

# Application of an ISE procedure in understanding the contribution of milk protein compounds to predict cheese yield coupling near infrared and chemical data

Tiziana M.P. Cattaneo<sup>1,2\*</sup>, Maria Chiara Casolin<sup>3</sup> and Riccardo Aleandri<sup>4</sup>

<sup>1</sup>CRA-FLC, Research Centre for Fodder Crops and Dairy production, Lodi, 26900, Italy

<sup>2</sup>CRA-IAA, Food Technology Research Unit, Milan, 20133, Italy

<sup>3</sup>DICTFA, Dept. Chemistry and Pharmaceutical Technology, University of Genoa, Genoa, Italy

<sup>4</sup>CRA, Agriculture Research Council, Rome, Italy

\*Corresponding author: tiziana.cattaneo@entecra.it

## Introduction

Milk protein content is one of the most important parameters considered in the cheese-making process. The amount of total crude protein and the relative concentrations of each protein fraction can affect cheese production and have a great influence on the final cheese yield.<sup>1-2</sup> Cheese yield is defined as the kilograms of cheese obtained from 100 kg of milk. The economic rewards for higher cheese yield make it a very important parameter to maximise.

Calculating the effects that each milk component (i.e. fat and casein) can have on cheese yield is equally important; such calculations would permit a milk quality payment system that could remunerate each parameter for its actual value.<sup>3</sup> Casein and fat content,<sup>4,6</sup> and casein polymorphism, can substantially influence cheese yield.<sup>7</sup> Further, Aleandri et al.<sup>8</sup> observed that curd firmness is the only coagulation parameter correlated with cheese yield. The two parameters, curd firmness and cheese yield, have a non-linear relationship. In particular, the influence of curd firmness on cheese yield is more pronounced if the fat content is low. Curd firmness has previously been linked to the “quality” of caseins (i.e. their genetic type), where genotypes such as  $\kappa$ -casein B correspond in general to a greater  $\kappa$ -casein and total casein content, as reported in literature.<sup>7</sup> As the total casein content is directly related to cheese yield, the  $\kappa$ -casein content influences the size of casein micelles; increased  $\kappa$ -casein content results in smaller micelles and significantly improves the rennet-coagulation properties of cheese.

Cheese-making would substantially benefit from a rapid method that estimated the final cheese yield from the composition of milk: this could give a cheese-maker continuous information on the efficiency of operations, and potentially estimate the influence that particular strategies could have on the entire process of cheese-making. Several formulae for predicting cheese yield have been proposed, with some based on the chemical characteristic of milk, and others using coefficients calculated from a large number of cheese-making trials.<sup>9-11</sup>

The main objective of this study was to build a fast and suitable prediction model for evaluating cheese yield, in order to optimise milk selection for Grana Padano cheese. In order to reach this goal, multivariate techniques were applied to select adequate predictors that were correctly related to cheese yield. NIR signals were identified and used to improve the model.

## Materials and Methods

### Samples

Raw milk samples were collected from two local farms on 13 different occasions. Samples were divided into two sets at a cheese-making firm (Soresina, Italy) producing Grana Padano cheese, and each subsample was entered into six different vats (selected as representative of the total daily transformed milk), for a total of (13×2×6) 156 samples. Cheese yield (kg cheese × kg<sup>-1</sup> milk [%]) and fat and total casein contents (%) were determined.

### Near infrared spectroscopy

Milk spectra were collected using a FT-NIR (BUCHI, Assago, Milan, Italy) spectrometer equipped with a Petri dish accessory for liquids (transmission mode, thickness: 0.3 mm). Spectra were collected from 1100 to 2500 nm using the following operative conditions: 32 scans, 8 cm<sup>-1</sup> resolution (1501 points) and 5 replicates. Standard normal variate (SNV), and conversion from transmission to absorbance, were applied as spectra pretreatments.

## Data analysis

The iterative stepwise elimination (ISE) method was applied to select predictors relevant to cheese yield. ISE performs a partial least squares (PLS) regression with cross-validation many times: each time eliminating one or more predictors (absorbances) with the least importance. PLS regression was then applied to chemical and NIR data to establish a prediction model for cheese yield. The calibration model for the cheese yield was calculated considering both the whole milk sample spectra (1501 points), and the most important variables selected using the iterative stepwise elimination (ISE). The best performing model was identified from the minimum standard error of prediction (SEP) and retained. Cross validations were made by considering the samples of the same cheesemaking process as a block.

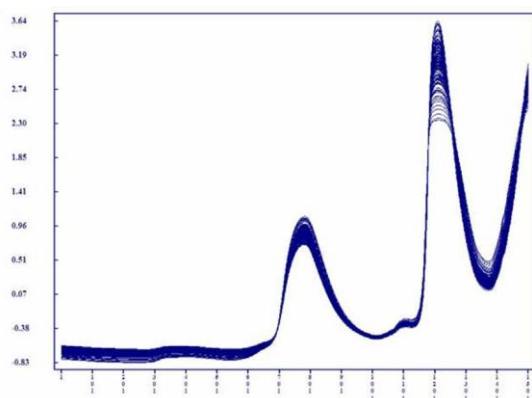
## Results and Discussion

Fat and protein concentration ranges, and cheese yield estimates, are reported in Table 1.

**Table 1.** Variability range for cheese yield %, fat and protein content.

Parameter (%)	Range	
	min	max
Cheese yield	7.94	8.59
Casein	2.47	2.64
Fat	2.35	2.69

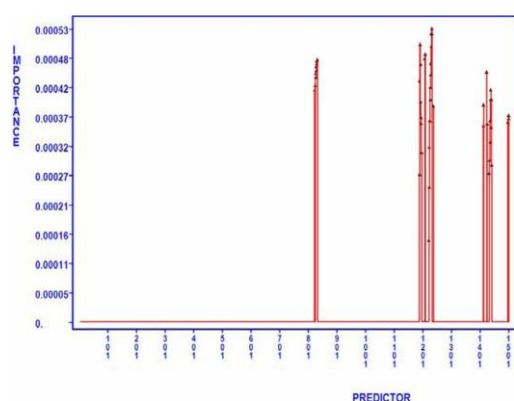
The values of fat content were in agreement with the official protocol used for the production of Grana Padano cheese, a protected designation of origin (PDO) Italian hard cheese, made from half fat milk. An example of a series of the spectra belonging to the same sample is shown in Figure 1; spectra were averaged before data processing for building up the PLS prediction model. The x-axis represents the spectral variables in progressive order (from 1 to 1501).



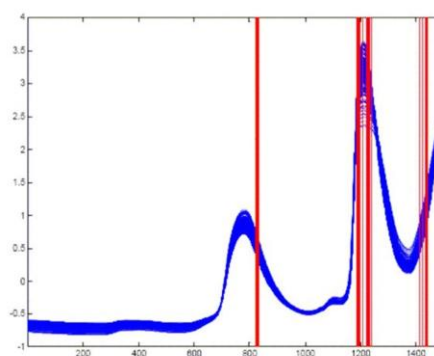
**Figure 1.** NIR absorbance of SNV pretreated spectra.

The ISE method suggested retaining 49 variables for calculating a PLS regression model for cheese yield. The resulting model was able to explain 53.8% of cross validation variance with a SEP 0.12, and using 6 latent variables (LV). The ISE selected variables are reported in Figure 2, and an overlay of the ISE selected variables on NIR spectra is shown in Figure 3. It is evident that the position of some ISE selected variables corresponds well with the absorption bands of “protein” (i.e. -NH bonds; caseins). Some other predictors were found to correspond to water (1900 nm) and fat (2300 nm) absorptions bands.

A PLS model to predict cheese yield was calculated using chemical parameters (milk fat and casein contents) determined by reference methods. This model was able to explain 82.18 % of cross validation variance using 1 LV. NIR variables selected by the ISE procedure and corresponding to the 49 selected predictors were then coupled with the chemical data and a new PLS model was built. Results are reported in Table 2.



**Figure 2.** Variables selected by ISE procedure.



**Figure 3.** Variables selected by ISE procedure, and superimposed on NIR spectra.

The range of casein and fat contents was sufficient for building a satisfactory prediction model for cheese yield; indeed, more than 80% of the explained variance was associated with chemical data. Information from NIR spectral variables improved the model performance and raised the variance explained by cross-validation from 82 to 85%.

**Table 2.** Comparison of the PLS models performance. A) chemical model, and B) chemical+NIR model.

Variables	LV	% of the explained variance in cross-validation
A) Casein+Fat	1	82.18
B) Casein+Fat+NIR	2	85.02

## Conclusion

Coupling NIR spectral variables with chemical data improved the prediction of cheese yield at the milk collection point. NIR determinations of fat and casein contents could provide timely and cost efficient predictions of cheese yield. These results could be confirmed with the addition of new samples, improving the model's robustness. The application of this model in checking the aptitude to coagulate of individual milk samples could be useful for selecting an appropriate milk source for Grana Padano cheese.

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