Elemental and isotopic characterisation of typical Italian alpine cheeses

L. Bontempo [a, *], R. Larcher [a], F. Camin [a], S. Hölzl [b], A. Rossmann [c], P. Horn [b], G. Nicolini [a]

[a] IASMA Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[b] Bavarian States Collection for Palaeontology and Geology, Richard-Wagner Strasse 10, D-80333 München, Germany

[c] Isolab GmbH, Woelkestrasse 9, D-85301 Schweitenkirchen, Germany

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[*] Corresponding author. Tel.: +39 461 615138; fax: +39 461 615200.

E-mail address: luana.bontempo@iasma.it (L. Bontempo).

Abstract

Seven kinds of cow milk cheese (Asiago, N=16; Fontina, N=16; Montasio, N=16; Puzzone, N=14; Spressa, N=15; Toma, N=16; Vezzena, N=16) produced in alpine and pre-alpine Italian areas are described using the isotopic ratios of C, N, O, S and Sr and the contents of 49 mineral elements. A multivariate discriminant analysis based on Ba, Ca, K, Mg, Rb, $\delta^{13}C_{casein}$, $\delta^{15}N$ and $\delta^{18}O$ correctly classified 94 % of the 109 samples with a maximum of 100% in the case of Fontina and Puzzone. $\delta^{13}C_{casein}$ and $\delta^{13}C_{glycerol}$ allowed to estimate the maize uptake in the animal's diet and PDO protocols observance.

1. Introduction

The stable isotope ratios of light and heavy elements, in combination with the content of several mineral elements, have been used in the last few years as a powerful tool to characterise several food commodities: *e.g.* olive oils (Camin et al, 2010a; Camin et al., 2010b), orange juices (Rummel, Hoelzl, Horn, Rossmann & Schlicht, 2010) and tea (Pilgrim, Watling & Grice, 2010). For milk and dairy products, elements and stable isotopes ratios have frequently been used but very rarely in combination.

Coni and co-workers (Coni, Bocca & Caroli, 1999; Coni, Bocca, Ianni & Caroli, 1995) highlighted that curdling and salting, as well as releases from manufacturing equipment, are the main critical points in the uneven distribution of elements in the different sub-products (e.g. whey, curd). Fresno and co-workers (Fresno, Prieto, Urdiales & Sarmiento, 1995) reported significant differences in the content of P, K, Mg, Zn, Fe and Mn in goat's and cow's milk, whereas Benincasa et al. (Benincasa, Lewis, Sindona & Tagarelli, 2008) were able to discriminate milk supplied by cows and water buffaloes using Ca, P, Ga, Zn, Mn, B and S content. In a few studies elemental composition has also been used to distinguish the geographical origin of dairy products. Brescia and co-workers

(Brescia, Monfreda, Buccolieri & Carrino, 2005) identified Li and K as useful markers for distinguishing milk and mozzarella cheeses from Apulia and Campania, whereas Sacco et al. (2009) distinguished Southern Italian milk from Central European samples using the combination of some elements and basic compositional parameters.

Isotopic ratios have been used for the identification of the type of diet by Camin et al. (Camin, Perini, Colombari, Bontempo & Versini, 2008; Camin, Wietzerbin, Cortes, Haberhauer, Lees & Versini, 2004), Knobbe et al. (2006) and Kornexl et al. (Kornexl, Werner, Rossmann, & Schmidt, 1997), confirming that the $\delta^{13}C$ of bulk milk, as well as of casein and glycerol extracted from milk or cheese, is related to the amount of C3 and C4 plants in animal diet. On the contrary, the reliability of milk and casein $\delta^{15}N$ for reconstructing dietary composition is lower, being affected not only by dietary composition (the presence of N-fixing plants) but also by pedology, aridity, distance from the sea and fertilisation practices adopted in the area where the feed was grown. Crittenden et al. (2007) were able to distinguish cow's milk from different regions in Australasia using the $\delta^{18}O$ isotope ratios of milk water and the $\delta^{13}C$ of skim milk and casein, confirming the isotope fractionation patterns predicted on the basis of latitude and climate. Camin et al. (2004), considering cheeses from Spain, Italy and France, found that the $\delta^{15}N$ and $\delta^{34}S$ of casein are mostly influenced by the geographic and climatic conditions in the area (aridity, closeness to the sea, altitude). Fortunato and co-workers (2004) demonstrated that the $^{87}Sr/^{86}Sr$ ratio in cheeses from different sources (alpine and pre-alpine areas, Bretagne, Finland, Canada and Australia) agreed with local geological properties. Very recently, the stable isotope ratios of light elements have also been recognised as authentication markers for Grana Padano cheese (EC 2009/C 199/11).

To date, only Pillonel et al. (2003) have used isotopes and elements jointly in the dairy sector, specifically to authenticate Emmental-type cheeses produced in Switzerland, Germany, France, Austria and Finland. Their very encouraging results showed that $\delta^{13}C$, δD, $\delta^{15}N$ and $^{87}Sr/^{86}Sr$ stable isotope ratios allowed good discrimination between geographically distant regions, whereas elemental composition contributed more towards distinguishing relatively close regions. The same authors consider the combination of different analytical approaches a very effective tool for compositional characterisation and geographical location of milk and dairy products. This combination could be particularly helpful in the case of fresh milk and PDO cheeses, for which Italian and European law (DM 14 January 2005, EC 2081/92) requires indication of the origin of milk and raw materials used for manufacture on the label.

Our study investigates the possibility of using isotopic and elemental analysis to distinguish cheeses produced in alpine and pre-alpine Italian areas.

2. Material and methods

2.1. Sampling

We considered seven kinds of 'semicotto di media stagionatura' (curd cooked at 40-48 °C, semi-matured) cheeses, five with PDO status (Asiago, EC 1107/96 and 1200/07; Fontina, EC 1107/96; Montasio, EC 1107/96; Spressa, EC 2275/03; Toma, EC 1107/96) and two traditional products (Puzzone and Vezzena, with PDO request underway), produced in alpine/pre-alpine Italian regions (Friuli, Piemonte, Trentino, Valle d'Aosta and Veneto) exclusively using cow's milk. This type of cheese is manufactured by setting the curd at about 32-37 °C, breaking down and cooking it at about 40-48 °C and finally pressing the curd in moulds. This production procedure is traditional in alpine regions and allows an effective whey purge process at about 25 °C in 12-24 hours. After a few days, the cheeses are immersed in a brine solution or processed with dry salt for a period ranging from a few hours (14h) to 3 months. After the salting process, the cheeses are matured for a period ranging from 15 days to 18 months.

Authentic cheeses (N=109) were collected directly from the producers at technological maturity over a period of 3 years in March, June, September and December, in order to include the maximum variability of production and changes in animal diet (percentages of *e.g.* grass, maize silage, flour).

For each cheese, a radial segment of about 1 kg was sampled, with the crust removed, carefully homogenised with an electric mixer (Type 4249, Braun GmbH, Kronberg, Germany), vacuum-packed and stored at -20 °C up to the time of analysis.

The elemental composition and $\delta^{13}C$ and $\delta^{15}N$ in casein, $\delta^{13}C$ and $\delta^{18}O$ in glycerol were measured in all the samples, whereas $\delta^{34}S$ in casein (N=85) and $^{87}Sr/^{86}Sr$ in bulk (N=69) were quantified only in a reduced subset.

2.2. Elemental analysis

Around 0.65 g cheese was weighed directly into a quartz vessel (QXP-1500, CEM, Matthews, USA), with the addition of 4mL HNO_3 (Superpure 65%, Merck, Darmstadt, Germany), 2 mL H_2O_2 (Superpure 30%, Merck) and 1 mL Y internal standard solution (500 mg L^{-1}, Merck) for volume correction. The samples were mineralized in closed vessels using a microwave oven digestor (Mars5, CEM).

Analysis of Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Sr and Zn was carried out with an ICP–OES (Inductively Coupled Plasma – Optical Emission Spectrometer, Optima 3300 Dual View, Perkin Elmer, Norwalk, USA) using a solution of Tb 2 mg L^{-1} (BDH-Aristar, BDH Laboratory Supplies, Pool, UK) as on–line internal standard. Ag, Be, Cd, Ce, Co, Cs, Dy, Er, Eu, Ga, Gd, Ge, Ho, Ir, La, Li, Lu, Nb, Nd, Pb, Pr, Pt, Rb, Re, Ru, Sb, Sm, Ta, Te, Tl, Tm, U, V and Yb were analysed using an ICP–MS (Inductively Coupled Plasma – Mass Spectrometer, HP–4500 series, Hewlett–Packard Co., Corvallis, USA) with an on–line internal standard solution of Sc 1 mg L^{-1}, Rh 0.5 mg L^{-1} and Tb 0.25 mg L^{-1} all supplied by BDH-Aristar.

The accuracy of the whole process was assured using the certified material 'Whole milk powder'(SRM 8435, NIST-National Institute of Standards & Technology, Gaithersburg, MD, USA) in each analytical batch. Mean recoveries for the certified elements were: 80% for Pb, 100% for Ba, Ca, Cu, Mg, Na, Zn, K and Sr, 110% for B and Mn, 120% for Fe and Mo.

The precision (RSD%) of the process, evaluated by preparing and analysing a cheese sample 10 times, was under 10%, with the exclusion of Ir (11%), Mn (11%), Pt (11%), Ga (13%), Ni (14%), Ce (15%), Cr (16%), Ru (16%), Fe (18%), Al (21%).

2.3. Isotopic analysis

$\delta^{13}C$ and $\delta^{15}N$ were determined in the casein extracted from cheese (Manca et al., 2001) whereas $\delta^{13}C$ and $\delta^{18}O$ were measured in the glycerol extracted (Camin et al., 2004), using an IRMS (Isotopic Ratio Mass Spectrometer; Delta plus XP ThermoFinnigan, Bremen, Germany) after total combustion or pyrolysis of the sample (about 0.6 mg casein and 0.8 mg glycerol) in an Elemental Analyser (EA Flash 1112 ThermoFinnigan) or Pyrolyser (TC/EA, ThermoFinnigan) as described in Camin et al. (2004).

$\delta^{34}S$ was determined in casein using an IRMS (Isoprime, AP2003, GV Instruments Ltd., Manchester, UK) after combustion (about 3 mg casein) in an Elemental Analyser (Vario EL III Elementar Analysensysteme GmbH, Hanau, Germany) (Manca et al., 2006).

The isotope ratios were expressed relatively to international standards (Vienna - Pee Dee Belemnite for $\delta^{13}C$; Vienna - Air for $\delta^{15}N$; Vienna – Standard Mean Ocean Water for $\delta^{18}O$; Canyon Diablo Triolite for $\delta^{34}S$) in δ‰ according to the general formula:

$\delta(‰)=[(R_{sample}-R_{standard})/R_{standard}]* 1000$,

where R is the ratio between the heavier isotope and the lighter one.

The values were calculated against international reference materials (NBS-22 fuel oil for $\delta^{13}C$; IAEA-NO3 nitrate for $\delta^{15}N$; glycerol used in the European project SMT4-CT98-2236 for $\delta^{18}O$; NBS-123 ZnS for $\delta^{34}S$) provided by the International Atomic Energy Agency (Vienna, Austria). The precision of measurement, expressed as 2 times the standard deviation when measuring the same sample 10 times, was 0.2, 0.3, 0.5 and 0.3‰ respectively for $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$.

For strontium isotope analysis aliquots of about 100 mg of cheese were dried under a infrared lamp and then ashed in a muffle oven for 2 hours at 850 ºC. Remaining ashes were dissolved in 0.5 mL of 9N HNO_3. Purification and accumulation of Sr was achieved using PTFE columns filled with 0.025 g of Sr-specific crown-ether resin (Sr-Spec®, EIChroM Industries, Darien, IL, USA; recipe modified from Horwitz, Chiarizia & Dietz, 1992 and Pin & Bassin, 1992). About 50 mg of Sr was loaded in a mixture of $TaCl_5$, HF, HNO_3, H_3PO_4 and H_2O (“Birk's solution”) on single-band W filaments. Strontium isotope ratios were measured in static mode using a TIMS (Thermal Ionisation Mass Spectrometer, MAT 261.5, ThermoFinnigan). Measured isotope values were normalized for mass fractionation using the naturally invariant value for $^{88}Sr/^{86}Sr$ of 8.37521 and the exponential fractionation law. Accuracy and precision of the mass spectrometer runs were controlled by analysing reference material $SrCO_3$ NBS 987 and natural in house standards. During the period of analyses the mean $^{87}Sr/^{86}Sr$ of NBS 987 was 0.710231 ± 0.000027 (1 standard deviation, N=22). The accuracy of measured samples was estimated to be around ± 0.000040 or better. Total blanks for strontium were in the pico gram range and so had no significant influence on measured isotope values.

2.4. Statistical analysis

The data were statistically evaluated using Statistica v8 software (StatSoft Inc., Tusla, Oklahoma, USA) procedures (Kolmogorov-Smirnov test, Box-Cox transformation, ANOVA, Honestly Significantly Different Tukey’s test for unequal N, Kruskall Wallis test, multiple bilateral comparison, Pearson’s correlation, multivariate Canonical Discriminant Analysis).

3. Results and discussion

3.1. Elemental and isotopic composition

Table 1 shows the distribution statistics for elemental and isotopic composition. The element content was expressed as dry matter in order to correct the effects of different degrees of moisture and ripening. Of the 49 elements analysed, only the distribution of Al, B, Ba, Ca, Co, Cr, Cs, Cu, Fe, Ga, K, Mg, Mn, Mo, Na, Rb, Sr, V and Zn are given, these elements being detectable in at least 65% of the samples. Ag, Cd, Ce, Dy, Er, Eu, Gd, Ge, La, Li, Lu, Nb, Nd, Ni, Pb, Pr, Re, Ru, Sb, Sm, Ta, U and Yb were present in detectable amounts only in a lower % of samples, whereas none of the samples showed detectable amounts of Be, Ho, Ir, Pt, Te, Tl and Tm. All the data were normally distributed (Kolmogorov-Smirnov test) in each of the seven types of cheese, with the exception of B, Ce, Cu, Mo and V, which were normalised using Box-Cox transformation before any further statistical evaluation.

Table 2 shows the correlation coefficients, slopes and intercepts of the regression lines between parameters that were shown to be statistically significant ($P < 0.001$) in Person's correlation test.

The $\delta^{13}C$ of casein and glycerol were shown to be closely correlated, confirming the previous results of Camin et al. (2004) and Pillonel et al. (2003). Higher δ-values in casein as compared to glycerol could be explained by ^{13}C depletion occurring during the synthesis of lipids. The $\delta^{15}N$ and $\delta^{34}S$ values, affected by similar variability factors such as fertilisation practices, climatic conditions, closeness to the sea *etc*. were not significantly correlated, confirming previous results found in cow's milk from alpine regions (Rossmann, Kornexl, Versini, Pichlmayer & Lamprecht, 1998). This significant correlation ($r = 0.77$) had been observed on European scale for cow's and sheep cheeses from France, Italy and Spain by Camin et al. (2004).

Both $\delta^{13}C_{casein}$ and $\delta^{13}C_{glycerol}$ correlated significantly, positively with.$\delta^{15}N$ and negatively with $^{87}Sr/^{86}Sr$ ($P < 0.001$). No statistically significant correlations were found between isotopic and elemental parameters.

Some 'technological' elements correlated with each other, as expected (Coni et al., 1999; Fresno et al., 1995) and in particular Fe correlated positively with Al and Mn, as did Zn with Cr. Some of the elements of the I and II groups of the periodic table correlated with each other as reported by Cichoscki et al. (Cichoscki, Valduga, Valduga, Tornadijo & Fresno, 2002) and Fresno et al. (1995) for Spanish cheeses. In particular Cs and Rb correlated positively with Ba, K positively with Mg, Na negatively with Sr.

3.2. Product differentiation

Table 1 shows the statistically significant differences ($P < 0.001$) between cheeses in relation to values for 12 parameters. Such differences were confirmed by applying both parametric statistical approaches (ANOVA, HSD Tukey's test for unequal N) and non-parametric statistical approaches (Kruskall Wallis and multiple bilateral comparison tests, applied to raw data). In particular, statistically lower Ca content was found in Toma, Ca and Mg in Fontina, Ba, Ga and Rb in Montasio and K in Puzzone. According to Coni et al. (1999), Fresno et al. (1995) and Pillonel et al. (2003), such differences may depend on manufacturing processes, in particular coagulation conditions, salting, releases from manufacturing equipment and concentrates fed to the animals, as well as geographical origin.

Both $\delta^{13}C_{casein}$ and $\delta^{13}C_{glycerol}$ made it possible to discriminate Fontina from Montasio. As regards the former cheese, on the basis of the isotope values and using the relation given by Camin et al. (2008), we can estimate that the maize uptake in the animals' diet was at maximum/no more than 10%, fully complying with the 20% level established in the PDO production protocol. As regards Montasio, no limits have been established for maize in the PDO protocol and the higher values observed by us suggest a contribution of about 45-70%. For Asiago, Puzzone and Toma no limits have been established in the production protocol and the estimated contributions ranged between 20% and 40%, 16% and 20% and 40% and 60% respectively. Spressa and Vezzena production protocols provide for a maximum use of fodder, usually with a high but not legally established maize content, of 50% in the diet and the estimated contributions were 10-17% and 19-25% respectively.

Asiago and Montasio showed statistically higher $\delta^{18}O$ values than Fontina, Puzzone and Spressa, probably because the latter cheeses are produced at higher altitude and with a lower contribution of C4 plants, which have higher $\delta^{18}O$ and $\delta^{13}C$ values (Camin et al., 2008; Schmidt, Werner & Rossmann, 2001).

$\delta^{15}N$ made it possible to distinguish Toma and Vezzena, the latter with significantly lower values, confirming what has previously been observed in cheeses (Pillonel et al., 2003) and lamb meat samples (Camin et al., 2007) produced at high altitudes as compared to products from lower lying areas.

$^{87}Sr/^{86}Sr$ significantly distinguished Asiago and Montasio from Spressa, Fontina and Toma ($P < 0.001$) at lower values. Old acidic rock such as granite shows the highest $^{87}Sr/^{86}Sr$ ratios, whereas mafic and carbonate-rich rocks have the lowest (Figure 1) (Pillonel et al., 2003). Indeed, the lowest $^{87}Sr/^{86}Sr$ values were measured in Asiago and Montasio cheeses, the ratio of the former being clearly influenced by Tertiary basalt underlying pasture, while that of the latter is influenced by the river deposit sediment deriving from Mesozoic and Tertiary basalt in the Carnic Alps. In the area of Lavarone, where Vezzena is produced, Mesozoic carbonate-rich rocks with low $^{87}Sr/^{86}Sr$ values prevail, whereas values close to zero (Puzzone), are typical for cheeses from alluvial plain terraces with large catchment areas.

Standard multivariate Canonical Discriminant Analysis (CDA) was performed on the data in order to check for the possibility of discriminating between cheeses. $^{87}Sr/^{86}Sr$ and $\delta^{34}S$ were not considered because they were only measured in a subset of samples. Twelve parameters (Ba, Ca, Cu, Ga, K, Mg, Rb, Zn, $\delta^{13}C_{casein}$, $\delta^{15}N$, $\delta^{13}C_{glycerol}$, $\delta^{18}O$) were shown to be significantly different at HSD for unequal N Tukey and multiple bilateral comparison tests.

To make the model more robust against interference due to Cu and Zn releases from dairy equipment, we did not use these elements in the CDA. We also removed $\delta^{13}C_{glycerol}$ as it is significantly correlated (Pearson's correlation coefficient r = 0.966) with $\delta^{13}C_{casein}$ and we chose to retain the latter because casein is more easily extractable from the bulk cheese. Finally, Ga was removed by the CDA model as not statistically significant.

The first 3 canonical variables (Rad. 1, Rad. 2, Rad. 3) explained 91 % of total variability (Figures 2a and 2b). Rad.1 was loaded positively with $\delta^{13}C_{casein}$, Ba and $\delta^{18}O$ (standardised coefficients 1.58, 1.39 and 0.64 respectively) and negatively with Ca (-0.59). Rad. 2 was mainly loaded positively with $\delta^{13}C_{casein}$ (0.58) and Mg (0.57) and negatively with $\delta^{15}N$ (-1.18), K (-0.60) and Ba (-0.53) whereas Rad. 3 was loaded positively by Rb (1.09) and Mg (0.65) and negatively by Ba (-0.78).

Overall, 94 % of the samples were correctly classified (Table 2), with a re-classification percentage of 100 % in the case of Fontina and Puzzone.

Model predictivity and stability were proved by re-calculating 3 different models after removing 20 randomly selected cheeses (corresponding to 20% of the original database) each time and applying these models to the original data-set, obtaining correct classification for at least 90%.

In conclusion, the paper defined the compositional characteristics of authentic traditional alpine and pre-alpine Italian cheeses using multi-element stable isotope ratios and mineral elements. Such analytical parameters can effectively contribute towards distinguishing different kinds of cheese and supporting the demand for or the existence of a Protected Designation of Origin.

Acknowledgements

This work was funded entirely by the Provincia Autonoma di Trento - Italy (MIROP Project)

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Table 1

Elemental and isotopic composition of the cheeses.

	ASIAGO (N=16)		FONTINA (N=16)		MONTASIO (N=16)		PUZZONE (N=14)		SPRESSA (N=15)		TOMA (N=16)		VEZZENA (N=16)	
	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.	Mean	Std.Dev.
Al (mg kg^{-1})	2.35	1.01	2.63	0.79	2.88	1.49	2.26	1.03	2.38	1.30	2.79	1.50	1.90	0.88
B (mg kg^{-1})	0.37	0.21	0.41	0.24	0.53	0.26	1.09	1.21	0.46	0.33	0.56	0.26	0.56	0.31
Ba (mg kg^{-1})	1.16^{ab}	0.43	0.74^{ac}	0.28	0.53^{c}	0.13	1.22^{ab}	0.22	1.57^{b}	0.28	1.23^{ab}	0.67	0.97^{abc}	0.27
Ca (mg kg^{-1})	14.4^{ab}	1.56	12.9^{a}	2.01	13.3^{ab}	1.25	12.7^{ab}	1.63	15.9^{b}	1.27	12.3^{a}	1.18	14.6^{ab}	1.94
Co (mg kg^{-1})	7.43	2.99	6.45	2.15	6.66	3.32	9.57	5.46	8.09	3.59	7.39	4.18	8.31	3.50
Cr (mg kg^{-1})	0.26	0.10	0.25	0.08	0.25	0.10	0.29	0.18	0.38	0.22	0.24	0.09	0.27	0.09
Cs (mg kg^{-1})	4.68	2.43	6.67	2.49	3.15	1.82	6.69	4.08	8.98	4.61	7.24	6.08	6.95	2.88
Cu (mg kg^{-1})	5.20^{ab}	4.03	6.19^{ab}	5.33	1.38^{a}	3.40	3.35^{a}	5.79	13.8^{c}	2.90	0.45^{a}	0.36	8.90^{bc}	2.93
Fe (mg kg^{-1})	1.85	0.63	2.25	0.33	2.03	0.69	2.07	1.01	2.36	2.09	2.02	0.52	1.95	0.32
Ga (mg kg^{-1})	51.0^{ab}	28.5	38.6^{ab}	25.2	21.3^{a}	8.18	64.0^{ab}	28.1	80.9^{b}	36.8	60.9^{ab}	32.4	43.9^{ab}	26.3
K (mg kg^{-1})	1.69^{a}	0.17	1.73^{ab}	0.23	1.69^{ab}	0.22	1.49^{b}	0.41	1.95^{a}	0.22	1.78^{a}	0.21	1.80^{a}	0.21
Mg (mg kg^{-1})	0.54^{a}	0.05	0.43^{b}	0.07	0.49^{ab}	0.06	0.46^{ab}	0.11	0.56^{a}	0.06	0.45^{ab}	0.06	0.55^{a}	0.10
Mn (mg kg^{-1})	0.28	0.05	0.31	0.06	0.27	0.05	0.29	0.09	0.32	0.05	0.37	0.11	0.33	0.07
Mo (mg kg^{-1})	0.23	0.12	0.45	0.14	0.20	0.12	0.30	0.14	0.20	0.08	0.19	0.13	0.32	0.13
Na (mg kg^{-1})	12.5	3.05	11.6	3.13	11.2	3.66	9.66	2.82	13.2	2.59	13.3	2.98	11.6	2.18
Rb (mg kg^{-1})	2.57^{ab}	1.34	3.29^{ab}	1.18	1.41^{a}	0.44	2.07^{ab}	0.63	5.37^{b}	2.81	4.02^{ab}	3.32	4.74^{b}	2.04
Sr (mg kg^{-1})	4.76	1.53	4.73	1.55	4.45	1.94	6.31	2.67	5.72	1.33	4.52	0.87	3.55	0.86
V (mg kg^{-1})	11.9	8.13	12.2	7.08	12.3	7.90	11.2	6.83	12.5	7.03	18.3	8.38	8.18	4.77
Zn (mg kg^{-1})	65.0^{ab}	5.11	57.4^{a}	5.96	59.9^{a}	5.00	65.7^{ab}	8.42	74.0^{b}	5.96	56.4^{a}	5.65	74.5^{b}	7.68
$\delta^{34}S_{casein}$	5.2	0.5	4.5	0.6	5.2	0.5	4.6	0.4	5.0	0.3	5.4	0.9	4.9	0.4
$^{87}Sr/^{86}Sr$	-1.748^{a}	0.942	0.572^{b}	0.896	-2.209^{a}	1.933	-0.332^{ab}	0.257	0.718^{b}	0.143	0.458^{b}	0.925	-0.695^{ab}	0.177
$\delta^{18}O_{glycerol}$	18.0^{a}	0.9	14.9^{b}	2.6	17.9^{a}	0.8	15.5^{ab}	1.1	15.8^{ab}	0.8	17.0^{ab}	1.1	16.2^{ab}	1.4
$\delta^{13}C_{casein}$	-20.0^{ab}	1.4	-23.3^{c}	0.6	-18.1^{a}	0.6	-21.7^{bc}	0.5	-22.2^{c}	0.5	-20.1^{ab}	2.4	-22.0^{c}	0.8
$\delta^{15}N_{casein}$	4.9^{a}	0.4	4.6^{abc}	0.3	4.8^{abc}	0.4	4.3^{bc}	0.2	4.6^{ac}	0.2	5.2^{a}	0.4	4.2^{b}	0.4
$\delta^{13}C_{glycerol}$	-25.1^{a}	1.6	-29.5^{d}	0.7	-22.6^{b}	0.9	-26.8^{ac}	0.8	-27.7^{cd}	0.8	-25.1^{ab}	3.2	-27.7^{cd}	1.2

Table 2

Pearson' correlation coefficient (r), intercept (I) and slope (S) of the correlations between the analytical parameters. Only the statistically significant relationships ($P < 0.001$) are shown.

		Al	B	Ba	Ca	Co	Cr	Cs	Fe	Ga	K	Mg	Mn	Na	$\delta^{13}C_{casein}$	$\delta^{13}C_{glycerol}$	$^{87}Sr/^{86}Sr$
Cr	r				0.44												
	I				-0.06												
	S				0.02												
Fe	r	0.42															
	I	1.66															
	S	0.24															
Mg	r		-0.42		0.75						0.43						
	I		0.55		-0.04						0.22						
	S		-0.06		0.04						0.17						
Cs	r			0.56													
	I			-0.87													
	S			6.26													
Ga	r			0.77		0.50		0.64									
	I			-5.52		15.5		32.2									
	S			50.4		6.28		3.74									
Mn	r			0.38				0.40	0.36								
	I			0.28				0.30	0.25								
	S			0.04				0.004	0.04								
Rb	r			0.65				0.75		0.64			0.40	0.37			
	I			0.80				1.99		1.66			-0.89	-0.20			
	S			2.18				0.22		0.03			13.6	0.31			
Zn	r				0.68		0.38					0.64					
	I				26.4		57.2					39.6					
	S				2.79		28.2					49.6					
Sr	r													-0.36			
	I													7.74			
	S													-0.23			
$\delta^{15}N$	r														0.54	0.53	
	I														7.38	7.18	
	S														0.13	0.10	
$\delta^{13}C_{glycerol}$	r														0.98		-0.45
	I														0.94		-26.7
	S														1.30		-0.82
$\delta^{13}C_{casein}$	r																-0.45
	I																-21.4
	S																-0.63

Table 3

Results of the classification matrix of the cheeses based on Ba, Ca, K, Mg, Rb, $\delta^{13}C_{casein}$, $\delta^{15}N$ and $\delta^{18}O$, achieved applying Reclassification Discriminant Analysis

	% correctly classified	ASIAGO	FONTINA	MONTASIO	PUZZONE	SPRESSA	TOMA	VEZZENA
ASIAGO	88	14	0	0	0	1	1	0
FONTINA	100	0	16	0	0	0	0	0
MONTASIO	94	1	0	15	0	0	0	0
PUZZONE	100	0	0	0	14	0	0	0
SPRESSA	93	0	0	0	0	14	0	1
TOMA	81	1	0	1	0	1	13	0
VEZZENA	100	0	0	0	0	0	0	16
Total	94	16	16	16	14	16	14	17

Fig. 1. Synoptic diagram showing the $^{87}Sr/^{86}Sr$ variances (mean values and standard deviations) of Asiago, Fontina, Montasio, Puzzone, Spressa, Toma and Vezzena cheeses.

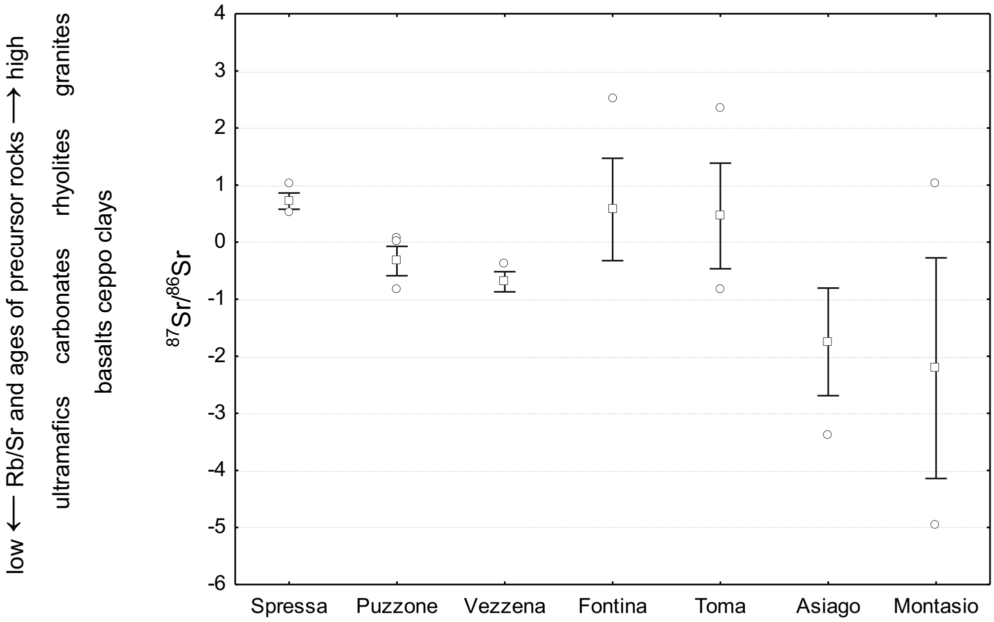

Fig. 2a. Canonical Discriminant Analysis of the isotopic and elemental composition of the cheeses. Scatterplot of the first two canonical variables.

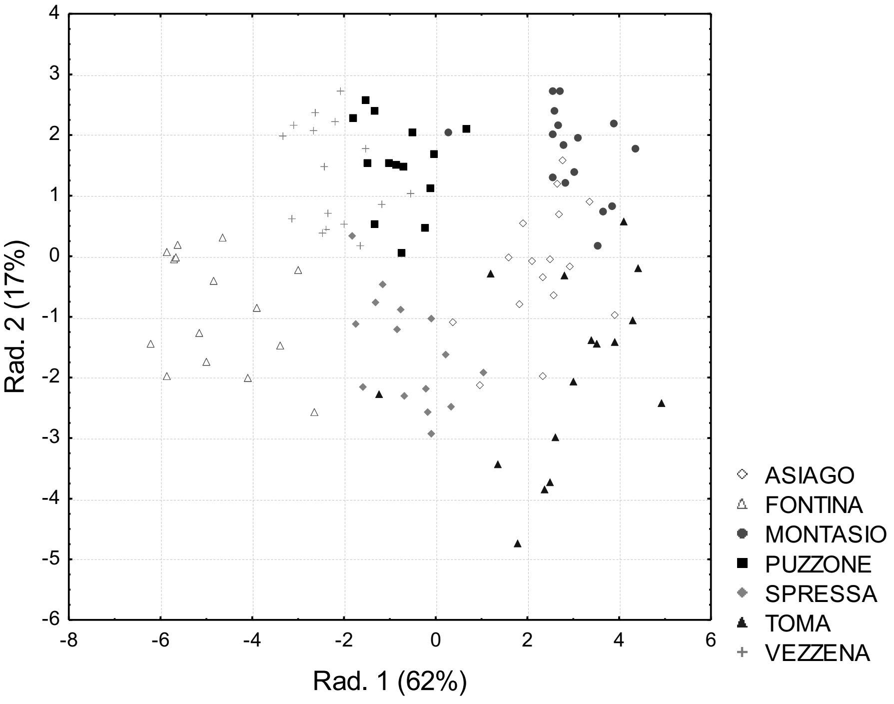

Fig. 2b. Canonical Discriminant Analysis of the isotopic and elemental composition of the cheeses. Scatterplot of the first and the third canonical variables.