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Certification of the crude protein, fat, lactose and ash content of whole milk powder and the crude protein and fat content of skim milk powder BCR-380R and BCR-685

M. Staniszewska, R. Koeber, T. Linsinger, R. Zeleny,
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BCR information
REFERENCE MATERIALS

Certification of the crude protein, fat, lactose and ash content of whole milk powder and the crude protein and fat content of skim milk powder

BCR-380R & BCR-685

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Summary

This report describes the preparation of two milk powder reference materials and the measurement exercises that led to the certification of the content (mass fraction) of the crude protein (Kjeldahl-N x 6.38), fat, lactose and ash in whole milk powder (BCR-380R) and crude protein (Kjeldahl-N x 6.38) and fat in skim milk powder (BCR-685).

The certified values are presented in Tables I and II. The results of the certification exercise, which involved material characterisation by a collaborative study involving several experienced European laboratories, are presented and discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1]. The stated uncertainties include contributions regarding the results of the characterisation measurements and uncertainties due to potential inhomogeneity and potential instability of the materials. The values carried by the certified reference materials are traceable to methods standardised by the International Dairy Federation (IDF), the International Organization for Standardization (ISO) and AOAC International.

Table I: Certified mass fraction of main components in whole milk powder – BCR-380R

Parameters	Mass fraction in g/100 g 1)		Relative uncertainty 3) in %	No. of accepted sets of results
	Certified value 2)	Uncertainty 3)		
Crude protein (Kjeldahl-N x 6.38)	28.66	0.28	1.0	6
Fat	26.95	0.16	0.6	6
Lactose (anhydrous)	37.1	1.0	2.7	11
Ash	6.00	0.13	2.1	8

1) Results corrected for dry mass.

2) Unweighted mean value of the means of accepted sets of results, each set being obtained in a different laboratory applying relevant methods of analysis standardised by IDF/ISO and AOAC The certified values are traceable to the methods used for certification.

3) Expanded uncertainty with a coverage factor of k = 2, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

Table II: Certified mass fraction of main components in skim milk powder BCR-685

Parameters	Mass fraction in g/100 g 1)		Relative uncertainty 3) in %	No. of accepted sets of results
	Certified value 2)	Uncertainty 3)		
Crude protein (Kjeldahl-N x 6.38)	38.2	0.4	1.0	7
Fat	0.96	0.12	12.6	6

1) Results corrected for dry mass.

2) Unweighted mean value of the means of accepted sets of results, each set being obtained in a different laboratory applying relevant methods of analysis standardised by IDF/ISO and AOAC. The certified values are traceable to the methods used for certification.

3) Expanded uncertainty with a coverage factor of k = 2, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

Table of contents

Glossary

ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
BCR	Community Bureau of Reference
CRM	certified reference material
GUM	Guide to the Expression of Uncertainty in Measurement
HPLC	high performance liquid chromatography
IDF	International Dairy Federation
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
JRC	Joint Research Centre
KFT	Karl Fischer titration
k	coverage factor
MS_{between}	mean squares between-bottles
MS_{within}	mean squares within-bottles
n	number of replicate determinations
s_{bb}	between-bottle standard deviation
SI	International Systems of Units
s_{r}	repeatability standard deviation
s_{R}	reproducibility standard deviation
u^{*}_{bb}	upper limit of inhomogeneity that can be hidden by method repeatability
u_{bb}	uncertainty due to potential material inhomogeneity
u_{c}	combined uncertainty
U_{CRM}	expanded uncertainty of certified value
u_{lts}	uncertainty due to long-term storage instability of the material
u_{meas}	measurement uncertainty
u_{sts}	uncertainty due to short-term storage instability of the material
v_{MSwithin}	degrees of freedom of MS_{within}

1 Introduction

Whole and skim milk powder are the most important commodities in international dairy trade. Product standards for these agro-industrial products have been issued by the Codex Alimentarius in close cooperation with the International Dairy Federation (IDF). Methods of analysis for the major components of milk powder (crude protein, fat, lactose, ash, dry matter) have been standardised to allow checking whether products conform to the specifications laid down in the Codex standards; acceptance or rejection of a lot depends whether specifications are met. Manufacturing processes in the dairy industry rely also on an accurate knowledge of the product composition in order to optimise the performance of a production plant, otherwise financial losses can not be avoided.

Besides manufacturing related aspects knowledge of the nutrient content of foods is necessary to study the relation between diet and health, for planning of diets, in official food control, and for food labelling purposes. Nutrition research and counselling also rely heavily on analytical data for the nutrient content of food. This information is compiled in nutrient data banks. Many countries have taken steps to improve the dietary habits of their populations, by publishing guidelines for a healthy diet. Nutritional labelling is essential for those consumers who use these guidelines to select a balanced diet.

The importance of reliable consumer information in the EU is reflected in the issuing of relevant Community legislation. In general, nutrition labelling is governed by Council Directive 90/496/EC [2], as amended by Commission Directive 2003/120/EC [3], whereas general provisions on the labelling of foodstuffs to be delivered to the consumer is laid out in European Parliament and Council Directive 2000/13/EC [4]. Official and private food control laboratories are charged to verify that the information provided is correct. However, analysis of the proximate composition of foodstuffs (crude protein, fat, carbohydrate, etc.) may produce widely different values, even when performed by experienced laboratories, as these properties are defined by the method itself (= defining methods). Analysts are required to follow strictly a pre-described protocol; any deviation from this will result in more or less pronounced differences in the results obtained. Standardisation is the only useful approach to ensure comparability of method dependent properties. Even if standardised procedures are made available, laboratories need to verify that they are capable to apply the method correctly. For this purpose matrix certified reference materials (CRMs), which have been certified by using the same standardised method, are needed.

The European Commission's Community Bureau of Reference (BCR) issued a whole milk powder CRM (BCR-380) in 1992, which ran out of stock and is now replaced by BCR-380R. As the content of crude protein and fat are the most important quality parameters for international trade in dairy products a skim milk powder material has also been prepared and certified for crude protein and fat content (BCR-685).

The methods of analysis used in this certification project were standardised by a joint working group of IDF, the International Organization for Standardization (ISO) and AOAC International, and are mutually recognised by each organisation. The equivalence of the standardised methods issued by the three standardisation bodies [5-20] is given in Table 1. All certified values are traceable to these standardised procedures.

Table 1: Equivalence of standardised methods used for the determination of main components in dairy products

	International Organization for Standardization (ISO)	International Dairy Federation (IDF)	AOAC International
Crude protein (Kjeldahl-N x 6.38)	ISO 8968-1:2001 ISO 8968-2:2001 ISO 8968-3:2004	IDF Standard 20-1:2001 IDF Standard 20-2:2001 IDF Standard 20-3: 2004	Official Method 991.20
Fat	ISO 1736:2000	IDF Standard 9C:1987	Official Method 932.06
Lactose	ISO 5765-1:2002 ISO 5765-2:2002	IDF Standard 79-1:2002 IDF Standard 79-2:2002	
Ash			Official Method 930.30
Dry matter		IDF Standard 26A:1993	

2 Participants

- **Preparation of the candidate reference material**
  - European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
- **Homogeneity tests**
  - Rijkskwaliteitsinstituut voor Land- en Tuinbouwproducten (RIKILT), Wageningen, NL
- **Stability tests**
  - Rijkskwaliteitsinstituut voor Land- en Tuinbouwproducten (RIKILT), Wageningen, NL
- **Certification measurements**
  - Rijkskwaliteitsinstituut voor Land- en Tuinbouwproducten (RIKILT), Wageningen, NL
  - Direct Laboratory Services Limited, Wolverhampton, UK
  - Eurofins Scientific, Schönenwerd, CH
  - Nestlé Research Center, Quality Assurance Department, Lausanne, CH
  - Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Maisons-Alfort, FR
  - Centrum voor Landbouwkundig Onderzoek, Departement Kwaliteit van Dierlijke Producten en Transformatietechnologie (CLO), Melle, BE
  - Institut Provincial d'Hygiène et de Bactériologie du Hainaut, Mons, BE
  - Centraal Orgaan voor Kwaliteitsaangelegenheden in de Zuivel (COKZ), Leusden, NL
  - National Veterinary and Food Research Institute, Helsinki, FI
- **Organisation of the certification exercise**
  - European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, BE
- **Statistical analysis and preparation of the report**
  - European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, BE

3 Processing of the materials

The spray-dried milk powder materials (whole milk and skim milk) were delivered in 25 kg units (packed in protected paper bags) from Lactoprot, Werk Hartberg, AT. Both were stored at room temperature till processing. Representative samples were taken for moisture determination by Karl Fischer-titration (KFT). Both milk powder materials were further dried under vacuum in a freeze dryer at -20 °C to lower their moisture content to 2.5 %. The homogenisation of the materials was done with a Turbula mixer T200 for 2 hours in a 200 L polyethylene container. Bottling was accomplished by using a PTFE-shielded feeder to exclude metal contamination. BCR-380R (whole milk powder) was filled in amber glass bottles (nominal volume 280 mL) in amounts of 100 g and BCR-685 (skim milk powder) was filled in amber glass bottles (nominal volume 125 mL) in amounts of 50 g. After filling the bottles were closed with a polyethylene insert and a screw cap and 174 units of BCR-380R and 195 units of BCR-685 set aside by using a stratified random sampling scheme. This guaranteed that samples truly representative for the whole lot were available for homogeneity, stability and characterisation study purposes.

Particle size measurements by laser diffraction were carried out using a Sympatec Helos particle size analyser (Sympatec GmbH, DE). The top particle size for both materials was less than 365 µm.

4 Homogeneity

An experimental design which allowed evaluation of the obtained results by analysis of variance (ANOVA) was used to quantify uncertainty contributions due to potential inhomogeneity of the batch. This study was performed by one laboratory that analysed 20 units (bottles) in duplicate for crude protein (Kjeldahl-nitrogen x 6.38), fat, lactose, ash, and dry matter for the whole milk powder material (BCR-380R), and crude protein (Kjeldahl-nitrogen x 6.38), fat and dry matter for the skim milk powder material (BCR-685) under repeatability conditions.

The following methods, standardised by IDF/ISO and AOAC, were used:

- crude protein: IDF 20-1:2001; the obtained nitrogen content was multiplied by a factor of 6.38 to obtain crude protein [12]
- fat: IDF 9C:1987 [11]
- lactose: IDF 79-1:2002 [9]
- ash: ashing to constant mass at 550 °C (according to AOAC 930.30) [5]
- moisture content: IDF 26A:1993 [8]

Data were dry-mass corrected before statistical data evaluation for BCR-380R, while for BCR-685 moisture data were not available. However, as only relative changes of a certain parameter are relevant for testing a batch of a bottled material for homogeneity, the uncorrected data were deemed equally valid. The results of the homogeneity study are plotted in Figure 1 to Figure 6; raw data are listed in Tables A-1 to A-2 in the Annex A. Normal probability plots for each of the properties in both materials confirmed that the data followed essentially a normal distribution (data not shown); no multi-modality of data was detected. Unimodality of data is an essential prerequisite for data evaluation by ANOVA. Regression analysis relating bottling sequence to property values did not reveal significant trends due to bottling sequence, except for crude protein and ash in BCR-380R. However, the figures for the slope of the regression functions were small (0.0001 for crude protein and 0.0029 for ash) compared to the absolute level of the property value, and therefore considered to be of practical insignificance.

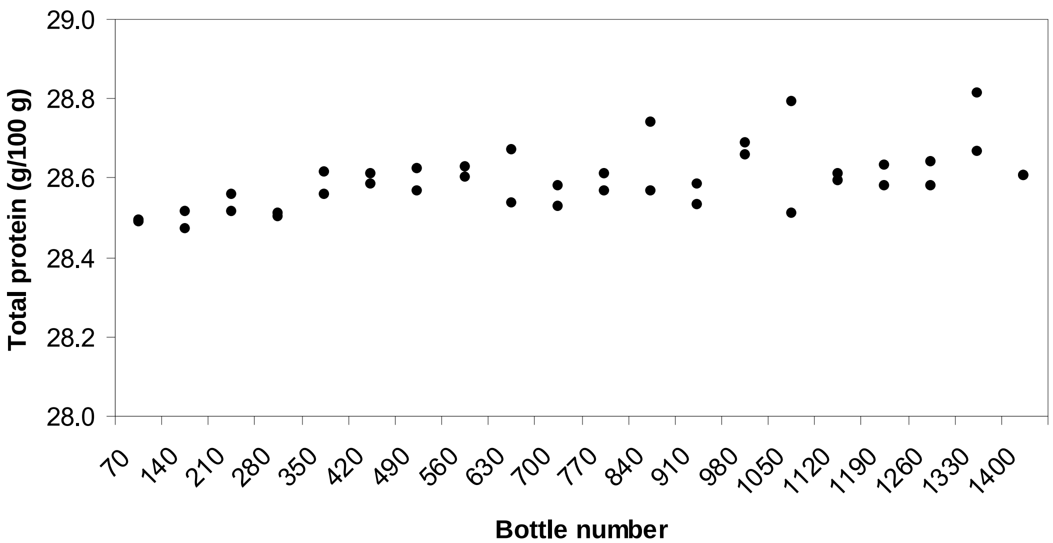

Figure 1: Results of the homogeneity study for crude protein (Kjeldahl-N x 6.38) content in BCR-380R (whole milk powder)

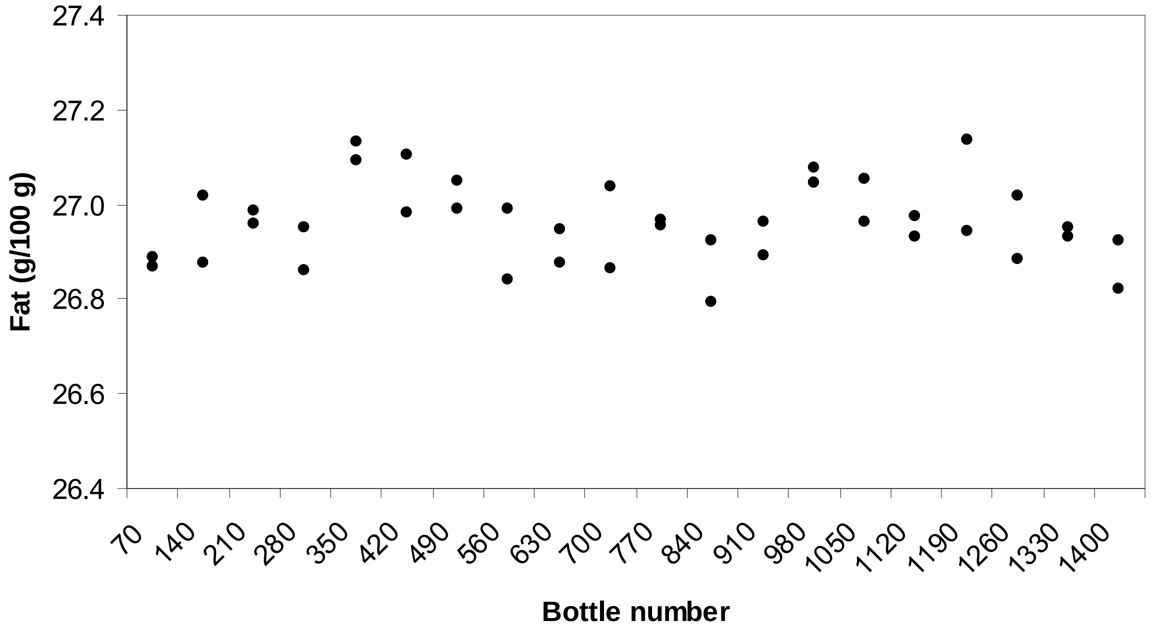

Figure 2: **Results of the homogeneity study for fat content in BCR-380R (whole milk powder)**

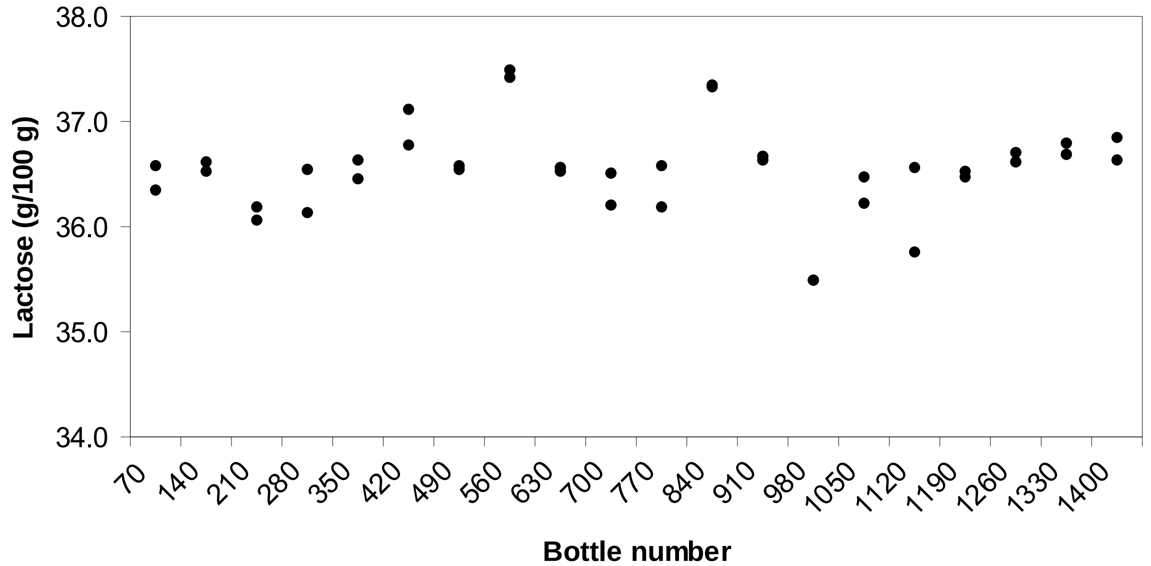

Figure 3: **Results of the homogeneity study for lactose content in BCR-380R (whole milk powder)**

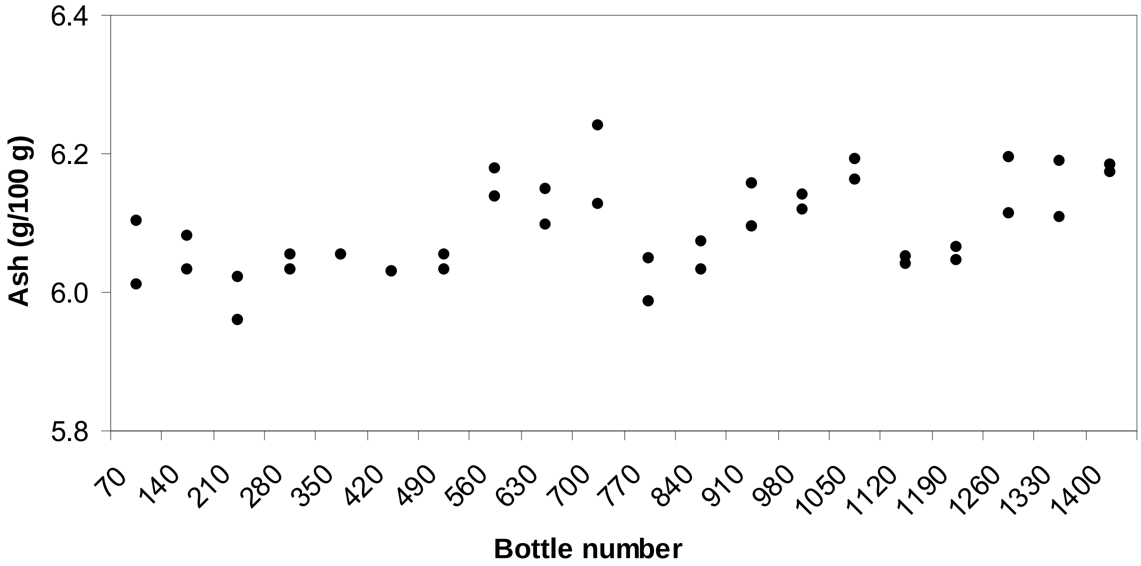

Figure 4: **Results of the homogeneity study for ash content in BCR-380R (whole milk powder)**

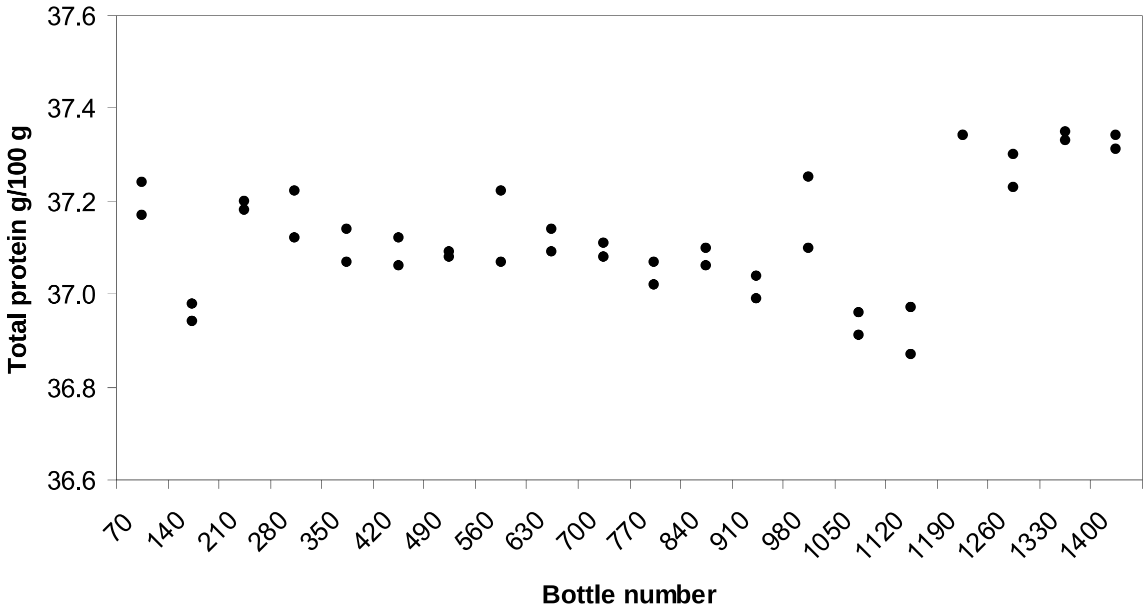

Figure 5: **Results of the homogeneity study for crude protein (Kjeldahl-N x 6.38) content in BCR-685 (skim milk powder)**

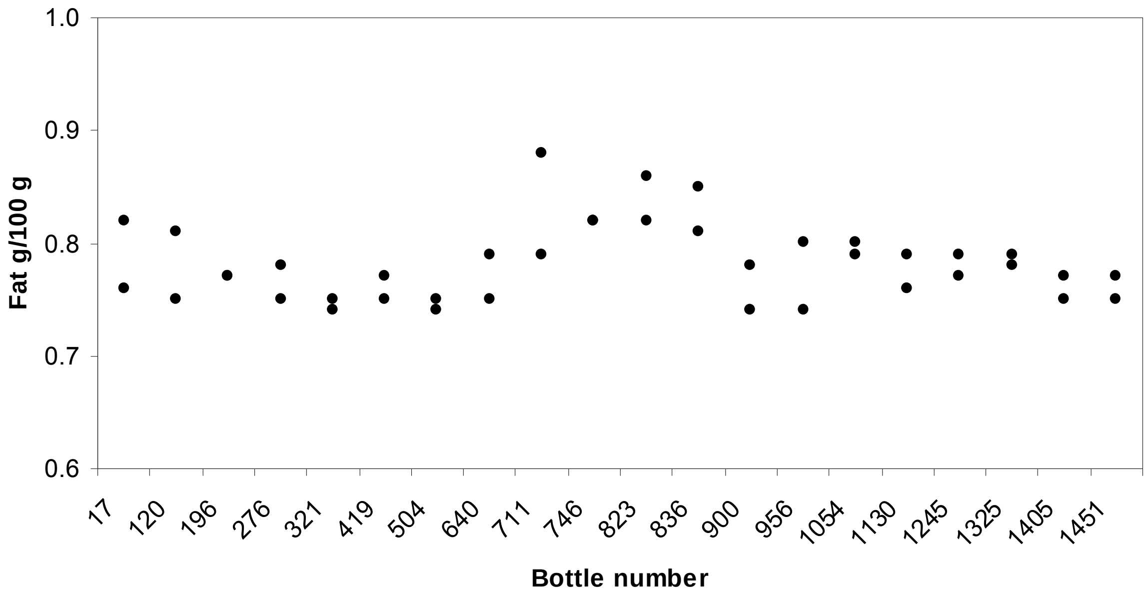

Figure 6: Results of the homogeneity study for fat content in BCR-685 (skim milk powder)

Uncertainty contributions due to inhomogeneity (u_{bb}) were quantified by using an ANOVA approach. In general, the between-bottle standard deviation (s_{bb}) is used as an estimator of the contribution of potential inhomogeneity to the total uncertainty of the certified property value. It was computed as *(1)*:

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \qquad (1)$$

s_{bb}	=	between-bottle standard deviation
$MS_{between}$	=	mean squares between bottles
MS_{within}	=	mean squares within bottles
n	=	number of replicate determinations

In case that MS_{within} is larger than $MS_{between}$, s_{bb} can not be determined. As method repeatability, which equals the square root of MS_{within}, may exert a profound influence on s_{bb}, the upper limit of inhomogeneity that can be hidden by method repeatability (u^*_{bb}) was estimated according to Linsinger et al. [21] *(2)*:

$$u^*_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (2)$$

$\nu_{MSwithin}$	=	degrees of freedom of MS_{within}

According to ISO Guide 35 [22] the numerically larger value of either s_{bb} or u^*_{bb} was used as a conservative estimate of u_{bb}.

Table 2 and Table 3 summarise the outcome of the statistical data evaluation for BCR-380R and for BCR-685, respectively. In all cases s_{bb} was adopted as the potential between-bottle inhomogeneity contribution for both materials and all properties studied. As u_{bb} was mostly < 1 %, BCR-380R and BCR-685 were considered to be homogeneous.

Table 2: Uncertainty contributions due to inhomogeneity of BCR-380R (whole milk powder) as obtained by ANOVA

	Crude protein	Fat	Lactose	Ash
Average (g/100 g)	28.59	26.96	37.26	6.09
Repeatability [%]	0.23	0.27	0.51	0.62
s_{bb} [%]	0.14	0.16	1.09	0.91
u^*_{bb} [%]	0.09	0.11	0.20	0.25
u_{bb} [%]	**0.14**	**0.16**	**1.09**	**0.91**

Table 3: Uncertainty contributions due to inhomogeneity of BCR-685 (skim milk powder) as obtained by ANOVA

	Crude protein	Fat
Average (g/100 g)	37.13	0.78
Repeatability [%]	0.13	3.45
s_{bb} [%]	0.32	2.74
u^*_{bb} [%]	0.05	1.37
u_{bb} [%]	**0.32**	**2.74**

5 Stability studies

The stability of the materials at various storage temperatures was assessed by using an isochronous measurement scheme [21]. This test design allows the assessment of the material property values under repeatability conditions. The same standardised methods were used as in the homogeneity study. Data evaluation was done by regression analysis, relating storage time to the tested material properties. The material properties were considered stable over time when the slopes of the regression functions did not significantly differ from zero.

5.1 Short-term stability

Short-term stability studies should indicate whether special care must be taken during the transport (shipping) of the materials. Short-term stability of the candidate CRMs was tested at 40 °C, 18 °C, and 4 °C, -20 °C for storage periods of 0, 1, 2 and 4 weeks (5 independent measurements at each time-point). Samples stored at -70 °C served as reference, as no changes in the property values were expected at that temperature.

The results are presented in the Annex B (Tables A-3 to A-8). No deterioration of the material was detectable. The slopes of the regression lines relating storage time to the content of the individual parameters were tested for significance and were found to be not significantly different from zero in most cases. Only for the crude protein content in BCR-380R the slopes were different from zero, although inconsistencies in the temperature data matrix suggested irregularities during testing. Therefore, the apparent instability was not further taken into account.

Although no instability at 40 °C and 18 °C was detected, the materials will be shipped cooled as a precautionary measure.

5.2 Long-term stability

For long-term storage 4 °C was considered to be a suitable storage temperature. BCR-380 was stored at 4 °C for more than 10 years without deterioration, as evidenced by IRMM's post-certification monitoring programme. Samples stored at -70 °C served as reference, as no changes in the property values were expected at that temperature.

Linear regression analysis of the long-term stability data was used to test whether the data followed a temporal trend. In case that the slope of the regression line did not significantly differ from zero the property value was considered to be stable at the selected storage temperature. The standard error of the slope of the regression line relating storage time to changes in the property value was adopted as an uncertainty contribution resulting from long-term storage. To factor-in the expected maximum storage time (shelf-life) of the candidate CRM where the certified values are valid, the standard error of the slope has to be multiplied with the foreseen shelf-life of the material to estimate the long-term stability uncertainty (u_{lts}).

The initial long-term stability study lasted for 24 months and was designed according to an isochronous measurement scheme. As further stability monitoring data became available, the two studies were combined to give a total study duration of 50 months (samples stored at 4 °C).

The outcome of the long-term stability studies (4 °C) are shown in Figure 7 to Figure 10 for BCR-380R and Figure 11 and Figure 12 for BCR-685. The relative figures (individual values related to the mean of all values) are depicted for better comparability. The solid lines shown represent the "uncertainty triangle" obtained by multiplication of the standard error of the slope with storage time (in months). Dotted lines in the graph show the long-term stability uncertainty contribution of the CRMs calculated for a storage time of 60 months. The validity of the certificate will be extended when additional data will support this measure.

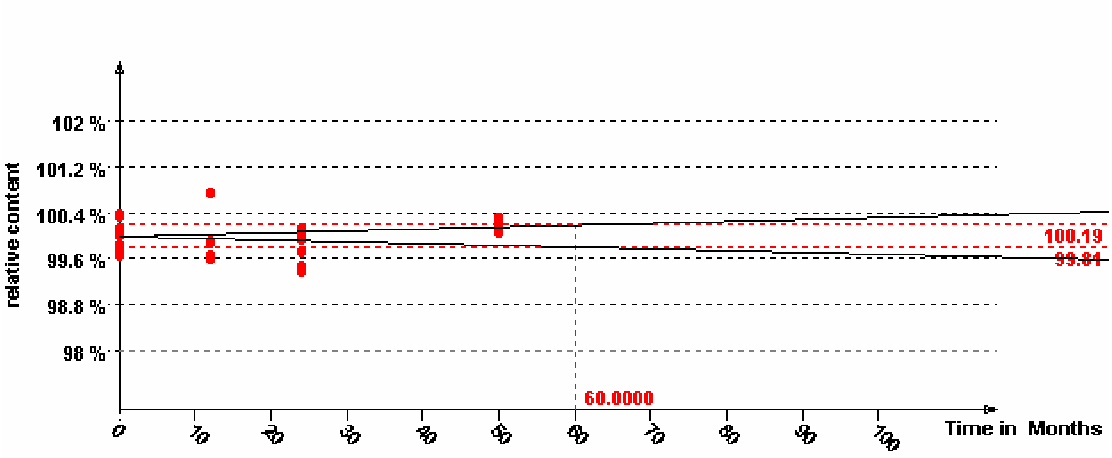

Figure 7: Results of the long-term stability study at 4 °C for the fat content (dry mass corrected) in BCR-380R

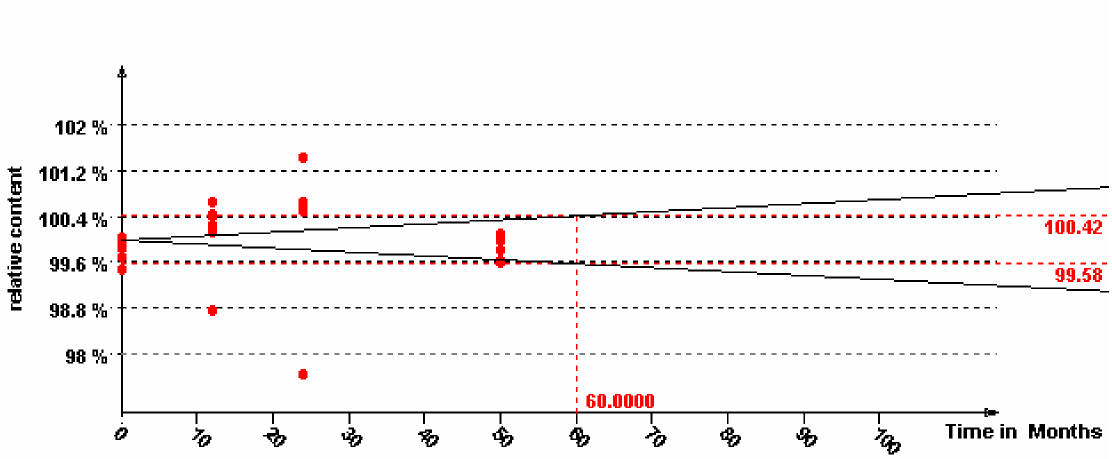

Figure 8: Results of the long-term stability study at 4 °C for the crude protein content (dry mass corrected) in BCR-380R

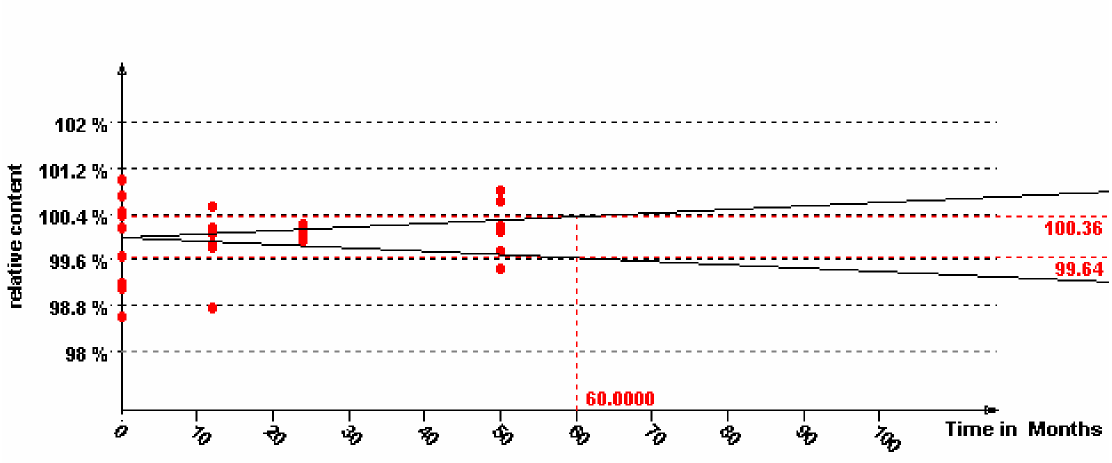

Figure 9: Results of the long-term stability study at 4 °C for the lactose content (dry mass corrected) in BCR-380R

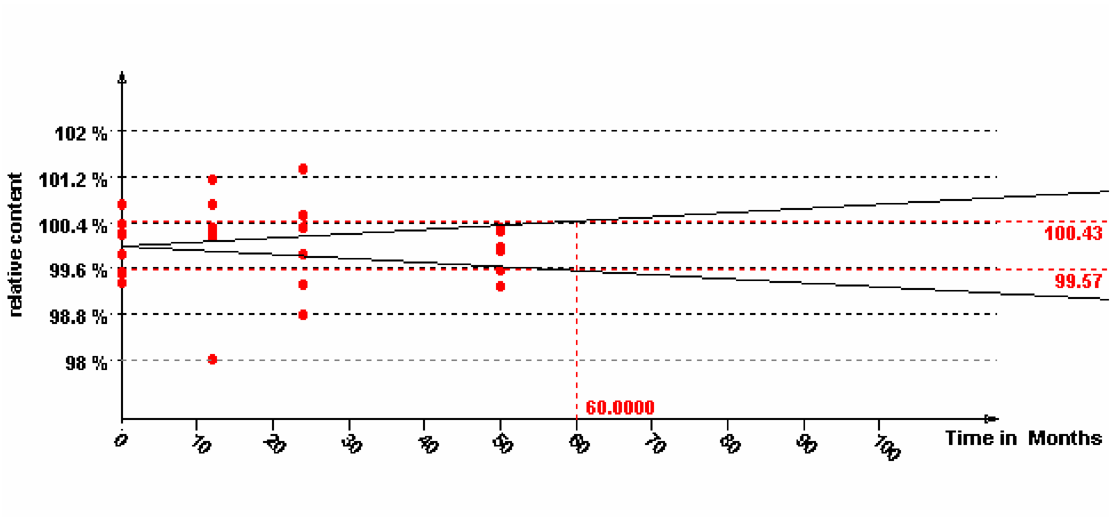

Figure 10: **Results of the long-term stability study at 4 °C for the ash content (dry mass corrected) in BCR-380R**

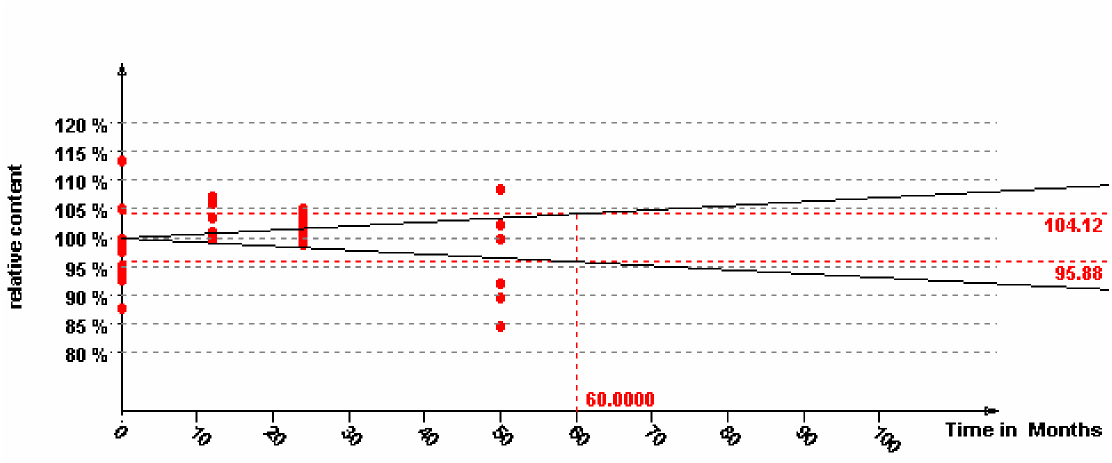

Figure 11: **Results of the long-term stability study at 4 °C for the fat content (dry mass corrected) in BCR-685**

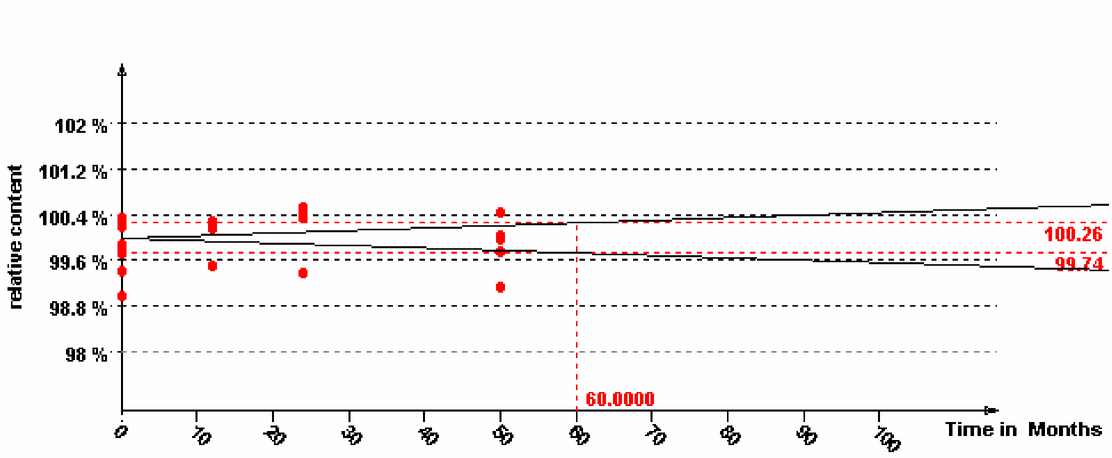

Figure 12: **Results of the long-term stability study at 4 °C for the crude protein content (dry mass corrected) in BCR-685**

Individual results of the long-term stability studies are tabulated in the Annex C (Tables A-9 and A-10). The results of the regression analyses and the estimation of u_{lts} for a storage period of 60 months are summarised in Table 4. u_{lts} was < 1 % for all parameters except for fat content in the skim milk powder (BCR-685).

Table 4: Uncertainty contributions due to long-term storage at 4 °C for BCR-380R and BCR-685

	Crude protein	**Fat**	**Lactose**	**Ash**
BCR-380R				
Slope	0.0002	0.0009	0.0020	-0.0001
Standard error of slope	0.0021	0.0009	0.0022	0.0004
P-value	0.94	0.32	0.39	0.80
u_{lts} (%) for 60 months shelf-life	**0.42 %**	**0.19 %**	**0.36 %**	**0.43 %**
BCR-685				
Slope	0.0006	-0.0005		
Standard error of slope	0.0017	0.0006		
P-value	0.74	0.41		
u_{lts} (%) for 60 months shelf-life	**0.26 %**	**4.12 %**		

6 Certification

6.1 Design of the characterisation exercise

Participants were selected based on documented expertise in the analysis of the gross composition of whole milk and skim milk powders. They were requested to use the following methods for the analysis of the candidate reference materials BCR-380R and BCR-685:

- crude protein: IDF 20-1:2001 [12], the obtained nitrogen content was multiplied by a factor of 6.38 for converting nitrogen to crude protein
- fat: IDF 9C:1987 [11]
- lactose: IDF 79:2002 [9]
- ash: ashing to constant mass at 550 °C (according to AOAC 930.30) [5]
- moisture content: IDF 26A:1993 [8]

As a minimum performance criterion participants had to fulfil the method precision requirements as specified in the standardised methods of analysis.

As an alternative to IDF 79:2002 participants were allowed to apply a validated HPLC method for the determination of lactose in BCR-380R, provided the performance of the HPLC method was equivalent to the IDF standardised method. Table 5 summarises the methods used.

Each participant received three bottles of BCR-380R (whole milk powder) and three bottles of BCR-685 (skim milk powder). The three bottles of each candidate CRM had to be analysed in duplicate on separate working days (6 independent determinations in total). Sub-sampling (weighing) for the individual determinations had to be carried out in the shortest time-span possible and at the same time as sub-sampling was done for the moisture determinations (dry mass correction). Balances used for the weighing operations were calibrated and the calibration status checked regularly.

In case that HPLC was used for determining the lactose content instrument calibration had to be performed on each of the three days separately.

Table 5: HPLC methods used by the participants for the determination of lactose in BCR-685R

Participant	Column	Mobile phase	Detector
1	Lichrosphere 100 NH2 (250 mm x 5 mm)	$CH_3CN:H_2O$, 4:1	Refractive index
4	HPX-87P (300 mm x 7.8 mm)	Water	Refractive index
5	HPX-87P (300 mm x 7.8 mm)	Water	Refractive index
7	Meta Carb Pb Plus (300 mm x 7.8 mm)	Water	Refractive index
8	HPX-87P (300 mm x 7.8 mm)	Water	Refractive index

The participants had to provide evidence for the proper execution of the analyses and submit their results in electronic format by using a fixed format spreadsheet programmed in MS Word (result form) accompanied with a methods questionnaire form.

6.2 *Technical evaluation of the results submitted*

The individual results as submitted by the participants can be found in the Annex D (Tables A-11 and A-12). For BCR-380R (whole milk powder) 8 data sets were received for the crude protein content, 8 data sets for fat, 7 data sets for lactose determined by IDF Standard 79:2002, 5 data sets for lactose determined by HPLC and 8 data sets for ash; for BCR-685 (skim milk powder) 7 data sets were received for crude protein and for fat.

Before submitting the data to statistical analysis they were scrutinised for completeness and technical plausibility.

Laboratory 3 had neither analysed BCR-380R nor BCR-685 for dry matter; therefore the other data provided by this laboratory could not be dry matter corrected. Consequently, the data from laboratory 3 were excluded.

Laboratory 8 submitted only 4 independently obtained results for the lactose by IDF Standard 79:2002 in BCR-380R instead of the requested 6. Therefore, the lactose by IDF Standard 79:2002 from this laboratory were not included in the data evaluation.

For each remaining data set, identified by a laboratory code, the arithmetic mean value and the within-laboratory standard deviation were calculated. They are given in graphical form in Figures A-1 to A-6 in the Annex E. Results were only accepted for certification, if the concerned laboratory fulfilled the method precision criteria as outlined in the respective IDF/ISO/AOAC standards. The repeatability (absolute difference between the results of two single determinations, carried out simultaneously or in rapid succession by the same operator under the same conditions on identical test material) and the reproducibility (absolute difference between two single and independent results, found by two operators working in different laboratories on identical test material) figures as specified in these standard methods are summarised in Table 6.

Table 6: Precision parameters of the standardised IDF/ISO/AOAC methods to characterise BCR-380R and BCR-685.

	IDF 20:2001 (crude protein)	IDF 9C:1987 (fat content)	IDF 79:2002 (lactose)	AOAC 930.30 (ash)	IDF 26A:1993 (moisture content)
Repeatability	0.13 g/100 g	0.2 g/100 g (whole milk powder) 0.1 g (skim milk powder)	3 % (of the arithmetic mean)	not available	0.2 g/100 g
Reproducibility	0.19 g/100 g	0.3 g/100 g (whole milk powder) 0.2 g (skim milk powder)	6 % (of the arithmetic mean)	not available	0.4 g/100 g

Inspection of Figures A-1 to A-6 revealed that Laboratory 9 did not meet the requirements for repeatability as set out in the respective IDF/ISO/AOAC standards (Table 6) for crude protein and fat content in BCR-380R, and Laboratory 7 for fat content in BCR-685. Therefore, these data were not included in the final statistical evaluation.

6.3 Statistical evaluation of the results submitted

The data sets accepted on technical grounds were subjected to the following statistical tests:

- Dixon's, Nalimov's and Grubbs' tests to detect outlying laboratory mean values
- Cochran's test to detect outlying values in the within-laboratory variances
- Bartlett's test for homogeneity of variances
- One-way analysis-of-variance (ANOVA) to determine within-data sets and between-data sets variability

The results of the statistical analysis for BCR-380R are given in Table 7and for BCR-685 in Table 8.

Table 7: Statistical evaluation of the accepted characterisation data for BCR-380R (whole milk powder)

	Crude protein [1)]	Fat	Lactose (anhydrous)	Ash
Accepted data sets (labs)	6	6	11	8
Analysed samples (total)	36	36	66	48
Mean of lab means (g/100 g)	28.66	26.95	37.06	6.00
Standard deviation of lab means (g/100 g)	0.130	0.104	0.608	0.054
Standard error of lab means (g/100 g)	0.053	0.043	0.183	0.019
Half-width of 95 % confidence interval (g/100 g)	0.136	0.109	0.409	0.0452
TESTING FOR OUTLYING LAB MEANS				
Dixon test	no	no	no	no
Nalimov t-test	no	no	lab 1	no
Grubbs test (single)	no	no	no	no
Grubbs test (double)	no	no	no	no
TESTING OF LAB VARIANCES				
Cochran test (outlying lab variances)	lab 7	lab 8	no	lab 2
Bartlett test (homogeneity of lab variances)	no	no	no	no
Pooling of data	no	no	no	no
DISTRIBUTION OF LAB MEANS				
Skewness & kurtosis test	[2)]	[2)]	normal	normal
ANOVA				
Between-lab standard deviation (g/100 g)	0.123	0.086	0.509	0.051
Within-lab standard deviation (g/100 g)	0.104	0.142	0.814	0.046
Differences between labs statistically significant?	yes	yes	yes	yes

[1)] Kjeldahl-N x 6.38
[2)] insufficient data (number of labs < 7)

Table 8: Statistical evaluation of the accepted characterisation data for BCR-685 (skim milk powder)

	Crude protein [1]	Fat
Accepted data sets (labs)	7	6
Analysed samples (total)	42	36
Mean of lab means (g/100 g)	38.18	0.96
Standard deviation of lab means (g/100 g)	0.291	0.092
Standard error of lab means (g/100 g)	0.110	0.038
Half-width of 95 % confidence interval (g/100 g)	0.269	0.097
TESTING FOR OUTLYING LAB MEANS		
Dixon test	no	no
Nalimov t-test	lab 9	no
Grubbs test (single)	lab 9	no
Grubbs test (double)	-	no
TESTING OF LAB VARIANCES		
Cochran Test (outlying lab variances)	no	no
Bartlett test (homogeneity of lab variances)	no	no
Pooling of data	no	no
NORMALITY OF DISTRIBUTION		
Skewness & kurtosis test	normal	[2]
ANOVA		
Between-lab standard deviation (g/100 g)	0.283	0.084
Within-lab standard deviation (g/100 g)	0.166	0.095
Differences between labs statistically significant?	yes	yes

[1] Kjeldahl-N x 6.38
[2] insufficient data (number of labs < 7)

One laboratory mean value was indicated by Nalimov's t-test as outlier in case of BCR-380R and one laboratory mean was flagged as an outlier by Nalimov's t-test as well as Grubbs' test in case of BCR-685. As no technical reason for the outlying result could be identified the values was retained. Laboratories showing outlying variances were considered of less importance, as all of them still conformed to the performance criteria as set out in the respective IDF/ISO/AOAC standards.

The assumption that the laboratory means were normally distributed could in most cases not be substantiated by testing skewness and kurtosis, as the number of data was insufficient (number of accepted data sets < 7). Therefore, normal probability plots were used instead. As proven in Figures A-7 to A-9 (Annex E) the distribution essentially followed a normal distribution. Consequently, arithmetic mean value and standard deviation were appropriate to describe the location (central tendency) and the dispersion of the data. These features are requirements to adopt the arithmetic mean of the concerned property as the certified value and the standard error of the mean as measure of uncertainty for the characterisation of the property values.

7 Certified values and their uncertainties

The certified values for the properties concerned were calculated as the arithmetic mean of the mean values of the data sets accepted for certification. The uncertainty contributions to the certified value of a CRM can be written as *(3)*:

$$U_{CRM} = k \cdot \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2} \qquad (3)$$

U_{CRM}	=	expanded uncertainty contribution to the certified value of a CRM
k	=	coverage factor (k = 2, to give a level of confidence of 95 %)
u_{char}	=	uncertainty of the certified property of the batch (characterisation)
u_{bb}	=	uncertainty contribution of between-bottle inhomogeneity
u_{lts}	=	uncertainty contribution of long-term stability (storage)
u_{sts}	=	uncertainty contribution of short-term stability (transport)

The individual uncertainty contributions as quantified in previous sections of this report were quadratically added and expanded by a coverage factor of two to give the expanded uncertainty (U_{CRM}) of the certified property value. Uncertainty contributions resulting from short-term stability (u_{sts}) were assumed to be negligible, as the material will be shipped cooled and no degradation is expected to happen during this short time. The estimation of u_{lts} was derived from regression analysis; 60 months was chosen as a suitable shelf-live of the material. It will be extended as soon as additional stability data become available.

The individual uncertainty components, the combined standard uncertainties and the expanded uncertainties for the certified properties are listed in Table 9 for BCR-380R and Table 10 for BCR-685. The certified values are valid until 01/2009. The validity will be extended, if further stability tests do not indicate degradation.

Table 9: Certified values and individual uncertainty contributions for the property values of BCR-380R (values have been rounded in accordance with relevant rounding rules [23])

	Mass fraction (g/100g)			
	Crude protein 1)	**Fat**	**Lactose (anhydrous)**	**Ash**
Certified value	28.66	26.95	37.1	6.00
Combined uncertainty	0.14	0.08	0.5	0.06
Expanded uncertainty (*k* = 2) 2)	0.28	0.16	1.0	0.13
Expanded uncertainty [%] (*k* = 2)	1.0	0.6	2.7	2.1
characterisation [% of combined uncertainty]	24.8	30.6	25.3	19.0
homogeneity [% of combined uncertainty]	18.8	31.7	56.2	55.2
long-term stability [% of combined uncertainty]	56.4	37.7	18.5	25.8

1) Kjeldahl-N x 6.38
2) confidence level 95 %

Table 10: Certified values and individual uncertainty contributions for the property values of BCR-685 (values have been rounded in accordance with relevant rounding rules [23])

	Mass fraction (g/100 g)	
	Crude protein [1)]	**Fat**
Certified value	38.2	0.96
Combined uncertainty	0.19	0.06
Expanded uncertainty (*k*=2) [2)]	0.4	0.12
Expanded uncertainty [%] (*k* = 2)	1.0	12.6
characterisation [% of combined uncertainty]	33.5	40.4
homogeneity [% of combined uncertainty]	36.5	23.0
long-term stability [% of combined uncertainty]	30.0	36.6

1) Kjeldahl-N x 6.38
2) confidence level 95 %

8 Metrological traceability

The certified quantities are method dependent; the measurands are defined by analysis conditions specified in the standardised IDF/ISO and AOAC methods used in the characterisation exercise. All relevant influence quantities of the applied methods have been calibrated using appropriate measurement standards. The values carried by the certified reference materials are traceable to methods standardised by the International Dairy Federation (IDF), the International Organization for Standardization (ISO) and AOAC International.

9 Instructions for use

9.1 Description of material and storage

BCR-380R is supplied in units of 100 g of whole milk powder and BCR-685 in units of 50 g of skim milk powder packaged in amber glass bottles. The samples must be stored unopened at 4 °C.

9.2 Instructions for use and minimum sample intake

The unopened sample shall be equilibrated to room temperature prior to use and the contents of the unopened bottle shall be mixed by inversion and swirling for at least 1 min before sub-sampling.

The minimum sample intake shall conform to requirement as specified in the standardised IDF/ISO/AOAC methods.

9.3 Intended use

BCR-380R and BCR-685 are intended as tools for checking the performance of the same standardised IDF/ISO/AOAC methods, which have been used in the characterisation of the materials, in the laboratories of users of these materials. For that purpose users

- shall demonstrate that the repeatability standard deviation of the applied methods (s_r) conforms to the requirements as set forth in the respective IDF/ISO/AOAC standards
- take the reproducibility standard deviation of the respective IDF/ISO/AOAC standard (s_R) as an estimation of the measurement uncertainty of the applied methods (u_{meas}).
- take the combined uncertainty of the certified property value (u_{CRM})
- combine u_{meas} and u_{CRM} to obtain $u_c = \sqrt{u_{meas}^2 + u_{CRM}^2}$

The measurement is consider to be unbiased if the difference between the arithmetic mean of a suitable number of replicates ($n \geq 5$) obtained on the CRM using the respective IDF/ISO/AOAC method and the certified value is less than 2 x u_c, which corresponds to a confidence level of approximately 95 %.

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ANNEX A – Homogeneity data

Table A-1: Results of the homogeneity study for the content of crude protein (Kjeldahl-N x 6.38), fat, lactose, ash and dry matter in BCR-380R (whole milk powder); duplicate analyses were carried out for each property; results are given in g/100 g

Bottle no.	Crude protein		Fat		Lactose		Ash		Dry matter	
70	27.96	27.97	26.37	26.39	36.34	36.58	5.99	5.90	98.13	98.16
140	27.99	27.94	26.38	26.52	36.52	36.61	5.92	5.97	98.18	98.13
210	28.03	28.00	26.46	26.49	36.18	36.05	5.91	5.85	98.11	98.20
280	27.98	27.97	26.36	26.45	36.13	36.53	5.94	5.92	98.14	98.13
350	28.03	27.98	26.54	26.58	36.62	36.45	5.93	5.93	97.94	97.98
420	28.02	28.04	26.57	26.45	37.10	36.77	5.91	5.91	98.04	98.01
490	28.04	27.99	26.44	26.5	36.58	36.54	5.93	5.91	97.95	97.98
560	28.05	28.06	26.47	26.32	37.41	37.49	6.02	6.06	98.11	98.02
630	27.99	28.12	26.43	26.36	36.51	36.55	6.03	5.98	98.08	98.08
700	27.98	28.03	26.52	26.35	36.20	36.5	6.12	6.01	98.09	98.08
770	28.01	28.05	26.44	26.43	36.57	36.18	5.87	5.93	98.05	98.04
840	28.03	28.21	26.29	26.42	37.34	37.32	5.92	5.96	98.1	98.15
910	27.99	28.05	26.45	26.38	36.63	36.66	5.98	6.04	98.07	98.13
980	28.13	28.10	26.52	26.55	35.49	35.48	6.00	6.02	98.05	98.05
1050	27.95	28.23	26.52	26.43	36.46	36.22	6.07	6.04	98.02	98.04
1120	28.07	28.08	26.48	26.44	35.75	36.55	5.93	5.94	98.19	98.15
1190	28.09	28.05	26.62	26.43	36.52	36.47	5.95	5.93	98.06	98.14
1260	28.06	28.00	26.47	26.34	36.69	36.61	5.99	6.07	97.98	97.97
1330	28.11	28.26	26.43	26.41	36.78	36.67	6.07	5.99	98.04	98.08
1400	28.04	28.03	26.39	26.29	36.63	36.84	6.05	6.06	98.04	97.99

Table A-2: Results of the homogeneity study for the content of crude protein (Kjeldahl-N x 6.38) and fat in BCR-685 (skim milk powder); duplicate analyses were carried out for each property; results are given in g/100 g

Bottle no.	Crude protein		Bottle no.	Fat	
70	37.24	37.17	**17**	0.76	0.82
140	36.94	36.98	**120**	0.81	0.75
210	37.18	37.20	**196**	0.77	0.77
280	37.22	37.12	**276**	0.75	0.78
350	37.14	37.07	**321**	0.74	0.75
420	37.12	37.06	**419**	0.75	0.77
490	37.09	37.08	**504**	0.75	0.74
560	37.22	37.07	**640**	0.75	0.79
630	37.09	37.14	**711**	0.79	0.88
700	37.08	37.11	**746**	0.82	0.82
770	37.02	37.07	**823**	0.82	0.86
840	37.06	37.10	**836**	0.81	0.85
910	37.04	36.99	**900**	0.78	0.74
980	37.10	37.25	**956**	0.74	0.80
1050	36.96	36.91	**1054**	0.79	0.80
1120	36.87	36.97	**1130**	0.79	0.76
1190	37.34	37.34	**1245**	0.79	0.77
1260	37.23	37.30	**1325**	0.78	0.79
1330	37.33	37.35	**1405**	0.75	0.77
1400	37.34	37.31	**1451**	0.77	0.75

ANNEX B – Short-term stability data

Table A-3: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of crude protein (Kjeldahl-N x 6.38) in BCR-380R (whole milk powder)

Storage time	Storage temperature			
(weeks)	40 ºC	18 ºC	4 ºC	-20 ºC
0	28.82	28.82	28.82	28.82
0	28.69	28.69	28.69	28.69
0	28.78	28.78	28.78	28.78
0	28.71	28.71	28.71	28.71
0	28.76	28.76	28.76	28.76
1	28.65	28.79	28.71	28.76
1	28.76	28.58	28.69	28.63
1	28.76	28.72	28.66	28.70
1	28.69	28.68	28.70	28.74
1	28.77	28.76	28.58	28.79
2	28.71	29.22	28.62	28.67
2	28.65	28.71	28.64	28.64
2	28.75	28.71	28.70	28.64
2	28.70	28.63	28.67	28.61
2	28.77	28.88	28.69	28.69
4	28.74	28.73	28.58	28.77
4	28.68	28.66	28.67	28.76
4	28.72	28.62	28.64	28.74
4	28.62	28.64	28.62	28.77
4	28.61	28.77	28.63	28.58
Slope	**-0.02**	**-0.01**	**-0.03**	**-0.01**
P-value	**0.03**	**0.60**	**0.00**	**0.48**

Table A-4: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of lactose in BCR-380R (whole milk powder)

Storage time (weeks)	Storage temperature			
	40 ºC	**18 ºC**	**4 ºC**	**-20 ºC**
0	35.63	35.63	35.63	35.63
0	36.76	36.76	36.76	36.76
0	34.99	34.99	34.99	34.99
0	36.23	36.23	36.23	36.23
0	36.20	36.20	36.20	36.20
1	35.73	35.12	37.23	35.77
1	36.30	37.56	36.78	35.82
1	36.10	37.84	37.21	36.83
1	37.07	36.39	36.27	36.85
1	35.33	36.46	35.12	36.85
2	35.43	36.60	38.11	37.36
2	34.48	35.89	36.83	36.02
2	35.50	34.06	37.28	36.92
2	36.15	34.28	36.59	35.67
2	36.71	35.49	36.29	35.66
4	36.77	36.75	35.70	36.82
4	36.50	35.48	34.77	36.61
4	35.11	36.08	37.26	37.26
4	34.70	37.21	35.82	35.26
4	37.20	36.35	36.88	36.58
Slope	**0.003**	**0.025**	**0.014**	**0.111**
P-value	**0.98**	**0.87**	**0.92**	**0.29**

Table A-5: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of fat in BCR-380R (whole milk powder)

Storage time	Storage temperature			
(weeks)	40 ºC	18 ºC	4 ºC	-20 ºC
0	27.09	27.09	27.09	27.09
0	26.87	26.87	26.87	26.87
0	27.11	27.11	27.11	27.11
0	26.90	26.90	26.90	26.90
0	27.03	27.03	27.03	27.03
1	26.83	27.07	26.97	26.98
1	27.01	26.87	26.87	26.96
1	26.97	27.05	26.92	26.93
1	26.82	26.87	26.92	27.13
1	27.02	26.94	26.92	27.08
2	26.94	26.84	26.82	26.87
2	26.89	26.98	26.80	26.99
2	26.92	27.02	27.01	26.87
2	27.09	26.96	26.84	26.93
2	26.95	27.08	26.87	26.97
4	26.94	26.88	26.92	27.02
4	26.76	26.84	27.02	26.84
4	27.06	26.94	26.89	26.92
4	26.97	27.04	26.96	26.99
4	26.90	27.06	26.97	26.91
Slope	**-0.014**	**-0.010**	**0.013**	**-0.020**
P-value	**0.33**	**0.50**	**0.46**	**0.13**

Table A-6: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of ash in BCR-380R (whole milk powder).

Storage time (weeks)	Storage temperature			
	40 ºC	**18 ºC**	**4 ºC**	**-20 ºC**
0	5.95	5.95	5.95	5.95
0	6.11	6.11	6.11	6.11
0	6.02	6.02	6.02	6.02
0	5.97	5.97	5.97	5.97
0	5.96	5.96	5.96	5.96
1	6.01	5.87	5.93	6.05
1	5.87	6.03	5.93	5.95
1	6.01	6.06	6.02	6.07
1	5.96	5.94	6.05	5.85
1	5.99	nd	5.95	6.05
2	6.13	6.16	5.97	5.97
2	6.03	6.12	6.01	6.09
2	6.06	6.09	6.09	6.11
2	5.92	5.93	5.98	6.13
2	6.02	5.89	5.90	6.06
4	5.96	5.93	6.02	6.00
4	6.13	6.05	6.00	5.96
4	6.09	5.95	5.89	6.13
4	5.98	5.89	6.01	6.02
4	nd	5.90	6.05	nd
Slope	**0.014**	**-0.012**	**0.000**	**0.010**
P-value	**0.24**	**0.38**	**0.99**	**0.44**

nd = not determined

Table A-7: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of crude protein (Kjeldahl-N x 6.38) in BCR-685 (skim milk powder)

Storage time (weeks)	Storage temperature 40 ºC	18 ºC	4 ºC	-20 ºC
0	37.41	37.41	37.41	37.41
0	37.53	37.53	37.53	37.53
0	37.37	37.37	37.37	37.37
0	37.50	37.50	37.50	37.50
0	37.44	37.44	37.44	37.44
1	37.51	37.43	37.37	37.40
1	37.47	37.42	37.46	37.37
1	37.39	37.31	37.49	37.35
1	37.44	37.46	37.46	37.41
1	37.47	37.52	37.48	37.46
2	37.39	37.43	37.42	37.41
2	37.46	37.38	37.53	37.44
2	37.40	37.42	37.42	37.54
2	37.35	37.34	37.59	37.41
2	37.36	37.45	37.30	37.41
4	37.44	37.39	37.31	37.30
4	37.36	37.48	37.41	37.38
4	37.34	37.55	37.47	37.50
4	37.24	37.47	37.44	37.48
4	37.45	37.27	37.41	37.42
Slope	**-0.024**	**-0.004**	**-0.011**	**-0.005**
P-value	**0.02**	**0.72**	**0.33**	**0.64**

Table A-8: Results (g/100 g, dry mass corrected) of the short-term stability study for the content of fat in BCR-685 (skim milk powder)

Storage time (weeks)	Storage temperature 40 ºC	18 ºC	4 ºC	-20 ºC
0	0.85	0.85	0.85	0.85
0	0.82	0.82	0.82	0.82
0	0.79	0.79	0.79	0.79
0	0.82	0.82	0.82	0.82
0	0.86	0.86	0.86	0.86
1	0.85	0.83	0.86	0.76
1	0.88	0.84	0.87	0.86
1	0.87	0.86	0.90	0.87
1	0.88	0.87	0.89	0.79
1	0.87	0.85	0.81	0.77
2	0.84	0.83	0.85	0.78
2	0.86	0.83	0.88	0.81
2	0.83	0.80	0.87	0.84
2	0.86	0.81	0.80	0.84
2	0.82	0.86	0.89	0.86
4	0.77	0.88	0.78	0.83
4	0.84	0.76	0.82	0.81
4	0.83	0.88	0.88	0.85
4	0.81	0.82	0.85	0.77
4	0.80	0.88	0.86	0.76
Slope	**-0.008**	**0.002**	**0.000**	**-0.005**
P-value	**0.09**	**0.67**	**0.98**	**0.41**

ANNEX C – Long-term stability data

Table A-9: Results (g/100 g, dry mass corrected) of the long-term stability study at 4 °C for the content of fat in BCR-380R (whole milk powder)

Storage time (months)	Crude protein	Fat	Lactose	Ash
0	28.62	26.90	37.45	6.05
0	28.53	26.89	37.18	6.06
0	28.62	26.88	37.47	6.08
0	28.57	27.01	37.36	6.03
0	28.59	27.07	37.58	6.03
0	28.53	27.06	37.68	6.01
0	28.46	26.97	36.79	6.01
0	28.60	27.06	36.97	6.00
0	28.58	26.93	37.01	6.05
12	28.69	26.94	37.50	6.05
12	28.65	27.17	37.37	5.92
12	28.26	26.95	37.24	6.08
12	28.74	26.94	36.84	6.05
12	28.80	26.88	37.33	6.06
12	28.73	26.86	37.26	6.11
24	28.76	26.89	37.29	6.07
24	27.94	26.98	37.39	6.00
24	28.78	27.01	37.34	6.03
24	28.78	26.80	37.30	6.06
24	29.03	26.95	37.33	6.12
24	28.80	26.83	37.30	5.97
50	28.51	27.01	37.54	6.00
50	28.56	26.99	37.38	6.06
50	28.65	27.03	37.61	6.04
50	28.61	27.06	37.35	6.01
50	28.62	27.04	37.10	6.05
50	28.50	27.03	37.21	6.03

Table A-10: Results (g/100 g, dry mass corrected) of the long-term stability study at 4 °C for the content of fat in BCR-685 (skim milk powder)

Storage time (months)	Crude protein	Fat
0	38.20	0.83
0	38.26	0.82
0	38.25	0.94
0	38.08	0.79
0	38.03	0.77
0	37.74	0.87
0	38.03	0.73
0	37.90	0.81
0	38.02	0.78
12	38.23	0.86
12	38.18	0.88
12	38.20	0.89
12	38.22	0.83
12	38.18	0.84
12	37.94	0.89
24	38.26	0.83
24	38.27	0.87
24	38.26	0.84
24	37.89	0.86
24	38.33	0.85
24	38.29	0.82
50	38.11	0.82
50	38.04	0.74
50	37.80	0.76
50	38.11	0.70
50	38.30	0.85
50	38.14	0.90

ANNEX D – Characterisation data

Table A-11: Results (g/100 g) of the property characterisation study for BCR-380R (whole milk powder)

Lab no.	Bottle no.	Crude protein *)		Fat		Lactose (IDF 79:2002)		Lactose (HPLC)		Ash		Dry matter	
1	160	27.50	27.51	26.19	26.18			35.80	35.59	5.72	5.74	97.10	97.10
	266	27.77	27.68	26.45	26.45			38.00	38.25	5.78	5.75	97.18	97.21
	1114	27.79	27.70	26.40	26.45			37.99	37.27	5.78	5.78	97.25	97.34
2	425					36.74	35.60			5.88	5.89	95.71	95.72
	743					35.65	35.02			5.76	5.75	96.84	96.86
	1325					35.09	34.87			5.90	5.89	97.35	97.38
3	796	27.88	27.82	26.23	26.13								
	1167	27.50	27.50	26.19	26.20								
	1220	27.56	27.63	26.13	26.12								
4	319	27.91	27.94	26.22	26.38	36.14	36.06	35.80	35.87	5.83	5.87	97.50	97.46
	637	27.96	27.92	26.32	26.35	36.50	36.40	35.71	35.70	5.85	5.88	97.44	97.48
	1008	27.85	27.80	26.14	26.19	36.13	36.12	35.44	35.51	5.81	5.87	97.01	97.02
5	372	27.94	27.88	26.16	26.03	35.66	35.08	35.35	35.28	5.76	5.72	96.85	96.93
	690	27.94	27.88	26.28	26.21	35.72	36.24	35.46	35.46	5.82	5.85	97.05	97.18
	902	27.94	28.01	26.28	26.23	36.06	36.27	35.52	35.52	5.75	5.86	97.44	97.45
6	3	27.88	27.88	25.93	25.95	36.44	33.92			5.79	5.78	97.09	97.00
	54	27.63	27.56	25.83	25.77	35.84	35.34			5.74	5.73	96.16	96.15
	213	27.82	27.88	26.22	26.21	34.46	34.43			5.84	5.84	97.64	97.55
7	533	27.69	27.50	26.23	26.17	35.41	35.80	35.22	35.33	5.85	5.79	97.24	97.26
	955	27.82	27.82	26.08	26.02	37.14	37.87	36.69	37.08	5.84	5.82	96.83	96.80
	1061	27.82	27.56	26.04	26.05	36.94	37.25	36.41	34.48	5.81	5.88	96.69	96.66
8	107	28.07	28.07	25.84	25.70	35.58	35.25	36.36	35.41	5.80	5.80	97.25	97.28
	584	28.14	28.14	26.34	26.43			36.03	36.37	5.87	5.88	97.66	97.67
	849	28.07	28.01	26.40	26.40	38.76	38.18	36.27	35.23	5.87	5.86	97.33	97.30
9	478	27.61	27.53	25.33	25.29	36.31	36.43			5.89	5.87	97.03	97.06
	1273	25.30	24.67	24.26	24.42	36.84	37.66			5.90	5.94	96.86	96.89
	1379	26.22	26.25	25.66	25.28	34.90	35.80			5.93	5.95	96.86	97.03

*) Kjeldahl-N x 6.38

Table A-12: Results (g/100 g) of the property characterisation study for BCR-685 (skim milk powder)

Lab no.	Bottle no.	Crude protein *)		Fat		Dry matter	
1	3	36.77	36.93	0.90	0.88	96.97	96.97
	435	36.51	36.65	1.17	1.21	96.69	96.69
	543	36.97	36.86	1.08	1.07	96.74	96.68
3	705	36.69	36.43	0.87	0.89		
	867	37.13	37.07	0.85	0.85		
	1299	36.75	36.88	0.84	0.86		
4	219	37.04	37.03	0.86	0.79	96.89	96.84
	651	37.11	37.06	0.96	0.82	97.10	97.09
	1081	36.95	36.91	0.90	0.90	96.66	96.57
5	327	37.13	37.13	0.82	0.85	97.06	96.98
	921	37.07	37.26	0.80	0.83	97.01	97.00
	1407	37.20	37.20	0.83	0.81	97.02	96.98
6	381	37.51	37.51	0.85	0.85	97.11	97.22
	489	36.88	36.94	0.90	0.89	96.93	96.83
	597	36.94	37.00	0.84	0.84	96.97	96.90
7	1029	36.94	37.20	0.48	0.51	97.22	97.24
	1137	37.07	37.13	0.49	0.50	96.70	96.74
	1353	37.45	37.39	0.87	0.94	97.02	97.05
8	57	37.39	37.39	0.81	0.81	96.92	96.90
	166	37.39	37.39	1.08	1.10	97.32	97.32
	273	37.32	37.45	1.10	1.04	97.23	97.21
9	111	36.46	36.25	1.04	1.06	96.80	96.87
	759	36.63	36.16	0.89	0.84	96.83	97.01
	1244	36.70	36.65	1.02	0.98	97.18	97.19

*) Kjeldahl-N x 6.38

ANNEX E – Characterisation data (graphs)

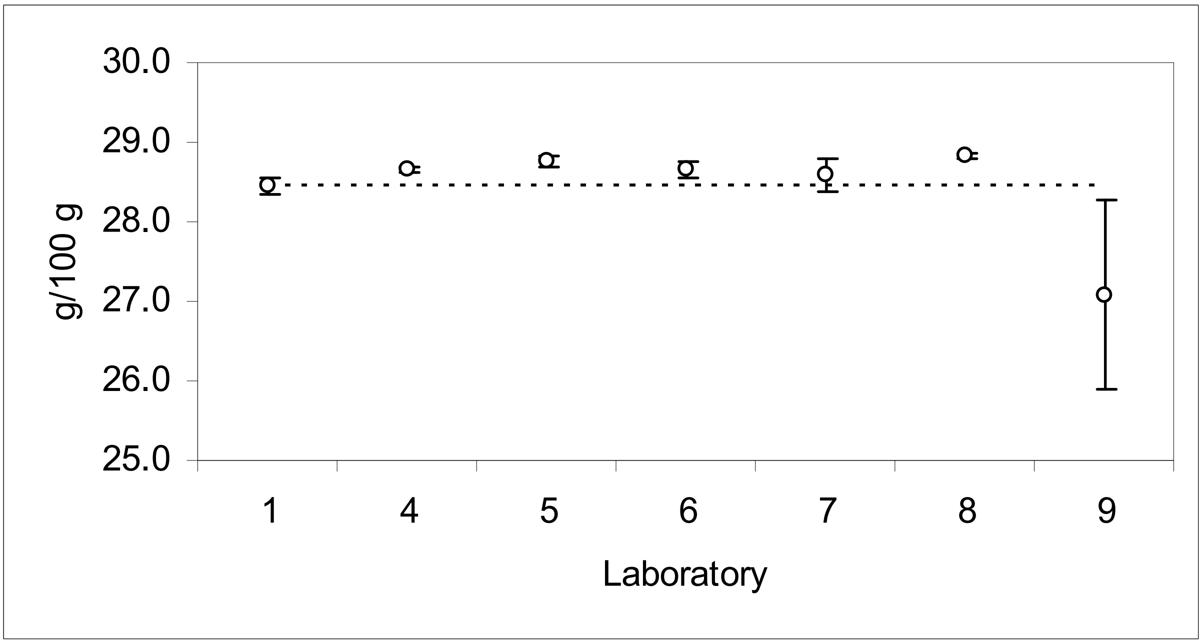

Figure A-1: Laboratory mean values ± standard deviation for the crude protein content (Kjeldahl-N x 6.38; dry mass corrected)) of BCR-380R (dashed line, mean of laboratory means = 28.43 g/100 g)

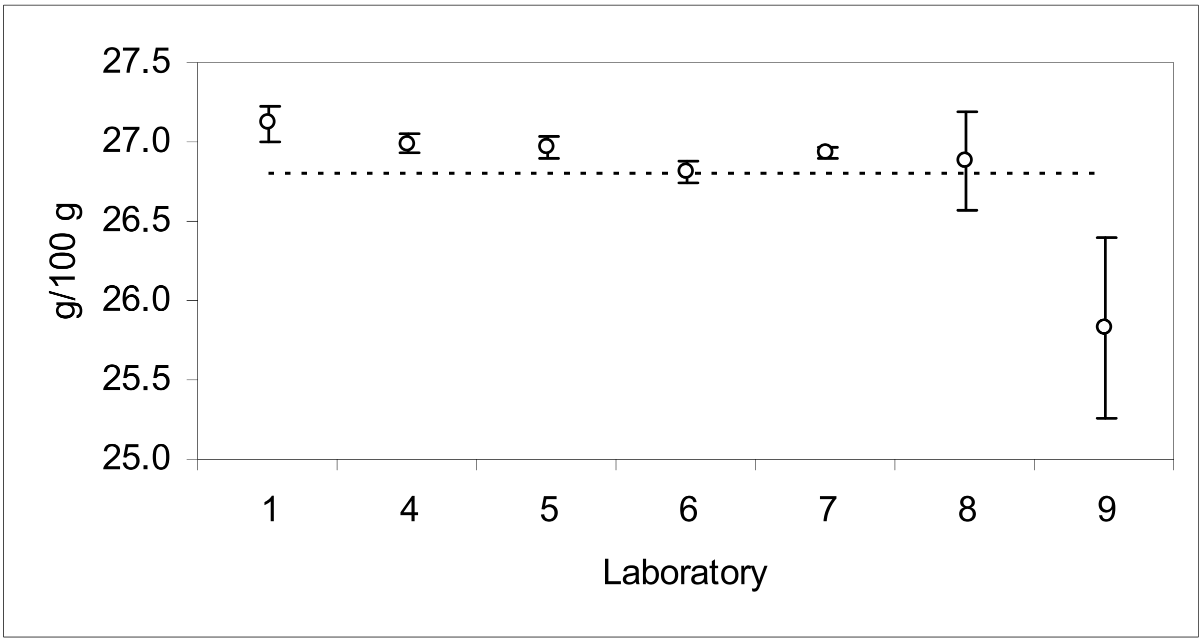

Figure A-2: Laboratory mean values ± standard deviation for the fat content (dry mass corrected) of BCR-380R (dashed line, mean of laboratory means = 26.79 g/100 g)

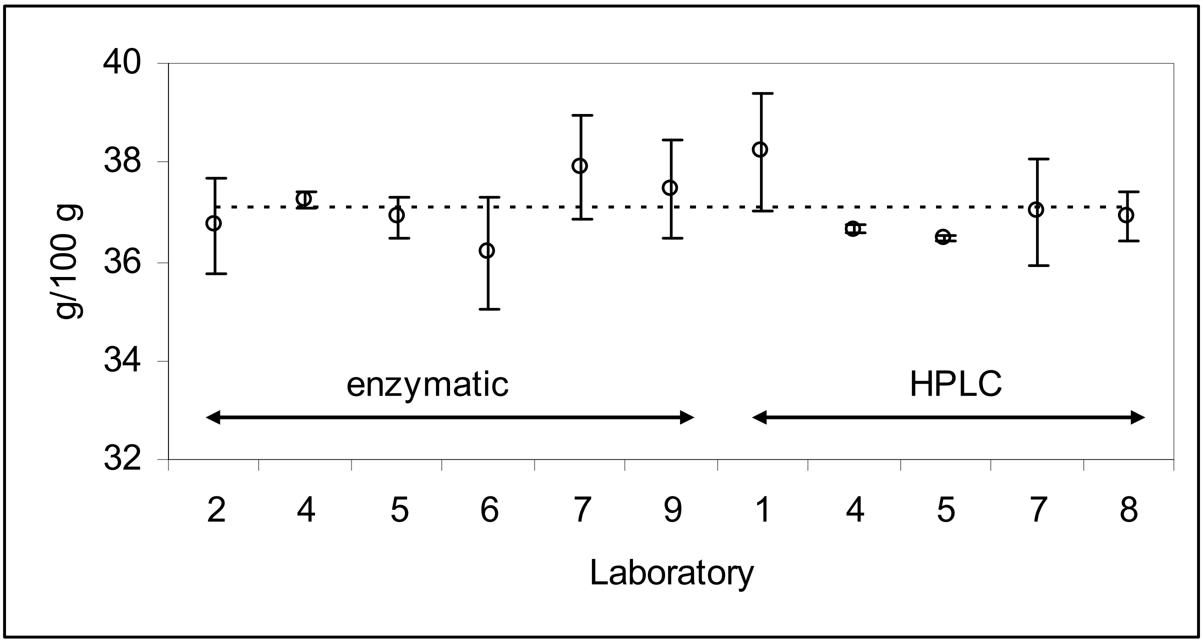

Figure A-3: Laboratory mean values ± standard deviation for the lactose (anhydrous) content (dry mass corrected) of BCR-380R (dashed line, mean of laboratory means = 37.06 g/100 g)

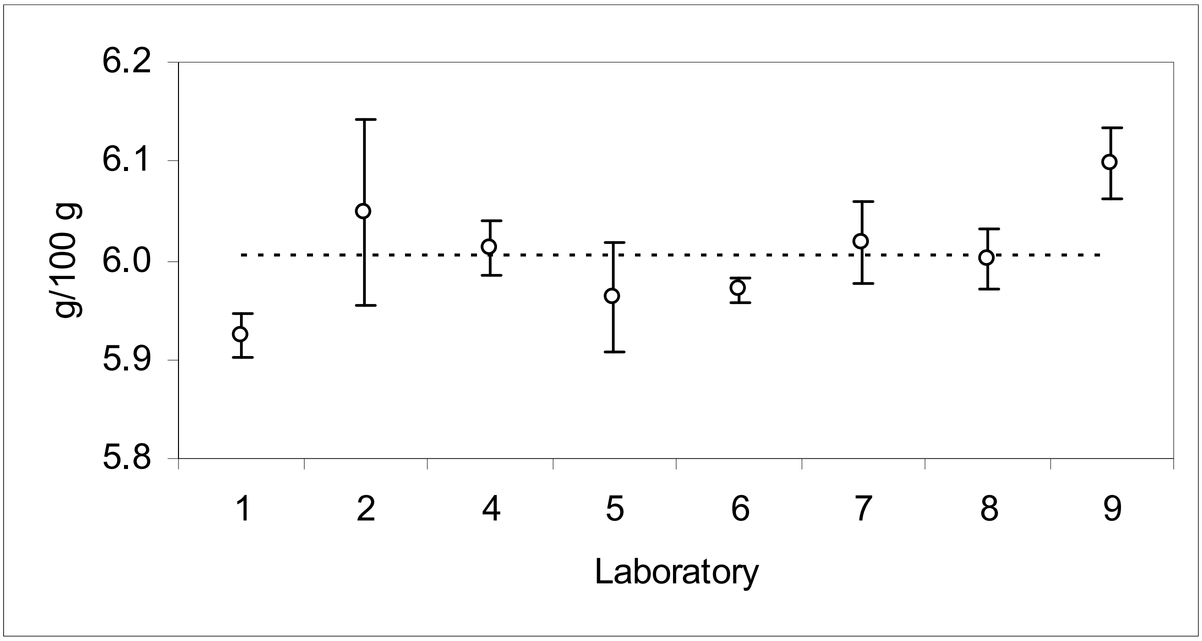

Figure A-4: Laboratory mean values ± standard deviation for the ash content (dry mass corrected) of BCR-380R (dashed line, mean of laboratory means = 6.00 g/100 g)

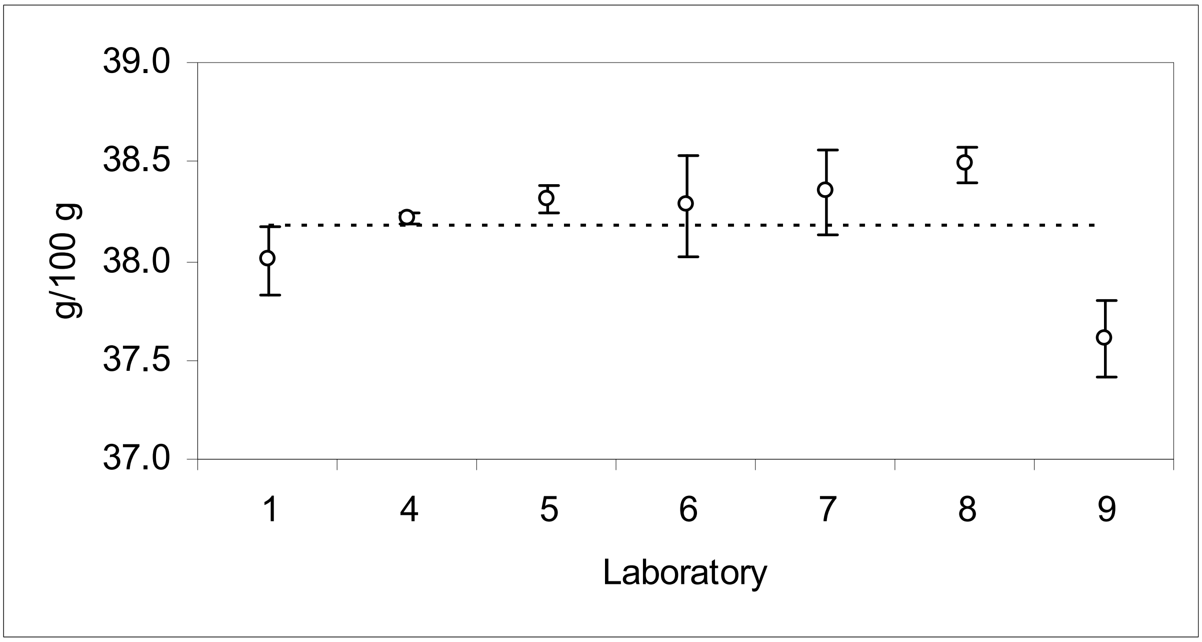

Figure A-5: Laboratory mean values ± standard deviation for the crude protein content (Kjeldahl-N x 6.38; dry mass corrected) of BCR-685 (dashed line, mean of laboratory means = 38.18 g/100 g)

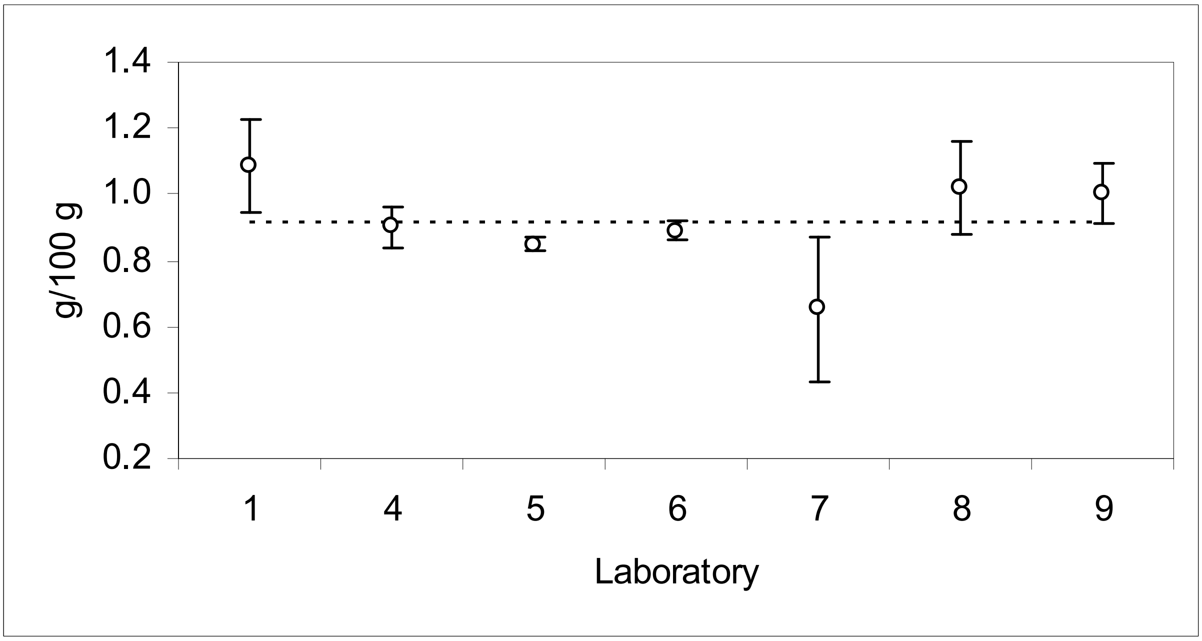

Figure A-6: Laboratory mean values ± standard deviation for the fat content (dry mass corrected) of BCR-685 (dashed line, mean of laboratory means = 0.91 g/100 g).

ANNEX F – Characterisation data (normal probability plots)

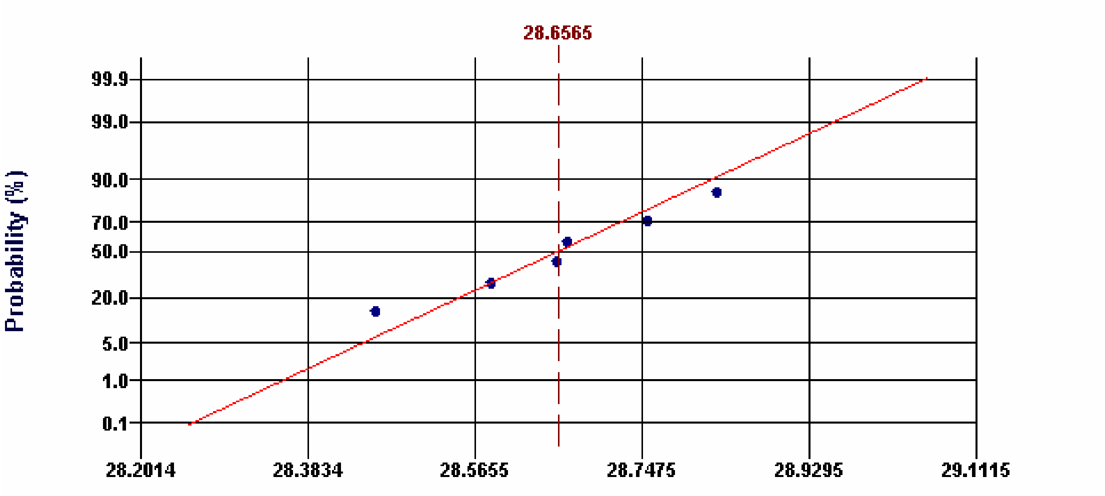

Figure A-7: Normal probability plot for the crude protein content (Kjeldahl-N x 6.38; dry mass corrected) mean values reported by the retained laboratories for BCR-380R

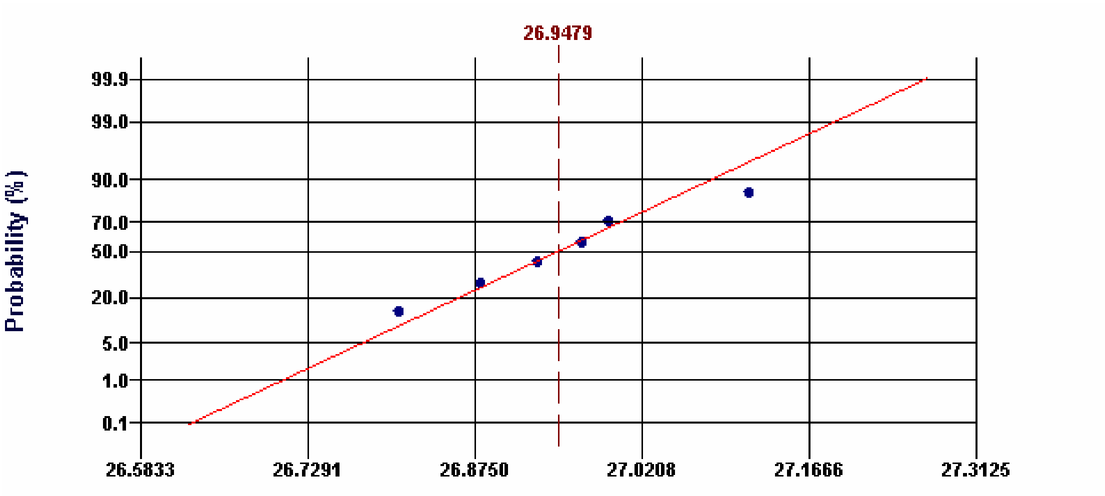

Figure A-8: Normal probability plot for the fat content (dry mass corrected) mean values reported by the retained laboratories for BCR-380R

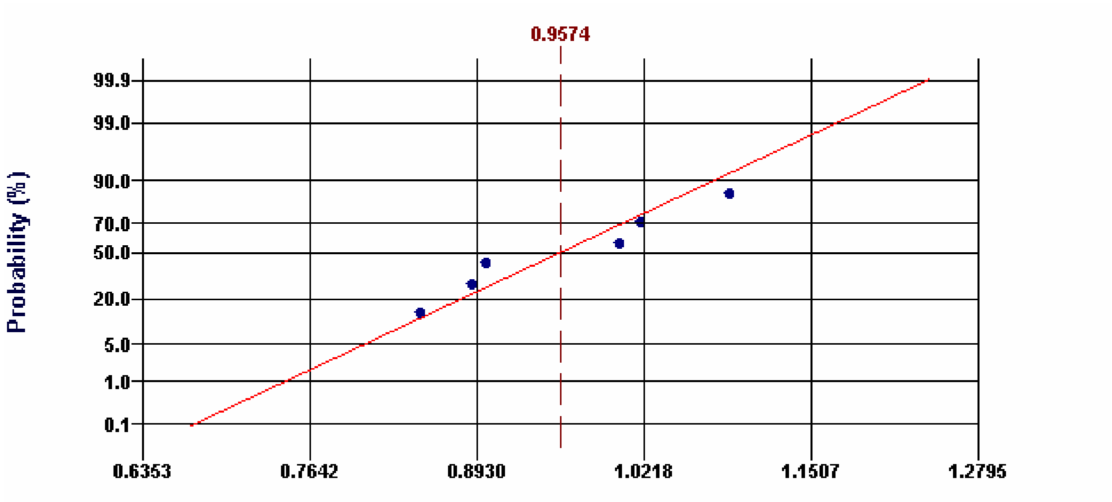

Figure A-9: Normal probability plot for the fat content (dry mass corrected) mean values reported by the retained laboratories for BCR-685.

European Commission

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Abstract

This report describes the preparation of two milk powder reference materials and the measurement exercises that led to the certification of the content (mass fraction) of the crude protein (Kjeldahl-N x 6.38), fat, lactose and ash in whole milk powder (BCR-380R) and crude protein (Kjeldahl-N x 6.38) and fat in skim milk powder (BCR-685).

The certified values are presented in Tables I and II. The results of the certification exercise, which involved material characterisation by a collaborative study involving several experienced European laboratories, are presented and discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1]. The stated uncertainties include contributions regarding the results of the characterisation measurements and uncertainties due to potential inhomogeneity and potential instability of the materials. The values carried by the certified reference materials are traceable to methods standardised by the International Dairy Federation (IDF), the International Organization for Standardization (ISO) and AOAC International.

Table I: Certified mass fraction of main components in whole milk powder – BCR-380R

Parameters	Mass fraction in g/100 g [1)]		Relative uncertainty [3)] in %	No. of accepted sets of results
	Certified value[2)]	Uncertainty [3)]		
Crude protein (Kjeldahl-N x 6.38)	28.66	0.28	1.0	6
Fat	26.95	0.16	0.6	6
Lactose (anhydrous)	37.1	1.0	2.7	11
Ash	6.00	0.13	2.1	8

1) Results corrected for dry mass.

2) Unweighted mean value of the means of accepted sets of results, each set being obtained in a different laboratory applying relevant methods of analysis standardised by IDF/ISO and AOAC The certified values are traceable to the SI.

3) Expanded uncertainty with a coverage factor of k = 2, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

Table II: Certified mass fraction of main components in skim milk powder BCR-685

Parameters	Mass fraction in g/100 g [1)]		Relative uncertainty [3)] in %	No. of accepted sets of results
	Certified value[2)]	Uncertainty [3)]		
Crude protein (Kjeldahl-N x 6.38)	38.2	0.4	1.0	7
Fat	0.96	0.12	12.6	6

1) Results corrected for dry mass.

2) Unweighted mean value of the means of accepted sets of results, each set being obtained in a different laboratory applying relevant methods of analysis standardised by IDF/ISO and AOAC The certified values are traceable to the SI.

3) Expanded uncertainty with a coverage factor of k = 2, according to the Guide to the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.

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