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CERTIFICATION REPORT

The Certification of the Mass Fractions of Proximates and Essential Elements in Rye Flour and Wheat Flour

Certified Reference Materials ERM®-BC381 and ERM®-BC382

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The Certification of the Mass Fractions of Proximates and Essential Elements in Rye Flour and Wheat Flour

Certified Reference Materials ERM®-BC381 and ERM®-BC382

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Disclaimer

Summary

This report describes the preparation of the rye flour and wheat flour matrix reference materials ERM®-BC381 and ERM®-BC382, respectively, and the certification of the contents (mass fractions) of four proximates and four essential elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the materials, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation. The certified values and their uncertainties are listed in Tables I and II:

Table I: Certified mass fractions of proximates and essential elements and their uncertainties in rye flour (ERM®-BC381)

Proximates and essential elements	Certified value 1)	Uncertainty 2)	Number of accepted sets of results
Kjeldahl nitrogen 3)	1.562 g/100 g	0.014 g/100 g	10
Total fat 4)	1.36 g/100 g	0.16 g/100 g	11
Ash 5)	1.08 g/100 g	0.11 g/100 g	10
Starch 6)	72.2 g/100 g	1.9 g/100 g	7
K	3.35 mg/g	0.11 mg/g	11
Mg	0.567 mg/g	0.013 mg/g	10
Ca	0.32 mg/g	0.04 mg/g	9
P	2.01 mg/g	0.07 mg/g	11

1) These values are related to dry mass and are based on the unweighted mean of accepted results
2) The uncertainties are the expanded uncertainties (k = 2) of the certified values
3) Protein can be derived by multiplying Kjeldahl nitrogen with an appropriate factor (e.g. see ISO 20483 [2])
4) Total fat determined after acid hydrolysis, solvent extraction and subsequent gravimetry
5) Ashing at 550 °C ± 25 °C
6) Starch determined by polarimetric method

Table II: Certified mass fractions of proximates and essential elements and their uncertainties in wheat flour (ERM®-BC382)

Proximates and essential elements	Certified value 1)	Uncertainty 2)	Number of accepted sets of results
Kjeldahl nitrogen 3)	1.851 g/100 g	0.017 g/100 g	10
Total fat 4)	1.39 g/100 g	0.17 g/100 g	11
Ash 5)	0.60 g/100 g	0.10 g/100 g	10
Starch 6)	81.2 g/100 g	1.7 g/100 g	7
K	1.88 mg/g	0.08 mg/g	11
Mg	0.247 mg/g	0.010 mg/g	10
Ca	0.210 mg/g	0.018 mg/g	9
P	1.19 mg/g	0.07 mg/g	11

1) These values are related to dry mass and are based on the unweighted mean of accepted results
2) The uncertainties are the expanded uncertainties (k = 2) of the certified values
3) Protein can be derived by multiplying Kjeldahl nitrogen with an appropriate factor (e.g. see ISO 20483 [2])
4) Total fat determined after acid hydrolysis, solvent extraction and subsequent gravimetry
5) Ashing at 550 °C ± 25 °C
6) Starch determined by polarimetric method

The assigned values and their uncertainties are based on minimum sample intakes varying from 7.5 g for starch, 2 g each for dry mass, total fat and ash, 1 g each for potassium, magnesium, calcium and phosphorus and 0.5 g for Kjeldahl nitrogen.

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Glossary

α	confidence level
AAS	atomic absorption spectrometry
ANOVA	analysis of variance
b	slope of linear regression
CRM	certified reference material
d_f	degree of freedom (regression)
DM	dry mass
ERM®	European Reference Material®
GUM	Guide to the Expression of Uncertainty in Measurement
ICP	inductively coupled plasma
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
JRC	European Commission's Joint Research Centre
KFT	Karl Fischer titration
LOQ	limit of quantification
$MS_{between}$	mean of squares between groups (ANOVA)
MSI	minimum sample intake
MS_{within}	mean of squares within groups (ANOVA)
n	number of replicates
n.c.	not calculable
n.d.	not determined
n.r.	not reported
OES	optical emission spectrometry
p	level of significance
PSA	particle size analysis
RSD	relative standard deviation
RSD_{stab}	relative standard deviation of all results of stability study
s	standard deviation
s_{bb}	between-bottle heterogeneity standard deviation
s_{wb}	within-bottle heterogeneity standard deviation
se_b	standard error of slope b of linear regression
SI	International Systems of Units
u_{bb}	relative standard uncertainty due to between-bottle heterogeneity
u^*_{bb}	relative standard uncertainty due to heterogeneity that can be hidden by method repeatability
u_{char}	relative standard uncertainty of characterisation exercise
u_{CRM}	combined standard uncertainty of certified value
$u_{CRM, rel}$	combined relative standard uncertainty of certified value
U_{CRM}	expanded uncertainty of certified value
$U_{CRM, rel}$	expanded relative uncertainty of certified value
u_{lts}	relative standard uncertainty of long-term stability
u_{meas}	standard uncertainty of measurement result
u_{sts}	relative standard uncertainty of short-term stability
u_Δ	combined standard uncertainty of certified value and measured value
U_Δ	expanded uncertainty of certified value and measured value
t_{sl}	pre-defined shelf life
x_i	result at time point i in an isochronous stability study
$\overline{x}$	average result of all time points in an isochronous stability study
$\overline{y}$	average of all results of a homogeneity study
Δ	difference between two measurement results
Δ_m	difference between measured and certified value
$\nu_{MSwithin}$	degrees of freedom (ANOVA)

1 Introduction

1.1 Background

This report describes the development of two flour reference materials, which will replace the exhausted CRMs BCR-381 (rye flour) and BCR-382 (wheat flour).

Knowledge of the nutritional content of foods is necessary to study the relation between diet and health, for planning of diets, in official food control, for food labelling purposes and for the manufacture of food products.

There is a growing awareness that dietary factors are important in the development of certain diseases, for example, the relationship between high fat consumption and heart disease in western societies. Many countries have therefore 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 establish a balanced diet.

The food industry relies on quality control programmes involving the measurement of components in food products. Nutrition research and counselling rely heavily on analytical data for the component content of foods. This information is compiled in national food tables and component databases, frequently supported by governmental surveillance programmes to determine whether recommended dietary intakes are met within the population or segments of the European Community.

The importance of reliable consumer information in the Community is reflected in the issuing of Directive 90/496 on nutrition labelling for foodstuffs [3] amended by Regulation 1882/2003 [4] and Directive 2003/120 [5] as well as by Directive 2000/13 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs [6]. Official Food Control laboratories are charged with verification that the information provided is correct. Thus, chemical analyses of components in foods form the basis of much of the science and practice of nutrition and dietetics and is required for enforcement of Community legislation.

Similar to the exhausted CRMs, it was intended to certify the mass fractions of Kjeldahl nitrogen, total fat, ash, starch, dietary fibre, potassium, sodium, magnesium, calcium, chlorine and phosphorus. The two flour reference materials will provide a basis for quality control of the measurements of the nutrient components most commonly measured in flours.

1.2 Expression of results

The results for the major components are expressed in g/100 g to be consistent with Directive 90/496 [3] on food labelling and food composition tables.

Throughout this report, results are expressed as a mass fraction on a dry mass basis. For practical purposes, the dry mass is established by determining the "loss of mass on drying" under carefully defined conditions (see also Sections 6.1 and 9.2). It should be noted that determination of the dry mass correction factor under conditions other than specified in this report might lead to results, which are incompatible with the certified values.

2 Participants

Project management and evaluation

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(Work performed under ISO Guide 34 accreditation; BELAC No. 268-TEST)

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(Measurements performed under ISO/IEC 17025 accreditation; DAP-PL-2566.99)

3 Processing of the material

3.1 Material selection

As it is difficult to find finely ground rye flour on the market, whole rye flour was purchased from Joosen-Luyckx N.V., (Turnhout, BE) in three 25 kg paper sacks (in total 75 kg). The wheat flour "Type 405" was purchased in 1 kg paper bags at a local grocery store in Mol, BE. It was produced in Gent for Lidl Benelux and had a nominal water content of maximum 15.5 % as stated on the package. In total 75 kg of wheat flour were purchased, whereof 65 kg were used for preparation of the final material. Both materials were stored at 18 °C prior to processing.

One important objective was to obtain final products with similar water contents as the former materials (BCR-381 and BCR-382). At IRMM, generally, powder materials are dried to a water content of approximately 3 %, but for flour, this would create an over-dried material that has little resemblance with commercially available flours, which have water contents ranging from 13 to 15 %. For stability reasons, it was decided to partially dry the materials to a target water content of about 12 % (relative mass fraction).

3.2 Rye flour processing

The whole rye flour was milled using a UPZ 100 mill (Hosokawa Alpine AG, Augsburg, DE). Then it was sieved (≤ 250 µm) using an industrial sieve (Russel Finex Ltd., London, GB) and homogenised in a Turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, CH). A sub-batch of about 50 % of the homogenised rye flour was partially dried under vacuum in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE). Thereafter, the partially dried batch and the untreated batch were mixed in a Turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, CH) to reach the target water content and filled under argon using an AccuRate feeder (Schenck AccuRate Inc., Whitewater, WI, US) into 100 mL amber glass vials. The vials were placed in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE), which was evacuated. After filling the freeze-drying chamber with argon, the vials were closed with rubber stoppers thereby providing an inert atmosphere. Then the vials were manually capped with crimp caps and labelled. All vials were stored at -20 °C. In total, 1197 units were produced, each containing about 37 g of rye flour.

3.3 Wheat flour processing

The wheat flour was homogenised in a Turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, CH). A sub-batch of about 50 % of the homogenised wheat flour was partially dried under vacuum in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE). Thereafter, the partially dried batch and the untreated batch were mixed in a Turbula mixer (Willy A. Bachofen AG Maschinenfabrik, Basel, CH) and filled using an AccuRate feeder (Schenck AccuRate Inc., Whitewater, WI, US) into 100 mL amber glass vials. The vials were placed in a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, DE), which was evacuated. After filling the freeze-drying chamber with argon, the vials were closed with rubber stoppers thereby providing an inert atmosphere. Then the vials were manually capped with crimp caps and labelled. All vials were stored at -20 °C. In total, 1197 units were produced, each containing about 37 g of wheat flour.

3.4 Additional characterisation measurements

Water content of base, intermediate and final materials was determined by Karl Fischer titration (KFT) [7]. From each of the final materials, ten vials were chosen for KFT measurements following a random stratified sample-picking scheme and analysed in duplicate. The determined mean water content of the rye flour material was 11.9 g/100 g (s = 0.5 g/100 g) and 12.3 g/100 g (s = 1.6 g/100 g) for the wheat flour material.

Particles size analysis (PSA) on the final material was performed using laser diffraction spectrometry. From each of the final materials five vials were chosen using a random stratified sample-picking scheme and analysed over a range of 0.5 to 875 µm using a Helos laser light scattering instrument (Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, DE). The determined top particle size for the rye flour material was 515 µm. About 50 % of all particles were smaller than 45 µm and approximately 1 % of all particles were smaller than 1 µm. The determined top particle size for the wheat flour material was 365 µm. About 50 % of all particles were smaller than 75 µm and approximately 1 % of all particles were smaller than 5 µm.

4 Homogeneity studies

4.1 Design of homogeneity studies

For the homogeneity studies, 30 vials (~ 2.5 % of the total batch) of ERM®-BC381 (rye flour) and 30 vials (~ 2.5 % of the total batch) of ERM®-BC382 (wheat flour) were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity per unit, the analyses per vial were split into two groups. In the first group (15 vials) triplicate determinations of Kjeldahl nitrogen and total fat content were performed. In the second group (15 vials) triplicate determinations of ash, starch, potassium, magnesium, calcium and phosphorus content were performed. Details for the analytical methods used are given in Tables 7 and 8 (see Section 6.1; lab code 2). As the contents of dietary fibre, sodium and chlorine could not be certified due to technical reasons (see Sections 6.3 and 6.4), no results from the homogeneity studies are reported here.

Samples were measured in a random order (predefined at IRMM and communicated to the laboratory) to allow distinction between an analytical trend and a trend in the filling sequence. As all required measurements per measurand could not be performed within one day, they were split over three days (for each of the vials one replicate measurement per day). In order to exclude the influence of the day-to-day variance, two-way analysis of variance (ANOVA) was applied. In each of the 30 vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination. Individual results can be seen in the Annex (Tables A1 to A16).

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying individual results as well as outlying bottle averages. Regression analyses were performed to detect possible trends regarding analytical or filling sequence. The uncertainty contribution from possible heterogeneity was estimated by a one-way analysis of variance (ANOVA) [8]. Method repeatability (s_{wb}) expressed as a relative standard deviation is given in equation 1:

$$s_{wb} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad (1)$$

MS_{within} = mean square within a bottle from an ANOVA

$\bar{y}$ = average of all results of a homogeneity study

Between-unit variability (s_{bb}) expressed as a relative standard deviation is given by equation 2:

$$s_{bb} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad (2)$$

$MS_{between}$ = mean square among bottles from an ANOVA

n = average number of replicates per bottle

Heterogeneity that can be hidden by method repeatability is defined in equation 3:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (3)$$

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

The larger value of s_{bb} or u^*_{bb} was used as uncertainty contribution for heterogeneity, u_{bb} (see Tables 1 and 2 for a summary of results, values were converted into relative uncertainties).

4.2 Results of the homogeneity study for ERM®-BC381 (rye flour)

One outlier (individual results; single Grubbs test) was detected for ash, but no technical reasons for exclusion could be given. Moreover, resulting uncertainty contributions for heterogeneity after outlier exclusion did not improve significantly, therefore, entire data set was kept to calculate the respective u_{bb}.

No significant slopes were found for analytical nor for filling sequences. In conclusion, the distribution of all eight proximates and essential elements in this material can be considered as homogeneous.

Table 1: Evaluation of homogeneity study for proximates and essential elements in ERM®-BC381 (rye flour)

Proximates	Kjeldahl N	Total fat	Ash	Starch
Mean [g/100 g]	1.53	1.05	1.17	72.53
RSD [%]	0.59	10.72	14.61	2.24
MS_{within}	0.00003	0.00819	0.01303	0.51549
$MS_{between}$	0.00007	0.00467	0.01252	0.23076
s_{wb} [%]	0.36	8.65	9.72	0.99
s_{bb} [%]	0.25	n.c. 1)	n.c. 1)	n.c. 1)
u^*_{bb} [%]	0.11	2.58	2.90	0.30
u_{bb} [%]	**0.25**	**2.58**	**2.90**	**0.30**

Essential elements	K	Mg	Ca	P
Mean [mg/g]	3.14	0.53	0.33	1.87
RSD [%]	1.92	2.17	12.79	1.80
MS_{within}	0.00308	0.00010	0.00078	0.00053
$MS_{between}$	0.00246	0.00007	0.00172	0.00062
s_{wb} [%]	1.77	1.90	8.41	1.23
s_{bb} [%]	n.c. 1)	n.c. 1)	5.37	0.28
u^*_{bb} [%]	0.53	0.57	2.51	0.37
u_{bb} [%]	**0.53**	**0.57**	**5.37**	**0.37**

1) n.c. = not calculable because $MS_{between} < MS_{within}$

4.3 Results of the homogeneity study for ERM®-BC382 (wheat flour)

One outlier (individual results; single Grubbs test) was detected for calcium, but no technical reasons for exclusion could be given. Moreover, resulting uncertainty contributions for heterogeneity after outlier exclusion did not improve significantly. Therefore, entire data set was kept to calculate the respective u_{bb}.

No significant slopes were found for analytical nor for filling sequences. In conclusion, the distribution of all eight proximates and essential elements in this material can be considered as homogeneous.

Table 2: Evaluation of homogeneity study for proximates and essential elements in ERM®-BC382 (wheat flour)

Proximates	Kjeldahl N	Total fat	Ash	Starch
Mean [g/100 g]	1.81	1.13	0.63	81.89
RSD [%]	0.52	11.48	14.13	1.76
MS_{within}	0.00003	0.01494	0.00774	0.04558
$MS_{between}$	0.00008	0.01605	0.00405	0.19337
s_{wb} [%]	0.30	10.83	13.91	0.26
s_{bb} [%]	0.22	1.70	n.c. 1)	0.27
u^*_{bb} [%]	0.09	3.23	4.15	0.08
u_{bb} [%]	**0.22**	**3.23**	**4.15**	**0.27**

Essential elements	K	Mg	Ca	P
Mean [mg/g]	1.76	0.23	0.22	1.08
RSD [%]	1.19	2.81	11.08	2.04
MS_{within}	0.00050	0.00003	0.00043	0.00037
$MS_{between}$	0.00030	0.00002	0.00029	0.00027
s_{wb} [%]	1.27	2.46	9.63	1.79
s_{bb} [%]	n.c. 1)	n.c. 1)	n.c. 1)	n.c. 1)
u^*_{bb} [%]	0.38	0.73	2.87	0.53
u_{bb} [%]	**0.38**	**0.73**	**2.87**	**0.53**

1) n.c. = not calculable because $MS_{between}$ < MS_{within}

4.4 Minimum sample intake

The minimum sample intakes for both flour materials were established based on the sample intakes used for the measurements for the homogeneity and stability studies as well as for the characterisation (if accepted data sets were submitted). For details, see also Tables 7 and 8 in Section 6.1.

The minimum sample intakes are 7.5 g for starch, 2 g each for dry mass, total fat and ash, 1 g each for potassium, magnesium, calcium and phosphorus and 0.5 g for Kjeldahl nitrogen.

5 Stability studies

5.1 Short-term stability study

5.1.1 Design of short-term stability studies

A four weeks isochronous study [9] was performed to evaluate stability of the two flour materials during transport. For the short-term stability study, 28 vials each of ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour) were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity per unit, the 28 vials were split into two groups. In the first group (14 vials) duplicate determinations of dry mass, Kjeldahl nitrogen and total fat content were performed. In the second group (14 vials) duplicate determinations of dry mass, ash, starch, potassium, magnesium, calcium and phosphorus content were performed. Details for the analytical methods used are given in Tables 7 and 8 (see Section 6.1; lab code 2). As the contents of dietary fibre, sodium and chlorine could not be certified due to technical reasons (see Sections 6.3 and 6.4), no results from the short-term stability studies are reported here.

Samples were stored at 18 °C and 60 °C as well as at a reference temperature of -20 °C. Two vials were stored at each temperature for 0, 1, 2 and 4 weeks. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under intermediate precision conditions in the order predefined at IRMM (randomised sample order) using the same methods as for the homogeneity study. In each of the 28 vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination.

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to shipping conditions (see Tables 3 and 4 for a summary). The observed slopes were tested for significance using a *t*-test, with $t_{\alpha,df}$ being the critical *t*-value (two-tailed) for a confidence level α = 0.05 (95 % confidence level) and for a confidence level α = 0.05 (95 % confidence level). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Graphs can be found in Annexes B1 and B2.

5.1.2 ERM®-BC381 (rye flour)

One outlier (individual results; single Grubbs test) was detected for phosphorus (18 °C). No technical reasons for exclusion could be given. The resulting uncertainty contributions for short-term stability after outlier exclusion did not improve significantly and no influence could be found on the significance of the slopes. Therefore, entire data sets were kept to calculate the respective uncertainty contribution from short-term stability (u_{sts}).

No statistically significant slopes were detected at 99 % confidence level. A statistically significant slope (95 % confidence level) was detected at 18 °C for phosphorus, but none at 60 °C. No significant slope was detected at 18 °C after removing of the outlier. Thus, it can be concluded that this analyte is stable at 18 °C as well as at 60 °C. For Kjeldahl nitrogen a statistically significant positive slope (95 % confidence level) was detected at 60 °C, but as the uncertainty contribution from short-term stability during dispatch at 60 °C for 1 week was very small (u_{sts} = 0.07 %), the potential degradation can be assumed to be negligible. Also for calcium a statistically significant slope (95 % confidence level) was detected at 60 °C, which was not the case after removing the outlier from the data set. Moreover, it is rather unlikely that there would be a degradation of only one of the essential elements at 60 °C. It should also be mentioned that no significant slopes were found for Kjeldahl nitrogen and calcium at both tested temperatures for the similar material ERM®-BC382 (wheat flour; see Section 5.1.3). In general, it was concluded that the uncertainties of the short-term stability (u_{sts}) can be assumed to be negligible, if sample shipment is carried out at ambient temperature, which therefore shall be the dispatch condition for sample shipment to the customer.

Table 3: Evaluation of short-term stability study for proximates and essential elements in ERM®-BC381 (rye flour)

Proximates	Kjeldahl N		Total fat		Ash		Starch	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	1.73	2.22	1.31	0.20	2.12	0.01	0.86	1.85
Outlier (99 % confidence level)	none	none	none	none	none	none	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	yes	no	no	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no	no	no	no	no
u_{sts} [%/week]	0.15	0.07	1.35	1.43	1.11	0.98	0.12	0.17

Essential elements	K		Mg		Ca		P	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	1.12	0.67	0.16	0.58	0.85	2.57	2.32	0.12
Outlier (99 % confidence level)	none	none	none	none	none	none	yes	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no	no	yes	yes	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no	no	no	no	no
u_{sts} [%/week]	0.37	0.54	0.29	0.33	1.10	0.84	0.30	0.22

1) $t_{0.05;14}$ = 2.145
2) $t_{0.01;14}$ = 2.977

5.1.3 ERM®-BC382 (wheat flour)

One outlier (individual results; single Grubbs test) was detected at both tested temperatures for calcium. In both cases no technical reasons for exclusion could be given. Although, resulting uncertainty contributions for short-term stability significantly improved after outlier exclusion, no influence could be found on the significance of the slopes. Therefore, entire data sets were kept to calculate the respective uncertainty contribution from short-term stability (u_{sts}).

No statistically significant slopes were detected at 99 % confidence level. One statistically significant slope (95 % confidence level) was detected at 18 °C for phosphorus, but none at 60 °C. Thus, it can be concluded that this analyte is stable at 18 °C as well as at 60 °C. In general, it was concluded that the uncertainties of the short-term stability (u_{sts}) can be assumed to be negligible, if sample shipment is carried out at ambient temperature, which therefore shall be the dispatch condition for sample shipment to the customer.

Table 4: Evaluation of short-term stability study for proximates and essential elements in ERM®-BC382 (wheat flour)

Proximates	Kjeldahl N		Total fat		Ash		Starch	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	0.17	0.91	0.50	1.24	0.62	0.15	0.89	1.09
Outlier (99 % confidence level)	none	none	none	none	none	none	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no	no	no	no	no
u_{sts} [%/week]	0.09	0.11	1.70	1.67	1.69	1.17	0.08	0.07

Essential elements	K		Mg		Ca		P	
	18 °C	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*	*18 °C*	*60 °C*
$\|b\|/se_b$	0.23	0.91	0.07	1.40	2.05	0.21	2.69	2.14
Outlier (99 % confidence level)	none	none	none	none	yes	yes	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no	no	no	yes	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no	no	no	no	no
u_{sts} [%/week]	0.63	0.50	0.52	0.47	1.50	2.14	0.15	0.20

1) $t_{0.05;14}$ = 2.145
2) $t_{0.01;14}$ = 2.977

5.2 Long-term stability study

5.2.1 Design of long-term stability studies

A 12 and a 24 months isochronous study [9] were performed to evaluate stability of the two flour materials during storage. Twenty-four vials each of ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour) and per study were chosen using a random stratified sample picking scheme and analysed. Because of the limited sample quantity per unit, the 24 vials per study were split into two groups. In the first group (12 vials) duplicate determinations of dry mass, Kjeldahl nitrogen and total fat content were performed. In the second group (12 vials) duplicate determinations of dry mass, ash, starch, potassium, magnesium, calcium, chlorine and phosphorus content were performed. Details for the analytical methods used are given in Tables 7 and 8 (see Section 6.1; lab code 2). As the contents of dietary fibre, sodium and chlorine could not be certified due to technical reasons (see Sections 6.3 and 6.4), no results from the long-term stability studies are reported here.

Samples were stored at 4 °C as well as at a reference temperature of -20 °C. Three vials were stored at each temperature for 0, 6, 8 and 12 months and for 0, 8, 16 and 24 months. After the indicated storage periods, the samples were transferred to storage at -20 °C until analysis. Samples were analysed under intermediate precision conditions in the order predefined at IRMM (randomised sample order) using the same methods as for the homogeneity study. The results from both studies were combined. In order to exclude the influence of the day-to-day variance the results were normalised to the mean of the results on the particular day versus the mean of all results. In each of the 24 vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination.

Grubbs tests on 99 % confidence levels were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to storage conditions (see Tables 5 and 6 for a summary). The observed slopes *b* were tested for significance using a *t*-test, with $t_{\alpha,df}$ being the critical *t*-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence level) and for a confidence level $\alpha = 0.05$ (95 % confidence level). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Finally, the uncertainty of stability u_{lts} [10] was calculated for a pre-defined shelf life of 24 months applying equation 4:

$$u_{lts} = \frac{RSD_{stab}}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl} \qquad (4)$$

with RSD_{stab} being the relative standard deviation of all 48 individual results of the relevant stability study, x_i being the time point for each replicate, $\bar{x}$ being the average of all time points and t_{sl} being the pre-defined shelf-life. Graphs can be found in Annexes C1 and C2.

5.2.2 ERM®-BC381 (rye flour)

One outlier (individual results; single Grubbs test) was detected each for calcium as well as for phosphorus. In both cases no technical reasons for exclusion could be given. Moreover, resulting uncertainty contributions for long-term stability after outlier exclusion did not improve significantly. Therefore, entire data sets were kept to calculate the respective u_{lts}.

For none of the analytes significant slopes (95 % confidence level) were detected, which demonstrates the stability of the material under these conditions. As storage temperature for the whole batch, 4 °C was chosen.

Table 5: Evaluation of combined and normalised 12 months and 24 months long-term stability studies at 4 °C for proximates and essential elements in ERM®-BC381 (rye flour)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**	**Starch**
$\|b\|/se_b$	1.17	0.97	1.10	0.39
Outlier (99 % confidence level)	none	none	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no
u_{lts} [%/24 months]	**0.19**	**3.41**	**2.69**	**0.22**

Essential elements	**K**	**Mg**	**Ca**	**P**
$\|b\|/se_b$	0.37	1.58	0.63	1.10
Outlier (99 % confidence level)	none	none	yes	yes
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no
u_{lts} [%/24 months]	**0.65**	**0.52**	**0.65**	**0.60**

1) $t_{0.05;46} = 2.013$
2) $t_{0.01;46} = 2.687$

5.2.3 ERM®-BC382 (wheat flour)

One outlier (individual results; single Grubbs test) was detected each for Kjeldahl nitrogen, total fat as well as for magnesium. In none of the cases technical reasons for exclusion could be given. Therefore, entire data sets were kept to calculate the respective u_{lts}.

For none of the analytes significant slopes (95 % confidence level) were detected, which demonstrates the stability of the material under these conditions. As storage temperature for the whole batch, 4 °C was chosen.

Table 6: Evaluation of combined and normalised 12 months and 24 months long-term stability studies at 4 °C for proximates and essential elements in ERM®-BC382 (wheat flour)

Proximates	Kjeldahl N	Total fat	Ash	Starch
$\|b\|/se_b$	0.80	0.09	1.67	1.44
Outlier (99 % confidence level)	yes	yes	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no
u_{lts} [%/24 months]	**0.13**	**3.50**	**6.03**	**0.16**

Essential elements	K	Mg	Ca	P
$\|b\|/se_b$	1.24	1.92	0.69	1.89
Outlier (99 % confidence level)	none	yes	none	none
Statistical significance of the slope (95 % confidence level) 1)	no	no	no	no
Statistical significance of the slope (99 % confidence level) 2)	no	no	no	no
u_{lts} [%/24 months]	**0.98**	**1.29**	**1.57**	**1.15**

1) $t_{0.05;46}$ = 2.013
2) $t_{0.01;46}$ = 2.687

6 Characterisation

6.1 Design of characterisation studies

The certification exercise was performed in 2007. Eleven laboratories were carefully selected to perform the analytical measurements. Validated methods were an indispensable requirement for participation; an accredited method was considered an asset. The laboratories had to prove their measurement capabilities and had to demonstrate previous experience in the analysis of proximates and essential elements in comparable matrices.

Each laboratory was provided with six vials each of ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour). Because of the limited sample quantity per unit, the analyses per vial were split into two groups. In the first group (three vials) duplicate determinations of Kjeldahl nitrogen and total fat content were performed. In the second group (three vials) duplicate determinations of ash, starch, dietary fibre, potassium, sodium, magnesium, calcium, chlorine and phosphorus content were performed. The measurements per analyte were spread over two days. In each of the six vials duplicate determinations of dry mass content were performed. All results per vial were related to the mean of the respective duplicate dry mass determination. Details for the minimum sample intakes and the analytical methods used are given in Tables 7 and 8.

Table 7: Minimum sample intakes (MSI) in gram and methods used for determination of proximate contents in ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour)

Lab code	DM 1)	Kjeldahl N	Total fat 2)	Ash 3)	Starch 4)		Dietary fibre 5)
	MSI	MSI	MSI	MSI	MSI	Method	MSI
1	2.0	0.5	5.0	2.5	2.0	enzymatic	2.5
2	5.0	1.0	2.0	5.0	7.5	polarimetry	1.0
3	2.0	1.0	2.0	2.0	0.3	enzymatic	1.0
4	5.0	2.0	5.0	5.0	2.5	polarimetry	1.0
5	2.0	0.2	3.0	1.0	0.2	enzymatic	1.0
6	5.0	1.0	5.0	2.5	7.5	polarimetry	1.0
7	1.0	1.0	2.0	3.5	7.5	polarimetry	2.0
8	2.0	0.8 6)	2.0	5.0	7.5	polarimetry	1.0
9	2.0	0.4	5.0	2.0	0.1	enzymatic	1.0
10	3.0	1.0	3.0	3.0	5.0	polarimetry	1.0
11	4.0 7)	1.0	4.0	4.0	2.5	polarimetry	1.0

1) Dry mass determination to be performed at 130 °C ± 3 °C (1 h) according to procedure AOAC 925.10
2) Total fat determination (gravimetric) to be performed after acid hydrolysis and solvent extraction [11]
3) Ash determination to be performed at 550 °C ± 25 °C
4) Polarimetric starch determination: specific optical rotation value to be applied is +184.0 ° for rye flour and +182.7 ° for wheat flour as specified in ISO 10520 [12]
5) Dietary fibre determination to be performed according to procedure AOAC 985.29
6) Nitrogen content was determined by Dumas method
7) Dry mass determination was performed at 102 °C (3 h)

The following variations of the Kjeldahl nitrogen determination were noticed: Different digestors (Büchi, Foss, Gebhardt) were used. Digestion was performed using sulphuric acid with or without H_2O_2 and different catalyst combinations ($CuS0_4$ + TiO_2, Se + K_2SO_4, $CuSO_4$ + K_2SO_4) with varying digestion times (1 to 3 h).

The following variations of the total fat determination were noticed: Hydrolysis was performed using different concentrations of hydrochloric acid (10 to 50 %) with varying hydrolysis times (1 to 6 h). Extraction was performed using petrol ether or a mixture of petrol ether with diethyl ether with varying extraction times (1 to 8 h).

Table 8: Minimum sample intakes (MSI) in g and methods used for determination of essential element contents in ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour)

Lab code	K		Na		Mg		Ca		Cl		P	
	MSI	Method	MSI	Method	MSI	Method	MSI	Method	MSI	Method	MSI	Method
1	0.2	flame OES	0.2	flame OES	0.2	flame AAS	0.2	flame AAS	2.0	potentio-metry	0.2	spectro-photometry
2	5.0	ICP-OES	5.0	ICP-OES	5.0	ICP-OES	5.0	ICP-OES	5.0	ICP-OES	5.0	ICP-OES
3	1.0	ICP-OES	1.0	ICP-OES	5.0	ICP-OES	5.0	ICP-OES	n.d.	n.d.	1.0	ICP-OES
4	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	5.0	potentio-metry	1.0	ICP-OES
5	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	2.5	potentio-metry	1.0	ICP-OES
6	2.5	flame photometry	2.5	flame photometry	2.5	flame AAS	2.5	flame photometry	5.0	potentio-metry	2.5	spectro-photometry
7	3.0	flame photometry	3.0	flame photometry	3.5	flame AAS	3.5	flame AAS	0.5	ion chro-matography	3.0	spectro-photometry
8	5.0	ICP-OES	5.0	flame photometry	5.0	ICP-OES	5.0	ICP-OES	5.0	potentio-metry	5.0	ICP-OES
9	2.0	flame OAS	2.0	flame OAS	2.0	flame AAS	2.0	flame AAS	2.0	potentio-metry	2.0	spectro-photometry
10	5.0	flame photometry	5.0	flame photometry	5.0	flame AAS	3.0	gravimetry	1.0	potentio-metry	1.0	spectro-photometry
11	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	ICP-OES	1.0	titrimetry	1.0	ICP-OES

n.d. = not determined

6.2 Results and technical evaluation – Principles

After receipt of the data sets, the results were subjected to technical evaluation. The accepted sets of results were submitted to the following statistical tests:

- Scheffe multiple *t*-test to check if the means of two labs are significantly different
- Dixon test to detect outlying laboratory means
- Nalimov *t*-test to detect outlying laboratory means
- Grubbs test to detect single and double outliers
- Cochran test to check for outlying laboratory variances
- Bartlett test to check for homogeneity of laboratory variances
- ANOVA to assess between laboratory and within laboratory variances and test their significance employing the Snedecor *F*-test
- Skewness and kurtosis tests to assess the normality of the lab means distribution

6.3 Results and technical evaluation – ERM®-BC381 (rye flour)

The results of the statistical tests of the finally considered data for ERM®-BC381 (rye flour) are summarised in Table 9. Individual results and corresponding graphs can be found in Annex D1.

All participants submitted technically accepted data sets (11 data sets containing 66 individual results) for total fat, potassium and phosphorus.

Kjeldahl N Lab 8 submitted a data set obtained by the Dumas method, although the results fit very well into the data sets of the other participants, only data sets obtained by the Kjeldahl method were kept as this was previously requested. Therefore, ten data sets were taken into account for certification.

Ash The data set from Lab 11 was excluded because it did not meat the specifications of the lab. Therefore, ten data sets were taken into account for certification.

Starch Only those seven data sets were kept for certification, which were obtained by the polarimetric method. Labs 1, 3, 5 and 9 applied a method based on the enzymatic determination of glucose after hydrolysis. These data sets are very heterogeneous and two data sets showed significantly lower results. As now technical reasons could be found for these findings, it was decided to eliminate those data sets obtained by the enzymatic method.

Dietary fibre All 11 participants submitted complete data sets, but because of the heterogeneity of the received data sets, the dietary fibre content was not taken into account for certification.

Magnesium Data set from Lab 3 was eliminated, as the results obtained with two emission lines were significantly different, which may indicate a technical problem during the required measurements. Therefore, ten data sets were taken into account for certification.

Calcium Lab 6 reported that the content was below the lab's limit of quantification (LOQ), therefore no data set was submitted. Lab 10, the only lab using a gravimetric method, confirmed that the provided sample quantity was not sufficient for this method. Therefore this data set was excluded for technical reasons. Therefore, nine data sets were taken into account for certification.

Chlorine Lab 3 did not measure this analyte. Labs 9 and 11 reported that the content was below the labs' LOQ, therefore no data sets were submitted. Because of the heterogeneity of the remaining data sets, the chlorine content was not taken into account for certification.

Sodium In addition, the participants were also asked to measure the sodium content. Labs 2, 5 and 6 reported that the content was below the labs' LOQ. Therefore no data sets were submitted. For the same reason Labs 8 and 11 reported fewer than the requested six results. Because of the heterogeneity of the remaining data sets, the sodium content was not taken into account for certification.

In all cases, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Moreover, it was decided to keep those data sets, which have outlying laboratory variances (Cochran test) as no technical reasons could be given.

Table 9: Summary of statistical evaluation for proximates and essential elements in ERM®-BC381 (rye flour)

Proximates	Kjeldahl N	Total fat	Ash	Starch
Number of data sets	10	11	10	7
Number of replicate measurements	60	66	60	42
Mean of means [g/100 g]	1.56	1.36	1.08	72.22
Relative standard deviation of mean of means [%]	0.97	12.47	8.89	3.17
Relative standard error of mean of means (u_{char}) [%]	0.31	3.76	2.81	1.20
All data sets compatible two by two? (Scheffe test)	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	none	yes (*Lab 8)	yes (*Lab 4)	yes (*Lab 8)
Lab variances homogeneous? (Bartlett test; p = 0.01)	yes	no	no	no
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes

Essential elements	K	Mg	Ca	P
Number of data sets	11	10	9	11
Number of replicate measurements	66	60	54	66
Mean of means [mg/g]	3.35	0.57	0.32	2.01
Relative standard deviation of mean of means [%]	4.47	2.67	5.24	4.95
Relative standard error of mean of means (u_{char}) [%]	1.35	0.85	1.75	1.49
All data sets compatible two by two? (Scheffe test)	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	yes (*Lab 2, Lab 4, Lab 8 Lab 9)	yes (*Lab 9)	yes (*Lab 8)	yes (*Lab 4, *Lab 8)
Lab variances homogeneous? (Bartlett test; p = 0.01)	no	no	no	no
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes

* p = 0.01

6.4 Results and technical evaluation – ERM®-BC382 (wheat flour)

The results of the statistical tests of the finally considered data for ERM®-BC382 (wheat flour) are summarised in Table 10. Individual results and corresponding graphs can be found in Annex D2.

All participants submitted technically accepted data sets (11 data sets containing 66 individual results) for total fat, potassium and phosphorus.

Kjeldahl N Lab 8 submitted a data set obtained by the Dumas method, although the results fit very well into the data sets of the other participants, only data sets obtained by the Kjeldahl method were kept as this was previously requested. Therefore, ten data sets were taken into account for certification.

Ash The data set from Lab 11 was excluded because it did not meat the specifications of the lab. Therefore, ten data sets were taken into account for certification.

Starch Only those seven data sets were kept for certification, which were obtained by the polarimetric method. Labs 1, 3, 5 and 9 applied a method based on the enzymatic determination of glucose after hydrolysis. These data sets are very heterogeneous and two data sets showed significantly lower results. As now technical reasons could be found for these findings, it was decided to eliminate those data sets obtained by the enzymatic method.

Dietary fibre All 11 participants submitted complete data sets, but because of the heterogeneity of the received data sets, the dietary fibre content was not taken into account for certification.

Magnesium Data set from Lab 3 was eliminated, as the results obtained with two emission lines were significantly different, which may indicate a technical problem during the required measurements. Lab 6 submitted results around the lab's limit of quantification (LOQ), therefore this data set was eliminated. Therefore, nine data sets were taken into account for certification.

Calcium Lab 6 reported that the content was below the lab's limit of quantification (LOQ), therefore no data set was submitted. Lab 10, the only lab using a gravimetric method, confirmed that the provided sample quantity was not sufficient for this method, therefore this data set was excluded on technical reasons. Therefore, nine data sets were taken into account for certification.

Chlorine Lab 3 did not measure this analyte. Labs 9 and 11 reported that the content was below the labs' LOQ, therefore no data sets were submitted. Because of the heterogeneity of the remaining data sets, the chlorine content was not taken into account for certification.

Sodium In addition, the participants were also asked to measure the sodium content. Labs 2, 5 and 6 reported that the content was below the labs' LOQ, therefore no data sets were submitted. For the same reason Labs 8 and 11 reported fewer than the requested six results. Because of the heterogeneity of the remaining data sets, the sodium content was not taken into account for certification.

In all cases, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Moreover, it was decided to keep those data sets, which have outlying laboratory variances (Cochran test) as no technical reasons could be given.

Table 10: Summary of statistical evaluation for proximates and essential elements in ERM®-BC382 (wheat flour)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**	**Starch**
Number of data sets	10	11	10	7
Number of replicate measurements	60	66	60	42
Mean of means [g/100 g]	1.85	1.39	0.60	81.19
Relative standard deviation of mean of means [%]	1.12	12.28	10.03	2.62
Relative standard error of mean of means (u_{char}) [%]	0.35	3.70	3.17	0.99
All data sets compatible two by two? (Scheffe test)	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	yes (Lab 4, Lab 11)	yes (Lab 8)	none	yes (*Lab 7, Lab 8, *Lab 11)
Lab variances homogeneous? (Bartlett test; p = 0.01)	yes	yes	yes	no
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes

Essential elements	**K**	**Mg**	**Ca**	**P**
Number of data sets	11	9	9	11
Number of replicate measurements	66	54	54	66
Mean of means [mg/g]	1.88	0.25	0.21	1.19
Relative standard deviation of mean of means [%]	6.20	3.82	8.13	7.44
Relative standard error of mean of means (u_{char}) [%]	1.87	1.27	2.71	2.24
All data sets compatible two by two? (Scheffe test)	no	no	no	no
Outlying means? (Dixon test; p = 0.05)	none	none	none	none
Outlying means? (Grubbs test; p = 0.05)	none	none	none	none
Outlying lab variances? (Cochran test; p = 0.05)	yes (*Lab 4, *Lab 6, *Lab 9)	yes (*Lab 4)	yes (*Lab 4)	yes (*Lab 4, *Lab 6, *Lab 8)
Lab variances homogeneous? (Bartlett test; p = 0.01)	no	no	no	no
Variances between labs significantly different? (Snedecor F-test; p = 0.01)	yes	yes	yes	yes
Distribution of means normal (p = 0.01)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes

* p = 0.01

7 Certified values and uncertainties

The certified values for ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour) are calculated as the mean of means of the accepted data sets. The standard error of the mean of means was used as an estimation of the uncertainty contribution of the characterisation exercise. The standard error is calculated as the standard deviation divided by the square root of the number of accepted data sets.

The combined standard uncertainty of the certified value includes contributions from the between-bottle heterogeneity, long-term storage and the characterisation study. The relative combined standard uncertainty is calculated according to equation 5:

$$u_{CRM} = \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2} \qquad (5)$$

Tables 11 and 12 summarise the individual uncertainty contributions and the resulting expanded uncertainties as well as the certified values and their uncertainties after rounding for ERM®-BC381 (rye flour) and ERM®-BC382 (wheat flour).

Table 11: Certified values and uncertainties for ERM®-BC381 (rye flour)

Proximates	**Kjeldahl N**	**Total fat**	**Ash**	**Starch**
u_{bb} [%]	0.25	2.58	2.90	0.30
u_{lts} [%] 1)	0.19	3.41	2.69	0.22
u_{char} [%]	0.31	3.76	2.81	1.20
$u_{CRM, rel}$ [%]	0.44	5.69	4.86	1.25
$U_{CRM, rel}$ (k = 2) [%]	0.88	11.39	9.71	2.51
Certified value [g/100 g]	**1.562**	**1.36**	**1.08**	**72.2**
U_{CRM} (k = 2) [g/100 g]	**0.014**	**0.16**	**0.11**	**1.9**

Essential elements	**K**	**Mg**	**Ca**	**P**
u_{bb} [%]	0.53	0.57	5.37	0.37
u_{lts} [%] 1)	0.65	0.52	0.65	0.60
u_{char} [%]	1.35	0.85	1.75	1.49
$u_{CRM, rel}$ [%]	1.58	1.14	5.68	1.65
$U_{CRM, rel}$ (k = 2) [%]	3.17	2.29	11.36	3.30
Certified value [mg/g]	**3.35**	**0.567**	**0.32**	**2.01**
U_{CRM} (k = 2) [mg/g]	**0.11**	**0.013**	**0.04**	**0.07**

1) Shelf life 24 months

Table 12: Certified values and uncertainties for ERM®-BC382 (wheat flour)

Proximates	Kjeldahl N	Total fat	Ash	Starch
u_{bb} [%]	0.22	3.23	4.15	0.27
u_{lts} [%] 1)	0.13	3.50	6.03	0.16
u_{char} [%]	0.35	3.70	3.17	0.99
$u_{CRM, rel}$ [%]	0.43	6.04	7.98	1.04
$U_{CRM, rel}$ (k = 2) [%]	0.87	12.07	15.95	2.07
Certified value [g/100 g]	**1.851**	**1.39**	**0.60**	**81.2**
U_{CRM} (k = 2) [g/100 g]	**0.017**	**0.17**	**0.10**	**1.7**

Essential elements	K	Mg	Ca	P
u_{bb} [%]	0.38	0.73	2.87	0.53
u_{lts} [%] 1)	0.98	1.29	1.57	1.15
u_{char} [%]	1.87	1.27	2.71	2.24
$u_{CRM, rel}$ [%]	2.14	1.96	4.25	2.58
$U_{CRM, rel}$ (k = 2) [%]	4.29	3.91	8.50	5.16
Certified value [mg/g]	**1.88**	**0.247**	**0.210**	**1.19**
U_{CRM} (k = 2) [mg/g]	**0.08**	**0.010**	**0.018**	**0.07**

1) Shelf life 24 months

8 Metrological traceability

The measurement results for assigning nitrogen mass fraction values are method dependent (Kjeldahl). They were obtained by different digestion procedures and subsequent quantification by Kjeldahl methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning total fat mass fraction values are method dependent. They are obtained by different procedures for acid hydrolysis and solvent extraction and subsequent quantification by gravimetric methods. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning ash mass fraction values are method. They are obtained by gravimetric methods based on ashing at 550 °C ± 25 °C. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning starch mass fraction values are method dependent. They are obtained by acid hydrolysis, clarification and filtration and subsequent quantification by polarimetry. The certified mass fractions are traceable to the specific optical rotation values as specified in ISO 10520 [12].

The measurement results for assigning potassium, magnesium and calcium mass fraction values are obtained by different digestion and extraction procedures and subsequent quantification by ICP-OES, flame OES, flame AAS and flame photometric methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

The measurement results for assigning phosphorus mass fraction values are obtained by different digestion and extraction procedures and subsequent quantification by ICP-OES and spectrophotometric methods based on calibrants of known purity and concentration. The certified mass fractions are traceable to the International System of Units (SI).

9 Instructions for use and intended use

9.1 Safety precautions

The usual laboratory safety precautions apply.

9.2 Use of materials

- Allow the vial to warm up to ambient temperature before opening.
- Shake vial before aliquotation.
- Certified values are based on dry mass.
- Dry mass determination should be performed at least in duplicate.
- To determine dry mass weigh accurately an aliquot of approximately 2 g on an analytical balance. The weighing should be performed immediately after opening of the vial to minimise potential water uptake or release by the flour material. Drying has to be performed at 130 °C ± 3 °C for at least 1 h (according to procedure AOAC 925.10).

9.3 Intended use

This material is intended to be used for method performance control and validation purposes. For assessing the method performance, the measured values of the CRMs are compared with the certified values following a procedure described by Linsinger [13]. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}) according to equation 6:

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad (6)$$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two ($k = 2$), corresponding to a confidence level of approximately 95 %.
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

9.4 Storage conditions

The materials should be stored at a temperature of 4 °C ± 3 °C. However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially after opening of the vials.

10 Acknowledgements

The authors would like to thank B. Toussaint and S. Voorspoels (IRMM, BE) for internal review of this report as well as P. Gowik (Federal Office of Consumer Protection and Food Safety, DE), P. Finglas (Institute of Food Research, GB) and J. de Boer (Institute for Environmental Studies, VU University Amsterdam, NL) as members of the Certification Advisory Panel for reviewing the certification documents and for their constructive comments.

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Annex A1 ERM®-BC381 (rye flour) – Results of the homogeneity study

Table A1: Data of homogeneity study measurements of Kjeldahl nitrogen content in ERM®-BC381 (related to dry mass)

Kjeldahl nitrogen mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	1.54	1.54	1.53
149	1.53	1.54	1.51
186	1.53	1.54	1.53
278	1.52	1.53	1.52
380	1.53	1.54	1.53
412	1.53	1.54	1.52
523	1.51	1.54	1.51
603	1.53	1.54	1.53
707	1.54	1.53	1.53
762	1.53	1.54	1.53
854	1.53	1.53	1.52
900	1.53	1.53	1.52
1003	1.54	1.53	1.52
1075	1.53	1.54	1.53
1151	1.52	1.53	1.51

Table A2: Data of homogeneity study measurements of total fat content in ERM®-BC381 (related to dry mass)

Total fat mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
25	1.11	1.11	0.79
132	1.07	1.16	1.01
189	1.10	1.18	0.79
294	1.07	1.17	0.79
358	1.07	1.23	1.01
418	1.08	1.16	0.90
519	1.00	1.19	1.12
569	1.09	1.13	0.90
701	1.08	1.09	1.01
774	1.08	1.13	1.01
829	1.08	1.18	0.90
887	1.10	1.04	0.79
1002	1.05	1.03	1.12
1036	1.04	0.91	1.01
1169	1.06	1.15	1.01

Table A3: Data of homogeneity study measurements of ash content in ERM®-BC381 (related to dry mass)

Ash mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	1.13	1.04	1.12
149	1.21	1.00	1.23
186	1.12	1.07	1.35
278	1.01	0.99	1.23
380	1.21	1.02	1.57
412	1.15	1.03	1.46
523	1.11	1.15	1.23
603	1.09	1.09	1.23
707	1.17	0.92	1.23
762	1.18	1.01	1.35
854	1.10	1.04	1.23
900	1.13	0.96	1.79
1003	1.14	1.08	1.57
1075	1.22	1.02	1.23
1151	1.15	1.08	1.34

Table A4: Data of homogeneity study measurements of starch content in ERM®-BC381 (related to dry mass)

Starch mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
25	74.11	74.00	70.08
132	72.24	74.25	71.49
189	73.58	74.61	70.38
294	72.46	72.77	70.37
358	73.54	73.47	70.83
418	72.76	73.99	70.19
519	72.99	74.47	70.15
569	73.29	74.07	70.45
701	73.84	74.11	70.26
774	73.81	74.51	70.22
829	73.13	74.31	70.30
887	72.92	74.21	70.44
1002	72.51	73.98	69.92
1036	73.79	71.30	71.57
1169	73.36	74.23	70.42

Table A5: Data of homogeneity study measurements of potassium content in ERM®-BC381 (related to dry mass)

Potassium mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	3.26	3.10	3.07
149	3.05	3.13	3.16
186	3.18	3.09	3.18
278	3.05	3.07	3.19
380	3.18	3.16	3.24
412	3.02	3.12	3.23
523	3.06	3.11	3.09
603	3.07	3.12	3.20
707	3.11	3.17	3.16
762	3.21	3.15	3.15
854	3.15	3.11	3.18
900	3.06	3.09	3.12
1003	3.06	3.12	3.20
1075	3.11	3.11	3.25
1151	3.15	3.07	3.23

Table A6: Data of homogeneity study measurements of magnesium content in ERM®-BC381 (related to dry mass)

Magnesium mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	0.53	0.52	0.52
149	0.52	0.52	0.54
186	0.53	0.52	0.54
278	0.53	0.50	0.55
380	0.53	0.53	0.54
412	0.53	0.52	0.56
523	0.52	0.53	0.53
603	0.52	0.52	0.54
707	0.52	0.53	0.54
762	0.54	0.54	0.53
854	0.55	0.52	0.54
900	0.52	0.52	0.53
1003	0.52	0.53	0.54
1075	0.53	0.53	0.53
1151	0.53	0.52	0.54

Table A7: Data of homogeneity study measurements of calcium content in ERM®-BC381 (related to dry mass)

Calcium mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	0.43	0.33	0.30
149	0.31	0.29	0.30
186	0.31	0.29	0.34
278	0.40	0.29	0.30
380	0.37	0.30	0.33
412	0.36	0.28	0.35
523	0.44	0.36	0.32
603	0.36	0.29	0.31
707	0.33	0.31	0.34
762	0.44	0.36	0.34
854	0.31	0.29	0.30
900	0.34	0.29	0.31
1003	0.34	0.29	0.30
1075	0.45	0.29	0.34
1151	0.35	0.30	0.31

Table A8: Data of homogeneity study measurements of phosphorus content in ERM®-BC381 (related to dry mass)

Phosphorus mass fraction in ERM®-BC381 (rye flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
45	1.85	1.83	1.89
149	1.86	1.89	1.90
186	1.86	1.86	1.92
278	1.87	1.83	1.94
380	1.89	1.88	1.93
412	1.88	1.83	1.93
523	1.84	1.85	1.87
603	1.87	1.88	1.90
707	1.82	1.85	1.91
762	1.86	1.85	1.90
854	1.87	1.84	1.92
900	1.85	1.80	1.88
1003	1.86	1.86	1.94
1075	1.85	1.91	1.86
1151	1.85	1.83	1.93

Annex A2 ERM®-BC382 (wheat flour) – Results of the homogeneity study

Table A9: Data of homogeneity study measurements of Kjeldahl nitrogen content in ERM®-BC382 (related to dry mass)

Kjeldahl nitrogen mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	1.81	1.82	1.80
101	1.82	1.83	1.81
196	1.81	1.82	1.81
278	1.81	1.81	1.80
337	1.81	1.82	1.81
454	1.81	1.82	1.81
532	1.81	1.82	1.80
599	1.82	1.82	1.80
708	1.82	1.81	1.80
761	1.82	1.83	1.81
830	1.81	1.81	1.80
946	1.81	1.81	1.80
1022	1.82	1.82	1.80
1083	1.82	1.82	1.80
1150	1.82	1.82	1.80

Table A10: Data of homogeneity study measurements of total fat content in ERM®-BC382 (related to dry mass)

Total fat mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
3	1.04	1.16	1.13
80	1.07	1.30	0.79
221	1.26	1.22	1.01
276	1.14	1.13	1.24
341	1.27	1.28	1.24
411	1.14	1.18	1.23
511	1.11	0.96	0.90
591	1.09	1.09	1.01
648	1.16	1.16	1.01
727	1.11	1.38	1.12
811	1.16	1.03	1.24
870	1.24	1.16	0.90
995	1.07	1.20	1.12
1029	1.10	1.24	0.79
1112	1.31	1.06	1.24

Table A11: Data of homogeneity study measurements of ash content in ERM®-BC382 (related to dry mass)

Ash mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	0.61	0.48	0.79
101	0.63	0.49	0.67
196	0.60	0.53	0.56
278	0.56	0.75	0.67
337	0.66	0.61	0.79
454	0.67	0.47	0.67
532	0.67	0.57	0.56
599	0.64	0.72	0.56
708	0.69	0.48	0.67
761	0.62	0.61	0.67
830	0.65	0.69	0.56
946	0.66	0.63	0.67
1022	0.63	0.53	0.67
1083	0.70	0.53	0.90
1150	0.56	0.61	0.79

Table A12: Data of homogeneity study measurements of starch content in ERM®-BC382 (related to dry mass)

Starch mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
3	82.46	84.06	80.23
80	80.88	83.34	80.13
221	82.05	83.58	79.98
276	82.22	83.55	80.27
341	82.09	83.83	80.14
411	81.84	83.47	80.02
511	81.94	83.55	80.22
591	82.17	83.54	80.68
648	82.20	84.12	80.56
727	82.20	83.54	80.11
811	81.65	83.22	79.73
870	81.70	83.28	79.78
995	81.84	83.08	80.02
1029	81.96	83.67	80.00
1112	82.19	83.69	80.34

Table A13: Data of homogeneity study measurements of potassium content in ERM®-BC382 (related to dry mass)

Potassium mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	1.74	1.73	1.81
101	1.74	1.73	1.80
196	1.76	1.77	1.75
278	1.76	1.78	1.79
337	1.74	1.77	1.77
454	1.73	1.76	1.80
532	1.75	1.76	1.73
599	1.74	1.76	1.72
708	1.78	1.78	1.76
761	1.77	1.72	1.75
830	1.76	1.75	1.74
946	1.74	1.77	1.74
1022	1.74	1.76	1.75
1083	1.77	1.76	1.76
1150	1.74	1.79	1.78

Table A14: Data of homogeneity study measurements of magnesium content in ERM®-BC382 (related to dry mass)

Magnesium mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	0.22	0.21	0.25
101	0.22	0.22	0.24
196	0.22	0.22	0.22
278	0.24	0.22	0.24
337	0.22	0.22	0.24
454	0.22	0.22	0.24
532	0.24	0.22	0.22
599	0.22	0.22	0.22
708	0.24	0.22	0.24
761	0.22	0.22	0.24
830	0.24	0.22	0.22
946	0.22	0.22	0.24
1022	0.22	0.22	0.24
1083	0.24	0.22	0.24
1150	0.22	0.22	0.24

Table A15: Data of homogeneity study measurements of calcium content in ERM®-BC382 (related to dry mass)

Calcium mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	0.20	0.18	0.26
101	0.26	0.19	0.22
196	0.20	0.21	0.24
278	0.30	0.21	0.20
337	0.25	0.19	0.20
454	0.21	0.21	0.21
532	0.22	0.20	0.20
599	0.24	0.19	0.21
708	0.27	0.20	0.21
761	0.24	0.19	0.21
830	0.22	0.19	0.24
946	0.21	0.19	0.20
1022	0.21	0.19	0.20
1083	0.22	0.21	0.22
1150	0.21	0.19	0.21

Table A16: Data of homogeneity study measurements of phosphorus content in ERM®-BC382 (related to dry mass)

Phosphorus mass fraction in ERM®-BC382 (wheat flour) [g/100 g]			
Sample #	**Day 1**	**Day 2**	**Day 3**
15	1.08	1.06	1.11
101	1.05	1.08	1.11
196	1.06	1.10	1.07
278	1.06	1.07	1.12
337	1.05	1.08	1.10
454	1.07	1.12	1.12
532	1.09	1.04	1.05
599	1.06	1.09	1.06
708	1.07	1.06	1.11
761	1.07	1.08	1.10
830	1.07	1.07	1.08
946	1.06	1.10	1.08
1022	1.06	1.07	1.09
1083	1.06	1.08	1.10
1150	1.07	1.09	1.10

Annex B1 ERM®-BC381 (rye flour) – Results of the short-term stability study

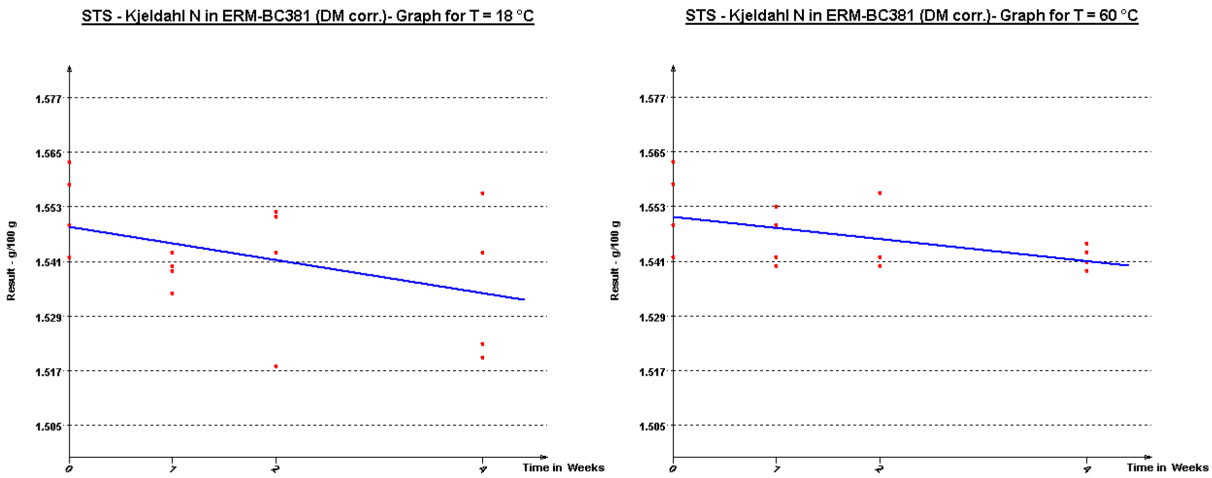


Figure B1: Short-term stability of Kjeldahl nitrogen content in ERM®-BC381 at 18 and 60 °C.

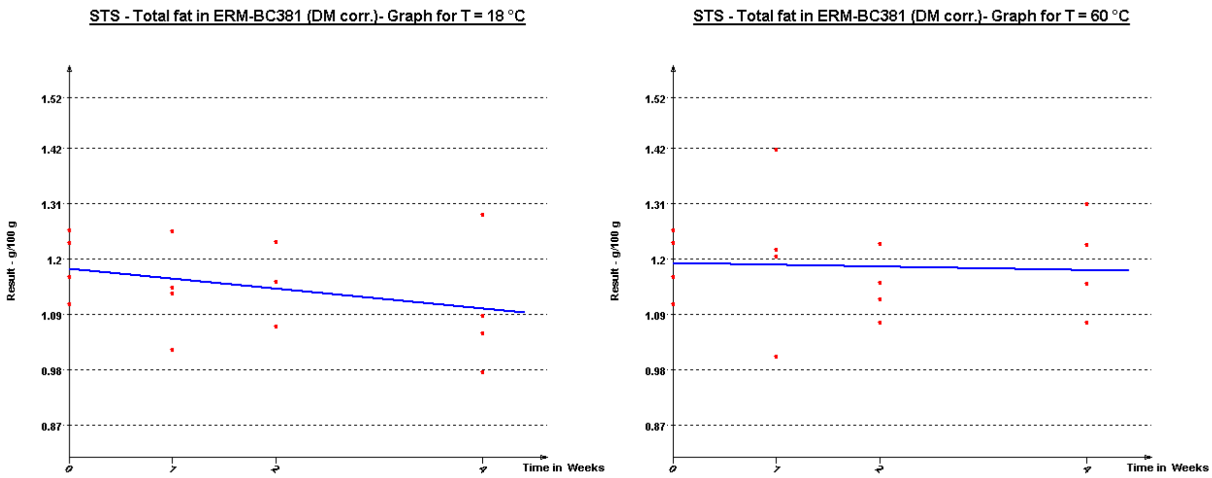


Figure B2: Short-term stability of total fat content in ERM®-BC381 at 18 and 60 °C.

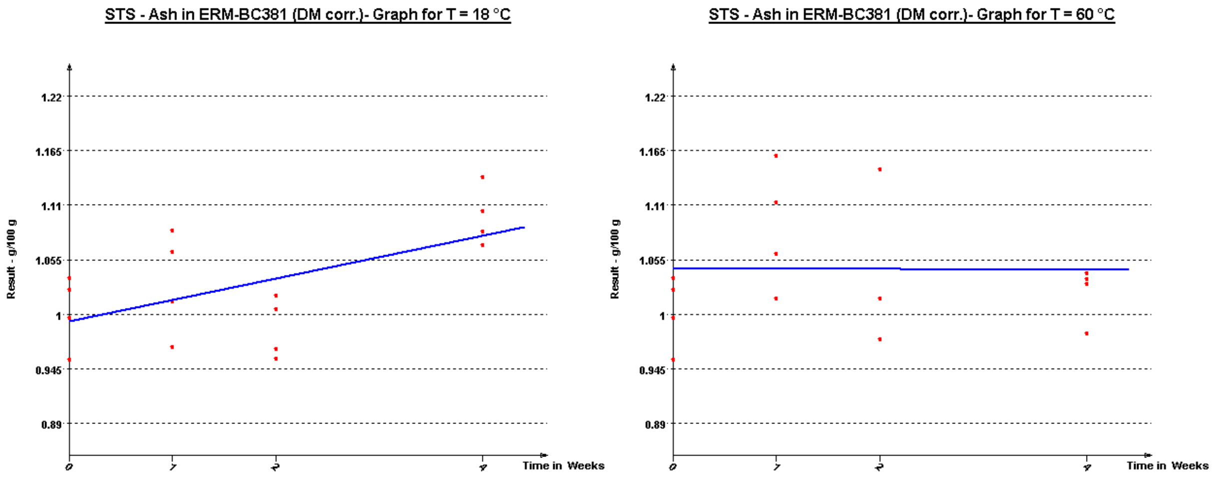


Figure B3: Short-term stability of ash content in ERM®-BC381 at 18 and 60 °C.

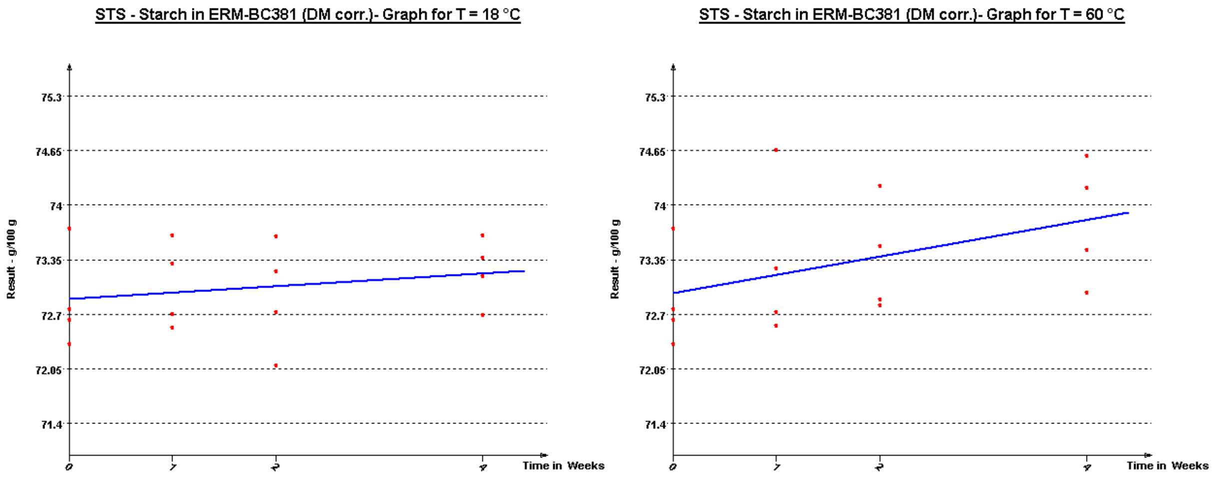


Figure B4: Short-term stability of starch content in ERM®-BC381 at 18 and 60 °C.

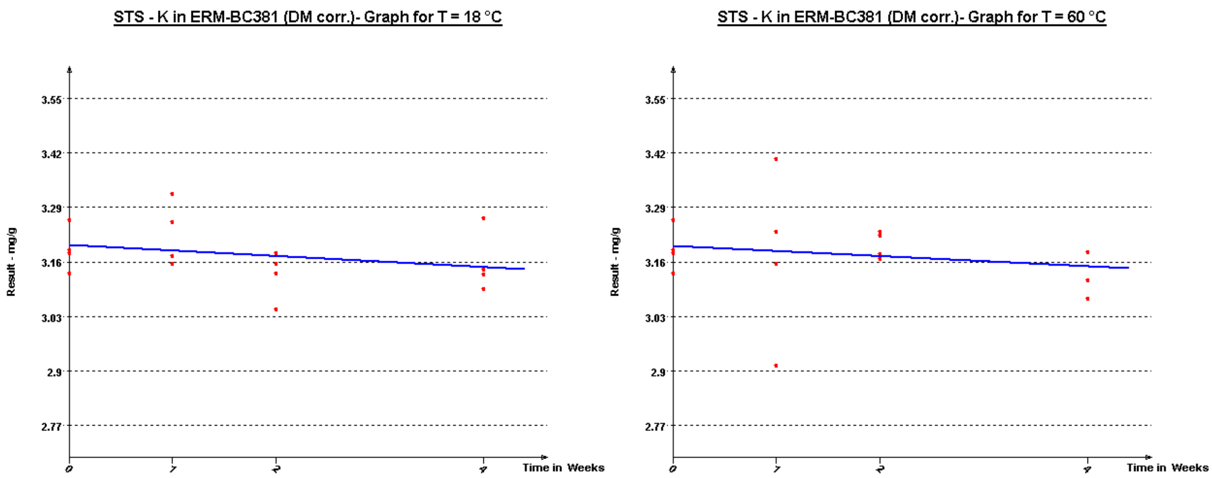


Figure B5: Short-term stability of potassium content in ERM®-BC381 at 18 and 60 °C.

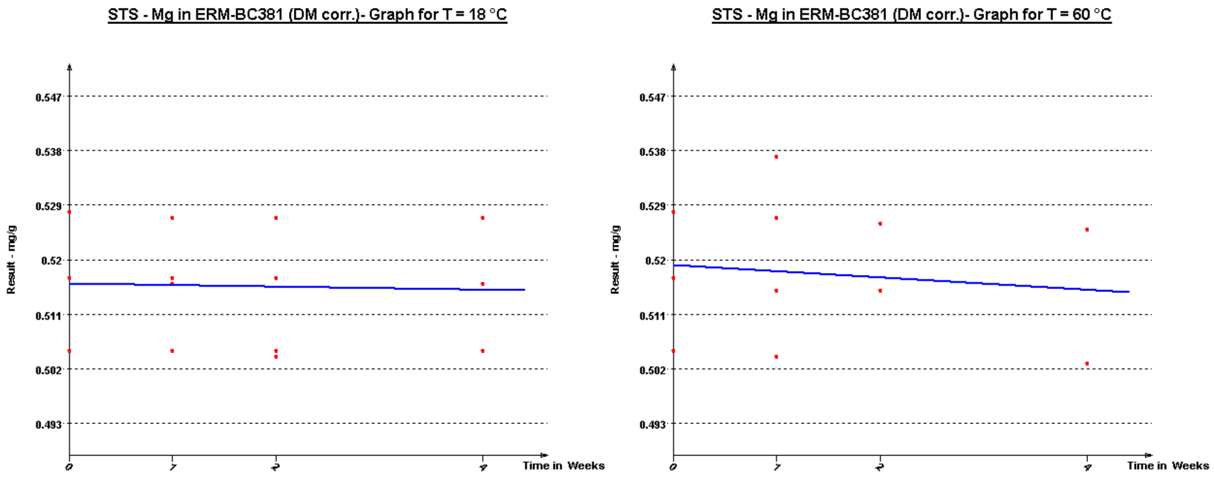


Figure B6: Short-term stability of magnesium content in ERM®-BC381 at 18 and 60 °C.

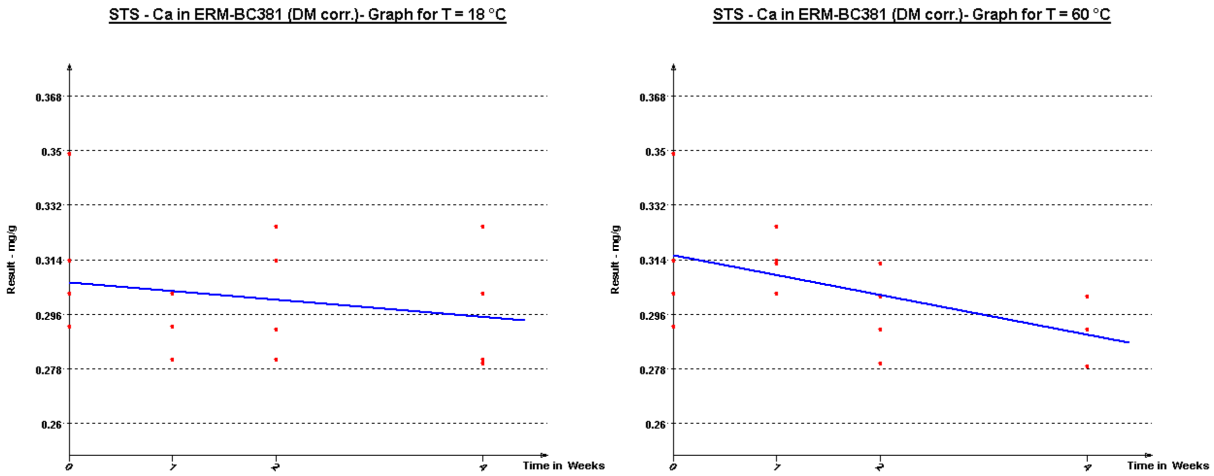


Figure B7: Short-term stability of calcium content in ERM®-BC381 at 18 and 60 °C.

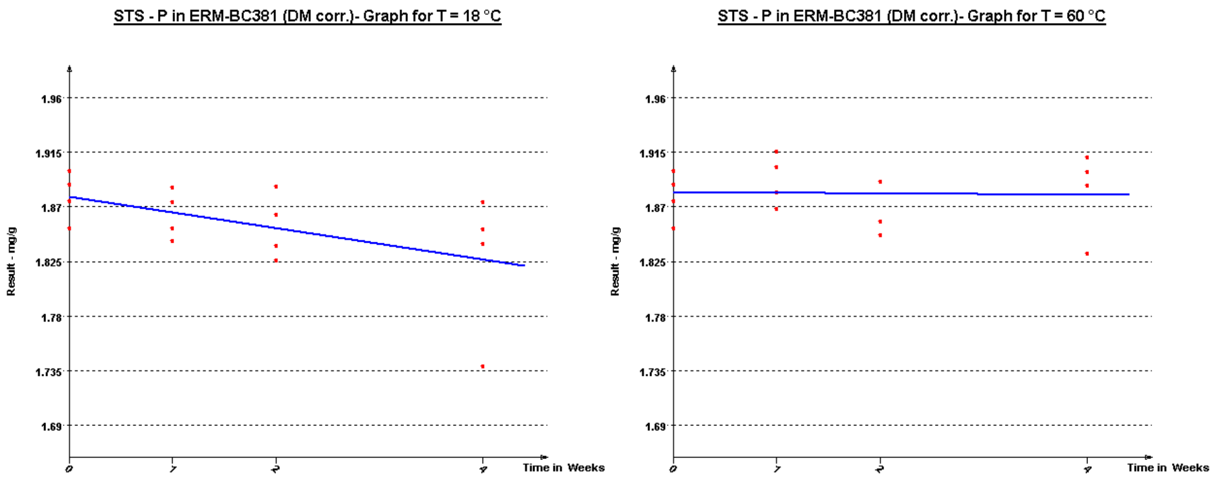


Figure B8: Short-term stability of phosphorus content in ERM®-BC381 at 18 and 60 °C.

Annex B2 ERM®-BC382 (wheat flour) – Results of the short-term stability study

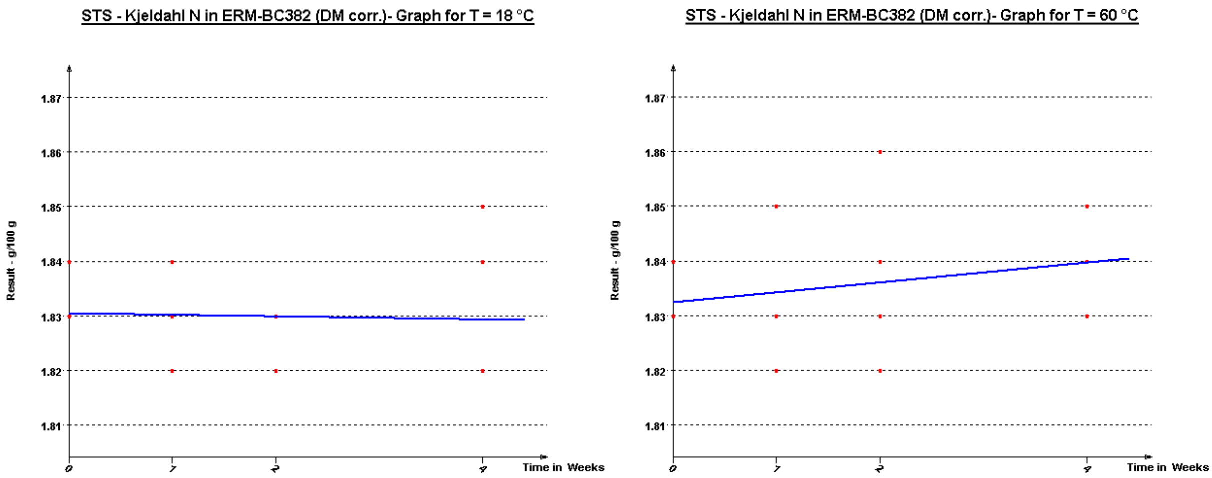


Figure B9: Short-term stability of Kjeldahl nitrogen content in ERM®-BC382 at 18 and 60 °C.

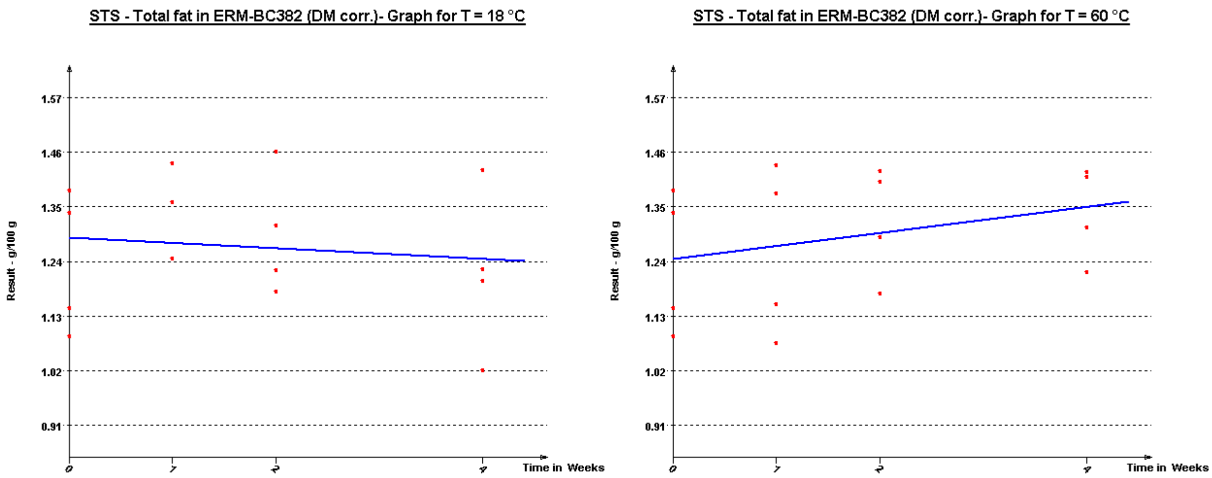
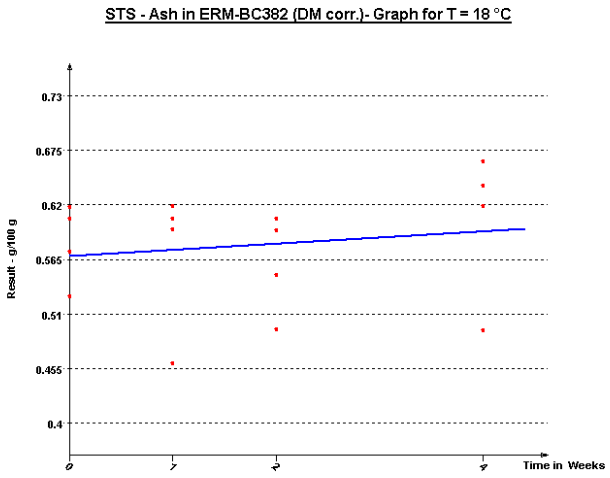


Figure B10: Short-term stability of total fat content in ERM®-BC382 at 18 and 60 °C.



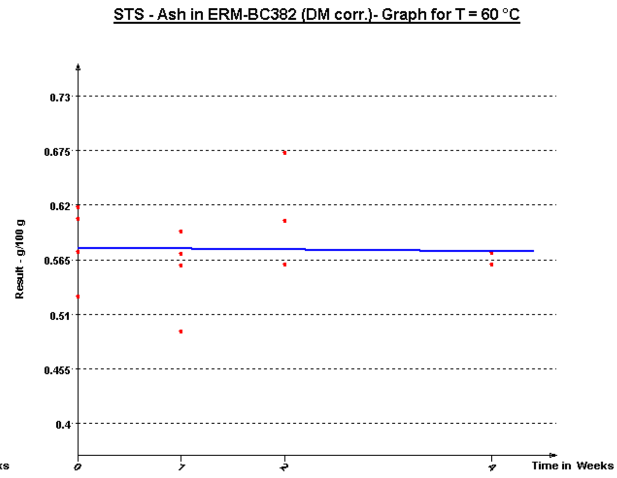
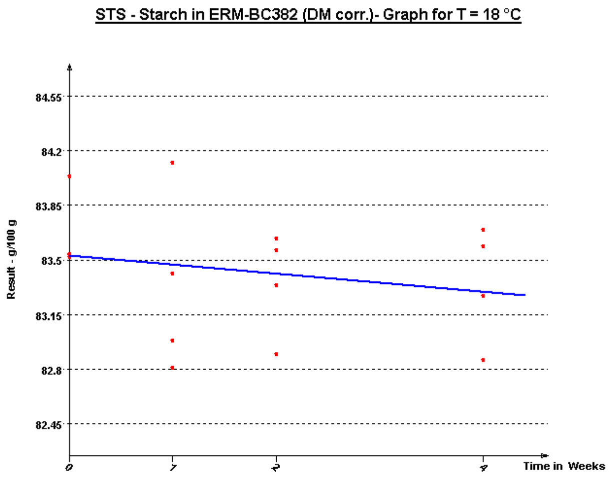


Figure B11: Short-term stability of ash content in ERM®-BC382 at 18 and 60 °C.



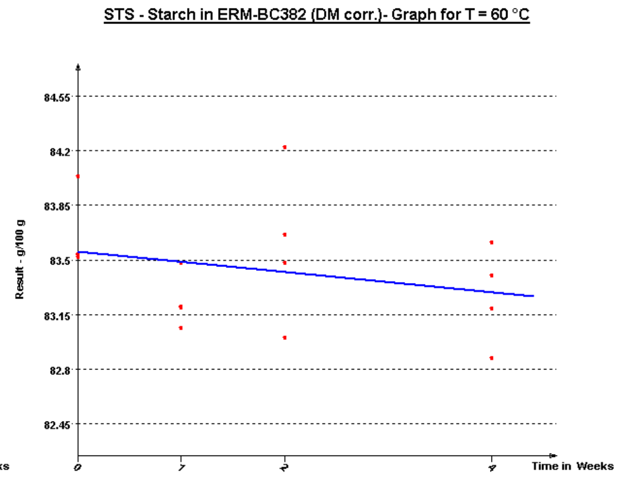
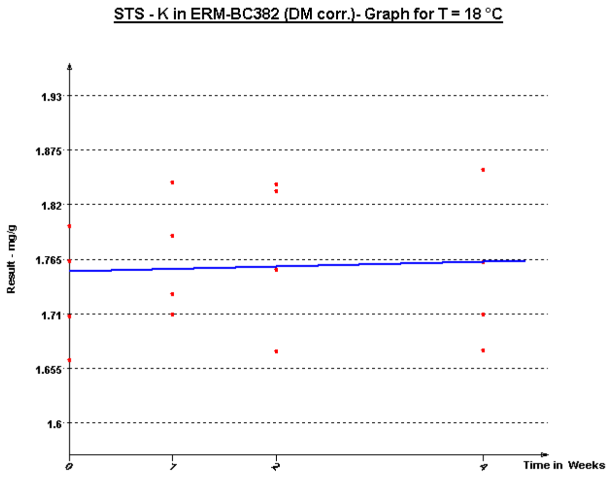


Figure B12: Short-term stability of starch content in ERM®-BC382 at 18 and 60 °C.



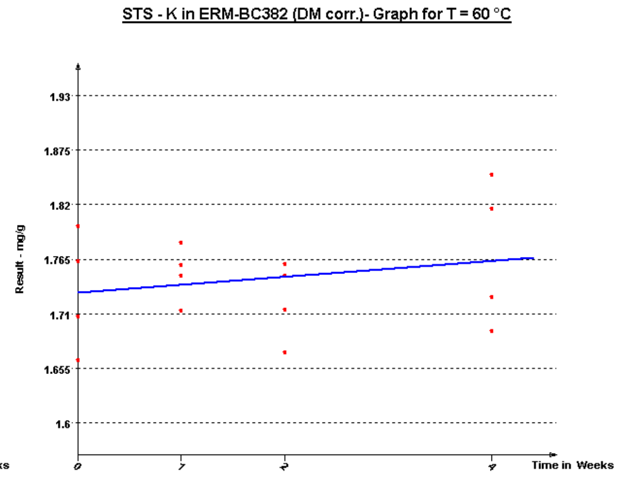


Figure B13: Short-term stability of potassium content in ERM®-BC382 at 18 and 60 °C.

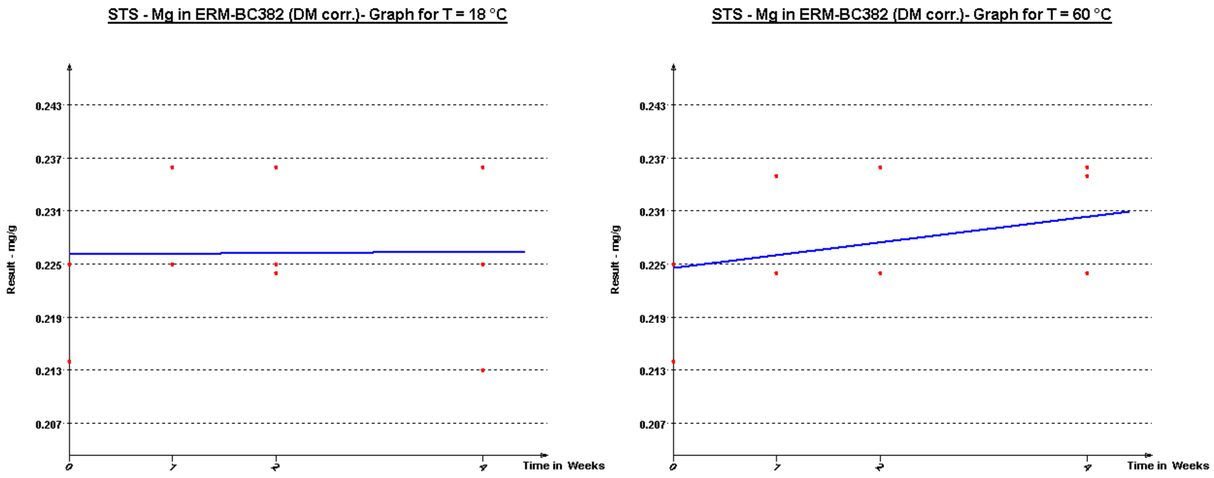


Figure B14: Short-term stability of magnesium content in ERM®-BC382 at 18 and 60 °C.

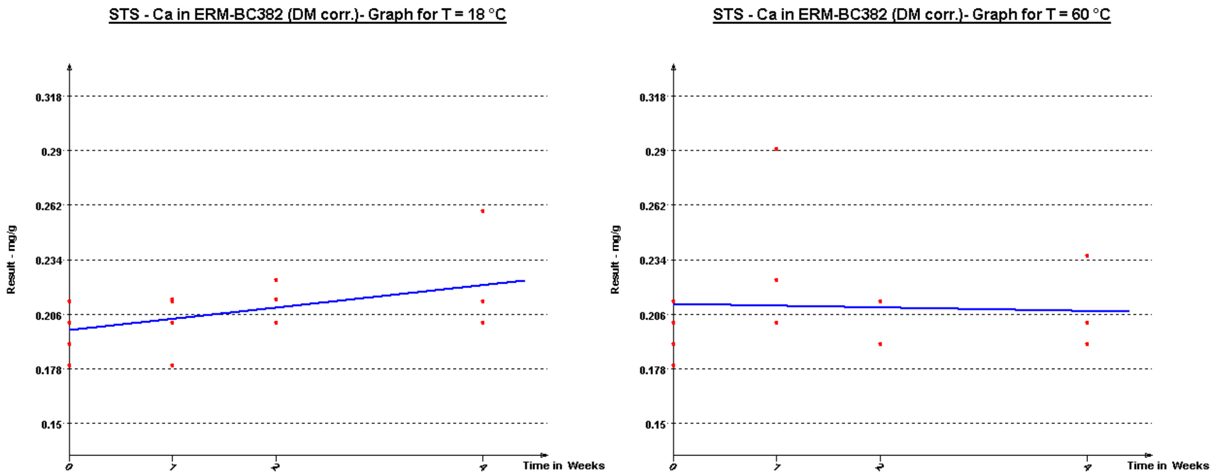


Figure B15: Short-term stability of calcium content in ERM®-BC382 at 18 and 60 °C.

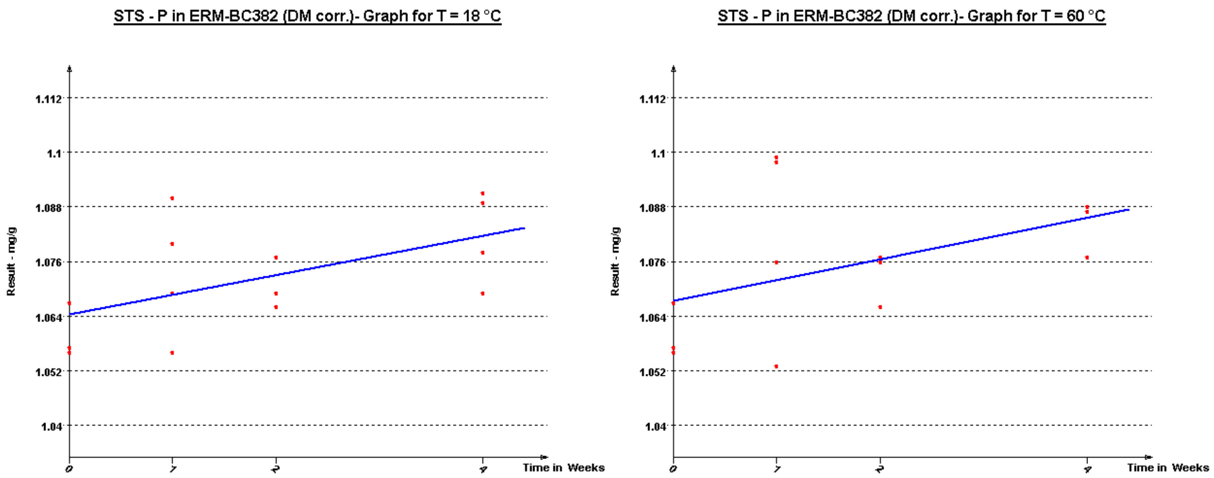
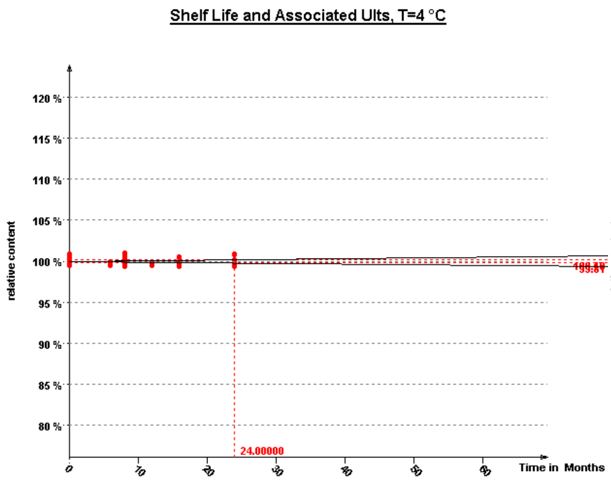


Figure B16: Short-term stability of phosphorus content in ERM®-BC382 at 18 and 60 °C.

Annex C1 ERM®-BC381 (rye flour) – Results of the long-term stability study



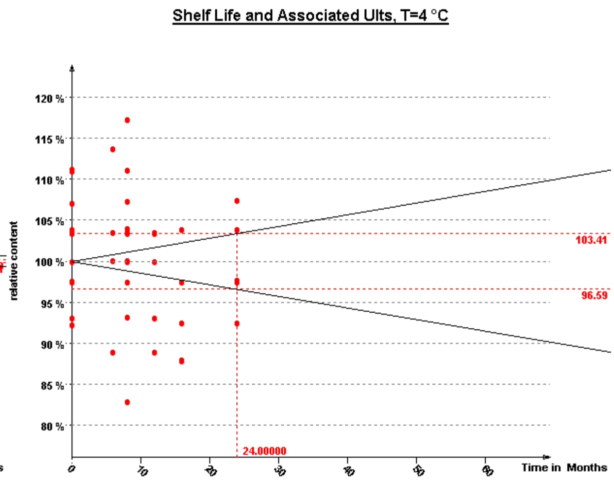
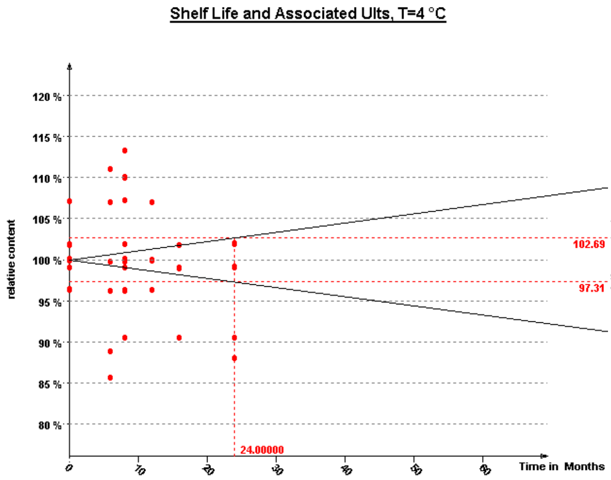


Figure C1 (left): Long-term stability of Kjeldahl nitrogen content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C2 (right): Long-term stability of total fat content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.



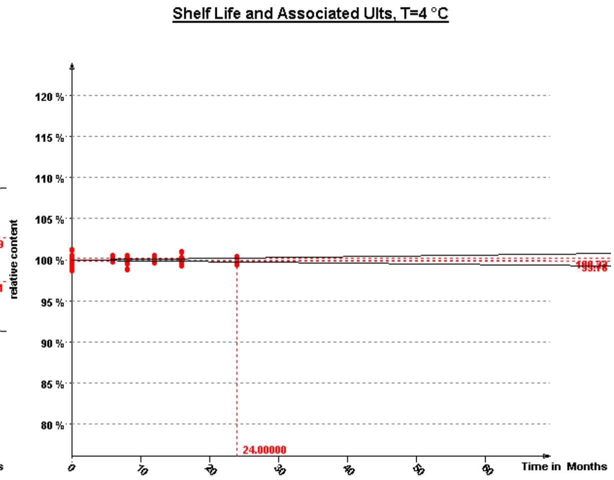
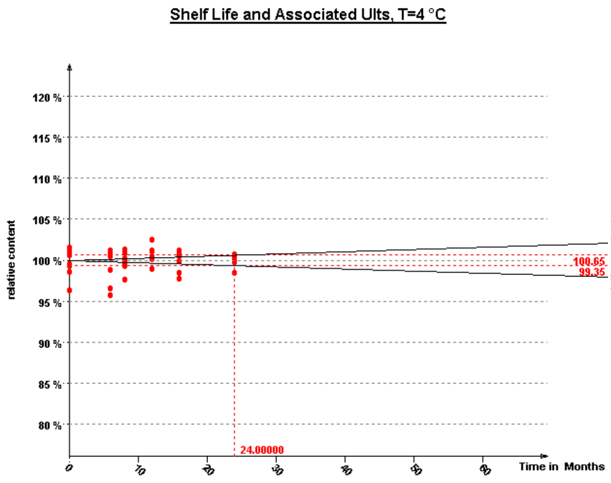


Figure C3 (left): Long-term stability of ash content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C4 (right): Long-term stability of starch content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.



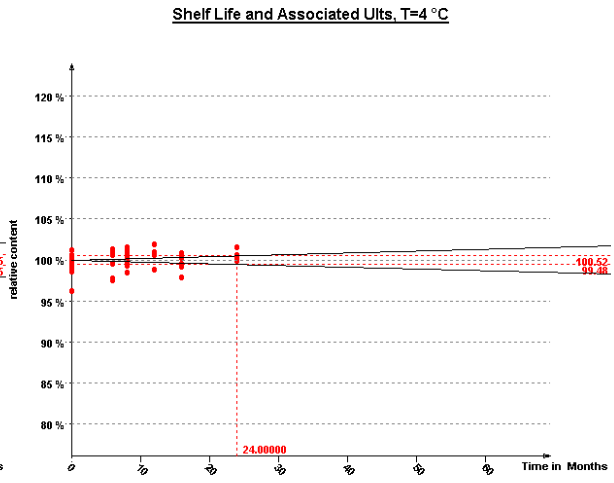
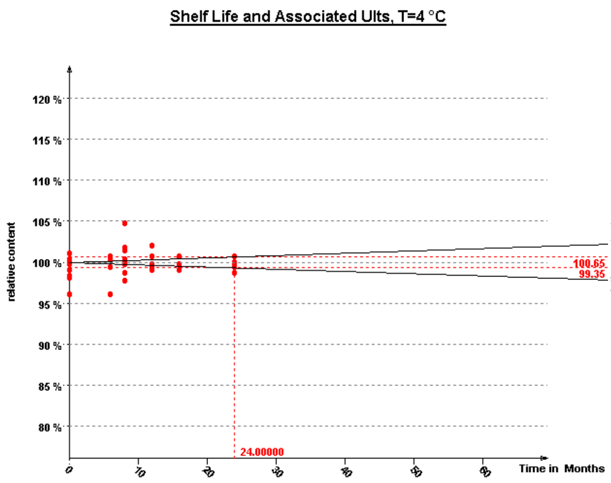


Figure C5 (left): Long-term stability of potassium content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C6 (right): Long-term stability of magnesium content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.



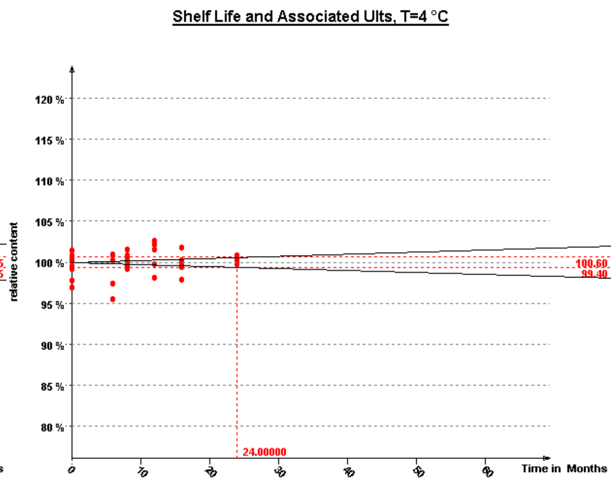
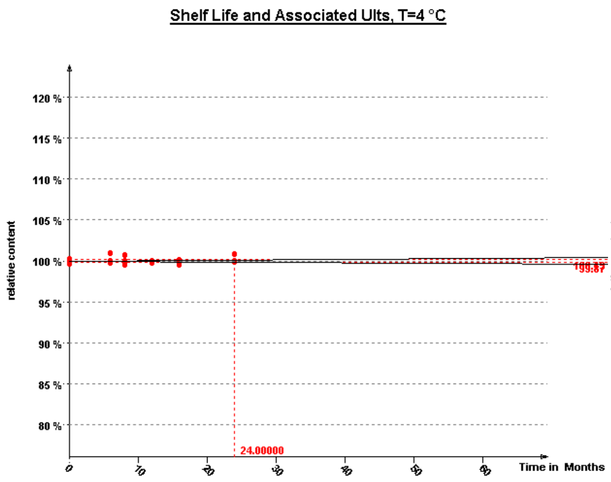


Figure C7 (left): Long-term stability of calcium content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C8 (right): Long-term stability of phosphorus content in ERM®-BC381 at 4 °C with associated u_{lts} for storage period of 24 months.

Annex C2 ERM®-BC382 (wheat flour) – Results of the long-term stability study



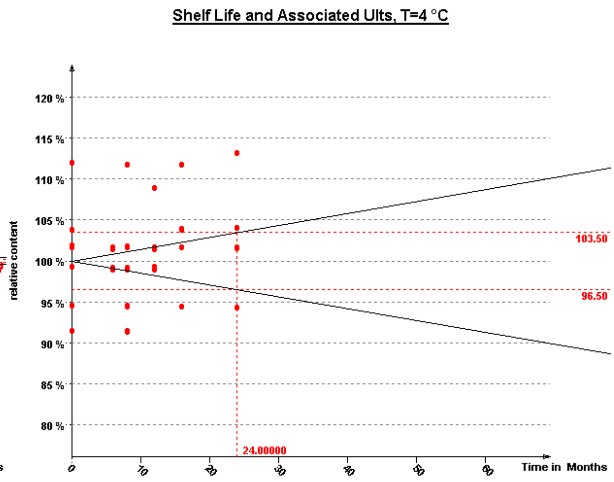
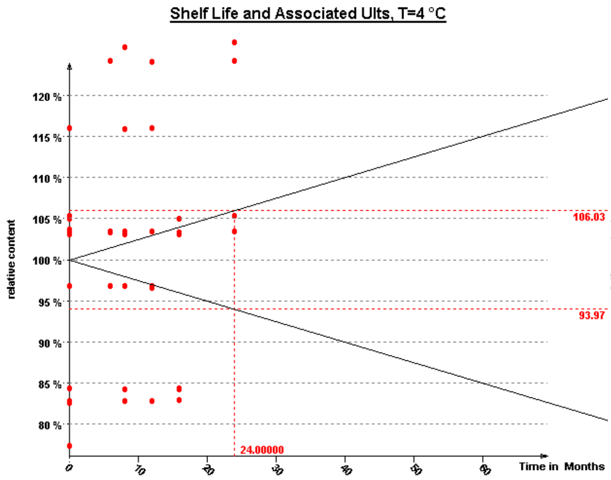


Figure C9 (left): Long-term stability of Kjeldahl nitrogen content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C10 (right): Long-term stability of total fat content in ERM®-BC382 at 4 °C with~ associated u_{lts} for storage period of 24 months.



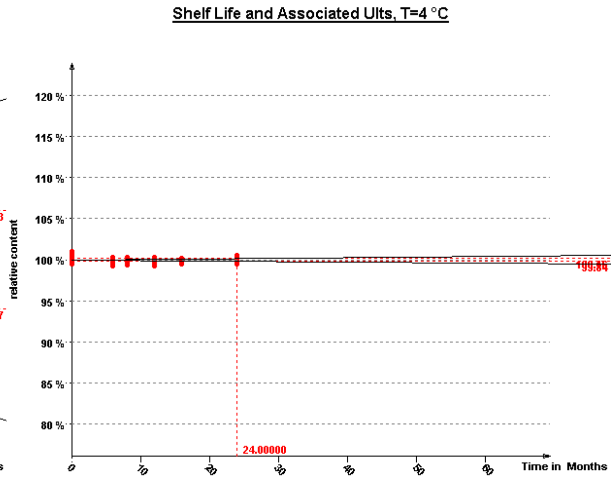
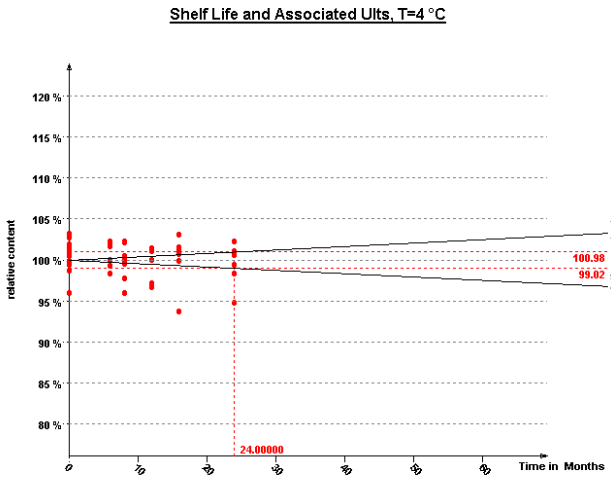


Figure C11 (left): Long-term stability of ash content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C12 (right): Long-term stability of starch content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.



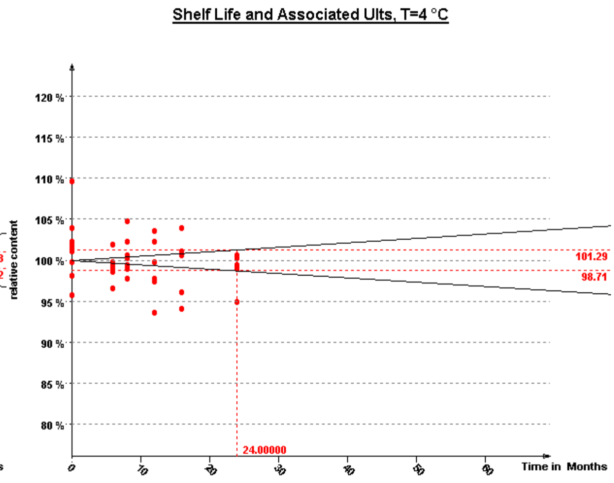
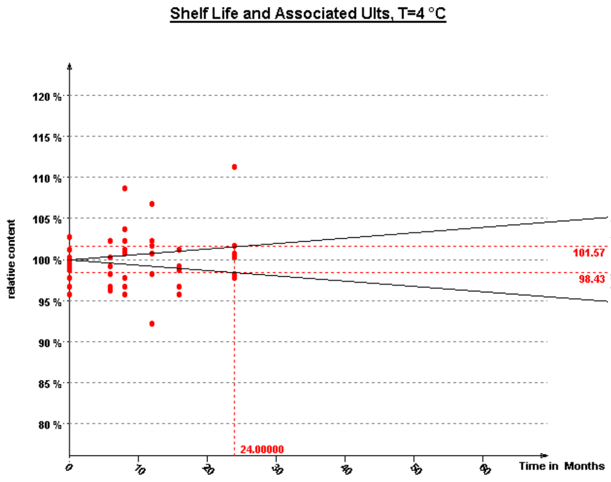


Figure C13 (left): Long-term stability of potassium content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C14 (right): Long-term stability of magnesium content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.



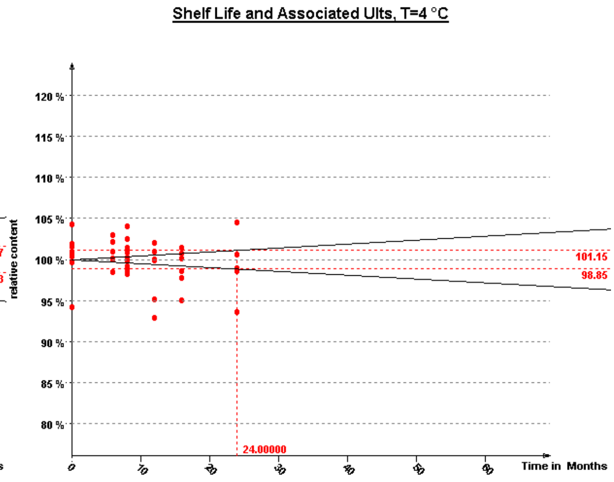


Figure C15 (left): Long-term stability of calcium content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.

Figure C16 (right): Long-term stability of phosphorus content in ERM®-BC382 at 4 °C with associated u_{lts} for storage period of 24 months.

Annex D1 ERM®-BC381 (rye flour) – Characterisation data

Table D1: Results of characterisation measurements of Kjeldahl nitrogen content in ERM®-BC381 (related to dry mass)

Lab code	Kjeldahl nitrogen mass fraction in ERM®-BC381 (rye flour) [g/100 g]					
	Day 1			Day 2		
1	1.57	1.55	1.55	1.55	1.55	1.58
2	1.54	1.56	1.56	1.58	1.57	1.56
3	1.57	1.55	1.56	1.57	1.56	1.57
4	1.59	1.58	1.58	1.57	1.58	1.58
5	1.59	1.59	1.59	1.58	1.57	1.57
6	1.54	1.55	1.55	1.55	1.55	1.55
7	1.56	1.54	1.54	1.55	1.56	1.54
9	1.53	1.53	1.53	1.54	1.54	1.55
10	1.61	1.58	1.59	1.56	1.58	1.58
11	1.58	1.56	1.56	1.55	1.56	1.56

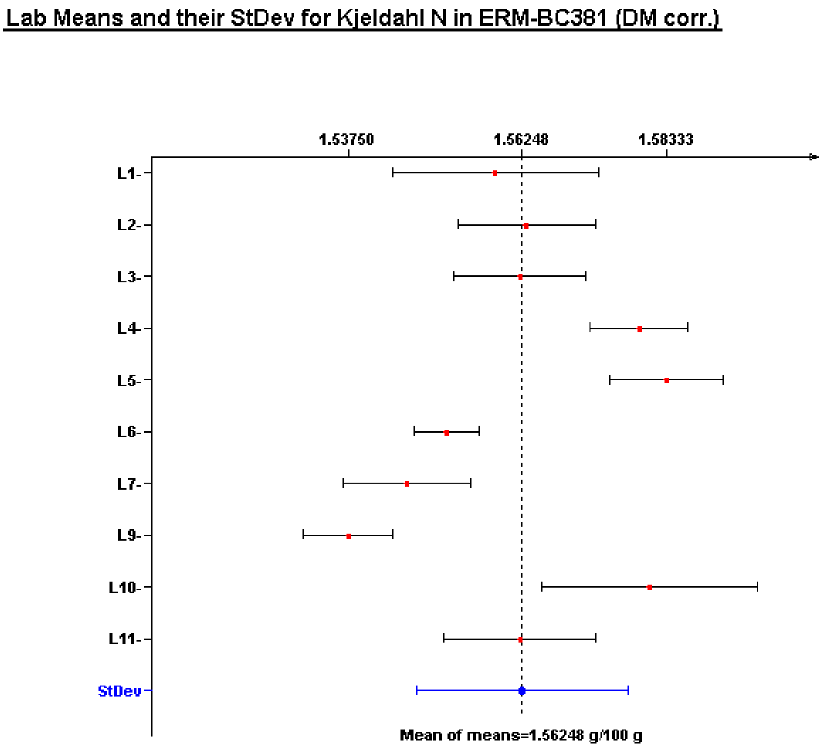


Figure D1: Laboratory means, mean of means and corresponding standard deviations for Kjeldahl nitrogen in ERM®-BC381 (related to dry mass)

Table D2: Results of characterisation measurements of total fat content in ERM®-BC381 (related to dry mass)

Lab code	Total fat mass fraction in ERM®-BC381 (rye flour) [g/100 g]					
	Day 1			Day 2		
1	1.24	1.27	1.30	1.26	1.30	1.26
2	1.13	1.19	1.18	1.20	1.31	1.17
3	1.42	1.39	1.38	1.53	1.47	1.51
4	1.32	1.32	1.20	1.37	1.26	1.23
5	1.29	1.30	1.34	1.28	1.30	1.37
6	1.44	1.44	1.37	1.46	1.44	1.41
7	1.54	1.46	1.64	1.51	1.47	1.65
8	1.01	0.95	0.94	1.24	1.13	1.36
9	1.11	1.28	1.24	1.26	1.14	1.12
10	1.62	1.50	1.36	1.58	1.58	1.50
11	1.73	1.64	1.55	1.66	1.70	1.56

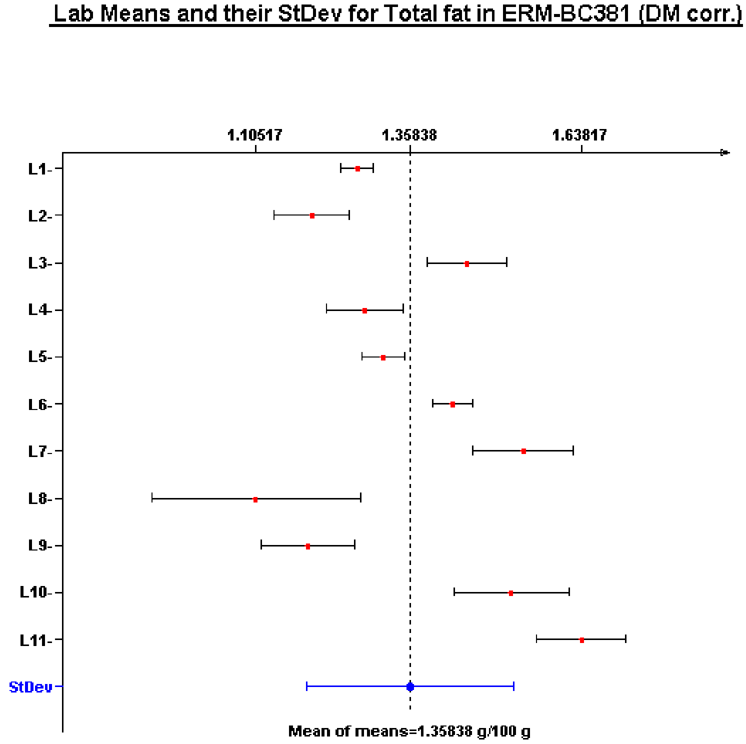


Figure D2: Laboratory means, mean of means and corresponding standard deviations for total fat in ERM®-BC381 (related to dry mass)

Table D3: Results of characterisation measurements of ash content in ERM®-BC381 (related to dry mass)

Lab code	Ash mass fraction in ERM®-BC381 (rye flour) [g/100 g] Day 1			Day 2		
1	1.18	1.13	1.16	1.13	1.15	1.11
2	1.28	1.26	1.28	1.12	1.12	1.17
3	0.99	1.00	0.97	1.00	1.03	0.92
4	0.97	0.73	1.06	0.93	0.79	1.11
5	1.17	1.04	1.11	1.06	0.95	1.05
6	0.95	0.99	1.04	1.13	1.17	1.08
7	1.23	1.23	1.23	1.22	1.21	1.22
8	1.06	1.04	1.09	1.10	1.06	1.10
9	1.10	1.14	1.12	1.07	1.08	1.10
10	0.92	1.01	1.01	0.99	0.98	0.98

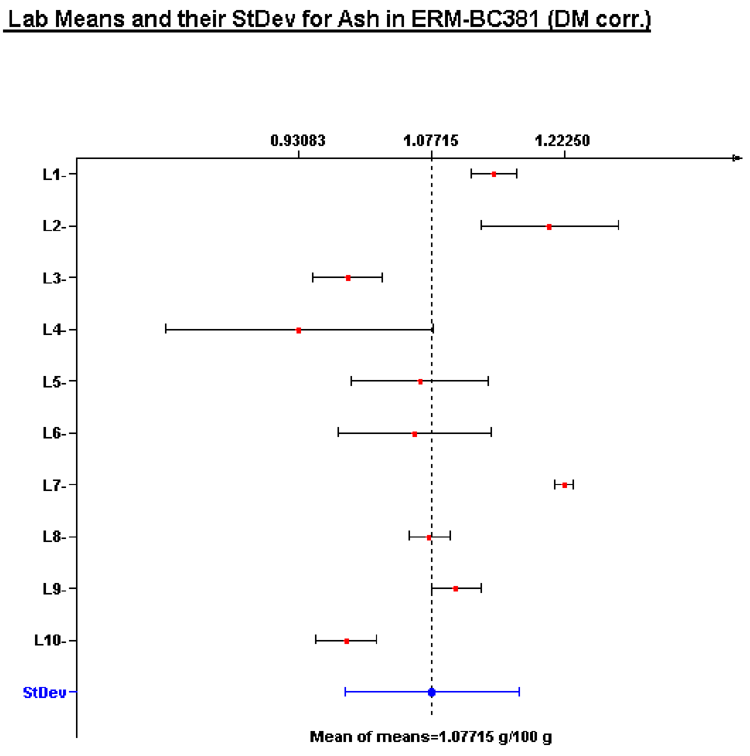


Figure D3: Laboratory means, mean of means and corresponding standard deviations for ash in ERM®-BC381 (related to dry mass)

Table D4: Results of characterisation measurements of starch content in ERM®-BC381 (related to dry mass)

	Starch mass fraction in ERM®-BC381 (rye flour) [g/100 g]					
Lab code	**Day 1**			**Day 2**		
2	73.16	73.27	73.16	73.48	73.15	73.31
4	72.13	72.28	72.36	71.34	71.04	71.35
6	70.27	70.37	70.47	70.63	70.62	70.83
7	73.11	74.21	74.17	74.05	74.32	74.26
8	73.83	73.50	73.23	74.84	74.97	74.93
10	73.84	73.59	73.79	73.50	73.81	74.12
11	68.57	67.40	67.46	68.01	68.46	68.23

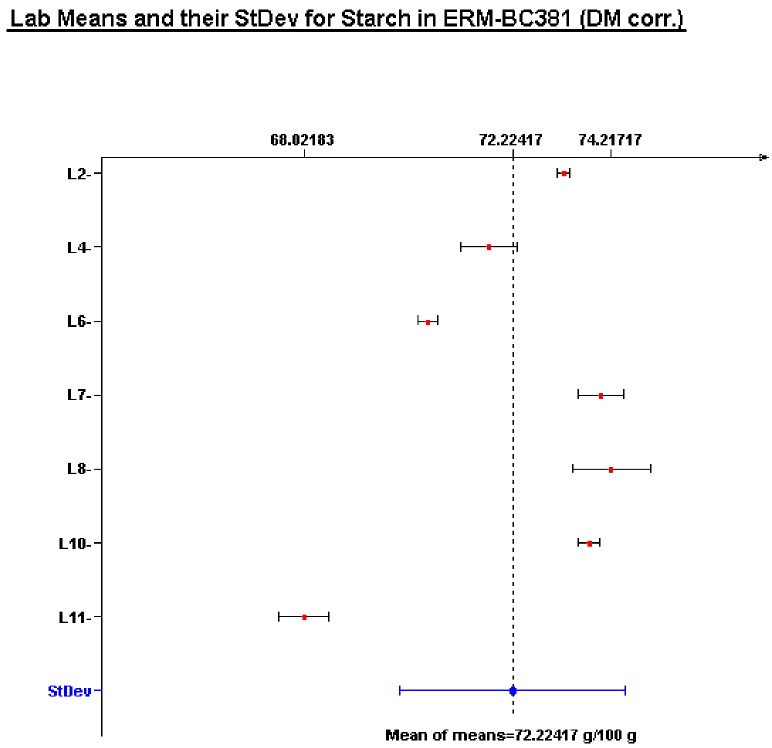


Figure D4: Laboratory means, mean of means and corresponding standard deviations for starch in ERM®-BC381 (related to dry mass)

Table D5: Results of characterisation measurements of potassium content in ERM®-BC381 (related to dry mass)

	Potassium mass fraction in ERM®-BC381 (rye flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	3.22	3.33	3.28	3.29	3.27	3.31
2	3.11	2.94	3.38	3.44	3.39	2.96
3	3.37	3.33	3.32	3.26	3.32	3.30
4	3.18	3.35	2.98	3.17	3.53	3.35
5	3.34	3.32	3.31	3.41	3.38	3.37
6	3.60	3.60	3.60	3.49	3.49	3.60
7	3.19	3.25	3.17	3.13	3.11	3.04
8	3.21	3.10	3.34	3.38	3.37	3.47
9	3.44	3.41	3.40	3.29	3.15	3.08
10	3.70	3.69	3.62	3.64	3.68	3.49
11	3.50	3.57	3.55	3.45	3.38	3.39

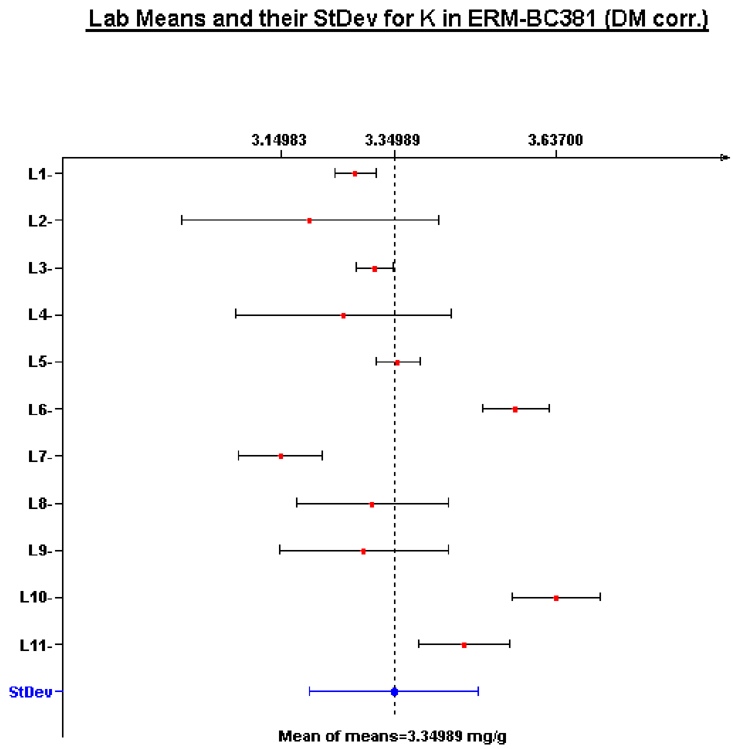


Figure D5: Laboratory means, mean of means and corresponding standard deviations for potassium in ERM®-BC381 (related to dry mass)

Table D6: Results of characterisation measurements of magnesium content in ERM®-BC381 (related to dry mass)

	Magnesium mass fraction in ERM®-BC381 (rye flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	0.57	0.57	0.55	0.57	0.56	0.55
2	0.57	0.57	0.56	0.57	0.56	0.58
4	0.53	0.55	0.50	0.52	0.58	0.55
5	0.56	0.55	0.55	0.57	0.56	0.57
6	0.56	0.56	0.56	0.56	0.56	0.56
7	0.54	0.54	0.53	0.59	0.58	0.59
8	0.60	0.60	0.60	0.58	0.53	0.57
9	0.68	0.58	0.56	0.58	0.58	0.58
10	0.56	0.56	0.56	0.54	0.57	0.55
11	0.58	0.60	0.59	0.58	0.58	0.59

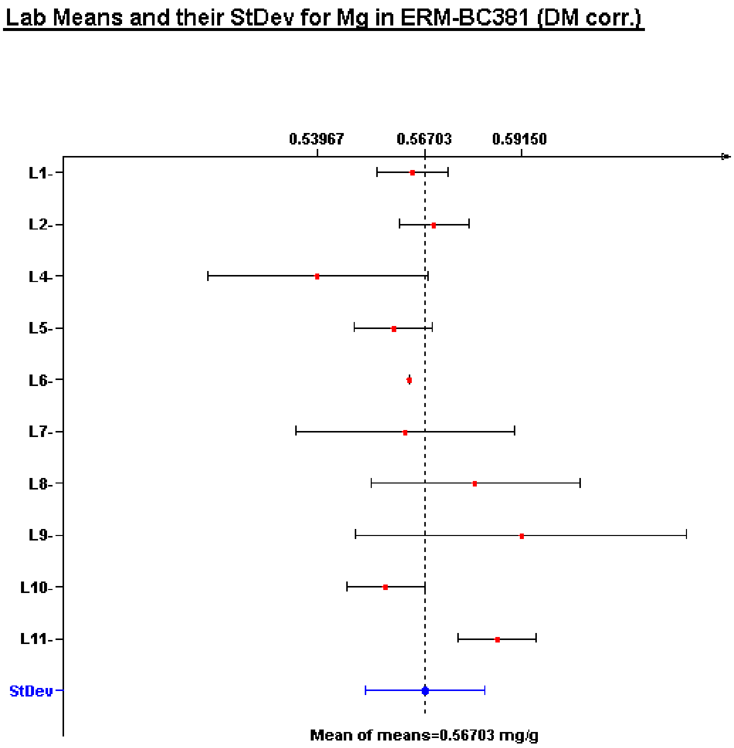


Figure D6: Laboratory means, mean of means and corresponding standard deviations for magnesium in ERM®-BC381 (related to dry mass)

Table D7: Results of characterisation measurements of calcium content in ERM®-BC381 (related to dry mass)

	Calcium mass fraction in ERM®-BC381 (rye flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	0.33	0.34	0.33	0.33	0.37	0.33
2	0.31	0.30	0.31	0.30	0.30	0.31
3	0.31	0.30	0.30	0.30	0.30	0.30
4	0.28	0.32	0.28	0.31	0.28	0.32
5	0.30	0.30	0.29	0.30	0.30	0.31
7	0.34	0.33	0.34	0.31	0.32	0.32
8	0.31	0.28	0.30	0.39	0.32	0.32
9	0.31	0.32	0.33	0.31	0.29	0.29
11	0.35	0.34	0.35	0.33	0.35	0.33

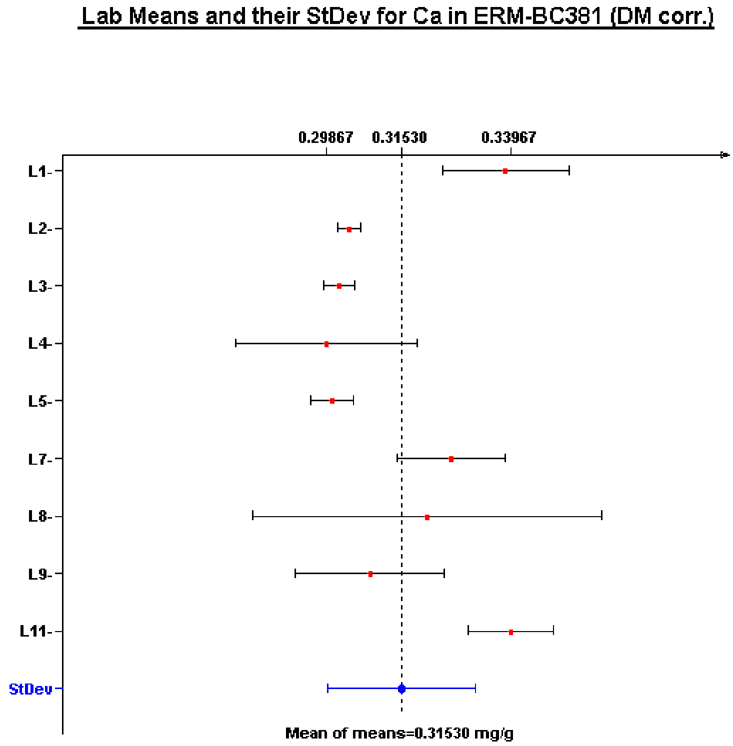


Figure D7: Laboratory means, mean of means and corresponding standard deviations for calcium in ERM®-BC381 (related to dry mass)

Table D8: Results of characterisation measurements of phosphorus content in ERM®-BC381 (related to dry mass)

Lab code	Phosphorus mass fraction in ERM®-BC381 (rye flour) [mg/g]					
	Day 1			Day 2		
1	1.95	1.92	1.97	1.95	1.92	1.94
2	1.99	1.96	1.95	1.95	1.93	2.04
3	1.94	1.91	1.93	1.94	1.95	1.96
4	1.83	1.95	1.73	1.76	1.85	1.85
5	2.03	2.06	2.02	2.08	2.05	2.05
6	2.03	2.03	2.03	2.03	2.03	2.03
7	2.02	2.06	1.94	2.04	2.05	2.03
8	2.18	2.00	2.13	2.26	2.25	2.25
9	2.03	2.01	1.97	2.01	2.00	2.00
10	2.16	2.15	2.18	2.16	2.16	2.18
11	2.01	2.04	2.02	1.98	1.96	1.99

Lab Means and their StDev for P in ERM-BC381 (DM corr.)

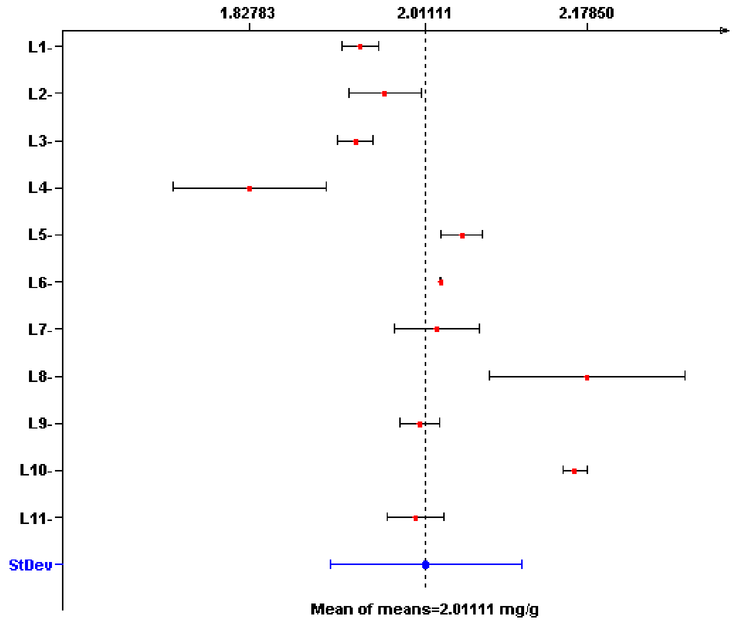


Figure D8: Laboratory means, mean of means and corresponding standard deviations for phosphorus in ERM®-BC381 (related to dry mass)

Annex D2 ERM®-BC382 (wheat flour) – Characterisation data

Table D9: Results of characterisation measurements of Kjeldahl nitrogen content in ERM®-BC382 (related to dry mass)

Lab code	Kjeldahl nitrogen mass fraction in ERM®-BC382 (wheat flour) [g/100 g]					
	Day 1			Day 2		
1	1.84	1.85	1.84	1.86	1.85	1.86
2	1.84	1.83	1.85	1.85	1.87	1.83
3	1.85	1.85	1.84	1.86	1.85	1.84
4	1.93	1.88	1.87	1.86	1.85	1.87
5	1.87	1.88	1.88	1.86	1.87	1.88
6	1.85	1.86	1.84	1.86	1.86	1.86
7	1.81	1.81	1.81	1.84	1.81	1.81
9	1.84	1.80	1.80	1.83	1.82	1.82
10	1.86	1.86	1.88	1.88	1.87	1.86
11	1.84	1.84	1.85	1.84	1.85	1.90

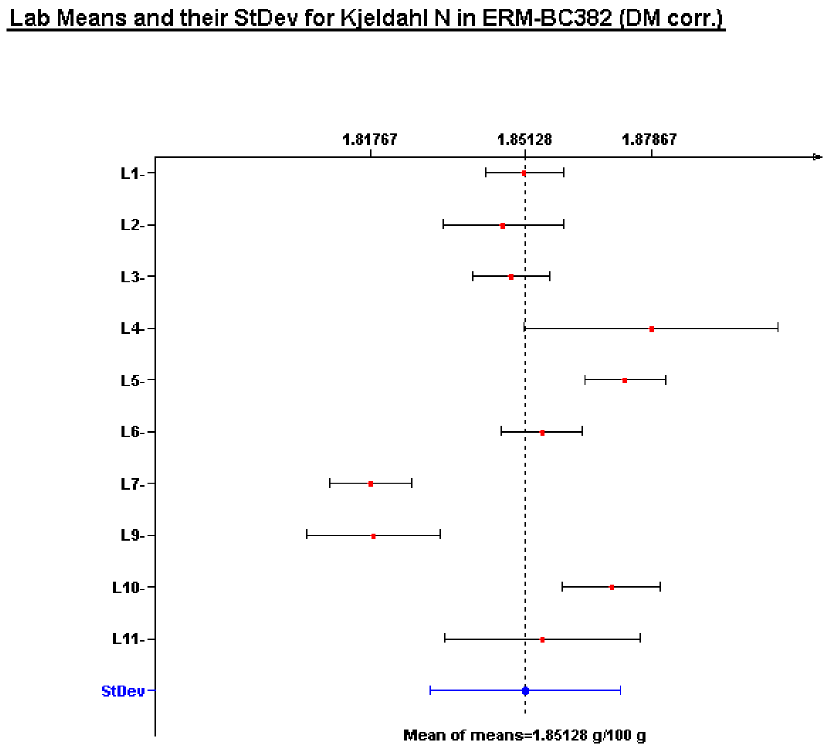


Figure D9: Laboratory means, mean of means and corresponding standard deviations for Kjeldahl nitrogen in ERM®-BC382 (related to dry mass)

Table D10: Results of characterisation measurements of total fat content in ERM®-BC382 (related to dry mass)

Lab code	Total fat mass fraction in ERM®-BC382 (wheat flour) [g/100 g]					
	Day 1			Day 2		
1	1.12	1.09	1.08	1.18	1.15	1.15
2	1.29	1.42	1.29	1.28	1.31	1.32
3	1.34	1.36	1.37	1.52	1.45	1.49
4	1.31	1.33	1.29	1.28	1.49	1.22
5	1.42	1.44	1.45	1.46	1.42	1.40
6	1.54	1.58	1.46	1.58	1.55	1.63
7	1.27	1.37	1.46	1.23	1.24	1.39
9	1.24	1.04	0.92	1.36	1.25	1.24
10	1.37	1.43	1.34	1.21	1.42	1.37
11	1.55	1.66	1.55	1.70	1.50	1.69

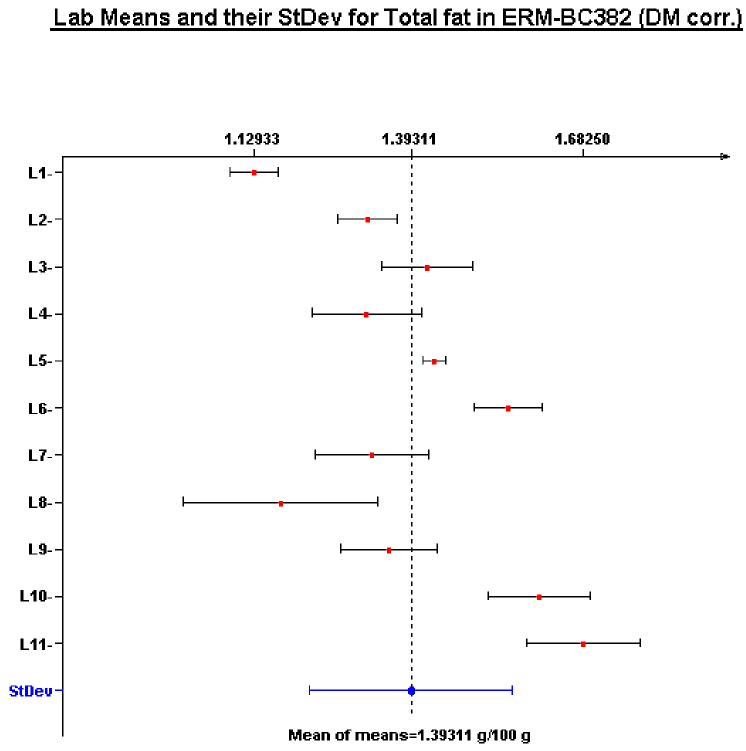


Figure D10: Laboratory means, mean of means and corresponding standard deviations for total fat in ERM®-BC382 (related to dry mass)

Table D11: Results of characterisation measurements of ash content in ERM®-BC382 (related to dry mass)

Lab code	Ash mass fraction in ERM®-BC382 (wheat flour) [g/100 g]					
	Day 1			Day 2		
1	0.61	0.66	0.65	0.59	0.68	0.62
2	0.74	0.81	0.65	0.63	0.70	0.63
3	0.45	0.45	0.56	0.55	0.56	0.54
4	0.52	0.49	0.61	0.55	0.56	0.67
5	0.67	0.66	0.67	0.52	0.61	0.58
6	0.59	0.50	0.63	0.68	0.63	0.54
7	0.63	0.68	0.64	0.63	0.66	0.63
8	0.59	0.63	0.60	0.61	0.62	0.61
9	0.65	0.64	0.64	0.58	0.62	0.62
10	0.52	0.47	0.45	0.50	0.51	0.51

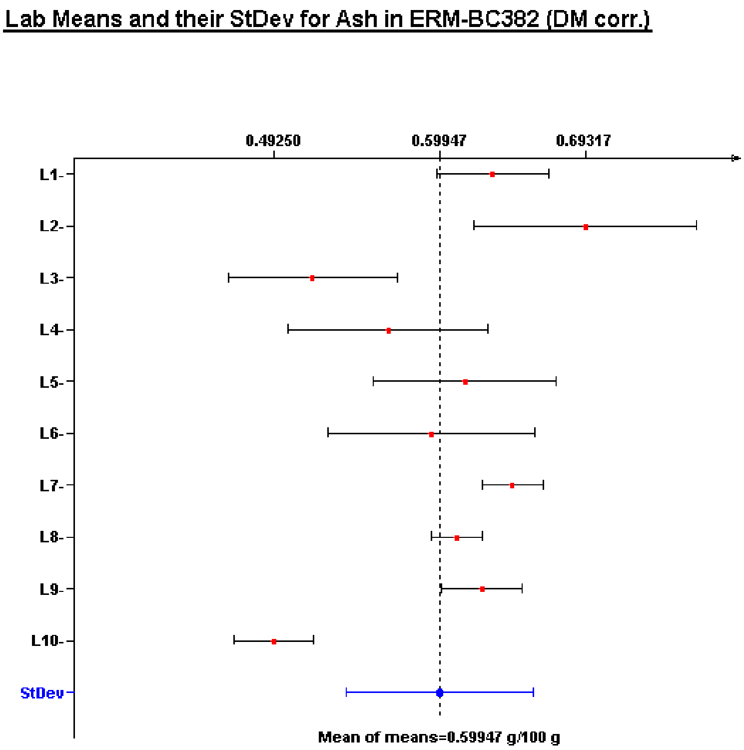


Figure D11: Laboratory means, mean of means and corresponding standard deviations for ash in ERM®-BC382 (related to dry mass)

Table D12: Results of characterisation measurements of starch content in ERM®-BC382 (related to dry mass)

Lab code	Starch mass fraction in ERM®-BC382 (wheat flour) [g/100 g]					
	Day 1			Day 2		
2	81.99	82.08	82.16	81.40	82.13	82.27
4	79.58	79.91	80.01	80.09	79.45	79.90
6	80.50	80.38	80.42	80.14	80.01	80.53
7	84.77	84.38	82.82	84.93	84.72	82.93
8	81.29	81.95	82.37	81.85	84.10	83.84
10	82.11	81.62	81.73	82.23	81.73	81.96
11	76.74	77.12	77.50	78.54	77.34	78.28

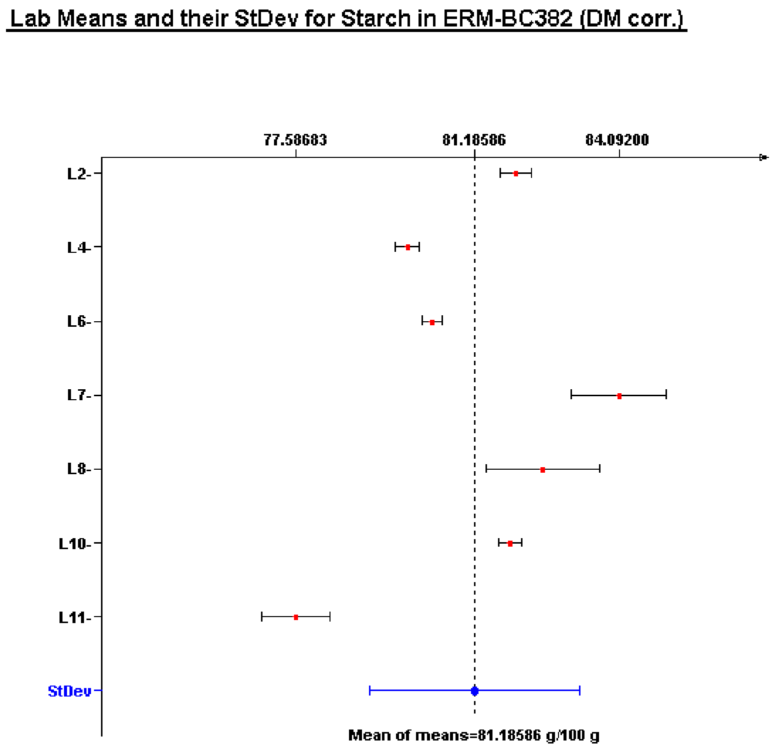


Figure D12: Laboratory means, mean of means and corresponding standard deviations for starch in ERM®-BC382 (related to dry mass)

Table D13: Results of characterisation measurements of potassium content in ERM®-BC382 (related to dry mass)

	Potassium mass fraction in ERM®-BC382 (wheat flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	1.77	1.78	1.85	1.81	1.78	1.79
2	1.89	1.87	1.87	1.91	1.88	1.82
3	1.88	1.89	1.85	1.85	1.84	1.87
4	1.77	2.02	1.92	2.16	2.04	2.10
5	1.85	1.89	1.82	1.83	1.84	1.85
6	2.03	1.92	1.92	2.03	2.37	1.92
7	1.79	1.71	1.74	1.62	1.66	1.64
8	1.80	1.80	1.85	1.79	1.82	1.80
9	1.84	1.85	1.83	1.65	1.64	1.62
10	2.09	2.12	2.10	1.97	2.01	2.01
11	1.98	1.97	1.98	1.86	1.90	1.90

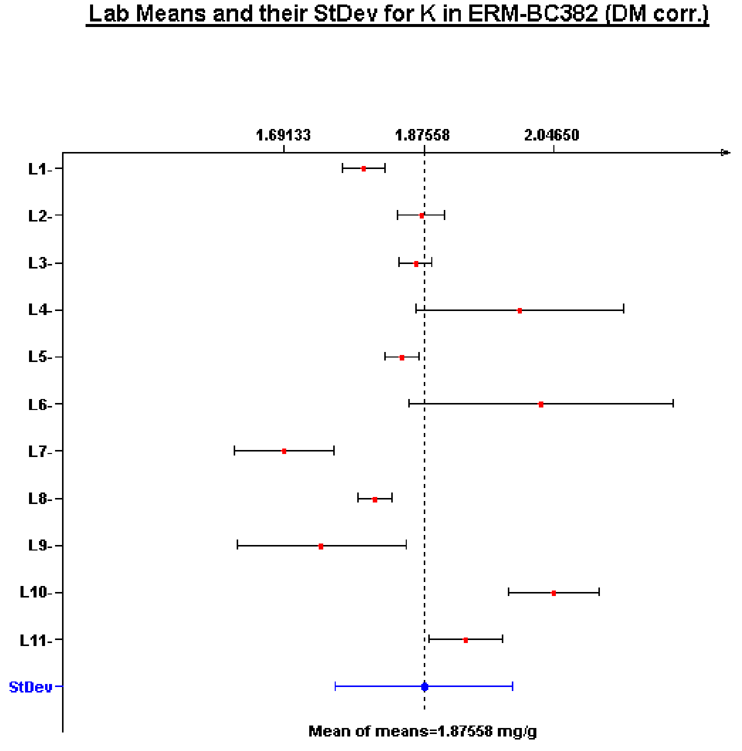


Figure D13: Laboratory means, mean of means and corresponding standard deviations for potassium in ERM®-BC382 (related to dry mass)

Table D14: Results of characterisation measurements of magnesium content in ERM®-BC382 (related to dry mass)

	Magnesium mass fraction in ERM®-BC382 (wheat flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	0.23	0.23	0.23	0.24	0.24	0.25
2	0.24	0.24	0.24	0.24	0.24	0.24
4	0.23	0.26	0.26	0.28	0.26	0.27
5	0.23	0.24	0.24	0.24	0.24	0.24
7	0.23	0.23	0.24	0.24	0.24	0.24
8	0.26	0.26	0.26	0.25	0.25	0.25
9	0.26	0.25	0.25	0.24	0.26	0.24
10	0.25	0.24	0.24	0.24	0.24	0.25
11	0.26	0.26	0.26	0.25	0.26	0.26

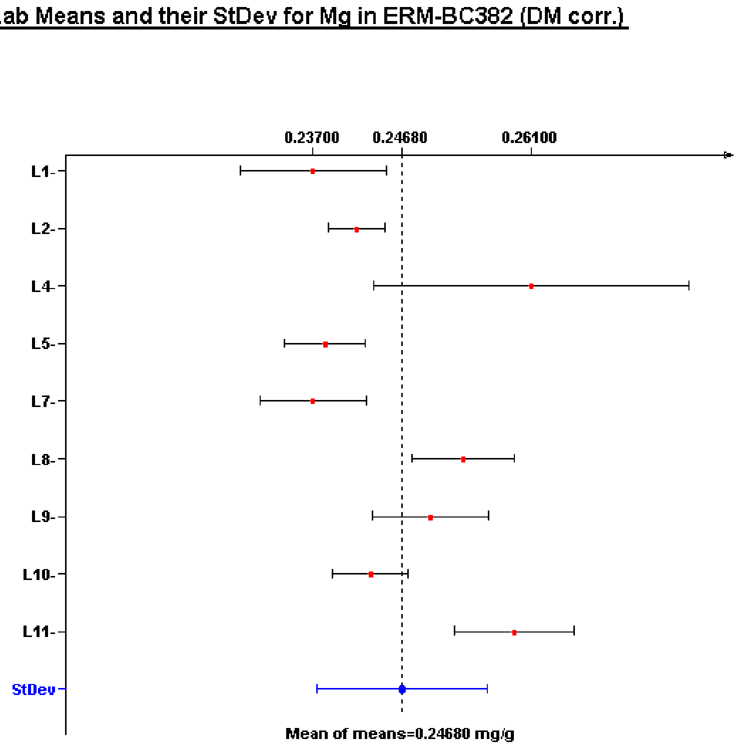


Figure D14: Laboratory means, mean of means and corresponding standard deviations for magnesium in ERM®-BC382 (related to dry mass)

Table D15: Results of characterisation measurements of calcium content in ERM®-BC382 (related to dry mass)

Lab code	Calcium mass fraction in ERM®-BC382 (wheat flour) [mg/g]					
	Day 1			Day 2		
1	0.19	0.20	0.20	0.20	0.18	0.19
2	0.20	0.20	0.23	0.20	0.20	0.19
3	0.20	0.20	0.19	0.20	0.20	0.20
4	0.21	0.23	0.22	0.28	0.28	0.24
5	0.19	0.19	0.19	0.19	0.20	0.20
7	0.21	0.21	0.22	0.20	0.20	0.20
8	0.22	0.22	0.21	0.21	0.20	0.20
9	0.22	0.20	0.22	0.20	0.23	0.19
11	0.23	0.22	0.24	0.23	0.23	0.24

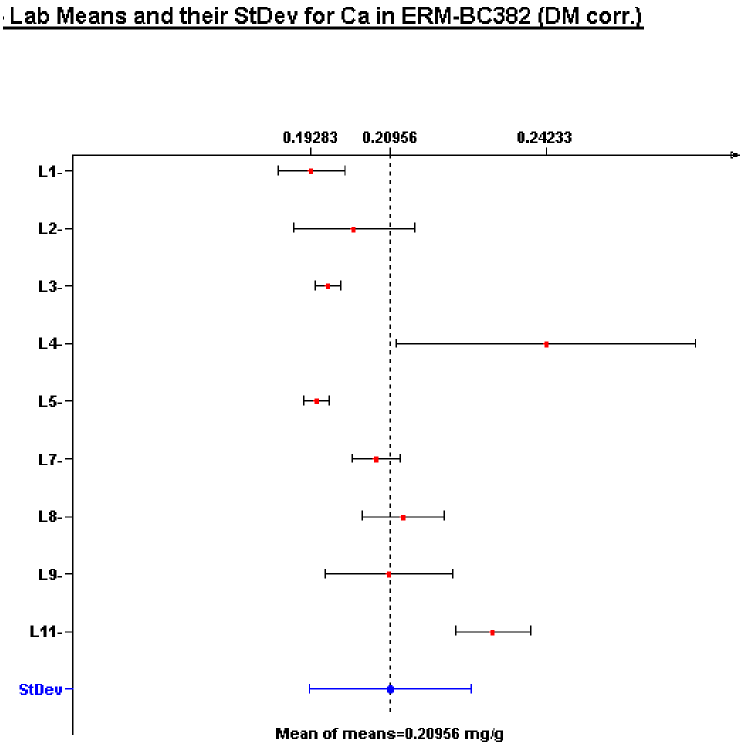


Figure D15: Laboratory means, mean of means and corresponding standard deviations for calcium in ERM®-BC382 (related to dry mass)

Table D16: Results of characterisation measurements of phosphorus content in ERM®-BC382 (related to dry mass)

	Phosphorus mass fraction in ERM®-BC382 (wheat flour) [mg/g]					
Lab code	**Day 1**			**Day 2**		
1	1.11	1.13	1.13	1.13	1.14	1.13
2	1.11	1.12	1.11	1.14	1.12	1.08
3	1.13	1.13	1.11	1.13	1.13	1.17
4	0.93	1.04	1.09	1.16	1.07	1.24
5	1.16	1.19	1.16	1.17	1.18	1.18
6	1.24	1.24	1.24	1.24	1.47	1.24
7	1.20	1.21	1.20	1.20	1.18	1.20
8	1.36	1.38	1.37	1.26	1.28	1.29
9	1.16	1.15	1.14	1.19	1.16	1.14
10	1.39	1.33	1.35	1.38	1.35	1.36
11	1.18	1.19	1.20	1.12	1.18	1.17

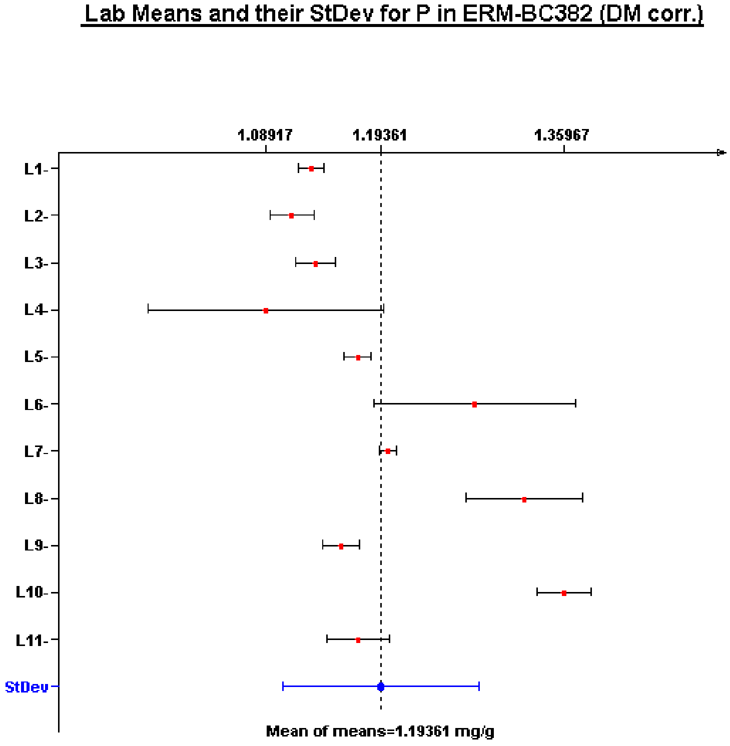


Figure D16: Laboratory means, mean of means and corresponding standard deviations for phosphorus in ERM®-BC382 (related to dry mass)

European Commission

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Title: The Certification of the Mass Fractions of Proximates and Essential Elements in Rye Flour and Wheat Flour - Certified Reference Materials ERM®-BC381 and ERM®-BC382
Author(s): A. Bernreuther, H. Emteborg, H. Schimmel, M. Staniszewska, K. Teipel, F. Ulberth
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Abstract
This report describes the preparation of the rye flour and wheat flour matrix reference materials ERM®-BC381 and ERM®-BC382, respectively, and the certification of the contents (mass fractions) of four proximates and four essential elements. All results are expressed as a mass fraction on a dry mass basis.

The preparation and processing of the materials, homogeneity studies, stability studies and characterisation are described hereafter and the results are discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and from characterisation.

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