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.

News about CECALAIT

For 9 years, Cecalait has been offering a variety of services : ringtests, secondary reference materials, education and training, documentation…Of course , these will go on and be further developped, but, a few months ago, CECALAIT's board of Directors also decided to create a new department in CECALAIT devoted to research, development and international programs.

INTERESTING RECENT EEC REGULATION

➢ As usual, regulation n° 2377/90 of the Council concerning maximum residue limits of veterinary drugs in foods of animal origin has been amended.

Annexes I, II and III were amended by regulations 2953/1999 of 1999/12/8 (JOCE L 315 of 1999/12/9), 2728/1999 of 1999/12/20 (JOCE L 328 of 1999/12/22)

Annexes I and II were amended by regulation 2757/1999 of 1999/12/22 (JOCE L 331 of 1999/12/23)

Annex II was amended by regulation 2758/1999 of 1999/12/22 (JOCE L 331 of 1999/12/23)

New residue limits were then inserted in the tables (annexes I and III). There were also other substances, not subject to MLRs, inserted in annex II.

Commission Directive 1999/91/EC of 23 November 1999 amending Directive 90/128/EEC relating to plastic materials and articles intended to come into contact with foodstuffs (JOL 310 of 1999/11/23).

Commission Regulation (EC) No 49/2000 of 10 January 2000 amending Council Regulation (EC) No 1139/98 concerning the compulsory indication on the labelling of certain foodstuffs produced from genetically modified organisms of particulars other than those provided for in Directive 79/112/EEC (JOL 6 of 2000/1/11).

Commission Regulation (EC) No 50/2000 of 10 January 2000 on the labelling of foodstuffs and food ingredients containing additives and flavourings that have been genetically modified or have been produced from genetically modified organisms (JOL 6 of 2000/1/11).

⇒ also issued
White Paper on food safety.
European Commission. Brussels : 2000/1/12, DOC/00/1, COM (1999) 719, 32 pages.

This White Paper contains a three year comprehensive strategy on food safety. The Commission intends to implement its strengthened powers of intervention in matters of food and health safety and to treat food safety as an integral part of health. By strengthening legislation and controls, making these more accessible to the general public, the Commission hopes to increase consumer confidence in the food industry. It also lays out proposals for the creation of an independent European food agency. Comments from whom the White Paper may concern (Community members or candidates, general public…)are welcome until the end of april.

Official Journals of the European Communities of the last 45 days may be consulted on Internet http://europa.eu.int/eur-lex
Older texts may be ordered on Internet http://www.eudor.com

FORTHCOMING EVENTS

➲ REMINDER		CONTACT
3 – 7 april 2000 LJUBLJANA, SLOVENIA IDF/ISO/AOAC Analytical week + Symposium on "Dairy product safety"	FIL Secrétariat 41, square Vergote B-1030 BRUXELLES BELGIQUE	Tel : 32/2.733.1690 Fax : 32/.2.733.04.13 e-mail : info@fil-idf.org http://www.fil-idf.org
13 – 14 april 2000 NICOSIA, CYPRUS Symposium on development strategy for the sheep and goat dairy sector	Mr G. Psathas 39, Dem. Severis Ave. PO Box 22418 1521, NICOSIA *Or* IDF	fax : +357/2.667313 e-mail : cmio@cytanet.com.cy
8 - 10 may 2000 VELDHOVEN, NETHERLANDS Euroresidue IV International Conference on veterinary drug residues in food	EuroResidue Foundation Dr L. A Van Ginkel c/o RIVM PO Box 1 NL-3720 BA BILTHOVEN	tel : +31 30 274 2613 fax : +31 30 274 4403 e-mail : euroresidue@rivm.nl Internet : http://www.rikilt.dlo.nl/euroresidue/

11 – 14 june 2000 STRESA, ITALY IDF seminar on udder defences and immunology	New Team Via Ghiretti, 2 43100 PARMA *or* IDF	tel : +39 0521.29.39.13 fax : +39.0521.29.40.36 e-mail : newteam@paramiol.it http://www.newteam.it
26 - 27 june 2000 NANTES, FRANCE, FOODSIM 2000 1st International Conference on simulation in food and bio industry	Society for Computer Simulation International	tel : +33. 2.51.78.54.54 fax : +33 .251.78.54.55 e-mail : ICSFBI@enitiaa.nantes.fr
28 - 30 june 2000 MELBOURNE, AUSTRALIA Dairy Ingredients Science 2000	OzAccom Conference Services PO Box 164 Fortitude Valley QLD Australie 4006 *ou* DIAA National Office Kristine MANSER Level 6, 84 William Street Melbourne VIC Australie 3000	tel : +61 (0)7 3854 1611 fax : +61 (0)7 3854 1507 e-mail : ozaccom@ozaccom.com.au tel : +61 (0)3 9670 0422 fax : +61 (0)3 9642 8144 e-mail : diaa@dairy.com.au

➲ OTHER IDF EVENTS

16 – 20 september 2000 DRESDEN, GERMANY 84th IDF annual sessions	IDF	

➲ OTHER EVENTS

27 – 29 march 2000 PARIS, FRANCE environmental, sanitary and financial risk management 5th Qualibio national sessions	QUALIBIO	tel : + 33/2.99.78.40.40
12 – 14 april 2000 PORTO, PORTUGAL Nutritionists meet food scientists and technologists.	NMFST Conference Secretariat Philippa ORME 12 Church street, West Hanney, Nr Wantage OXON OX12 0LN UNITED KINGDOM	tel : 44/(0)1235868811 fax : 44/(0)1235868811 e-mail : p.orme@dial.pipex.com http://www.elsevier.nl/locate/nmfst2000
24 – 28 july 2000 BALTIMORE, MD, USA Rencontres annuelles ADSA – ASAS *American Dairy Science Association / American Society of Animal Science*	2000 ADSA/ASAS Joint meeting 111 North Dunlap Avenue Savoy IL 61874 USA	tel : 1/217.3563182 fax : 1/217.3984119 e-mail : adsa@assochq.org asas@assochq.org
6 – 9 august 2000 ATLANTA, USA 87th IAMFES annual meeting *International Association of Milk, Food and Environmental Sanitarians*	IAMFES 6200 Aurora Ave Suite 200W DES MOINES IA 50322-2863 USA	Tel : +1/515.2763344 Fax : +1/515.276.8655 e-mail : iamfes@iamfes.org http://www.iamfes.org
10 – 14 september 2000 PHILADELPHIA, USA 114th AOAC International annual meeting *Association of Official Analytical Chemists*	IDF	

INTERESTING NEW STANDARDS

IDF STANDARDS

IDF 73B :1998. MILK AND MILK PRODUCTS. Enumeration of coliforms.

This provisional standard replaces the 1985 version. It also describes a colony count technique and a MPN technique, both at 30°C, without resuscitation

IDF 83A :1998. MILK AND MILK-BASED PRODUCTS. Detection of thermonuclease produced by coagulase-positive staphylococci in milk and milk-based products. This standard replaces the 1978 provisional version.

FIL 181 :1998. DRIED MILK PRODUCTS Enumeration of *Bacillus cereus* : MPN technique. It is a new provisional standard

IDF just began to circulate these new standards. So we cannot give you more details yet.

EUROPEAN (and french) STANDARD

EN ISO 1854, december 1999 (ICS 67.100.30 ie « cheese») WHEY CHEESE Determination of fat content. Gravimetric reference method

EN ISO 2450, december 1999. (ICS 67.100.99 ie « other milk products») CREAM. Determination of fat content. Gravimetric reference method

EN ISO 7208, february 2000, (ICS 67.100.01 ie « *milk and milk products* »). SKIM MILK, WHEY AND BUTTERMILK. Determination of fat content. Gravimetric reference method

There are mostly editing differences between these three standards and the previous versions, issued respectively in 1989, 1988 and 1985. However an important methodological difference is that now the operator may use pentane instead of light petroleum.

LIST OF BIBLIOGRAPHIC REFERENCES

You will find enclosed the list of references that we found in our litterature survey over the past months and that we decided to put into our data base on dairy analytical techniques. Should you be interested in any of these references, please, contact us.

➢ We also noticed

• Animal Production Service, FAO Animal Production and Health Division. Manual on the use of the LP-system in milk handling and preservation. 1998, 31 pages. *ISBN 92-5-104254-3*
Il is the report of an international programme involving FAO, WHO, IDF, the university of Uppsala…about the lactoperoxidase-system in 80 countries around the world.

• CHAVERON H. Introduction à la toxicologie nutritionnelle. Editions Tec & Doc. Paris : Lavoisier, 1999, 224 pages.

• les amines biogènes dans les aliments. Editions Yves Dacosta : Paris, 1999, 211 pages

• MOSSOBA, M.M. (Ed.) Spectral methods in food analysis. New-York : Marcel Dekker Inc., 1999, ISBN 0-8247-0223-9

• RYSER E.T. ; MARTH E.H. (Eds). *Listeria*, listeriosis and food safety. New-York : Marcel Dekker Inc., 1999, *ISBN 0-8247-0235-2*

• a special report about biosensors in the magazine Analusis, 1999,vol. 27, n° 7