ARTICLE

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.

MARCH	**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.

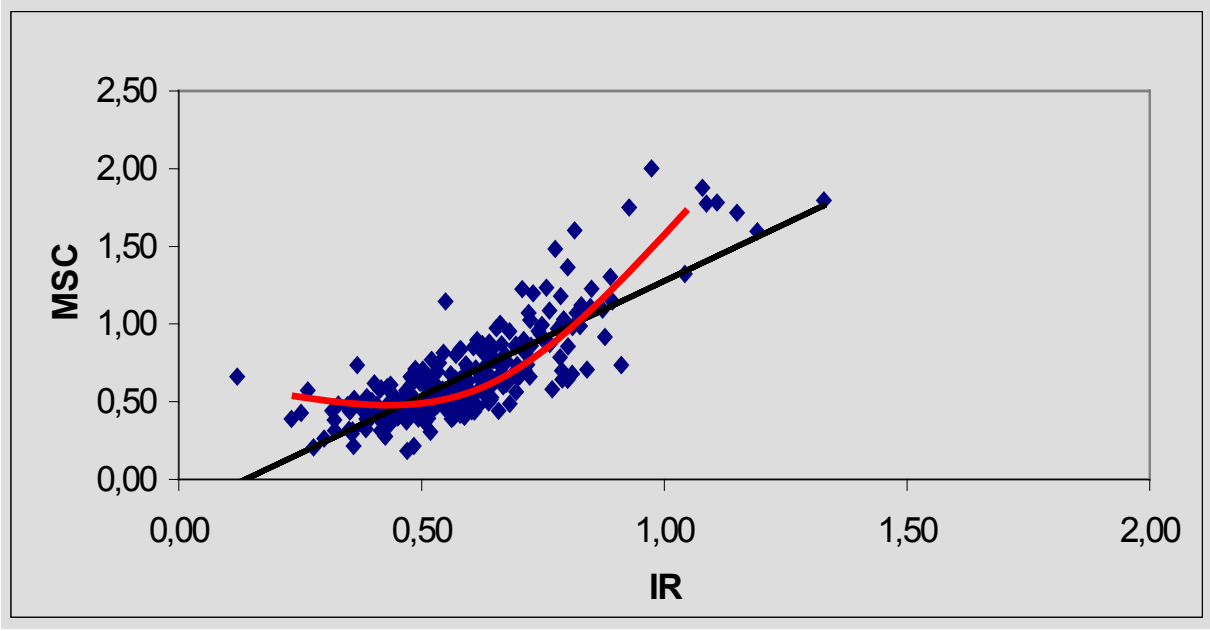
3.3.1- Crude results

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.