Comparison of methods of NIR analysis for goat milk: reflectance vs folded transmission

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Introduction

One of the problems that we face when trying to use near infrared (NIR) technology to analyse food and feeding products is to find a reproducible mode of presentation of samples which minimise the influence of factor, such as sample temperature and humidity, differences among operators, etc, which reduce the spectra repeatability.[1] An additional problem, specific of analysing liquid products is that most of their spectra information comes from water. In order to solve the last problem, Alfaro and Meurens (1989)[2] proposed the DESIR method, a protocol for drying liquid samples in a fibre glass filters before presenting them to NIR instruments. The reflectance mode is used for obtaining spectra of these dried samples. We have used this method to analyse goat and sheep milk, and we have obtained reasonably good calibrations for predicting fat, protein and casein contents in these products.[3] However, we have obtained very poor results when validating these calibration equations with different set of samples collected in different moments and prepared and presented to NIR by different operators. After testing the homogeneity of reference laboratory values of calibration and validations sets of samples, we presumed that the poor validation results were due to small differences in the way of applying the DESIR[2] protocol. This drove us to search for a mode of obtaining more repeatable spectra, analysing liquid samples of milk through folded transmission.[1,3]

The objective of this work is to compare statistics of calibration equations developed to predict protein and casein contents in goat's milk, analysed through reflectance (dried milk) and folded transmission (liquid milk).

Material and methods

To complete this task 106 samples of milk were collected from individual goats in different herds at different moments of their lactation. Samples, then, cover the known range of values of the components analysed (protein and casein). They were transported refrigerated to the laboratory and kept no longer than 24 hours in a refrigerator before they were analysed and dried out to be presented to NIR. Before their analysis, milk samples were heated to 40°C and shaken in order to homogenise fat. After that, each sample was split in three parts, one for the laboratory analysis and the other two for the two modes of NIR analysis.

Reference laboratory values of protein and casein contents (%) were obtained for each sample by Kjeldahl method.[4] Analysis were repeated twice for each sample.

Spectra were obtained with a near infrared spectrophotometer, Foss-NIRystems 6500 System I (with a spinning module) connected to a computer controlled with the Software ISI NIR 3 ver. 3.11

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

(Infrasoft International, Port Matilda, PA, USA).Two methods were used to obtained the spectra from each sample:
(a) Reflectance (R): A glass fibre filter was prepared for each sample using DESIR[2] method. This filter was placed in a standard 3,5 cm diameter circular capsule for solid products, totally transparent to the infrared radiation.
(b) Double transmission (DT): 0,335 mL of milk were dropped in a capsule for semi-liquid and liquid products, with a reflectance material inside (gold). Two different capsules were prepared for each milk sample. The average of the spectra obtained with both capsules was used to develop calibration equations.

Software WINISI II ver. 1.04 (Infrasoft International) was used. Previous to the development of calibration equations, the Center algorithm, in order to detect possible outliers, the Standard normal variate and detrending mathematical treatments, to correct the scatter effect, and four derivatives, to reduce noise, were applied to spectra information in the range of 1100–2500 nm.

Calibration equations were developed using the modified partial least squares regression (MPLS) method. Best calibrations equations were chosen for each constituent analysed, comparing the calibration statistics: lowest cross-validation standard error (*SECV*), lowest coefficient of variation (*CV*), highest coefficient of determination of cross-validation (r^2), ratio of standard deviation of reference values to *SECV* (*RPD*) larger than three and ratio of range of reference values to *SECV* (*RER*) larger than ten

Results and discussion

Statistics of reference laboratory values for the calibration set are presented in Table 1.

Table 1. Statistical descriptors of laboratory analysis for protein and casein content (%) of the calibration set of samples of goat milk.

Constituent	*N*	Mean	Maximum	Minimum	Standard deviation	*SEL*
Protein	100	3.483	4.256	2.881	0.329	0.064
Casein	84	2.800	3.617	2.258	0.292	0.100

N: sample size. *SEL*: standard error of laboratory values

Statistics of the best calibration equation for protein and casein contents, chosen according to the criteria formerly described in Material and Methods, are shown in Table 2.

Table 2. Statistics of best calibration equations for protein and casein contents of goat milk obtained under reflectance and folded transmission modes.

Constituent	Mode	*N*	Mean	Range	*SECV*	r^2	*CV*	*RPD*	*RER*
Protein	R	98	3.484	2.88–4.26	0.102	0.902	2.939	3.195	13.476
	FT	93	3.485	2.88–4.26	0.067	0.959	1.930	4.924	20.505
Casein	R	82	2.803	2.26–3.62	0.130	0.795	4.647	2.213	10.437
	FT	81	2.806	2.30–3.26	0.126	0.801	4.522	2.229	10.401

N: Sample size. *SECV*: Standard error of cross-validation. *r*2: Coefficient of determination of cross-validation. *CV* (%): Coefficient of variation. *RPD*: Ratio of standard deviation of reference values to SECV, *RER*: Ratio of range of reference values to *SECV*, R: reflectance. FT: folded transmission.

It can be observed that the *SECV* value for the best calibration equation for protein content obtained with the folded transmission mode are almost half of that corresponding to the reflectance mode. This explains why the rest of the statistics are also better for the folded transmission mode, being particularly important the difference between the r^2 values.

Very slight differences, probably non significant, are found between the statistics of the calibration equations obtained with the folded transmission and the reflectance modes for casein content.

Conclusions

Calibration equations developed using reflectance and folded transmission modes of analysis, present a high predictive capacity for the determination of protein and total casein content in goat milk. Folded transmission renders, however, better calibrations statistics than reflectance for protein content prediction. Moreover this mode of analysis has advantages in terms of simplicity and quickness of sample preparation. Therefore, higher repeatability of spectra obtained with this method should be expected. This will be tested through independent validations in further experiments.

Acknowledgements

This work was supported by the Spanish Ministry of Science and Technology (Project1FD 1997-1052-C0-01) and was carried out using an NIR instrument and software belonging to SCAI (NIR/MIT Unit) of the University of Córdoba (Spain). The authors would like to thank Ms Francisca Baena of the Animal Production Department (ETSIAM-UCO) for technical assistance.

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