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Minor and trace element content in sheep and goat milk and dairy products

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The concentration ranges of certain health-related elements in milk and cheese are closely dependent upon animal species and feeding, time of year of sample collection, environmental conditions and manufacturing processes. The present study deals with the assessment of selected trace elements such as Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn in sheep and goat milk as well as in typical cheese, and concludes a series of studies previously carried out on cow's milk and related products. Samples of milk, curd and cheese following salting and after ripening for 1, 4 and 8 weeks were analysed. Quantitative determinations were performed mainly by inductively coupled plasma atomic emission spectrometry. The results obtained show considerable differences among the trace element contents of sheep and goat milk and related products. Furthermore, significant differences were also found when comparing the present data on sheep and goat milk with those previously obtained for cow's milk. This sheds further light on the influence of animal species, feeding characteristics and environmental conditions on the distribution of trace elements in dairy products. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The rationalization of production processes and identification of quality markers for milk and milk products is of great importance for the protection and evaluation of typical dairy products. Consequently, as a safeguard, the first objective is to undertake a careful and thorough assessment of all mechanisms by which feedstuff quality, manufacturing processes and environmental conditions, including health parameters, can influence milk and cheese properties.

From this standpoint, a project named '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 Innovations in the Agricultural System) project supported by the National Research Council. One of the topics considered during the development of this project was the assay of trace elements in milk and dairy products. In general, the existing literature on trace element composition of milk and cheese is rather scarce at the international level (International Dairy Federation, 1978; World Health Organization, 1989). This is all the more true in Italy, although studies have recently been published in which elemental analysis of the most characteristic commercial cheese products in Italy are exhaustively reported (Favretto *et al.*, 1987; Gabrielli Favretto *et al.*, 1989; Gabrielli Favretto, 1990; Vojnovic *et al.*, 1991).

In this context, rigorous experimental protocols and reliable analytical measurements are essential to monitor influences from the environment on the levels of trace elements in the matrices of interest, to control possible secondary contamination or essential element losses during processing, storage and packaging of milk and milk products and to estimate the role of trace element contents with regard to product quality or health risks (Garfield, 1980, 1984; Tölg, 1988).

Recent investigations on raw bulk cow's milk and cheese samples were undertaken in Italy 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, as well as to elucidate any possible relationship between animal feeding, environmental conditions, time of year of sample collection, manufacturing process and distribution of elements in cow's milk and cheese (Coni *et al.*, 1994, 1995).

Along similar lines, the aim of this work was to extend previous investigations to milk and cheese of sheep and goat origin. To plan the research properly and thus guarantee the reliability of data, three livestock farms were chosen in the Perugia area (Umbria region), all of them raising flocks on a closed-cycle basis in a well-defined restricted geographical zone and producing typical local cheese. Two farms raised sheep, whereas the third one raised goats.

The analytical technique used for the quantification of elements was, in almost all cases, inductively coupled plasma atomic emission spectrometry (ICP-AES) given its inherent suitability (multi-elemental capability, high detection power, wide dynamic range and reduced dependence on matrix composition) for this kind of investigation (Caroli, 1988). Electrothermal atomization atomic absorption spectrometry (ETA-AAS) measurements were also performed to check data for certain elements, namely Cr and Ni, that are particularly prone to spectral interferences with ICP-AES. ETA-AAS was also used to determine elements such as Cd and Pb, for which ICP-AES could not provide adequate detection power at the expected levels in the matrices investigated (Mumcu & Aras, 1988; Larsen & Rasmussen, 1991). Finally, for Pt analysis, on the basis of results obtained in previous studies, it was necessary to resort to inductively coupled plasma mass spectrometry (ICP-MS) because of the very low levels envisaged for this analyte.

MATERIALS AND METHODS

Sampling procedure

Sample collection at all three farms was carried out in two different periods of the year to better evaluate the influence of different feeding patterns on the levels of trace elements in milk. In each case, raw milk, curd and cheese after salting and ripening for 1, 4 and 8 weeks were sampled. The six lots of samples thus obtained had the following characteristics: (a) sheep of farm no. 1 fed with composite pasture; (b) sheep of farm no. 1 fed with grass pasture; (c) sheep of farm no. 2 fed with composite pasture; (d) sheep of farm no. 2 fed with wheat bran and composite pasture; (e) goats of farm no. 3 fed with composite pasture; (f) goats of farm no. 3 fed with maize and composite pasture.

Raw bulk milk samples were collected with rigorous precautions to minimize any possible source of exogenous contamination, and using normal working conditions to evaluate 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 agreement with the procedure prescribed by Italian Official Analytical Methods (Ministerial Decree, 1986).

Along with milk and cheese sampling, samples of water and food used to feed the flock were also collected. In particular, analyses were performed on pasture, bran, maize and water samples from wells placed in the geographical area of the farms.

As described in previous studies, sampling and storage steps were optimized so as to reduce all possible contamination, loss or alteration that could negatively affect reliability of data (Coni *et al.*, 1990*a*,*b*; Stacchini *et al.*, 1992). Details on the experimental protocol adopted are reported elsewhere (Coni *et al.*, 1994, 1995).

Sample treatment

For practical reasons all raw bulk milk samples under examination were first lyophilized within the same storage containers. This greatly facilitated the subsequent digestion of organic compounds, while at the same time allowing dilution to be minimized. Organic matrix destruction was tested in each sample by performing dry and wet ashing. After a systematic comparison of their respective performances, a dry ashing procedure at a relatively low temperature, but with a high efficiency of combustion and low risk of contamination, was chosen. In summary, aliquots of about 5 g of the freeze-dried milk and of about 2 g for all other samples were weighed into decontaminated quartz crucibles. Samples were then dried in an oven at 120°C for 6 h and the final weight was ascertained. Crucibles were transferred into a muffle furnace, the internal walls of which were completely lined with laminar quartz to exclude any possibility of release of elements from the refractory material. The temperature was increased at a rate of about 50°C h^{-1} up to 300°C. This value was kept constant for 2 h, then the temperature was increased again at the same rate as above up to 420°C, whereby combustion was continued for 6 h. If, at the end of the thermal treatment, ashes still contained black particles of carbon, 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 by a new ashing cycle (from 300°C to 420°C in 3 h and treatment at this temperature for 30 min). This operation was repeated until the ashes contained no traces of carbon. Dissolution of white ashes was then performed by adding 1 ml of double-distilled water and 1 ml of 65% HNO_3, with subsequent careful heating on a hotplate. Finally, solutions were transferred quantitatively into a 25 ml flask and double-distilled water was added 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 above steps to the final concentration values, as discussed below.

Analytical determinations

Details of the spectrometric apparatas used and working conditions adopted are given in Tables 1 and 2.

The entire analytical procedure was tested for both measurement accuracy and precision in order to estimate the degree of reliability of the data. Accuracy was

Table 1. Instrumentation and working conditions for ICP-AES

Instrumentation	
Spectrometer	JY 32 + 38 (VHR Instruments, France)
RF generator	Durr-JY 3848, frequency 56 MHz, nominal output 2.2 kW
Induction coil	5 turns, outer diameter 32 mm, height 30 mm
Torch	INSA, demountable
Monochromator	HR 1000 M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves mm^{-1} holographic plane grating, linear dispersion in the first order 0.27 nm mm^{-1}, spectral range 170–450 nm
Polychromator	HR 1000 M, focal length 0.5 m, Paschen-Runge mounting, equipped with a 3600 grooves mm^{-1} holographic concave grating, linear dispersion in the first order 0.55 nm mm^{-1}, spectral range 170–410 nm
Software	Jobin-Yvon ESS
Nebulizer	Meinhard with Scott-type nebulizer chamber
Working conditions	
Spectral lines (nm)	
Monochromator	Al(I) 237.3, Cd(I) 226.5, Cr(II) 206.1, Pb(II) 220.4
Polychromator	Ba(II) 233.5, Mg(II) 279.6, Zn(I) 213.8, Co(II) 238.9, Mn(II) 257.4, Cu(I) 324.7, Ni(II) 231.6, Fe(II) 259.9, Sr(II) 407.7
Air flow rates (litre min^{-1})	Plasma 18, coating 0.9, carrier 0.1
Slit width (μm)	40 (entrance and exit for monochromator), 50 (entrance and exit for polychromator)

Table 2. Instrumentation and working conditions for ETA-AAS

Instrumentation	
Spectrometer	Perkin-Elmer 5100 with Zeeman corrector (PE Instruments, USA)
Monochromator	Czerny-Turner mounting, equipped with a holographic plane grating with 2880 grooves mm^{-1} in the UV region and 1440 grooves mm^{-1} in the visible region, linear dispersion in the first order 0.65 nm mm^{-1} (UV) or 1.30 nm mm^{-1} (visible), spectral range 170–900 nm
Furnace	Perkin-Elmer HGA 600 with autosampler AS-60
Lamp type	Hollow cathode
Working conditions	
Spectral lines (nm)	Cd 228.8, Cr 357.9, Ni 232.0, Pb 283.4
Matrix modifier (mg)	$0.2NH_4H_2PO_4 + 0.01Mg(NO_3)_2$ for Cd and Pb; $0.05Mg(NO_3)_2$ for Ni and Cr
Thermal programme	Drying: 110°C for Cd, Cr, Ni and Pb Ashing: 700°C for Cd, 1650°C for Cr, 1000°C for Ni, 850°C for Pb Atomization: 1600°C for Cd, 2600°C for Cr, 2300°C for Ni, 1800°C for Pb
Slit width (nm)	0.7 nm for Cd, Cr and Pb, 0.2 nm for Ni

constantly monitored by tests carried out on three series of Certified Reference Materials (CRMs) supplied by the EC Measurements and Testing Programme (formerly BCR, Brussels, Belgium), as these represented the type of matrix under consideration, namely CRM 063 (skim milk powder) and CRM 150 and CRM 151 (two different spiked skim milk powders). Precision, in turn, was ascertained by replicating (ten times) the entire analytical cycle, including the pre-treatment steps.

RESULTS AND DISCUSSION

Analytical data for all three CRMs were acceptable and recovery varied from 91% to 109% depending on the element. Precision was also found to be more than sufficient, with relative standard deviations always between 1% and 7%. There were only three elements (Cd, Co and Ni) for which the concentrations measured were very close to the detection limits of the technique, with ensuing worsening of precision (17–20%). Results for both accuracy and precision are reported in Table 3.

Data obtained in this work refer to six complete cycles of cheese production (four for sheep cheese production and two for goat cheese production). Results of spectrometric analysis of raw bulk milk (collected under normal working conditions), cheese and intermediate products are shown in Tables 4–9, whereas Table 10 summarizes data pertaining to samples of water and feed.

As regards the dependence of trace element levels (in raw sheep and goat milk and cheese) on environmental conditions and manufacturing processes and equipment, results obtained in this work do confirm the findings of previous studies (Coni *et al.*, 1994, 1995). In fact, careful analysis of all data pertaining to the various steps carried out during cheese production corroborates the assumption that the manufacturing process considerably influences element concentrations in consequence of both chemical and physical treatments involved and processing equipment employed (Wong *et al.*, 1976; Wong & La Croix, 1978).

It is appropriate to briefly consider the methods of production of the two types of cheese examined in this investigation. In sheep cheesemaking, raw milk is

Table 3. Analysis of certified reference materials (CRMs)

Element	Concentration (μg g⁻¹)					
	CRM 063		CRM 150		CRM 151	
	Certified[a]	Found[b]	Certified[a]	Found[b]	Certified[a]	Found[b]
Cd[c]	0.0029 ± 0.0012	0.003 ± 0.0006 (103%, 20%)	0.0218 ± 0.0014	0.022 ± 0.002 (101%, 9%)	0.101 ± 0.008	0.095 ± 0.002 (94%, 2%)
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	0.530 ± 0.025 (97%, 5%)	2.23 ± 0.080	2.10 ± 0.10 (94%, 5%)	5.23 ± 0.080	5.19 ± 0.15 (99%, 3%)
Fe	2.06 ± 0.25	2.25 ± 0.10 (109%, 5%)	11.8 ± 0.6	11.5 ± 0.6 (97%, 5%)	50.1 ± 1.3	50.5 ± 2.0 (101%, 4%)
Mg	1120 ± 30	1079 ± 42 (96%, 4%)				
Mn	0.226	0.211 ± 0.011 (93%, 5%)	0.236	0.220 ± 0.011 (93%, 5%)	0.223	0.204 ± 0.010 (91%, 5%)
Ni[c]	0.0112	0.011 ± 0.002 (98%, 18%)	0.0615	0.059 ± 0.005 (96%, 8%)	0.056	0.054 ± 0.006 (96%, 11%)
Pb[c]	0.1045 ± 0.003	0.110 ± 0.006 (105%, 5%)	1.000 ± 0.040	1.06 ± 0.054 (106%, 5%)	2.002 ± 0.026	2.12 ± 0.062 (106%, 3%)
Zn	42.0	41.6 ± 1.95 (99%, 5%)	49.5	51.3 ± 2.02 (104%, 4%)	50.4	52.7 ± 1.78 (105%, 3%)

Results are the mean of ten independent sample preparations.

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

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

Table 4. Concentrations of elements (μg g⁻¹ 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	3.87	2.57	0.162	0.014	0.014	0.453	4.37	644	0.339	0.063	0.109	0.430	5.98	26.0
Curds	4.58	3.00	0.168	0.036	0.037	0.781	4.64	512	0.717	0.050	0.125	0.550	4.58	43.4
Cheese after salting	5.79	2.77	0.139	0.018	0.020	0.729	4.07	488	0.707	0.031	0.086	0.447	4.88	39.3
Cheese after ripening for 1 week	3.91	1.82	0.103	0.017	0.025	0.456	3.34	391	0.609	0.010	0.096	0.346	3.67	25.3
Cheese after ripening for 4 weeks	3.96	1.73	0.104	0.013	0.027	0.469	3.03	290	0.522	0.009	0.098	0.307	2.99	29.2
Commercial cheese (8 weeks)	4.29	1.38	0.097	0.015	0.032	0.519	2.65	250	0.449	0.008	0.115	0.220	2.98	16.8

[a] Sheep cheese: summer production.

Table 5. Concentrations of elements (μg g⁻¹ 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	2.57	2.76	0.128	0.029	0.020	0.681	3.71	588	0.276	0.061	0.059	0.417	5.44	21.2
Curds	3.86	3.54	0.150	0.036	0.026	1.09	4.63	486	0.626	0.056	0.071	0.449	4.16	33.3
Cheese after salting	4.46	3.42	0.126	0.029	0.017	0.872	4.12	441	0.579	0.039	0.044	0.427	3.68	33.0
Cheese after ripening for 1 week	5.72	3.39	0.120	0.027	0.019	1.02	4.45	400	0.552	0.027	0.062	0.346	3.28	30.2
Cheese after ripening for 4 weeks	2.94	2.59	0.111	0.021	0.020	0.904	3.23	305	0.448	0.026	0.075	0.325	2.89	24.8
Commercial cheese (8 weeks)	2.55	1.79	0.076	0.018	0.036	0.932	2.31	213	0.339	0.016	0.082	0.274	2.74	15.5

[a] Sheep cheese: winter production.

Table 6. Concentrations of elements ($\mu g\ g^{-1}$ 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	3.61	3.91	0.183	0.025	0.025	0.545	4.27	653	0.333	0.040	0.078	0.455	6.24	26.0
Curds	5.40	6.47	0.229	0.030	0.028	0.843	6.25	580	0.671	0.031	0.098	0.488	6.22	45.9
Cheese after salting	6.89	5.74	0.215	0.028	0.025	1.21	6.08	501	0.613	0.029	0.071	0.407	5.55	49.6
Cheese after ripening for 1 week	6.93	5.47	0.198	0.028	0.022	0.779	5.48	440	0.552	0.023	0.085	0.294	4.91	37.7
Cheese after ripening for 4 weeks	9.08	5.09	0.199	0.019	0.024	0.786	6.39	511	0.518	0.016	0.098	0.356	4.71	34.1
Commercial cheese (8 weeks)	8.75	4.96	0.183	0.016	0.025	0.908	5.56	443	0.489	0.016	0.115	0.298	4.54	22.9

[a] Sheep cheese: summer production.

Table 7. Concentrations of elements ($\mu g\ g^{-1}$ 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	1.52	3.00	0.181	0.015	0.015	0.784	2.74	619	0.297	0.064	0.094	0.422	4.72	22.7
Curds	2.29	3.77	0.200	0.040	0.018	1.94	3.30	610	0.545	0.055	0.113	0.554	4.49	39.0
Cheese after salting	2.68	3.56	0.190	0.037	0.016	1.94	3.28	526	0.496	0.041	0.085	0.405	6.56	42.2
Cheese after ripening for 1 week	2.86	3.39	0.181	0.029	0.021	1.81	4.30	518	0.461	0.032	0.091	0.355	5.88	40.6
Cheese after ripening for 4 weeks	2.87	3.25	0.123	0.029	0.037	1.99	2.91	421	0.417	0.026	0.099	0.459	3.55	31.1
Commercial cheese (8 weeks)	3.11	2.18	0.112	0.023	0.049	1.61	2.55	381	0.411	0.020	0.107	0.342	3.65	22.4

[a] Sheep cheese: winter production.

Table 8. Concentrations of elements ($\mu g\ g^{-1}$ 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	1.35	1.72	0.161	0.020	0.030	0.619	2.94	757	0.111	0.023	0.050	0.519	4.80	16.8
Curds	2.66	3.27	0.226	0.023	0.062	1.31	4.81	540	0.221	0.019	0.069	0.566	4.23	34.5
Cheese after salting	1.14	3.06	0.171	0.023	0.041	1.37	3.42	485	0.205	0.015	0.038	0.324	5.93	30.4
Cheese after ripening for 1 week	1.02	2.90	0.168	0.017	0.051	1.28	3.40	465	0.204	0.012	0.040	0.299	5.48	24.8
Cheese after ripening for 4 weeks	1.18	2.16	0.140	0.013	0.062	1.36	3.73	407	0.202	0.013	0.045	0.278	4.82	22.0
Commercial cheese (6 weeks)	1.09	2.12	0.137	0.012	0.067	1.45	2.87	445	0.192	0.011	0.058	0.307	4.73	17.0

[a] Goat cheese: summer production.

Table 9. Concentrations of elements ($\mu g\ g^{-1}$ 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	0.850	2.08	0.130	0.020	0.039	0.605	2.42	752	0.145	0.015	0.049	0.520	4.60	19.3
Curds	1.81	2.77	0.142	0.031	0.045	1.56	3.79	518	0.264	0.013	0.075	0.546	3.96	37.8
Cheese after salting	2.91	2.44	0.107	0.024	0.035	0.749	3.26	417	0.241	0.012	0.050	0.369	4.26	28.9
Cheese after ripening for 1 week	1.79	2.20	0.105	0.020	0.042	0.765	3.10	372	0.236	0.006	0.068	0.324	3.89	26.6
Cheese after ripening for 4 week	1.24	1.45	0.089	0.020	0.051	0.771	2.36	306	0.199	0.004	0.080	0.285	3.10	25.4
Commercial cheese (6 weeks)	1.80	1.23	0.090	0.013	0.056	0.759	2.01	284	0.194	0.003	0.096	0.257	2.97	19.2

[a] Goat cheese: winter production.

Table 10. Mean concentrations of elements in samples of water ($\mu g\ ml^{-1}$) and feed for flock breeding ($\mu g\ g^{-1}$ dry weight) ($n = 10$ 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	0.019	0.079	0.0008	0.0009	0.0003	0.003	0.044	21.5	0.007	0.0006	0.003	0.014	0.153	0.082
Farm no. 2	0.023	0.102	0.0009	0.0007	0.0003	0.011	0.021	18.6	0.0007	0.0001	0.004	0.011	0.159	0.068
Farm no. 3	0.010	0.037	0.001	0.0006	0.0002	0.005	0.023	24.4	0.004	0.0003	0.001	0.015	0.160	0.098
Summer feeding														
Farm no. 1	0.209	5.41	0.281	0.291	0.060	1.22	10.7	849	12.1	0.019	0.468	0.668	2.50	6.62
Farm no. 2	0.287	3.24	0.302	0.277	0.101	2.56	9.55	765	10.5	0.028	0.519	0.608	1.12	7.45
Farm no. 3	0.069	0.950	0.855	0.339	0.093	2.05	11.2	681	9.78	0.012	0.709	0.908	0.145	4.71
Winter feeding														
Farm no. 1	0.169	4.07	0.309	0.391	0.097	2.98	14.2	862	11.8	0.016	0.578	0.789	1.87	8.71
Farm no. 2	0.200	3.14	0.296	0.265	0.128	2.71	8.78	986	14.9	0.018	0.602	0.745	0.897	6.92
Farm no. 3	0.073	1.24	1.398	0.401	0.109	3.67	19.7	945	8.46	0.017	0.804	0.897	0.545	7.56

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

poured into stainless steel vats where it is clarified for 12 h at about 5°C. Thereafter, the milk is transferred into a copper boiler and, after addition of the necessary amounts of starter culture and rennet, it is heated at 37°C for 30 min. The temperature is then increased to 42°C for a few minutes; the resultant curd is pressed for a short time in the same copper boiler, then cut into blocks and placed into special PVC moulds. After about 18 h, during which time the curd loses water and some whey and acquires the desired shape, a 36-h immersion in salt brine follows. Subsequently, the cheese is put on wooden boards where it is allowed to ripen for a period of about 8 weeks. The process of goat cheese manufacture is very similar and the only significant differences are to be found in the salting procedure, which is performed with dry salt, and in the ripening time.

Curdling and salting are the two phases of production that cause the most important variations in element concentrations. The reasons for this strong influence are that, in the case of curdling, an uneven distribution of elements occurs between curd and whey depending on the different element binding forms in the milk, whereas, during salting, osmotic phenomena occur with subsequent variations in the levels of unbound minerals. More specifically, it has been observed that curdling gives rise to increased concentrations of some elements, such as Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Pb, Pt and Zn, in the curds compared to raw milk, whereas the opposite is true for Mg, Ni and Sr (see the first two rows of Tables 4–9). This may be ascribed to the fact that the elements of the former group are preferentially bound to caseins and fat and consequently shift mostly to the curds, whereas the latter elements concentrate especially in whey because in milk they are bound to components with a relatively low molecular weight (whey proteins, citrates, lactose, mineral salts, etc.). This assumption is also corroborated by previous studies carried out on trace element speciation in milk (Brätter *et al.*, 1988; Dunemann & Schwedt, 1988; Coni *et al.*, 1996).

The salting step, in turn, causes a more or less pronounced decrease in the concentration of all elements with the exclusion of Al, the level of which turns out to be higher after cheese salting (see the second and third rows of Tables 4–9). Apparently, the strong concentration of NaCl gives rise to osmosis with subsequent release of elements that are partially present in the curds as cations. Moreover, this effect continues, albeit to a lesser extent and only for a few elements, during the ripening period.

This general behaviour is analogous, for both curdling and salting, to that encountered in previous studies on cow cheese production. The only exception appears to be Al, since during the curdling of raw cow milk the concentration of this element decreased passing from milk to curds. A possible explanation for this apparent discrepancy is that, during cow's milk collection, a greater release of Al in the form of unbound element may occur from milking equipment and elements in cationic form are prone to pass into the whey. In fact, a prompt analysis revealed that the milk containers used in bovine farms, as a rule, were made of aluminium, whereas those employed in ovine and goat farms were made of steel. This shows unequivocally that, as a result of the contact of milk with the equipment employed, often also at relatively high temperatures, a release may occur of all those elements (e.g. Al, Cd, Cr, Cu, Fe, Mn and Ni) that are normally contained in the metal alloys with which the tools and containers used to process milk are made. Moreover, the high increases of Cu found in all the six cycles of cheese production are mostly due to the abundant and traditional use of copper containers, typical for the production processes considered.

Further evidence shows that the levels of some toxic elements (e.g. Al and Pb) can be increased by local environmental contamination during the phase of cheese ripening.

Finally, in terms of feed intake, no conclusive correlation could be established between feeding and milk element concentrations. In fact, Table 10 shows that, unlike what had been observed in the case of cow feeding, there were small differences in terms of element contents among the six groups of feedstuffs analysed.

It is interesting to compare the data reported in this study with those regarding the cow's milk sampling already accounted for in previous studies. From a general viewpoint, it is clear that significant differences exist between the three sets of data for raw bulk milk. Some examples are illustrated in Figs 1 and 2, from which it can be clearly seen that there are large differences in trace element concentrations in the three kinds of samples analysed. Maximum differences in the range of values within each series of samples are very small and hence the differences noted in the various series are significant and actually reflect different distributions of trace elements in the three types of raw bulk milk. Finally, it should be stressed that the above data refer to the same year period of sample collection (summer) and, consequently, feeding type (pasture).

Further analysis of these differences and of all other available information allows two interesting considerations of general validity to be made. The first regards the essential elements, some of which are present at very different levels in the various types of milk. The phenomenon is quite evident when data on cow's milk are compared to those for sheep and goat's milk. In particular, the levels of elements such as Cu, Fe, Cr and Ni are higher in the cow's milk samples, whereas for Ba and Sr the opposite holds, the concentration of these latter analytes being much higher in the sheep and goat milk samples. Since a comparison between the essential element contents of feeds for cow, sheep and goat clearly shows that there are no striking differences in the concentrations of almost all elements, the reasons for the differences can probably be ascribed to the fact that the concentrations of essential elements in milk strongly reflect metabolic differences in the three animal species. In fact, for different animal species, bioavailability, absorption, mobility, accumulation and excretion of a given element are not the same and, therefore, the same intake does not result in the same element contents in milk (Mills *et al.*, 1985; Underwood, 1977). Sometimes, within the same animal species, element metabolism can also be affected by the genetic make-up of the single animal (Field, 1984).

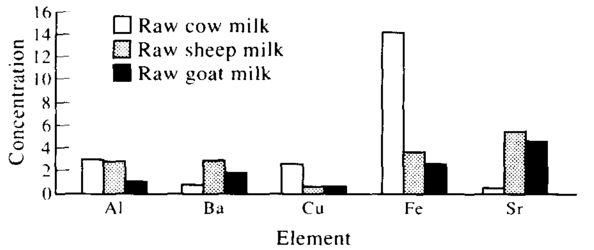

Fig. 1. Trace element content ($\mu g\ g^{-1}$ dry weight) of milk as a function of animal species.

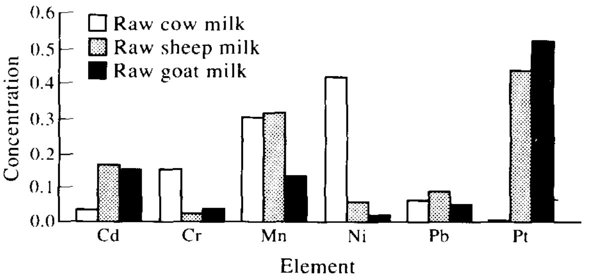

Fig. 2. Trace element content ($\mu g\ g^{-1}$ dry weight) of milk as a function of animal species.

Further support for this hypothesis comes from the fact that, when there are element level differences in feeding (e.g. for Fe, Ba and Sr), these rank in an opposite manner to that observed for raw milk samples. For example, in cow feed Fe is present in the range 0.567–2.42 $\mu g\ g^{-1}$ (dry weight), whereas in sheep and goat feed the range is 8.78–19.7 $\mu g\ g^{-1}$ (dry weight). In spite of this, the concentrations of Fe in raw milk are 4.36–24.6 $\mu g\ g^{-1}$ (dry weight) for cow's milk, 2.74–4.37 $\mu g\ g^{-1}$ (dry weight) for sheep's milk and 2.42–2.94 $\mu g\ g^{-1}$ (dry weight) for goat's milk.

Another consideration is the influence of environmental pollution on the levels of some potentially toxic elements, such as Al, Cd, Pb and Pt. For these latter elements the variations observed in the three types of milk do not seem to reflect differences in animal species, but rather a different environmental situation (i.e. local contamination), which can have a negative impact on toxic element levels in water and feed for flock breeding (Krelowska Kulas, 1990; Stevens, 1991). In this regard, among all toxic elements taken into account in this research, Pt deserves particular mention. The current use of Pt catalytic converters in controlling vehicle exhaust emissions may result in its release into the atmosphere and ensuing possible contamination of food (Zeisler & Greenberg, 1988). Additional evidence of this risk is provided by the present data. In fact, it has been possible to ascertain that Pt is present at relatively high levels both in sheep and goat feed and milk, whereas its levels are very low in cow feed and virtually nil in cow milk (the concentrations detected never exceeded the value of 0.0001 $\mu g\ g^{-1}$). This can be ascribed to the fact that the three sheep and goat farms were situated in a zone (Perugia area) where vehicular traffic is certainly more intense compared to that in the zone of the three cow farms (Valsassina area).

This notwithstanding, human exposure to toxic elements through milk and dairy products does not appear, at present, to be a source of risk (Coni *et al.*, 1994). Attention, however, must be paid to situations in which continuous emission of exogenous elements into the environment, as for the Pt case, may represent a source of risk in the near future. There is thus a clear need for continued monitoring of certain toxic elements in food and the environment coupled with research on the effect on man of long-term exposure to low levels of such elements.

The experimental evidence gained in this work together with the wealth of information acquired in the previous two studies shed further light on the influence of the animal species, feedstuff, year period of sample collection, environmental conditions and manufacturing processes on the concentration ranges of certain health-related elements in cow, sheep and goat milk and typical cheese products. This constitutes a notable starting point for sound and effective action to enhance safety and quality of milk products.

Finally, the experimental approach and analytical method developed in this and in former studies appear adequate for the purpose and can therefore be recommended for other similar investigations.

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