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DETERMINATION OF THE MILK LIPOLYSIS INDEX BY INFRARED SPECTROSCOPY

The CNIEL, in collaboration with CECALAIT and interprofessional laboratories, are studying the feasibility of using infrared spectrophotometry for the determination of the lipolysis index within the context of milk payment.

1/- OBJECT

The infrared scanner FT6000 from FOSS possesses a module permitting the determination of milk fat acidity. The purpose of this study is to analyse the feasibility of this method within the context of milk payment. Infrared (IR) spectroscopy is envisaged as an alternative to the copper soap method (MSC) actually validated and used.

The tests concerning this evaluation on the LIPOLYSIS criteria were performed by 3 interprofessional laboratories using the same type of scanner (FT 6000) and the same version of the basic « LIPOLYSIS » calibration set up by the manufacturer FOSS Electric: version 1.1.0 (system 4000) or version 1.2.1 (Foss integrator).

2/- STUDY PROTOCOL

An identical population of producers' milk samples was analysed over 4 consecutive months (March to

June 2007) in 3 interprofessional laboratories according to the following principle:

MARCH	APRIL	MAY	JUNE
3 IR analyses (a-b-c)	3 IR analyses (a-b-c)	3 IR analyses (a-b-c)	3 IR analyses (a-b-c)
1 MSC analysis	/	/	1 MSC analysis
MSC/IR comparative	MSC/IR comparative	MSC/IR comparative	MSC/IR comparative
BDI SRM analysis	BDI SRM analysis	BDI SRM analysis	BDI SRM analysis
Daily repeatability	Daily repeatability	Daily repeatability	Daily repeatability
Daily stability	Daily stability	Daily stability	Daily stability

3/- RESULTS

3.1/- Initial calibrations

From a standard calibration set up by FOSS, a comparative analysis of a set of producers' milk

samples was carried out using the MSC and IR methods to establish a specific calibration per laboratory. The results obtained are summarised in table 1:

	LAB N° 1	LAB N° 2	LAB N° 3
N	198	220	208
X (meq/l)	0,26	0,22	0,26
Sx (meq/l)	0,17	0,08	0,14
Regression equation	0,857x + 0,11	0,794x - 0,10	0,850x - 0,07
Sy,x (meq/l)	0,09	0,04	0,07

Table 1: Summary of the initial calibrations of the infrared scanners

N: number of samples, *X* and *Sx*: mean and standard deviation of MSC results, *Sy,x*: residual standard deviation of MSC regression = $b(IR) + a$

It can be noted that the calibration equations are relatively close between laboratories 1 and 3 whereas laboratory 2 presents a weaker regression slope (about 5 to 6 %). The values of the residual standard deviation of regression are significantly different between the 3 laboratories (0.04 to 0.09 meq/l).

3.2/- Comparative tests

To follow the adjustment of the infrared method to the copper soap method, the comparative tests between both methods were performed each month from March to June.

3.2.1- Crude results

A comparison per laboratory between the infrared results (with the help of the laboratory's initial calibration) and the copper soap method results was carried out in March and June on milk samples from the laboratories' collecting zones.

For March, the regression slopes obtained are globally very close to 1.00, showing a good accuracy of the instrument when using the initial calibration equation.

In June, concerning the instrument accuracy evaluations for laboratories 2 and 3, it can be observed that the regression slopes obtained are significantly different to 1.00 (1.48 and 1.36 respectively), indicating a strong underestimation of the infrared results in comparison to the copper soap method. The instrument accuracy of laboratory 1 is satisfactory when using the initial calibration (slope = 1.01).

These observations are linked to the composition of the milk analysed between both periods (February-March and June). Indeed, a strong increase (about 40 %) in the mean lipolysis results between both periods for laboratories 2 and 3 (0.48 and 0.47 respectively in March compared to 0.68 and 0.67 in June) was observed, whereas the mean of laboratory 1 remains stable between both periods for the samples studied.

The increase in levels observed for the milk samples analysed by laboratories 2 and 3 are probably connected to the increase in lipoprotein lipase activity during these periods, a function of the lactation period and animal gestation.

For laboratories 2 and 3, it can thus be observed that the initial calibrations no longer correspond to the composition of the samples analysed in June.

It can also be said that the FOSS basic calibration model does not take into account the variations in

composition observed by both these laboratories, not directly compensating for them.

3.2.2- MSC corrected results

Each laboratory followed the accuracy of its scanner monthly, and carried out a comparative analysis of a milk sample population using the infrared method and the copper soap method.

With the help of the adjustment equations for each month, the infrared data was rectified for March and June in order to study the improvements of the relation between both methods using this monthly adjustment of the scanners.

For March and June, the correction of the infrared data using the adjustment equations did not improve the scanner's accuracy. However, it can be noted that the accuracy performance of the laboratories was already very good using the initial calibration.

3.2.3- BDI SRM corrected results

The reference materials supplied by CECALAIT and used to calibrate the copper soap method (BDI SRM) were tested on the scanner to study the possibility of a direct monthly calibration (without the use of the copper soap method).

A correction equation (BDI vs. IR) was calculated for each month from March to June. Table 2 presents the results of the adjustment equations obtained in March for the three laboratories.

The infrared results from the initial calibrations were then corrected with the help of the equation obtained as above and compared to the copper soap results by simple linear regression.

<u>MARCH</u>	LAB N° 1	LAB N° 2	LAB N° 3
N	6		
X (meq/l)	0,36		
Mini-maxi (meq/l)	0,157-0,462		
Regression equation	2,088x – 0,44	2,352x – 0,56	1,487x – 0,07
Sy,x (meq/l)	0,02	0,02	0,03

Table 2: Summary of the adjustments to infrared scanners in March

N: number of samples, X and Sx; mean and standard deviation of MSC results, Sy,x: residual standard deviation of MSC regression = b(IR) + a

The slopes obtained with the correction equations are very different from 1.00 and the intercept is strongly negative for laboratories 1 and 2.

The linear regressions realised using the corrected data from the BDI SRMs present slopes significantly different to 1.00. Due to the strongly negative intercept, a very high number of data are negative for laboratories 1 and 3 after correction.

By studying these comparison test results and the "crude" infrared results of BDI SRMs, we can say that these samples are not appropriate for the calibration and/or the adjustment of infrared scanners for the lipolysis criteria.

The sample production technique and notably lipolysis induction by addition of biological effectors and their heat stabilisation explain their specific

infrared signal and their non-representativeness in relation to producers' milks.

3.3/- Results classification

A comparison of the classification of producers' milk analysed using the copper soap method and the infrared method was performed for the 4 months of the study, with a limit of 0.89 meq/100 g of fat.

To study the influence of the number of infrared values taken into account per quarter for the concordance classification with the copper soap method, the classification was performed taking into account:

- An infrared value obtained at the same time as the copper soap value
- The mean of 3 infrared values from March, April and May
- The mean of 6 infrared values from March and April
- The mean of 9 infrared values from March, April and May.

3.3.1- Crude results

The proportion of the results over the limit always seems lower with the infrared method than with the copper soap method, and that for analyses in March and June.

These results can probably be explained by the visual reports of non-linearity observed on all the comparisons realised between the copper soap and infrared methods. These observations corroborated by the observations on the results obtained during the initial evaluation of the FT 6000 scanner by CECALAIT on the lipolysis criteria (with the BDI method as the reference) lead to the conclusion that the non-linearity observed stems from the initial calibration model of the FT 6000 scanner (Basic calibration FOSS).

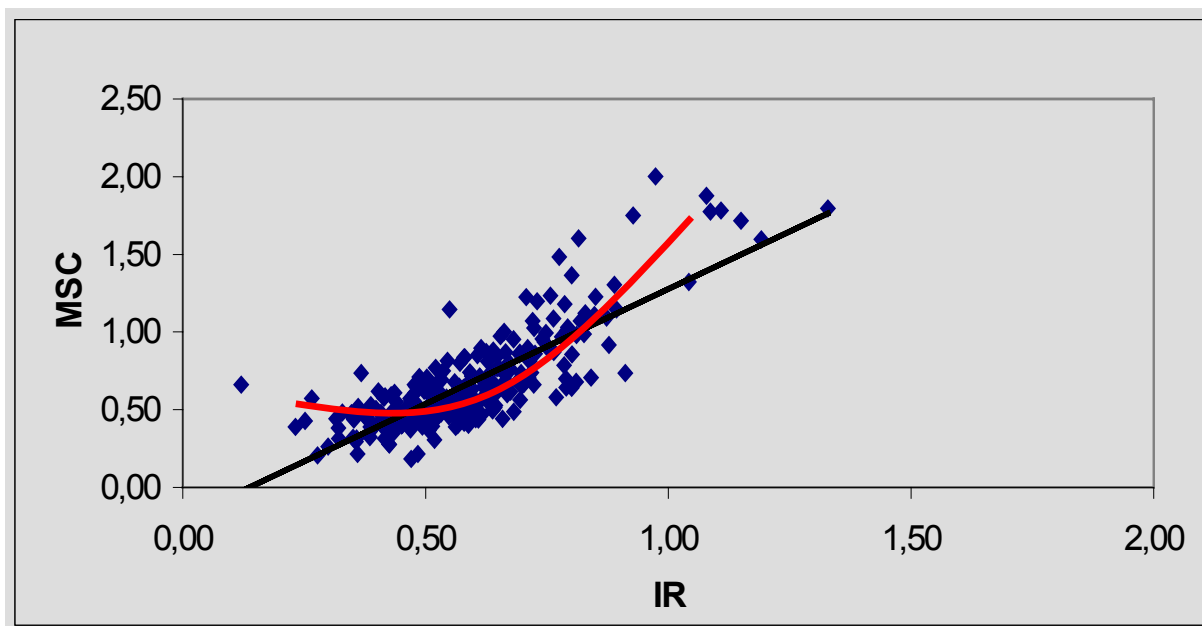
Thus, it can be observed that the infrared calibration has a tendency to underestimate low and high results and overestimate median results.

Specific tests would be necessary to statistically confirm this hypothesis.

It is therefore difficult to conclude on the improvement provided by multiple infrared analyses (each decade) in relation to a quarterly analysis due to this problem of linearity and the very low percentage of samples out of limit during the period from March to May.

3.3.2- MSC corrected results

After correction of the infrared data using the adjustment equations obtained in March and June respectively, overall the same tendencies can be observed as with the "crude" results.



3.4/- Evaluation of the repeatability

The repeatability was evaluated daily by analysis of a series of 10 samples in non-consecutive duplicate.

The monthly mean standard deviations of repeatability (in meq/l) are noted in the following table:

	LAB N° 1	LAB N° 2	LAB N° 3
MARCH	0,026	0,017	0,020
APRIL	0,025	0,015	0,019
MAY	0,028	0,015	0,019
JUNE	0,029	0,016	0,018

Table 3: summary of the monthly mean standard deviations of repeatability (meq/l)

We can observe a variability of the standard deviation of repeatability due to an “instrumental” effect.

The results, except for laboratory 2, are superior to the reference limit of the copper soap method, which is 0.018 meq/l.

It can also be noted that the differences observed between the laboratories can mainly explain the differences observed in the residual standard deviations of regression (initial calibration); the

repeatability error has a direct impact on the “accuracy of estimation” of the infrared method.

3.5/- Stability

The stability was evaluated daily by analysis of a series of 8 duplicates of the same frozen milk.

It can be observed that the mean monthly values per laboratory are very close to the target values determined at the beginning of the month, and this for the 4 months for all the laboratories.

CONCLUSION

- We noticed that the scanner accuracy on the lipolysis criteria was satisfactory (slope not significantly different to 1.00) when the calibration was performed with milk representative of routine samples (case of analyses in March 2007). However, the monthly adjustment system used during these tests did not permit to ensure a good correlation of the calibration to the fine composition of the milk samples, especially during periods of variation (notably at the beginning of the summer).
- The concordance of the milk classification, whether the copper soap or infrared method is used, is not today ensured (systematic underestimation of high levels by the infrared method), probably due to a problem of non-linearity of the model used (basic calibration FOSS).
- The use of BDI SRMs, perfectly satisfactory for the calibration or the verification of the chemical methods (copper soap method and BDI), can certainly not be a way of replacing calibrations and/or scanner adjustment using a chemical transfer method (copper soap).
- In view of these results, the determination of the lipolysis index by the infrared method is not yet applicable within the context of milk payment. Complementary tests will be led with the objective to produce a more robust initial calibration of the scanners and to palliate the variations of the fine composition of milk.

**MODIFICATION OF REGULATION (CE) N° 2073/2005 CONCERNING
MICROBIOLOGICAL CRITERIA**

Following many opinions issued by the Scientific Panel on Biological Hazards (BIOHAZ Panel) of the European Food Safety Authority (EFSA), **the annex I to Regulation (EC) n° 2073/2005** on microbiological criteria for foodstuffs **was replaced** by the text in **the annex to the Regulation (EC) n° 1441/2007 of 5 December 2007**. Many new microbiological criteria, described in the tables below, were added:

Food safety criteria

Food category	Micro-organisms	Sampling plan ⁽¹⁾		Limits ⁽²⁾		Analytical reference method ⁽³⁾	Stage where the criterion applies
		n	c	m	M		
Dried follow-on formulae	<i>Salmonella</i>	30	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life

Process hygiene criteria – Milk and dairy products

Food category	Micro-organisms	Sampling plan ⁽¹⁾		Limits ⁽²⁾		Analytical reference method ⁽³⁾	Stage where the criterion applies	Action in case of unsatisfactory results
		n	c	m	M			
Dried follow-on formulae	<i>Enterobacteriaceae</i>	5	0	Absence in 10 g		ISO 21528-1	End of the manufacturing process	Improvements in production hygiene to minimise contamination
Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age	Presumptive <i>Bacillus cereus</i>	5	1	50 cfu/g	500 cfu/g	EN/ISO 7932	End of the manufacturing process	Improvements in production hygiene. Prevention of recontamination. Selection of raw material

NEW EU REGULATIONS

Classification is established in alphabetical order of the first keyword

O.A.P. / P.G.I. / CHEESE

O.J.E.U. C 232, 4th October 2007 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Slovenska Bryndza (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_232/c_23220071004en00170022.pdf

O.J.E.U. C 236, 9th October 2007 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs ["Gamoneu" or "Gamonedo" (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_236/c_23620071009en00130017.pdf

O.J.E.U. L 271, 16th October 2007 – Commission regulation (EC) n° 1200/2007 of 15 October 2007 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Asiago (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_271/l_27120071016en00030004.pdf

O.J.E.U. C 268, 10th November 2007 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Afuega'l Pitu (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_268/c_26820071110en00280032.pdf

O.J.E.U. C 278, 21st November 2007 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Jihoceska niva (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_278/c_27820071121en00130016.pdf

O.J.E.U. C 278, 21st November 2007 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Laguiole (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_278/c_27820071121en00170021.pdf

O.J.E.U. C 279, 22nd November 2007 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Epoisses (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_279/c_27920071122en00190023.pdf

O.J.E.U. C 291, 5th December 2007 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Rocamadour (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_291/c_29120071205en00140020.pdf

O.J.E.U. C 298, 11th December 2007 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Bleu de Gex Haut-Jura ou Bleu de Septmoncel (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_298/c_29820071211en00210027.pdf

O.J.E.U. C 298, 11th December 2007 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Roquefort (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_298/c_29820071211en00280033.pdf

O.J.E.U. L 330, 15th December 2007 – Commission Regulation (EC) n° 1485/2007 of 14 December 2007 registering certain names in the Register of protected designations of origin and protected geographical indications [Pecorino di Filiano (cheese) (PDO)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_330/l_33020071215en00130014.pdf

O.J.E.U. C 308, 19th December 2007 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) N° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Slovensky Ostiepok (cheese)]

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/c_308/c_30820071219en00280032.pdf

STANDARDS - REGULATIONS

FOOD INGREDIENTS

O.J.E.U. L 310, 28th November 2007 – Commission directive 2007/68/EC of 27 November 2007 amending Annex IIIa to directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_310/l_31020071128en00110014.pdf

MICROBIOLOGICAL CRITERIA

O.J.E.U. L 322, 7th December 2007 – Commission regulation (EC) n° 1441/2007 of 5 December 2007 amending regulation (EC) n° 2073/2005 on microbiological criteria for foodstuffs

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_322/l_32220071207en00120029.pdf

PRESERVED MILK

O.J.E.U. L 258, 4th October 2007 – Council Directive 2007/61/EC of 26 September 2007 amending Directive 2001/114/EC relating to certain partly or wholly dehydrated preserved milk for human consumption

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_258/l_25820071004en00270028.pdf

TRADITIONAL SPECIALITIES GUARANTEED

O.J.E.U. L 275, 19th October 2007 – Commission regulation (EC) n° 1216/2007 of 18 October 2007 laying down detailed rules for the implementation of Council regulation (EC) n° 509/2006 on agricultural products and foodstuffs as traditional specialities guaranteed

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_275/l_27520071019en00030015.pdf

VETERINARY MEDICAL PRODUCTS / RESIDUE LIMITS

O.J.E.U. L 303, 21st November 2007 – Commission regulation (EC) n° 1353/2007 of 20 November 2007 amending Annex I to Council regulation (EC) n° 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, as regards Monensin, Lasalocid and Tylvalosin

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_303/l_30320071121en00060008.pdf

O.J.E.U. L 310, 28th November 2007 – Corrigendum to Commission regulation (EC) n° 2796/95 of 4 December 1995 amending Annex II of Council regulation (EEC) n° 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_310/l_31020071128en00220022.pdf

STANDARDS, DRAFT STANDARDS

Classification in alphabetic order by theme

ISO published standards

LINEAR CALIBRATION

LINEAR CALIBRATION	ISO 11843-2/Cor 1:2007 October 2007	Capability of detection Part 2: Methodology in the linear calibration case Technical corrigendum 1
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MILK AND MILK POWDER

MILK / MILK POWDER / AFLATOXIN M1	ISO 14501:2007 October 2007	MILK AND MILK PRODUCTS Determination of aflatoxin M1 content – Clean-up by immuno-chromatography and determination by high-performance liquid chromatography
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MILK AND MILK PRODUCTS

MILK / MILK PRODUCTS / LACTOSE CONTENT	ISO 22662:2007 September 2007	MILK AND MILK PRODUCTS Determination of lactose content by high-performance liquid chromatography (Reference method)
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BOOKSHOP: LATEST PUBLICATIONS

The classification in alphabetic order of the first keyword allows you to consult the references according to your interests. The web site allows you to know more, or to order the book.

MASS SPECTROMETRY

WATSON J.T.; SPARKMAN O.D. – **Introduction to mass spectrometry: instrumentation, applications, and strategies for data interpretation, 4th edition** –Wiley Edition – October 2007 – ISBN 978-0-470-51634-8 – 862 pages

<http://eu.wiley.com/WileyCDA/>

This 4th edition, completely revised and updated, presents the concept of mass spectrometry, its potentials and limitations. Many examples of qualitative and quantitative applications of mass spectrometry are described in this book.

FORTHCOMING EVENTS

Classified in chronological order

CHEESE / RIPENING

9-13 March 2008
Berne, Switzerland

5th IDF Symposium on cheese ripening

<http://www.cheese2008.ch>

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

PESTICIDES / ANTIBIOTICS / MILK

Alternative methods proposed to detect pesticides and antibiotics in water and natural food

http://www.innovations-report.com/html/reports/agricultural_sciences/report-91185.html

► This article presents the results of a thesis concerning the development of new methods to detect pesticides and antibiotics in food (in particular water and foods of animal origin). Many new methods proposed constitute a routine analysis alternative to the analysis used until now.

SALMONELLA / RAPID METHOD

Rapid culture method for *Salmonella* in food

<http://www.laboratorytalk.com/news/oxo/oxo352.html>

► Oxoid announces the launch of a new culture method for isolation, differentiation and identification of *Salmonella* species in food. The Oxoid *Salmonella* rapid culture method provides presumptive positive colonies in just 42 hours, without the need for specialised equipment.

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