Stable isotope ratios, major, trace and radioactive elements in emmental cheeses of different origins

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Abstract

Twenty Emmental cheeses from six European regions (Allgäu (D), Bretagne (F), Finland, Savoie (F), Switzerland and Vorarlberg (A)) were analysed for stable isotope ratios such as $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, D/H and $^{87}Sr/^{86}Sr$, as well as major (Ca, Mg, Na, K), trace (Cu, Mn, Mo, I) and radioactive elements (^{90}Sr, ^{234}U, ^{238}U). The discriminating potential of these parameters was evaluated using difference tests of mean values and principal component analysis (PCA). "Finland", "Bretagne" and "Savoie" cheeses were well separated using $\delta^{13}C$-, $\delta^{15}N$-, δ^2H- and $\delta^{87}Sr$-values. Concentrations of molybdenum and sodium allowed the groups "Switzerland", "Vorarlberg" and "Allgäu" to be separated. ^{90}Sr activity was highly correlated with the altitude of the production zone. Using this parameter, "Finland" and "Bretagne" were separated from "Vorarlberg" cheese. The results of the current screening test will help selecting the best tracers of origin for the remainder of the project.
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1. Introduction

Analysis of stable isotopes has often been used to determine the authenticity of different food products, e.g. fruit juices, wines, spirits, essential oils, milk and milk products, honey and olive oil (Hener et al., 1998; Rossmann, 2001). In milk products, this technique was basically used for the origin assignment of milk (Kornexl, Werner, Rossmann, & Schmidt, 1997; Rossmann, Kornexl, Versini, Pichlmayer, & Lamprecht, 1998), butter (Rossmann et al., 2000) and cheese (Manca et al., 2001). The stable isotope ratios of various elements provide complementary information, which can be combined with multivariate statistics.

The $^{13}C/^{12}C$ ratio for both milk fat and cheese protein gives information on the type of forage fed to the cows. The carbon isotope value of plants depends on their photosynthetic cycles for CO_2 fixation (Smith & Epstein, 1971). C_4 plants such as maize show higher $\delta^{13}C$ values than C_3 plants, which constitute the major part of a cow's fodder.

Differences in the $^{15}N/^{14}N$ ratio also result essentially from forage. Organic fertilisers and intensive farming methods increase the level of ^{15}N in the soil and consequently in the plants, in milk, and in cheese (Mariotti et al., 1981). In addition, climate and soil conditions can influence $^{15}N/^{14}N$ in soil (Farrell, Sandercock, Pennock, & Van Kessel, 1996) and therefore the type of plants growing on it. Nitrogen-fixing plants (leguminosae) show lower $\delta^{15}N$ than non-nitrogen-fixing plants (Delwiche & Steyn, 1970).

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The $^{18}O/^{16}O$ ratio of milk depends on the water ingested and the proportion of fresh vs. dry fodder. Isotope ratios of precipitation and groundwater depend largely on temperature, latitude, altitude and distance from the sea (e.g. Moser & Rauert, 1980). In grass proper, enrichment in ^{18}O occurs due to fractional evaporation of water. Therefore, during summer, when cows feed almost exclusively on fresh grass, a higher $^{18}O/^{16}O$ ratio is observed (Kornexl et al., 1997; Rossmann et al., 1998). Similarly, D/H gives the same climate and weather information as does $\delta^{18}O$.

The $^{34}S/^{32}S$ ratio is more difficult to interpret due to the numerous factors influencing it: nature of soil, industrial emissions and sulphur-containing fertilisers (Rossmann, 2001). It was not investigated in the current study.

The $^{87}Sr/^{86}Sr$ ratio can also be useful for origin assignments as it is dependent only on the types of rocks and soils, and not on human activity, climate or season of production (Rossmann et al., 2000). ^{87}Sr is produced from ^{87}Rb by radioactive β-decay, whereas the abundance of primordial ^{86}Sr remains virtually constant in a given rock. Old acidic rocks such as granite show the highest ratios, mafic and carbonate-rich rocks the lowest.

Several major and trace elements have also been used successfully for determination of the authenticity of wine (e.g. Baxter, Crews, Dennis, Goodall, & Anderson, 1997). In cheese, trace elements mainly have been measured for descriptive purposes (Favretto, 1990; Gambelli, Belloni, Ingrao, Pizzoferrato, & Santaroni 1999; Karadjova, Girousi, Iliadou, & Stratis 2000). Coni, Bocca, Ianni, and Caroli (1994) and Coni, Caroli, Ianni, and Bocca (1995) investigated trace elements in milk and dairy products to find correlations between animal fodder, time of year, environmental conditions, and level of elements in raw milk. Concentrations of Ba, Cd, Co, Cr, Pb and Sr were highest in winter samples; those of Al and Mn were lowest. No particular trend was observed for Cu, Mg, Ni and Zn. In a further study, significant differences in the selenium content of nonfat dry milk in various countries were found (Varo et al., 1984). Favretto, Marletta, Bogoni, and Favretto (1989) observed correlations between some trace elements and the geographic origin of Italian milk.

Measurement of radioactive constituents may also give important information on the geographic origin of a food product. For instance, environmental ^{90}Sr in Central Europe stems essentially from atmospheric nuclear bomb tests performed in the second half of the 20th century and to a lesser extent from the Chernobyl accident.[1] Sr follows calcium in the food chain and could therefore be an interesting element in cheese (Geering, Friedli, & Lerch, 1990). Various deposition intensities have been observed depending on the geomorphology of the country and weather conditions (Froidevaux, Geering, Schmittler, Barraud, & Valley, 2001, Chap. 7.1; Gastberger, Steinhäusler, Gerzabeck, & Hubmer, 2001). Further uranium series disequilibria such as $^{230}Th/^{234}U$ or $^{234}U/^{238}U$ are highly dependant on the geology of soil and rock alteration processes (e.g. Asmerom & Edwards, 1995; Chabaux, O'nions, Cohen & Hein, 1997, Suksi et al., 2001, and references therein) and could probably also be used as indicators of the geographic origin of cheese.

Authentication procedures based on inorganic parameters as described above are fundamentally different and complementary to ''organic'' parameters (e.g. microbiology, volatile compounds, organic acids). With the exception of some trace elements, such as Cu, the former are not influenced by cheese manufacture (milk transformation process) and hence are primary geographic indicators. The latter parameters are highly dependant on the culture used, the manufacturing process, etc. and can be considered as secondary geographic *indicator*s because the technology used in the cheese making process is bound to regional or national traditions.

The current work belongs to the first part of a broad screening test in a 3-years study into the authenticity of Emmental cheese and its geographic traceability (Bosset, 2001; Pillonel et al., 2002a). During the first year, a great number of analytical methods were tested with respect to their discriminating potential using only two to six cheese samples per region (Pillonel et al., 2002a–c; Pillonel et al., 2003a, b). It is therefore obvious that the analytical results obtained from such a modest number of cheese samples per region can only give trends, which should be confirmed later if they appear valuable for discriminating cheeses produced in Switzerland from those produced in other countries. The aim of the present paper is to find out whether stable isotope ratios, trace elements and radioactive elements allow a discrimination between the various geographic origins of Emmental cheese samples to be made.

2. Material and methods

Details of reagents and analytical instruments are not given where a reference to these methods is available. The values of the isotope ratios are expressed in δ (‰) and correspond to international standards (V-SMOW for $\delta^{18}O$ and δ^2H, V-PDB for $\delta^{13}C$, AIR for $\delta^{15}N$, and a laboratory water standard with $^{87}Sr/^{86}Sr = 0.7093$ for calculating $\delta^{87}Sr$-values) according to the relation:

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

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[1] The much lower temperatures encountered during the emission of radioactive material in Chernobyl, in contrast to a nuclear explosion, produced strontium oxides with relatively high solubility. They therefore moved faster from the soil surface into deeper zones.

where R represents the ratio of the higher mass to the lower mass isotopes.

The absolute reproducibilities of the isotope measurements were: $\pm 0.4‰$ for $\delta^{18}O$ in cheese water and $\pm 0.1‰$ in glycerol, $\pm 0.1‰$ for $\delta^{13}C$ in glycerol and pH 4.3-insoluble fraction, $\pm 0.2‰$ for $\delta^{15}N$, $\pm 2‰$ for $\delta^{2}H$ and $\pm 0.04‰$ for $\delta^{87}Sr$, all in pH 4.3-insoluble fraction.

2.1. Origins and selection of the cheese samples

The main framework of this study and sampling have been described in detail by Pillonel et al. (2002a). Table 1 summarises origin, date of manufacture and ripening time of the samples. Emmental cheeses were collected at different ripening times according to the availability in the market. Each region supplies cheese with typical characteristics depending among other factors on the ripening time which can vary from several weeks to several months. Three samples came from each region of Bretagne, Savoie, Vorarlberg and Allgäu, two samples came from Finland and six from Switzerland.

2.2. Preparation and measurement of $^{18}O/^{16}O$ ratios in cheese water

Fifty grams of grated cheese were lyophilised using a freeze-dryer lyolab G-GII (LSL Secfroid, Aclens, Switzerland). The quantitatively extracted water was cold-trapped for analysis. The measurements were done using a Finnigan Delta-Plus XL mass spectrometer equipped with a gas bench II system. The $\delta^{18}O$ analyses were performed according to the water equilibration method (Klimmek, Preiss-Weigert, & Wittkowski, 2000).

2.3. Preparation and measurement of $^{18}O/^{16}O$ and $^{13}C/^{12}C$ in glycerol

Fat was extracted from the above-mentioned lyophilised cheese with two times 100 mL of diethyl ether. After evaporation of the solvent, glycerol was obtained from the fat extract by hydrolysis according to Weber, Kexel, and Schmidt (1997) and Fronza et al. (1998).

Measurements were carried out on a Micromass MM 903 IRMS with an on-line coupled PDZ Europa Roboprep CN elemental analyser modified to perform pyrolysis of organic samples for oxygen isotope analysis on CO at $1100°C$ using glassy carbon as catalyst (Werner, Kornexl, Rossmann, & Schmidt, 1996).

2.4. Preparation and measurement of $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{2}H/^{1}H$ and $^{87}Sr/^{86}Sr$ ratio in pH 4.3-insoluble fraction

The pH 4.3-insoluble fraction was obtained by acidifying the above-mentioned defatted lyophilised cheese to pH 4.3, washing the solid phase with water and drying it in vacuo (Manca et al., 2001). Measurements were carried out using a Micromass MM 903 IRMS with an on-line coupled PDZ Europa CN elemental analyser with standard operating combustion furnace at $1020°C$. Quantities of ~ 0.8 and ~ 1.6 mg of pH 4.3-insoluble fraction for the measurement of $\delta^{15}N$ and $\delta^{13}C$, respectively, were weighed in 4×6 mm tin capsules and introduced into the elemental analyser, as described by Kornexl et al. (1997).

The $^{13}C/^{12}C$ ratio was investigated in two further fractions: (i) fat was extracted from the fresh cheese samples by centrifugation and (ii) carbon dioxide was extracted from cheese body and precipitated as barium carbonate according to Pauchard, Flückiger, Bosset, and Blanc (1980). However, the standard deviations within a region were much higher in these extracts than in pH 4.3-insoluble fraction or glycerol. The corresponding results were, therefore, not presented.

The $^{15}N/^{14}N$ ratio was also measured in a further fraction: total nitrogen from a Kjeldahl digestion fixed as ammonium chloride. However, the results (not shown) were not interesting because of their strong scattering within a region.

The $^{2}H/^{1}H$ ratio for pH 4.3-insoluble fraction was determined using a high temperature pyrolysis system working at $1450°C$ on the basis of ceramic tubes with glassy carbon reactor filling coupled with a Thermo Finnigan Delta-Plus XL IRMS system, designed for continuous flow isotope ratio mass spectrometry (CF-IRMS) of hydrogen isotopes in helium carrier gas. Three replicates of a sample, $250–300$ μg each, were weighed into silver capsules. Measurements were made using calibration of the system with reference hydrogen gas and working standard material (sucrose) of known isotopic composition in regular repetitions (Kelly, Parker, Sharman, & Dennis, 1998).

For the strontium measurements, 0.2 g from the pH 4.3-insoluble fraction were ashed in a resistance furnace at $650°C$. After dissolving the ash in nitric acid (3 mol/L), strontium was separated by SrSpec® (Eichrom Industries, USA). Isotope ratios were measured in a thermion mass spectrometer (Finnigan MAT261). All reagents

Table 1
Origin and ripening time of the 20 cheese samples investigated

Sample	Region (country)	Date of manufacture	Ripening time (months)
AL 1-3	Allgäu (D)	20.12.2000	4
BR 1-3	Bretagne (F)	16.02.2001	2.5
CH 1-6	Switzerland (CH)	26.12.2000	4
FI 1-2	Middle Finland (FI)	05.02.2001	3
SA 1-3	Savoie (F)	08.02.2001	3
VO 1-3	Vorarlberg (A)	02.02.2001	3

were prepared by sub-boiling. Analytical blanks were negligible.

2.5. Major and trace elements measured by atomic absorption spectroscopy (AAS)

The cheese samples were grated with a plastic rasp. For the determination of calcium, magnesium and sodium, approximately 1 g of material was digested in 5 mL suprapur nitric acid (650 g/kg) at normal pressure. For copper, potassium and zinc, 2.5 g of material were used. The solutions were analysed with an air–acetylene flame spectrometer (Badertscher & Kuhn, 1998a; Badertscher & Kuhn, 1998b, FAM accredited method). For the determination of manganese, 0.5 g of material were digested in 5 mL suprapur nitric acid (650 g/kg) under pressure in Teflon bombs. The solutions were analysed in a graphite furnace spectrometer (Badertscher & Liniger, 1999, FAM accredited method). CV were below the following values: Ca, 2.3%; Na, 4.7%; Cu, 4.8%; Zn, 1.0%; Mn, 3.6%; K, 6.4%; Mg, 2.4%.

2.6. Preparation and measurement of trace elements using inductively coupled plasma mass spectrometry (ICP-MS)

Grated cheese (plastic rasp, 0.4 g) was placed with 8 mL sub-boiled nitric acid (650 g/kg) and 4 mL ultra-pure water in a quartz vessel. The vessels, closed with their lids, were placed in a Teflon bomb containing 4 mL of the same sub-boiled nitric acid, 2 mL ultrapure water and 0.5 mL oxygenated water (300 g/kg). Samples were digested under pressure in a microwave oven MLS ETHOS Plus up to 170°C for 20 min. The blue-green solutions obtained were diluted to 20 mL with ultrapure water and the solutions were transferred into PFA (perfluoro alkoxyl alkane) tubes for analysis. The microwave oven could process ten pressure bombs at once. For each batch, six samples, two references (milk powders, BCR-063R and BCR-150, IRMM, Geel, Belgium) and two blanks were prepared.

The solutions were analysed using a Perkin Elmer ELAN 6000 ICP-MS, calibrated with a ICP Multielement Standard Solution VI (Merck No 1.10580.0100). A solution of 0.05 mg/L Rh was added on-line (1:1) to the calibration and sample solutions as an internal standard with a peristaltic pump. Major operating conditions were as follow: RF power, 1100 W; plasma gas flow, 15 L/min; auxillary gas flow, 1 L/min; nebulizer gas flow, 1 L/min; solution pump rate, 0.5 mL/min; sample introduction system, micro-uptake concentric nebulizer, mini-cyclonic spray chamber; rinse time, 120 s at 12 rpm; sample uptake time, 15 s at 24 rpm; equilibration time, 120 s at 12 rpm; scanning mode, peak hopping; dwell time, 25 ms.

Analyses were carried out with the analytical program TotalQuant® (Perkin Elmer) which provides semi-quantitative results with a typical measurement uncertainty ($P = 0.95$) of about $\pm 10\%$. The precision was assured by three replicates, allowing a comparison between the different regions of origin. Therefore, only elements showing highly significant inter-region differences (ANOVA, $P \leqslant 0.001$) are reported.

2.7. Preparation and measurement of radioactive elements

γ-spectra (^{137}Cs) were recorded on a Canberra HPGe GCW4523 well detector in a 250 mL cylindrical geometry on fresh samples or in a 4 mL well geometry on ashed samples. After calcinations at 550°C for 24 h, half of the ash was used for the analysis of ^{90}Sr and half for ^{234}U/^{238}U.

^{90}Sr activity was determined by measuring its daughter product, ^{90}Y (Geering et al., 1990). Briefly, a pure strontium source was obtained after dissolution of ash in concentrated hydrochloric acid, followed by a separation on a Dowex AG 50w x8 cation exchange column. After a 15 days growing period for ^{90}Y to reach secular equilibrium, yttrium was separated on a cation exchanger, precipitated as yttrium oxalate and counted in a gas proportional counter (Tennelec LB 4100w, 0.2 cpm background). Natural strontium and yttrium were used as carrier and measured by atomic absorption at the end of the chemical separation to determine the overall yield of the separation procedure. The precision was better than 0.008 Bq. Calcium and strontium have similar chemical properties. The results were normalised by the calcium concentrations to minimise the influence of the fabrication (e.g. curdle washing).

^{238}U and ^{234}U activity was determined by alpha spectrometry (Canberra Alpha Analyst) after microwave dissolution of the samples of ash (Milestone Ethos Plus) in 8 mol/L nitric acid, extraction on an Eichrom UTEVA cartridge (2 mL) and electrodeposition on a stainless steel disk in sulphate media at pH 1.9 (1.2 A, 6 V). ^{232}U (50 mBq) was used as an internal standard to determine the overall yield of the separation process (for more details on uranium determination by α-spectrometry see Haldimann et al., 2001). Coefficients of variation were smaller than 15% except for "Finland", with values up to 30%. The results were normalised by the calcium concentrations too. They are quite representative for the ash content and easier to handle.

2.8. Statistical analyses

The averages and standard deviations for cheeses from the same region were calculated for each parameter. Descriptive statistics, analysis of variance (ANOVA), pairwise comparisons of mean values with

Fisher's LSD test and principal component analyses (PCA) were performed with Systat for Windows version 9.0 (SPSS Inc., Chicago, IL). Natural grouping was visualised by PCA. The goal of the current study was not to build a model for the prediction of a group assignment, but to check the capacity of a series of parameters to discriminate Emmental cheeses according to their region of origin. Therefore, trained classification techniques such as linear discriminant analysis were not used because they require a larger data set for reliability.

3. Results and discussion

3.1. Stable isotope ratios

The results of 8 different isotope ratios are listed in Table 2. $\delta^{13}C$ values for pH 4.3-insoluble fraction and glycerol were strongly correlated ($r = 0.98$), indicating that they may have the same origin. As already observed for butter, δ-values were systematically higher in pH 4.3-insoluble fraction than in fats (Rossmann et al., 2000). The depletion of ^{13}C during the synthesis of lipids is a well-known phenomenon (Melzer & Schmidt, 1987).

Bretagne was the only region where maize is fed during winter months. Accordingly, samples from this region had by far the highest $\delta^{13}C$-values. Significant differences between the other regions were also found. Especially, δ-values in "Finland" were significantly lower than the others. An explanation for this shift may be the colder climate and the higher latitude of this country. Results on this point are, however, contradictory in the literature. Troughton and Card (1975) found no pronounced trends in the $\delta^{13}C$ values of C3 plants as a function of temperature (14–40°C). Measured $\delta^{13}C$ values in tree rings gave a temperature coefficient of +2.4‰/°C (Libby & Pandolfi, 1974) while Smith, Herath, and Chase (1973) observed a coefficient

of −1.0‰/°C in C3 plants at low temperature (14°C) but no effect over the range 18–30°C.

Nitrogen ratios were investigated in the pH 4.3-insoluble fraction. "Bretagne" and "Finland" showed the highest $\delta^{15}N$ values, which reflect the conditions of more extensive use of organic fertilisers in these parts of Europe. The significantly lower values obtained in "Savoie" may be explained by extensive agricultural practices typical for such regions of the Pre-Alps or by a higher proportion of leguminosae in the fodder.

Oxygen ratios were investigated in two different fractions. In glycerol no significant differences were observed between the regions of origin. In cheese water, the ratios were significantly higher in "Bretagne". The proximity to the sea is responsible for the higher $\delta^{18}O$ in the rain and hence in ground water. The samples originating from the Alpine regions (Allgäu, Savoie, Switzerland and Vorarlberg) were depleted in ^{18}O due to their higher altitude and greater distance from the sea. The values in "Finland" were even lower, confirming results already obtained for butter (Rossmann et al., 2000). The analysis of cheese water is not very robust for authentication goals as it would be very easy to add water with a specific $\delta^{18}O$ value during cheese-making. An alternative is offered by D/H ratios, which generally follow the same trend as $^{18}O/^{16}O$, and can be measured in pH 4.3-insoluble fraction.

The D/H ratio of pH 4.3-insoluble fraction was determined by CF-pyr-IRMS. A good correlation was found with the results from oxygen in water ($r = 0.85$). Because the pH 4.3-insoluble fraction is also required for ^{13}C and ^{15}N measurements, ^{2}H measurements would be preferred to ^{18}O measurements.

The stable isotope ratios mentioned above all undergo seasonal variations which will have to be taken into account within the next step of the project. The isotope ratio of the element strontium ($^{87}Sr/^{86}Sr$) provides means by which regions may be subdivided further

Table 2
Stable isotope ratios in the 20 Emmental cheeses investigated

Region ($n =$)	ANOVA	AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	x	s_x	x	s_x	x	s_x	x	s_x	x	s_x
Stable isotope ratios (δ‰[a])													
$\delta^{13}C$ in pH 4.3-insoluble fraction	***	−25.2[C]	0.29	−17.8[A]	0.77	−24.8[C]	0.52	−26.38[D]	0.05	−23.9[B]	0.35	−24.9[BC]	0.14
$\delta^{13}C$ in glycerol	***	−31.9[CD]	0.14	−21.6[A]	0.28	−31.6[C]	0.42	−32.4[D]	0.13	−29.7[B]	0.39	−31.31[C]	0.04
$\delta^{15}N$ in pH 4.3-insoluble fraction	***	5.3[AB]	0.37	6.4[A]	0.72	5.8[AB]	0.70	6.4[A]	0.33	3.8[C]	0.16	5.1[B]	0.20
$\delta^{18}O$ in cheese water	**	−7.8[B]	1.0	−4.9[A]	1.7	−8.8[BC]	1.0	−11.2[C]	0.92	−8.1[BC]	1.0	−7.7[B]	1.2
$\delta^{18}O$ in glycerol	ns	16	1.2	19.9	0.69	17	1.1	16.0	0.86	17	1.7	18	2.1
$\delta^{2}H$ in pH 4.3-insoluble fraction	***	−121[BC]	1.1	−102[A]	4.1	−122[C]	1.9	−132[D]	6.3	−115[B]	1.8	−122.8[C]	0.57
$\delta^{87}Sr$ in pH 4.3-insoluble fraction	***	−1.6[B]	0.42	−1[B]	1.6	−1.2[B]	0.39	4.9[A]	0.70	−1.1[B]	0.26	−1.6[B]	0.24

x = mean value, s_x = standard deviation, ANOVA, ns = not significant, $*p \leqslant 0.05$, $** \ p \leqslant 0.01$, $***p \leqslant 0.001$.
Production sites: A > B > C > D (= significantly different contents) or AB = A and B overlap by using a univariate discriminant analysis.
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg.
[a] δ‰ = 1000[R_{sample}− $R_{standard}$]/$R_{standard}$, where R represents the ratio of the higher mass to the lower mass isotopes.

according to their geological conditions, independent of the season. The mean δ^{87}Sr values laid between –1.64‰ and –1.00‰ for all regions except for "Finland" with a mean value of 4.85‰. Finland is the only region investigated which is covered homogeneously with an old acidic rock bed. This led to this significantly higher ratio.

Combining the results of the four values, δ^{13}C, δ^{15}N, δ^2H and δ^{87}Sr (all for pH 4.3-insoluble fraction), in a principal component analysis (PCA), "Finland", "Bretagne" and "Savoie" were distinctly separated (Fig. 1). On the other hand, the regions "Switzerland", "Allgäu" and "Vorarlberg" were too similar (climate, geology, agriculture) to be differentiated.

3.2. Major and trace elements

Results obtained for the 7 elements analysed by AAS are listed in Table 3. All elements showed significant differences according to the geographic origin of the cheeses. The calcium content of cheeses from the two
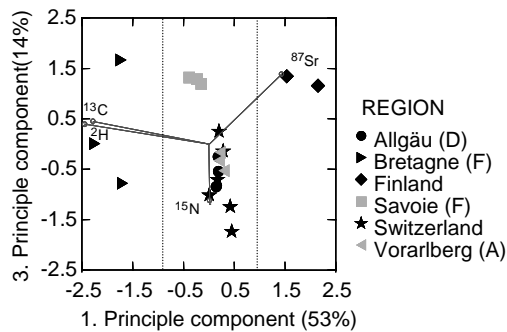
French regions (SA and BR) were the highest, those from Finland the lowest. This may be explained by the fact that no curd washing is applied in France whereas the washing rate is the highest in Finland with up to 26% water addition.

The regions Bretagne and Finland have in common that their Emmental cheese is manufactured in stainless-steel vats instead of the traditional copper vats encountered in most European regions. The copper content was therefore the lowest in "Bretagne". In "Finland", the values found were biased by the addition of copper sulphate into the milk during manufacture. It was not possible to explain the relatively low copper concentration in "Vorarlberg".

Differences obtained for magnesium and potassium were less significant than for the other elements and more difficult to interpret. Manganese and zinc were found at highest concentrations in "Bretagne" followed by "Finland". These higher concentrations could be explained by a different diet fed to the cows (type of concentrates). Sodium content correlated well with chloride concentration ($r = 0.95$, Pillonel et al., 2002a), confirming its origin as sodium chloride from the salt bath.

From the ICP-MS analyses carried out, four elements were retained for their high significance (Table 3). Two of them, copper and manganese, were already analysed by AAS. The results from both techniques were in very good agreement for copper ($r = 0.99$) and satisfactory for manganese ($r = 0.82$).

Iodine was a further interesting element. Significantly lower values were found in "Allgäu" and in "Vorarlberg", and significantly higher values in "Finland". The iodine content of milk products varies according to the season of the year and to the geographic origin of the milk (Larsen, Knuthsen, & Hansen, 1999). The

Fig. 1. PCA of the parameters δ^{13}C, δ^2H, δ^{15}N, δ^{87}Sr. Separation of the groups "Finland", "Savoie" and "Bretagne".

Table 3
Major and trace elements in µg/g cheese in the 20 Emmental investigated

Region ($n=$)	ANOVA	AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	x	s_x	x	s_x	x	s_x	x	s_x	x	s_x
Elements by AAS													
Calcium	***	9496[B]	153	10498[A]	209	9472[B]	193	8929[C]	136	10020[A]	357	9345[BC]	134
Copper	***	9.0[AB]	2.0	< 1[C]	—	8.5[B]	1.6	13.1[A]	2.7	8.0[B]	1.3	4.0[C]	1.4
Magnesium	**	385[A]	11	390[A]	4	348[B]	18	358[AB]	21	365[AB]	13	336[B]	11
Manganese	***	0.28[B]	0.02	0.40[A]	0.03	0.26[B]	0.03	0.30[AB]	0.08	0.27[B]	0.07	0.21[B]	0.02
Potassium	*	931[AB]	120	953[AB]	63	837[B]	77	800[B]	12	977[A]	38	868[AB]	3
Sodium	***	2475[A]	329	1700[AB]	630	1316[B]	217	2561[A]	39	1482[B]	375	1316[B]	355
Zinc	***	43.5[C]	1.3	47.8[A]	2.5	42.2[C]	0.8	47.1[AB]	0.5	44.4[BC]	1.5	41.3[C]	0.7
Elements by ICP-MS[a]													
Copper	***	8.12[B]	2.06	0.32[C]	0.12	8.18[B]	1.97	12.74[A]	2.42	7.34[BC]	0.89	3.59[C]	1.38
Iodine	***	0.01[C]	0.00	0.07[BC]	0.01	0.10[AB]	0.05	0.17[A]	0.03	0.06[BC]	0.01	0.02[C]	0.00
Manganese	***	0.21[B]	0.00	0.34[A]	0.05	0.21[B]	0.03	0.20[B]	0.00	0.21[B]	0.04	0.20[B]	0.01
Molybdenum	***	0.11[B]	0.03	0.07[C]	0.00	0.15[A]	0.01	0.07[BC]	0.00	0.08[BC]	0.02	0.07[BC]	0.01

See Table 2.
[a] The results of the ICP-MS are semi-quantitative.

values are higher in winter, probably due to the use of iodine-enriched fodder during these months. The geographic variation can be ascribed to differences in the natural iodine content of potable water. In cheese, the brine represents a further source of iodine.

Finally, molybdenum was very interesting for separating "Switzerland" from the other regions as its concentration was significantly higher only in this country. The origin of these differences will be investigated in future work.

By reporting the concentration of molybdenum against sodium, it was possible to distinguish between the three geographically very close regions, "Switzerland", "Allgäu" and "Vorarlberg". "Switzerland" was even clearly separated from all other cheese samples (Fig. 2).

3.3. Radioactive elements

No ^{137}Cs activity could be detected in the 20 cheese samples analysed. Activities of the three isotopes, ^{90}Sr, ^{234}U and ^{238}U, as well as ^{234}U/^{238}U ratios are presented in Table 4.

The activity of ^{90}Sr, mainly due to aerosol deposition, depends on the geomorphology encountered. In the northern alpine regions, geomorphology may be expressed as altitude. A correlation coefficient of $r = 0.71$ was found between the $^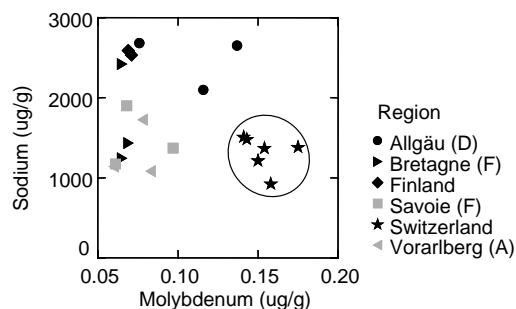{90}$Sr activity in cheese and the altitude of the production zones (Fig. 3). However, it only allowed a clear separation to be made of "Vorarlberg" from "Bretagne" and "Finland".

Preferential leaching of ^{234}U due to alpha recoil leads to ^{234}U/^{238}U ratios less than those in soil. As a result, groundwaters are ^{234}U enriched, as well as the uranium accumulation zones (Suksi et al., 2001). Surprisingly, all samples showed a ^{234}U/^{238}U ratio higher than one, in contrast to values generally found in soil and vegetation. This ^{234}U enrichment of cheese is not yet fully understood, even if these results tend to prove that the cow's drinking water must be the main pathway of contamination of the milk with uranium. Further details may be found in Froidevaux, Geering, Pillonel, Bosset, & Valley (submitted).

The concentration of uranium in all analysed samples was lower than 32 mBq/kg cheese ($= 2.6 \mu g/kg$). "Switzerland" showed the highest values. The Finnish samples had unusually low uranium concentrations considering the high uranium content of soil and water in Finland.

4. Conclusion

Primary geographic *indicator*s for the origin of Emmental cheese were investigated in this screening
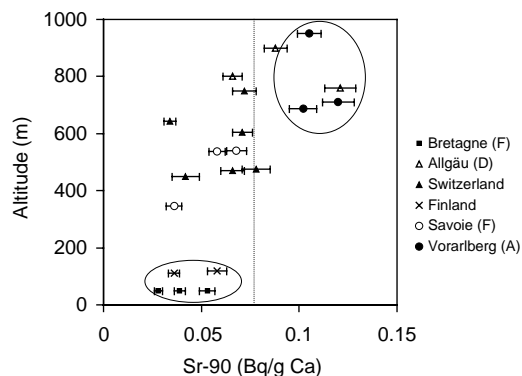

Fig. 2. Concentration of molybdenum and sodium by ICP-MS and AAS, respectively. Separation of "Switzerland" from all other samples. "Vorarlberg" and "Allgäu" are distinctly separated one from the other using these two parameters.

Fig. 3. Relation between the ^{90}Sr activity and the altitude of the production zone. "Finland" and "Bretagne" are separated from "Vorarlberg".

Table 4
Radioactive elements in the 20 Emmental cheeses investigated

Region ($n =$)	ANOVA	AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	x	s_x	x	s_x	x	s_x	x	s_x	x	s_x
Radioactive elements													
Bq/g calcium													
^{90}Sr	**	0.09[AB]	0.028	0.04[C]	0.013	0.06[BC]	0.018	0.05[BC]	0.016	0.05[BC]	0.016	0.11[A]	0.010
^{234}U	**	1.2[BC]	0.27	1.7[ABC]	0.76	2.5[A]	0.73	0.36[C]	0.057	1.3[ABC]	0.18	2.1[AB]	0.73
^{238}U	*	0.90[AB]	0.17	1.1[AB]	0.52	1.9[A]	0.76	0.275[B]	0.007	1.1[AB]	0.23	1.7[A]	0.65
^{234}U/^{238}U	ns	1.33	0.06	1.53	0.09	1.4	0.23	1.3	0.17	1.2	0.12	1.20	0.09

See Table 2.

study. With only 20 samples analysed, the goal of the current study was only to establish potential indicators, and not to build a model for prediction. This will be the next objective of this 3-years project. Analyses of the stable isotope ratios in pH 4.3-insoluble fraction made it possible to separate geographically distant regions. PCA of the parameters $\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$, $\delta^{87}Sr$ delivered a good separation of the groups "Finland", "Bretagne" and "Savoie". Major and trace elements such as calcium, copper, molybdenum, iodine, manganese, sodium and zinc showed highly significant differences. Copper concentrations were strongly correlated with the technology used (stainless steel vs. copper vat). Using concentrations of molybdenum and sodium, it was possible to separate the three relatively close regions "Switzerland", "Vorarlberg" and "Allgäu". Stable isotope ratios and major/trace elements are complementary and very useful for the determination of origin. Most of these parameters are however influenced by the season due to variation in the forage composition. Seasonal effects will be studied in the next step of this project. Information obtained from the radioactivity results were of limited interest for authentication except for the correlation between ^{90}Sr and the altitude of the production zone in the northern Alps. ^{137}Cs was not detected in cheese.

Acknowledgements

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