Detection of cows' milk in goats' milk and cheese

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Introduction

Although Europe has only 18% of the world census of goats it produces a large part of the milk produced by this specie in the world.[1] Only the European Union, with less than 3% of the world census produces 22% of this milk. Furthermore, goat dairy products have played throughout history an important role in the diet of the Mediterranean people.

However, to be competitive in present markets, both production and transformation techniques of goat milk must be improved.[2] They demand a technical updating and better economic and financial organisation, in the same way as it has happened in the cow dairy sector. They should, particularly, adapt to the market requirements of quality and to the regulations of products labelling.

Food adulteration is, in general, a serious consumer fraud. In particular, milk adulterations in dairy industry are a real problem,[3-6] which demands techniques to detect them in a fast, cheap and easy way. Mixing cows (cheaper milk), and goats milk to produce cheese labelled "pure goat" is one of the most frequent frauds[7–9] practised in cheese industries.

Nowadays, in Spain, polyacrilamide gel electrophoresis is the official method to detect these frauds.[10]This is an expensive and time consuming technique, which needs sophisticated laboratory equipment and specialised operators;[11] it consists on extracting whey proteins at pH 8.3 and detecting electrophoretically the presence of cows bands. Furthermore, its power for detecting mixture in cheese is limited to a minimum of 3% of cow milk.

The use of near infrared (NIR) spectroscopy to determine the chemical composition of dairy products, including those from goats, has been studied in several works.[12–14]

The objective of this work is to test the potential of NIRS to detect and quantify the content of cow milk in goat milk and cheese.

Material and methods

Experimental material

Eighteen mixtures of 3,6 litres volume, made with goat milk and varying proportions of cow milk, ranging from 3 to 51%, were prepared with milk collected once a week during 12 weeks in 6 herds of Murciano–Granadina goats and 1 herd of Holstein cows in two different seasons (spring and autumn).

Before cheese-making, a sample of 30 mL of milk was collected from each milk's mixture, and it was kept at 4°C with a 18 mg tablet of "RE Panreac 174798.1260" (wide spectrum micro-tablet).

Two cheeses were made with each of these mixtures of milk (1,8 l/cheese) every week. A first group of 468 cheeses were made in 13 elaborations carried out during the spring and summer of

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2001. A second group of 432 cheeses were made in 12 elaborations carried out in autumn and winter of 2001 and winter 2002. In this way the inter-year, inter-season and inter-herd variability was presumably covered. The cheese-making process used[15] was based on traditional handmade methods of Andalusian cheeses.

Fresh cheeses were store at vacuum at 4°C and hard cheeses were matured for 21 days under conventional ripening conditions (9°C and 80-88% of relative humidity), until their NIR analysis.

Near infrared reflectance spectra

NIR reflectance spectra were obtained from each mixture of milk and each cheese at two maturation stages (fresh and 21 days). A monochromator Foss-NIRSystems model 6500 System-I scanning, equipped with a spinning module, was used. Folded transmission cups (0,1 mm thick with reflecting material bottom IH-0345) were used to present milk samples. Cheese samples were presented intact in small circular sample cups with quartz windows (3,75 cm in diameter and 10 mm in depth).

Each ample was analysed twice. Spectra were collected over the 400–2500 nm region with 2 nm intervals, totalling 1050 absorbance values per spectrum. Spectra were recorded as log (1/*R*), being R reflected energy.

Spectra obtained from 332 milk samples; from 309 fresh cheese samples and from 168 mature cheese samples were used for the calibrations.

Calibration and validation

Quantitative prediction

Calibration and validation analyses were carried out using WINISI II software (Infrasoft International), version 1.04. Previous to the development of calibration equations, the CENTER algorithm, in order to detect possible outliers, the SNV and de-trending mathematical treatments, to correct the scatter effect, and four derivatives, to reduce non informative parts of the spectra, were applied to spectra information.

Calibration equations were developed using modified partial least squares (MPLS) as calibration method[16–19] and the performances of best calibration equations found were externally validated.

Qualitative prediction: discriminant analysis

To allocate a sample in a given mixture group two types of models by discriminant equations were used: One with regression by partial least squares (RPLS) were carried out with WINISI software. Lineal discriminant analysis (LDA) models by matrix of covariance (WINDISCRIM software), where samples are classified in the group to which had a litter Mahalanobis distance (H), were also performed.

All empirical equations and models were developed for milk, fresh cheese and hard cheese. They were also validated with external samples (which had not been used to carry out calibrations). To do this, a training and a test samples set were selected. The training set was used to build equations or models and the test set was used to validate the performance of equations/models.

Results and discussion

Quantitative prediction: calibration equations

The best calibration equation, that with lowest calibration standard error (*SEC*), lowest cross-validation standard error (*SECV*), highest coefficients of determination of calibration (R^2) and cross-

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validation (r^2) were chosen for each of the analysed products. Statistics of these calibration equations are shown in Table 1.

Table 1. Better calibration equations obtained for milk, fresh cheese and mature cheese.

Product	Mean	Standard deviation	Sample size	λ number	PLS factors	*SEC*	R^2	*SECV*	r^2
Milk	25.536	15.630	332	206	16	3.333	0.955	4.100	0.931
Fresh	25.680	15.532	309	693	12	6.999	0.797	8.479	0.704
Mature	24.482	16.397	168	671	12	6.088	0.866	7.895	0.769

λ: wavelengths. *SEC*: standard error of calibrations. *SECV*: standard error of cross-validation. R^2 and r^2:coefficients of determination of calibration and cross-validation, respectively.

Cross-validation statistics obtained for milk were sufficiently good as to expect good predictions from the calibration equation. Results from the cheese calibration were somewhat worst. Coefficients of determination are lower and *RPD* and *RER* values under the limits set as acceptable (3 and 10, respectively). However, these results indicate that the adulteration level can be accurately predicted because all R^2 and r^2 are larger than 0.7 and are not too high in relation with the range of values covered. They are, however, relatively high in order to predict low (under 4% in milk and 9% in cheese) contents of cow milk.

Obviously milk presented the best results, since it is a raw material and, therefore, a simplest product. Cheese is a manufactured product with more structural complexity, that explains the poorest results of their calibration equations.

All calibration equations were validated with external samples, i.e. the test sets. Validation statistics of the three best equations are presented in Table 2:

Table 2. External validation of selected calibration equations.

Product	*SEP*	Mean	Standard deviation	BIAS	*SEP*(*c*)	SLOPE	BIAS Limit	*SEP*(*c*) Limit	*RSQ*
Milk	4.220	26.788 28.047	14.431 13.899	–1.259	4.405	0.997	2.460	5.330	0.921
Fresh	7.857	27.167 24.508	14.855 14.183	2.658	7.428	0.911	5.387	11.671	0.757
Mature	10.108	25.518 25.910	14.567 13.351	–0.393	10.130	0.807	4.737	10.264	0.548

SEP: Standard error of predictions. *SEP*(*c*): corrected standard error of predictions. *RSQ*: coefficient of determination.

Table 2 shows good values for all statistics except for the *RSQ* of mature cheese. Determination coefficient obtained for validation of calibration equation for milk was high. Determination coefficient of validation for fresh cheese was lower, but the slope of the regression of predicted and reference values was close to one, which means that the calibration equations obtained presents a sufficiently good predicting capacity.

Qualitative prediction: discriminant equations and LDA models

Having into account that there were 18 different types of mixtures, it was not possible to carry out a good discriminant equation for all types with the software used. Therefore, five groups of mixtures, with percentages of cow milk (0, 12, 27, 42 51%) covering the whole range of values, were selected to develop the equations.

In the case of milk, the discriminant equation found could classify correctly 76,3% of the samples. LDA models obtained were not acceptable for fresh and mature cheeses.

Conclusions

- -Successful calibration equations haven been obtained for a fast and simple determination of mixtures of cow and goat milk in liquid milk and fresh and mature cheese.
- The potential of NIRS technology to determine milk mixtures in milk and cheese has been demonstrated. However, further research is needed to improve the power of these equations to detect low percentages of cow milk in mixtures (particularly in cheese) and to validate these calibration equations with mixtures of cow and goat milk collected in other regions and moments.

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