SUMMARY OF « LA LETTRE DE CECALAIT », N°29 (APRIL 1999)

DETERMINATION OF MILK PROTEIN

PART 1 : EVALUATION OF THE ATL 33 SPECTROPHOTOMETER

In France, the official method for protein determination, for milk payment purposes is the Amido Black dye-binding method. Many dairy laboratories also use it, either directly, or as as secondary reference method for the calibration of infrared instruments.

A spectrophotometer is necessary to perform the method. Until now, French laboratories have mainly been using three types Vital 33, Promilk and Astor. As the first two are no longer manufactured, new ones have been developped and marketed. In view of the importance of the Amido Black method as a secondary reference method for the calibration of infra red instruments, French authorities decided that these new spectrophotometers had to be subjected to a national procedure of approval. This requires evaluation tests by an expert laboratory. CECALAIT has therefore recently evaluated the analytical characteristics of two new spectrophotometers ATL 33 (distributed by Humeau) and CECIL 2000 (distributed by Grosseron).

In this issue of « La Lettre de CECALAIT », we report the evaluation of the ATL 33. In the next issue, we will deal with CECIL 2000.

APPARATUS

ATL 33 is a spectrophotometer , equiped with a flow-through cell with an optical path length of 1 mm. It has a network that allows it to operate within the wavelength range of 330 to 999 nm, but the usual wavelength chosen for the method is 620 nm. The ATL 33 is composed of three separate items : the photometer, a microcomputer running the apparatus and a printer.

TESTS PERFORMED

Tests were performed in November 1998, using the following procedure :

- milk volume : 0.8 ml
- addition of 16 ml of Amido Black solution
- swirling for 10 mn
- centrifugation (350g) for 5 mn.
- measuring of the absorbance of the supernatant at 620 nm.

The following characteristics were evaluated, according to IDF standards 128 :1985 and 135B :1991 :

- stability
- carry-over effect
- linearity
- repeatability
- accuracy

❶ **Stability** was evaluated by analysing a set of four amido-black solutions diluted at 4 distinct levels, every 15 mn for half a day. The results show a standard deviation of reproducibility of : **0.03 to 0.06 g/kg or 0.10 to 0.24%,** which corresponds to a good stability.

❷ CARRY-OVER EFFECT

This was evaluated by analysing amido black solutions and water, 10 times, in the following sequence : water-solution 1-solution2, at three different concentrations.

The carry-over effect (Tc %) was estimated with following equation :

$$Tc\ \% = [\Sigma(\text{SOLUTION 2}) - \Sigma(\text{SOLUTION 1}) / \Sigma(\text{SOLUTION 2})] \times 100$$

Tc belongs to the interval **0.45 to 0.51%.**

These values comply with the maximum limit of 1% allowed in routine methods of determination of milk composition, used for milk payment purposes.

❸ LINEARITY

Linearity was evaluated by analysing :

- a set of diluted amido black solutions within the usual absorbance range.
- a set of milk samples, with protein content ranging from 20 to 45 g NP/kg

Linearity was estimated by using simple linear regression. The results obtained on the set of milk samples are shown in Figure 1, in « La Lettre de CECALAIT ».

The spectrophotometer is linear in the range 25 to 36 g NP/kg. This result was obtained using the linear calibration method, given in the basic configuration of the ATL 33. Non linear calibration, which is also possible, is not necessary.

❹ REPEATABILITY

Repeatability was evaluated by duplicate analysis of 100 individual milk samples (preserved with 0.02% bronopol).The results are given in table 1 in « La Lettre de CECALAIT ».

These values are very close the ones obtained with the former spectrophotometers, such as VITAL 33.

❺ ACCURACY

Accuracy was evaluated by duplicate analysis of 100 individual milk samples, using ATL 33 and VITAL 33 (CECALAIT's instrument), in parallel. The instruments were calibrated by using CECALAIT commercial Amido Black SMRs at three concentrations : 25, 30 and 26 g NP/kg.

Accuracy was estimated by using the residual standard deviation of the regression where Y represents the values given by VITAL 33 and X the values given by ATL 33. Figure 2 in « La Lettre de CECALAIT » shows the results in the 25 to 36 g NP/kg range.

- **The mean bias, not significant, is – 0.017 ,**
- **the regression slope is not significantly different to 1 ,**
- **the residual standard deviation of the regression is 0.199**.

This shows a good agreement between the results given by the two instruments.

➢ **In conclusion**, the analytical characteristics of ATL 33 have been found satisfactory. Therefore, it received an official national approval for protein determination for milk payment purposes.

abbreviations : MAP (Fr) = NP = protein nitrogen
SMR : secondary reference material

Bibliographic references are in « La Lettre de CECALAIT »