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# Toward the Determination of the Geographical Origin of Emmental(er) Cheese via High Resolution MAS NMR: A Preliminary Investigation

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We tested the potential of High-Resolution MAS NMR spectroscopy to study 20 samples of Emmental cheeses from 7 different geographical regions. Principal component analysis (PCA) and discriminant analysis (DA) were used to analyze the data set of $^1$H HRMAS NMR spectra and succeeded in grouping the studied samples according to their geographical origins.

**KEYWORDS: HRMAS NMR; Emmental cheese; geographical origin; chemometrics**

## INTRODUCTION

Emmental cheese is very popular in many countries and more particularly so in Europe. Switzerland, France, and Germany are the most important producers of this kind of cheese. However, the originality of a cheese depends on the milk used, which is itself influenced by the climate, geology, or foliage of the country in which it is produced as well as by the cheesemaking process. In the case of neighboring countries, the differences can be difficult to bring to the fore. The opening of the European dairy market has led to producers finding analytical methods to certify the authenticity of their cheese and to obtain the protected designation of origin (PDO) label. Several investigations have been carried out to characterize Emmental cheese from Austria, France, Switzerland, Germany, and Finland and show their specificities (*1*−*6*) by performing microbiological, biochemical, color, and sensory analyses associated with PCA (*7*). Gas chromatography alone or coupled with mass spectrometry (*1*, *8*−*9*), high performance liquid chromatography (*10*), mid- and near-infrared spectroscopy, fluorescence (*3*, *5*, *11*), and isotopic studies (*4*, *12*) associated with statistical models have also been used but none tested the potential of NMR spectroscopy.

We showed in a previous work (*13*) that HRMAS NMR spectroscopy combined with principal component analysis (PCA) and discriminant analysis (DA) succeeded in characterizing Parmigiano Reggiano cheese according to its age of ripening. We tested this protocol here on a set of 20 Emmental cheese samples that had been previously analyzed in Pillonel's academic thesis (*14*) from seven different regions. In this preliminary investigation, we show that this method could discriminate the samples according to their geographical origin and that it was possible to determine some compounds defining the originality of Swiss Emmental cheese.

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**Table 1.** Geographical Origin of the 20 Cheese Samples Studied

| country | region | code | no. of samples | date of manufacture | heat hreatment |
|---|---|---|---|---|---|
| Austria | Vorarlberg | VO | 3 | 02/02/2001 | raw |
| Finland | middle Finland | FI | 2 | 04/02/2001 | heat treated |
| France | Bretagne | BR | 3 | 20/02/2001 | heat treated |
| France | Savoie | SA | 3 | 05/02/2001 | raw |
| Germany | Allgaü | AL | 3 | 25/12/2000 | raw |
| Switzerland | Bern | BE | 3 | 26/12/2000 | raw |
| Switzerland | St Gall | SG | 3 | 26/12/2000 | raw |

## MATERIALS AND METHODS

**Samples. Table 1** summarizes the geographic origin of the twenty Emmental cheeses from five different countries, which were the basis of this study.

These samples have already been studied by L. Pillonel for his academic thesis including detailed descriptions of sampling (*14*). The samples were frozen at −20 °C. The night before the analysis, a small piece of sample was cut and stored at 4 °C under conditions of slow unfreezing.

For each sample, about 20 mg of Emmental cheese was packed into a 4 mm MAS rotor of 50 $\mu$L total volume. To prevent the pH-dependent variation of some signals, 45 $\mu$L of 0.1 M phosphate buffer at pH 7 (prepared from 1 M phosphate buffer solution at pH 7(*13*)) with DSS (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt) was used as the internal reference. This mixture was homogenized directly in the rotor with a spatula.

**Proton HRMAS NMR Spectroscopy.** All proton HRMAS NMR spectra were recorded on a Bruker AVANCE 400 instrument operating at 400.13 Hz for $^1$H and using a 4 mm HRMAS $^1$H/$^{13}$C probehead. The samples were spun at 5000 Hz. All spectra were performed with presaturation of the water peak (*15*). A typical $^1$H HRMAS NMR spectrum consisted of 1024 transients using 16 k data points over a 4800 Hz spectral width with 5.95 $\mu$s of 90° pulse. A recycle time of 2 s was used. Each spectrum was phased and baseline corrected using a polynomial function. Five $^1$H HRMAS spectra, corresponding to five

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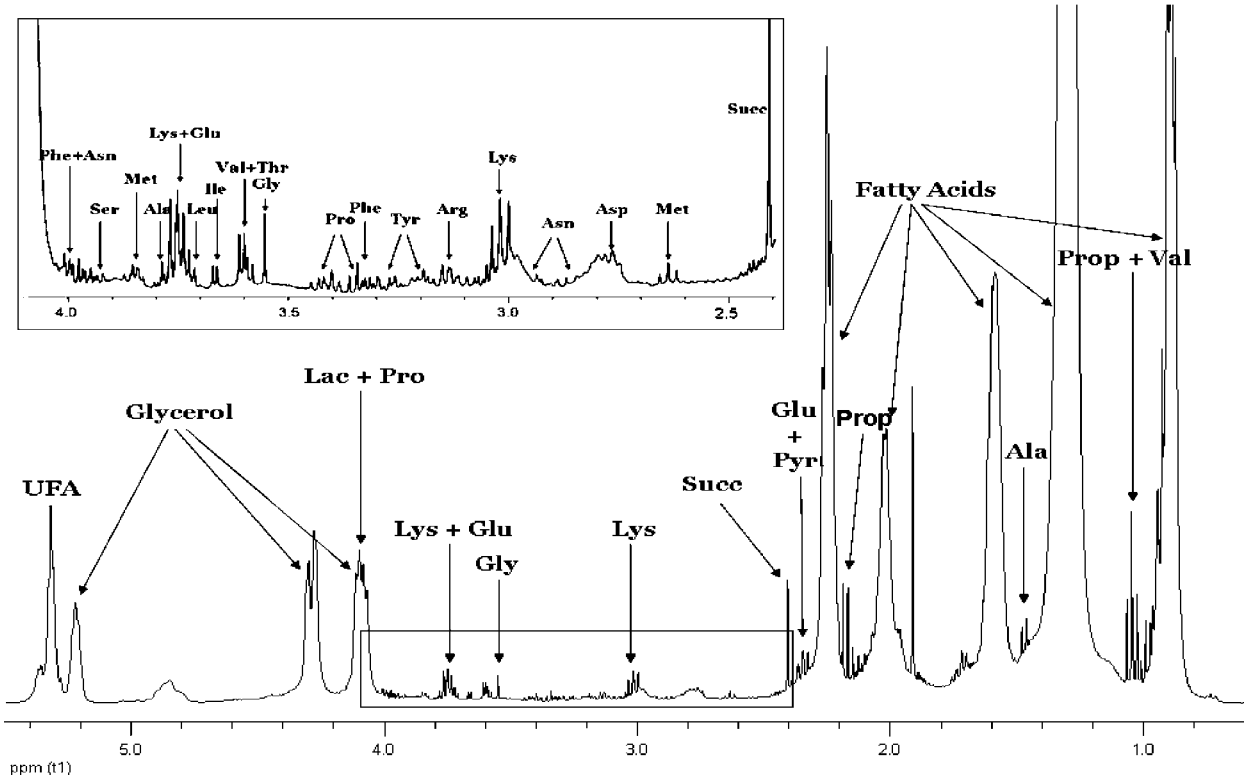


**Figure 1.** Portion of $^1$H HRMAS NMR spectrum of Finnish Emmental cheese with the assignment of some compounds. An expansion of the framed region is shown in the top window. (Uncommon abbreviations: UFA, unsaturated fatty acids; Succ, succinate; Pyr, pyroglutamic acid; Prop, Propionate.)

independently prepared rotors of the same sample, were recorded for each sample.

**Data Pretreatment.** Following our published protocol (*13*), the frequency region between 0.5 and 9.5 ppm of each spectrum was split into 162 buckets (integrated regions) of 0.05 ppm. The range between 4.70 and 5.10 ppm equivalent to the water suppression region was removed, and each spectrum was normalized to the whole spectrum without considering the water peak domain.

The 100 spectra × 162 buckets data matrix was imported into STATISTICA v.7.1 software (Statsoft, Inc.) for statistical analyses.

**Chemometrics.** *One-way ANOVA.* One-way ANOVA was used to reduce the number of initial variables and to select the most statistically significant ones (with a $p$-level $<0.01$, which means a probability of error of 1%) and the most discriminant ones by computing the $F$ factor, which is the ratio of the between-group variance over the within-group variance. The larger this ratio, the larger the discriminant power of the corresponding variable. We chose to keep the variables with an $F$ factor greater than 5, which is the limit yielding a good compromise between the number of variables and the power of their discrimination in PCA. The selection of the variables was carried out on the basis of the discrimination of the seven groups (AL, BE, BR, FI, SA, SG, and VO).

*Principal Component Analysis.* This unsupervised method allows us to obtain the linear combination of the selected variables that maximize the variability among groups. First, a principal component analysis (PCA) on the whole (100 × 162) data matrix was performed to find possible outliers. A second principal component analysis was performed on the remaining objects, and some variables were selected by ANOVA to reduce the dimensionality of multivariate data and to permit a first overview of the data.

*Forward Stepwise Discriminant Analysis (DA).* This is a supervised method in which we know a priori the membership group of the observations. The discriminant power of the variables is evaluated using Wilks' lambda, $F$, and $p$-level parameters. Wilks' lambda is computed as the ratio of the determinant of within-group variance/covariance matrix to the determinant of the total variance/covariance matrix. Its value ranges from 1 (no discriminant power) to 0 (high discriminant power). To select these variables, we used the forward stepwise algorithm, which retains the variables with the lowest Wilks' lambda. To test the robustness of the model, the data set was split into calibration (about 70% of samples) and validation sets (30% of samples). All replicates of one sample were considered as indissociable so that calibration and validation sets were completely independent. The process was repeated three times to ensure that all samples were excluded at least once.

Then, a graphical representation was performed using canonical variates, which are linear combinations of the selected initial variables, so that the rate of variance between groups to the variance intragroup are maximized. It is possible to visualize, on a plane formed by two canonical variates, the difference between the groups. Moreover, the standardized correlation coefficients within the canonical variates show the contributions of each initial variable to the discrimination. The higher the coefficient, on a given canonical axis, the more discriminant the variable.

## RESULTS AND DISCUSSION

A portion of the $^1$H HRMAS NMR spectrum of a Finnish Emmental sample is shown in **Figure 1**. The assignments (a complete table, in the Supporting Information section, is provided) were carried out on the basis of the study of $^1$H-$^1$H TOCSY, $^1$H-$^{13}$C HMQC, and $^1$H-$^{13}$C HMBC HRMAS NMR spectra of Emmental cheese (not shown), the previous NMR assignments performed on a Parmigiano Reggiano sample (*16*), and the literature (*17*−*19*).

The comparison of the $^1$H HRMAS NMR spectra of two samples (for instance, Swiss and Austrian cheeses, see **Figure 2**) bring to the fore the variations in the intensity of some signals.

It is therefore interesting to test statistical methods applied to HRMAS NMR data to try to discriminate our different samples.

A principal component analysis, based on the correlation matrix (variables centered and scaled to the unit variance to give the same weight to high and low peaks) and calculated on the whole data matrix, showed that two spectra of the Swiss

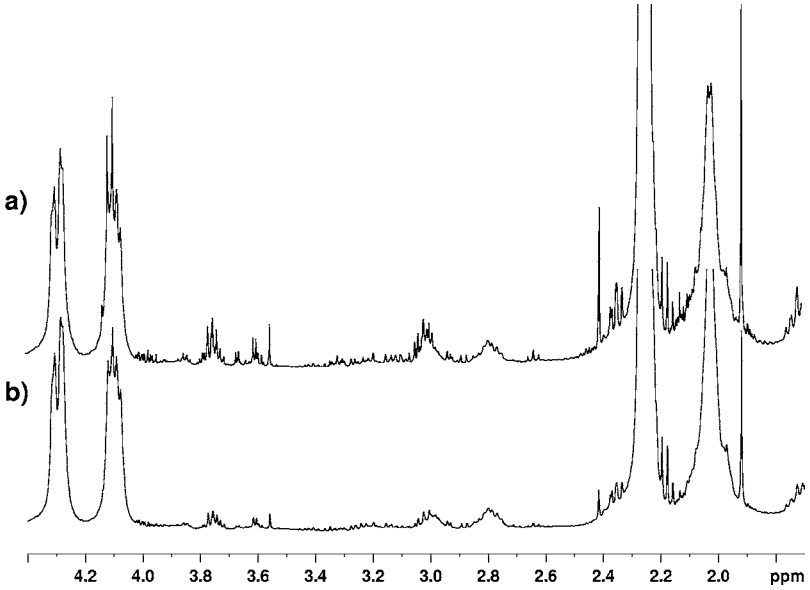


**Figure 2.** Expansions of $^1$H HRMAS NMR spectra of (**a**) Austrian and (**b**) Swiss Emmental cheeses.

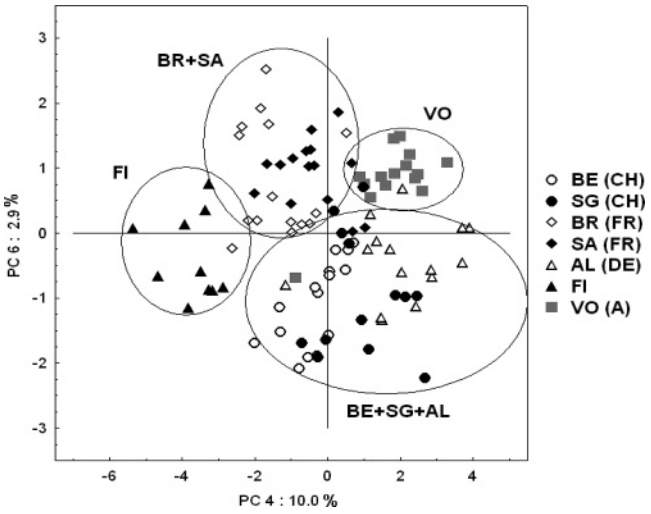


**Figure 3.** Principal component analysis: projections of 98 observations of the 7 groups on the PC4 vs PC6 plane.

group (SG) were outliers. Consequently, one-way ANOVA was performed for the 98 remaining experiments, and it selected the 39 most statistically significant variables (see Materials and Methods section) on the 98 × 162 initial data matrix. These variables were employed to perform a new PCA (based on the correlation matrix). The first six principal components were significant (i.e., with an eigenvalue greater than 1) and explained 91% of the total variance. The study of the sample projections on all of the first six principal component axes allowed us to choose the PC4 and PC6 axes as those yielding the best discrimination of the different groups, as shown in **Figure 3**.

These two PC axes represent 12.9% of the total variance. We observed a high dispersion of the observations within the same group because of sample heterogeneity (*13*), but we were able to distinguish four groups: Finnish (FI), Austrian (VO), and French (BR and SA) groups, and a remaining set composed of Swiss (BE and SG groups) and German (AL) samples.

The coefficients of the PC4 and PC6 loadings are shown in **Table 2**.

The Finnish and French groups are located on the negative side of the PC4 axis, whereas the Austrian and German groups are located on the positive one. The Swiss samples have very dispersed scores on this axis, and we cannot conclude on the contribution of this latter group on the PC4 axis. Moreover, the fourth principal component loadings show mainly a positive correlation (coefficient of 0.76) with the 2.75−2.80 ppm spectral domain, corresponding to the bucket of unsaturated fatty acids B and aspartic acid signals. On the PC6 axis, the French and Austrian group scores are mainly positive, those of Swiss and German samples are mainly negative, and the Finnish group scores are close to zero on average. These latter samples are thus very weakly influenced by this axis. The loadings of the sixth principal component have mainly positive correlations with the 2.40−2.45 ppm spectral domain corresponding to the pyroglutamic acid and succinate signals (coefficient of 0.46). This component is also negatively correlated (coefficient of −0.41) with the 1.95−2.00 ppm bucket constituted by unsaturated fatty acids A, proline, and lysine signals. Thus, the PC4 scores and loadings suggested that the Austrian and German cheeses studied presented higher relative amounts of unsaturated fatty acids B and/or aspartic acid (bucket "2.75−2.80 ppm") than the other ones. Moreover, the PC6 scores and loadings showed that the French and Austrian cheeses studied had higher relative amounts of pyroglutamic acid and/or succinate (bucket "2.40−2.45 ppm") and lower relative amounts in unsaturated fatty acids A and/or proline and/or lysine than the other ones.

In a previous analysis, Pillonel et al. (*8*) showed that Austrian and German cheeses have the highest concentration in fat and the French samples (Bretagne) the lowest one. Swiss cheeses have the lowest concentration in succinate and Finnish and French (Savoie) samples the highest one, in agreement with our results.

To find a model able to discriminate all of the 7 groups, we performed a forward stepwise discriminant analysis on the 39 previously selected variables, out of which 10, with the smallest Wilks' lambda factor, were kept (see **Table 3**). These variables allowed us to correctly group 100% of the observations (including replicates).

The squared Mahalanobis distances (*20*) reported in **Table 4** show that the closest groups (19.03) were the VO (Austria) and AL (Germany) groups, whereas the BR (France) and VO (Austrian) groups were the farthest away.

To test the robustness of this classification model, we used the validation method explained in the Materials and Methods

**Table 2.** Principal Component Analysis: Coefficients of Correlations between Initial Variables and Principal Components[a]

| buckets (in ppm) | compds | PC4 loading | PC6 loading | buckets (in ppm) | compds | PC4 loading | PC6 loading |
|---|---|---|---|---|---|---|---|
| 8.20–8.25 | unknown compound A | –0.47 | 0.06 | 3.95–4.00 | asparagine + serine + tyrosine | –0.08 | 0.06 |
| 7.30–7.35 | phenylalanine | 0.07 | –0.06 | 3.90–3.95 | serine | 0.01 | –0.04 |
| 7.25–7.30 | phenylalanine | 0.24 | –0.13 | 3.85–3.90 | aspartic acid | 0.12 | –0.06 |
| 6.25–6.30 | lipid A | 0.53 | –0.10 | 3.00–3.05 | lysine + asparagine | 0.13 | 0.36 |
| 5.85–5.90 | lipid A | 0.48 | –0.06 | 2.80–2.85 | UFA[b]B + asparagine | 0.52 | –0.14 |
| 5.60–5.65 | lipid A | 0.03 | 0.06 | 2.75–2.80 | **UFA[b]B + aspartic acid** | **0.76** | –0.22 |
| 5.55–5.60 | lipid A | 0.19 | –0.02 | 2.40–2.45 | **pyroglutamic acid + succinate** | –0.38 | **0.46** |
| 5.50–5.55 | lipid A+ lipid C | –0.09 | 0.04 | 2.25–2.30 | FA[b] | 0.15 | 0.01 |
| 5.45–5.50 | lipid C | –0.18 | 0.04 | 2.20–2.25 | FA[b] | 0.05 | –0.07 |
| 5.40–5.45 | lipid C + UFA A | –0.16 | –0.01 | 2.15–2.20 | propionate | –0.46 | –0.15 |
| 5.35–5.40 | UFA[b]A | 0.32 | –0.26 | 2.05–2.10 | UFA[b]A + methionine + proline + glutamic acid | 0.21 | –0.09 |
| 5.30–5.35 | UFA A and UFA[b]B | 0.11 | –0.17 | 2.00–2.05 | UFA[b]A+ methionine + proline + isoleucine | –0.49 | –0.29 |
| 5.20–5.25 | glycerol (triglyceride) | 0.44 | 0.07 | 1.95–2.00 | **UFA[b]A+ proline + lysine** | 0.04 | **–0.41** |
| 5.15–5.20 | glycerol (triglyceride) | –0.03 | –0.09 | 1.60–1.65 | FA[b]+ lysine | 0.05 | –0.02 |
| 4.30–4.35 | glycerol (triglyceride) | 0.13 | 0.07 | 1.35–1.40 | FA[b] | –0.31 | –0.15 |
| 4.25–4.30 | glycerol (triglyceride) | 0.47 | 0.13 | 1.05–1.10 | propionate | –0.55 | –0.24 |
| 4.20–4.25 | glycerol (triglyceride) | –0.10 | –0.03 | 1.00–1.05 | propionate + valine | –0.45 | 0.03 |
| 4.10–4.15 | glycerol (triglyceride) + lactate + proline | 0.35 | 0.35 | 0.95–1.00 | isoleucine + leucine | –0.02 | 0.11 |
| 4.05–4.10 | glycerol (triglyceride) + lactate | 0.43 | 0.16 | 0.90–0.95 | isoleucine + leucine | 0.05 | –0.10 |
| 4.00–4.05 | glycerol (triglyceride) + asparagine + phenylalanine | –0.11 | –0.04 | | | | |

[a] The variables with the greatest correlation coefficients are shown in bold type. [b] Abbreviations: FA, fatty acid; UFA, unsaturated fatty acid.

**Table 3.** Discriminant Analysis: Wilks' Lambda and *p*-Level for the Ten Selected Discriminant Variables

| bucket (in ppm) | compds | Wilks' lambda | *p*-level |
|---|---|---|---|
| 5.55–5.60 | lipid A | 0.000081 | 0.000000 |
| 0.95–1.00 | isoleucine + leucine | 0.000086 | 0.000000 |
| 2.25–2.30 | FA | 0.000090 | 0.000000 |
| 5.45–5.50 | lipid C | 0.000091 | 0.000000 |
| 2.40–2.45 | pyroglutamic acid + succinate | 0.000100 | 0.000000 |
| 1.00–1.05 | propionate + valine | 0.000113 | 0.000000 |
| 2.80–2.85 | UFA[a] B + asparagine | 0.000139 | 0.000000 |
| 2.00–2.05 | UFA A + methionine + proline + isoleucine | 0.000177 | 0.000000 |
| 3.90–3.95 | serine | 0.000190 | 0.000000 |
| 2.75–2.80 | UFA B + aspartic acid | 0.000195 | 0.000000 |

[a] UFA: unsaturated fatty acid.

**Table 4.** Discriminant Analysis: Squared Mahalanobis Distances from Group Centroids[a]

| | BE | SG | BR | SA | AL | FI | VO |
|---|---|---|---|---|---|---|---|
| BE | 0.00 | 69.15 | 236.32 | 52.55 | 122.83 | 190.45 | 134.07 |
| SG | 69.15 | 0.00 | 489.42 | 32.55 | 52.85 | 414.70 | 33.50 |
| BR | 236.32 | 489.42 | 0.00 | 377.41 | 528.42 | 62.89 | **607.14** |
| SA | 52.55 | 32.55 | 377.41 | 0.00 | 44.80 | 294.71 | 40.99 |
| AL | 122.83 | 52.85 | 528.42 | 44.80 | 0.00 | 423.24 | **19.03** |
| FI | 190.45 | 414.70 | 62.89 | 294.71 | 423.24 | 0.00 | 510.50 |
| VO | 134.07 | 33.50 | **607.14** | 40.99 | **19.03** | 510.50 | 0.00 |

[a] The highest and lowest distances are shown in bold type.

section. For each validation set, we calculated the number of correctly reclassified samples (corresponding to a posteriori probability greater than 85% for the correct group), which was 89.5%. Although the small number of samples used for this preliminary investigation does not allow a definite conclusion, the model seems to be robust, and the 10 selected variables may be used to classify a new dataset in a future investigation.

To further clarify the differences in the composition of these cheeses, we performed a canonical analysis on the 98 × 10 data set matrix. The first two canonical variates (93% of the total variance) led to a good discrimination of all groups. The projection of the observations on the CV1 vs CV2 plane is shown in **Figure 4**.

The CV1 axis showed a separation between the Finnish and French (BR) samples on the positive side and the other groups on the negative side. The examination of the standardized canonical coefficients of each variable on the first canonical axis (**Table 5**) suggested that this separation was due to the spectral domains, positively correlated with CV1, located at 3.90−3.95 and 5.45−5.50 ppm corresponding to the serine and lipid C signals, respectively, and to the spectral domain, negatively correlated with CV1, situated at 2.75−2.80 ppm and corresponding to the unsaturated fatty acid B and aspartic acid signals. This implies that Finnish and French (BR) samples have higher relative content of serine and/or lipid C and lower relative contents of unsaturated fatty acid B and/or aspartic acid. Pillonel et al. (*8*) have already highlighted the low concentration of some groups of fatty acids (C18:2, polyunsaturated, conjugated

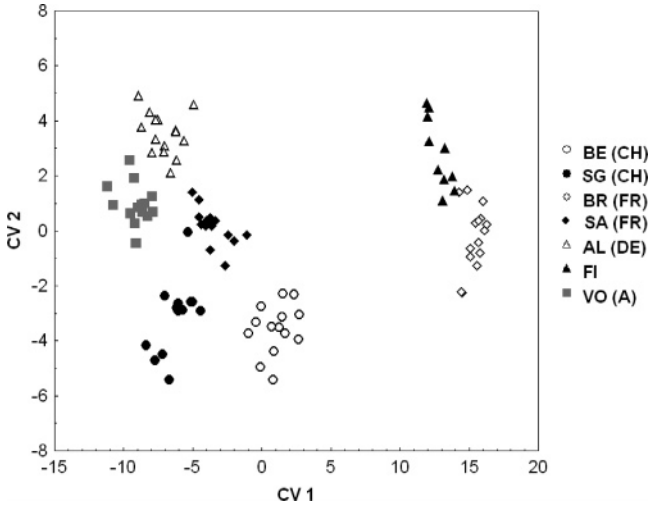


**Figure 4.** Canonical analysis: projections of the 98 observations on the CV1 vs CV2 plane.

**Table 5.** Canonical Analysis: Standardized Canonical Correlation Coefficients of the 10 Variables with CV1 and CV2

| bucket (ppm) | compds | CV1 loadings | CV2 loadings |
|---|---|---|---|
| 5.55–5.60 | lipid A | –3.42 | 1.53 |
| 0.95–1.00 | isoleucine + leucine | –1.67 | 1.61 |
| 2.25–2.30 | FA[a] | –1.08 | 0.66 |
| 5.45–5.50 | lipid C | 4.44 | –1.05 |
| 2.40–2.45 | pyroglutamic acid + succinate | 0.36 | 1.40 |
| 1.00–1.05 | propionate + valine | –1.16 | –1.84 |
| 2.80–2.85 | UFA[a] B + asparagine | 2.11 | –5.23 |
| 2.00–2.05 | UFA[a] A + methionine + proline + isoleucine | 1.37 | 0.22 |
| 3.90–3.95 | serine | 4.41 | 2.07 |
| 2.75–2.80 | UFA[a] B + aspartic acid | –4.43 | 1.94 |
| | eigenvalue | 86.93 | 6.45 |
| | cumulative proportion | 0.87 | 0.93 |

[a] Abbreviations: FA, fatty acid; UFA, unsaturated fatty acid.

linoleic acids, and $\omega$-3) for the samples from Finland and Bretagne and more generally in fat (307 g/kg for FI, 300 g/kg for BR against 322, 318, 306, and 342 g/kg for AL, CH, SA, and VO, respectively). Moreover, the free amino acid ratios calculated in these samples showed a very small percentage of aspartic acid in the FI samples (0.16% of the total free amino acid content (*10*)). The similarities between the Finnish and French (BR) groups may be also due to the difference in milk heat treatment prior to manufacturing. Indeed, these two groups are the only ones manufactured from heat treated milk instead of raw milk like the other analyzed specimens (*3*). The CV1 axis can thus be considered as the discriminant axis for the milk treatment.

The second canonical variate can be interpreted as the canonical variate characterizing the Swiss Emmental cheese compared to those from Austria, France, Finland, or Germany; considering the two Swiss sample sets (SG and BE groups) like distinct groups, their contribution on the CV2 axis is identical and original (the only groups with negative contribution on this axis). In a previous work (*8*), the content of fat in Swiss samples is given as 318 g/kg against 322 g/kg and 342 g/kg for German and Austrian samples, respectively. Moreover, the sum of polyunsaturated fatty acids is given as 3.97 g/100 g of fat for

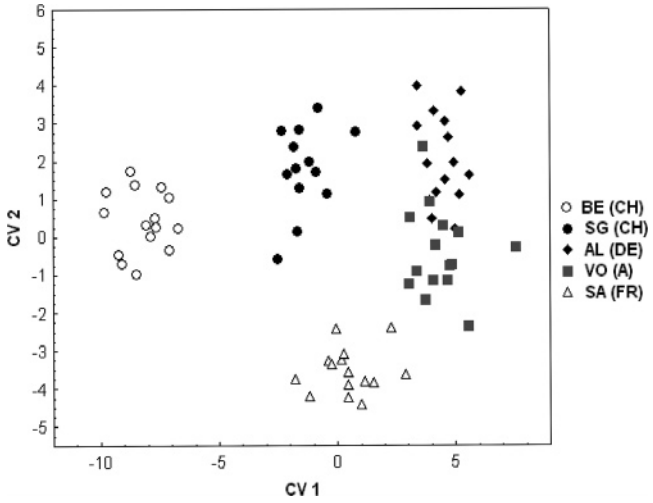


**Figure 5.** Canonical analysis on only the raw milk samples: projections of the 75 observations on the CV1 vs CV2 plane. The CVs axes are different from those in **Figure 4**.

Swiss samples against 4.61 g/100 g of fat for German samples and 4.32 g/100 g of fat for Austrian samples. However, the content of asparagine is given at 7.06%, with respect to the total amount in free amino acids, for the Swiss samples against 3.08, 4.63, 1.35, 2.51, and 4.77% for AL, BR, FI, SA and VO groups respectively (*10*)). Thus, we assumed that the statistically relevant bucket at 2.80−2.85 ppm may be more influenced by the asparagine than by the unsaturated fatty acids.

Because of the evident differences in the pretreatment of milk, we performed the same kind of analysis on the samples manufactured with only raw milk. We proceeded in the same way on the corresponding 75 × 39 dataset, and a new set of 10 variables was found by a forward stepwise variable selection. Because the new dataset is more homogeneous, the canonical analysis is likely to provide a more straightforward chemical interpretation of the group spectral differences.

Three successive validation processes of the new discriminant model led to 87.7% of correct reclassification on average. **Figure 5** shows the projections of the raw milk samples on the new CV1 versus CV2 plane, and the corresponding loadings are given in **Table 6**.

The CV1 axis allowed the separation of the Swiss cheeses (negative side) from the German and Austrian cheeses (positive side). The CV1 loadings suggested that this separation is influenced by the spectral domain containing unsaturated fatty acids A and B signals (bucket 5.30−5.35 ppm). The positive correlation between this bucket and the CV1 axis (coefficient of 9.04) suggested that the Swiss cheeses have lower relative amounts of unsaturated fatty acids. These results are in agreement with previous results (*8*).

The CV2 loadings and scores suggested that the bucket at 2.40−2.45 ppm, corresponding to the pyroglutamic acid and succinate signals with a negative coefficient of −1.82 and that at 5.30−5.35 ppm corresponding to unsaturated fatty acids A and B signals with a positive coefficient of 1.84 allowed us to discriminate samples from Savoie (on the negative side of CV2 axis) and Swiss and German samples (on the positive side). The Austrian samples have not been considered because their scores are close to zero on average on this axis. The samples studied from Savoie, thus, have lower relative amount of unsaturated fatty acids (306 g/kg of fat and 3.76 g/100 g of fat for the sum of polyunsaturated fatty acids) and higher content in succinate (12.5 mmol/kg against 3.2 and 6.7 mmol/kg for Swiss and German samples, respectively) (*7*−*8*).

**Table 6.** Canonical Analysis of Only the Raw Milk Samples: Standardized Canonical Correlation Coefficients of the 10 Variables with CV1 and CV2

| buckets (ppm) | compds | CV 1 loadings | CV 2 loadings |
|---|---|---|---|
| 5.35–5.40 | UFA[a] A | –1.69 | –0.66 |
| 4.25–4.30 | glycerol (triglyceride) | –1.83 | –0.77 |
| 2.00–2.05 | UFA[a] A + methionine + proline + isoleucine | –4.92 | –0.90 |
| 2.40–2.45 | pyroglutamic acid + succinate | 2.48 | –1.82 |
| 2.15–2.20 | propionate | –4.621 | 0.05 |
| 5.85–5.90 | lipid A | –1.25 | 1.21 |
| 8.20–8.25 | unknown compd A | 0.21 | –0.72 |
| 1.95–2.00 | UFA[a] A + proline + lysine | 3.96 | 1.30 |
| 5.30–5.35 | UFA A[a] and UFA B | 9.04 | 1.84 |
| 7.30–7.35 | phenylalanine | 3.59 | 0.90 |
| | eigenvalue | 23.95 | 4.37 |
| | cumulative proportion | 0.73 | 0.87 |

[a] UFA: unsaturated fatty acid.

Despite the small number of studied samples, $^1$H HRMAS NMR spectra combined with statistical models allowed us to discriminate European Emmental cheeses according to their geographical origin with 89.5% correct reclassification, although these countries are geographical neighbors. We showed that it was possible to point out several spectral domains, suitable for this discrimination, at 2.75−2.80 ppm (unsaturated fatty acid B and aspartic acid signals), 5.45−5.50 ppm (olefinic proton of lipid C), 3.90−3.95 ppm (serine signals), and 2.80−2.85 ppm (unsaturated fatty acid B and asparagine signals). It was also possible to find a direction clarifying the discrimination of the samples according to their milk pretreatment (CV1 axis). A second discriminant analysis, performed only on samples from raw milk, also permitted a geographical discrimination of the samples. We showed that in this case the spectral domain at 5.30−5.35 ppm (unsaturated fatty acids A and B signals) can be used as a marker of Swiss Emmental cheese. All of these results are in agreement with previous studies on the same sample set relying on a complete chemical analysis, and most notably, the same markers are highlighted. However, because of the limited number of analyzed samples, our results should be considered as a test of feasibility, and they should be confirmed by the analysis of larger sets of samples. Nonetheless, HRMAS NMR appears to be a quick and reliable method for classification studies, providing analogous results as standard analysis protocols.

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**Supporting Information Available:** Peak assignments in Emmental cheese HRMAS NMR from TOCSY, HMQC, and HMBC. This material is available free of charge via the Internet at http://pubs.acs.org.

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