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ORIGINAL PAPER

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Analytical methods for the determination of the geographic origin of Emmental cheese: mid- and near-infrared spectroscopy

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Abstract Four different Fourier transform infrared spectroscopic techniques (near infrared diffuse reflection (NIR/DR), mid-infrared attenuated total reflection (mIR/ATR) using two different instruments and mid-infrared transmission (mIR/Tr) spectroscopy) in combination with multivariate chemometrics were investigated for their potential for discriminating Emmental cheese of various geographic origins. A total of 20 Emmental cheese samples produced in winter from Switzerland (n=6), Allgäu (Germany) (n=3), Bretagne (France) (n=3), Savoie (France) (n=3), Vorarlberg (Austria) (n=3) and Finland (n=2) were analysed. The normalised spectra or their 2nd derivatives were analysed by principal component analysis (PCA) and linear discriminant analysis (LDA) of the PCA-scores. Despite the few samples in this preliminary investigation clear trends were observed. The mIR transmission spectra achieved 100% correct classification in LDA when differentiating the Swiss Emmental from the other samples pooled as one group. The NIR/DR spectroscopy allowed a classification by the six regions of cheese origin.

Keywords Authenticity · Traceability · Emmental cheese · NIR · MIR · IR

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Introduction

Infrared (IR) spectroscopy is widely used for determination of organic constituents in foods and pharmaceutical products. The advantages of IR measurements are obvious: they are rapid, cheap, non-destructive and multiparametric. The use of IR spectrometry for the analysis of dairy products has been reviewed [1, 2, 3]. Mid- and near- infrared (mIR and NIR) spectroscopy of dairy products has in practice been restricted to measurements of main components such as fat, protein, moisture, lactose and urea. Some applications for cheese have been published [4, 5, 6, 7, 8, 9, 10]. In addition, Sorensen et al. [11] found correlation between certain sensory properties and NIR spectra. Dufour et al. [12] were able to discriminate between four different ripening stages by combining mIR and fluorescence spectra. Infrared spectroscopy found application for identification and/or authentication of various products such as meat [13, 14, 15], honey [16], coffee [17, 18, 19] and fruit juice [20, 21, 22].

The present investigation is part of a broad screening test, which is the first step in a 3-year study into the authenticity of Emmental cheese and its geographic traceability [23, 24]. At this point, a large number of analytical methods have been tested for their discriminating potential [24, 25, 26, 27] and the number of cheese samples for each region has been limited. It is therefore obvious that the analytical results obtained from such a modest number of cheese samples from each region can only give trends that should be confirmed later if they appear valuable for discriminating cheeses produced in different countries. The aim of the present paper is to find out whether NIR and mIR make possible a discrimination between the different geographic origins of Emmental cheese samples.

Materials and methods

The sampling procedure and the background of the project have been described in the first publication of the series [24]. Table 1

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

Abbreviation	Region (country)	Number of samples	Date of manufacture	Ripening time (months)
AL	Allgäu (D)	3	25 Dec 2000	4
BR	Bretagne (F)	3	20 Feb 2001	2.5
CH	Switzerland (CH)	6	26 Dec 2000	4
FI	Middle Finland (FI)	2	04 Feb 2001	3
SA	Savoie (F)	3	05 Feb 2001	3
VO	Vorarlberg (A)	3	02 Feb 2001	3

summarises the origin, the date of manufacture and the ripening time of the samples. They were chosen with different ripening time according to the reality of the market. Each region produces a cheese with typical characteristics, one of which is the ripening time, which can vary from 6 weeks to several months. Three samples originated from each of Bretagne, Savoie, Vorarlberg and Allgäu. Two samples originated from Finland and six from Switzerland. All instruments were of type Fourier transform.

NIR diffuse reflection (NID/DR)

The samples were kept at –18 °C until analysis. Approx. 150 g grated cheese were placed in a glass Petri dish and measured by diffuse reflection on a Büchi NIRLab N-200 spectrometer (Flawil, Switzerland). The Petri dish rotates on itself during the measurement. For each sample, 64 scans were recorded from 4000 cm^{-1} to 10 000 cm^{-1} with a spectral resolution of 2 cm^{-1}.

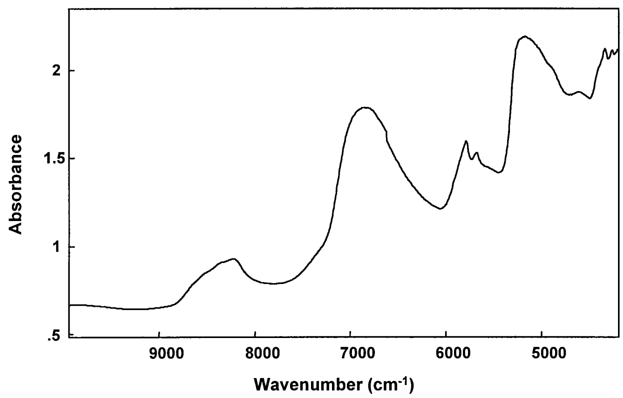

Fig. 1 Typical NIR/DR diffuse reflection absorption spectrum using a Büchi NIRLab N-200 instrument

mIR attenuated total reflection (mIR/ATR) using two different instruments

Slices of approx. 6×1×0.4 cm were cut from the fresh cheese samples avoiding eyes of the body. All analyses were carried out within a few days. The cheese slices were then pressed with the help of a clamp on the horizontal ZnSe crystal plate of an ATR sampling accessory (Graseby-Specac benchmark 6 reflection horizontal ATR with clamp assembly). The pressure applied on the cheese sample ensured an homogeneous contact between the cheese surface and the crystal. The measurements were carried out using an mIR spectrometer equipped with a DTGS detector (FTS-7, Bio-Rad Digilab) at room temperature.

The same analyses were carried out in another laboratory with a second instrument using an ATR accessory designed for 12 reflections (Nicolet Magna 750 FTIR spectrometer).

With both instruments the spectral resolution was 4 cm^{-1}, 32 scans were averaged for each spectrum in the range of 3500 cm^{-1} to 700 cm^{-1}. The spectra of three replicates of each cheese sample were recorded.

mIR transmission (mIR/Tr)

The analyses were carried out on fresh samples within a few days. Grated samples (5 g) were dispersed in 100 mL water with a Polytron PT2100 at 15 000 rpm for 60 s. An aliquot of 60 µL was applied to a polyethylene card (Spectra Tech Nicolet) and dried overnight. The prepared cards were analysed in transmittance on a Nicolet Magna 750 IR spectrometer.A total of 32 interferograms with a resolution of 4 cm^{-1} were recorded and averaged. Each cheese sample was analysed in triplicate and the average spectrum was introduced into the data set.

Statistical methods

For each type of spectrum, spectral regions were selected to eliminate zones with either low signal-noise ratios or no significant spectral information. All spectra were normalised between 0 and 1. For the spectra obtained using the Büchi NIR spectrometer and the Bio-Rad instrument, principal component scores from the correlation matrix were computed with PLSPlus/IQ Vs. 5.07 (Thermo Galactic, Salem, NH). Linear discriminant analysis (LDA) was performed on these scores with Systat Vs. 9.0 (SPSS Inc., Chicago, IL).

The spectra from the Nicolet instrument were not used directly. Their 2nd derivatives were first calculated before principal component scores were computed with Statistica VS 5.5 (Satasoft France, Paris). LDA was carried out with Systat.

Results

Typical IR spectra and the selected spectral windows are illustrated in Figs. 1, 2 and 3 for the three techniques mentioned above. The mIR spectra showed narrower and more abundant absorption zones than NIR, especially in the mIR/ATR technique. The interpretation of the results focused on the differentiation of "Switzerland" from the other regions. However, discrimination between the other regions was also possible.

Two grouping possibilities were investigated: in the first case, the Swiss samples were compared to a pool including all the others (two groups); in the second case, each region was one group within six groups. Principal component analysis was applied to each set of spectra to reduce the number of variables for further statistical analysis. Linear discriminant analyses were carried out on the principal component scores selected by stepwise backward elimination to evaluate the potential for separation between the groups. The goal was not to build a model for prediction but only to study the feasibility of

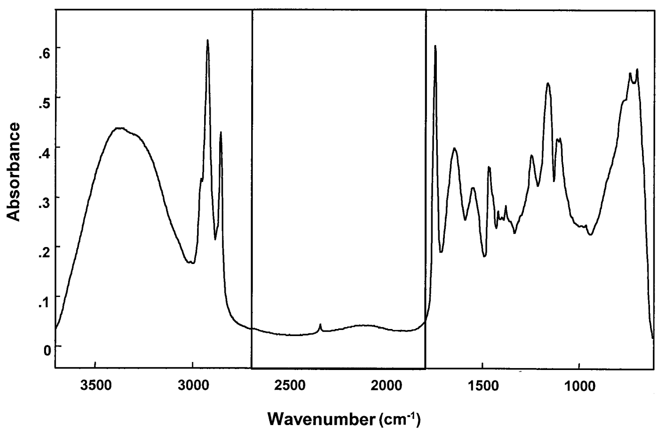

Fig. 2 Typical mIR/ATR attenuated total reflection absorption spectrum using a Bio-Rad FTS-7 instrument; range of interest 3700–2700 and 1800–620 cm^{-1}. Area between the *vertical lines* was omitted due to the low signal-noise ratio

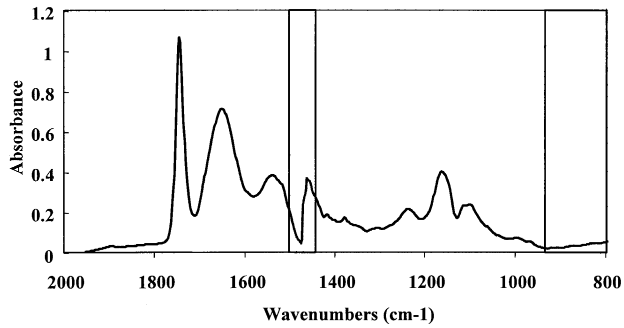

Fig. 3 Typical mIR/Tr transmission absorption spectrum using a Nicolet Magna 750 instrument; range of interest 900 – 1450 and 1500–1800 cm^{-1}. Area between the *vertical lines* was omitted due to the low signal-noise ratio

the method. The number of principal components used was reduced to a minimum by backward elimination to diminish the problem of model over-fitting. The percentage of correct classification, as well as the median, minimal, maximal and normalised Mahalanobis distances between the samples of one region and the centroid of the group "Switzerland", are listed for the four methods in Table 2.

The NIR/DR and mIR/ATR (Magna 750) spectra showed the best discrimination with 100% correct classification by individual region. "Finland" was always the easiest region to separate (highest median distance from Switzerland). It was not possible to classify all Swiss samples correctly (78%) using the mIR/ATR (FTS-7) spectra. To improve the comparison of the methods, the

Table 2 Classification rates by linear discriminant analysis according to two types of grouping

	Switzerland vs. other regions		Separation of each individual region					
	Switzerland	Others	Switzerland	Allgäu (D)	Bretagne (F)	Finland	Savoie (F)	Vorarlberg (A)
NIR/DR								
PC for LDA*	4		7					
% Correct	100	90	100	100	100	100	100	100
Distance**	3 (1, 5)	11 (4, 25)	8 (5, 12)	25 (17, 27)	38 (25, 48)	95 (67, 118)	25 (10, 32)	25 (7, 28)
Median normalised***	1	4	1	3	5	12	3	3
mIR/ATR(FTS7)								
PC for LDA*	1	9						
% Correct	72	81	78	89	100	100	100	89
Distance**	1 (0.1, 9)	5 (0.9, 19)	6 (3, 20)	24 (15, 54)	42 (25, 55)	295 (250, 318)	46 (29, 66)	12 (3, 23)
Median normalised***	1	5	1	4	7	49	8	2
mIR/ATR (Magna 750)								
PC for LDA*	3	10						
% Correct	100	71	100	100	100	100	100	100
Distance**	2 (0.7, 4)	5 (0.8, 14)	9 (4, 11)	150 (103, 156)	264 (242, 300)	439 (414, 464)	30 (12, 39)	20 (6, 27)
Median normalised***	1	3	1	17	29	49	3	2
mIR/Tr								
PC for LDA*	4	6						
% Correct	100	100	100	67	100	100	100	100
Distance**	2 (0.6, 8)	12 (4, 33)	5 (2, 8)	16 (9, 16)	23 (10, 32)	71 (40, 101)	22 (22, 25)	26 (15, 27)
Median normalised***	1	6	1	3	5	14	4	5

* Number of principal components used for the linear discriminant analysis
** Median distance to the centroid of the group "Switzerland" (minimum, maximum)
*** Median distance normalised by the median distance of "Switzerland"

median distances from “Switzerland” were normalised by the median distance of the Swiss samples from their centroid. With NIR/DR and mIR/Tr, the median distances normalised to Switzerland were generally lower than with the other techniques. However, the closest groups to “Switzerland” were at a distance of at least three in NIR/DR and mIR/Tr vs. two for the others techniques. This may explain the good discrimination ability of these two techniques when separating “Switzerland” from the others. The mIR/Tr classified 100% correctly, NIR/DR 100% for “Switzerland” and 90% for the non-Swiss groups. The mIR/ATR (FTS-7) spectra only achieved 72% correct classification for the Swiss samples.

Discussion and conclusion

Multivariate statistics is a very powerful tool for distinguishing groups of objects with very similar properties. The more properties (variables) used for classification, the more objects (samples) are needed to get a robust, unambiguous statistical model. With only 20 samples, the current model tends to suffer from over-fitting and is not very robust against the inclusion or exclusion of samples. However, the goal of the current work was only to evaluate the basic discriminating potential of IR spectroscopy with respect to cheese authenticity and not, at this point, to build a detailed model for the prediction of the origin. LDA made it possible to compare the various techniques used.

A simple method for checking the robustness of the discriminant functions of an LDA is the Jackknife classification test. In this re-sampling test, the discriminant functions are computed consecutively leaving out one sample. The group membership of the removed sample is then predicted by the classification functions obtained without the sample excluded in the training step (this procedure is also known as cross validation). If the model is robust, the classification pattern will not depend to a great extent on the inclusion or exclusion of a given sample in the analysis and the percentages of well-classified samples will be similar for both methods. Table 3 compares the results of the total correct classification rate in the normal and the Jackknife mode.

For the classification by regions, only the NIR/DR technique gave satisfactory results: 100% correct classifications were achieved in both modes. The very good classification obtained using mIR/ATR (Magna 750) broke down to 65% with the Jackknife test. Similar poor results were obtained using the two remaining techniques.

For the classification into two groups, the best results were achieved using mIR/Tr: 100% could be classified correctly in the normal mode and 85% in the Jackknife test. NIR/DR did not do badly with 93% and 90%, respectively. The mIR/ATR (FTS-7) technique is interesting because almost all variation is contained in only one principal component. However, the correct classification rate is poor.

Table 3 Percentage of total correct group assignments by normal and Jackknife linear discriminant classification

	By region		CH vs. Others	
	Normal	Jackknifed	Normal	Jackknifed
NIR/DR	100	100	93	90
mIR/ATR(FTS-7)-	90	78	88	85
mIR/ATR(Magna 750)	100	65	80	70
mIR/Tr	95	65	100	85

In conclusion, of the four techniques tested, two may be retained for their promising results. NIR/DR gave 100% discrimination by grouping into the six individual regions chosen for this preliminary study, whereas mIR/Tr achieved 100% correct classification when comparing “Switzerland” with the other regions pooled as one group. Both techniques made possible the analysis of grated cheese, which is an essential condition if one wants to test the authenticity of prepackaged grated Emmental cheese. Further analyses with more samples will be necessary to confirm these results and to build a validated model for prediction.

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