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ACTILAIT

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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

STANDARDS, DRAFT STANDARDS

Classification in alphabetical order by theme

ISO standards under development

STATISTICS	
ISO/DIS 16269-6 February 2013	Statistical interpretation of data – Part 6: Determination of statistical tolerance intervals

ISO published standards

MICROBIOLOGY OF FOOD AND ANIMAL FEED	
ISO/TS 13136 November 2012	MICROBIOLOGY OF FOOD AND ANIMAL FEED Real-time polymerase chain reaction (PCR)–based method for the detection of foodborne pathogens – Horizontal method for the detection of Shiga-toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26 and O145 serogroups
ISO/TS 6579-2 November 2012	MICROBIOLOGY OF FOOD AND ANIMAL FEED Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 2: Enumeration by a miniaturized most probable number technique
SENSORY ANALYSIS	
ISO 8586 December 2012	SENSORY ANALYSIS General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors
ISO 11132 November 2012	SENSORY ANALYSIS Methodology – Guidelines for monitoring the performance of a quantitative sensory panel
UNCERTAINTY OF MEASUREMENT	
ISO/IEC GUIDE 98-4 November 2012	UNCERTAINTY OF MEASUREMENT Part 4: Role of measurement uncertainty in conformity assessment

NEW EU REGULATIONS

Classification is established in alphabetical order of the first keyword

FOOD INGREDIENTS

O.J.E.U. L 327, 27th November 2012 – Commission Implementing Decision of 22 November 2012 authorising the placing on the market of bovine lactoferrin as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0046:0048:EN:PDF

O.J.E.U. L 327, 27th November 2012 – Commission Implementing Decision of 22 November 2012 authorising the placing on the market of duhydrocapsiate as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0049:0051:EN:PDF

STANDARDS - REGULATIONS

PHARMACOLOGICALLY ACTIVE SUBSTANCES

O.J.E.U. L 336, 8th December 2012 – Commission Implementing Regulation (EU) No 1161/2012 of 7 December 2012 amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance fenbendazole

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:336:0014:0016:EN:PDF

O.J.E.U. L 338, 12th December 2012 – Commission Implementing Regulation (EU) No 1186/2012 of 11 December 2012 amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance phoxim

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:338:0020:0022:EN:PDF

P.D.O. / P.G.I.

O.J.E.U. C 352, 16th November 2012 – Publication of an amendment application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Emmental français est-central (PGI) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2012:352:0017:0021:EN:PDF

O.J.E.U. C 353, 17th November 2012 – Publication of an application pursuant to Article 6(2) of Council regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Travia da Beira Baixa (PDO) (dairy product)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2012:353:0014:0017:EN:PDF

O.J.E.U. C 377, 7th December 2012 – Publication of an amendment application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Jihoceska Zlata Niva (PGI) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2012:377:0019:0023:EN:PDF

O.J.E.U. L 337, 11th December 2012 – Commission Implementing Regulation (EU) No 1173/2012 of 5 December 2012 entering a name in the register of protected designations of origin and protected geographical indications [Queso Camerano (PDO) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:337:0013:0014:EN:PDF

O.J.E.U. C 384, 13th December 2012 – Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Saint-Marcellin (PGI) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2012:384:0021:0025:EN:PDF

O.J.E.U. L 343, 14th December 2012 – Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:343:0001:0029:EN:PDF

O.J.E.U. L 347, 15th December 2012 – Commission Implementing Regulation (EU) No 1204/2012 of 14 December 2012 approving amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Castelmagno (PDO) (cheese)]

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:347:0008:0009:EN:PDF

O.J.E.U. L 10, 15th January 2013 – Council Decision of 3 December 2012 on the conclusion of the Agreement between the European Union and the Republic of Moldova on the protection of geographical indications of agricultural products and foodstuffs

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:010:0001:0002:EN:PDF

O.J.E.U. L 10, 15th January 2013 – Agreement between the European Union and the Republic of Moldova on the protection of geographical indications of agricultural products and foodstuffs

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:010:0003:0170:EN:PDF

AFNOR VALIDATIONS

During its November meeting, the Technical Committee of NF VALIDATION approved by vote:

Commercial name	Date	Certificate	Description
NEW VALIDATION			
3M™ MOLECULAR DETECTION ASSAY *SALMONELLA*	Validation date: 30 Dec 2012 **End of validity: 30 Nov 2016**	**3M-01/11-11/12**	**Detection of *Salmonella*** Meat products, dairy products (except milk powders), egg products
RENEWALS OF VALIDATIONS			
TRANSIA PLATE *SALMONELLA* GOLD	Validation date: 23 Mar 2001 Renewal: 3 Feb 2005, 2 July 2009 and 29 Nov 2011 Extension: 12 May 2011 **End of validity: 3 Feb 2017**	**TRA-02/08-03/01**	**Detection of *Salmonella*** All human and animal food products and production environment samples (except primary production stage environment)
ASSURANCE GDS *SALMONELLA*	Validation date: 26 Jan 2009 Renewal: 29 Nov 2012 **End of validity: 26 Jan 2017**	**TRA-02/12-01/09**	**Detection of *Salmonella*** All human and animal food products and production environment samples (except primary production stage environment)
RAPID' *E. COLI* 2	Validation date: 19 Nov 1997 Renewal: 7 Mar 2002, 2 Dec 2004, 28 Nov 2008 and 29 Nov 2012 **End of validity: 2 Dec 2016**	**BRD-07/01-07/93**	**Enumeration of *E. coli* at 44 °C** All human food products
RAPID' *E. COLI* 2	Validation date: 2 Dec 2004 Renewal: 28 Nov 2008 and 29 Nov 2012 **End of validity: 2 Dec 2016**	**BRD-07/07-12/04**	**Enumeration of *E. coli* at 37 °C** All human food products
RAPID' *E. COLI* 2	Validation date: 2 Dec 2004 Renewal: 28 Nov 2008 and 29 Nov 2012 **End of validity: 2 Dec 2016**	**BRD-07/08-12/04**	**Enumeration of coliforms at 37 °C** All human food products
AL DETECTION	Validation date: 26 Jan 2009 Renewal: 29 Nov 2012 Extension: 2 Feb 2012 **End of validity: 26 Jan 2017**	**BRD-07/16-01/09**	**Detection of *Listeria monocytogenes* and *Listeria* spp.** All human food products and environmental samples
AL ENUMERATION	Validation date: 26 Jan 2009 Renewal: 29 Nov 2012 **End of validity: 26 Jan 2017**	**BRD-07/17-01/09**	**Enumeration of *Listeria monocytogenes*** All human food products and environmental samples

AFNOR VALIDATIONS

TEMPO EC	Validation date: 4 Feb 2005 Renewal: 26 Jan 2009 and 30 Nov 2012 **End of validity: 4 Feb 2017**	**BIO-12/13-02/05**	**Enumeration of *E. coli*** All human food products and pet food products
LUMIPROBE 24 *SALMONELLA*	Validation date: 29 Nov 2000 Renewal: 8 Apr 2005, 18 May 2009 and 29 Nov 2012 Extension: 7 Mar 2002 **End of validity: 29 Nov 2016**	**EUR-15/02-11/00**	**Detection of *Salmonella*** All human and animal food products
PROLONGATIONS OF VALIDATIONS			
GENEDISC *SALMONELLA* SPP.	Validation date: 28 Nov 2008 Extension: 27 Jan 2009 and 4 Feb 2010 **End of validity: 28 Nov 2012** **Prolongation till 28 May 2013**	**GEN-25/05-11/08**	**Detection of *Salmonella*** Meat, dairy products and vegetables
GENEDISC *E. COLI* O157:H7	Validation date: 28 Nov 2008 Extension: 27 Jan 2009 and 4 Feb 2010 **End of validity: 28 Nov 2012** **Prolongation till 28 May 2013**	**GEN-25/06-11/08**	**Detection of *E. coli* O157:H7** All human food products and animal feeding

The validation certificates and the recapitulative list are available at the following website address:
http://www.afnor-validation.com/afnor-validation-validated-methods/validated-methods.html

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.

FOOD INGREDIENTS

SMITHERS G.W. ; AUGUSTIN M.A. – **Advances in dairy ingredients** –Wiley-Blackwell Editions – January 2013 – ISBN : 978-0-8138-2395-9 – 352 pages

http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0813823951.html

This book provides an international perspective on recent developments in the area of dairy ingredients and dairy technology. It reviews the market and manufacturing trends, the latest science tools, the functional foods, and gives a glimpse into the future of new dairy ingredients and foods on the horizon.

MICROBIOLOGY

DA SILVA N. ; TANIWAKI M.H. ; JUNQUEIRA V.C. ; SILVEIRA N. ; DE SILVA DO NASCIMENTO M. ; ROMEIRO GOMES R.A. – **Microbiological examination methods of food and water: A laboratory manual** – CRC Press Editions – December 2012 – ISBN : 9780415690867 – 484 pages

http://www.crcpress.com/product/isbn/9780415690867

This book presents the standardised methods and procedures for the microbiological analysis of food and water. All the microorganisms dealt in this manual are described with precision. All chapters provide schematic comparisons between the methods presented, highlighting the main differences and similarities. Moreover, the validated alternative quick methods, which, though not described in the book, can be used for the analysis of the microorganism dealt, are listed in each chapter.

YOGURT / FERMENTED MILKS

CHANDAN R.C. ; KILARA A. – **Manufacturing yogurt and fermented milks, 2nd edition** –Wiley-Blackwell Editions – February 2013 – ISBN : 978-1-1199-6708-8 – 496 pages

http://eu.wiley.com/WileyCDA/WileyTitle/productCd-1119967082,descCd-description.html

This book gives a complete description of the manufacturing stages of yogurt and fermented milks from the receipt of raw materials to the packaging of the products. Information about milk composition characteristics, dairy processing principles, regulatory requirements, analysis, starter cultures, packaging, fruit preparation, ingredients, cleaning, quality assurance, sensory analysis, health benefits… is available in this book.

FORTHCOMING EVENTS

Classified in chronological order

FOOD TECHNOLOGY

5-6 March 2013 Dublin, Ireland	11th world food technology and innovation forum 2013	http://www.foodinnovate.com/

STANDARDISATION

3-7 June 2013 Rotterdam, The Netherlands	IDF/ISO analytical week	http://www.idf-iso-analytical-week.org/ColumnsPage.php?siteID=1716&ID=1717

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

CONTAMINANTS

Scientific opinion on the presence of dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs) in commercially available foods for infants and young children

http://www.efsa.europa.eu/en/efsajournal/pub/2983.htm

► Following a request from the Federal Institute for Risk Assessment (BfR), the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the presence of dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and dioxin-like polychlorinated biphenyls (DL-PCBs) in commercially available foods for infants and young children.

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