metal materials.

This notwithstanding, human exposure to exogenous elements through cow's milk does not appear to be a source of risk. In fact, from the figures obtained in this investigation, an attempt was made to estimate the contribution of milk to the total dietary intake of toxic elements. This calculation is based on a nationwide dietary survey carried out by ISTAT (Italian Central Institute for Statistics). The total Italian dietary intake of Al, Cd and Pb is 30, 45 and 15% of the respective provisional tolerable intakes. The estimated intake of Al, Cd and Pb from milk is less than 3% of the Italian total dietary intake of these elements and is therefore toxicologically insignificant.

In this connection, among all the toxic elements taken into account in this research, Pt deserves a particular mention (Zeisler & Greenberg, 1988). In fact, although Pt concentration in cow's milk is still below detection limits, the widespread use of Pt catalyst converters in controlling vehicle exhaust emissions may result in it becoming a ubiquitous contaminant of food. Baseline data are, thus, needed for monitoring. To better grasp present levels and monitor future variations, Pt concentration in cow's milk samples was, as an exception, determined by inductively-coupled plasma mass spectrometry. The concentrations detected never exceeded the value of 0·0001 μg/g.

As regards the cheese production, unambiguous trends have been detected between manufacturing process and element concentration. In general, on the basis of the total variations observed between raw material (bulk milk) and finished food (seasoned cheese), the elements investigated can be roughly subdivided into three classes. The first includes elements such as Ba, Fe, Mg, Ni and Sr for which concentration levels show a general trend of decrease along the line milk-cheese. The second class regards elements such as Cr, Cu, Pb and Zn, which behave in an opposite way. For the remaining elements, there is no particular trend, in that the concentrations obtained in seasoned

Table 9. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 4)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	6·53	1·01	0·042	0·024	0·144	2·69	14·4	532	0·284	0·609	0·074	<0·000 1	0·735	36·8
Curds	1·06	1·10	0·084	0·071	0·451	9·54	7·90	348	0·314	0·312	0·084	<0·000 1	0·657	45·9
Cheese after moulding	0·675	1·15	0·082	0·065	0·447	10·0	11·5	324	0·316	0·223	0·090	<0·000 1	0·518	48·6
Cheese after salting	0·926	1·07	0·081	0·023	0·436	10·6	3·48	368	0·256	0·191	0·066	<0·000 1	0·594	44·5
Seasoned cheese	1·11	0·975	0·068	0·019	0·592	11·7	4·63	343	0·256	0·138	0·101	<0·000 1	0·468	44·7
Very seasoned cheese	1·73	0·914	0·049	0·024	0·950	13·2	5·23	366	0·505	0·120	0·115	<0·000 1	0·683	46·3

[a] 'Quartirolo' cheese: summer production (July 1992).

Table 10. Concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products[a] (Livestock farm no. 5)

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	1·42	1·46	0·046	0·060	0·160	1·03	18·5	642	0·614	0·343	0·064	<0·000 1	0·718	30·1
Curds	0·875	2·85	0·081	0·079	0·704	16·5	8·54	551	1·19	0·108	0·088	<0·000 1	0·650	53·3
Cheese after moulding	0·946	3·25	0·080	0·010	0·704	17·1	7·63	529	1·21	0·084	0·088	<0·000 1	0·630	79·8
Cheese after salting	2·02	3·19	0·074	0·038	0·817	19·9	5·38	590	1·25	0·070	0·055	<0·000 1	0·688	87·7
Seasoned cheese	2·11	3·06	0·043	0·031	0·875	18·9	7·81	479	1·30	0·058	0·070	<0·000 1	0·568	89·6
Very seasoned cheese	2·90	2·26	0·042	0·058	1·227	19·0	7·91	664	1·55	0·048	0·095	<0·000 1	0·699	90·8

[a] 'Semigrasso' cheese: summer production (July 1992).

cheese slightly fluctuate around those measured in milk.

Meticulous analysis of all data relating to the various steps of cheese production shows, on the other hand, that curdling and salting are the two phases of production that give rise to the greatest variations in the element levels. This can be ascribed to the fact that, during curdling, the elements partition in an unequal way between curd and whey on the basis of their different binding ability. Salting causes osmotic loss of liquid from the cheese and this affects the concentration of unbound minerals.

Processing equipment too, seems to play a key role in determining the final concentrations of trace elements in finished products. It is not surprising, in fact, that the levels of elements like Al, Cd, Cr, Cu, Fe, Mn and Ni can be influenced by the metal materials with which milk and intermediate products come into contact. In this connection, it should be stressed that the high increases of Cu found in all the eight cycles of cheese production are clearly linked to the large and traditional use of copper containers typical for the regions considered.

Furthermore, on the basis of the figures obtained in this investigation, it is possible to state that the level of some toxic elements may be increased by local contamination during the period of cheese maturity. This holds true especially for elements such as Al and Pb whose presence in the atmosphere is of a ubiquitous nature.

Finally, in terms of feed intake, no unambiguous relationships could be detected between feeding and element concentration in milk. In fact, the data listed in Table 11 show, for some elements, significant differences between the five groups of feed samples under test. In spite of this, the aforesaid discrepancies are not reflected in trace element contents of the respective bulk milk samples. A possible explanation for this behaviour may be identified by the fact that the homeostatic mechanisms of control are able to account for small variations in the element intake and thus maintain the elements within well-defined concentration intervals. From a general point of view, the inability to find clear links among these parameters may be frustrating. Nevertheless, the problem is complex and data are needed for winter periods to show overall patterns. In fact, the feeding completely changes during the year and, only when information on the different levels of elements in the various matrices is obtained, can one attempt identification of more specific trends.

Table 11. Mean concentration values of elements in samples of water (μg/ml) and feed for cattle breeding (μg/g dry weight); 10 for each group and matrix

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Water[a]														
Farm no. 1	0·022	0·025	0·000 9	0·000 1	0·000 4	0·003	0·015	7·14	0·000 4	0·000 2	0·005	0·004	0·161	0·027
Farm no. 2	0·025	0·021	0·000 9	0·000 2	0·000 3	0·005	0·099	6·13	0·000 2	0·000 3	0·006	0·004	0·162	0·081
Farm no. 3	0·016	0·022	0·000 6	0·000 5	0·000 2	0·005	0·012	11·8	0·000 4	0·000 1	0·005	0·006	0·057	0·012
Farm no. 4	0·015	0·034	0·000 9	0·000 4	0·000 3	0·010	0·044	1·64	0·000 7	0·001 0	0·007	0·001	0·035	0·053
Farm no. 5	0·030	0·098	0·001 1	0·000 1	0·000 3	0·007	0·017	2·34	0·001 0	0·000 1	0·004	0·001	0·042	0·036
Forage														
Farm no. 1	0·430	342	2·19	0·743	0·949	7·41	2·42	0·797	23·1	0·063	0·620	0·072	162	2·79
Farm no. 2	0·126	250	0·490	0·285	0·305	5·94	0·576	0·338	7·54	0·048	0·590	0·041	106	1·03
Farm no. 3	0·215	299	0·521	0·947	0·182	2·81	0·822	0·685	10·2	0·015	0·630	0·052	151	1·69
Farm no. 4	0·073	83	0·327	6·69	0·153	1·13	0·220	0·860	3·57	0·013	0·660	0·046	122	0·38
Farm no. 5	0·120	496	0·773	0·692	0·173	2·59	1·25	0·840	17·3	0·013	0·710	0·067	171	1·96

[a] Measurements were carried out on preconcentrated solutions. Actual values were then referred to the original samples.

In conclusion, this research provides information on concentration values for trace elements in cow's milk and cheese products which should be regarded as reference ranges. The analytical method developed in this study appears adequate for the purpose and can therefore be recommended for other similar investigations.

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