# Casein by FTIR Lactoscope

**CASEIN IN BULK MILK, VIA OPTIMIZED TRUE PROTEIN MEASUREMENT BY FTIR WITH SLOPE/BIAS ADAPTATION**

**Introduction**

Casein constitutes the main part of milk proteins. The rest is what is called whey protein, including some non protein components. The average casein content of milk expressed as a percentage on total milk protein or true protein is approx. 78% and 82% respectively.

The former ratio is known as the casein number (cas.no), in formula:

Cas-no. = [casein]/[total protein] = [casein-N]/([true protein-N] + [NPN])

where, NPN stands for non protein nitrogen.

Knowledge about the actual casein content of milk is especially important to cheese-makers, since: 1) the higher the casein content of the milk supplied the higher the cheese yield and 2) the casein to fat ratio of the cheese milk has to be optimum in order to achieve a maximum cheese yield. Given standard (reference) methods of analysis for casein in milk are laborious and rather error prone, a common method of determining the casein content is to multiply results for total protein or true protein by the averages for the above ratio's respectively. The latter approach has the advance of eliminating errors connected to variations in NPN content. The basic IR determination of protein resides on the measurement of signals related to true protein (peptide backbone bonds), which signals can be fairly readily discerned from most non protein nitrogen component signals. In contrast spectra for true whey protein and casein are much more alike. As a consequence the discrimination among casein and true whey protein is much less straight forward. In simple matrices like for example protein retentates derived from specific filtration processes this is possible, as field studies carried out by Delta Instruments have revealed. But, with a complex matrix like milk the discrimination is strongly hampered by interferences from variations in organic acids (e.g. citric acid), non protein nitrogen components (read urea), lipolysis (FFA), phosphate, etc. For those reasons the measurement of Casein by FTIR, via slope/bias adaptation of IRresults for true protein, based on an optimized full spectrum model, currently appears the best approach. As follows from the results presented below of a field study after the composition of bulk milk and results obtained for the full spectrum model of true protein, this at least holds with bulk milk.

**Casein in bulk milk**

In 2001 a study after the casein content and true protein content of bulk milk was carried out by “Centre Recherches Agronomiques de Gembloux” (Belgium). Over a period of one year 131 milk samples were collected in 7 dairy plants in South Belgium. Each sample was made up of aliquots out of 6 tankers and was representative of about 125,000 liters. Samples were analyzed for total protein (TN*6.38, “Prot”), non casein nitrogen (NCN) and non protein nitrogen (NPN), using standard (reference) methods of analysis. Casein and true protein contents were calculated by subtraction:(TN-NCN)*6.38 and (TN-NPN)* 6.38 respectively. Table 1 summarizes the regression results for the prediction of casein (Cas) on the basis of the true protein reference values (TrueP). The standard error of calibration (SEC), including all samples (N=131) amounts only 0.020%m/m. The SEC even reduces to 0.016%m/m when 5 samples, which might be outliers, are left out from the calibration (N=126). Shown in figures 1a and 1b are the Y<>X plot and the residual difference plot of the calibration respectively. Figure 2 provides further information regarding the distribution of samples, the variation in casein number (:%cas/prot), as well as the ratio’s %Cas/ TrueP and %NPN/Prot over the year. Given the results obtained, the conclusion from this study can be that the casein content of bulk milk (100.000 liters and more) can be estimated with an extreme high accuracy from an estimate of the true protein content of the milk, via simple slope/bias adaptation.

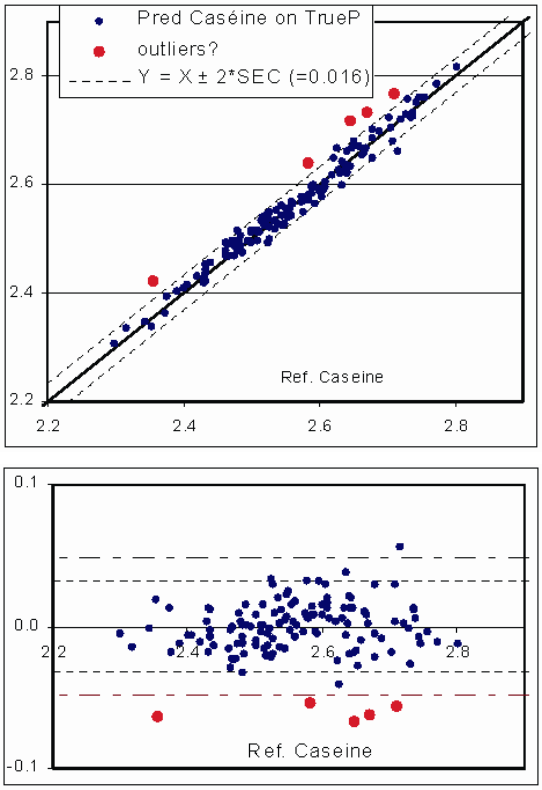
| X | SEC (%m/m) | Coeff | Std Err | N |
|---|---|---|---|---|
| True P<br>Intercept | 0.020 | 0.78<br>0.13 | 0.013<br>0.04 | 131 |
| True P<br>Intercept | 0.016 | 0.79<br>0.09 | 0.011<br>0.04 | 126 |

*Table 1: Prediction of casein*

**True protein in tank milk by Lactoscope FTIR**

In close cooperation with Cecalait (France) a model for the measurement of true protein of milk has been developed in autumn of 2001 on the Lactoscope FTIR. Over a period of 4 months (Sep to Dec) 273 tank milk samples taken for milk payment purposes, were analyzed for true protein by Amido Black and concurrently on the Lactoscope FTIR. A full spectrum calibration model for TrueP was established based on 8 pls-factors. Figures 3a and 3b illustrate the calibration results in the form of an X<>Y plot and a residual difference plot of the cross validation (by cycling, leaving out 1 sample at a time). A further validation of the TrueP model derived, was obtained from results for 79 tank milk samples taken over the period March-April 2002. A bias correction of 0.011% m/m was found to improve the results. An X<>Y plot and a residual difference plot for this validation set are presented in figures 4a and 4b. The standard error of cross validation (SECV) obtained with the calibration set of 2001, (leaving out a single sample only (N=272)) was found to amount 0.016%. With the validation set of 2002 the standard error of prediction (SEP) was 0.017%m/m and even only 0.012%m/m when corrected for the systematic bias mentioned above.

A further model evaluation (not elaborated on here) revealed that the full spectrum model for TrueP includes corrections of IR-protein readings for variations in NPN (read urea), lipolysis (free fatty acids) and organic acids (e.g. citric acid). From these results it thus can be concluded that the true protein content of tank milk can be predicted with extreme high accuracy on the Lactoscope FTIR using the full spectrum model developed.




*Figures 1a and 1b: Y<>X plot and residual difference plots of the regression of reference values for casein on reference values for true protein in bulk milk (study Gembloux)*

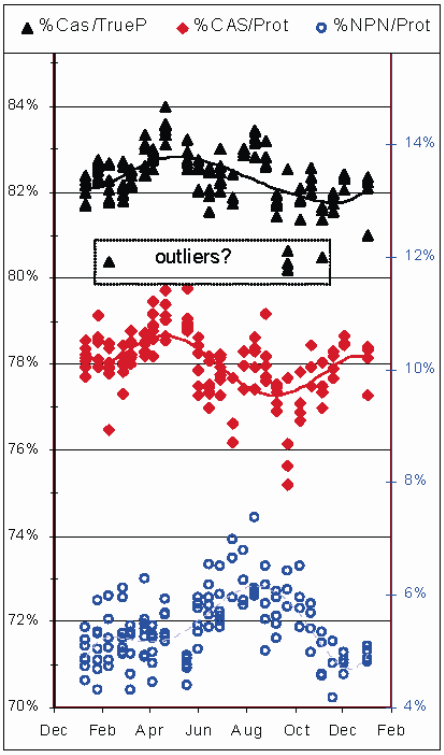



*Figure 2: Plot displaying the variation in casein number (=% Cas/prot), %Cas/TrueP and %NPN/prot over the year for the set of bulk milk samples. The Y-axis on the right handed side corresponds to the ratio %NPN/Prot.*

**Conclusion**

From the combination of the above results it can be concluded that using the full spectrum model for true protein, the casein content of bulk milk can be predicted at very high precision applying slope/bias adaptation only.

**Acknowledgements**

Hereby we want to thank Mr. J. Laloux and Dr. A. van Reusel of the Centre de Recherches Agronomiques (Gembloux, Belgium) for providing us the data on the field study after the protein composition bulk milk and Mr. P. Trossat of Cecalait (Polygny, France) for the research carried out after the optimized model for true protein.

**Note:**

The full spectrum model for true protein available from the LSMmodel set for milk, parm022.

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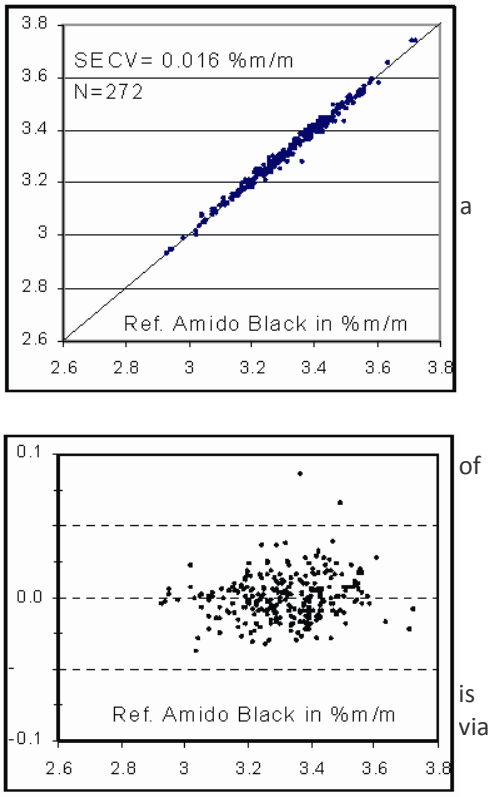
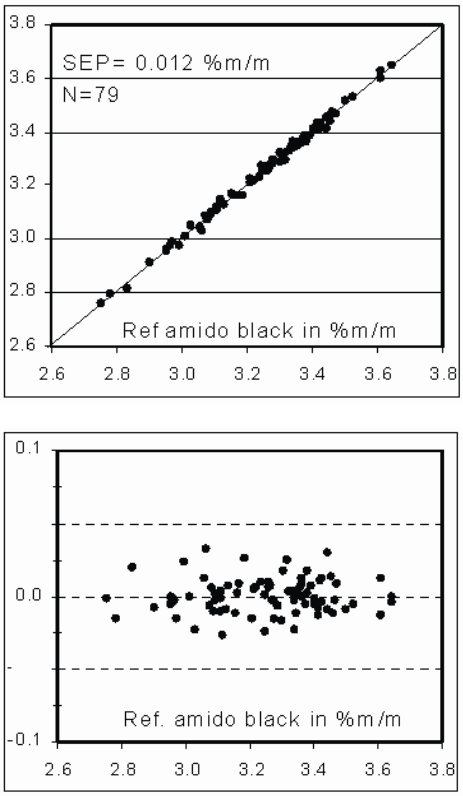



*Figures 3a 3b: Y<>X plot and a residual difference plot of the cross validation of the optimized full spectrum model of true protein.*




*Figures 4a and 4b: Y<>X plot and a residual difference plot of the validation of the same model.*



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