SUMMARY OF LA LETTRE DE CECALAIT, N° 32 (1st quarter 2000)

Evaluation of the ChemSpec 150

About 95% of milk nitrogen is proteinic. Urea may represent up to half of the non proteinic nitrogen (NPN). So variations in urea explain most of the variations of NPN. Determining the urea content of milk is interesting both for milk and dairy producers. The available analytical methods are either colorimetric, enzymatic (french reference method), spectroscopic (IR) or pH-metric. However, very few are automatic or can be automatized (see La Lettre de CECALAIT, n°19).

Some time ago (see La Lettre de CECALAIT n° 23), we described a differential pH-metric analyser, which seemed an interesting option to avoid the practical difficulties of enzymatic methods. Since then, other analysers have been marketed ; among them the Bentley Instruments (USA) ChemSpec 150. CECALAIT has recently evaluated its analytical characteristics

APPARATUS

ChemSpec 150 is an automatic analyser for the urea content of milk, run by micro-computer for analyses and calibration. Its analytical speed is 150 samples/h. Its principle is based on following reactions :

➢ enzymatic hydrolysis : urea $\xrightarrow{\text{urease}}$ ammonia + CO_2

➢ formation of a colored complex « ammonia – colorant » in the presence of an activating agent

➢ colorimetric detection of the intensity of the green coloration of the complex.

For each sample, the analyse is also performed without urease in order to determine its initial ammonia content, which is substracted from the result given by the analyse in the presence of urease.

TESTS PERFORMED

Tests were performed from february to april 1999.

The following characteristics were evaluated, according to IDF standard 141B and AFNOR V 04-217 standard :

- stability
- carry-over effect
- linearity
- influence of the preservating agent
- repeatability
- accuracy

❶ STABILITY

The stability was evaluated by analysing automatically and in double, a set of three milks, corresponding to the usual range of urea levels every 15 mn for half a day. The results show a standard relative deviation of reproducibility of : **1.69 to 3.53 %.** These are less than the value given in the reference method, which is **6.2% .**

Moreover, the reproducibility values obtained here are about as high as the repeatability values of this test, which shows a good analytical stability.

❷ CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing the same individual milk and distilled water, 20 times, in the following sequence : milk – milk – water - water.

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

$$Tc \% = [(S(\text{water 1}) - S(\text{water 2}))/(S(\text{milk 2}) - S(\text{water 2}))] \times 100$$

Tc values are in the interval of **0.28% to 0.52%.**

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

❸ INFLUENCE OF THE PRESERVATIVE

The test was performed on 38 individual cow milks, from two different herds and the results obtained in following cases were compared:

- on raw milk,
- on milk + Bronopol 0.02 %.

For each milk, samples with and without preservative were analysed one after the other, to avoid a drifting effect.

The results are significantly different (at the 1 % limit) between preserved and unpreserved milk. However this difference is rather low, about **–0.5 mg/dl (**-1.4 %).

In fact, as with all other routine methods, the calibration samples must be preserved in the same way as the test samples.

❹ **LINEARITY**

Linearity was evaluated by a manual analysis in triplicate, without stirring, of a set of 11 urea solutions, with increasing concentrations from 0 to 100 mg urea / dl.

Linearity was estimated by using simple linear regression. The results between 0 and 100 mg/dl give a residual standard deviation of the regression of about **0.6 mg urea /dl** which corresponds to the sum of all theoretical random errors of the method. So, the analyser is linear in the range 0-100 mg urea /dl.

❺ **REPEATABILITY**

Repeatability was evaluated by duplicate automatic analysis of the following samples, all preserved with 0.02% bronopol :

• 150 individual milk samples, among which 15 had been supplemented with urea to increase their urea content by about 30 mg/dl,

• 50 herd milks.

Finally, samples had urea contents ranging from 1.2 to 61.2 mg/dl. The stability of the analyser was checked during the tests.

The results are given in table 1, page 3, in « La Lettre de CECALAIT ».

The standard deviation of repeatability varies from **0.457 to 0.580 mg/dl,** or from **2.82 to 3.04 %** for relative standard deviation. Anyhow, they remain far below the manufacturer's specifications (Sr < 1 mg/dl).

❻ **ACCURACY**

Accuracy was evaluated, as in ❺, by duplicate automatic analysis of :

• 102 individual milk samples,

• 50 herd milks.

The instrument was calibrated using a calibration sample from Bentley, the reference value (31.75 mg/dl of milk) having been determined by differential pH-metry.

The reference method used in this study is the AFNOR enzymatic method (standard V 04-217).

Accuracy was estimated by using :

• the mean bias to the reference values (*moyennes des écarts*),
• the standard deviation of the differences (*écarts types des écarts*),
• the residual standard deviation (Sy,x),
• the equations of the estimated linear regressions,

Figure 1 and 2, page 3, in « La Lettre de CECALAIT » show the results on individual and herd milks.

✎ The mean biases are :

• **-2.4 mg/dl** for individual milks,

• **-1.9 mg/dl** for herd milks.

✎ the residual standard deviations are :

• **2.59 mg/dl**, ie an estimation precision of about ± 4.220 mg/dl, for individual milks,

• **1.60 mg/dl** for herd milks.

The differences between the two methods may come from the fact that the reference method used here is enzymatic, whereas the value of the manufacturer's calibration sample was determined by differential pH-metry.

In conclusion, the analytical characteristics of ChemSpec 150 : instrumental stability, carry-over effect, linearity, repeatability, accuracy, have all been found satisfactory.

Errata

In the last « Lettre de CECALAIT » (n° 32). On page 1, 7th line, lefthand column, it was « pour des teneurs d'urée anormalement basses ou élevées »

< 18 mg**/dl** ou >27 mg**/dl** instead of « mg/l ».

We apologize for this mistake