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Analytical Methods for the Determination of the Geographic Origin of Emmental Cheese. Summary of a Screening Study*

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Introduction

Food authenticity is a broad analytical challenge for food chemists, including many different types of misrepresentations (1). Determination of the geographic origin is one aspect which is receiving more attention with the increasing mobility and the low transport costs of our society. Consumers are used to agricultural products coming from all over the world, but what about Emmental cheese from Bretagne, Finland, Ireland or even New Zealand? It may sound strange to most Swiss consumers but less than 10 % of the Emmental produced in Europe is Swiss (2).

The imminent opening of the Swiss cheese market will promote importation of less expensive cheeses produced in countries with low-cost milk. Emmental cheese, often called "Swiss cheese", is the example par excellence of a cheese variety that is produced in many countries around the world. It is often difficult or sometimes impossible to differentiate a prepacked young Emmentaler SwitzerlandTM from a foreign one by sensory analyses. The risk of finding mislabelled cheeses in stores should therefore not be underestimated. For the protection of the consumers, canton chemists need efficient analytical tools, in addition to rigorous accounting controls, to check the origin of cheese. The goal of the current 3-years research project, carried out at the Federal Dairy Research Station with the financial support of the

*Paper dedicated to Mrs. Helene Griessen on the occasion of her retirement in gratitude for her valuable processing of many submitted manuscripts.

Swiss Federal Office of Public Health (3), is to deliver such analytical tools to canton chemists.

Many analytical methods have been proposed for solving problems of authenticity (4). For instance, the techniques used in meat authentication were recently reviewed (5). The matrix of cheese is very complex and no single method could solve the problem. In order to find which is the best set of analytical methods, a preliminary study in the form of a screening test was carried out in a first step. This work, which focuses on Emmental cheese, may be extended in the future to other cheese types such as raclette or PDO (protected denomination of origin) cheeses.

Twenty Emmental cheese samples from six regions were investigated using more than 30 analytical methods. All results have been or will be published in various journals (6–11). This paper examines the use of these methods for the differentiation between Emmentaler Switzerland™ and those Emmental cheeses produced in other countries. The results of this preliminary study are shortly presented here along with the methods that will be used for the remainder of the project.

Material and methods

Table 1 summarises the origin, the date of manufacture and the ripening time of the samples used in the screening test. Six samples originated from Switzerland, three each from Allgäu (D), Vorarlberg (A), Bretagne (F) and Savoie (F) and two from Finland. The cheese samples were chosen with different ripening time (2.5–4 months) according to availability at the retailers in the corresponding regions. For most regions, this means a ripening time of less than three months. In Switzerland however, a 4-months old Emmentaler is still considered very young. We limited our investigations to young Emmental because there the risk of confusion is highest. For more details see (6).

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

<i>Abbreviation</i>	<i>Region (country)</i>	<i>Number of samples</i>	<i>Date of manufacture</i>	<i>Ripening time (months)</i>
AL	Allgäu (D)	3	25.12.2000	4
BR	Bretagne (F)	3	20.02.2001	2.5
CH	Switzerland (CH)	6	26.12.2000	4
FI	Middle Finland (FI)	2	04.02.2001	3
SA	Savoie (F)	3	05.02.2001	3
VO	Vorarlberg (A)	3	02.02.2001	3

The analytical methods tested are listed in tables 2–4. For details, see the corresponding references indicated therein.

To evaluate the potential of each parameter to discriminate between cheeses, a difference test on the mean value of each region was calculated. Furthermore princi-

Table 2
Methods which will no longer be used

<i>Methods/Parameters</i>	<i>Reason for rejection</i>	<i>Reference</i>
Biogenic amines	Strong variation within a region	7
OPA ¹	Redundance with the NPN-fraction	6
Propionic acid bacteria	No significant differences	6
Enterococci, facultative hetero-fermentative und salt tolerant lactobacilli	Poor significant differences	6
Water, alkaline phosphatase, citrate, vitamin A und E	No significant differences	6, 7
Rheology	No significant differences except for penetration depth	7
Colour measurement	Strong dependence on the season	6
Fat chemistry, triglycerides, free fatty acids	Time-consuming, strong dependence on the season	9
Radioactive compounds	Poor significant differences, high cost	11
ICP-MS ²	High cost	11

¹ o-phthalaldehyd-value

² Inductively Coupled Plasma Mass Spectrometry

Table 3
Promising but time-consuming methods

<i>Methods or parameters</i>	<i>Separated</i>	<i>Not separated</i>	<i>Reference</i>
HPLC ¹ – peptide profile	FI, (CH+VO), (BR+SA)	AL	7
SDS-PAGE ²	(BR, FI)	all others	7
Volatile compounds using GC ³	CH, AL, VO, SA, FI	BR	10
Free amino acids	CH, VO, BR, AL, FI	SA	7

AL=Allgäu, BR=Bretagne, CH=Switzerland, FI=Finland, SA=Savoie, VO=Vorarlberg

Regions within brackets are not separated from one another

¹ High Performance Liquid Chromatography

² Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

³ Gas Chromatography

pal component analysis (PCA) was carried out to visualise the natural groupings generated by the selected parameters.

Results and discussion

Two types of geographic indicators may be defined: the primary and secondary ones. Primary indicators are independent of the technology used. They are only

Table 4
Selected methods for the remainder of the project

Methods or parameters	Separated	Not separated	Reference
Infrared spectroscopy ¹	All regions		8
Volatile compounds using electronic nose	All regions ²		10
Obligate heterofermentative Lb.	CH ²	All others	6
<i>Lb. helveticus</i>	CH	All others	6
pH-value, L-lactate, succinate, pyruvate, N-fractions ³ , fat, sodium chloride	VO, FI, BR, CH, AL ² , SA ²		6
Volatile short-chain acids	BR, FI ²	AL, CH, SA, VO	6
Atomic absorption spectroscopy ⁴	BR, FI, CH ² , VO ² , AL ²	SA	11
Isotope ratio mass spectrometry ⁵	FI, BR, SA	CH, VO, AL	11

¹ Using a linear discriminant analysis on the spectra.

² A clear grouping tendency was observed for these regions. They are however not perfectly separated.

³ including total nitrogen, water soluble nitrogen and non protein nitrogen

⁴ including the elements calcium, copper, magnesium, manganese, molybdenum, sodium and zinc.

⁵ D/H, ¹³C/¹²C, ¹⁵N/¹⁴N, ¹⁸O/¹⁶O and ⁸⁷Sr/⁸⁶Sr

influenced by the origin and the composition of the milk. Forage and drinking water, influenced by e.g. nature of soil, climatology and human activity, are the two factors influencing these indicators. Stable isotope ratios, radioactive elements and certain trace elements belong to this group. The secondary indicators are dependant on the technology used. They include all fabrication steps, starters and non-starters bacteria and ripening conditions. Each region produces a typical Emmental that, hand-made or industrial, expresses a character of origin which may be defined by secondary indicators. This group contains mainly organic compounds such as peptides, free amino acids, organic acids, enzymes, volatile compounds and microorganisms.

Primary geographic indicators

Stable isotope ratios have already been successfully used for the determination of origin of Swiss wines (12). In the current investigation, the following five isotope ratios were investigated using isotope ratio mass spectrometry (IRMS): D/H, ¹³C/¹²C, ¹⁵N/¹⁴N, ¹⁸O/¹⁶O and ⁸⁷Sr/⁸⁶Sr. "Bretagne" showed the highest ¹³C/¹²C ratio because of the maize forage fed to the cows during the winter months. Maize belongs to the group of C4-plants which are enriched in ¹³C because of their specific pathway for fixing carbon dioxide. Levels of D/H and ¹⁸O/¹⁶O are often related to each other and depend on the distance from the sea, the altitude and the climatic conditions. They both presented the highest values in "Bretagne" due to the prox-

imity to the sea. The $^{15}\text{N}/^{14}\text{N}$ ratio is normally higher when organic fertilisers are intensively used. This ratio was lower in "Savoie", probably relating to the more extensive use of the pastures in this prealpine region. The four parameters mentioned above, though independent of the manufacturing process, undergo seasonal variation which must be taken into account. $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is complementary and very useful for origin assignment as it is only dependent on the type of rock where the cows grazed and not on human activity, climate or season of production. The supplementation with imported forage (maize, concentrates, etc.) can, of course, modify the results. The Sr-ratio was the highest in "Finland" because of the old acidic rock bed (granite) underlying this country. The three geographically close regions "Switzerland", "Allgäu" and "Vorarlberg" could not be separated using stable isotope ratios (fig. 1).

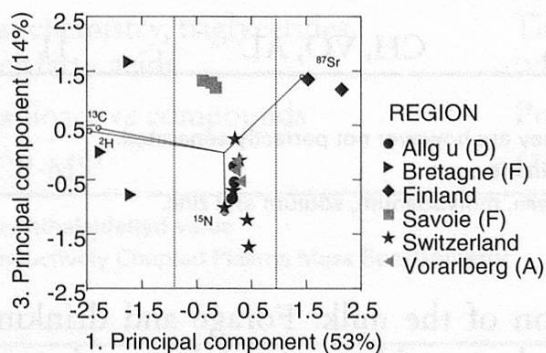


Figure 1 Principal component analysis of the parameters $\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{15}\text{N}$, $\delta^{87}\text{Sr}$. Clear separation of the groups "Finland", "Savoie" and "Bretagne" from the other regions

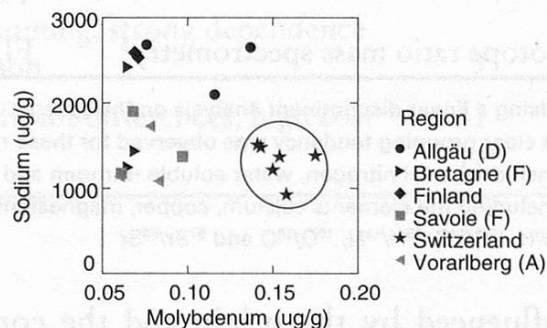


Figure 2 Concentration of molybdenum and sodium by ICP-MS and AAS respectively. Separation of Emmentaler SwitzerlandTM from all other samples. "Vorarlberg" and "Allgäu" are distinctly separated one from the other using these two parameters

Some trace elements also showed significant differences. The concentrations of sodium and molybdenum made possible the discrimination of the three latter regions. Emmentaler SwitzerlandTM was even completely separated from the other samples (fig. 2). The radioactive isotopes ^{137}Cs , ^{90}Sr , ^{234}U and ^{238}U showed few significant differences between the regions.

Secondary geographic indicators

This category contains more than 20 methods or parameters. The microbiological analyses delivered interesting information on the Emmentaler SwitzerlandTM.

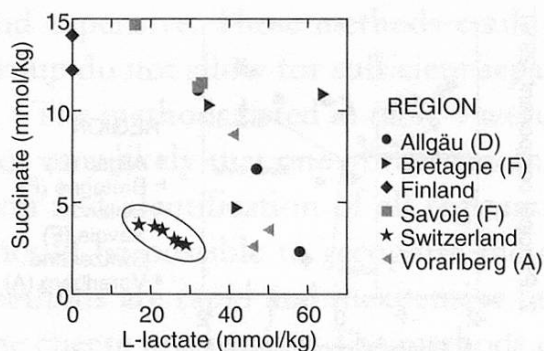


Figure 3 **Discrimination of the Emmentaler Switzerland™ according to L-lactate and succinate concentrations**

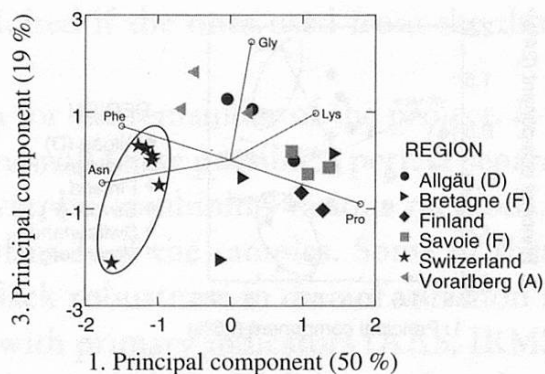


Figure 4 **Principal component analysis of the relative concentration of asparagine, glycine, lysine, phenylalanine and proline (total free amino acids = 100 %). Separation of the regions "Switzerland"**

Significantly fewer obligate heterofermentative *Lactobacilli* and *Lb. Helveticus* were found in this cheese. The former are kept low with specific cultures while the latter are not used in starters at all because both have a high proteolytic activity that may lead to failures in texture and aroma. With the two parameters L-lactate and succinate, it was possible to clearly separate the Swiss products from the others (fig. 3).

Combining the five free amino acids asparagine, glycine, lysine, phenylalanine and proline by principal component analysis, a good separation of the group "Switzerland" was achieved (fig. 4).

Further parameters showed significant differences between the regions. A PCA of the values obtained for pyruvate, L-lactate, fat and pH-value led to the separation of "Finland", "Vorarlberg" and "Bretagne" (fig. 5).

Infrared spectroscopy made possible a 100 % correct classification of all groups using linear discriminant analysis (LDA) of the principal component scores. However, the separation is not visible on the 2-dimensional representation. Furthermore, it must be emphasised that trained classification techniques such as LDA require a large data set to be reliable. These promising results must be confirmed by further investigations.

Fat composition, which is strongly related to the type of forage, was less interesting because of the high seasonal variations and the difficulty of interpreting the results. For certain parameters or groups of parameters such as the conjugated linoleic acid (CLA), a correlation between the altitude of production zone and the concentration in cheese was however observed.

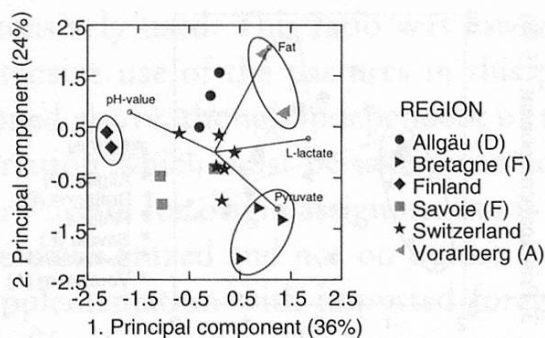


Figure 5 Principal component analysis of the parameters fat, L-lactate and pyruvate as well as pH-value. Separation of the groups "Finland", "Vorarlberg" and "Bretagne"

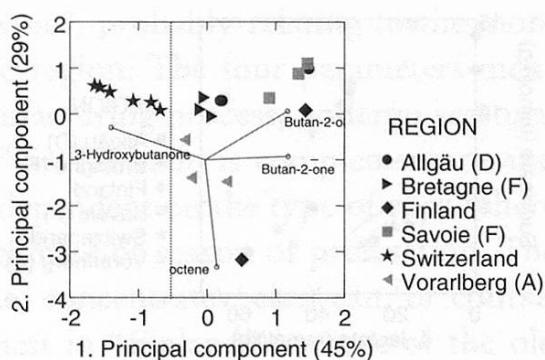


Figure 6 Principal component analysis using the concentrations of butan-2-one, 3-hydroxybutanone, butan-2-ol and octene. 100% separation of the Emmentaler Switzerland™

Valuable information was obtained from the volatile compounds investigated using gas chromatography. A combination of the peak heights of the four compounds butan-2-one, 3-hydroxybutanone, butan-2-ol and octene by PCA made possible a good separation of the Emmentaler Switzerland™ (fig. 6). However such analyses are time-consuming and expensive. The performance of a mass spectrometry-based electronic nose was therefore tested. The results, not presented here, were promising and could be enhanced in the future by incorporating a pre-concentration step of the volatiles prior to injection into the spectrometer. Further investigations will be carried out along these lines.

Finally, some major and trace elements may be assigned to the group of secondary indicators. The best example is copper, which has very different concentrations depending on the type of vat used. In Bretagne, where Emmental is made in stainless steel closed vats, copper concentrations are approximately one order of magnitude lower than in Emmental manufactured in the traditional copper vats.

Conclusions

To select the criteria to be used for the remainder of the project, the methods tested within the current screening programme were divided into three groups according to the results obtained. The first group contains the methods which will no longer be used (table 2). The differences were either not significant enough or the dependence on the season was too strong. ICP-MS (Inductively Coupled Plasma Mass Spectrometry) will no longer be applied because of the difficulty of finding appropriate reference materials and its high cost. The interesting molybdenum concentrations will be investigated using atomic absorption spectroscopy (AAS).

Table 3 lists the methods that provided good results but were time-consuming and expensive. These methods could be exploited if the ones used from the third group do not allow for sufficient separation.

The methods listed in table 4 were chosen for the remainder of the project. It is not very likely that one of these methods alone will make possible a perfect separation and identification of all regions. However, by combining various methods it should be possible to recognise the origin of each of the samples. Some of these methods are rapid and inexpensive but may lack robustness in case of variation in the cheese production. The methods dealing with primary indicators (AAS, IRMS) require more time and skill but are less subject to variations. Both types of methods are therefore complementary and should be considered jointly. The investigations on the geographic situation and the manufacturing method together warrant a high confidence level for the classification of the origin.

During the year 2002–2003, the selected parameters will be tested on a new set of samples from the same six regions. This time, approximately 80 samples from winter and summer production will be investigated. This will then make possible the evaluation of any seasonal influence on the individual parameters measured. Also, powerful statistical analyses such as linear discriminant analysis or artificial neural networks will be used to classify the 160 samples analysed. A mathematical model for the control of the origin (Swiss or non-Swiss) will be proposed.

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Summary

The current paper presents the summary of results obtained during the first part of a 3-year research project on the authenticity and geographic traceability of Emmental cheese. The aim of this study was to select the best methods or parameters for the discrimination between the different regions of origin. A screening test was carried out on more than 30 analytical methods or parameters with a limited number of samples ($n=20$) from six regions in Europe: Bretagne (F), Savoie (F), Allgäu (D), Vorarlberg (A), Finland and Switzerland. The following methods or parameters were selected for the remainder of the project: infrared spectroscopy, MS-based electronic nose, short-chain fatty acids as well as other organic acids, major and trace elements, stable isotope ratios, pH-value, gross chemical composition as well as microbiology, especially with regard to *Lb. helveticus*.

Zusammenfassung

Diese Arbeit stellt die Synthese der Resultate dar, die während des ersten Teils eines Forschungsprojektes über drei Jahre zur Authentizität und geographischen Rückverfolgbarkeit von Emmentaler Käse erhalten wurden. Das Ziel war, die besten Methoden oder Kriterien auszuwählen, um die verschiedenen Ursprungsregionen unterscheiden zu können. Ein Screeningtest wurde mit mehr als 30 analytischen Methoden oder Kriterien an einer begrenzten Anzahl von Proben ($n=20$) aus sechs Regionen Europas durchgeführt: Bretagne (F), Savoyen (F), Allgäu (D), Vorarlberg (A), Finnland und Schweiz. Die folgenden Methoden oder Parameter wurden für den weiteren Verlauf des Projektes ausgewählt: Infrarot-Spektroskopie, elektronische Nase basierend auf der Massenspektrometrie, kurzkettige Fettsäuren und andere organische Säuren, Mineralstoffe, Spurenelemente, Verhältnis der stabilen Isotope, pH-Wert, grobchemische Zusammensetzung und die Mikrobiologie, im speziellen *Lb. helveticus*.

Résumé

Cette publication présente la synthèse des résultats obtenus durant la première partie d'un projet de recherche de trois ans portant sur l'authenticité et la traçabilité géographique de fromages Emmental. Le but de cette première étude était de sélectionner les méthodes et critères les plus discriminants pour reconnaître des fromages d'origines différentes. Un test de criblage a été effectué avec plus de 30 techniques ou critères analytiques mais sur un nombre limité d'échantillons ($n=20$) provenant de six régions en Europe: Bretagne (F), Savoie (F), Allgäu (D), Vorarlberg (A), Finlande et Suisse. Les méthodes ou paramètres suivants ont été retenus pour la suite du projet: spectroscopie infrarouge, nez électronique basé sur la spectrométrie de masse, acides gras à courte chaîne et autres acides organiques, éléments minéraux et de traces, rapports d'isotopes stables, valeur de pH, composition chimique globale ainsi que microbiologie, spécialement pour *Lb. helveticus*.

Key words

Emmental cheese, Authenticity, Analytical traceability, Screening test

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