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Preliminary evaluation of the factors influencing the trace element content of milk and dairy products

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As part of an overall monitoring programme launched by the Istituto Superiore di Sanità (Italian National Institute of Health) within the framework of the RAISA (Advanced Researches for the Innovation of Agricultural Systems) project supported by the National Research Council, an investigation was undertaken with the following goals: (i) assessment of the content of selected trace elements such as Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn in raw cow's milk and cheese; (ii) identification of possible correlations between animal feeding, year period of sample collection, environmental conditions 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 mainly by inductively coupled plasma atomic emission spectrometry. The results obtained show considerable differences among the levels of trace elements in raw milk samples collected during different periods of the same year. Furthermore, differences occur also among the trace element contents of raw milk and related products. All this provides evidence that animal feeding, year period of sample collection, environmental conditions and manufacturing processes all play a key role in the distribution of trace elements.

INTRODUCTION

The last few decades have witnessed a rapidly expanding consciousness of the growing significance of scientific research, especially upstream research, in developing innovative processes and supplying foodstuffs competitive in international markets. Also, in Italy scientific research has recently been applied to the agri-food system, and remarkable progress has been made. Nevertheless, some traditional Italian products still suffer from market constraints, and those with good trading prospects are strongly affected by structural and technological deficiencies.

From this point of view, Italian dairying is one of the most penalized industrial sectors and consequently needs new strategies for the development of alternative production methods aimed at satisfying the demand for better and more marketable foodstuffs. The rationalization of manufacturing processes and identification of quality markers for milk and milk products is therefore of great importance for Italian dairy products. A further requirement is a thorough examination of processes by which feedstuff quality, manufacturing processes and environmental factors, including sanitary conditions, affect milk and cheese properties.

As regards this last aspect, a project entitled 'Study on feeding and technological factors affecting the quality of milk of cow, sheep and goat as well as of typical cheese: environmental and treatment residues' was launched by the Istituto Superiore di Sanità (Italian National Institute of Health) within the framework of the RAISA (Advanced Researches for the Innovation of Agricultural Systems) project supported by the National Research Council. One of the several issues considered during the development of this project dealt with the assessment of trace elements in milk and dairy products. The obtainment of baseline figures for a number of essential and potentially toxic elements is compulsory for the assessment of the nutritional integrity of these foodstuffs as well as of their compliance with general health requirements (Nielsen, 1974; Passmore *et al.*, 1974; Langa & Norseth, 1977; Underwood, 1977; International Dairy Federation, 1978; Mills *et al.*, 1985; WHO, 1989). To face the problem correctly, five livestock farms were selected in the Valsassina area (Lombardy region), all of them raising cattle on a closed-cycle basis in a well-defined restricted geographical area and producing typical local cheese such as 'Quartirolo del Monte' and 'Semigrasso del Monte'.

A previous investigation (Coni *et al.*, 1995), centred on raw bulk milk and cheese samples drawn during a summer period, was undertaken in order to measure

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, as well as to elucidate any possible correlation between animal feeding, environmental situation, manufacturing process and levels of elements in raw milk and cheese.

Much along the same lines, the objective of the work described hereafter was to establish the dependence (if any) between time of year of sample collection and levels of trace elements in raw milk and, consequently, in cheese. To this end, the previous sampling campaign at five livestock farms in the Valsassina area was extended to the winter period.

The technique chosen in this study for the quantification of elements has been in the majority of cases inductively coupled plasma atomic emission spectrometry (ICP-AES) given its suitability for this kind of investigation (Caroli, 1988). Also, atomic absorption spectrometry with electrothermal atomization (ETA-AAS) measurements were performed to check the reliability of data for certain elements, namely Cr and Ni, particularly prone to spectral interferences with ICP-AES, as well as to determine elements such as Cd and Pb, whose quantification by ICP-AES was significantly affected by inadequate detection power for measuring their expected levels in the matrices investigated (Mumcu & Aras, 1988). Finally, for Pt determination it has been necessary to resort to inductively coupled plasma mass spectrometry (ICP-MS) in consequence of the very low levels expected for the said analyte.

MATERIALS AND METHODS

Sampling procedure

The following specimens were examined: (i) raw bulk milk; (ii) curds; (iii) cheese after moulding; (iv) cheese after salting; (v) seasoned cheese; and (vi) very seasoned cheese.

The raw bulk milk samples were both collected following strict precautions, in order to minimize any possible source of exogenous contamination, and under normal working conditions to evaluate better the influence of mechanical milkers and milk metallic containers on concentration levels of the elements under test. Cheese and intermediate product samples were collected in accordance with the procedure prescribed by Italian Official Analytical Methods (Ministerial Decree, 21 April 1986).

In addition to milk and cheese sampling, samples of water and food used to feed the cattle were also collected. In particular, analyses were performed on unifeed, mineral integrator and water samples from wells situated in the geographical area of the farms.

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 that could affect the reliability of the data. Details on the experimental protocol adopted are reported elsewhere (Coni *et al.*, 1995).

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

	ICP-AES
Spectrometer consisting of:	Instruments SA 32 + 38 VHR (France)
Polychromator	HR 1000 M, focal length 0·5 m, Paschen-Runge mounting, equipped with a 3600 grooves/mm holographic concave grating, linear dispersion in the first order 0·55 nm/mm, spectral range 170–410 nm, entrance and exit slit widths 50 μm.
Monochromator	HR 1000 M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic plane grating, linear dispersion in the first order 0·27 nm/mm, spectral range 170–450 nm, entrance and exit slit widths 40 μm
RF generator	Durr-JY 3848, frequency 56 MHz, nominal output 2·2 kW
Induction coil	5 coils, o.d. 32 mm, height 30 mm
Torch	INSA, demountable, with plasma argon flow 18 litres /min, coating argon flow 0·9 litres/min and carrier argon flow 0·1 litres/min.
Nebulizer	Meinhard-type with Scott type nebulizer chamber
Computer	IBM PS/2 55SX with Jobin-Yvon ESS software
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 consisting of:	Perkin-Elmer 5100 with Zeeman corrector (USA)
Monochromator	Czerny-Turner mounting, equipped with a holographic plane grating with 2880 grooves/mm in the UV region and 1400 grooves/mm in the Vis 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), atomization 1600°C (Cd) and 1800°C (Pb)
Slit width	0·7 nm
Spectral line (nm)	Cd 228·8, Pb 283·4

Table 2. Analysis of certified reference materials (CRMs). 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]
Cd	0·0029 ± 0·0012	0·003 ± 0·0006 (103%, 20%)	0·0218 ± 0·0014	0·020 ± 0·001 (92%, 5%)	0·101 ± 0·008	0·094 ± 0·006 (93%, 6%)
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%)
Cu	0·545 ± 0·030	0·540 ± 0·020 (99%, 4%)	2·23 ± 0·080	2·12 ± 0·09 (95%, 4%)	5·23 ± 0·080	5·15 ± 0·13 (98%, 2%)
Fe	2·06 ± 0·25	2·05 ± 0·10 (99%, 5%)	11·8 ± 0·6	11·5 ± 0·4 (97%, 3%)	50·1 ± 1·3	49·9 ± 1·0 (100%, 2%)
Mg	1120 ± 30	1108 ± 30 (99%, 3%)	—	—	—	—
Mn	0·226	0·218 ± 0·008 (96%, 4%)	0·236	0·225 ± 0·010 (95%, 4%)	0·223	0·215 ± 0·009 (96%, 4%)
Ni	0·0112	0·012 ± 0·002 (107%, 16%)	0·0615	0·065 ± 0·005 (106%, 8%)	0·056	0·057 ± 0·004 (102%, 7%)
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	41·9 ± 0·40 (100%, 1%)	49·5	50·0 ± 0·50 (101%, 1%)	50·4	49·5 ± 0·60 (98%, 1%)

[a]Each mean value is accompanied by standard deviation. Concentrations given without standard deviation are only qualified.
[b]Each mean value is accompanied by standard deviation. Values in brackets are, in order, the recovery percentage and the relative standard deviation (RSD).

Sample treatment

Prior to analysis, raw bulk milk samples were subjected to lyophilization within the same containers in which the specimens were stored. This operation greatly expedited the subsequent mineralization of the organic matrix, while at the same time it allowed dilution to be minimized.

The procedure adopted for the mineralization was chosen after some preliminary attempts and consisted in dry ashing at a relatively low temperature, but with a high efficiency of combustion and low risk of contamination. In more detail, aliquots of about 5 g of the freeze-dried milk and of about 2 g for all other samples were weighed into a quartz crucible. Residual humidity was removed in an oven at 120°C for 6 h and the final weight recorded. The crucible was transferred into a muffle furnace, the internal walls of which were completely lined with laminar quartz to eliminate any possibility of release of elements from the refractory material. The temperature was increased at a rate of about 50°C per hour up to 300°C. This value was kept constant for 2 h and then the temperature was increased again at the same rate as above up to 420°C, whereby combustion went on for 6 h. If ashes still contained carbon (black particles), 1 ml of double distilled water and 1 ml of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany) were added, followed by evaporation on a hot-plate and a new ashing cycle (from 300°C to 420°C in 3 h and treatment at this temperature for 30 min). This procedure was repeated until the ashes contained no more traces of carbon. Ashes were dissolved by adding 1 ml of double distilled water and 1 ml of 65% HNO_3 and careful heating on a hot-plate. The solution was transferred quantitatively with double distilled water into a 25 ml flask.

The validity of the entire procedure was tested in all possible ways by evaluating the blank contribution of each of the above steps to the final concentration values, as discussed below.

Analytical determinations

Details of the spectrometric apparatus used and working conditions adopted are given 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 attached to the data generated in 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 (skim milk powder) and CRM 150 and CRM 151 (both spiked skim 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 pre-treatment steps, an adequate number of times (in all cases, 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 92 to 107% depending on the element. Precision was also found to be more than satisfactory, with relative standard deviations (RSDs) always between 1 and 5% (n = 10). Only in three cases (Cd, Co and Ni), for which the 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 seven complete cycles of cheese production (February and March for two farms and February for the other three) 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 working conditions), cheese and intermediate products are shown in Tables 3–9, whereas Table 10 reports the data pertaining to samples of water

Table 3. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 1

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	1·44	3·75	0·066	0·059	0·244	2·76	5·15	544	0·187	0·484	0·106	<0·0001	1·29	27·0
Curds	0·743	5·93	0·085	0·077	0·436	6·56	6·69	416	0·239	0·464	0·135	<0·0001	1·11	44·7
Cheese after moulding	1·91	5·07	0·055	0·070	0·466	6·79	12·9	306	0·233	0·322	0·138	<0·0001	1·58	45·4
Cheese after salting	1·53	4·47	0·046	0·056	0·490	7·45	5·30	289	0·235	0·329	0·099	<0·0001	2·88	44·7
Seasoned cheese	3·16	2·87	0·045	0·031	0·539	7·75	7·11	253	0·304	0·185	0·150	<0·0001	2·46	40·6

[a]'Quartirolo' cheese: winter production (February 1993).

Table 4. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 2

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	1·55	1·11	0·099	0·047	0·205	1·35	3·97	537	0·139	0·513	0·108	<0·0001	1·30	27·9
Curds	1·02	1·69	0·105	0·056	0·258	3·83	5·50	436	0·222	0·470	0·130	<0·0001	1·20	45·2
Cheese after moulding	0·801	2·00	0·105	0·062	0·426	4·20	8·37	382	0·293	0·458	0·140	<0·0001	1·89	58·4
Cheese after salting	0·927	1·44	0·064	0·108	0·915	4·48	5·99	341	0·296	0·441	0·095	<0·0001	2·38	52·4
Seasoned cheese	2·60	0·333	0·051	0·092	1·19	3·80	6·60	221	0·314	0·372	0·125	<0·0001	2·06	51·1

[a]'Quartirolo' cheese: winter production (February 1993).

Table 5. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 3

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	5·14	1·57	0·070	0·027	0·282	0·988	14·2	517	0·014	0·513	0·098	<0·0001	1·04	21·6
Curds	3·41	1·71	0·083	0·086	0·314	4·20	7·86	415	0·245	0·449	0·110	<0·0001	1·53	49·1
Cheese after moulding	4·25	1·81	0·048	0·058	0·364	5·61	7·27	329	0·246	0·380	0·115	<0·0001	1·38	42·6
Cheese after salting	1·08	1·19	0·048	0·044	0·351	5·54	5·09	332	0·284	0·314	0·079	<0·0001	1·83	35·5
Seasoned cheese	2·75	0·511	0·037	0·013	0·427	5·51	6·32	339	0·316	0·272	0·105	<0·0001	1·07	31·0

[a]'Quartirolo' cheese: winter production (February 1993).

Table 6. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 4

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	4·05	2·01	0·087	0·056	0·271	2·25	2·15	671	0·138	0·712	0·134	<0·0001	4·26	34·8
Curds	2·04	5·29	0·123	0·143	0·966	2·74	10·14	515	0·269	0·693	0·167	<0·0001	3·92	48·4
Cheese after moulding	0·454	5·67	0·120	0·115	1·07	4·36	23·19	443	0·359	0·340	0·182	<0·0001	3·00	62·9
Cheese after salting	0·484	5·89	0·119	0·068	1·44	4·83	3·80	448	0·272	0·339	0·101	<0·0001	3·10	65·3
Seasoned cheese	1·71	4·78	0·119	0·018	1·39	4·59	3·04	412	0·277	0·124	0·130	<0·0001	2·87	71·3
Very seasoned cheese	5·40	4·26	0·084	0·028	1·35	4·01	3·33	462	0·290	0·117	0·192	<0·0001	2·83	72·4

[a]'Semigrasso' cheese: winter production (February 1993).

Table 7. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 5

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	1·96	1·99	0·070	0·025	0·198	12·1	8·19	707	0·233	0·672	0·095	<0·0001	5·83	30·7
Curds	1·17	3·96	0·089	0·051	0·245	24·0	7·71	366	0·335	0·547	0·121	<0·0001	1·45	43·2
Cheese after moulding	0·643	6·61	0·085	0·061	0·238	27·0	7·58	495	0·361	0·569	0·135	<0·0001	2·53	76·4
Cheese after salting	0·697	6·38	0·076	0·078	0·264	24·3	4·13	402	0·285	0·509	0·092	<0·0001	2·25	60·5
Seasoned cheese	2·09	5·81	0·064	0·053	0·392	24·8	5·84	483	0·332	0·327	0·115	<0·0001	5·59	61·3
Very seasoned cheese	3·45	5·69	0·056	0·041	0·453	26·1	5·44	475	0·347	0·253	0·148	<0·0001	5·54	61·0

[a]'Semigrasso' cheese: winter production (February 1993).

Table 8. Mean concentration values of elements (μg/g dry weight) in samples of raw milk and related dairy products;[a] five for each matrix. Livestock farm no. 4

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	5·94	2·54	0·130	0·010	0·284	0·873	12·3	785	0·233	0·527	0·160	<0·0001	10·1	32·7
Curds	3·23	4·80	0·146	0·043	0·479	3·89	8·99	681	0·443	0·342	0·170	<0·0001	9·14	40·8
Cheese after moulding	1·26	3·95	0·089	0·064	0·453	4·14	5·02	475	0·318	0·114	0·178	<0·0001	6·42	52·1
Cheese after salting	1·12	3·34	0·064	0·054	0·475	4·07	3·44	423	0·264	0·075	0·120	<0·0001	5·91	53·2
Seasoned cheese	1·99	3·02	0·059	0·052	0·527	4·07	3·59	419	0·298	0·073	0·148	<0·0001	6·05	58·2
Very seasoned cheese	2·54	2·96	0·051	0·049	0·525	4·11	3·87	418	0·304	0·069	0·168	<0·0001	6·13	59·7

[a]'Semigrasso' cheese: winter production (March 1993).

Table 9. Mean concentration values of elements (μg/g dry weight) in samples of raw materials and related dairy products;[a] five for each matrix. Livestock farm no. 5

Sample	Element													
	Al	Ba	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Pt	Sr	Zn
Raw milk	3·65	1·88	0·115	0·079	0·277	8·55	5·14	638	0·157	0·162	0·133	<0·0001	5·62	41·5
Curds	2·15	3·56	0·180	0·139	0·359	21·2	7·71	596	0·302	0·153	0·150	<0·0001	9·58	45·7
Cheese after moulding	2·98	7·65	0·134	0·122	0·362	38·9	9·88	535	0·315	0·141	0·155	<0·0001	13·3	48·3
Cheese after salting	3·63	4·03	0·070	0·092	0·433	36·9	5·97	453	0·309	0·066	0·104	<0·0001	8·65	50·0
Seasoned cheese	3·96	3·75	0·063	0·085	0·497	35·9	6·03	449	0·348	0·061	0·139	<0·0001	8·24	51·3
Very seasoned cheese	4·33	3·54	0·065	0·080	0·505	36·0	6·15	450	0·352	0·049	0·155	<0·0001	8·77	53·6

[a]'Semigrasso' cheese: winter production (March 1993).

and feed for cattle breeding. As regards correlations between environmental situation, manufacturing process and equipment and levels of elements in raw milk and cheese, the results obtained in the previous study (Coni *et al.*, 1995) were systematically confirmed in the present one. Meticulous analyses of all data relating to the various steps of cheese production confirm that the manufacturing process considerably influences element concentrations both by chemical and physical treatments involved and by processing equipment employed. In particular, curdling and salting are the two phases of production that bring about the greatest variations in the element concentrations. As regards the second aspect, levels of elements such as Al, Cd, Cr, Cu, Fe, Mn and Ni can be increased by release of metals from containers and tools, with which milk and intermediate products come into contact. Finally, it is confirmed that the levels of some toxic elements (e.g. Al and Pb) are increased by local environmental contamination during the period of cheese seasoning.

More interesting and novel information can be deduced by comparing the data reported in this study with those pertaining to the summer sampling already reported. From an overall point of view, it is clear that, as regards raw bulk milk, significant differences exist between the two sets of data. To frame the phenomenon better, two explanatory examples, representative of a general behaviour, are illustrated in Figs 1 and 2. The former shows the seasonal variation of trace element levels in raw bulk milk employed for 'Quartirolo' cheese production, whereas the latter refers to raw bulk milk employed for 'Semigrasso' cheese production. It is obvious that, in both cases, livestock farm and cattle were the same. On the basis of the elemental variations

Table 10. 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	0·012	0·036	0·0007	0·0002	0·0003	0·006	0·025	5·43	0·0006	0·0001	0·004	0·003	0·121	0·035
Farm no. 2	0·020	0·026	0·0009	0·0003	0·0003	0·005	0·089	6·01	0·0003	0·0003	0·005	0·004	0·155	0·060
Farm no. 3	0·010	0·025	0·0005	0·0003	0·0002	0·005	0·016	10·7	0·0005	0·0001	0·004	0·005	0·049	0·015
Farm no. 4	0·009	0·035	0·0008	0·0003	0·0002	0·008	0·044	1·34	0·0008	0·0009	0·002	0·001	0·046	0·040
Farm no. 5	0·025	0·088	0·0010	0·0001	0·0003	0·009	0·027	2·54	0·0009	0·0002	0·003	0·001	0·045	0·030
Feed														
Farm no. 1														
Unifeed	4·11	9·53	0·194	0·281	0·351	9·89	93·2	1314	8·23	0·936	0·745	0·101	1·70	74·0
Integrator	128	21·5	0·387	0·669	0·817	20·48	352	1325	8·01	1·48	1·01	0·116	9·03	81·7
Farm no. 2														
Unifeed	5·78	2·89	0·178	0·245	0·405	7·87	85·6	1280	7·85	1·24	0·784	0·069	0·985	81·6
Integrator	75·2	10·86	0·399	0·401	1·57	12·4	205	1370	8·08	1·35	1·15	0·110	6·43	98·1
Farm no. 3														
Unifeed	6·75	0·786	0·157	0·187	0·487	5·46	80·2	1235	7·80	1·69	0·890	0·023	0·241	92·7
Integrator	63·0	8·42	0·339	0·230	4·19	7·89	185	1312	8·84	1·18	1·13	0·112	7·69	152
Farm no.4														
Unifeed	4·47	0·480	0·098	0·204	0·398	6·58	75·7	1320	6·68	1·54	0·770	0·057	0·996	50·2
Integrator	80·1	9·79	0·287	0·350	2·70	9·56	200	1410	7·59	1·44	1·01	0·146	8·41	101
Farm no. 5														
Unifeed	5·74	3·56	0·148	0·239	0·361	8·45	90·0	1298	7·11	1·35	0·670	0·045	0·818	66·8
Integrator	59·8	15·7	0·300	0·478	3·89	8·99	278	1410	9·00	1·46	1·12	0·128	8·09	90·1

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

observed for all five farms between raw bulk milk samples taken during the summer and the winter periods, the elements investigated can be roughly subdivided into three classes. The first includes elements such as Ba, Cd, Co, Cr, Pb and Sr for which concentration levels are higher in the winter milk samples. The second group regards elements such as Al, Mg and Mn, which behave the opposite way. For the remaining elements (Cu, Mg, Ni and Zn), there is no particular trend, in that the concentration ranges obtained for winter and summer milk samples overlap fairly well.

The true meaning of the patterns observed cannot be entirely elucidated, though feeding differences may be the main cause. This is justified by the fact that a com-

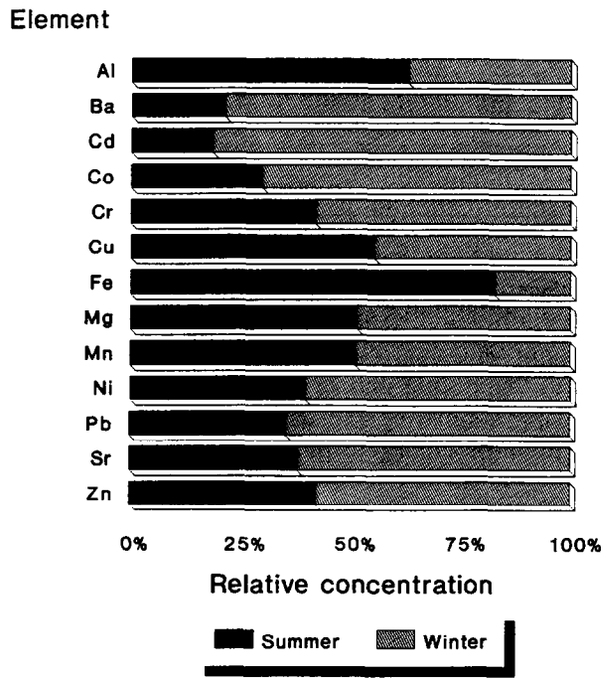

Fig. 1. Seasonal variations of trace elements' levels in raw cow's milk employed for 'Quartirolo' cheese production. Livestock farm no. 1. The relative concentration is normalized to 100% for all elements.

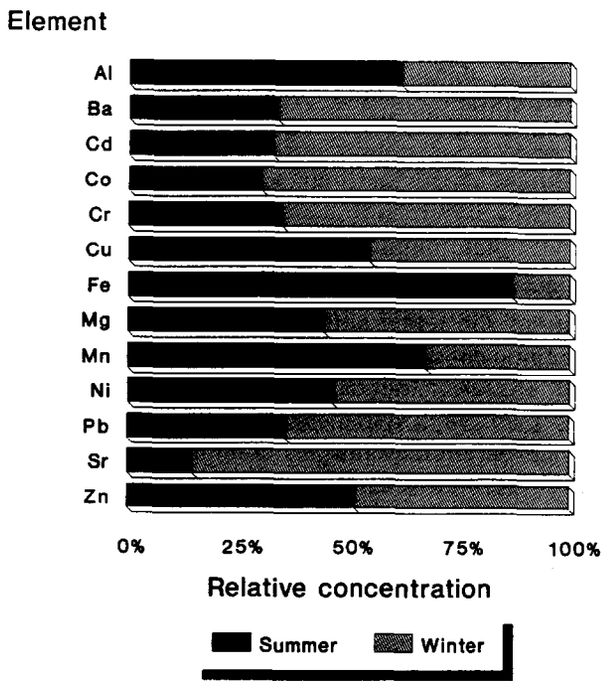

Fig. 2. Seasonal variations of trace elements' levels in raw cow's milk employed for 'Semigrasso' cheese production. Livestock farm no. 4. The relative concentration is normalized to 100% for all elements.

parison between the element contents of winter feeding and summer feeding clearly shows that there are striking differences in the concentrations of almost all elements. In particular, the levels of elements such as Al, Cr, Cu, Fe, Mg, Ni, Pb, Pt and Zn are higher for the winter feeding, whereas for Ba and Sr the contrary holds, the concentration of these latter being much higher in the summer feeding. Since the homeostatic mechanisms of control are able to counterbalance only small variations in the intakes of essential elements, too small or too large contents in feedstuffs will result in significant alterations of the element contents of cow's milk.

Furthermore, the results obtained suggest that absorption of several trace elements may well depend not only on the total amount in the feeding, but also on the chemical forms in which they occur. In fact, bioavailability, absorption, mobility, accumulation and excretion of a given element can be different and, therefore, the same intake does not ensure the same element content in milk. Therefore, the different natures of the two kinds of feeding can also explain some apparent anomalies that occur for elements such as Al, Ba, Fe, Sr and Zn. The data relative to these latter elements show that there is no direct relation between element intake and element content in milk. On the other hand, in some cases, a greater intake corresponds to a lower level and vice versa.

It is also worth mentioning that the winter feed samples, especially the integrators, have decidedly high contents of toxic elements (Al, Cd, Cr, Ni, Pb and Pt). This is so marked that it would seem to support the hypothesis that bovine intake of these elements is virtually entirely through the consumption of unifeed and integrators. In consequence of this, much attention should be paid to feedstuffs that are not produced locally, but that come from a variety of sources. This is essential to guarantee the quality and safety of milk and typical cheese products. Furthermore, the information obtained in this investigation shows that the above-mentioned integrators need urgent reassessment as do the essential trace elements. In fact, almost none of the mineral salts employed show an acceptable bioavailability.

As regards cheese production, it is possible to state that some elements, even when present at equivalent total concentrations in milk collected in winter and in summer, respectively, may behave differently during the manufacturing process with consequent differentiation of the concentration levels of those elements in finished goods (seasoned cheese). A possible explanation for this apparent discrepancy is that, in raw milk an element may occur in different chemical forms according to the kind of feeding. It is thus self-evident that an element may behave in different ways during the sequence of biochemical processes taking place during cheese production. Moreover, local and physiological factors can influence the metabolic fate of an element and, therefore, account for the pattern observed.

The validity of the assumptions reported herein on the precautions mandatory for studies focused on trace elements in foodstuffs is substantiated by the experimental evidence attained so far. The considerable improvement, during recent years, in element transfer mechanisms, along the line environment–feeding–cow's milk–cheese, proves the correctness of the overall approach.

This work provides important information on safety and quality standards of milk and typical cheeses and constitutes a noticeable step forward for sound and effective action in the safeguard of food safety and, consequently, human health.

Finally, it should be emphasized that the experimental approach described above is not only an appropriate strategy for a better compliance with nutritional demands, but also the only way to guarantee the survival of many Italian traditional foodstuffs.

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