Testing the ability of FT-NIR to determine the α_{s1}-casein and β-casein content in individual milk samples

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Introduction

Milk protein content is one of the main parameters considered for the cheese-making process. The amount of total crude proteins, and in particular of each single protein fraction, can affect cheese production and has a great influence on the final cheese yield.[1,2] Caseins and whey proteins are the two principal groups forming the nitrogen compounds of milk. Among caseins, which are globular proteins associated into micelles and are able to maintain themselves as a stable suspension in milk, the amount of α_{s1}-fraction is a major factor responsible for milk coagulation. The ability to determine its content by a fast and accurate method would be very useful, in order to predict the aptitude of milk to make a stable and firm protein network after clotting. Polymorphism associated with casein fractions is another factor affecting cheese yield.[3] The relative amount of the β-casein fraction is also important for the cheese-making process and cheese-yield, because of its capacity to link whey proteins, in particular β-lactoglobulin, during the clotting phase in forming stable co-precipitates.[4]

This study aimed at verifying the ability of Fourier transform near infrared (FT-NIR) spectroscopy in predicting the concentration of these two main protein fractions in individual milk samples, with the identification of fast and suitable tools for evaluating the milk clotting aptitude as the final objective. The paper has focused on the determination of single milk protein fractions, on the basis of the satisfactory results obtained in a previous work,[5] using FT-NIR for the prediction of the total protein, casein and whey protein contents in individual milk samples.

Materials and methods

One-thousand, five-hundred individual fresh milk samples were collected in association with the Italian Farmers Association. High-performance liquid chromatography (HPLC)[6] was used as the reference method to estimate the content of single protein fractions on all samples. At

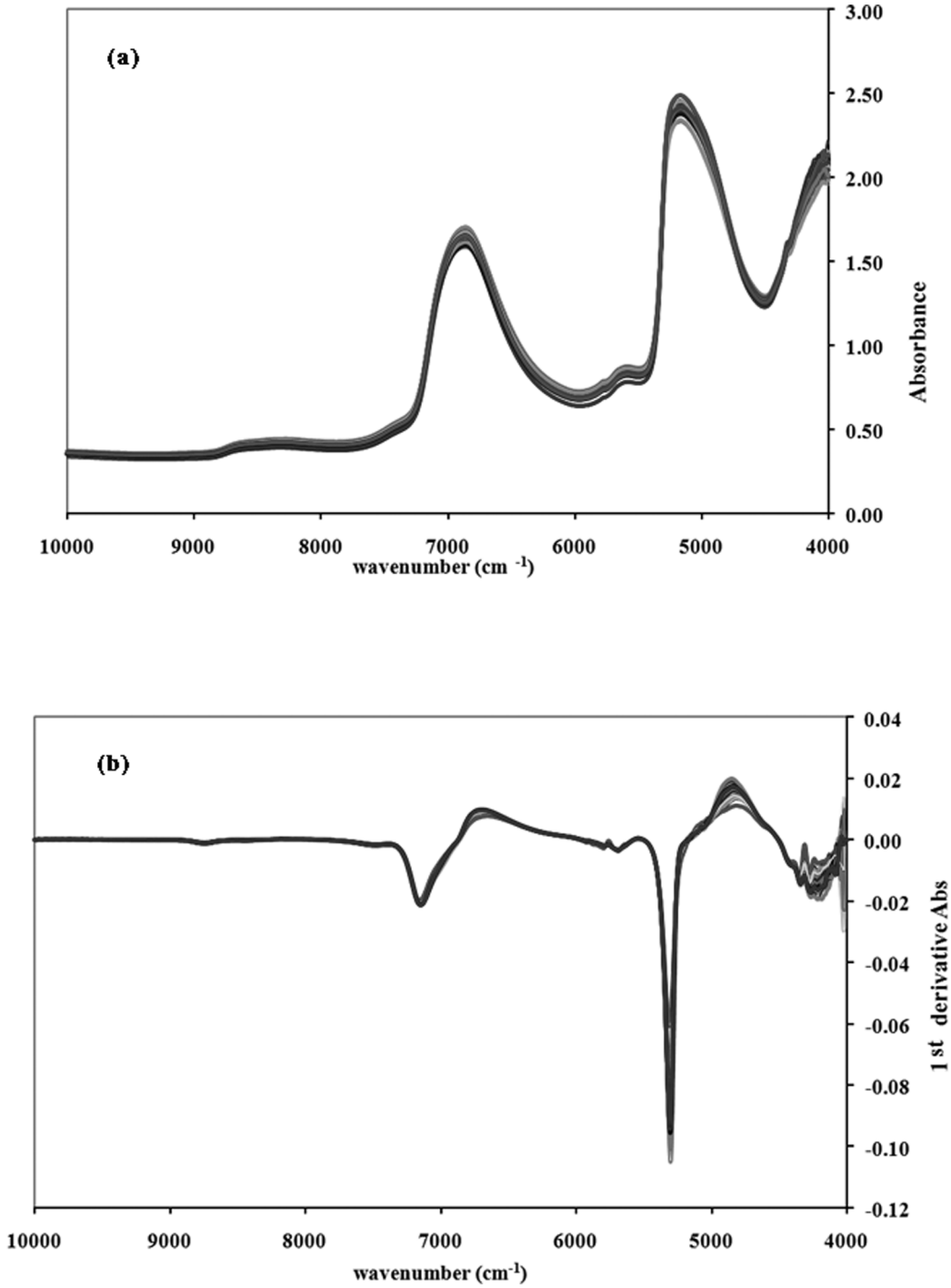

Figure 1. Individual milk samples: (a) examples of raw and (b) pre-treated milk spectra.

Table 1. Statistical parameters associated with calibration and prediction curves for α_{s1}-casein and β-casein determination. Cal: calibration; Pred: prediction; LV: latent variables.

α_{s1}-casein g kg^{-1}	Samples	*LV*	R^2	*RMSEC*	*RMSEP*
Cal/Pred	130/60	15	0.853	0.443	0.597
β-casein g kg^{-1}	Samples	*LV*	R^2	*RMSEC*	*RMSEP*
Cal/Pred	130/60	15	0.938	0.441	1.01

the same time, NIR spectra were collected (32 scans, four times for each sample, at intervals of 4 cm^{-1} for a total of 1501 points, with a resolution of 8 cm^{-1}) at room temperature (20°C ± 1°C), over the NIR range (4000–10000 cm^{-1}). The instrument was a NIRFlex N500 (BUCHI Italia, Assago, Milan, Italy) equipped with a transflectance optical probe (optical distance: 0.16 mm). Spectra were processing by using NIRCal v.5.21 software (BUCHI Italia). First derivative, using the Savitzky–Golay (SG) smoothing filter (five points, polynomial regression of 2nd order), was applied as spectral pre-treatment. Calibration and prediction curves were made on separate sets of samples (130 calibration samples; 60 prediction samples) using partial least squares (PLS) with 15 LV. Using the model developed from these samples, the concentration of α_{s1}-casein and β-casein in the other 1310 samples were predicted, and results compared with those obtained by the HPLC reference method.

Results and discussion

The concentrations of α_{s1}-casein and β-casein in the analyzed individual milk samples ranged respectively from 7.20–14.46, and 8.42–22.72 g kg^{-1}.

Examples of raw and pre-treated FT-NIR spectra of individual milk samples are reported in Figures 1(a) and (b), respectively. The main characteristic absorption bands for –OH, -NH, -C=O, and –CH groups are visible and well highlighted when 1st derivative was applied as pre-treatment [Figure 1(b)].[7]

Table 1 shows the calibration and prediction results obtained using 200 samples, that substantially confirm the ability of FT-NIR in estimating the α_{s1}-casein and β-casein content with satisfactory accuracy. As shown in Table 1, the *RMSEC* values were about double in comparison with the standard error (*SE*) associated with the HPLC reference method used for this specific application (mean $SE = 0.23$; mean recovery 93 %), but they are capable of improvement by increasing the number of samples used in both the calibration and the prediction sets. In particular, a larger *RMSEP* was found for β-casein estimation, probably due to a shortage of samples with high β-casein content.

Figures 2, and 3 show the relationships between HPLC reference and FT-NIR predicted values for α_{s1}-casein and β-casein concentration, respectively.

The results confirmed a satisfactory agreement between HPLC and FT-NIR results for both parameters. The good correlation obtained for α_{s1}-casein can possible be ascribed to: (i) better sample distribution along the concetration range, (ii) its primary structure, (iii) its hydrophylic characteristics, which are higher than those of β-casein fraction, and could have a certain influence on the absorbance in the NIR range.

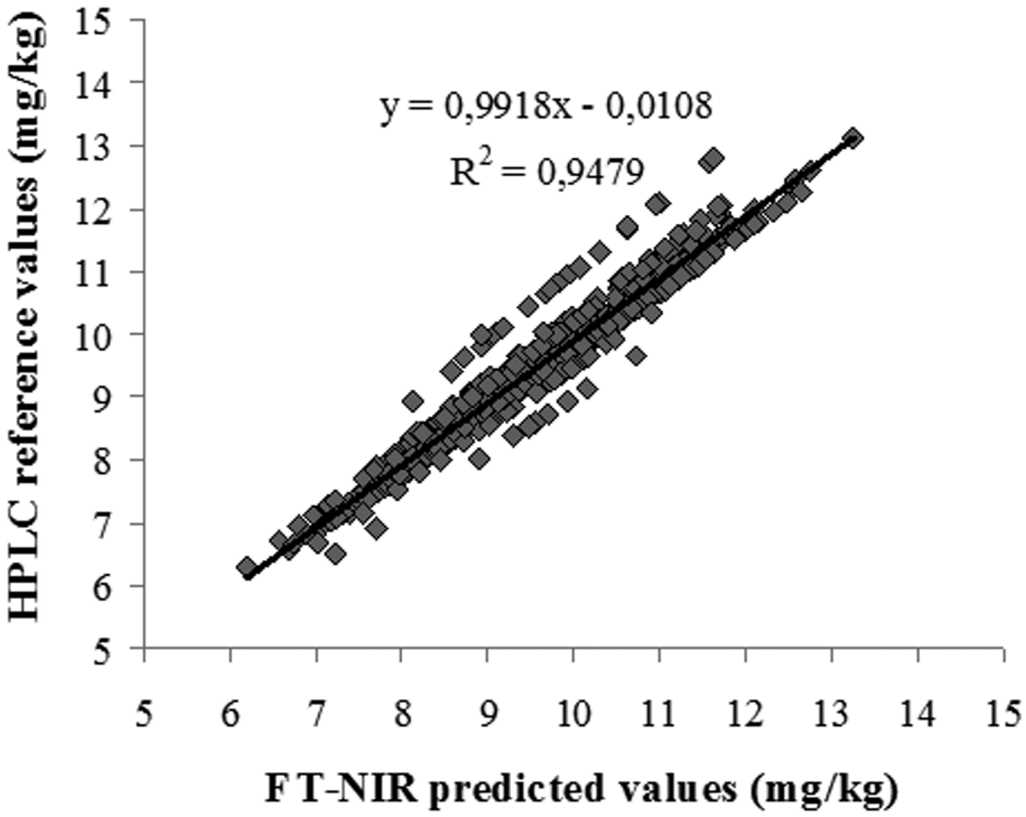

Figure 2. Individual milk samples: relationship between HPLC and FT-NIR data for α_{s1}-casein content determination (1310 unknown samples).

Conclusions

These encouraging results suggest the possibility to analyze a large number of samples for very specific parameters in a short time. These data offer the possibility of developing further studies related to the genetic influence on protein synthesis and phenotype, with positive influence on the genetic selection, facilitating the animals selection on the basis of milk proteins quality and composition. Further investigations will be addressed to evaluating the FT-NIR ability for the determination of κ-casein fraction, which is the third very important parameter to estimate the clotting aptitude of milk. This fraction is present in milk at lower concentration than the α_{s1}-casein and β-casein, so for the present paper the work was firstly addressed to the two main milk protein components.

The data base developed will be used in the near future, in cooperation with the Italian Association of Frisona Breed, to study the presence of genetic factors influencing the synthesis of single milk protein fractions, in order to improve the animal selection.

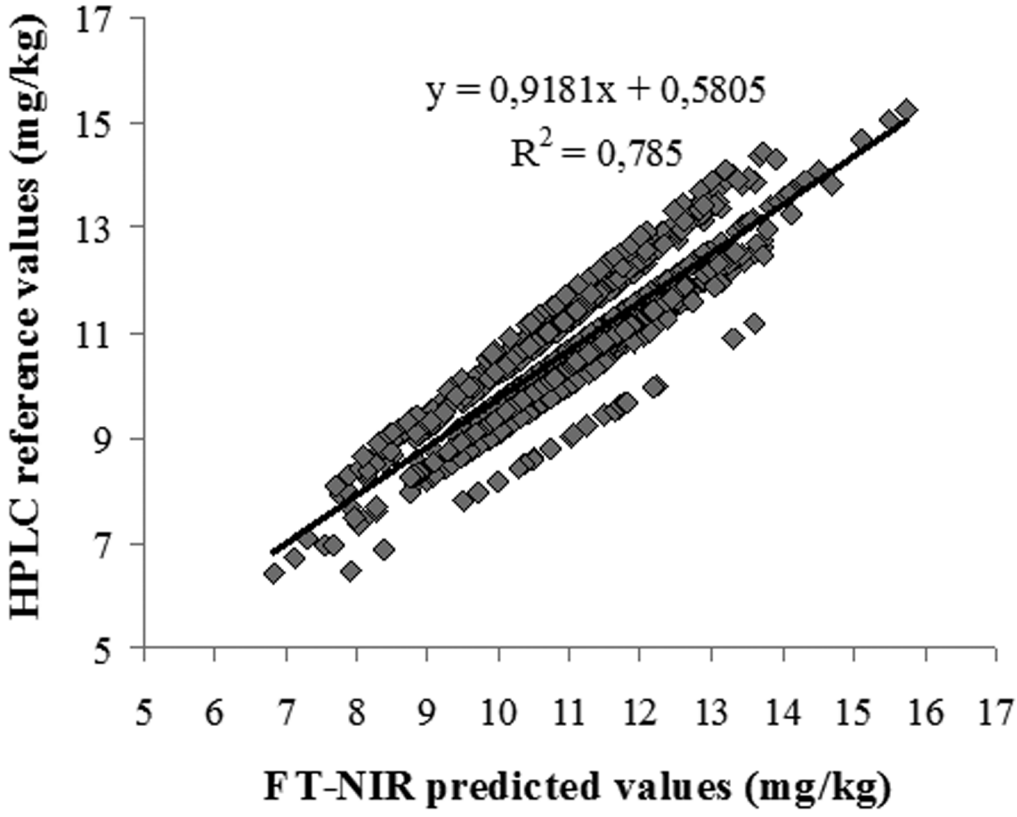

Figure 3. Individual milk samples: relationship between HPLC and FT-NIR data for β-casein content determination (1310 unknown samples).

Acknowledgements

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References

1. M.J. Auldist, B.J. Walsh and N.A. Thomson, *J. Dairy Res.,* **65,** 401 (1998).
2. E. Jakob, and Z. Puhan, *Int. Dairy J.* **2,** 157 (1992).
3. P. Martin, M. Szymanowska, L. Zwierzchowski and C. Leroux, *Reprod. Nutr. Dev.* **42,** 433 (2002).
4. P. Walstra, T.J. Geurts, A. Noomen, A. Jellema and M.A.J.S. van Boekel, in *Dairy Technology,* Ed by O.R. Fennema, M. Karel, G.W. Sanderson, S.R. Tannenbaum, P. Walstra, and J.R. Whitaker. Marcel Dekker, Inc. New York, USA, p. 620 (1999).

5. S. Barzaghi, E.V. Panarelli, K. Cremonesi and R. Giangiacomo, *CD-ROM Proceedings of III Italian Symposium on NIR,* 22-23 May 2008*,* Ed by University of Ferrara, Lazise, Italy, (2008).
6. G. Bordin, F. Cordeiro Raposo, B. De La Calle and A.R. Rodriguez, *J. Chromatogr. A* **928,** 63 (2001).
7. I. Murray and P.C. Williams, in: *Near-Infrared Technology in the Agricultural and Food Industries*, Chapter 2, Ed by P. Williams and K. Norris. AACC, Inc., St Paul, Minnesota, USA, p. 17 (1987).