ARTICLE

EVALUATION OF SIGMA® REAGENTS

This objective of this study was to evaluate the adequacy of certain reagents manufactured by SIGMA and distributed by HUMEAU, for the realisation of tests on milk and cheese. The following test-reagents couples were evaluated:

- Sulfuric acid 90% (ref. 84722) and amylic alcool (ref. 59090) for the determination of fat in milk according to the acido-butyrometric method NF V 04-210[(1)] (Gerber) ;
- Acetic acid (ref. 33209) and perchloric acid 60% (ref. 311413) for the determination of fat in cheese according to the acido-butyrometric method NF V 04-287[(2)] (part 2 Heiss) ;
- Ammoniac 25% (ref. 30501), ethanol 96%(ref. 32294), diethyl oxyde (ref. 31671) and petroelum ether 40-60 (ref. 32299) for the determination of fat in milk according to the extraction method NF EN ISO 1211[(3)] (Röse-Gottlieb).

The tests were performed from July to October 2012 in Actilait-Cecalait®'s physico-chemical laboratory at Poligny (39 - France). The reagents used by Actilait for the study are listed at the end of this article.

1. Procedure

The following types of tests were performed according to the methods tested:

- *Acido-butyrometric method: NF V 04-210 (Gerber):*
  - Test, in duplicate, on 4 reference samples (Gerber SRMs) over 2 consecutive months (July and August 2012);
  - Comparative test, in duplicate, on 10 samples of mixtures of raw milk in relation to the reagents used by Actilait.

- *Acido-butyrometric method: NF V 04-287 (part 2 Heiss):*
  - Comparative tests, in duplicate, on 10 samples of cheese (see list at the end of this article) in relation ot the reagents used by Actilait.

- *Extraction method: NF ISO 1211 (Röse Gottlieb):*
  - Test, in duplicate, on 8 reference samples (Extraction SRMs) from August to September 2012;
  - Comparative test, in duplicate, on 10 samples of mixtures of raw milk in relation to the reagents used by Actilait.

2. Results

2.1. Reference samples (SRMs)

The following tables present the results obtained on the reference samples. The results observed correspond to:

➢ *for the acido-butyrometric method NF V 04-210 (Gerber)*, the mean of 8 repetitions performed. The reference value correspond to the SRM's assigned value, from the results of the expert laboratories.

SRM 07 LGER	RESULT OBSERVED (g/l)	REFERENCE VALUE (g/l)	SRM 08 LGER	RESULT OBSERVED (g/l)	REFERENCE VALUE (g/l)
X	36.813	36.84	**X**	37.850	37.78
Sx	0.083		**Sx**	0.053	
d	-0.03		**d**	0.07	

Table 1: Results of the « Gerber » tests realised on 2 consecutive SRMs
X: arithmetic mean of the results, Sx: standard deviation of the results, d: mean deviation between the results observed and the reference value.

We can note that the mean deviations obtained are low in relation to the reference values on the two sets tested.

➢ *for the extraction method NF ISO 1211 (Röse-Gottlieb)*, the mean of 6 (SRM 08) and 8 (SRM 09) repetitions performed. The reference value correspond to the SRM's assigned value, from the results of the expert laboratories.

SRM 08 LEXT	RESULT OBSERVED (g/kg)	REFERENCE VALUE (g/kg)	SRM 09 LEXT	RESULT OBSERVED (g/kg)	REFERENCE VALUE (g/kg)
X	34.815	34.96	**X**	33.884	33.80
Sx	0.120		**Sx**	0.069	
d	-0.14		**d**	0.08	
CD 95	0.28				

Table 2: Results of the «extraction» tests realised on 2 consecutive SRMs
X: arithmetic mean of the results, Sx: standard deviation of the results, d: mean deviation between the results observed and the reference value, CD 95: critical deviation according to ISO 5725-6

The mean deviation observed are low and below the maximal limit of acceptability, which corresponds to the calculated critical deviation (CD 95).

2.2. Samples of milk and cheese

The following tables present the results obtained on milk and cheese samples. The results observed correspond, for the three methods, to the mean of 2 repetitions performed in repeatability conditions (deviations between duplicates < 0,25 g/l).

➢ **« Gerber » acido-butyrometric method on milk samples**

ID	1	2	3	4	5	6	7	8	9	10	X	Sx	d	Sd
RESULT OBSERVED (g/l)	38.60	36.30	36.95	37.00	37.10	36.95	39.45	40.10	37.00	39.20	**37.865**	**1.335**	**0.06**	**0.09**
ACTILAIT RESULT (g/l)	38.50	36.20	36.90	37.00	37.00	36.95	39.20	40.10	37.10	39.10	**37.805**	**1.304**		

Table 3: Results of the « Gerber » tests realised on mixture of raw milk samples
X and Sx: arithmetic mean and standard deviation of the results, d: deviation mean between the results observed and Actilait's results

It can be noted that the mean deviation between the two analytical sets is low and not statistically significant.

The comparison with a « critical deviation CD 95 » criteria, as a maximal limit of acceptability, calculated according to ISO 5725-6 from standardised values (r = 0,5 g/l et R = 1,0 g/l), was not used because it was not appropriate considering the real performances of the method and its use.

➢ **« Röse-Gottlieb » extraction method on milk samples**

ID	1	2	3	4	5	6	7	8	9	10	X	Sx	d	CD 95
RESULT OBSERVED (g/kg)	37.50	38.51	40.03	34.38	36.86	40.86	39.59	41.63	39.88	36.53	**38.577**	**2.243**	**0.06**	**0.14**
ACTILAIT RESULT (g/kg)	37.46	38.30	40.09	34.07	36.96	40.77	39.53	41.54	39.95	36.50	**38.517**	**2.290**		

Table 4: Results of the « extraction» tests realised on mixture of raw milk samples
X and Sx: arithmetic mean and standard deviation of the results, d: deviation mean between the results observed and Actilait's results, CD 95: critical deviation according to ISO 5725-6

The mean deviations between the two sets is low and below the maximal limit of acceptability, , which corresponds to the critical deviation.

➢ **« Heiss » acido-butyrometric method on cheese samples**

ID	1	2	3	4	5	6	7	8	9	10	X	Sx	d	CD 95
RESULT OBSERVED (g/100 g)	6.5	8.50	2.88	25.00	12.50	33.00	25.88	36.00	28.50	27.50	**20.626**	**11.893**	**-0.03**	**0.09**
ACTILAIT RESULT (g/100 g)	6.50	8.38	3.00	25.00	12.50	33.00	26.00	36.00	28.50	27.63	**20.651**	**11.901**		

Table 5: Results of the « Heiss » tests realised on cheese samples
X and Sx: arithmetic mean and standard deviation of the results, d: deviation mean between the results observed and Actilait's results, CD 95: critical deviation according to ISO 5725-6

The mean deviation between the two sets is low (not statistically significant) and below the critical deviation calculated from the performances of the standardised method.

3. Conclusion

All the reagents tested [sulfuric acid 90% (ref. 84722) and amylic alcool (ref. 59090); acetic acid (ref. 33209) and perchloric acid 60% (ref. 311413); ammoniac 25% (ref. 30501), ethanol 96%(ref. 32294), diethyl oxyde (ref. 31671) and petroleum ether 40-60(ref. 32299)], permit to obtain results equivalent to these obtained with other reagents available on the market.

Bibliography:

(1) Standard AFNOR NF V 04-210 : 2000 « Lait- Détermination de la teneur en matière grasse - Méthode acido-butyrométrique ».
(2) Standard AFNOR NF V 04-287 : 2002 « Fromages - Détermination de la teneur en matière grasse - Méthode acido-butyrométrique ».
(3) Standard NF EN ISO 1211 : 2010 « Lait - Détermination de la teneur en matière grasse - Méthode gravimétrique ».

List of the reagents used by Actilait for this study:

- Acetic acid - Panréac® ref. 131008
- Ammoniac 25% - Merck® ref. 1133.1000
- Amylic acid - Panréac® ref. 125715
- Diethyl oxyde - Prolabo® ref. 23806
- Ethanol 96% - Prolabo® ref. 20824
- Perchloric acid 60% - Panréac® ref. 131054
- Petroleum ether 40-60 - Prolabo® ref. 23835.
- Sulfuric acid 90% - Panréac® ref. 121010

List of the cheese analysed:

- Cheese strainer - Fromage blanc (x2) - Camembert
- Camembert light –Soft and square cheese - Comté
- Cantal
- Emmental.

According to the Sigma® reagents evaluation report – X. QUERVEL and Ph. TROSSAT – October 2012