Near infrared analysis of cheese-making properties of goat's milk

C. Angulo, M. Analla, I. Jiménez, A. Muñoz, A. Moraga and J.M. Serradilla

Laboratorio de Fisiogenética Animal, Departamento de Producción Animal, Universidad de Córdoba, Apdo. 3048, 14080 Córdoba, Spain.

Introduction

Near infrared (NIR) reflectance spectroscopy is being used for the analysis of milk and dairy products, such as butter, yoghurt, cheese, whey products etc. However, most NIR reflectance spectroscopy applications to dairy products are related to the analysis of conventional major constituents such as moisture, fat, protein, lactose, ashes etc.[1,2] More recently, we have looked into the possibilities of using NIR reflectance spectroscopy to quantify milk proteins' fractions; α_{S1}, β and κ caseins,[3] pH, salt, water solubility of cheese and even a categorical classification of cheese ripening time.[4]

Goat's milk is used in Spain mainly for cheese production.[5] Cheese yield and certain cheese qualities, as well as the efficiency of the cheese making process, depend on milk composition, mainly caseins and fat contents and some physical (rheological) characteristics of milk during clotting process. The cheese industry uses these chemical and physical parameters to predict the cheese making behaviour of different lots of milk, in order to set up their price and to monitor manufacturing conditions. This industry is very interested in NIR reflectance spectroscopy for the quantitative and qualitative analysis of milk and dairy products as it offers the advantages of low cost, rapidity, reliability and accuracy.[4] NIR reflectance spectroscopy technology offers also many possibilities in milk recording schemes.[3]

Literature shows that NIR reflectance spectroscopy can be used to predict functional and sensory properties of foods[6] and that, at least in some cases, the equations have a sufficient accuracy to be used in breeding programs.[7] However, rheological parameters of milk, like pH, clotting time and curd firmness, which can be used to predict cheese yields and qualities, have not been yet analyzed using NIR reflectance spectroscopy.

The objective of this work is to show the possibility of NIR reflectance spectroscopy to determine the cheese-making properties of goats' milk. NIR reflectance spectroscopy will be used for the routine analysis of milk samples coming from a breeding program on the "Malagueña" breed carried out in Southern Spain. [8]

Materials and methods

Milk sample collection

One hundred millilitres of milk from goats of the Malagueña breed were collected monthly, throughout seven months (from January to June).

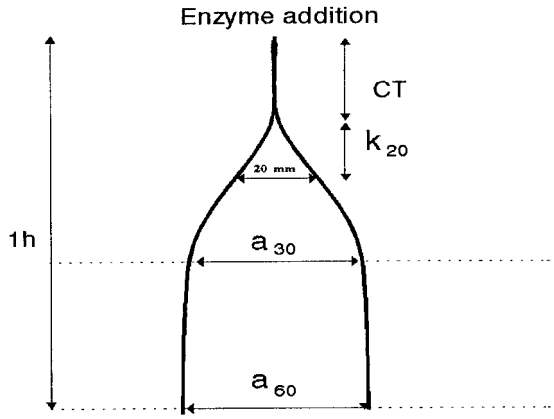

Figure 1. Diagram of coagulation and curd firmness as a function of time as recorded with the Formagraph. CT—clotting time; k_{20}—curd firming time; a_{30}— curd firmness after 30 min. and a_{60}—curd firmness after 60 min.

Sample were kept cold (near 5°C) with no preservative that could influence the cheese-making properties.

Chemical analyses

Twenty four hours after collection (this is the average time elapsed between milk collection and milk processing in the cheese industry), samples were analyzed with a Formagraph (Foss Electric). Previously, samples were heated to 32ºC (clotting temperature). pH measurements were taken with a pH meter (Crison micropH 2001). The Formagraph was operated according to McMahon's[9] instructions and using the traditional Spanish goat's cheese making recipe. The purified calf rennet solution used was obtained from Marshall (Rhone-Poulenc Groups, Spain) and had a nominal clotting activity of 940 CU g^{-1}.

A diagram of firmness versus time[9] is obtained (Figure 1). The following variables are measured in that diagram:

- CT: clotting time or gel formation. This is the value obtained by measuring distance from origin to the point where the baseline is about 1 mm width (expressed in minutes).
- k_{20}: curd firming rate. Curd firmness adequate for cutting of cheese curd. This is the time from CT until a width of 20 mm (expressed in minutes).
- a_{30}: curd firmness at 30 min. This is the width of the graph 30 minutes after the process started (expressed in mm).
- a_{60}: curd firmness at 60 min is the width of the graph 60 minutes after the process started (expressed in mm).
- TY: total yield. After 1 hour clotting, curd was cut and centrifuged for 30 minutes at 3500 rpm and solids were separated from the whey and weighed. TY is the ratio of total milk weight to solid weight (expressed as a percentage).

Samples were split in the two sub-samples which were analyzed simultaneously.

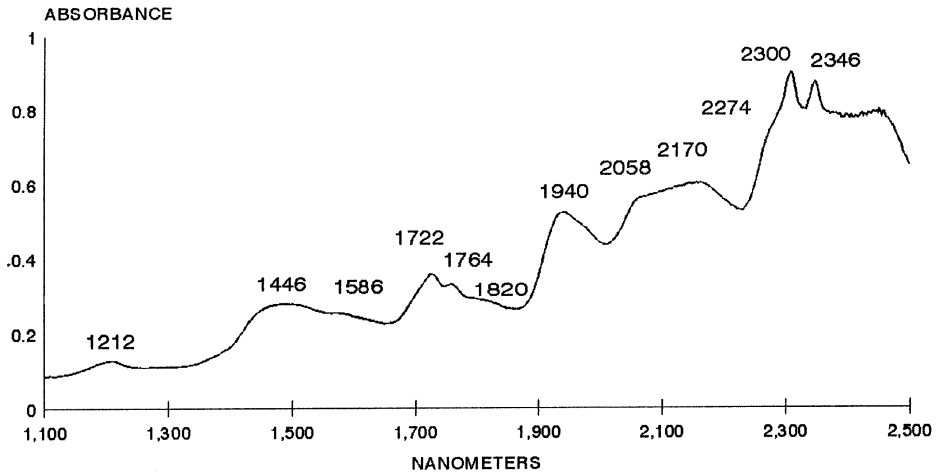

Figure 2. Goat's milk NIR spectrum.

NIR analyses and statistical methods

Liquid milk is one of the most difficult products to analyze using NIR reflectance spectroscopy because of the high level of moisture (>88%). Therefore, a sample pre-treatment, to get a dry sample presentation in a glass fibre filter disk, is used. [3]

All samples were scanned with a NIR reflectance monochromator (NIRSystems 6500). Each sample was placed into a sample cell having a quartz-glass cover, avoiding physical contact between the sample and the glass. Reflectance measurements of monochromatic light were made from 1100 nm to 2500 nm at 2 nm intervals, to generate a spectrum with 700 data points. Reflectance from a ceramic reference is divided by reflectance of the sample. The result is expressed as log(1/*R*), where 1 is the reference and *R* is the sample reflectance at each wavelength. Data analysis was performed using ISI software.[10] A typical NIR spectrum of goat's milk dried out on glass fibre filter disk is shown in Figure 2.

Mahalanobis' distances (*H* values) were computed with raw spectral data, previously to use them for calibrations, in order to discard outliers (center selection). The limit for *H* was set at 3.0

Calibrations were generated after scatter correction of the spectra[10] and using three different mathematical treatments: log (1/*R*), first and second derivate. Derivates were computed using a smoothing segment of four data points. Common parameters are shown in Table 1.

Table 1. Common parameters for the three calibrations models.

Scatter correction: SNV and detrend	Cross-validation groups: 4
Maximum number of terms: 10	Number of outliers elimination passes: 3
Numbers of variables: 698	Critical "T' outliers value: 2.50
Downweight outliers: no	Critical "H" outliers value: 10.00

Table 2. Mean, standard deviation and range of laboratory data for each parameter within the calibration set (*n* = 222).

Variable	*n*	Mean	Std deviation	Minimum	Maximum
pH	193	6.3534	0.2079	5.5	6.77
TY	209	18.4040	5.4827	9.09	36.43
CT	200	26.8033	9.8461	7.5	59.50
k_{20}	178	7.8468	3.9070	1.75	27.50
a_{30}	209	15.6064	15.4606	0.0	60.00
a_{60}	209	36.3012	14.4075	0.0	66.00

Calibration equations were obtained using modified partial least squares (MPLS). This regression method uses both the whole spectral information and laboratory data. It is very useful in cases where data are strongly correlated.[12]

The standard error of prediction (*SEP*) was stimated by cross-validation using four groups and is noted as *SECV*. Calibration equations with the lowest *SECV* are retained.

Results and discussion

A total number of 227 samples have been analyzed. After a first raw spectral data analysis (center selection), five samples were discarded as outliers (*H* values larger than 3).

Data from laboratory analyses are shown in Table 2. Parameters TY, a_{30} and a_{60} are highly variable. pH is the least variable. All values obtained are slightly higher than those obtained by other authors.[13, 14]

Table 3. Data set of differences between two duplicate laboratory analyses. Mean, standard deviation, range and standard errors for each parameter within the calibration set (*n* = 222) are given.

Variable	*n*	N_{miss}	Mean	USS	DSD	Min.	Max.
pH	193	30	0.0580	1.7571	0.0957	0	0.32
TY	209	14	1.6044	1608.07	2.7805	0	16.79
CT	200	23	2.1634	4044.29	4.5081	0	31.25
k_{20}	178	45	1.1489	775.75	2.0935	0	12.00
a_{30}	209	14	2.9043	6840.50	5.7347	0	34.00
a_{60}	209	14	3.5909	8471.43	6.3818	0	41.50

N_{miss}: missing data values.
USS: Uncorrected sums of squares.
DSD: Difference standard deviation.

Table 4. MPLS regression. Calibration equation statistics. Raw data.

	SEC	R^2	*SECV*	1-*VR*	#
pH	0.108	0.675	0.120	0.602	212
TY	2.863	0.665	3.012	0.628	212
CT	6.177	0.345	6.336	0.313	199
k_{20}	2.676	0.221	2.843	0.125	190
a_{30}	13.99	0.166	13.849	0.157	221
a_{60}	9.726	0.314	10.003	0.277	209

SEC: Standard error of calibration.
SECV: Standard error of cross-validation.
VR: Variance ratio (explained variance divided by total variance).

Table 3 shows mean, maximum and minimum values, uncorrected sums of squares and standard deviations of the differences between replicated analyses of the same sample. Statistics were carried out by SAS package.[15] The difference standard deviations (DSD) gives an estimation of laboratory errors. The highest mean, the highest range of values and the highest DSD correspond to CT, a_{30} and a_{60} variables. The lowest values of all these statistics correspond to pH variable.

Results of cross-validations are shown in Tables 4, 5 and 6. The final calibration equation was that having the lowest *SECV* and *SEC* errors and with no more than 10 terms.

A general comment on the equations obtained is, that only two of the six parameters studied (pH and TY) appear to be predictable with a reasonable accuracy. For pH and TY, lowest *SECV* and highest R^2 were obtained when using derivatives of the spectral data. The *SECV* value obtained for these parameters (Tables 5 and 6) compares quite well with their DSD values (Table 2) and, in the case of pH, it is similar to the *SEP* (0.08) reported by Frankhuizen[7] on a data set of 100

Table 5. MPLS regression. Calibration equation statistics. First derivate.

	SEC	R^2	*SECV*	1-*VR*	#
pH	0.098	0.738	0.110	0.668	216
TY	2.635	0.670	2.860	0.610	208
CT	6.362	0.359	6.793	0.267	202
k_{20}	2.768	0.230	2.938	0.132	192
a_{30}	14.032	0.142	14.389	0.098	222
a_{60}	9.068	0.398	9.857	0.294	208

SEC: Standard error of calibration.
SECV: Standard error of cross-validation.
VR: Variance ratio (explained variance divided by total variance).

Table 6. MPLS regression. Calibration equations statistics. Second derivate.

	SEC	R^2	*SECV*	1-*VR*	#
pH	0.096	0.750	0.112	0.661	217
TY	2.066	0.789	2.415	0.771	201
CT	5.500	0.402	5.984	0.296	195
k_{20}	2.655	0.140	2.788	0.047	185
a_{30}	13.069	0.259	14.337	0.108	220
a_{60}	9.024	0.253	9.536	0.177	202

SEC: Standard error of calibration.
SECV: Standard error of cross-validation.
VR: Variance ratio (explained variance divided by total variance).

cheese samples. The low R^2 values obtained for pH variable by Frankhuizen[7] and by us (0.71 and 0.75 respectively) could be explained by its relatively low DSD (Table 3).

Of the five rheological parameters (CT, TY, k_{20}, a_{30} and a_{60}) only the equation for TY showed acceptable *SECV* (2.41) and R^2 (0.77) values (Table 6). It is apparent from the preliminary results obtained for CT, k_{20}, a_{20} and a_{60} and from previous attempts made by others authors in measuring quality parameters in others commodities (flour water absorption, loaf and sedimentation volume and Farinograph and Alveograph characteristics of wheat) that it will be very difficult to get equations with a high accuracy for milk rheological parameters. One explanation for the poor performance of the equations could be that the reference data used had very high errors (Table 3).

It should be noted, that the results presented on Tables 4, 5 and 6 are all preliminary results. The short time available between the end of the milk samples collection (June 95) and the writing of this paper, made a deeper NIR data analysis imposible. Work is in progress to study the influence on the calibrations statistics of the following factors:

- Reducing DSD values for each rheological parameter by using four replicates of each sample.
- Increasing the size of the calibration set, adding samples from a second collection period (July–December 95).
- Using different calibrations subsets according to low and high values for each parameter. Studying other mathematical treatments, (discriminant analysis, neural network etc.).

Conclusions

Results obtained up to now showed that NIR reflectance spectroscopy predictions of pH and TY had sufficient accuracy to be used in our dairy goats breeding program. Further work is needed before NIR reflectance spectroscopy can be of interest to the dairy industry for predicting cheese making properties of milk.

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