EVALUATION OF THE BENTLEY FTS™ INFRARED ANALYSER ON EWE AND GOAT MILK MATRIX

The FTS is an infrared spectrophotometer (mid infrared: 2-10 µm) manufactured by Bentley Instruments (USA) and commercialized in the Western Europe by Bentley Instruments SARL. It is used for the determination of the principal components in milk samples.

This instrument uses a high resolution industrial infrared spectrometer based on Fourier transform (FTIR). The interferometer is referenced by a laser and placed in an anti-vibration, temperature regulated airtight enclosure. The complete infrared spectrum is collected and recorded for each sample. Using a MLR or PLS calibration, standard components (fat, protein and lactose) and other criteria such as the freezing point and urea can be determined.

The apparatus is connected to a computer that ensures the running of the instrument and the signal treatment.

The tests:

The evaluation tests were performed in Actilait-Cecalait's physico-chemistry laboratory (reference and infrared analyses) in January and February 2010 on ewe and goat mixed milk samples. They concerned fat (equivalent fat filter B), protein (MP) and freezing point (FPD). The calibration (ewe milk), the repeatability and the accuracy were evaluated.

The appreciation criteria of the estimated parameters were taken from ISO 9622 / IDF 141 C:2000 "Guide for the operation of mid-infrared instruments", or from the CNIEL/IE handbook concerning the use of infrared apparatus within the context of milk payment and milk control in France.

The following instrumental parameters were used:
- Rate: 500 samples / hour;
- No correction of contamination.

A- EWE MILK

A1- Evaluation of the calibration

A1.1- Procedure

The evaluation of the calibration for fat and protein, initially installed by the manufacturer, was performed with 13 commercial "high" infrared standard reference materials (SRM) produced by Actilait-Cecalait in January 2010. Each sample was analysed in duplicate.

A1.2- Results

The table below presents the results obtained:

	N	Min-max	Sr	d	Sd	Sl1	Sl3
Fat (g/l)	13	59-89	0.08	0.01	0.21	0.22	0.23
Protein (g/l)	13	45-67	0.04	0.00	0.06	0.05	0.05

Table 1: FTS calibration parameters for fat and protein "high" contents
N: number of standards, min and max: minimum and maximum values, Sr: standard deviation of repeatability, d and Sd: mean and standard deviation of deviations (instrument-reference), Sl1 and Sl3: residual standard deviation of simple linear regression (reference vs instrument) or multiple (reference vs MG, MP and lactose).

It can be noted that the residual standard deviations of regression are low and equivalent to the standard deviations of deviations. The residual interactions are not significant.

A1.3- Conclusion

The residual standard deviations of the linear regression obtained for fat and protein are in agreement with the recommendations of the CNIEL/IE handbook (below 0.30 and 0.20 g/l respectively), which corresponds to the content in ewes' milk.

A2- Evaluation of repeatability and accuracy

A2.1- Samples

The tests were performed on about 100 samples of milk from the Roquefort region (12). Bronopol was added to the samples to give a final concentration of 0.02 %.

A2.2- Repeatability

A2.2.1- Procedure

The repeatability of the instrument was evaluated using all the milk samples for fat, protein and freezing point. The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 10 samples according to the following sequence: (Set 1 rep 1 - Set 1 rep 2 - Set 2 rep 1 - Set 2 rep 2 … Set n rep 1 - Set n rep 2). A control milk was analysed every 30 samples to verify the stability of the analyser.

A2.2.2- Results

The table below presents the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/l)	106	55.64	85.36	66.66	5.75	0.13	0.20	0.37
Wide fat (g/l)	106	55.92	85.21	66.88	5.67	0.13	0.20	0.37
Protein (g/l)	106	44.77	64.63	50.66	4.17	0.08	0.15	0.22
Wide protein (g/l)	106	44.77	64.63	50.66	4.17	0.08	0.15	0.22
PLS protein (g/l)	106	45.78	65.31	51.60	4.08	0.06	0.11	0.16
FPD (m°c x-1)	80	550	567	561	3.4	0.94	0.17	2.61

Table 2: FTS repeatability criteria for fat, protein and freezing point in ewe milk samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr%: absolute and relative standard deviation of repeatability; r: maximum deviation of repeatability in 95% of cases.
Wide: results obtained after calibration with "median" and "high" SRM. PLS: results obtained after PLS calibration

A2.2.3- Conclusion

For fat and protein content, FTS presents a relative standard deviation of repeatability (Sr%) in accordance with the recommendations of the CNIEL/IE handbook (Sr ≤ 0.45%). Concerning the freezing point, the standard deviation of repeatability (Sr) obtained is in accordance with the recommendations of the CNIEL/IE handbook (Sr ≤ 2 m°c), which corresponds to cows' milk.

A2.3- Evaluation of the accuracy

A2.3.1- Procedure

The accuracy of the analyser was evaluated using 80 samples of ewe milk for fat, protein and freezing point. The quantitative analyses were performed in accordance with the evaluation of repeatability (cf A2.2). For fat and protein, the evaluation concerns the values obtained after calibration of the instrument with commercial SRMs produced by Actilait-Cecalait (cf A1). For FPD, the instrumental values are from a calibration carried out by the manufacturer.

The following reference methods were used:
- Fat: Acido-butyrometric method according to NF V 04-155 (single test);
- Protein: Amido black method according to NF V 04-216 (test in duplicate);
- Freezing point: Thermistor cryoscopic method according to ISO 5764/IDF 108 (single test).

A2.3.2- Results

The following tables and figures present the results obtained:

	Fat (g/l)	Fat (g/l) (wide)	Protein (g/l)	Protein (g/l) (wide)	Protein (g/l) (PLS)	Freezing point (m°C x –1)
n	80	80	80	80	80	80
Min	57.60	57.60	47.33	47.33	47.33	542
Max	86.40	86.40	68.71	68.71	68.71	572
Y	67.98	67.98	53.12	53.12	53.12	561
X	66.31	66.55	50.97	50.74	51.70	561
Sy	5.85	5.85	4.60	4.60	4.60	5.5
d	-1.67	-1.44	-2.16	-2.38	-1.42	-0.5
Sd	0.52	0.52	0.48	0.45	0.48	4.2
Sy,x	0.51	0.52	0.41	0.42	0.39	4.2
Sy,x %	0.74	0.76	0.8	0.78	0.79	0.75
b	0.977	0.993	1.057	1.040	1.066	1.068
a	3.23	1.92	-0.77	0.33	-1.97	-37.55

Table 3: FTS accuracy parameters for fat, protein and freezing point in ewes' milk samples
n, min, max: number of results, minimum and maximum value; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x and Sy,x%: absolute and relative residual standard deviation; b, a: slope and intercept of the linear regression.
Wide: results obtained after calibration with "median" and "high" SRMs. PLS: results obtained after PLS calibration

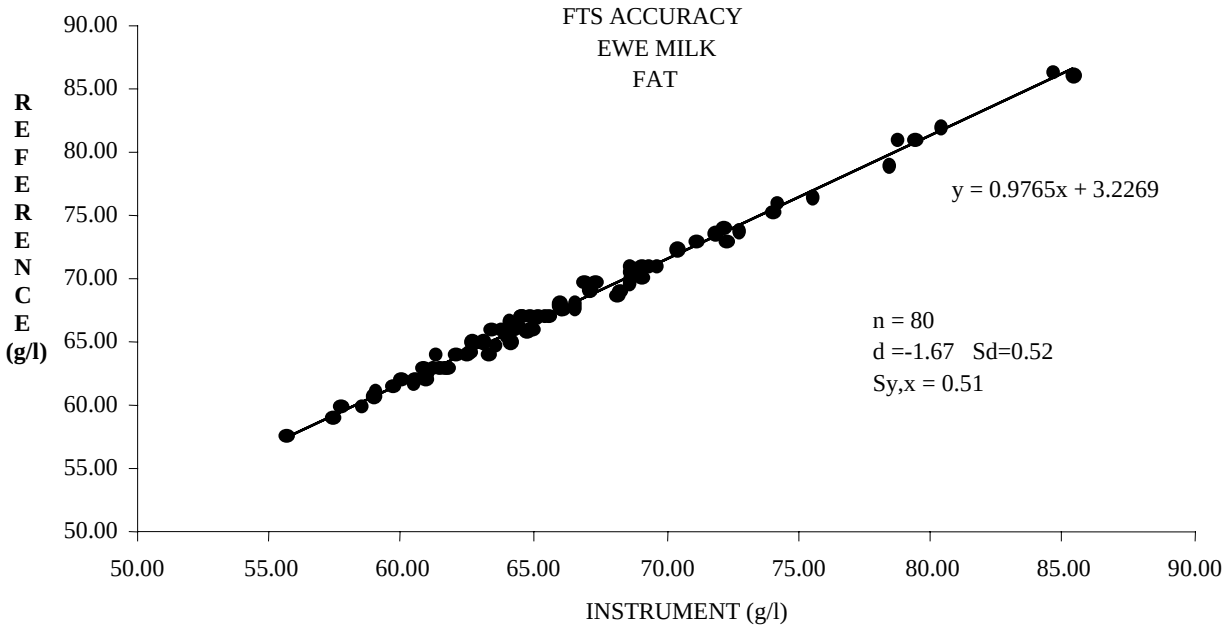

Figure 1: Relation between FTS and reference results for fat in ewe's milk samples

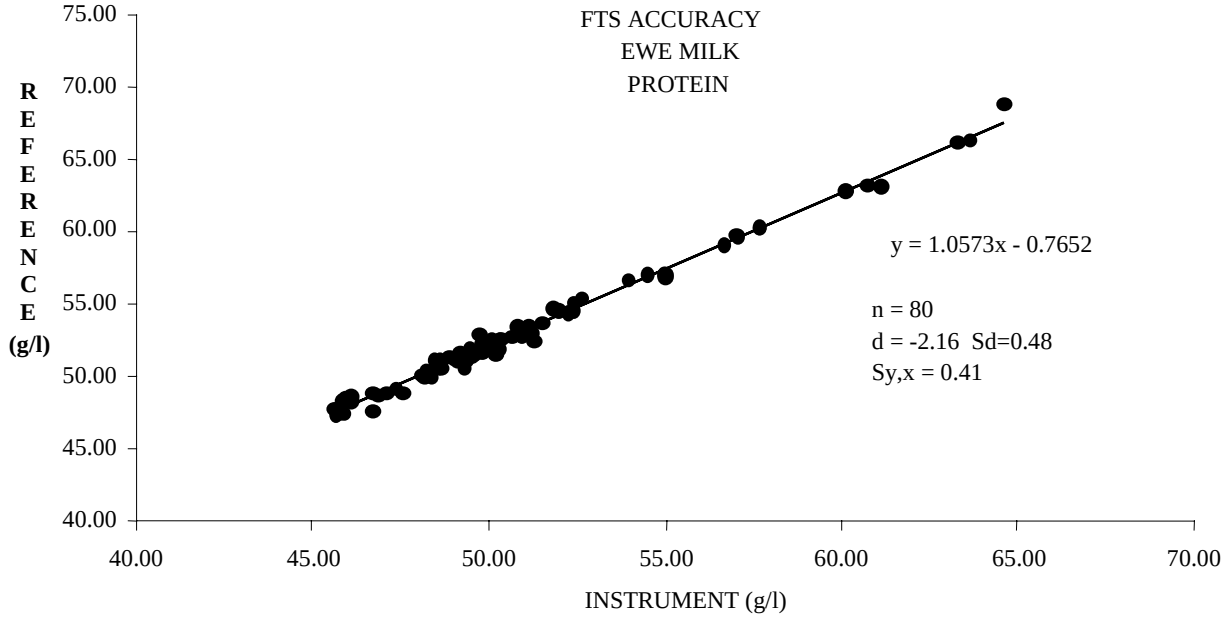

Figure 2: Relation between FTS and reference results for protein in ewe's milk samples

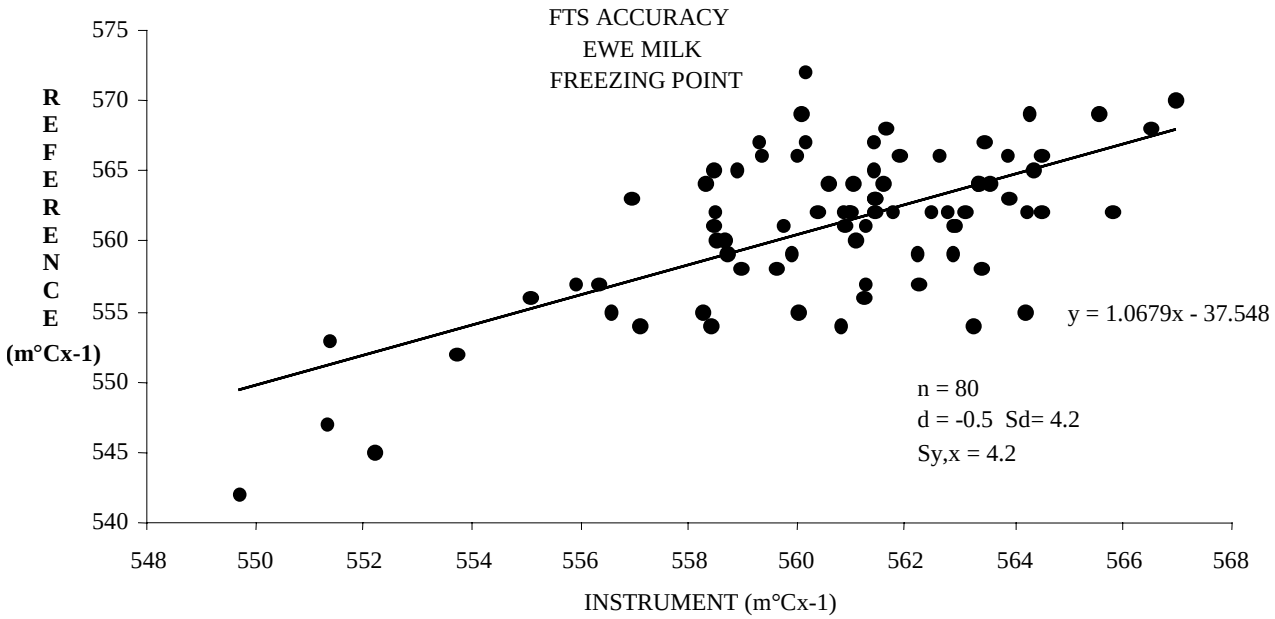

Figure 3: Relation between FTS and reference results for freezing point in ewe's milk samples

It can be noted that:
- For fat, the mean and standard deviation of deviations are –1.67 and 0.52 g/l respectively. The regression line obtained is significantly different from 1.00 (P = 5%) and the intercept is significantly different from zero (P = 1%). The residual standard deviation of regression is equal to 0.51g/l.
- For protein, the mean and the standard deviation of deviations are –2.16 and 0.48 g/l respectively. The regression line obtained is significantly different from 1.00 (P = 1%) and the intercept is significantly different from zero (P = 1%). The residual standard deviation is equal to 0.41g/l.
- For freezing point, the mean and standard deviation of deviations are –0.5 and 4.2 (m°C x –1) respectively. The regression line obtained is significantly different from 1.00 (P = 1%) and the intercept is significantly different from zero (P = 1%). The residual standard deviation is equal to 4.2 (m°C x –1).

The results obtained from wide calibrations give residual standard deviations equivalent to slope and mean values closest to 1 and 0.

A2.3.3- Conclusion

With no regulation or standard limits for this type of milk, it can be noted that for the both components criteria, the standard deviations obtained are lower than the accuracy limit of the standardised value of the method ISO 9622 / IDF 141 for cow herd milk samples, which is 0.7 g/L. However, it can be noted that the slope values are statistically different from 1.00 for fat and protein on this type of milk. The composition of ewe milk is probably in relation with this observation. It will be necessary to proceed to slope and intercept adjustments on specific milk samples

B- GOAT MILK

B1- Samples

The tests were performed on 100 samples of milk from Poitou-Charentes Region.

B2- Evaluation of repeatability

B2.1- Proceure

The repeatability of the instrument was evaluated using all the milk samples for fat, protein and freezing point. The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 10 samples according the following sequence: (Set 1 rep 1 - Set 1 rep 2 - Set 2 rep 1 - Set 2 rep 2 … Set n rep 1 - Set n rep 2). A control milk was analysed every 30 samples to verify the stability of the analyser.

B2.2- Results

The table below presents the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
Fat (g/l)	100	32.81	56.47	42.66	5.05	0.08	0.19	0.23
Wide fat (g/l)	100	32.62	55.91	42.49	5.01	0.09	0.20	0.24
Protein (g/l)	100	29.76	47.32	35.18	3.18	0.10	0.29	0.28
Wide protein (g/l)	100	30.50	48.44	35.82	3.33	0.07	0.19	0.19
PPLS protein (g/l)	100	30.67	47.10	35.49	3.19	0.05	0.15	0.14
FPD (m°C x-1)	100	536	565	552	5.5	0.89	0.16	2.47

Table 4 : FTS repeatability criteria for fat, protein and freezing point in goat milk samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr%: absolute and relative standard deviation of repeatability; r: maximum deviation of repeatability in 95% of cases.
Wide: results obtained after calibration with "median" and "high" SRM. PLS: results obtained after PLS calibration

B2.3- Conclusion

For fat and protein content, FTS presents a standard deviation of repeatability (Sr) in accordance with the recommendations of the CNIEL/IE handbook (Sr ≤ 0.14 g/l).
Concerning the freezing point, the standard deviation of repeatability obtained is in accordance with the recommendations of the CNIEL/IE handbook (Sr ≤ 2 m°c), which corresponds to cows' milk.

B3- Evaluation of accuracy

B3.1- Procedure

The accuracy of the analyser was evaluated using about 80 samples of goat milk (74 for fat after elimination of absurd results and 67 for protein after elimination of the results higher to 39 g/l) for fat, protein and freezing point. The quantitative analysed were performed in accordance with the evaluation of repeatability (cf B2.1). For fat and protein, the evaluation concerns the values obtained after calibration if the instrument with commercial SRMs produced by Actilait-Cecalait. For FPD, the instrumental values are from a calibration carried out by the manufacturer.

The following reference methods were used:
- Fat: Acido-butyrometric method according to NF V 04-210 (single test);
- Protein: Amido Black method according to NF V 04-216 (test in duplicate);
- Freezing point: Thermistor cryoscopic method according to ISO 5764/IDF 108 (single test).

B3.2- Results

The following tables and figures present the results obtained:

	Fat (g/l)	Fat (g/l) (wide)	Protein (g/l)	Protein (g/l) (wide)	Protein (g/l) (PLS)	Freezing point (m°C x –1)
n	74	74	67	67	67	79
Min	33.80	33.80	30.82	30.82	30.82	535
Max	56.00	56.00	38.74	38.74	38.74	560
Y	42.94	42.94	35.15	35.15	35.15	551
X	42.55	42.38	34.34	34.92	34.64	554
Sy	4.78	4.78	2.09	2.09	2.09	5.0
d	-0.39	-0.56	-0.81	-0.23	-0.51	2.6
Sd	0.40	0.42	0.39	0.33	0.33	4.0
Sy,x	0.38	0.41	0.27	0.32	0.29	3.6
Sy,x %	0.88	0.94	0.78	0.91	0.83	0.66
b	0.972	0.978	1.006	0.937	1.015	0.665
a	1.59	1.49	0.61	2.41	0.01	183

Table 5: FTS accuracy parameters for fat, protein and freezing point in goats' milk samples

n, min, max: number of results, minimum and maximum value; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x and Sy,x%: absolute and relative residual standard deviation; b, a: slope and intercept of the linear regression.

Wide: results obtained after calibration with "median" and "high" SRMs. PLS: results obtained after PLS calibration

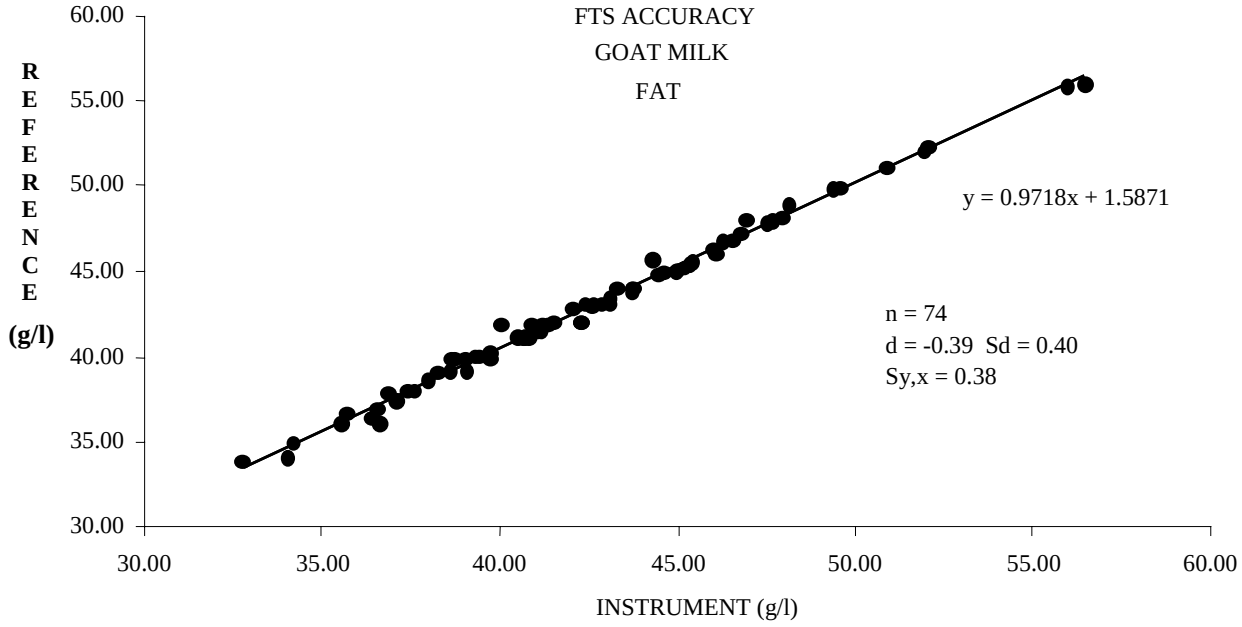

Figure 5: Relation between FTS and reference results for fat in goats' milk samples

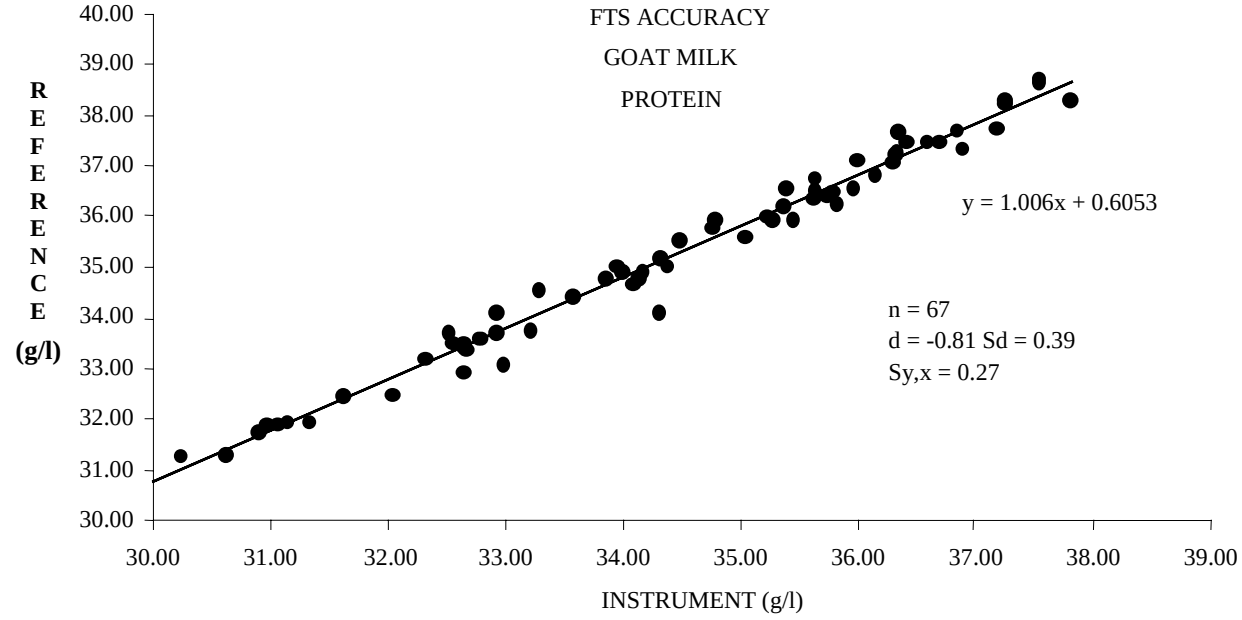

Figure 6: Relation between FTS and reference results for protein in goats' milk samples

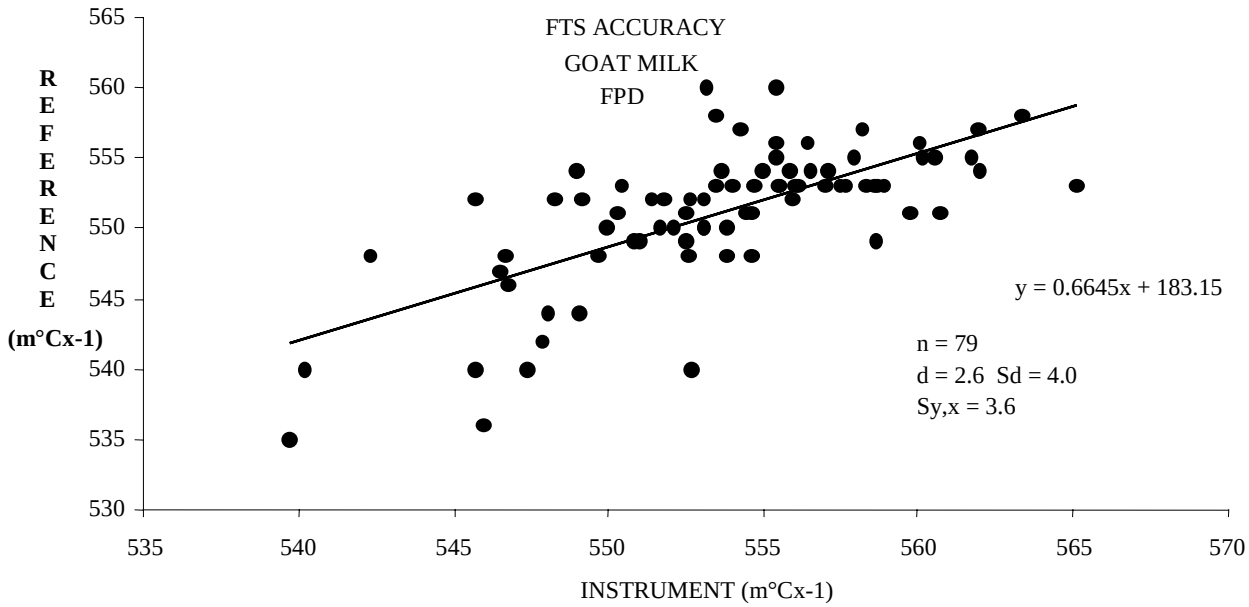

Figure 7: Relation between FTS and reference results for freezing point in goats' milk samples

It can be noted that:
- For fat, the mean and standard deviation of deviations are –0.39 and 0.40 g/l respectively. The regression line obtained is significantly different from 1 (P = 1%) and the intercept is significantly different from zero (P = 1%). The residual standard deviation of regression is equal to 0.38 g/l.
- For protein, the mean and standard deviation of deviations are –0.81 and 0.39 g/l respectively. The regression line obtained is not significantly different from 1 (P = 5%) and the intercept is significantly different from zero (P = 5%). The residual standard deviation of regression is equal to 0.27 g/l.
- For freezing point, the mean and standard deviation of deviations are 2.6 and 4.0 (m°C x –1) respectively. The regression line obtained is significantly different from 1 (P = 1%) and the intercept is significantly different from zero (P = 1%). The residual standard deviation of regression is equal to 3.6 (m°C x –1).

For fat and protein, the results obtained from the wide or PLS calibrations do not improve the residual standard deviations and the slopes.

B3.3.3- Conclusion

With no regulation or standard limits for this type of milk, it can be noted that, for the both composition criteria, the standard deviations obtained are lower than the accuracy limit of the standardised method ISO 9622 / IDF 141 for the cow herd milk samples, which is 0.7 g/L. However, it can be noted that the slope value is statistically different from 1.00 for fat on this type of milk. The composition of ewe milk is probably to be in relation with this observation, and it will be necessary to proceed to a specific slope adjustment.
For FPD determination, as if the slope deviation is important in relation to 1.00, the adjustment do not allow to improve clearly the prediction of the results (Sy,x near to Sd). It will be necessary to proceed to slope and intercept adjustments on specific milk samples.