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CECALAIT
NEWSLETTER

ABOUT IDF /ISO / AOAC ANALYTICAL WEEK

The IDF / ISO / AOAC Analytical Week was held from 19 to 22 May, in Holstebro, Denmark.

On the occasion of the general opening session, F. van Luin, MSSG Chairperson, reminded the audience that one of the primary objectives was the work on the methods of analysis on sheep and goat's milk.

Then the week went on with work groups on chemical and microbiological analyses methods and quality assurance, in which project leaders presented the state of advancement of the work to groups of experts.

Proceedings will be published by the IDF Secretariat.

Philipe TROSSAT

Abbreviation :

MSSG : Method Standard Steering Group, within the IDF

ADRESS BOOK

AOAC

American Organisation of Analytical Chemistry

http://www.aoac.org

ISO

International Standard Organisation

http://www.iso.ch

IDF

International Dairy Federation

http://www.fil-idf.org

ARTICLES TO COME IN NEXT ISSUES

METHOD

R&D in Dairy Analyses -
Enumeration of Butyric Spores in Milk : Application to Microrespirometry

MICROBIOLOGY

Bibliographic Synthesis on *Pseudomonas*

EVALUATION OF YORK® INFRARED ANALYSER ON FAT AND PROTEIN CONTENT

YORK® mid-infrared spectrophometer permits to determine fat content, protein matter and lactose, in milk and dairy products. CECALAIT physico-chemical laboratory conducted evaluation assays on apparatus stability, contamination between samples, linearity, repeatability and accuracy. This study concerned fat and protein criteria. The results obtained pertaining to stability, contamination, linearity, calibration, repeatability and accuracy, comply with the IDF standard 141C:2000. "Whole milk - determination of milk fat, protein and lactose content (Guidance for the operation of mid-infrared instruments)", and the CNIEL "Manuel d'utilisation des analyseurs infrarouges pour les laboratoires interprofessionnels et de contrôle laitier" requirements *(= User's Guide to Infrared Analysers for Interprofessional and Milk Control Laboratories").*

The YORK® mid-infrared spectrophotometer (2-10 µm) is a manual apparatus (without conveyor) with an analytical rate of 120 samples/h. It is designed for the determination of the composition of certain constituants in milk and dairy products. It is manufactured by On-Line Instrumentation (GB) and marketed by Swantech.

The apparatus works using a filter mono-beam infrared system. It is also equipped with a sample re-heating system. From a MLR* calibration, it determines the common constituants (fat, protein and lactose). It is coupled with a micro-computer which deals with running and signal processing.

TESTS

CECALAIT physico-chemical laboratory conducted the evaluation tests. They were concerned with reference and infrared analyses for fat and protein. The tests took place from December 2002 to June 2003 and delt with evaluating the apparatus stability. Then, after calibration of the device, the following characteristics were evaluated : contamination between samples, linearity, repeatability and accuracy.

While being evaluated, certain improvements were carried out on the apparatus. Among others, an on-line reheater was fitted. The evaluation criteria of estimated parameters were taken from IDF 141C:2000 "Whole milk - determination of milk fat, protein and lactose content (Guidance for the operation of mid-infrared instruments" and from the CNIEL's Guide.

STABILITY

The evaluation of stability was performed by analysis of duplicate milk samples every 20 minutes for half a day (representing 7 measurement cycles) according to the actual working conditions of a milk payment laboratory.

In order to evaluate the apparatus stability, repeatability and reproductibility parameters were calculated for each analytical crierion.

Criterion	M (g/l)	Sr (g/l)	SR (g/l)	Sr (%)	SR (%)	r (g/l)	R (g/l)
Fat	37.78	0.09	0.25	0.25	0.75	0.26	0.78
Protein	32.09	0.11	0.19	0.40	0.63	0.36	0.56

M : mean
Sr et SR : standard deviation of repeatability and reproducibility
Sr% et SR% : relative deviation of repeatability and reproducibility
r et R : maximal difference of repeatability and reproducibility within 95% of cases

Concerning the fat and protein criteria, the standard deviation of repeatability and reproducibility (SR) observed with the control session complies with those deduced from the IDF standard 141 C :2000 (SR ≤ L / 2,58 soit 0.27; L = control card limit at 99 % equals 0,7 g/l)

CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing one batch of milk and distilled water, 20 times for fat and protein content, in the following sequence : MILK (L1) - MILK (L2) - WATER (W1) - WATER (W2). The volume pumped was 12 ml.

The carry-over effect was estimated with the following formula :

$$Tc\ (\%) = 100 \times [\ \Sigma W1 - \Sigma W2\ ] / [\ \Sigma M2 - \Sigma M2\ ]$$

Criterion	Mean Level (g/l)	Carry-over (%)
Fat	37.88	0.00
Protein	31.83	0.00

The carry-over effect between successive samples is lower than 1% for each criterion tested (acceptability limit according to the IDF standard 141C:2000 and CNIEL Guide).

LINEARITY

In every case, **volume/volume** dilutions were performed by corrected weighing of the volumic mass. This corresponds to the infrared spectrophotometry dosage and to the French reference measurements which are equally volumic.

▪ Fat : a set of 14 evenly distributed milks set, from 0 to 120 g/l, was elaborated using a mixture of cream and skimmed milk.

▪ Protein : a set of 14 evenly distributed milks set, from 0 to 80 g/l was elaborated using a mixture of proteinic retentate and filtrate obtained by tangential ultrafiltration (cut off level : 10 KD).

Each set was evaluated in duplicate, following increasing, then decreasing levels of fat or protein matter.

▪ Fat :

Figure 1 : Linearity deviation distribution (whole range)

(See at the end of this article)

Figure 2 : Linearity deviation distribution (adjusted range from 22 to 56 g/l)

(See at the end of this article)

The above graphs represent the deviation distribution of linearity according to the different levels obtained from dilutions of cream in skimmed milk over the whole range (figure 1) or after digital adjustment (figure 2) on the apparatus calibration range (from sample 3 to sample 7 or from 22 to 56 g/l).

The residual standard deviations of regression are :

Type of regression	Whole range (g/l)	Calibration range (g/l)
1st Order	0.51	0.15
2nd Order	0.23	/
3rd Order	0.15	/

It can be noted that the apparatus is not linear over the whole range tested : after adjustment between 22 and 56 g/l, the residual deviation is about –0.8 g/l to 70g/l and from –2.4 g/l to 100 g/l. However, it is possible to observe two areas of linearity (between 20 and 60 g/l and between 60 to 100 g/l).
It is also possible to improve the linearity of the apparatus by applying a correction usinging a 3rd order polynomial.

▪ Protein content :

Figure 3: Linearity deviation distribution

(See at the end of this article)

Figure 4: Linearity deviation distribution (adjusted range from 25 to 40 g/l)

(See at the end of this article)

The above graphs represent the deviation distribution of linearity according to the different levels obtained with ultrafiltration retentate and filtrate dilutions over the whole range (figure 3) or after digital adjustment (figure 4) on the apparatus calibration range (from sample 4 to sample 7 or from 25 to 40 g/l).

The residual standard deviations of regression are :

Regression type	Whole range (g/l)	Calibration range (g/l)
1st Order	0.32	0.03
2nd Order	0.07	/
3rd Order	0.07	/

The results showed a minor linearity defect of the instrument over the whole range tested. After adjustment between 25 and 40 g/l, the residual deviation is about 0.5 g/l to 50 g/l and1.9 g/l to 80 g/l. Two areas of linearity can also be observed (between 0 and 40 g/l and between 40 to 80 g/l).
It is also possible to improve the linearity of the apparatus by applying a correction using a 2nd order polynomial.

For fat and protein contents, the linearity of the iinstrument s satisfactory in the range corresponding to mean contents (FM : 20 to 55 g/l ; PM : 20 to 40 g/l). However, it is possible, to widen the usable area, by using an equation to correct the results ; either
a 3rd order (FM) or a 2nd order polynomial (PM).

CALIBRATION

Calibration was evaluated using a range of 13 recombined milk samples in an orthogonal matrix of Fat and Protein contents complying with the IDF standard 141 C : 2000 requirements. Each sample was analysed in successive duplicates from 1 to 13. A control was set at the beginning and at the end in order to ensure stability. Calibration was performed on 03/06/2003.

The table below recapitulates the results obtained using simple and multiple linear regression.

Criterion	Y= b.x + a			
Fat	b	a	Sd (g/l)	Sy,x (g/l)
Protein	1.002	-0.14	0.19	0.19
	1.005	-0.17	0.09	0.09

Criterion	Y= a.C1 + b.C2 + c.C3 + d				
Fat	c	b	a	d	Sy,x(g/l)
Protein	-0.01	0.00	1.00	0.57	0.21
	0.01	0.00	1.00	-0.45	0.10

b and a : slope and bias of the simple linear regression
Sd : standard deviation
Sy,x : standard residual deviation
c, b, a ,d : multiple linear regression factors
(FM : REF = a.FM + b.PM + c.LA + d ;
PM : REF = a.PM + b.FM + c.LA + d)

Results of the simple linear regression are satisfactory since the residual standard deviations are lower than the acceptability limits set by the CNIEL Guide. (FM 20 g/l ; PM 0.15 g/l). There are no significant interactions between components.

REPEATABILITY

The repeatability was evaluated using 87 herd milk samples from the Jura county. Samples contained bronopol (0.02 %).

Dosages were performed in consecutive duplicates for each sample on 04/06/2003.

Criterion	n	Min (g/l)	Max (g/l)	M (g/l)	Sx (g/l)	Sr (g/l)	Sr (%)	R (g/l)
Fat	87	33.18	40.64	36.92	1.89	0.07	0.19	0.20
Protein	87	30.16	37.16	32.74	1.17	0.06	0.19	0.17

n : number of results
min and max : minimum and maximum values
M : mean results
Sx : standard deviation of results
Sr et Sr (%) : relative and absolute standard deviation of repeatability
r : maximal deviation of repeatability in 95% of cases

For fat matter and protein matter, the analyser shows a repeatability that complies with the requirements of the IDF standard 141C:2000 and with the CNIEL Guide ($r \leq 0.4$ g/l or $Sr \leq 0.14$ g/l).

ACCURACY

Samples were identical to those used in the evaluation of repeatability. Infrared analyses were performed using successive duplicates (means of duplicates were used for statistical treatments), a control milk was set every 20 samples in order to check the signal stability. The evaluation concerned values obtained after calibration. Analyses were performed on 04/06/2003.

Reference analyses :

▪ Fat content: *(Gerber method) ; single test performed but confirmed in the case of too much residue).*
NF standard V 04-210:2000 - Lait - Détermination de la teneur en matière grasse - Méthode acido-butyrométrique. = Milk - Determination of fat content - Gerber method - *(This standard IS NOT equivalent to ISO 2446:1976 though it deals with the same topic).*

▪ Protein content : *(in duplicate)*
IDF standard 98 A : 1985 - Milk - Determination of protein content -Amido black dye-binding method (routine method)

	FM	PM
n	86	87
Min (g/l)	33.00	29.87
Max (g/l)	40.00	37.12
Y (g/l)	36.69	32.29
X (g/l)	36.91	32.74
Sy (g/l)	1.74	1.26
d (g/l)	0.22	0.44
Sd (g/l)	0.40	0.22
Sy,x (g/l)	0.35	0.21
b	0.901	1.056
a	3.42	-2.26

n, min, max : number of results, minimum and maximum values
Y,X : mean results obtained by reference and instrumental method
Sy : standard deviation of results obtained by the reference method
d, Sd : mean and standard deviation of deviations
Sy,x : residual standard deviation
b, a : slope and bias of the simple linear regression

The table and graphs above show the results obtained :

(See at the end of this article)

- for fat matter, the mean result of deviations obtained is 0.22 g/l and the residual standard deviation of linear regression is equal to 0.35 g/l. The slope (0.901) and bias (3.42) are different from 1 and 0 (P=1%), respectively.

- for protein matter , the mean result of deviations obtained is 0.44 g/l and the residual standard deviation of linear regression is equal to 0.21 g/l. The slope (1.056) and bias (-2.26) are respectevely different from 1 and 0 (P=1%), respectively.

For fat and protein matter, the slope and bias deviations are particularly high and could be explained by :
- the sample composition for calibration (they came from a different geographic area)
- the gap between the age of the calibration range (June 2003 performed on 07/05/2003) and herd milk analysis (04/06/2003)
- the low amplitude of FM and PM rate.

Instability of the analyser is excluded, as the results obtained from the control milk used are similar (The control milk was identical during calibration and evaluation of accuracy).

However, regarding stability, carry-over effect, linearity, calibration, repeatability and accuracy, the analyser complies with the requirements of IDF 141 C : 2000 set at 0,7 g/l for herd milk, the residual standard deviation of regression values.

CONCLUSION

The results regarding stability, carry-over effect, linearity, calibration, repeatability and accuracy, comply with the requirements of IDF 141 C : 2000 141C and with the CNIEL "Manuel d'utilisation des analyseurs infrarouges pour les laboratoires interprofessionnels et de contrôle laitier" *(= User's Guide to Infrared Analysers for Interprofessional and Milk Control Laboratories')*.

X.QUERVEL, Ph. TROSSAT

ABREVIATIONS

CNIEL : Centre National Interprofessionnel de l'Economie Laitière = Interprofessional Centre for the Dairy Economy
FM, PM : fat matter, protein matter
LA : lactose

BIBLIOGRAPHY

Standards :

- NF V 04-210:2000 - **Milk - Determination of fat content - Gerber method** *(This standard IS NOT equivalent to ISO 2446:1976 though it deals with the same topic).*
- IDF 98A : 1985 - **Milk - Determination of protein content - Amido black dye-binding method (routine method)**
- IDF 141 C : 2000 - **Whole milk - determination of milk fat, protein and lactose content (Guidance for the operation of mid-infrared instruments)**

Guide and Report :

- CNIEL et Institut de l'élevage, **Manuel d'utilisation des analyseurs infrarouges pour les laboratoires interprofessionnels et de contrôle laitier**, (Référence : CNIEL PROC IR-04-05/00), 2000, 48 p.
- QUERVEL X., TROSSAT P. **Rapport d'évaluation de l'analyseur infrarouge YORK®**, CECALAIT, 18/08/2003, 8 p.

NEW ISO / IDF STANDARD

IDF 173 (ISO 15323:2002) DRIED MILK PROTEIN PRODUCTS - **Determination of Nitrogen Solubility Index**

ISO DRAFTS

Draft ISO/CD 10272- MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS - **Horizontal method for the detection and enumeration of Campylobacter growing at 41,5°C - Part 2 : colony count technique**

Draft ISO/CD 16649- MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS - **Enumeration of â-glucuronidase positive Escherichia coli - Part 3 : most probable number technique using 5-bromo-4-chloro-3-indolyl-â-D-glucuronic acid**

NEW EU REGULATION

PESTICIDE / MRL : maximum residue levels /

OJEU 30.09.2003 - Notice n° 2003/C 234/09 - Opinion of the European Economic and Social Committee on the "Proposal for a Regulation of the European Parliament and of the Council on **maximum residue levels of pesticides in products of plant and animal origin"** (COM(2003) 117 final — 2003/0052 (COD)) (4 p.)

To refer to and print regulations : http://europa.eu.int/eur-lex/en/oj/index.html

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AFNOR VALIDATION RENEWAL

This alternative methods list was **updated on June 24th 2003** and forwarded by AFNOR Certification to CECALAIT.

Name / Manufacturer	Date	Attestation number	Description
VALIDATIONS RENEWAL			
Listeria Rapid Test **OXOID** **6 route de Paisy** **BP13** **69572 Dardilly cedex**	Date of validation 11.04.1995 Renewals 11.04.1999 and 24.06.2003 **End of validity** **07.02.2007**	UNI-03/2-04/95A	***Listeria* detection test** **For all human foodstuffs**

IN REVIEWS - ON THE NET

LABORATORY

The following 4 articles resumes speechs presented at a symposium session organised by the IDF / ISO/ AOAC, in 2002 in Toledo (Spain).

ACCREDITATION / ISO 17025 /

Lopez-Fandino, Accreditation and Quality Assurance in Dairy Laboratories Following ISO 17025, Bulletin of the International Dairy Federation, n°380/2003; p. 33-36.

▶ Presentation of ISO 17025 and of the requirements applicable to milk industry laboratories wishing to get an accreditation following this standard.

IT : information technology / MILK /

Wilson G., Information Technology, Bulletin of the International Dairy Federation, n°380/2003; p. 37-46.

▶ Presentation of an information technology system by mean of a case study applied to the dairy industry.

IN-LINE ANALYSIS /

Holroyd S., In-line Analysis, Bulletin of the International Dairy Federation, n°380/2003; p. 51- 57.

▶ Presentation of New Zealand Dairy Research Institute with in-line analysis.

MILK CONTROL /

Bijgaart H. van den, Central Milk Testing Logistics - MCS on the Move, Bulletin of the International Dairy Federation, n°380/2003; p. 44-50.

▶ Presentation of the Netherlands Milk Control Station (MCS) experience .

WASTAGE

WASTAGE / PREVENTION /

Guide for Dairy Managers on Wastage Prevention in Dairy Plants, Bulletin of the International Dairy Federation, n°382/2003, 49 p.

▶ Revised edition of a guide published in 1980. In 4 parts, the "Guide for Dairy Managers on Wastage Prevention in Dairy Plants" "covers process description, types of waste, sources of loss, and waste reduction practices, together with advice on minimizing environmental impact and cost. Technical aspects and systematic wastage managment make up an overall environmemtal management suystem. Methods of measuring flow, sampling and methods of analysis are also covered".
[summary from the IDF Bulletin]

BOOKSHOP

PATHOGENS / ANALYSIS /

McLandsborough, Lynne. - **Food Microbiology Laboratory** - CRC Press, January 2004, 160 p.

"This manual presents a sequence of well-tested, student-proven, and thoroughly outlined experiments that incorporate traditional testing techniques as well as rapid method testing techniques. Covering topics such as *E.* coli, *Staph*, and *Salmonella* detection, thermal destruction of microorganisms, and using PCR to confirm *Listeria* monocytogenes, the manual provides readers with hands-on experience with methods often used by industry for enumerating microorganisms."

www.crcpress.com

FORTHCOMING EVENTS

Events in chronological order

CHEESE / RIPENING / CHARACTERISATION / CHEESE TECHNOLOGY /

21 - 25 March 2004 Prague Czech Republic	IDF Symposium on Cheese : Ripening, Characterization & Technology	http://www.fil-idf.org

DAIRY PRODUCTS /

18-22 September 2005 Vancouver, Canada	IDF World Dairy Summit	http://www.fil-idf.org

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