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Application of strontium isotope abundance ratios measured by MC-ICP-MS for food authentication

G. Fortunato,[a] **K. Mumic,**[a] **S. Wunderli,**[a] **L. Pillonel,**[b] **J. O. Bosset**[b] **and G. Gremaud**[c]

[a] *Swiss Federal Laboratories for Materials Testing and Research EMPA, 9014 St. Gallen, Switzerland. E-mail: giuseppino.fortunato@empa.ch; Fax: ++41 (0)71 274 77 88; Tel: ++41 (0)71 274 76 77*
[b] *Swiss Federal Dairy Research Station FAM, 3003 Berne, Switzerland*
[c] *Swiss Federal Office of Public Health BAG, 3003 Berne, Switzerland*

Naturally occurring isotopes of such elements as strontium (Sr) have proved to be good tools for detecting trends in the soil-vegetation system and the tracing of a variety of objects. Multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has been used for the precise determination of variations in the isotopic composition of Sr. The method described has been applied to the establishment of the potential and limits to determine the geographical origins of different Emmental-type cheese samples. The sample preparation consists of *(i)* a freeze-drying procedure to remove water, *(ii)* an extraction step to eliminate the fat components and to obtain the cheese casein fraction, *(iii)* a thermal decomposition of the latter, and *(iv)* a chromatographic matrix separation of the redissolved residue. The determination of the isotope abundance ratios $^{88}Sr/^{86}Sr$, $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ resulted in precisions of 0.002–0.01%. Simultaneously, the ion currents for krypton (^{83}Kr, ^{82}Kr) and rubidium (^{85}Rb) were measured to correct for interferences with the Sr isotopes 84, 86 and 87. These and further (argide) spectral interferences causing bias effects to the Sr isotope abundance ratios have been investigated and an adequate computational correction procedure has been assessed. The whole set of validation data has been used for the calculation of the combined standard measurement uncertainty of the isotopic abundance ratio, resulting in a value of 0.016%. Comparison of the measured $^{87}Sr/^{86}Sr$ data with thermal ionisation mass spectrometric (TIMS) results, determined on the same cheese samples, agreed within the stated measurement uncertainties, thus indicating that both the validation of the sample preparation procedures and the mass spectrometric measurements cause no evident bias effect with respect to the Sr isotope abundance values. The $^{87}Sr/^{86}Sr$ isotope abundance ratios in cheese originating from different regions (alpine, pre-alpine, Bretagne, Finland, Canada, Australia) accorded to local geological properties. No difference was found between "casein-bound" and "whole-cheese" Sr isotope abundance ratios within the stated measurement uncertainties.

Introduction

Strontium (Sr) has four naturally occurring stable isotopes: ^{88}Sr, ^{87}Sr, ^{86}Sr and ^{84}Sr. Natural variations of the isotopic composition of $^{87}Sr/^{86}Sr$ mainly arise from the natural β-decay of ^{87}Rb to ^{87}Sr ($t_{1/2} = 4.88 \times 10^{10}$ a). The Sr–Rb system has been widely studied in relation to a range of applications, *e.g.*, for both petrogenetic and environmental tracing as well as geochronological purposes. The $^{87}Sr/^{86}Sr$ isotope abundance ratio varies from place to place both on a global and local scale. Relative natural abundance variations given by IUPAC fall in a range of 82.29–82.75% for ^{88}Sr, 6.94–7.14% for ^{87}Sr, 9.75–9.99% for ^{86}Sr and 0.55–0.58% for ^{84}Sr, respectively.[1] Some exceptional specimens are known in which the element has an isotopic composition outside the reported range. Sr behaves almost identically to Ca in milk and dairy products due to its similar chemical properties. The mass ratio of Sr/Ca is in the range of approximately 2.5×10^{-4}.

Food authenticity is one aspect which is receiving more attention with the increasing mobility and low transport costs of our society. For the protection of consumers and producers analytical methods have to be validated[2–6] for the prevention of frauds and mislabelling and to guarantee the origin of the products. A number of different analytical techniques and parameters have been evaluated for the purpose of Emmentaler cheese authentication, such as infrared spectroscopy, light element stable isotope mass spectrometry, major and trace elemental analysis, pH-value determinations, *etc*. It was shown that one single method or one single parameter on its own is generally insufficient to achieve this task, and that results from several methods should be combined using multivariate statistical analysis in order to achieve a good differentiation of cheeses from various geographic origins.

The strontium isotopes represent one part of the local geological conditions and may therefore be linked to the origin of the raw cheese products. Values of Sr isotope abundance ratios in terrestrial vegetation will be dominated by the Sr isotopic composition of labile cations in the soil, which are themselves influenced by bedrock, soil water properties and atmospheric inputs.[7,8] The typical strontium mass content of mobile strontium in soil and in solution ranges from 0.2 to 20 mg kg^{-1} (μg Sr leached per g of soil).[9,10] For different types of geological samples the overall mass contents of Sr range from 1 up to 2000 mg kg^{-1} and from 0.01 to 7620 μg L^{-1} for hydrological samples (sea-water, rivers, rain …) and from 8 to 2500 mg kg^{-1} for biological samples (wood, roots …). Biological processes, whether involved in plant or animal metabolism, do not significantly fractionate strontium isotopes.[11] Sillen *et al.*[12] investigated the possible shift of the isotopic composition between labile Sr cations in the soil and plants in a well-defined geographic area. Interestingly, differences of up to 0.5% in the composition of $^{87}Sr/^{86}Sr$ have been found. It was suggested that this phenomenon could be explained by the presence of significant amounts of dust

Table 1 Possible interferences for Sr isotopes and approximative mass resolving power $^{m}/_{\Delta m}$ needed for the separation of the signals (10% valley definition)

Type of interference	Isotope $^{84}Sr^+$		$^{86}Sr^+$		$^{87}Sr^+$		$^{88}Sr^+$	
Isobaric	$^{84}Kr^+$	>10 000	$^{86}Kr^+$	>10 000	$^{87}Rb^+$	>10 000	—	—
Molecular	$^{48}Ti^{36}Ar^+$	>10 000	$^{46}Ti^{40}Ar^+$	>10 000	$^{51}V^{36}Ar^+$	>10 000	$^{53}Cr^{36}Ar^+$	>10 000
	$^{44}Ca^{40}Ar^+$	>10 000	$^{70}Ge^{16}O^+$	10 000	$^{47}Ti^{40}Ar^+$	10 000	$^{48}Ti^{40}Ar^+$	>10 000
	$^{68}Zn^{16}O^+$	>10 000	$^{70}Zn^{16}O^+$	10 000	$^{71}Ga^{16}Ar^+$	10 000	$^{48}Ca^{40}Ar^+$	>10 000
					$^{69}Ga^{18}Ar^+$	7000	$^{72}Ge^{16}O^+$	10 000
Doubly charged	$^{168}Er^{2+}$	2000	$^{172}Yb^{2+}$	2000	$^{174}Yb^{2+}$	2000	$^{176}Hf^{2+}$	2000
	$^{168}Yb^{2+}$	2000			$^{174}Hf^{2+}$	2000	$^{176}Yb^{2+}$	2000
							$^{176}Lu^{2+}$	2000

components, differential weathering of highly radiogenic rocks and an influence of whole soil constituents contributing to available soil Sr.

It is assumed that geological properties (*e.g.*, Sr isotope abundance ratios) are reflected directly in the cheeses when the cows are kept under a controlled dietary regime and are not fed with industrially produced feeds or feeds from geographically distant sources. Only contamination and blending of the dairy products with other Ca and Sr containing sources could then provide a shift in the Sr isotope ratios and lead to misinterpretation of results.[3]

MC-ICP-MS offers a way to establish accurate high precision isotope ratios as long as the influencing parameters are understood and under control. Typical internal precisions for $^{87}Sr/^{86}Sr$ are in a range of 0.001–0.002%.[13–15] Various parameters may influence the determination of Sr isotope abundance ratio by MC-ICP-MS. The main factors are believed to be isobaric and molecular interferences such as $^{87}Rb^+$, $^{44}Ca^{40}Ar^+$, $^{48}Ca^{40}Ar^+$ and doubly charged rare earth isotopes ($^{167}Er^{2+}$, $^{168}Yb^{2+}$, $^{171}Yb^{2+}$ ...) stemming from the sample, and $^{84}Kr^+$ and $^{86}Kr^+$ from the argon gas (Ar) used to operate the inductively coupled plasma, by which both the precision and the accuracy of the isotope abundance ratios can be affected (Table 1).

This paper describes means of preventing or controlling the influences of either type of interference using chemical separation methods or using measurement procedures with adequate computational correction of the contributions. The main objectives of the current study are: *(i)* to determine the Sr isotope ratios in cheese and thereafter to calculate and discuss the measurement uncertainties; and *(ii)* to investigate the distributions amongst the Sr isotope abundance values of the cheeses in order to discriminate between the geographical and geological origin of the investigated foods as a tool for confirming their authenticity and establishing their geographic traceability.

Experimental

Sample selection

The "Emmental" type cheese samples were received from the "Swiss Federal Dairy Research Station FAM" (Berne) and were all designated with proven geographic origin from Allgaeu (Germany), Bretagne and Savoie (France), Berne and St.Gallen (Switzerland), Vorarlberg and Salzburg (Austria), Middle Finland, Australia and Canada (Table 2). The cheeses were produced between the end of the year 2000 and the middle of 2002. The cheeses investigated were sampled at ages between 2.5–4 months of ripening, which corresponds to the age of that sold in stores. For the Canadian and Australian samples no data are available concerning their date of production and the manner of production itself.

The samples were supplied as slices from a whole block. The first 2 cm from the rind were discarded.

Table 2 Origin of "Emmental" type cheese samples used for Sr isotope abundance determination

Region	Abbreviation	Location	Altitude/ m	Date of production
Bretagne	BR1	Yffiniac	52	16.02.2001
Bretagne	BR3	St Méen-le-Grand	100	16.02.2001
Berne	BE1	Aarwangen	475	26.12.2000
Berne	BE3	Amsoldingen	644	26.12.2000
St-Gall	SG1	Schänis	750	26.12.2000
St-Gall	SG3	Altnau	450	26.12.2000
Finland	FI1	Lapinlahti	124	07.02.2001
Finland	FI104	Toholammi	108	22.08.2002
Savoie	SA1	Massingy	537	08.02.2001
Savoie	SA3	Avraissieux	540	08.02.2001
French Jura	TR1	Trevillier	800	11.06.2002
Vorarlberg	VO1	Schwarzenburg	950	02.02.2001
Vorarlberg	VO3	Lingenau	687	02.02.2001
Salzburg	SAL105	Unknown	—	15.10.2002
Allgäu	AL1	Unknown	760	20.12.2000
Allgäu	AL3	Unknown	900	20.12.2000
Australia	AUS1	Unknown	—	—
Canada	CAN101	Unknown	—	—

Sample preparation

The sample preparation procedure for the determination of "casein-bound" as well as "whole-cheese" Sr isotope abundance ratios are summarised in Fig. 1.

All operations, except the thermal decomposition and the freeze-drying procedures, were carried out under clean room conditions (class 100). All solutions were prepared using high purity deionised 18 MΩ water (Elix-Millipore, USA). Analytical grade nitric acid (Fluka, Switzerland) was purified by subboiled distillation with an MLS duo PUR (Milestone, Germany) system.

For each determination 1–2 g of cheese sample was weighed on an analytical balance Mettler AT201 (Mettler, Switzerland) and crushed into pieces of about 2 mm diameter after freezing with liquid nitrogen. The freeze drying process was performed on a Beta 1–16 (Christ, Germany) over 12 h at a condenser temperature of −50 °C and at a pressure of 0.3 mbar. The sample temperature was held at 10 °C. Typical water loss for weighed cheese was in the range of 25–35%. The extraction was performed in three steps: first, twice with 15 mL of high purity diethyl ether (Fluka, Switzerland) for fat extraction, then once with 15 mL hydrochloric solution adjusted to a pH of 4 using a pH-meter (692 pH/ion meter, Metrohm, Switzerland) to separate all non-casein bound Sr. The hydrochloric acid solution was prepared using concentrated acid (Traceselect, Fluka, Switzerland) and high purity deionised water. All operations were carried out in capped Teflon vials. The thermal decomposition was performed in a tubular furnace (Nabertherm, Switzerland), first for 2 h at 200 °C then for 1 h at 650 °C in covered quartz vessels. The residue was redissolved in 1.2 mL of 2% nitric acid. A strontium- and lead-selective extraction chromatographic Sr.Spec™ (EiChrom,
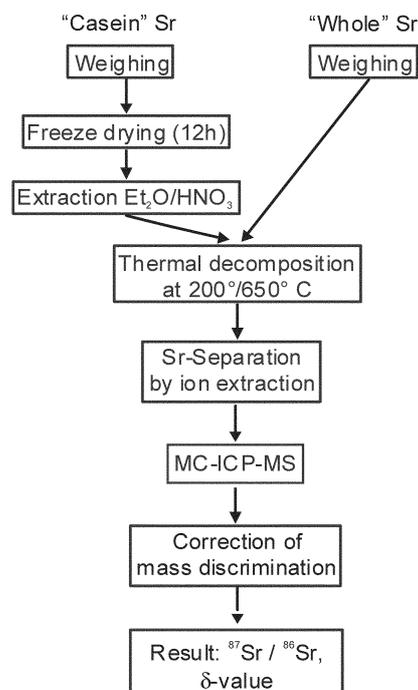
"Casein" Sr → Weighing

"Whole" Sr → Weighing

Weighing (Casein) → Freeze drying (12h) → Extraction Et₂O/HNO₃ → Thermal decomposition at 200°/650° C

Weighing (Whole) → Thermal decomposition at 200°/650° C

Thermal decomposition at 200°/650° C → Sr-Separation by ion extraction → MC-ICP-MS → Correction of mass discrimination → Result: ^{87}Sr / ^{86}Sr, δ-value

Fig. 1 Sample preparation and measurement scheme for Sr isotope abundance ratio determination in cheese.

USA) resin was used.[16–18] The separations were performed using Teflon micro-columns (1 mL volume) loaded with 35 mg of the resin. The resin was first washed and activated by rinsing with 1.5 mL of 8 M hydrochloric acid to precondition the resin for surface strontium complexation. The sample solution was carefully loaded onto the column and 0.4 mL of 2 M then 0.4 mL of 8 M nitric acid passed through. The strontium fraction was desorbed by using 1.4 mL of water; the lead fractions could be desorbed using 1.4 mL of 8 M hydrochloric acid. The total strontium and lead containing fractions were collected, water and the hydrochloric acid removed and the dried sample redissolved in 1 mL of 2% nitric acid. The Sr contents of the cheese samples were adjusted so that the intensity of ^{88}Sr on Faraday cup H2 ranged between 3×10^8–5×10^8 counts s^{-1} to obtain the optimum precision for the Sr isotope abundance ratios.

Mass spectrometry

All isotope ratio experiments as well as the trace analysis measurements were carried out on a VG AXIOM MC-ICP-MS (Thermo-Elemental, Winsford, England). This is a double focusing magnetic sector instrument equipped with an array of 10 variable Faraday collectors and a further fixed Faraday and photomultiplier detector. The normal operating conditions adopted for the mass spectrometer are summarised in Table 3.

The instrument is placed in a temperature $(17 \pm 1 \ °C)$ stabilised clean hood (class 100). Before high precision ratio measurement was started, the instrument was stabilized for about 1.5 h under normal working conditions until the temperature values within the mass spectrometer were constant $(\pm 0.2 \ °C)$. Air temperature in the clean hood as well as inside the instrument was monitored during the measurements. The solutions were aspirated by a TH1 nebuliser and introduced into an aerosol drying system (Aridus, CETAC, France) with 3 L min^{-1} Ar and 20 mL min^{-1} nitrogen (temperature 160 °C). Ar gas with stated purity of 99.996% was used (Carbagas, Switzerland). The liquid sample uptake rate was about 100 µl min^{-1}. The system was cleaned with 2% nitric acid for 2 min before each measurement.

Once a week a gain calibration was performed for the

Table 3 Analytically relevant operating conditions for the VG Axiom MC-ICP-MS

Rf-power	1250 W	
Plasma gas flow	14.0 L min^{-1} Ar	
Auxiliary gas flow	0.58 L min^{-1} Ar	
Nebuliser gas flow	1.0 mL min^{-1} Ar	
Sampling cone material	Pt	
Mass resolving power (10% valley) $m/\Delta m$	410	
Dwell time	50 ms	
Points per replicate	40	
Mass assignment to Faraday cup detectors	Kr	83	L4
	Sr/Kr	84	L2
	Rb	85	L1
	Sr/Kr	86	Ax
	Sr/Rb	87	H1
	Sr	88	H2
Aerosol drying unit	CETAC Aridus	
Nebuliser type	TH 1 concentric	
Typical liquid sample uptake rate	100 µL min^{-1}	
Air temperature in clean hood for instrument	(17 ± 1) °C	

multicollector system. Multicollector detector positions were adjusted while a 500 µg kg^{-1} Sr solution was aspirated.

Isotope ratios of the cheese samples were determined using the following sequence: 500 µg kg^{-1} Sr (NIST 987) – blank solution-sample 1 – sample 1-500 µg kg^{-1} Sr (NIST 987) – blank solution-sample 2 – sample 2.... Each measurement was followed by a wash sequence with 2% nitric acid lasting 2 min, which guaranteed a lack of Sr carryover. The set-up of this measurement protocol permits both internal correction of the isotope ratios ^{87}Sr/^{86}Sr and ^{84}Sr/^{86}Sr with ^{88}Sr/^{86}Sr and a standard–sample–standard bracketing method using the isotopic reference material SRM 987 (NIST, USA).

Trace analytical determinations used for the draw up of the matrix separation procedure were performed by an external three-point calibration using single element high purity reference solutions (Johnson–Matthey, Germany). For this purpose the mass spectrometer was operated in single collector mode. Isobaric and molecular interferences were separated using various resolving power settings $(m/\Delta m)$ of 10 000 for ^{39}K, 8000 for ^{88}Sr, 4000 for ^{44}Ca, 400 for ^{86}Sr and ^{208}Pb. The mass resolving power settings are according to the 10% valley definition.

Results and discussion

Separation of the matrix

In most cases the influence from the matrix with respect to the correct determination of the isotope abundance ratios is unknown. Therefore a matrix separation is an adequate method for eliminating possible bias effects. The main influences are caused by ^{87}Rb$^+$ on ^{87}Sr, ^{44}Ca^{40}Ar$^+$ on ^{84}Sr and ^{48}Ca^{40}Ar$^+$ on ^{88}Sr as the interferent elements are highly concentrated in the cheese samples (Table 1). Krypton, an impurity in the Ar gas used for the operation of the mass spectrometer, cannot be eliminated but has to be screened on mass ^{83}Kr. High purity Ar gas has to be used.

The relatively low sensitivity of Faraday detectors demands analyte contents, in our case, in the range of up to 300–500 ng g^{-1} strontium. Such contents permit us to obtain relative standard deviations for the isotope amount ratios in a range of 2×10^{-5} up to 10^{-4}. Furthermore, the removal of the matrix elements eliminates the danger of contamination of the instrument inlet system and therefore prevents long wash out times. It minimises long term contributions to signal drift caused by deposition of solids originating from the matrix on the surfaces of the instrument inlet system (nebuliser, torch, cones).

The validation of the matrix removal was performed either by using digested cheese samples to test for the separation

Table 4 Analyte mass determination of different fractions collected for a matrix separation of digested cheese samples[a]

	Na/μg[c]	K	Ca	Zn	Rb	Sr ($m/z = 86$)[b]	Sr ($m/z = 88$)[b]	Pb
Fraction 1	2337	852	3719	34	0.55	0.72	0.78	<0.005
Fraction 2	6.9	47.4	135	1.0	0.02	0.09	0.07	<0.005
Fraction 3	0.2	0.3	6.5	0.1	0.00	6.34	6.47	<0.005
Fraction 4	0.1	0.1	0.3	0.1	0.00	0.10	0.11	0.03
	Na (%)	K	Ca	Zn	Rb	Sr ($m/z = 86$)[b]	Sr ($m/z = 88$)[b]	Pb
Fraction 1	99.7	94.7	96.3	96.6	96.3	9.9	10.4	—
Fraction 2	0.3	5.3	3.5	2.9	3.2	1.2	1.0	—
Fraction 3	0.0	0.0	0.2	0.2	0.2	87.5	87.1	—
Fraction 4	0.0	0.0	0.0	0.3	0.2	1.4	1.5	101.0
mg kg^{-1} cheese	2570	986	4231	38	0.6	7.9	8.1	0.02

[a] Used sample mass for separation: 0.9 g cheese. [b] Measured isotope for mass determination. [c] Mass in the respective fraction.

efficiency of the involved analytes or by using the reference material SRM 987, including blank solutions, to check for mass dependent isotopic fractionation on the resin.

Table 4 shows the separation of Sr^{2+} for approximately 1 g of a cheese sample. Because the alkaline and alkaline earth element ions have only a very small affinity for the chromatographic resin, they pass through the column with the sample solution put onto the column and the fraction of 2 M nitric acid (Table 4: fraction 1). Then 0.4 ml of 8 M nitric acid is eluted (Table 4: fraction2). The separation of Sr^{2+} from Pb^{2+} is ensured by using water as the eluent for Sr^{2+} (Table 4: fraction 3). Finally, the lead ions can be desorbed using 1.4 mL of 8 M hydrochloric acid (Table 4: fraction 4). The extraction yield of Sr is about 70–90%. Possible Sr isotope fractionation on the chromatographic resin was investigated using SRM 987 (NIST, USA). 2 g of a solution containing 500 μg kg^{-1} of Sr SRM 987 was digested in the same manner as the cheese samples except for the freeze-drying and the extraction process. Mass dependent fractionation or a carry in of ubiquitous Sr would have been detected in that way. Sr in the blank solutions did not affect the result within the stated measurement uncertainties for the isotope abundance ratios. After separation and comparison of the measured isotope abundance ratios, $^{88}Sr/^{86}Sr$, $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$, with the values from non-separated Sr SRM 987, no significant difference was detectable. All isotope abundance values and their uncertainties overlap with the data given in the certificate of the reference material SRM 987.[19]

Correction of measured Sr isotope abundance ratios for mass discrimination and evaluation of the measurement uncertainties

Two different types of correction for the mass discrimination effect of the instrument were investigated: an internal and an external correction. For internal correction, the mass discrimination was corrected using the isotope abundance ratio $^{88}Sr/^{86}Sr$, assuming that the value is invariant in nature. For external calibration a standard–sample–standard bracketing method was used, whereby the isotope abundance ratio $^{88}Sr/^{86}Sr$ from the standard SRM 987 acted as the reference value. As mathematical model equation an exponential law was used with the mass discrimination per mass unit ε:[20]

$$\varepsilon = \ln\left(\frac{R_{cert}}{R_{meas}}\right) \cdot \frac{1}{\Delta m_{88}} \quad (1)$$

ε is consequently used to correct the measured isotope abundance ratios from the analyte in the samples. The corrected isotope abundance ratio can then be calculated using the model equation for the measurand R_{87}:

$$R_{87} = R'_{87} \times \exp(\varepsilon \times \Delta m_{87}) \quad (2)$$

The exponential law model gave the best overlap of the

values and their uncertainties for the measured Sr isotope abundance ratios $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ using the certified reference material SRM 987 (NIST, USA). The estimated combined measurement uncertainties, a crucial need for comparing results amongst different measurement techniques and laboratories, were calculated from the external repeatabilities, the correction of the mass discrimination, the variation of the blank values and from the influences from interferent elements. Contributions from Faraday detector cup efficiencies are not included in these calculations as the values are at least one order of magnitude smaller than the values of the major uncertainties. Applying the uncertainty propagation law[21] without using second order covariances

$$u_c^2(R_{87}) = \sum_{i=1}^{n} \left(\frac{\partial R_{87}}{\partial x_i}\right)^2 \cdot u^2(x_i) \quad (3)$$

the expression for the uncertainty of the isotope ratios becomes explicitly:

$$u_c(R_{87}) = \sqrt{\left(\exp(\varepsilon \cdot \Delta m_{87})^2 \cdot u^2(R'_{87}) + (R'_{87} \cdot \Delta m_{88} \cdot \exp(\varepsilon \cdot \Delta m_{88}))^2 \cdot u^2(\varepsilon)\right)} \quad (4)$$

The standard uncertainty $u(R'_{87})$ of the measured isotope ratio R'_{87} is estimated using the contributions of the isotope ratio precision (u_{prec}) of R_{87}, the variations of the blank values (u_{bl}) and the influences of the correction for the interference from the isotope ^{87}Rb (u_{Rb}). As an approximation, the values were combined by adding the square root of the squared uncertainties as independent contributions:

$$u(R'_{87}) = \sqrt{u_{prec}^2 + u_{bl}^2 + u_{Rb}^2} \quad (5)$$

By application of the uncertainty propagation law the combined uncertainty of ε is deduced using the fact that the mass difference Δm of the two isotopes involved is considered to be constant with negligible or zero uncertainty ($u(\Delta m) \equiv 0$):

$$u(\varepsilon) = \sqrt{\left(\frac{\partial \varepsilon}{\partial R_{cert}}\right)^2 \cdot u^2(R_{cert}) + \left(\frac{\partial \varepsilon}{\partial R_{meas}}\right)^2 \cdot u^2(R_{meas})}$$
$$= \sqrt{\left(\frac{1}{R_{cert}}\right)^2 \cdot u(R_{cert})^2 + \left(-\frac{1}{R_{meas}}\right)^2 \cdot u(R_{meas})^2} \quad (6)$$

Typical calculated measurement uncertainties $u_c(R_{87})$ of the analyte in the samples not involved in chemical processing (digestion, matrix separation, ...) result in relative expanded uncertainty values, *e.g.* for SRM 987 of 0.014% (coverage factor $k = 2$). The contributions account for 12% from $u(R'_{87})$ and 88% from $u(\varepsilon)$. For the results from cheese samples, where a chemical procedure has to be carried out, uncertainty contributions from the carry in of ubiquitous Sr into the samples has to be considered additionally. Mass fractionation effects during matrix separation can be neglected in this case as
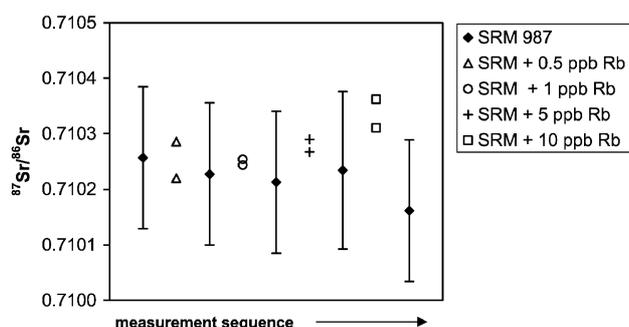
Fig. 2 $^{87}Sr/^{86}Sr$ isotope abundance values for SRM 987 doped with different increasing amounts of Rb.

the validation of the separation procedure indicated no significant bias.

Correction of Rb and Kr signals: influence of Ca

The measurement of the ^{87}Sr isotope is interfered by the ^{87}Rb isotope. The concentration of Rb in the Sr reference material (NIST 987) is small and the uncertainty contributions from the Rb interference are much smaller than uncertainty contributions from, for example, the repeatability. The influence of Rb was determined by doping a Sr reference solution with defined amounts of a Rb solution to obtain concentrations of $0.0\ \mu g\ L^{-1}$, $0.5\ \mu g\ L^{-1}$, $1\ \mu g\ L^{-1}$, $5\ \mu g\ L^{-1}$ and $10\ \mu g\ L^{-1}$ of Rb in $500\ \mu g\ L^{-1}$ Sr solution each (Fig. 2). The raw isotope abundance ratio $^{85}Rb/^{87}Rb$ was determined in a solution containing Rb only at a concentration of $10\ \mu g\ L^{-1}$. The correction to the interference with ^{87}Sr was performed point by point by subtracting the intensity from the value measured on the Faraday cup H1 (mass 87) from the measured value on cup L1 (mass 85) times the measured raw ratio of the single element Rb.

$$R'_{87} = \frac{\left(I_{87} - \frac{1}{R'_{85}} \cdot I_{85}\right)}{I_{86}} \qquad (7)$$

After mathematical calculation to remove the bias effect caused by ^{87}Rb (eqn. 7), and the correction for the mass discrimination, the value of R_{87} gives no evidence of a measurable influence from Rb. This means that the bias effect regarding the comparability of R_{87} for Rb separated and non-separated samples can be guaranteed within the used Rb amount contents. The isotope abundance ratio values overlap within the stated measurement uncertainties. The fact of the

small variability of the $^{85}Rb/^{87}Rb$ value found in nature can be neglected. Firstly, the influence of the correction factor (measured $^{85}Rb/^{87}Rb$) is small considering the subtraction of ^{87}Rb from ^{87}Sr and secondly the contents of Rb in the separated Sr fraction of the cheese samples are smaller than $2\ \mu g\ L^{-1}$. This value makes a subtraction necessary to maintain the required accuracy, but a change of the $^{85}Rb/^{87}Rb$ ratio of the sample used for the calculation of the fraction on mass 87 of 1% affects the result of R_{87} only in the $2\ \times\ 10^{-5}$ range.

Traces of Kr in the Ar gas used to operate the rf plasma flame causes interferences with the signals for the isotopes ^{84}Sr and ^{86}Sr. The signal of $m/z = 83$, corresponding to ^{83}Kr, was monitored simultaneously during the Sr measurements. As the Kr concentration in the Ar gas used was small, in general no correction was necessary. For higher amounts of Kr a reliable correction procedure is still lacking. It is suggested that this could be due to a lack of information concerning the mass discrimination behaviour of Kr in the mass spectrometer inlet system.

The influence of $^{44}Ca^{40}Ar^+$ and $^{48}Ca^{40}Ar^+$ with respect to the Sr isotope abundance ratios was investigated by means of the addition of variable amounts of Ca to a mixture of $0.5\ mg\ L^{-1}$ SRM 987 standard solution and $5\ \mu g\ L^{-1}$ Rb to obtain Ca concentrations of 1, 5, 10 and $100\ mg\ L^{-1}$. A serious effect that leads to loss of close comparability of measurement values and their uncertainties for different samples which have undergone the same validated preparation procedure was not detectable within the above Ca concentration range (Fig. 3). However, the precision of the isotope abundance ratio R_{87} became larger by a factor of 3. This indicates that the bimolecular argide ($^{44}Ca^{40}Ar^+$, $^{48}Ca^{40}Ar^+$) species are only formed to a very small extent and that the stability of the species is slight under the instrumental conditions chosen. The measured isotopic values indicate that a matrix separation for samples containing less than $100\ mg\ L^{-1}$ Ca is only necessary when precisions smaller than 0.003% (1 SD) have to be obtained. Nevertheless, the removal of a matrix with high Ca content is advisable as possible long term signal drift and contamination of cones can be avoided.

Sr in cheese samples, comparability to thermal ionisation mass spectrometric (TIMS) results

Similar precision values were measured for the isotopic ratios $^{87}Sr/^{86}Sr$ in the cheese samples and the reference material used (SRM 987). The reproducibility was investigated by comparing the isotope ratio results from independently prepared samples, for which the whole preparation process was applied to a specimen several times (Table 5). The relative standard deviation of the $^{87}Sr/^{86}Sr$ isotope ratio values, corrected
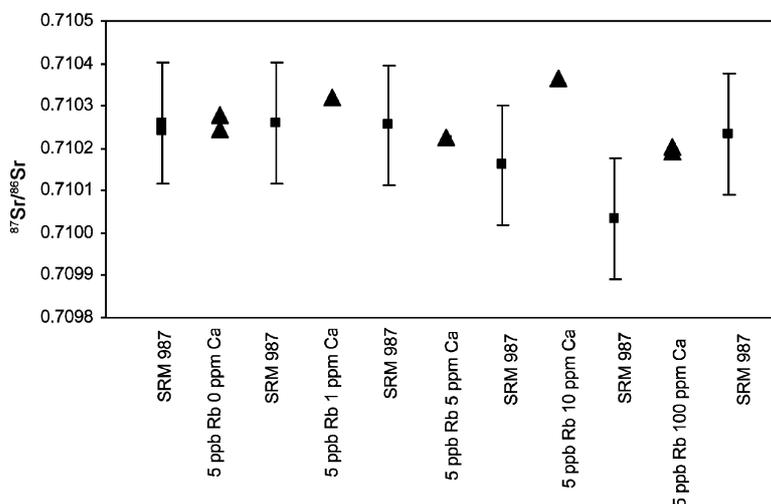

Fig. 3 Influence of Ca doping for $^{87}Sr/^{86}Sr$ isotope abundance ratios in SRM 987 (NIST, USA).

Table 5 "Casein"-Sr isotope abundance ratios for "Emmental" type cheese samples

	N^b	$^{87}Sr/^{86}Sr^c$ mean	RSD^d (%)	$^{87}Sr/^{86}Sr^e$ mean	RSD (%)		N^b	$^{87}Sr/^{86}Sr^c$ mean	RSD^d (%)
Berne BE3^a	4	0.707 88	0.003	0.707 87	0.005	Berne BE1^a	3	0.708 76	0.003
Savoie SA3	7	0.708 65	0.003	0.708 63	0.003	Savoie SA1	4	0.708 27	0.005
Vorarlberg VO3	4	0.708 12	0.005	0.707 76	0.008	Vorarlberg VO1	3	0.708 05	0.002
St. Gallen SG3	2	0.708 38	0.001	0.708 27	0.008	St. Gallen SG1	3	0.708 39	0.004
Finland Fl 104	2	0.709 27	0.012	—	—	Finland Fl1	3	0.712 93	0.010
Allgaeu AL3	4	0.708 33	0.008	0.708 14	0.004	Allgaeu AL1	3	0.707 89	0.001
Bretagne BR3	6	0.707 55	0.001	0.707 54	0.011	Bretagne BR1	3	0.709 75	0.001
Australia AUS1	2	0.709 50	0.002	—	—	Trevillier TR1	2	0.708 60	0.007
Canada CAN101	1	0.707 74	—	—	—	Salzburg SAL1	2	0.708 18	0.001

^{a} Numbers denote different sampling sites. ^{b} N, number of independent sample preparations. ^{c} Internally corrected for mass discrimination. ^{d} Relative standard deviation of results from independent prepared samples. ^{e} Externally corrected for mass discrimination.

internally using the $^{88}Sr/^{86}Sr$ isotope ratio, ranged from 0.001 to 0.012%. The standard deviation of the mean for the $^{87}Sr/^{86}Sr$ results can be used as the standard uncertainty of an overall parameter for the influence of sample preparation. The expression for the calculation of $u_c(R_{87})$ has to be expanded with the measured standard uncertainty $u(R_{cheese})$.

$$u(R_{87,cheese}) = \frac{}{\sqrt{(\exp(\varepsilon \cdot \Delta m))^2 \cdot u^2(R'_{87}) + \left((R'_{87} \cdot \Delta m \cdot \exp(\varepsilon \cdot \Delta m))^2 \cdot u^2(\varepsilon) \right) + u^2(R_{cheese})}} \quad (8)$$

The uncertainties for the isotope abundance ratios $R_{87, cheese}$ are in a range of 0.03 ± 0.005% (Table 6). The contributions of the influence parameters to the combined standard uncertainty of $R_{87, cheese}$ are approximately 9% from $u(R'_{87})$, 65% from $u(\varepsilon)$ and 26% from $u(R_{cheese})$. The results and their uncertainties guarantee a full comparability with different methods and laboratories. The values for the same cheese samples measured by thermal ionisation mass spectrometry (TIMS) overlap within the stated uncertainties (Table 7). The absolute differences for the $^{87}Sr/^{86}Sr$ isotope abundance values range between 4×10^{-5} and 2.8×10^{-4}.

The external standardisation method was used to verify the assumption of constant R_{88} values for the cheese samples. These were used for internal correction of mass discrimination. Isotopic fractionation of Sr caused by geochemical processes (*e.g.*, weathering of soils) or environmental influences (atmospheric transport of Sr or Rb) have to be considered.[22,23] Using the standard–sample–standard bracketing approach, constant K values $\left(\frac{R_{cert}}{R_{meas}} \right)$ from the reference material are used. Most of

Table 6 Uncertainty budget for $R_{87,cheese}$

	Estimate	Standard uncertainty	Uncertainty relative	Contribution to $u_c (R_{87})$ (%)
u_{prec}	0.708 53	0.000 01	0.002	
u_{bl}	0.708 53	0.000 03	0.002	
u_{Rb}	0.708 53	0.000 02	0.002	
$u_{R_{87}}$	0.708 53	0.000 03	0.005	8.9
u_ε	−0.021	0.0001	0.382	65.4
u_{cheese}	0.708 53	0.000 06	0.008	25.7
$u_c(R_{87})^a$	0.708 53	0.000 11	0.016	

^{a} Coverage factor $k = 1$.

the $^{87}Sr/^{86}Sr$ values of externally corrected isotope ratios agree with the values of the internally corrected ratios (Table 5). A significant difference was only found for the sample originating from Vorarlberg. The repeatabilities are larger by a factor of about two, which can be attributed to instrumental drift as the simultaneous measurement of all isotopes involved is not possible. The relative standard deviation of the corrected $^{87}Sr/^{86}Sr$ values (reference material SRM 987), determined over 1 month and used for the standard–sample–standard bracketing method, has a value of 0.007%. This partly explains the generally higher standard deviation of externally corrected Sr isotope abundance values with respect to internally corrected ratio results giving values of 0.002%. The comparison of externally and internally corrected $^{87}Sr/^{86}Sr$ isotope abundance values shows that $^{88}Sr/^{86}Sr$ isotope ratios are constant within the stated and required uncertainties and can be used for internal correction of instrumental mass discrimination.

The question of whether a casein separation is necessary or not was investigated by comparing the results of both sample preparation procedures (Fig. 1). Possible intake of Sr during the cheese preparation process (use of brine) may influence the results of the origin investigation. The $^{87}Sr/^{86}Sr$ isotope abundance ratio values of "casein" and "whole" Sr in cheese give no evidence of a measurable influence. The isotope abundance ratio values of Sr in samples from both preparation procedures range within the stated measurement uncertainties (internally corrected for mass discrimination).

Authentication

According to the investigations of Rossmann *et al.*[3] and Oda *et al.*[24] on butter and brown rice as well as origin studies on woods,[25] cements,[26] bones and ivories,[27] the measured $^{87}Sr/^{86}Sr$ isotope ratios for cheese samples show a similar behaviour with respect to the geological subsoil. The values reflect the mineral composition and the age of the rocks they have interacted with. Sr in mafic rock type terrain, or better Sr in its bioavailable soil moisture, has $^{87}Sr/^{86}Sr$ isotope abundance values ranging from 0.700–0.710: Sr in sedimentary carbonate rich rocks has even higher values of 0.710–0.750. Geochronologically, cheese samples from older crystalline areas (samples FI 1, FI 104, AUS 100) showed the highest $^{87}Sr/^{86}Sr$ values, whereas samples
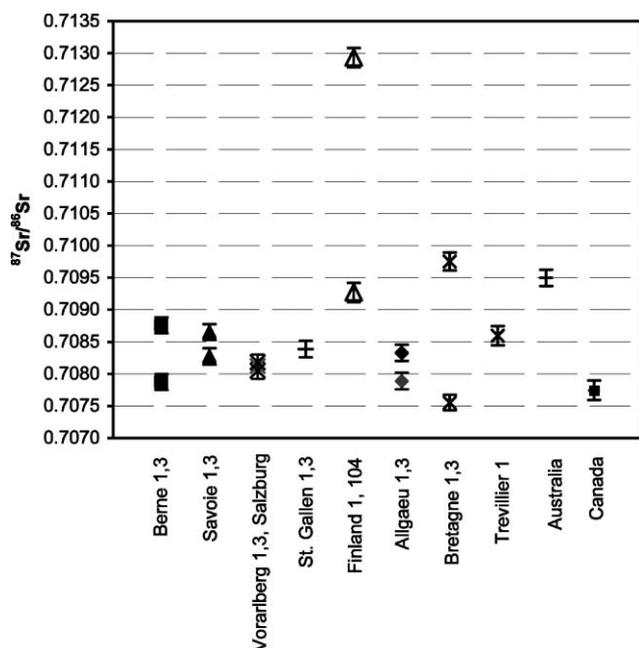
Table 7 Comparison of Sr isotope abundance values for MC-ICP-MS to TIMS

	$^{87}Sr/^{86}Sr^a$ mean	Uncertainty $u_c (R_{87})$	Deviation from TIMS value		$^{87}Sr/^{86}Sr^a$ mean	Uncertainty $u_c (R_{87})$	Deviation from TIMS value
Berne 3	0.707 88	0.000 28	−0.000 08	Berne 1	0.708 76	0.000 28	−0.000 05
Savoie 3	0.708 65	0.000 28	−0.000 08	Savoie 1	0.708 27	0.000 29	−0.000 13
Vorarlberg 3	0.708 12	0.000 29	−0.000 09	Vorarlberg 1	0.708 05	0.000 28	0.000 05
St. Gallen 3	0.708 38	0.000 28	−0.000 04	St. Gallen 1	0.708 39	0.000 28	−0.000 06
				Finland 1	0.712 93	0.000 29	−0.000 16
Allgäu 3	0.708 33	0.000 29	−0.000 09	Allgäu 1	0.707 89	0.000 28	−0.000 28
Bretagne 3	0.707 55	0.000 28	−0.000 07	Bretagne 1	0.709 75	0.000 28	−0.000 11

^{a} Internally corrected for mass discrimination.

Fig. 4 $^{87}Sr/^{86}Sr$ isotope abundance values and origins for different cheese samples.

from regions where rocks are high in carbonate, basaltic and/or young crystalline showed the lowest values (Fig. 4).

The isotopic data for the samples originating from Finland have an absolute difference of about 5 permil. FI 1 comes from the area of central Finland (gneissic bedrock) and FI 104 from the western coast in middle Finland (mica schist bedrock). The difference in the Sr isotope abundance can be due to different causes: bedrock composition and its weathering behavior or influences of precipitation.[9,10] Sr isotope values from the upper part of the soil located near a marine environment can be influenced by precipitation of aerosols composed of sea salts, depending on the prevailing meteorogical conditions. Thus, Sr isotope ratios of the upper part of the soil have values which represent the mixing of both soil and sea-water values. The whole set of the influences described underpins the difference in the observed isotopic values.

The samples originating from the sedimentary Swiss molasse basin (SG 1, SG 2, VO 1, VO 3, BE 1, SA 1) showed similar isotope $^{87}Sr/^{86}Sr$ values within a range of 0.780–0.788. The relatively large scatter of the values from cheese samples originating from the same region (*e.g.*, BE1, BE3) can be explained by locally derived geological distinctions (BE1 originates from the Swiss molasse basin, BE3 from the Prealps located between the molasse basin and the alpine helveticum). The comparison of the Sr isotope composition of basalts from the Prealps (Gets nappe)[28] with BE3 shows overlap of the values although the definitive soil composition or its bioavailable soil moisture of the cheese production sites was not taken into account. The $^{87}Sr/^{86}Sr$ ratios for the basalts are in the range of 0.7071 up to 0.7085, whereas for the cheese sample a value of 0.707 88 was found.

From the statistical point of view there are two main cornerstones to include: first the measurement uncertainty of the sample Sr abundance ratio values to differentiate the results from each other, and second the variations of the values from the soils where the strontium uptake took place. Fig. 4 shows evidence that the differentiation of samples, for example from the Swiss Molasse Basin (SG 1, SG 2, VO 1, VO 3, BE 1, SA 1), is not possible in the form of a general assignment and thus other parameters from the food samples have to be taken into account. On the other hand, samples from Finland, Australia and partially from Bretagne are distinguishable. This observation is comparable to origin studies of brown rice, where a

differentiation between products from Australia and products from Japan, China and Vietnam was possible.[24] The Australian rice gave the highest strontium isotope values (R_{87} from 0.715 to 0.717), followed by Chinese and Vietnamese rice (R_{87} = 0.710 to 0.711) and Japanese values (R_{87} = 0.706 to 0.709).

The scatter of the investigated isotope values implies a more general approach to the origin determination of food products. A more detailed Sr isotope geological mapping of the soils has to be generated to trace back the values directly to their geographical origin. This is one of the main promising attractions of the Sr isotope ratio method, as a direct "geographical" property is used for the origin assessment. The comparison with light element isotope ratio procedures allows the assumption that more stable Sr isotope values with respect to time dependent factors (climatic changes) will be generated.

Furthermore, complementary analytical methods and parameters (infrared spectroscopy, trace elemental contents, volatile compounds, isotope abundance ratios for other elements, ...)[2] have to be applied additionally to obtain more detailed information concerning the production process, to indicate the origin and to establish the traceability of foods. Using light element stable isotope ratio analysis for olive oil samples from different south European regions, and applying statistical procedures, partially good discrimination behaviour was observed.[29] It was concluded that the mathematical clustering of results from samples originating from the same region gives evidence to a differentiation due to climatic areas. The combination of light element stable isotope ratio (N, C, O) and free amino acid ratio results by multivariate data treatments showed the possibility for food authentication in casein cheese samples from Sardinia, Sicily and Apulia.[30] These results make it obvious that combining different methods seems to be the most promising procedure for origin determinations at the moment as various properties of the samples are taken into account. It indicates that the presented results from the investigated cheese samples by the strontium isotope ratio method have to be compared with other methods and procedures to obtain qualitative and quantitative values of the discrimination behaviour.

Cheese is a living system continuously undergoing microbial, enzymatic and chemical modifications and therefore the ripening time—which can be different from dairy to dairy—may play an important role in terms of the stability of values to be measured. It is suggested that Sr abundance isotope ratios may overcome this problems as they are not influenced by time dependent factors or any custodial handling. The method described offers a way of obtaining very stable instrumental responses and thus comparable results with defined and comparable measurement uncertainties. Using additional measured parameters relating to the origin of the investigated foods, discriminant analysis becomes much easier to interpret as the effects from the measurement procedure itself and real differences of the values found in nature are attainable.

As it is assumed that the strontium isotope ratio values found in the cheese samples reflect the geological properties of their place of production, the traceability of the dairy products can only be guaranteed if a strontium variation in the soil is observably larger than the stated measurement uncertainties. As for many other techniques under investigation in the field of food authentication, the results can be changed or blurred, *e.g.*, if supplementary forage or milk from very different locations has been used in quantities which may have different isotopic ratios.

With respect to accurate isotope analysis, other elements will be considered, such as boron and magnesium. These elements are candidates with known variability in nature and will give more specific information with respect to the soil properties, therefore enabling analysts to make more accurate distinctions between origins in a more accurate way.

Conclusions

Sr isotope abundance ratios have been used to assess the assignment of cheese products according to their geographic origin.

A correct and appropriate sample preparation procedure and high precision isotope ratio determinations provide isotope data with small combined measurement uncertainties to enable differentiation of the origin, providing precise geological Sr data are available (soil and/or soil moisture). The examination of the influencing parameters with respect to possible bias effects and an appropriate determination of the measurement uncertainties permit comparability of the measured values.

It becomes obvious that the Sr isotope ratio data can be used to assign the origin of the cheese in cases where there is a large enough difference in the Sr isotope ratios of the milk production site, that is to say different geology and age of rocks and soils.

Concerning the stability of the Sr isotope abundance ratios with time (summer *versus* winter production) the investigations have to be extended, to obtain results from the same locations.

For a more detailed discrimination two further approaches are available: the use of conventional methods of analysis, such as trace elemental analysis and infrared spectroscopy, or a more differentiated isotopic Sr mapping of soils and their bioavailable moisture. Such approaches may produce promising additional results.

Acknowledgements

Appendix

Symbols

R corrected isotope abundance ratio

R' measured isotope abundance ratio

R_{87} $\left(\frac{^{87}Sr}{^{86}Sr}\right)$ isotope abundance ratio, corrected for mass discrimination

R'_{85}, measured isotope amount ratio $\left(\frac{^{87}Rb}{^{85}Rb}\right)$

R_{cheese} isotope amount ratio of cheese samples

Δm_{88} difference of mass ^{88}Sr and the mass of ^{86}Sr

Δm_{87} difference of mass ^{87}Sr and the mass of ^{86}Sr

u_X standard measurement uncertainty of X

u_c combined measurement uncertainty

u_c $(R_{87, cheese})$, combined standard measurement uncertainty of $\left(\frac{^{87}Sr}{^{86}Sr}\right)$ isotope abundance ratios from cheese samples

s standard deviation

R_{cert} certified isotope amount ratio $\left(\frac{^{88}Sr}{^{86}Sr}\right)$ of the reference material

R_{meas}, observed amount ratio $\left(\frac{^{88}Sr}{^{86}Sr}\right)$ of the reference material

Δm mass difference of measured isotope pair or peak width at 10% peak height

ε mass discrimination correction factor per mass unit

I_{87} intensity of mass 87 measured on detector cup H1

I_{86} intensity of mass 86 measured on detector cup Ax

I_{85} intensity of mass 85 measured on detector cup L1

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