EVALUATION OF FISHER CHEMICAL® REAGENTS

The objective of this study was to evaluate different Fisher Chemical® reagents, manufactured by Thermo Fisher Scientific company, used for analysis on milk and cheese.

The following test-reagents couples were evaluated:

- Sulfuric acid (code: S/9360/PB15) and isoamyl alcohol (code: A/7000/15) for the determination of fat content in milk according to the Gerber method NF ISO 19662.
- Petroleum ether 40-60 °C (code: P/1760/17) and diethyl ether (code: D/2450/17) for the determination of fat content in milk according to the Rose-Gottlieb method NF EN ISO 1211.
- Sulfuric acid 98 % (code: S/9250/15), sodium hydroxide 40 % (code: S/4960/17) and boric acid solution 4 % (code: K/0205/15) for the determination of nitrogen content in milk according to the Kjeldahl method NF EN ISO 8968-1.
- Acetic acid (code: A/0400/PB15) and perchloric acid 60 % (code: P/1240/PB15) for the determination of fat content in cheese according to the acido-butyrometric method NF V04-287 (Heiss).

All the characteristics of the reagents, except isoamyl alcohol (code: A/7000/15), fulfil to the requirements of the standards. Isoamyl alcohol doen not fulfil requirements of the standardised method on the isomeric ratio (91 % ± 2 % of 3-methylbutan1-ol and 9 % ± 2 % of 2-methylbutan-1-ol). The quality certificate pointes out only the proportion of the 2 isomers in the product (≥ 98 %).

1- THE TESTS

The tests were performed, in duplicate, on 4 reference samples (ACTALIA Cecalait SRMs) over 2 consecutive months from September to October 2019 in ACTALIA cecalait chemistry laboratory according to the methods tested (Gerber method NF ISO 19962, Röse-Gottlieb method NF EN ISO 1211, Kjeldahl method NF EN ISO 8968-1° and Heiss method NF V04-287).

The comparative tests were performed, in duplicate, on 10 samples of raw milk and cheese (Heiss method) analysed both with ACTALIA Cecalait's and Thermo Fisher Scientific's reagents. The cheeses analysed in this test are 4 soft cheeses, 2 hard cheeses and 4 semi-hard cheeses.

The reagents used by ACTALIA Cecalait for this study are presented in the following table:

	Gerber method	Röse Gottlieb method	Kjeldahl method	Heiss method
Reagents	- Sulfuric acid 90-91% *Panreac® ref.121010* - Isoamyl alcohol *Panreac® ref.125715*	- Petroleum ether 40-60°C *VWR Chemicals® ref.23835* - Diethyl ether *VWR Chemicals® ref.23809*	- Sulfuric acid 98% *Panreac® ref.173163* - Sodium hydroxide *VWR Chemicals® ref.28248* - Boric Acid *Fluka® ref.31146*	- Acetic acid glacial *Panreac® ref.131008* - Perchloric acid 60% *Panreac® ref.131054*

Table 1: list of the reagents used by ACTALIA Cecalait

2- THE RESULTS

2-1. Reference samples (SRMs)

The following table presents the results obtained on the reference samples. The results correspond to the mean of 8 repetitions for the Gerber, Kjeldahl and Heiss methods and 7 repetitions for Röse-Gottlieb method (3 on SRM 10 LEXT and 4 on SRM 11 LEXT). The reference values are the SRM's assigned values, calculated from the results of a group of expert laboratories.

GERBER METHOD	**SRM 08 LGER**	**Results (g/l)**	**Reference value (g/l)**	**SRM 09 LGER**	**Results (g/l)**	**Reference value (g/l)**
	X	38.888	38.75	**X**	36.000	35.98
	Sx	0.048		**Sx**	0.041	
	d	0.137		**d**	0.020	
	CD 95	0.637		**CD 95**	0.637	
ROSE-GOTTLIEB METHOD	**SRM 10 LEXT**	**Results (g/kg)**	**Reference value (g/kg)**	**SRM 11 LEXT**	**Results (g/kg)**	**Reference value (g/kg)**
	X	35.64	35.57	**X**	35.02	35.02
	Sx	0.071		**Sx**	0.069	
	d	0.072		**d**	0.002	
	CD 95	0.308		**CD 95**	0.296	
KJELDAHL METHOD	**SRM 08 LKJEL**	**Results (gN/kg)**	**Reference value (gN/kg)**	**SRM 09 LKJEL**	**Results (gN/kg)**	**Reference value (gN/kg)**
	X	5.489	5.47	**X**	5.262	5.25
	Sx	0.005		**Sx**	0.006	
	d	0.019		**d**	0.012	
	CD 95	0.040		**CD 95**	0.040	
HEISS METHOD	**SRM 08 FFO**	**Results (g/100g)**	**Reference value (g/100g)**	**SRM 09 FFO**	**Results (g/100g)**	**Reference value (g/100g)**
	X	27.96	28.01	**X**	28.02	28.09
	Sx	0.186		**Sx**	0.120	
	d	-0.055		**d**	-0.074	
	CD 95	0.535		**CD 95**	0.535	

X: mean; Sx: standard deviation; d: mean deviation between the results and the reference value; CD 95: critical deviation according to ISO 5725-6.

Table 2: Results of the methods tests realised on 2 consecutive SRMs

It can be noted that for all the methods, the mean deviations for both samples batches obtained between the results and the reference values are lower than the critical deviation Cd_{95} according to ISO 5725-6 (§ 4.2.3). For the Gerber method, the mean deviation is also lower than the limit usually applied in dairy laboratories for checking pilot samples (± 0.20 g/l).

2-2. Milk and cheese samples

The following table presents the results obtained on raw milk and cheese (Heiss method) samples. The results observed correspond to the mean of 2 repetitions performed in repeatability conditions (deviations between duplicates < 0.5 g/l).

METHOD	ID	X	Sx	d	Sd	Sr	CD $_{95}$
GERBER METHOD	**Results (g/l)**	33.35	10.95	0.01	0.043	0.051	
	ACTALIA Cecalait results (g/l)	33.34	10.96			0.061	
ROSE-GOTTLIEB METHOD	**Results (g/kg)**	32.20	10.54	0.136	0.147	0.080	0.158
	ACTALIA Cecalait results (g/kg)	32.06	10.58			0.090	
KJELDAHL METHOD	**Results (g/kg)**	5.15	0.606	0.012	0.015	0.014	0.019
	ACTALIA Cecalait results (g/kg)	5.16	0.606			0.013	
HEISS METHOD	**Results (g/100 g)**	26.74	6.193	0.036		0.11	0.095
	ACTALIA Cecalait results (g/100 g)	26.71	6.262			0.14	

X, Sx: mean and standard deviation of the results; d: mean deviation between the results observed and the ACTALIA Cecalait's results; Sr: absolute standard deviation of repeatability; CD_{95}: critical deviation according to ISO 5725-6.

Table 3: Results of the methods on raw milk and cheese (Heiss method)

For the repeatability aspects, the absolute standard deviations are equivalent for the two analytical sets and lower than the limits of the reference methods for Gerber method (Sr = 0.18g/l), for Röse-Gottlieb method (Sr = 0.16g/kg), and for Kjeldahl method (Sr = 0.022 gN/kg), and closed to the limit of the reference method for Heiss method (Sr = 0.11 g/100g).

For accuracy, it can be noted that means are not statistically different (Student test at 5%) and the mean deviation for the Gerber method is very low (0.01 g/l).

In addition, we can observe that the calculated performances (d and Sd) are in accordance with the limits used in proficiency tests on determination of:

- fat content by Gerber method on milk (± 0.20 g/l for mean bias and 0.30 g/l for standard deviation of differences).
- fat content by extraction method on milk (± 0.20 g/kg for mean bias and 0.30 g/kg for standard deviation of differences).
- nitrogen content by Kjeldahl method on milk (± 0.04 gN/kg for mean bias and 0.03 gN/kg for standard deviation of differences).
- fat in cheese by Heiss method (± 0.30 g/100 g for mean bias and 0.30 g/100 g for standard deviation of differences).

Furthermore, we can pointed out that the mean deviations between the two analytical sets are lower than the critical deviations CD_{95}, calculated according to ISO 5725-6 (§ 4.2.1) from standardized values (r = 0.43 g/kg and R = 0.56 g/kg for Röse-Gottlieb method; r = 0.06 gN/kg and R = 0.077 gN/kg for Kjeldahl method; and r = 0.3 g/100g and R = 0.8 g/100g for Heiss method).

3- CONCLUSION

Except the amyl alcohol, all the reagents proposed by Thermo Fisher Scientific fulfil with the technical requirements of the standardized reference methods.

Concerning the performance tests conducted, all the reagents tested [sulfuric acid (Gerber test) (code: S/9360/PB15), isoamyl alcohol (code: A/7000/15), petroleum ether 40-60°C (code: P/1760:17), diethyl ether (code: D/2450/17), sulfuric acid 98% (code: S/9250/15), sodium hydroxide 40% (code: S/4960/17), boric acid solution 4% (code: K/0205/15), acetic acid glacial (code: A/0400/PB15) and perchloric acid 60% (code: P/1240/PB15)] allow to obtain equivalent results to those obtained with other reagents available on the market.

According to the evaluation report of the Fisher Chemical® reagents - A. OUDOTTE and Ph. TROSSAT – September to November 2019