# 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 % = [Σ(SOLUTION 2) - Σ(SOLUTION 1) / Σ(SOLUTION 2) ] x 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 »

## AFNOR VALIDATION

➢ AFNOR (French standardisation body) validated recently the following alternative methods :

- COLI-ID, (distributed by BioMérieux SA) an agar medium for the fast detection and enumeration of β-glucuronidase positive *Escherichia coli* in food.

- VIDAS ICS SLM AND ICS-box test kits for the detection of *Salmonella* in food. (distributed by BioMérieux SA)

- PETRIFILM high sensibility for the enumeration of gas-producing coliforms (distibuted by 3M)

➢ The validation of three other alternative methods was renewed for four years : Gene-Trak systems DNA GT609 (distributed by Diffchamb), Accuprobe (distributed by BioMérieux) and Oxoid *Listeria* rapid test, for the detection of *Listeria monocytogenes.*

➢ The Listerscreen alternative method for the detection of the *Listeria* genus is no longer validated.

## AOAC VALIDATION

AOAC International recently validated the EiaFoss analyzer (based upon an ELISA test) for the detection of *Salmonellae*

## INTERESTING NEW STANDARDS

### ↳ INTERNATIONAL STANDARDS

**ISO 11813 December 1998**. MILK AND MILK PRODUCTS Determination of zinc content. Flame atomic absorption spectrometric method.

**ISO 12081 December 1998**. MILK. Determination of calcium content. Titrimetric method

**ISO 6887-1 February 1999**. MICROBIOLOGY OF FOOD AND ANIMAL STUFFS Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1 : General rules for the preparation of the initial suspension and decimal dilutions

**ISO 6888-1 February 1999**. MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1 : technique using Baird-Parker agar medium

**ISO 6888-2 February 1999**. MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 2 : technique using rabbit plasma fibrinogen agar medium.

### ↳ EUROPEAN STANDARDS

**EN ISO 14501 February 1999 (ICS 67.100.10 Milk).** MILK AND MILK POWDER. Determination of aflatoxin M1 content. Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography

**FD CR 12739, March 1999 (ICS 07.080, biology, zoology, botanics)** BIOTECHNOLOGY. Research, development and analysis laboratories. Report on the selection of equipment needed for biotechnology laboratories according to the degree of hazard.

## FORTHCOMING EVENTS

**🕮 put this date in your diary**
**18th June 1999 :Cecalait's Annual General Meeting**

at the restaurant « TAC Salons de Bercy », 48, Bd de Bercy, Paris (12e)

In the morning : ordinary Annual General Meeting
in the afternoon, scientific-technical lectures

**➲ Reminder**

**💣 be careful : some dates have changed**

➢ **7 – 10th June 1999 :** IDF Symposium in SAINT MALO (France)

« New applications of membrane technology in the dairy industry. »

For information, please contact

Prof. J.L. MAUBOIS
Laboratoire de Recherches Laitières INRA
65, rue de Saint-Brieuc
35042 RENNES CEDEX

➢ **9 – 11th june 1999** : 5th International symposium on authenticity of foods, in LA BAULE (France)

For information, please contact

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➢ **1 – 4th August 1999** : IAMFES ANNUAL MEETING, in DEARBORN (Michigan, USA)

*IAMFES : International Association of Milk, Food and Environmental Sanitarians*
For information, please contact

IAMFES
6200 Aurora Ave
Suite 200W
DES MOINES
IA 50322-2863
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mel : iamfes@iamfes.org
http://www.iamfes.org

➢ **18 – 21 october 1999** : METROLOGIE 99, 9th International Congress of metrology in Bordeaux (France)

For information, please contact

Secrétariat Général Métrologie 99
Sandrine GAZAL
Maison de l'entreprise
429, rue de l'Industrie
34966 MONTPELLIER CEDEX 2
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**➲ IDF EVENTS**

**3 – 4th June 1999** : BUENOS-AIRES (Argentina)

« WTO and the prospects for dairying in the next negotiating round»

**14 – 18th September 1999** : ATHENS (Greece) 83rd annual sessions

**5 – 7th October 1999** : SAN FRANCISCO (USA), nutrition week

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41, square Vergote
B-1030 BRUXELLES
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**➲ OTHER EVENTS**

**26 – 30th September 1999** : HOUSTON, (Texas, USA), 113th AOAC International annual meeting

for information, please contact **IDF**

IUFoST Congress 10, GPO Box 128,
Sydney
NSW 2001
AUSTRALIA
Tel : +61 2 9262 2277
Fax : +61 2 9262 2323
e-mail : Tourhosts@tourhosts.com.au

**3 – 10th October 1999** : SYDNEY (Australia), 10th World Congress of Food Science and Technology

For information, please contact

## DETECTION OF β-HEMOLYTIC STREPTOCOCCI

### ➢ REGULATION

β-hemolytic streptococci are pathogens responsible for septic sore throat and scarlet fever in humans and for mastistis in cattle. However there exists no EC microbiological criteria concerning these microorganisms, the French regulation [1] specifies that **for raw milk intended for direct human consumption,**

**there should be no β-hemolytic streptococci in 0.1ml.**

It also specifies that the β-hemolytic streptococci belong to A, B, C, G or L Lancefield serological groups [2]

### ➢ NON STANDARDISED METHOD

An official text [3] lists the standardised methods applicable to all other microbiological criteria, but there is no recommanded method for the detection and enumeration of these streptococci.

The litterature [4] explains that « there exists no satisfactory selective medium for the quantitative estimation of β-hemolytic streptococci in foods ». However, it is claimed that the TKT medium, described in 1953 by Hauge and Ellingsen, quoted in [4], allows detection and selective growing of all β-hemolytic streptococci. [4] and [5]. This medium is a blood agar, containing thallium sulfate (T), crystal violet (K) and a titrated quantity of toxin from a *Staphylococcus* β-hemolytic culture (T). The toxin accentuates the hemolytic reaction of the streptococci looked for. A few years ago, this toxin was manufactured and allowed easy preparation of the medium. But production has stopped ; so the users have to prepare the toxin themselves, which is not easy. It seems that AFNOR therefore cancelled, a few years ago, a draft standard, based upon this medium.

At the present time, there is no alternative commercial medium. Identification strips imply prior isolation, whereas commercial tests kits for fecal streptococci are usually intended for those belonging to Lancefield group D, not concerned here.

### ➢ AFSSA METHOD

AFSSA recently developped a method. For isolation purposes, it recommends to use a classical blood agar medium, but with a slightly modified procedure. Indeed, this medium is not selective enough. Therefore, β-hemolytic streptococci may be completely undetectable because of overgrowth by other bacteria, particularly by those that grow rapidly and produce large colonies. That's why AFSSA recommends to streak 0.1 ml of the initial suspension on more than one Petri dish, in order to favour isolated colonies.

Colonies having β-hemolytic zones must then picked for confirmation and identification. First, they have to be examined microscopically. Typical cocci chains must then be prepared for :

- Gram staining : streptococci are Gram-positive,
- catalase test : streptococci are catalase-negative,
- for biochemical screening, such as the CAMP-test, esculin or hippurate hydrolysis, or sugar

fermentation screening. This can also be done by using identification strips for streptococci, which allow to identify rapidly pathogenic species of streptococci.

In some special cases, further serological tests may be necessary.

➢ **IN CONCLUSION**

As regulation exists and users demand it, it seems really necessary to define a reference method. The AFSSA method is applicable, but not well known. It would be highly interesting to launch a standardisation procedure based upon this method.

Abbreviations : AFNOR : French Standardization Body
AFSSA : French food safety agency, former CNEVA

*Bibliographic references are in « La Lettre de CECALAIT »*

## INTERESTING RECENT EEC REGULATION

**Regulation n° 175/99** of 1999/1/26, of the Commission modifying regulation EEC n°3942/92, EC n° 86/94, EC n° 1082/96 et CE n° 1459/98 establishing reference methods for the determination of some tracers (*ie sitosterol and stigmasterol*) in butter, butter-oil and cream (JOCE L 20 on 1999/1/27).

**Regulation n° 508/99** of 1999/3/4, of the Commission modifying annexes I to IV of regulation n° 2377/90 of the Council concerning maximum residue limits of veterinary drugs in foods of animal origin. (JOCE L60 on 1999/3/9)

This is a remarkable update of the regulation, which annexes have been modified very often (usually in order to add residue limits for other drugs) !

But as soon as 1999/4/16, the regulation was modified once again, with some new residue limits being inserted in the tables. (**Regulation n° 804/1999** in JOCE L102 on 1999/4/17)

**Regulation n° 568/99 of 1999/3/16** modifying regulation n° 577/97 concerning the application of regulation n° 2991/94 of the Council establishing standards for spreadable fat and of regulation n° 1898/87 of the Council concerning milk and dairy products denomination in marketing. (JOCE L70 on 1999/3/17)

**Commission Directive 1999/11 of 1999/3/8** adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (JOCE L77 on 1999/3/23)

**Commission Directive 1999/12 of 1999/3/8** adapting to technical progress for the second time the Annex to Council Directive 88/320/EEC on the inspection and verification of good laboratory practice (GLP) (JOCE L77 on 1999/3/23)

**➳ others texts**

➢ **Commission Decision of 23 February 1999** adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996 (JOCE L84 on 1999/3/27)

➢ **Directive 1999/2/EC** of the European Parliament and of the Council of 1999/2/22on the approximation of the laws of the Member States concerning foods and food ingredients treated with ionising radiation

## LIST OF BIBLIOGRAPHIC REFERENCES

It is the list of references that we noticed in our litterature survey during 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, contact us, please.

*NB : we remind you that we can copy neither book nor standard.*