

Geographic origin of European Emmental cheese: Characterisation and descriptive statistics

L. Pillonel^a, R. Badertscher^a, M. Casey^a, J. Meyer^a, A. Rossmann^b,
H. Schlichtherle-Cerny^{a,*}, R. Tabacchi^c, J.O. Bosset^a

^a*Agroscope Liebefeld-Posieux (ALP), 3003 Bern, CH Switzerland*

^b*Isolab GmbH, Schweitenkirchen D-85301, Germany*

^c*University of Neuchâtel, Neuchâtel CH-2007, Switzerland*

Abstract

To survey the authenticity of Emmental cheese from some of the main European countries of origin, samples of cheeses manufactured during winter (110 samples) and summer (73 samples) were collected. From a preliminary study, a series of promising analytical methods were selected and applied: total nitrogen, water soluble nitrogen (WSN), 12% TCA soluble nitrogen (TCA-SN), pH-value, volatile short-chain acids, chloride, organic acids, enterococci, obligate heterofermentative lactobacilli (OHL), *Lactobacillus helveticus*, sodium, copper, zinc, magnesium and stable isotope ratios ($\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). The data were analysed by univariate statistical methods according to the geographic origin and the season of production. Significant differences between the regions of origin were found for all parameters investigated ($P \leq 0.001$). Cheeses from some regions showed very specific properties. Seasonal differences were observed in certain regions for acetate, propionate, caproate, WSN, TCA-SN, pyruvate, OHL, zinc and $\delta^{13}\text{C}$ levels.

Keywords: Authenticity; Emmental cheese; Season effect; Stable isotope

1. Introduction

Food authenticity and traceability of origin have become subjects of great interest during the last decade. Economic pressures, combined with new manufacturing technologies and low transport costs, have led to increasing numbers of food scandals and frauds. Various products are subject to adulteration or false denomination, including meat, cereals, coffee, olive oil, dairy products, wine, fruit juices and honey (Lees, 1998).

Among cheeses, Emmental represents a very interesting case; also known as Swiss cheese, Emmental is a

widespread cheese type manufactured in almost all industrialised countries. Its added value depends on the technology used. Only a few regions such as Switzerland, the East part of France, Austria and South Germany manufacture Emmental by traditional methods using, for example, raw milk and copper vats. Some consumers are ready to pay more for these traditional/regional products. Switzerland is especially sensitive to the question of authenticity because of the high priced milk. Swiss Emmental is the most expensive, making fraudulent substitution lucrative (Bosset, 2001). Therefore it is necessary to have analytical means of checking whether the origin of a particular sample of Emmental corresponds with its labelling.

To achieve this goal, a screening study using more than 20 analytical methods was first carried out on 20 authentic cheese samples collected in France,

Corresponding author. Tel.: +41 31 323 81 67; fax: +41 31 323 82 27.
E-mail address: hedwig.schlichtherle-cerny@alp.admin.ch
(H. Schlichtherle-Cerny).

Switzerland, Germany, Finland and Austria (Pillonel, Badertscher Bütikofer, Casey, Dalla Torre, Lavanchy, Meyer, Tabacchi, & Bosset, 2002; Pillonel, Collomb, Tabacchi, & Bosset, 2002; Pillonel, Ampuero, Tabacchi, & Bosset, 2003; Pillonel, Luginbühl, Picque, Schaller, Tabacchi, & Bosset, 2003; Pillonel, Albrecht, Badertscher, Chamba, Bütikofer, Tabacchi, & Bosset, 2003; Pillonel, Badertscher, Froideveaux, Haberhauer, Jakob, Pfammatter, Piantini, Rossmann, Tabacchi, & Bosset, 2003). The aim of these preliminary studies was to select analytical methods or parameters suitable for discriminating the geographic origin of the samples analysed before using them in the large scale study described in this paper. The selection of the methods from the screening was presented in a further paper (Pillonel, Tabacchi, & Bosset, 2003).

The current study follows on from these preliminary studies, building on the knowledge acquired so far. A total of 110 samples from a winter production and 73 from a summer production were analysed using the tools selected from the screening study. The current discussion of the results is limited to descriptive statistics and difference tests on the mean values for each region. Appropriate mathematical models using multivariate statistical analysis and allowing the determination of the geographic origin are presented elsewhere (Pillonel, Bütikofer, Schlichtherle-Cerny, Tabacchi, & Bosset, 2005).

2. Materials and methods

2.1. Cheese samples

Table 1 summarises the geographic origin, ripening time and season of production of the 183 samples analysed. From the 110 winter samples, 20 originate from the preliminary study. To compare and discrimi-

nate the cheese samples according to their origin, the following seven regions were defined: the regions Switzerland (CH), France Savoie (FR), Germany Allgäu (D), Western Austria (A) where cheese was made using raw milk, and Finland (FI), France Brittany (FT_b) and France East-Central (FT_e) where cheese was made using thermised milk. In the last two regions, bacto-fugation was also carried out. The analytical results for the 20 samples used in the preliminary study (Pillonel, Tabacchi et al., 2003) were included in the current study for statistical analyses.

The ripening time (at point of sampling) of the selected samples varied greatly depending on their geographic origin. According to manufacturing usage, Emmental may be sold up to a maximum of 6–7 weeks of ripening (Brittany) or after a minimum of 3 months (Switzerland). The samples were selected on the basis of their ripening time to reflect what consumers may expect in the stores. The scattering of some results within a given region was partly explained by the diversity in ripening of the Emmental samples available (from young to very mature). In Switzerland, for instance, it was very difficult to find Emmental ripened for only 90 days. Most of the Swiss samples had a ripening time varying from 4 to 6 months, with some of them reaching 12 months or even more. We focused our attention on young and medium-aged samples (only 8 samples were ripened for more than 7 months) for which the risk of misidentification was highest.

2.2. Sample preparation

The samples were purchased as blocks and the outer 2 cm was discarded. The first five centimetres from the side were also not used. The samples to be delivered to the various laboratories were cut into slices across the whole height of the block. This helped to avoid misinterpretation of results due to gradients within the

Table 1
Origin, ripening time, season of manufacture and number of samples investigated

Country of origin	Abbr.	Processing of the milk	Median ripening time (days) (min., max.) ^a	Number of summer samples ^b	Number of winter samples ^c
Austria	A	Raw	80 (60, 155)	5	10
Switzerland	CH	Raw	175 (90, 260)	32	38
Germany	D	Raw	101 (71, 210)	6	17
Finland	FI	Therm ^d	90 (81, 90)	4	8
France	FR	Raw	106 (81, 215)	13	19
France	FT _b ^e	Therm, bact	53 (42, 73)	7	12
France	FT _e	Therm, bact	74 (41, 149)	6	6
Total	73	110			

^aAt point of sampling.

^bProduction 15 April, 2002–30 September, 2002.

^cProduction 15 October, 2001–30 March, 2002.

^dFT_b, from the region Brittany; FT_e, from the region East-central.

^eTherm, thermisation; bact, bacto-fugation.

block. Unless otherwise specified, the samples were deep-frozen (-20°C) until analysis.

2.3. Chemical and biochemical analyses

The following analyses were carried out: Volatile short-chain acids using gas chromatography (Badertscher, Liniger, & Steiger, 1993); Fat according to Gerber van Gulik (Anonymous, 1975); Total nitrogen (TN); Water-soluble nitrogen (WSN) and 12% trichloroacetic acid (TCA) soluble nitrogen (TCA-SN) by the Kjeldahl method (Collomb, Spahni-Rey, & Steiger, 1990); Sodium chloride with a potentiometric titration using a silver electrode (Anonymous, 1988); L- and D-lactate; Succinate and pyruvate with enzymatic test kits after extraction (Anonymous, 1989); Activity of L-leucine aminopeptidase (LAP) using L-leucine-4-nitroanilide as substrate by measuring the release of the product *p*-nitroaniline at 405 nm (Bergmeyer, 1974). The pH-value was determined at room temperature using a penetrometric glass electrode (Mettler-Toledo; No. 104063123).

2.4. Microbiological analyses

Enterococci (ECOC) (Mossel, 1978) and obligate heterofermentative lactobacilli (OHL) (Isolini, Grand, & Glättli, 1990) were measured in fresh samples. The occurrence of *Lactobacillus helveticus* was investigated by the polymerase chain reaction (PCR) after extraction of DNA using the High Pure PCR Template Preparation Kit (Roche, Switzerland); PCR amplifications were carried out using Taq DNA polymerase (Applied Biosystems). The specific primers for the detection of *Lb. helveticus* were 5'-CCGAAGGAACNCCTA-ATCTCTTA-3' and 5'-AGCAGATCGCATGATCAG-CT-3', which correspond to internal sequences of the 16S rDNA gene of this organism. The conditions for the PCR were as follows: 10 min at 95°C followed by 33 cycles (30 s at 95°C , 30 s at 55°C , 1 min at 72°C) and finally 10 min at 72°C . Quantification of the 854 bp amplicon was performed by electrophoresis using an Agilent 2100 Bioanalyser (Agilent, Waldbronn, Germany).

2.5. Inorganic compounds and isotope ratios

For the quantification of magnesium and sodium, approximately 1 g of grated cheese was digested with 5 mL nitric acid (650 g kg^{-1} , suprapur Merck AG, Darmstadt, Germany) at atmospheric pressure; for copper and zinc, 2.5 g of material were used. The solutions were analysed, after suitable dilution, with an air-acetylene atomic absorption flame spectrometer (Varian SpectrAA-800; Basel, Switzerland) using FAM accredited methods no. ME05101O.620

(Mg), ME3902O.620 (Na), ME04002O.620 (Cu), ME04202O.620 (Zn).

$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, $^2\text{H}/^1\text{H}$ and $^{34}\text{S}/^{32}\text{S}$ ratios were determined in a protein fraction obtained as follows: the grated cheese samples were freeze-dried and afterwards defatted with petroleum ether (Merck, analytical grade) in a Soxhlet apparatus. In the preliminary study (Pillonel, Badertscher et al., 2003), an older procedure with a further operation was used; the non-fat fraction was adjusted to pH 4.3 and the resultant insoluble fraction washed with water. However no significant differences were observed between the two preparation modes and thus the pH adjustment step was not used in this study. C, N and S were measured by Elementar elemental analyser Vario EL III (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IRMS) AP 2003 (GVI Instruments Ltd. Manchester, UK). D/H ratios were measured using a Delta XL plus IRMS coupled with a high temperature pyrolysis unit (Thermo Instruments GmbH, Dortmund, Germany).

The following standards with known ratios were used: standard casein (Sigma-Aldrich, analytical grade) which had been calibrated in a European research project (SMT4-CT2236-1998) for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, and later for $^2\text{H}/^1\text{H}$ and $^{34}\text{S}/^{32}\text{S}$ versus official reference materials (PEF-1 and NIST-22, V-CDT and silver sulphide, respectively). The values were reported in the δ -scale (‰) according to the corresponding international standards (PDB, NBS-22, V-CDT, air N_2). To check the reliability of the analyses, additional IHRM samples (in-house reference materials) of known isotopic composition were used (wheat flour, lactose, sucrose).

2.6. Statistical analyses

The base 10 logarithm of the bacterial counts was used for calculations. The averages and standard deviations by regions of origin were calculated for each parameter. Descriptive statistics, box plots, analysis of variance (ANOVA), pairwise comparisons of mean values with Fisher's LSD test and *t*-test were performed with Systat for Windows version 9.0 (SPSS Inc., Chicago, IL). Fisher's LSD test was used to compare the various regions of origin ($P \leq 0.001$) and *t*-tests were used to investigate the influence of the season ($P \leq 0.01$).

3. Results and discussion

Indicators of origin for manufactured products may be subdivided into primary and secondary indicators. Primary indicators are not influenced by the technology applied for manufacture; in the case of cheese, compounds acting as primary indicators are transferred from the forage and the water consumed by the herd

into the milk and hence the cheese. Primary indicators are not influenced by cheese making or ripening conditions but depend only on the feed of the cows, which undergoes natural variation over the year. Furthermore, some of the forage may be imported from distant countries, requiring careful interpretation of the results.

Secondary indicators do not depend directly on geographic origin but mainly on the technology used for the transformation of a product, i.e., the milk used. Cheese making is related to local, regional or national traditions leading to differences between cheeses of the same type but of different origin. Starters, heating temperature of the curd, brining and ripening time are some of the manufacturing parameters that are typical for a defined region and lead to chemical, physical or microbial secondary indicators.

In the following ANOVA related to geographic origin, only parameters with $P \leq 0.001$ are presented. In the pair-wise comparison, two groups were considered as different only if $P \leq 0.001$ in the Fisher's LSD test. Furthermore, a box plot was prepared for each parameter to visualise the data, with the aim of locating outliers that could lead to erroneous interpretations of the results of the Fisher's test.

3.1. Primary indicators

Two minerals were investigated in this category (Fig. 1). The magnesium concentration was significantly

higher in region FT_b than in all other regions, except for region D. Zinc also was present at the highest concentration in FT_b whereas the lowest levels were found in CH, D and FR. The origin of such variations between the regions is very difficult to determine. Investigations on the grass and concentrates used as feed in the respective regions would be necessary to help understand these differences. No information on such differences could be found in the literature.

Four isotopic ratios were measured ($^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$; Fig. 1). The water molecules containing heavy isotope (^2H , ^{18}O) drop as rain faster than the light molecules. The highest $\delta^2\text{H}$ values were therefore found where the first rainfalls from the clouds, e.g., in regions such as Brittany which are close to the ocean. The next highest values were logically found in samples from FT_e, a region located in central France. The values for FR and CH lay in the middle, the values for A and D being still lower, because of their greater distance from the sea. The high latitude of Finland played a much more important role than the proximity to the sea and explained the low $\delta^2\text{H}$ values found for samples from this country (Moser & Rauert, 1980). Similar results were obtained for ^{18}O by Rossmann, Haberhauer, Hölzl, Horn, Pichlmayer, & Voerkelius (2000).

The regions where maize silage is fed (i.e. FT_b, FT_e) were characterised by a significantly higher $\delta^{13}\text{C}$ value (Pillonel, Badertscher et al., 2003). Smaller differences were observed between the other regions, which can be

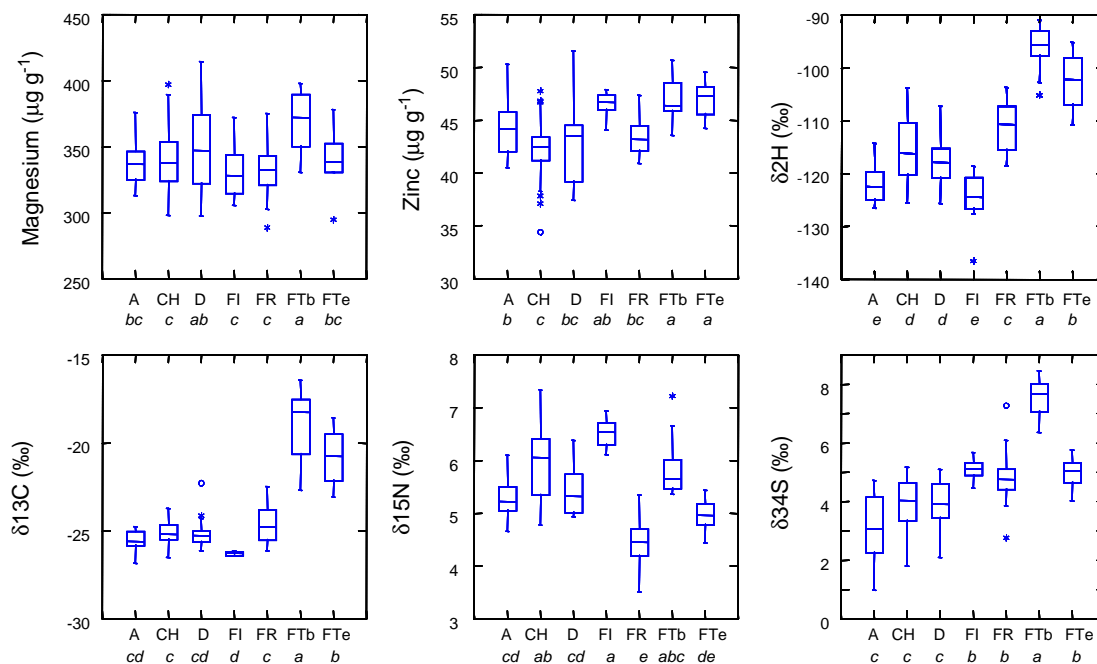


Fig. 1. Box plots of the primary indicators of origin. Regional mean values within a variable with at least one identical letter in cursive are not significantly different. $a > b > c > d$ (= significantly different contents) by using an univariate discriminant analysis. $\delta(\text{‰}) = 1000 [R_{\text{sample}} - R_{\text{standard}}] / R_{\text{standard}}$, where R represents the ratio of the higher mass to the lower mass isotopes. See Table 1 for cheese codes °outside value outside the inner fence; *far out value outside the outer fence.

explained by climatic conditions such as temperature and humidity, which are known to cause shifts in the carbon isotope ratios in plant materials between regions. However, one German sample showed a value too high (-22.3‰) to be only due to climate. Most probably, maize feeding was used there too. The $\delta^{15}\text{N}$ values were the highest in FI, possibly due to a lower proportion of leguminosae or a higher level of organic fertilizers (e.g., with animal manure) in these northern parts of Europe. The lowest values, found in D, A, and especially FR, probably reflected a low N-level fertilisation in these pre-Alpine regions (Rossmann, Kornxl, Versini, Pichlmayer, & Lamprecht, 1998) (Fig. 1).

There is no satisfactory explanation for the low $\delta^{15}\text{N}$ level found in FT_e. In the case of $\delta^{34}\text{S}$, FT_b showed the highest values due to sulphate seaspray. Marine sulphate is known to be highly enriched in ^{34}S as compared with soil sulphate (Krouse, Steward, & Grinenko, 1991). Further sources of ^{34}S fractionation are the nature of the bedrock (Rossmann et al., 1998) and to a lesser extent anthropogenic emissions (Pichlmayer, Schöner, Seibert, Stichler, & Wagenbach, 1998). The interpretation of the remaining $\delta^{34}\text{S}$ values was therefore more difficult.

3.2. Secondary chemical indicators

Fig. 2 presents the results of secondary chemical indicators. The formate concentrations in FT_b were significantly lower than in those from CH, D and FI. Formate is normally produced from citrate by facultatively heterofermentative bacteria (FHL). However, in the preliminary study, no correlation was found between levels of citrate and FHL (Pillonel, Badertscher et al., 2002). The highest acetate concentrations were found in FR and FI, and the lowest in FT_b and FT_e. Acetate is produced stoichiometrically with propionate during lactate fermentation; therefore propionate followed the same trend as acetate ($r = 0.80$). The correlations were, however, poor for the regions FI ($r = 0.58$) and FT_b ($r = 0.65$) when considered separately. Other pathways such as those described by Crow & Turner (1986) and Sebastiani & Tschager (1993) might therefore have an impact on the production of acetate and propionate. However, the current study was not designed for answering such questions in detail.

As expected, the highest butyrate concentrations were found in both FT_b and FT_e, regions where silage is allowed as feed and can be a source of contamination of milk with clostridia. In cheeses from Finland, though silage is also used, the concentrations encountered were not significantly different from the silage-free regions. This is probably due to a very strict production hygiene in the feeding and milking zones of Finnish farms. In all other regions, outliers were found, indicating the difficulty of producing spore-free milk. In normal cases, lipolysis accounts for about only 1 mmol butyrate

per kg Emmental (Collomb, Malke, Spahni, Sieber, & Bütikofer, 2003). Strong lipolysis would be accompanied by abnormally high caproate levels. High butyrate levels did not correlate with caproate values in the present study, except in two samples from Austria, where significant lipolysis probably occurred.

The content of sodium chloride, which originates almost exclusively from the brine, was significantly higher in cheese from region D than in cheeses from all other regions. The pH of CH and FT_b was significantly lower than in cheeses from the other regions except FT_e. No correlation was found between the sum of the investigated acids and the pH values, probably due to the buffering effect of free amino acids, levels of which are strongly dependent on the degree of proteolysis.

The TN content of the cheeses were quite similar. Only FR had significantly higher values than FT_b, CH and D. WSN and 12%TCA-SN showed much greater differences; the highest degree of proteolysis was found for CH, D and FR. In the case of CH and FR, these results could partly be explained by the longer ripening time. The lowest value was found in FT_b, where the blocks were ripened for only 6–7 weeks. Ratios such as WSN/TN and TCA-SN/WSN did not offer any additional information (not shown).

Among the minerals investigated, two belonged to the group of secondary indicators, i.e., copper and sodium. The copper concentration in cheese was naturally significantly higher in the regions still using traditional copper vats (A, CH, D, FR). However, some very low values were also found in D and in FR (Fig. 2). It is difficult to define a concentration limit for copper in cheese which would imply the use of copper vats, due to the lack of sufficient information from the individual manufacturers. The copper concentration also depends on the way the milk is stored before processing. In this study, FI samples contained even more copper because of addition of copper sulphate to the milk prior to cheese making. However, Emmental from certain Finnish manufacturers can also be made without addition of copper sulphate (H. Jatila, personal communication, 2003). This additive-free Finnish cheese was not included in the study. The outlier sample from Austria was probably also manufactured with copper sulphate addition. Sodium originated largely from brining and therefore was correlated with the chloride measurements ($r = 0.73$).

3.3. Secondary biological indicators

Results for the secondary biological parameters are shown in Fig. 3. Enterococci OHL are not desirable microorganisms in cheese because they increase the risk of secondary fermentation. The lowest number of enterococci was found in CH, FT_b and FI. OHL numbers were lowest in CH and FI. Both species can

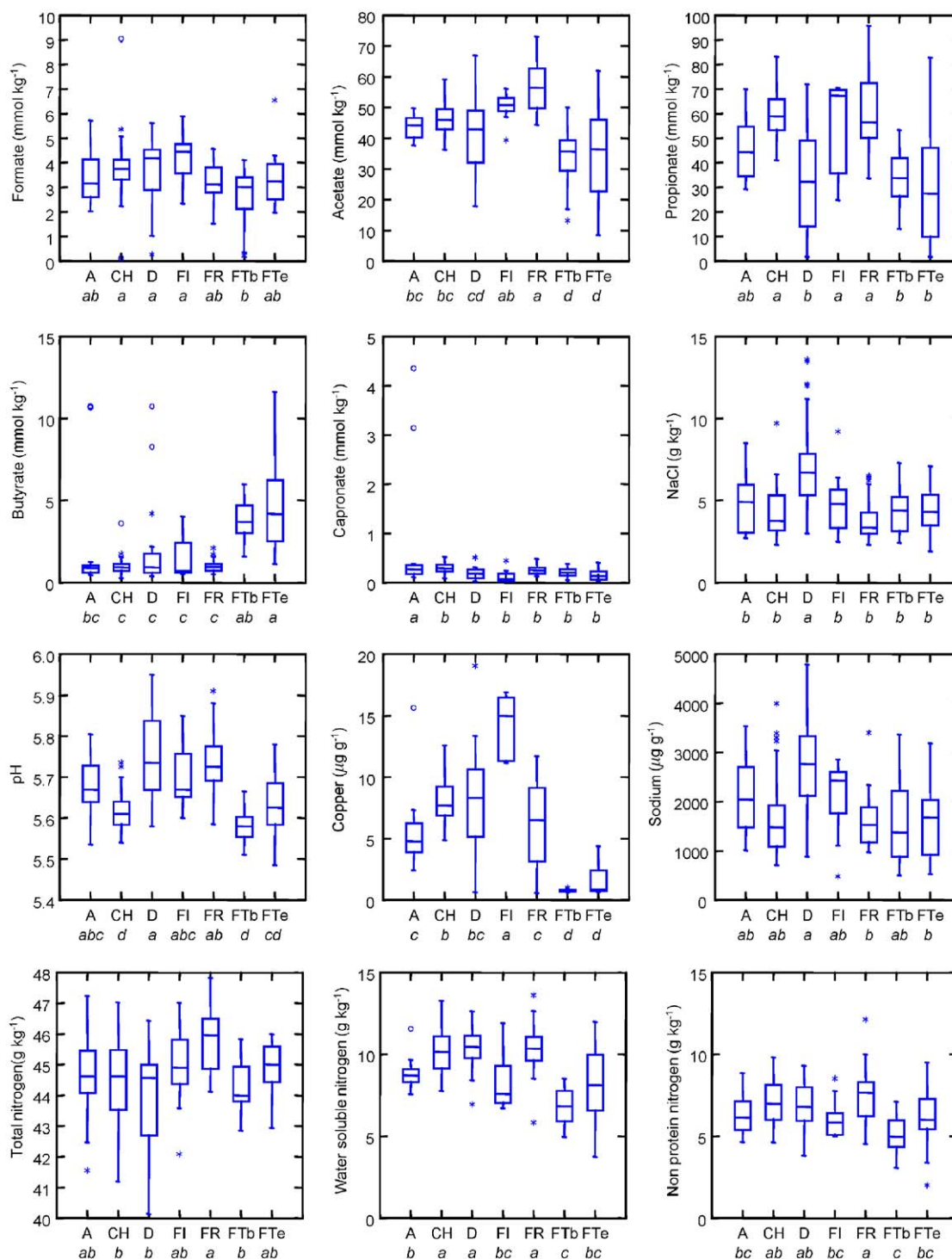


Fig. 2. Box plots of the secondary chemical indicators of origin. Regional mean values within a variable with at least one identical letter in cursive are not significantly different. $a > b > c > d$ (= significantly different contents) by using an univariate discriminant analysis. See Table 1 for cheese codes.

be kept at low level using selected starter cultures and strict production hygiene. No correlation between the ripening time of the samples and the number of enterococci or OHL was observed; this illustrates that the age of the cheese has a negligible importance on the counts when different samples are compared.

Lactate was negatively correlated with both acetate ($r = -0.60$) and propionate ($r = -0.73$), which are its metabolites. Except for two samples, FI no longer contained lactate, mainly due to a high level of curd washing (H. Jatila, personal communication, 2003). Also the values for pyruvate were extremely low in FI.

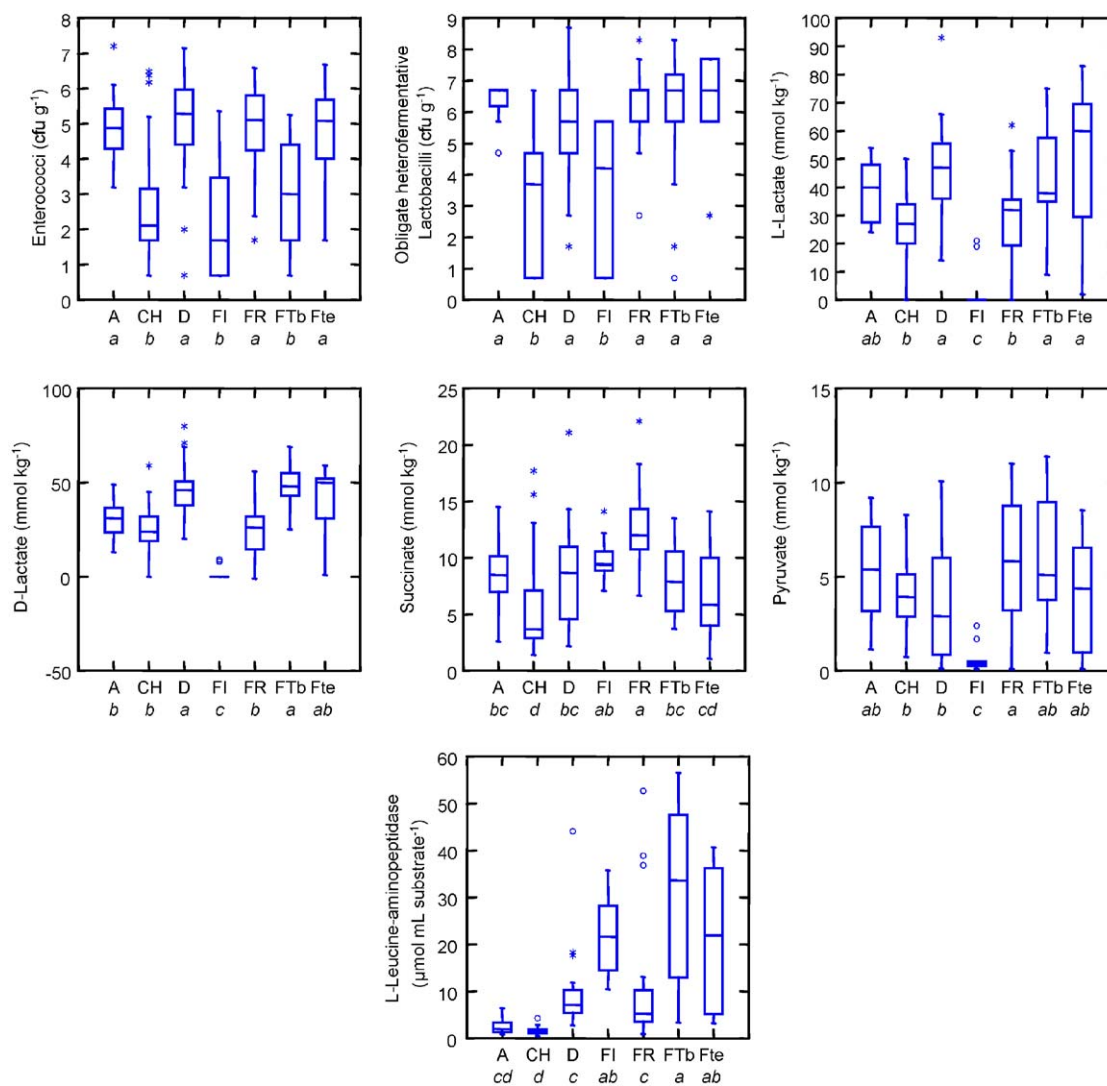


Fig. 3. Box plots of the secondary biological indicators of origin. Regional mean values within a variable with at least one identical letter in cursive are not significantly different. $a > b > c > d$ (= significantly different contents) by using an univariate discriminant analysis. See Table 1 for cheese codes.

The calculation of the L-/D-lactate ratio (data not shown) did not yield any additional information. Succinate is produced by certain propionic acid bacteria with strong aspartase activity (Fröhlich-Wyder, Bachmann, & Casey, 2002). A moderate aspartase activity may positively influence the quality of Emmental cheeses (e.g., openness and flavour intensity). CH showed significantly lower values for succinate than all other regions except FT_e, while FR had the highest concentration. It is difficult to interpret these results because no convincing correlation was found between aspartate and succinate in data obtained in the preliminary study (Pillonel, Badertscher et al., 2002).

The presence of *Lb. helveticus* was determined semi-quantitatively by specifically amplifying a fragment of the 16S rDNA gene. The information obtained was

more or less limited to a presence/absence response. In Switzerland, *Lb. helveticus* is never used in Emmental cheese starters because of its strong proteolytic activity. The amount of *Lb. helveticus* DNA measured in CH was below $1.2 \text{ ng } \mu\text{L}^{-1}$ in all samples and, in most of them, it was below the detection limit of $0.1 \text{ ng } \mu\text{L}^{-1}$. The values close to $1.0 \text{ ng } \mu\text{L}^{-1}$ indicated a natural contamination of the milk from the environment, which most probably occurred during milking. In other regions, Emmental is also sometimes manufactured without the use of *Lb. helveticus*. In some samples from FT_b, D and A, values for *Lb. helveticus* DNA below $1 \text{ ng } \mu\text{L}^{-1}$ were found. In Fig. 4, the results were classified into two groups ($< 3 \text{ ng } \mu\text{L}^{-1}$ and $\geq 3 \text{ ng } \mu\text{L}^{-1}$). In the second group, the direct addition of *Lb. helveticus* can be assumed; therefore, the samples in this group cannot be

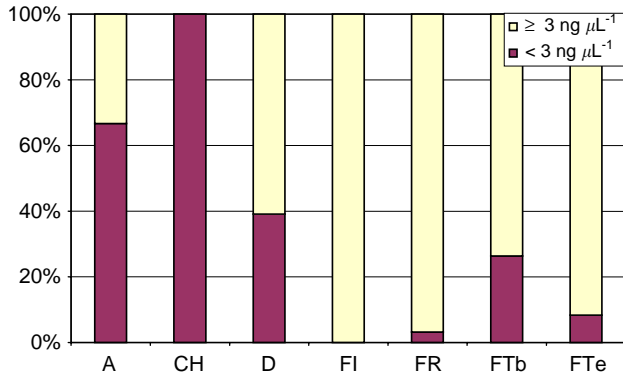


Fig. 4. Proportion of Emmental cheese samples above and under the limit of $3 \text{ ng } \mu\text{L}^{-1}$ DNA from *Lb. helveticus* by regions of origin. See Table 1 for cheese codes.

of Swiss origin. A limit of $3 \text{ ng } \mu\text{L}^{-1}$ was chosen to avoid any risk of erroneous positive classification of Swiss Emmental.

An indirect way of obtaining information on the presence of *Lb. helveticus* is the measurement of the LAP activity. This enzyme is produced in significantly higher amounts by *Lb. helveticus* than by *Lactobacillus delbrueckii* (Bouton, Guyot, Dasen, & Grappin, 1993). CH and A showed significantly lower LAP activities than the other groups. In CH, all values, except for one at 4.5 IU, were less than 3 IU; 75% of the cheese samples from the other regions showed values higher than 4 IU.

Mostly, the correlation between the LAP and the *Lb. helveticus* values within each region was poor. For instance in CH, the LAP values varied from 0.7 to 4.3 even when no *Lb. helveticus* was detected. The opposite phenomenon was also often observed, i.e., high numbers of *Lb. helveticus* for relatively low LAP values. The aminopeptidase activity of other microorganisms such as *Streptococcus thermophilus* and the difference in activity between strains of *Lb. helveticus* might explain these apparent discrepancies (Prost & Chamba, 1994).

3.4. Seasonal influence

Most of the indicators of the origin of Emmental cheese (both primary and secondary) were influenced by the season because the composition of the forage changes depending on the time of the year. Particularly where silage feeding is not allowed, the change from green fodder to hay could have important consequences on the results. Table 2 shows, for each separate region, the parameters that changed significantly with the season. Only the parameters dealt with in Figs. 1–3 were investigated. Interpretation of these results is made difficult because of the lack of precise information on

Table 2

Parameters significantly influenced by the season of manufacture ($P \leq 0.01$) (see Table 1 for cheese codes)

	A	CH	D	FI	FR	FT _b	FT _c
Formate	W ^a	—	—	—	—	—	—
Acetate	S	—	S	—	—	—	—
Propionate	S	—	S	W	—	—	—
Caproate	—	—	—	S	—	—	—
WSN	—	—	—	S	—	—	—
TCA—SN	S	—	—	—	S	—	—
Pyruvate	—	—	—	—	S	—	—
Obl. hetero lb ^b	—	—	—	—	—	—	—
Zn	S	—	S	—	S	—	—
$\delta^{13}\text{C}$	W	—	—	—	W	W	W

^aW, content significantly higher for the winter production (15 October–30 March); S, content significantly higher for the summer production (15 April–30 September).

^bObligate heterofermentative lactobacilli.

both manufacture and herd feeding practices. Moreover a year-to-year comparison would be needed for reliable conclusions.

Switzerland was the only region where no significant differences due to season were observed. No differences within the secondary indicators were found in both French regions where thermisation is applied (FT_b and FT_c). The raw milk French Emmental (FR) had higher level of pyruvate, TCA-SN and zinc during summer. Samples from A and D showed similar trends, with higher contents of acetate, propionate and zinc during the summer. Moreover the TCA-SN values were higher and the formate concentrations lower in summer than in winter in A. In FI, three compounds showed significant differences; propionate was higher in winter whereas caproate and WSN were higher in summer. The increase in the $\delta^{13}\text{C}$ value in winter in FT_b and FT_c was not surprising due to the increased proportion of maize fed during the cold season. The significant change in $\delta^{13}\text{C}$ observed in FR and A was difficult to explain; however, in both seasons the values remained below ($< -22\%$) those typical of maize feeding regions.

4. Conclusions

The objective of the current work was to statistically evaluate parameters that had been selected as likely to discriminate between various geographic origins of Emmental cheese. The following seven regions were defined: Austria, Finland, France Savoie, France Brittany, France East-Central, Germany and Switzerland. Some of these regions showed very characteristic properties. For instance, samples from Finland contained almost no lactate and pyruvate, and more copper than the other samples. Samples of Swiss origin had

significantly lower levels of *Lb. helveticus* and a lower LAP activity was also found. However, in all cases, multivariate statistical analysis will be required to approach 100% recognition or classification. The latter is subject of a further publication (Pillonel et al., 2005).

The influence of the season of production on the parameters and for each region was also investigated. Regions with a high level of milk standardisation such as France East-Central and France Brittany did not show any differences for secondary indicators of origin. The season also influenced the values for certain secondary indicators in Switzerland ($n = 1$), Austria ($n = 2$), Germany ($n = 2$), France Savoie ($n = 2$) and in Finland ($n = 4$). As far as the primary indicators of origin are concerned, the zinc levels were higher in summer in Germany and France Savoie and the $\delta^{13}\text{C}$ values were higher in winter in the region where maize silage is used as animal feed.

Acknowledgements

The authors thank the Swiss Federal Office of Public Health for its financial and logistical support, G. Urbach and G. Gremaud for their reviewing of the publication as well as J.F. Chamba (ITFF, France), M. Carl (MUVA Germany), H. Spring (Emmentaler SwitzerlandTM) and H. Jatila (Valio Ltd, Finland) for collecting the samples.

References

- Anon (1975). Cheese. *Determination of fat content*. Van Gulik method. ISO 3433.
- Anon (1988). Cheese and processed cheese products. *Determination of chloride content*. Potentiometric titration method. IDF Standard 88A.
- Anon (1989). *Methods of biochemical analysis and food analysis*. Boehringer Mannheim GmbH.
- Badertscher, R., Liniger, R., & Steiger, G. (1993). Bestimmung der flüchtigen Fettsäuren in Käse aus dem Wasserdampfdestillat mit "Headspace—GC/FID". *FAM Information*, 272.
- Bergmeyer, K.U. (1974). *Methoden der enzymatischen analyse*. Band I, 3. Auflage. (pp. 995–1000) Weinheim: Verlag Chemie.
- Bosset, J. O. (2001). Authenticity of Emmentaler cheese Switzerland. Project announcement. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, 92, 328–332.
- Bouton, Y., Guyot, P., Dasen, A., & Grappin, R. (1993). Proteolytic activity of lactobacilli strains isolated from starters and Comté. 1. Validation of laboratory tests on mini-cheeses. *Lait*, 73, 265–279.
- Collomb, M., Malke, P., Spahni, M., Sieber, R., & Bütikofer, U. (2003). Dosage des acides gras libres dans le fromage par chromatographie gaz-liquide: précision de la méthode et différences saisonnières de la lipolyse dans divers fromages suisses. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, 94, 212–229.
- Collomb, M., Spahni-Rey, M., & Steiger, G. (1990). Dosage de la teneur en azote selon kjeldahl de produits laitiers et de certaines de leurs fractions azotées à l'aide d'un système automatisé. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, 81, 499–509.
- Crow, V. L., & Turner, K. W. (1986). The effect of succinate production on other fermentation products in Swiss-type cheese. *New Zealand Journal of Dairy Science and Technology*, 23, 75–85.
- Fröhlich-Wyder, M. T., Bachmann, H. P., & Casey, M. G. (2002). Interaction between propionibacteria and starter/non-starter lactic acid bacteria in Swiss-type cheeses. *Lait*, 82, 1–15.
- Isolini, D., Grand, M., & Glättli, H. (1990). Selektivmedien zum Nachweis von obligat und fakultativ heterofermentativen Laktobazillen. *Schweizerische Milchwirtschaftliche Forschung*, 19, 57–59.
- Krouse, H. R., Steward, J. W. B., & Grinenko, V. A. (1991). SCOPE 43. In H. R. Krouse, & V. A. Grinenko (Eds.), *Stable isotopes: natural and anthropogenic sulfur in the environment* (pp. 267–306). Chichester: Wiley.
- Lees, M. (1998). *Food authenticity—issues and methodologies*. Eurofins Scientific: France.
- Moser, H., & Rauert, W. (Eds.). (1980). *Isotopenmethoden in der hydrologie*. Gebrüder Bornträger: Berlin, Stuttgart.
- Mossel, D. A. A. (1978). Streptococci of Lancefields group-D in foods—their significance, enumeration and control. *Archiv für Lebensmittel-Hygiene*, 29, 121–127.
- Pichlmayer, F., Schöner, W., Seibert, P., Stichler, W., & Wagenbach, D. (1998). Stable isotope analysis for characterization of pollutants at high elevation alpine sites. *Atmospheric Environment*, 32, 4075–4085.
- Pillonel, L., Albrecht, B., Badertscher, R., Chamba, J. F., Bütikofer, U., Tabacchi, R., & Bosset, J. O. (2003). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Parameters of proteolysis and rheology. *Italian Journal of Food Sciences*, 15, 49–62.
- Pillonel, L., Ampuero, S., Tabacchi, R., & Bosset, J. O. (2003). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Volatile compounds by GC/MS-FID and electronic nose. *European Food Research and Technology*, 216, 179–183.
- Pillonel, L., Badertscher, R., Bütikofer, U., Casey, M., Dalla Torre, M., Lavanchy, P., Meyer, J., Tabacchi, R., & Bosset, J. O. (2002). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. *European Food Research and Technology*, 215, 260–267.
- Pillonel, L., Badertscher, R., Froideveaux, P., Haberhauer, G., Jakob, A., Pfammatter, E., Piantini, U., Rossmann, A., Tabacchi, R., & Bosset, J. O. (2003). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Stable isotope ratios, major, trace and radioactive elements. *Lebensmittel Wissenschaft und Technologie*, 36, 615–623.
- Pillonel, L., Bütikofer, U., Schlichtherle-Cerny, H., Tabacchi, R., & Bosset, J. O. (2005). Geographic origin of European Emmentaler. Use of discriminant analysis and artificial neural network for classification purposes. *International Dairy Journal*, in press, doi:10.1016/j.idairyj.2004.10.010.
- Pillonel, L., Collomb, M., Tabacchi, R., & Bosset, J. O. (2002). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Free fatty acids, triglycerides and fatty acid composition of cheese fat. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, 93, 217–231.
- Pillonel, L., Luginbühl, W., Picque, D., Schaller, E., Tabacchi, R., & Bosset, J. O. (2003). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Mid- and Near-Infrared spectroscopy. *European Food Research and Technology*, 216, 174–178.

- Pillonel, L., Tabacchi, R., & Bosset, J. O. (2003). Analytical methods for the determination of the geographic origin of Emmental cheese. Summary of a screening study. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, *94*, 60–69.
- Prost, F., & Chamba, J. F. (1994). Effect of aminopeptidase activity of thermophilic lactobacilli on Emmentaler cheese characteristics. *Journal of Dairy Sciences*, *77*, 24–33.
- Rossmann, A., Haberhauer, G., Hölzl, H., Horn, P., Pichlmayer, F., & Voerkelius, S. (2000). The potential of multielement stable isotope analysis for regional origin assignment of butter. *European Food Research and Technology*, *211*, 32–40.
- Rossmann, A., Kornexl, B. E., Versini, G., Pichlmayer, F., & Lamprecht, G. (1998). Origin assignment of milk from alpine regions by multielement stable isotope ratio analysis (Sira). *Rivista di Scienza dell'Alimentazione*, *27*, 9–21.
- Sebastiani, H., & Tschager, E. (1993). Succinatbildung durch Propionsäurebakterien—Eine Ursache der Nachgärung von Emmentaler? *Deutsche Molkereizeitung*, *114*, 76–80.