DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report

proficiency test

DLA 36/2016

Dietary Fibre and Inulin

in cereal food

Dienstleistung Lebensmittel Analytik GbR
Waldemar-Bonsels-Weg 170
22926 Ahrensburg, Germany

proficiency-testing@dla-lvu.de
www.dla-lvu.de

Coordinator of this PT:
Dr. Matthias Besler

Inhalt / Content

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

2. Realisation

2.1 Test material

The test material is a common in commerce bread baking mix with addition of a customized semi-finished product rich in inulin from European suppliers. The materials were crushed, mixed and homogenized. Before homogenization microtracer particles were added in order to check the accuracy of mixing. After homogenization aliquots were taken for microtracer analysis (s. 2.1.1). Afterwards the samples were packaged lightproof in portions to approximately 20 g. The portions were numbered chronologically.

The composition of the samples is given in table 1. The fat content of the samples is < 10%.

Table 1: Composition of DLA samples

Ingredients	**Amount**
Breakfast Cereals Ingredients: Wheat flour, whole wheat flour, rye flour, sea salt, rye sourdough dried, dried yeast, wheat flour, milk protein powder, dietary fiber (apple fiber), acid regulator: malic acid. Nutrients per 100 g: fat 1,5 g, carbohydrates 64 g thereof sugar 2,6 g, fiber 6 g, protein 11 g	84,5 g/100g
Cereal semi-finished product with 25% inulin	15,5 g/100g

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT sample showed a probability of 28%. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat value of 1,4. The results of microtracer analysis are given in the documentation.

The **homogeneity of bottled numbered DLA-samples** was checked by 5fold determination of sodium by ICP-MS. The repeatability standard deviation is 10,8% and is in the range of the repeatability standard deviation of the German official method ASU §64 L 00.00-144 for sodium in wheat flour (8,15%). The results of the homogeneity test are given in the documentation.

The calculation of the **repeatability standard deviation S_r of the participants** was also used as an indicator of homogeneity. For total dietary fiber and inulin they are similar to the repeatability standard deviations of the official methods ASU §64 LFGB L 17.03-1 and ASU §64 LFGB L 00.00-94 (see Tab. 2) [19, 21]. The repeatability standard deviations of the participants' results are given in the documentation of homogeneity testing (5.2) and in the table of statistic data (see 4.1 and 4.7).

Furthermore, the homogeneity was characterized by the **trend line function of participants' results for chronological bottled single samples**. The maximum deviations from the mean value of the trend line was in the range of 30% of the target standard deviation σ_{pt} (s. 5.2 homogeneity) and can therefore be regarded as low.

If the criteria for sufficient homogeneity of the test material are not fulfilled on a particular parameter, the impact on the target standard deviation is checked and optionally the evaluation of the results of the participants will be done using the z'-score considering the standard uncertainty of the assigned value (see 3.8 and 3.11) [3]. Even though criteria were fulfilled for the evaluation of inulin the z'-score was applied.

2.2 Sample shipment and information to the test

Two portions of test material were sent to every participating laboratory in the 7th week of 2016. The testing method was optional. The tests should be finished at 1st April 2016 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

Notes on test:
The samples contain less than 10% fat.
Please do not add your possibly determined result for inulin to your result of the enzymatic-gravimetric test. "Total dietary fiber" in this PT means the result of the enzymatic-gravimetric test only.

2.3 Submission of results

The participants submitted their results in standard forms, which have been handed out with the samples (by email).

The finally calculated concentrations of the parameter as average of duplicate determinations of both numbered samples were used for the statistical evaluation. For the calculation of the repeatability- and reproducibility standard deviation the single values of the double determination were used.

Queried and documented were single results, recovery and the used testing methods.

From the 15 participants one participant submitted the results delayed in consultation with DLA. All other participants submitted the result in time.

3. Evaluation

3.1 Consensus value from participants (assigned value)

The robust mean of the submitted results was used as assigned value (X_{pt}) („consensus value from participants") providing a normal distribution. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

The condition is that the majority of the participants' results show a normal distribution or are distributed unimodal and symmetrically. To this end, an examination of the distribution is carried out, inter alia, using the kernel density estimate [3, 12].

In case there are indications for sources of higher variability such as a bimodal distribution of results, a cause analysis is performed. Frequently different analytical methods may cause an anomaly in results' distribution. If this is the case, separate evaluations with own assigned values (X_{pti}) are made whenever possible.

The statistical evaluation is carried out for all the parameters for a minimum of 7 values are present.

The actual measurement results will be drafted. Individual results, which are outside the specified measurement range of the participating laboratory (for example with the result > 25 mg/kg or < 2,5 mg/kg) or the indicating "0" will not be considered for the statistic evaluation [3].

3.2 Robust standard deviation

For comparison to the target standard deviation σ_{pt} (standard deviation for proficiency assessment) a robust standard deviation (S^x) was calculated. The calculation was done according to algorithm A as described in annex C of ISO 13528 [3].

3.3 Repeatability standard deviation

The repeatability standard deviation S_r is based on the laboratory´s standard deviation of (outlier free) individual participant results, each under repeatability conditions, that means analyses was performed on the same sample by the same operator using the same equipment in the same laboratory within a short time. It characterizes the mean deviation of the results within the laboratories [3] and is used by DLA as an indication of the homogeneity of the sample material.

The calculation of the repeatability standard deviation S_r, also known as standard deviation within laboratories S_w, is performed by: [3, 4].

The relative repeatability standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_r in the table of statistical characteristics in the results section.

3.4 Reproducibility standard deviation

The reproducibility standard deviation S_R represents a inter-laboratory estimate of the standard deviation for the determination of each parameter on the bases of (outlier free) individual participant results. It takes into account both the repeatability standard deviation S_r and the within-laboratory standard deviation S_S. Reproducibility standard deviations of PT´s may differ from reproducibility standard deviations of ring trials, because the participating laboratories of a PT generally use different internal conditions and methods for determining the measured values.

In the present evaluation, the specification of the reproducibility standard deviation, therefore, does not refer to a specific method, but characterizes approximately the comparability of results between the laboratories, assumed the effect of homogeneity and stability of the sample are negligible.

The calculation of the reproducibility standard deviation S_R is performed by: [3, 4].

The relative reproducibility standard deviation as a percentage of the mean value is indicated as coefficient of variation CV_R in the table of statistical characteristics in the results section. Its meaning is explained in more detail in 3.9.

3.5 Exclusion of results and outliers

Before statistical evaluation obvious blunders, such as those with incorrect units, decimal point errors, and results for a another proficiency test item can be removed from the data set [2]. All results should be given at least with 2 significant digits. Specifying 3 significant digits is usually sufficient.

Results obtained by different analytical methods causing an increased variability and/or a bi- or multimodal distribution of results, are treated separately or could be excluded in case of too few numbers of results. For this results are checked by kernel density estimation [3, 12].

Results are identified as outliers by the use of robust statistics. If a value deviates from the robust mean by more than 3 times the robust standard deviation, it is classified as an outlier [3]. Detected outliers are stated for information only, when z-score are < -2 or > 2. Due to the use of robust statistics outliers are not excluded, provided that no other reasons are present [3].

3.6 Target standard deviation (for proficiency assessment)

The target standard deviation of the assigned value σ_{pt} (= standard deviation for proficiency assessment) can be determined according to the following methods.

If an acceptable quotient S^*/σ_{pt} is present, the target standard deviation of the general model by Horwitz is preferably used for the proficiency assessment. It is usually suitable for for evaluation of interlaboratory studies, where different analytical methods are applied by the participants. On the other hand the target standard deviation from the evaluation of precision data of an precision experiment is derived from collaborative studies with specified analytical methods.

In cases where both above-mentioned models are not suitable, the target standard deviation is determined based on values by perception, see under 3.6.3.

For information the z-scores of both models are given in the evaluation, if available.

For the valuation of total dietary fiber and inulin the target standard deviation according to a precision experiment was applied (3.4.2, official German ASU §64 methods: 19, 21). For inulin the standard uncertainty was considered additionally by valuating with z'-score (see 3.6).
For all other parameters there were less than 7 quantitative results, thus no statistical valuation was done.

3.6.1 General model (Horwitz)

Based on statistical characteristics obtained in numerous PTs for different parameters and methods Horwitz has derived a general model for estimating the reproducibility standard deviation σ_R [6]. Later the model was modified by Thompson for certain concentration ranges [10]. The reproducibility standard deviation σ_R can be applied as the relative target standard deviation σ_{pt} in % of the assigned values and calculated according to the following equations [3]. For this the assigned value X_{pt} is used for the concentration c.

Equations	**Range of concentrations**	**corresponds to**
$\sigma_R = 0{,}22c$	$c < 1{,}2 \times 10^{-7}$	< 120 µg/kg
$\sigma_R = 0{,}02c^{0{,}8495}$	$1{,}2 \times 10^{-7} \leq c \leq 0{,}138$	≥ 120 µg/kg
$\sigma_R = 0{,}01c^{0{,}5}$	$c > 0{,}138$	> 13,8 g/100g

with c = mass content of analyte (as relative size, e.g. 1 mg/kg = 1 ppm = 10^{-6} kg/kg)

3.6.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment (collaborative trial or proficiency test) the target standard deviation σ_{pt} can be derived considering the number of replicate measurements m of participants in the present PT [3]:

$$\sigma_{pt} = \sqrt{\sigma_R^2 - \sigma_r^2\left(m - 1/m\right)}$$

The values given in Table 2 relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) were determined in collaborative trials using the specified methods.
The in the table indicated resulting target standard deviations σ_{pt} were used for evaluation of the results.
For information the target standard deviations according to Horwitz are given additionally.

Table 2: Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) from precision experiments and resulting target standard deviations σ_{pt} [19, 21]

Parameter	**Matrix**	**Mean values**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Total dietary fiber	Rye-wheat bread	8,83 %	2,49 %	5,10 %	4,6 %[1]	ASU §64 L 17.03-1
Unsoluble fiber	Rye-wheat bread	5,45 %	5,14 %	8,44 %	7,6 %	ASU §64 L 17.03-1
Soluble fiber	Rye-wheat bread	3,02 %	14,6 %	20,9 %	18,2 %	ASU §64 L 17.03-1
Inulin	Instant meal Infant food Chocolate	16,7 % 0,61 % 5,95 %	1,86 % 4,92 % 2,35 %	3,60 % 8,20 % 4,03 %	3,4 % 7,4 %[1] 3,7 %	ASU §64 L 00.00-94

[1] *used in evaluation (s. chapter 4)*

3.6.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve [3].

For the present evaluation the target standard deviation according to 3.6.2 was regarded suitable.

3.7 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation (σ_{pt}) the result (x_i) of the participant is deviating from the assigned value (X_{pt}) [3].
Participants' z-scores are derived from:

$$z_i = \frac{\left(x_i - x_{pt}\right)}{\sigma_{pt}}$$

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

3.7.1 Warning and action signals

In accordance with the norm ISO 13528 it is recommended that a result that gives rise to a *z*-score above 3,0 or below −3,0, shall be considered to give an "action signal" [3]. Likewise, a *z*-score above 2,0 or below −2,0 shall be considered to give a "warning signal". A single "action signal", or "warning signal" in two successive PT-rounds, shall be taken as evidence that an anomaly has occurred which requires investigation. For example a fault isolation or a root cause analysis through the examination of transmission error or an error in the calculation, in the trueness and precision must be performed and if necessary appropriate corrective measures should be applied [3].

In the figures of z-scores DLA gives the limits of warning and action signals as yellow and red lines respectively. According to ISO 13528 the signals are valid only in case of a number of ≥ 10 results [3].

3.8 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x) of the participant from the respective consensus value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux_{pt}) [3].

The calculation is performed by:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u_{(x_{pt})}^2}}$$

If carried out an evaluation of the results by means of z 'score, we have

defined below the expression in the denominator as a target standard deviation σ_{pt}'.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

For warning and action signals see 3.7.1.

3.9 Reproducibility coefficient of variation (CK_R)

The coefficient of variation (CV_R) of the reproducibility (= relative reproducibility standard deviation) is calculated from the standard deviation and the mean as follows [4, 13]:

$$CV_R = \frac{S_R * 100}{X}$$

In contrast to the standard deviation as a measure of the absolute variability the V_K gives the relative variability within a data region. While a low CV_R, e.g. <5-10% can be taken as evidence for a homogeneous set of results, a CV_R of more than 50% indicates a "strong inhomogeneity of statistical mass", so that the suitability for certain applications such as the assessment of exceeded maximum values or the performance evaluation of the participants possibly can not be done [3].

3.10 Quotient S*/σpt

Following the HorRat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation S* and target standard deviation σ_{pt} does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given [3].

3.11 Standard uncertainty

The consensus value has a standard uncertainty $U(X_{pt})$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories (P) and perhaps on other factors. The standard uncertainty of the assigned value ($U(X_{pt})$) for this PT is calculated as follows [3]:

$$u_{(x_{pt})} = 1{,}25 \times \frac{s^*}{\sqrt{p}}$$

If $U(X_{pt}) \leq 0{,}3\ \sigma_{pt}$ the standard uncertainty of the consensus value needs not to be included in the interpretation of the results of the PT [3]. A clear exceeded the value of 0,3 is an indication that the target standard deviation was possibly set too low for the standard uncertainty of the assigned value. The quotient $U(X_{pt})/\sigma_{pt}$ is reported in the characteristics of the test.

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

In the first table the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median
Robust mean(X_{pt})
Robust standard deviation (S^*)
Number with m replicate measurements
Repeatability standard deviation (S_r)
Coefficient of Variation (CV_r) in %
Reproducibility standard deviation (S_R)
Coefficient of Variation (CV_R) in %
Target range:
Target standard deviation σ_{pt} or σ_{pt}'
Target standard deviation for information
lower limit of target range ($X_{pt} - 2\sigma_{pt}$) or ($X_{pt} - 2\sigma_{pt}$') *
upper limit of target range ($X_{pt} + 2\sigma_{pt}$) or ($X_{pt} + 2\sigma_{pt}$´) *
Variation coefficient V_K in %
Quotient S^*/σ_{pt} *or* S^*/σ_{pt}'
Standard uncertainty $U(X_{pt})$
Quotient $U(X_{pt})/\sigma_{pt}$ *or* $U(X_{pt})/\sigma_{pt}$'
Number of results in the target range
Percent in the target range

* Target range is calculated with z-score or z'-score

In the second table the individual results of the participating laboratories are listed:

Auswerte-nummer **Evaluation number**	**Parameter [Einheit / Unit]**	**Abweichung** **Deviation**	**z-Score σpt**	**z-Score** (Info)	**Hinweis** **Remark**

4.1 Total Dietary Fiber in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	12
Number of outliers	0
Mean	8,15
Median	8,05
Robust Mean (X)	**8,15**
Robust standard deviation (S*)	**0,579**
Number with 2 replicates	11
Repeatability SD (S_r)	0,254
Repeatability (CV_r)	3,10%
Reproducibility SD (S_R)	0,556
Reproducibility (CV_R)	6,80%
Target range:	
Target standard deviation σ_{pt}	**0,390**
Target standard deviation Horwitz (for Information)	0,238
lower limit of target range	**7,37**
upper limit of target range	**8,93**
Quotient S/σ_{pt}*	*1,5*
Standard uncertainty $U_{(Xpt)}$	*0,209*
Quotient $U_{(Xpt)}/\sigma_{pt}$	*0,54*
Results in the target range	*12*
Percent in the target range	*100%*

Comments to the statistic data:

The target standard deviation was calculated according to the official method ASU §64 L 17.03-1.

The evaluation showed a normal variability of results. The quotient S*/σ_{pt} was clearly below 2,0. The robust standard deviation as well as the repeatability and reproducibility standard deviations were in the range of established values for the applied methods (see 3.6.2). The comparability of results is given.

The quotient $U_{(Xpt)}/\sigma_{pt}$ was 0,54. Although it was below 0,3 it is acceptable due to the other statistical data and the use of different analytical methods.

All results were in the target range.

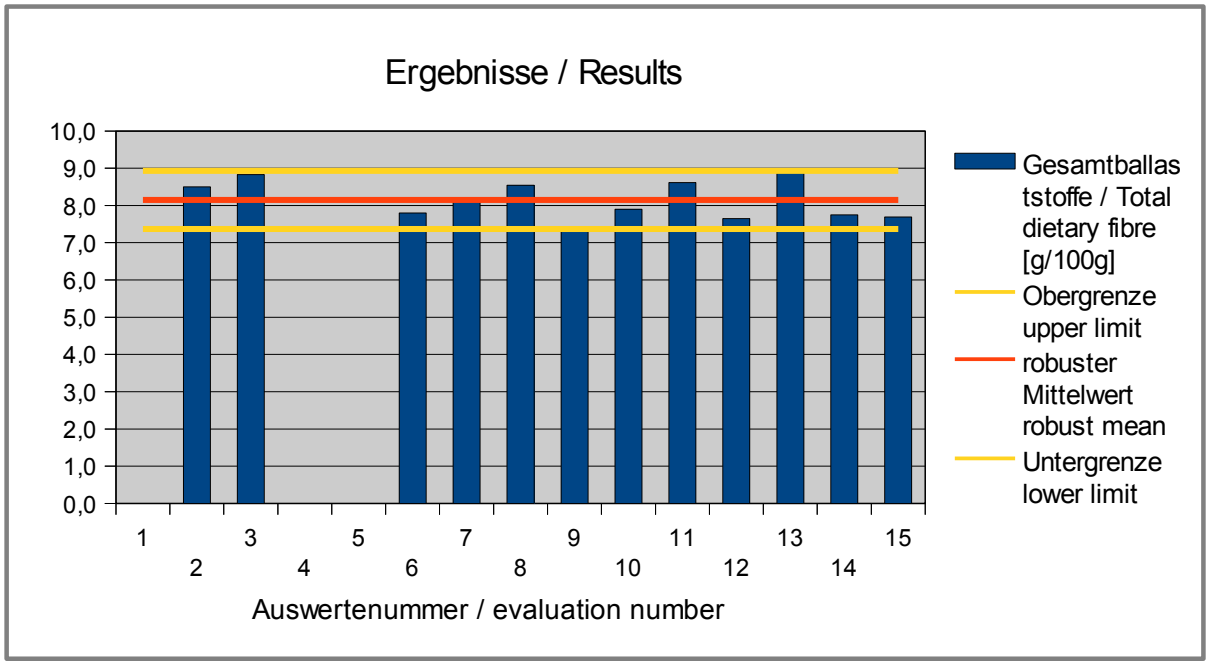

Abb. 1: Ergebnisse Gesamtballaststoffe
Fig. 1: Results total dietary fiber

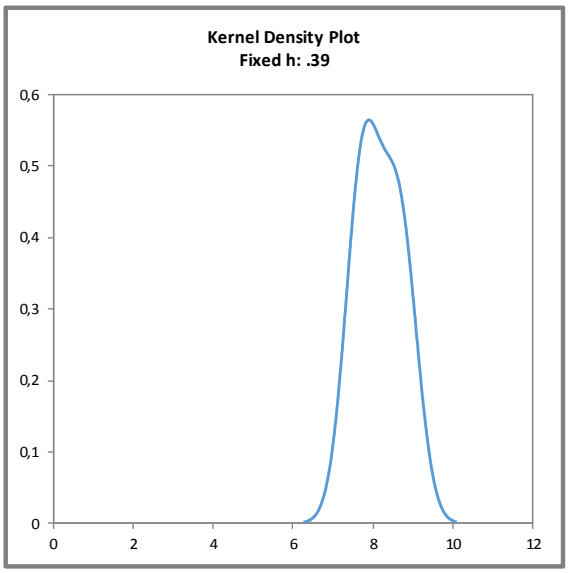

Abb. 2: Kerndichte-Schätzung der Ergebnisse für Gesamtballaststoffe (mit h = σ_{pt} von X_{pt})

Fig. 2: Kernel density plot of total fiber results (with h = σ_{pt} von X_{pt})

Comments:
The kernel density estimation shows nearly a normal distribution with a slight shoulder at approximately 8,9 g/100g (s. fig. 2).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Gesamtballaststoffe / Total dietary fibre [g/100g]	Abweichung [g/100g] Deviation [g/100g]	z-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1					
2	8,5	0,35	0,9	1,5	
3	8,83	0,68	1,7	2,9	
4					
5					
6	7,8	-0,35	-0,9	-1,5	
7	8,2	0,05	0,1	0,2	
8	8,54	0,39	1,0	1,6	
9	7,41	-0,74	-1,9	-3,1	
10	7,9	-0,25	-0,6	-1,0	
11	8,615	0,47	1,2	2,0	
12	7,65	-0,50	-1,3	-2,1	
13	8,9	0,75	1,9	3,2	
14	7,75	-0,40	-1,0	-1,7	
15	7,69	-0,46	-1,2	-1,9	

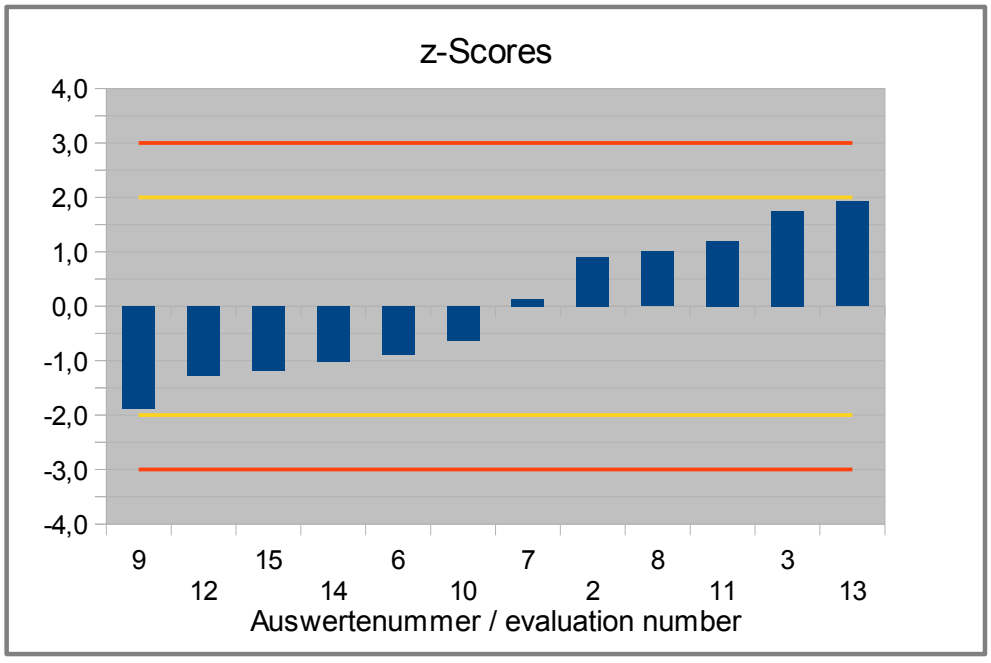

Abb. 3: Z-Scores Gesamtballaststoffe
Fig. 3: Z-Scores total dietary fiber

4.2 Soluble Fiber in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	4
Number of outliers	0
Mean	2,17
Median	2,00
Robust Mean (X)	**2,13**
Robust standard deviation (S*)	**0,380**

* Due to the low number of results (<7) no statistical evaluation was done.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Lösliche Ballaststoffe / Soluble dietary fiber [g/100g]	Abweichung [g/100g] Deviation [g/100g]	Hinweis Remark
1			
2			
3	2,77	0,64	
4			
5			
6			
7	1,9	-0,23	
8			
9			
10			
11			
12	1,95	-0,18	
13			
14			
15	2,05	-0,08	

4.3 Unsoluble Fiber in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	5
Number of outliers	0
Mean	6,06
Median	6,05
Robust Mean (X)	**6,06**
Robust standard deviation (S*)	**0,460**

* Due to the low number of results (<7) no statistical evaluation was done.

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Unlösliche Ballaststoffe / Unsoluble dietary fiber [g/100g]	Abweichung [g/100g] Deviation [g/100g]	Hinweis Remark
1			
2			
3	6,05	-0,01	
4			
5			
6			
7	6,3	0,24	
8			
9			
10			
11			
12	5,75	-0,31	
13	6,6	0,54	
14			
15	5,6	-0,46	

4.4 Total Dietary Fiber after Inulinase-Reaction in g/100g

Vergleichsuntersuchung / Proficiency Test

Only one result was submitted (participant 12: 7,2 g/100g).
Further details are given in the documentation.

4.5 Soluble Total Dietary Fiber after Inulinase-Reaction in g/100g

Vergleichsuntersuchung / Proficiency Test

No results were submitted.

4.6 Unsoluble Total Dietary Fiber after Inulinase-Reaction in g/100g

Vergleichsuntersuchung / Proficiency Test

No results were submitted.

4.7 Inulin in g/100g

Vergleichsuntersuchung / Proficiency Test

Statistic Data	
Number of results	15
Number of outliers	2
Mean	3,10
Median	3,20
Robust Mean (X)	**3,14**
Robust standard deviation (S*)	**0,524**
Number with 2 replicates	13
Repeatability SD (S_r)	0,108
Repeatability (CV_r)	3,41%
Reproducibility SD (S_R)	0,484
Reproducibility (CV_R)	15,2%
Target range:	
Target standard deviation σ_{pt}'	**0,288**
Target standard deviation (for Information)	0,106
lower limit of target range	**2,56**
upper limit of target range	**3,72**
Quotient S/σ_{pt}'*	*1,8*
Standard uncertainty $U_{(Xpt)}$	*0,169*
Quotient $U_{(Xpt)}/\sigma_{pt}$'	*0,59*
Results in the target range	*11*
Percent in the target range	*73%*

Comments to the statistic data:
The target standard deviation was calculated according to the official method ASU §64 00.00-94.

The evaluation showed a slightly increased variability of results. The quotient S^*/σ_{pt} was above 2,0 and the quotient $U_{(Xpt)}/\sigma_{pt}$ above 0,3. Therefore the evaluation was done using z'-score considering the standard uncertainty of the assigned value (s. 3.8 and 3.11).

The resulting quotient S^*/σ_{pt}' was below 2,0. The robust standard deviation as well as the repeatability standard deviation were in the range of established values for the applied methods, while the reproducibility standard deviation and the coefficient of variation CV_R were higher, respectively (see 3.6.2).

73% of results were in the target range. In comparison to the allowed tolerances of nutrient contents of the guidance document for the Food Information for Consumers EU-Regulation all results were within the recommendation of ±2g for amounts < 10 g/100g and except 3 results within ±20% (2,73 – 4,09%) [16].

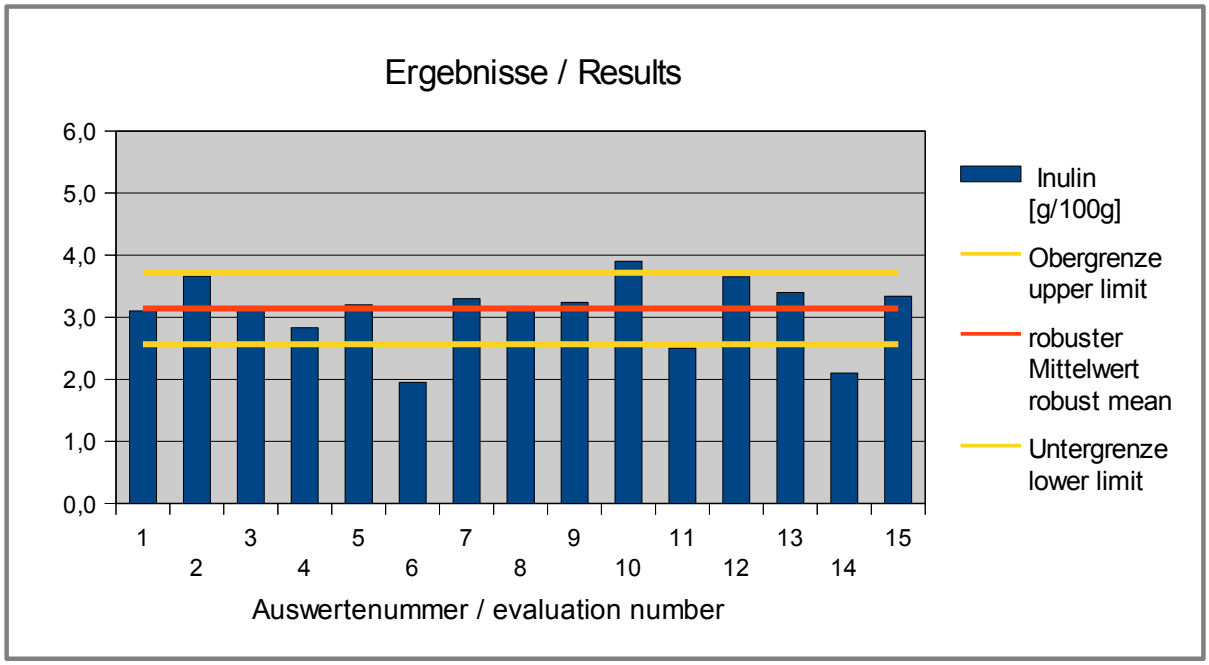

Abb. 4: Ergebnisse Inulin
Fig. 4: Results Inulin

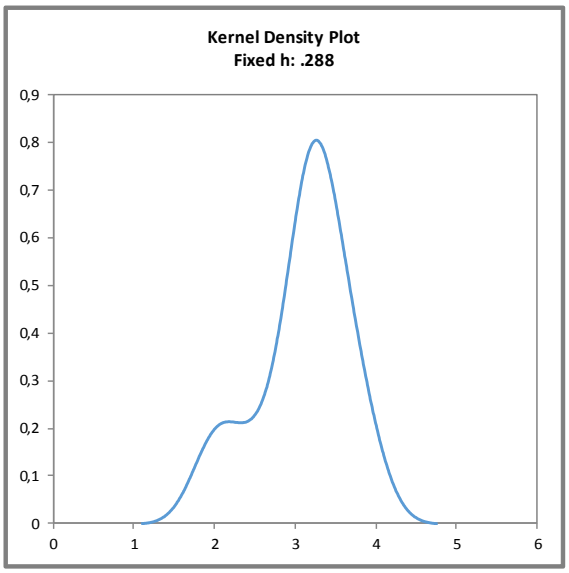

Abb. 5: Kerndichte-Schätzung der Ergebnisse für Inulin
(mit h = σ_{pt} von Xpt)

Fig. 5: Kernel density plot of inulin results (with h = σ_{pt} von Xpt)

Comments:
The kernel density estimation shows a normal distribution with a shoulder at approximately 2,0 g/100g due to two outliers (s. fig. 5).

Ergebnisse der Teilnehmer:
Results of Participants:

Auswerte-nummer Evaluation number	Inulin [g/100g]	Abweichung [g/100g] Deviation [g/100g]	z'-Score (σ_{pt})	z-Score (Info)	Hinweis Remark
1	3,1	-0,040	-0,1	-0,4	
2	3,66	0,520	1,8	4,9	
3	3,14	0,000	0,0	0,0	
4	2,83	-0,310	-1,1	-2,9	
5	3,2	0,060	0,2	0,6	
6	1,951	-1,189	-4,1	-11,2	Ausreißer / outlier
7	3,3	0,160	0,6	1,5	
8	3,125	-0,015	-0,1	-0,1	
9	3,24	0,100	0,3	0,9	
10	3,9	0,760	2,6	7,2	
11	2,5	-0,640	-2,2	-6,1	
12	3,65	0,510	1,8	4,8	
13	3,4	0,260	0,9	2,5	
14	2,1	-1,040	-3,6	-9,8	Ausreißer / outlier
15	3,34	0,200	0,7	1,9	

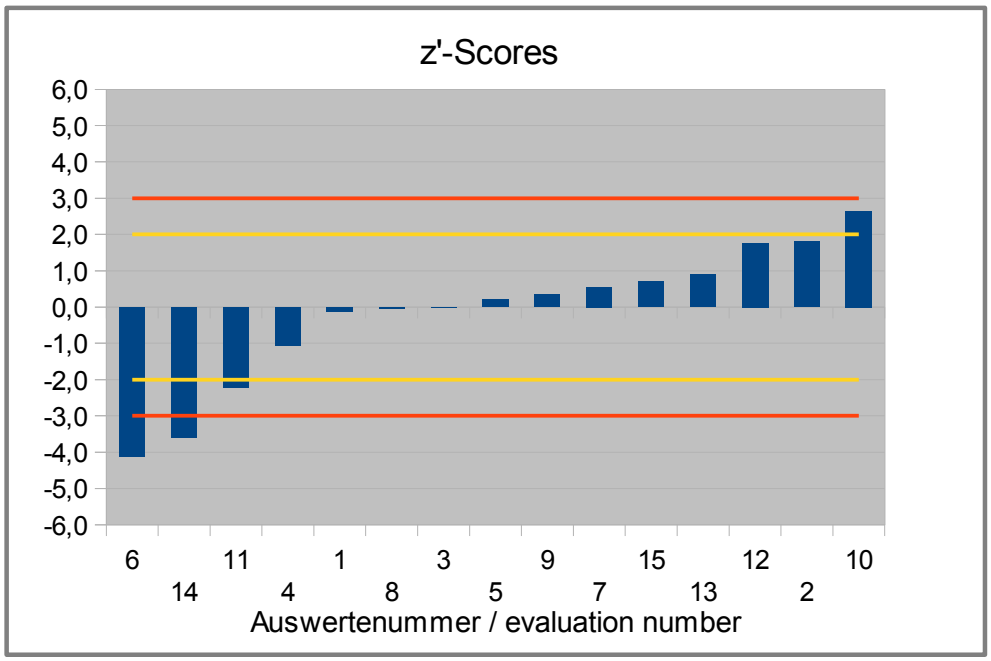

Abb. 6: Z'-Scores Inulin
Fig. 6: Z'-Scores Inulin

5. Documentation

5.1 Primary data

Parameter Analyte	Teilnehmer Participant	Einheit Unit	Proben-Nr. A Sample No. A	Proben-Nr. B Sample No. B	Ergebnis (Mittel) Result (Mean)	Ergebnis A Result A	Ergebnis B Result B
Gesamt-ballaststoffe / total dietary fiber	1	g/100g					
	2	g/100g	17	29	8,5	8,8	8,2
	3	g/100g	2	31	8,83	8,9	8,75
	4	g/100g					
	5	g/100g					
	6	g/100g			7,8		
	7	g/100g	10	41	8,2	7,9	8,4
	8	g/100g	18	46	8,54	8,65	8,43
	9	g/100g	12	30	7,41	7,29	7,53
	10	g/100g	39	50	7,9	7,8	7,9
	11	g/100g	33	57	8,62	8,63	8,6
	12	g/100g	6	28	7,65	7,7	7,6
	13	g/100g	43	71	8,9	8,5	9,3
	14	g/100g	5	26	7,75	7,8	7,7
	15	g/100g	11	36	7,69	7,72	7,65
Lösliche Ballaststoffe / soluble dietary fiber	1	g/100g					
	2	g/100g	17	29	N/A	N/A	N/A
	3	g/100g	2	31	2,77	2,69	2,84
	4	g/100g					
	5	g/100g					
	6	g/100g					
	7	g/100g	10	41	1,9	1,9	1,8
	8	g/100g	18	46			
	9	g/100g	12	30			
	10	g/100g	39	50			
	11	g/100g	33	57			
	12	g/100g	6	28	1,95	2,1	1,8
	13	g/100g	43	71	not tested	not tested	not tested
	14	g/100g	5	26			
	15	g/100g	11	36	2,05	2,06	2,04

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Ergebnis (Mittel)	Ergebnis A	Ergebnis B
Analyte	Participant	Unit	Sample No. A	Sample No. B	Result (Mean)	Result A	Result B
Unlösliche Ballaststoffe / unsoluble dietary fiber	1	g/100g					
	2	g/100g	17	29	N/A	N/A	N/A
	3	g/100g	2	31	6,05	6,11	5,98
	4	g/100g					
	5	g/100g					
	6	g/100g					
	7	g/100g	10	41	6,3	6	6,6
	8	g/100g	18	46			
	9	g/100g	12	30			
	10	g/100g	39	50			
	11	g/100g	33	57			
	12	g/100g	6	28	5,75	5,6	5,9
	13	g/100g	43	71	6,6	6,6	6,6
	14	g/100g	5	26			
	15	g/100g	11	36	5,6	5,69	5,5
Gesamtballast-stoffe nach Inulinase-Reaktion / total dietary fiber with Inulinase reaction	1	g/100g					
	2	g/100g	17	29	N/A	N/A	N/A
	3	g/100g	2	31			
	4	g/100g					
	5	g/100g					
	6	g/100g					
	7	g/100g	10	41			
	8	g/100g	18	46			
	9	g/100g	12	30			
	10	g/100g	39	50			
	11	g/100g	33	57			
	12	g/100g	6	28	7,2	7,2	7,2
	13	g/100g	43	71	not tested	not tested	not tested
	14	g/100g	5	26			
	15	g/100g	11	36			

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Ergebnis (Mittel)	Ergebnis A	Ergebnis B
Analyte	Participant	Unit	Sample No. A	Sample No. B	Result (Mean)	Result A	Result B
Lösliche Ballaststoffe nach Inulinase-Reaktion / soluble dietary fiber with Inulinase reaction	1	g/100g					
	2	g/100g	17	29	N/A	N/A	N/A
	3	g/100g	2	31			
	4	g/100g					
	5	g/100g					
	6	g/100g					
	7	g/100g	10	41			
	8	g/100g	18	46			
	9	g/100g	12	30			
	10	g/100g	39	50			
	11	g/100g	33	57			
	12	g/100g	6	28			
	13	g/100g	43	71	not tested	not tested	not tested
	14	g/100g	5	26			
	15	g/100g	11	36			
Unlösliche Ballaststoffe nach Inulinase-Reaktion / unsoluble dietary fiber with Inulinase reaction	1	g/100g					
	2	g/100g	17	29	N/A	N/A	N/A
	3	g/100g	2	31			
	4	g/100g					
	5	g/100g					
	6	g/100g					
	7	g/100g	10	41			
	8	g/100g	18	46			
	9	g/100g	12	30			
	10	g/100g	39	50			
	11	g/100g	33	57			
	12	g/100g	6	28			
	13	g/100g	43	71	not tested	not tested	not tested
	14	g/100g	5	26			
	15	g/100g	11	36			

Parameter	Teilnehmer	Einheit	Proben-Nr. A	Proben-Nr. B	Ergebnis (Mittel)	Ergebnis A	Ergebnis B
Analyte	Participant	Unit	Sample No. A	Sample No. B	Result (Mean)	Result A	Result B
Inulin	1	g/100g	20	59	3,1	3,1	3,1
	2	g/100g	17	29	3,66	3,49	3,82
	3	g/100g	2	31	3,14	3,11	3,16
	4	g/100g	25	63	2,83	2,84	2,82
	5	g/100g	16	58	3,2	3,2	3,2
	6	g/100g			1,95		
	7	g/100g	10	41	3,3	3,4	3,2
	8	g/100g	18	46	3,13	3,1	3,15
	9	g/100g	12	30	3,24	3,29	3,19
	10	g/100g	39	50	3,9	3,8	3,9
	11	g/100g	33	57	2,5	2,6	2,4
	12	g/100g	6	28	3,65	3,6	3,7
	13	g/100g	43	71	3,4	3,3	3,5
	14	g/100g	5	26	2,1	2	2,2
	15	g/100g	11	36	3,34	3,35	3,32

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test

Weight whole sample	1,77	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	3,5	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	20,32	31	3,1
2	19,84	43	4,3
3	20,62	43	4,2
4	15,46	39	5,0
5	18,71	35	3,7
6	21,53	36	3,3
7	20,65	33	3,2
8	23,46	40	3,4

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	37,9	Partikel
Standard deviation	6,83	Partikel
χ^2 (CHI-Quadrat)	8,61	
Probability	**28**	%
Recovery rate	107	%

Normal distribution		
Number of samples	8	
Mean	3,79	mg/kg
Standard deviation	0,68	mg/kg
rel. Standard deviaton	18,0	%
Horwitz standard deviation	13,1	%
HorRat-value	**1,4**	
Recovery rate	107	%

5.2.2 Homogeneity of bottled PT-samples

Homogeneity test of sodium (given as NaCl) by ICP-MS:

Independant samples	g/100g
1	1,76
2	1,88
3	2,23
4	1,75
5	1,77

Mean	1,88	
Repeatability Standard Deviation	0,204	10,8%

5.2.3 Repeatability standard deviation of replicate measurements of participants

The repeatability standard deviations S_r were calculated with the data documented in chapter 5.1 and given in the statistic data in 4.1 and 4.7.
It is 0,254 g/100g = 3,10 % of X for total dietary fiber.
It is 0,108 g/100g = 3,41 % of X for inulin.

5.2.3 Comparison of sample numbers / test results and trend line

By comparison of the increasing sample numbers and the measurement results, the homogeneity of the chronological bottled PT item can be characterized with the help of the trend line function:

Total dietary fiber
Sample numbers: 2 – 57 (without no. 43 and 71 outlier Horwitz)
Measurement results: 20
Trend line range: 8,09 ± 0,128 g/100g (= ± 0,33 x σ_{pt})
Maximum relative deviation to mean: ± 1,58%

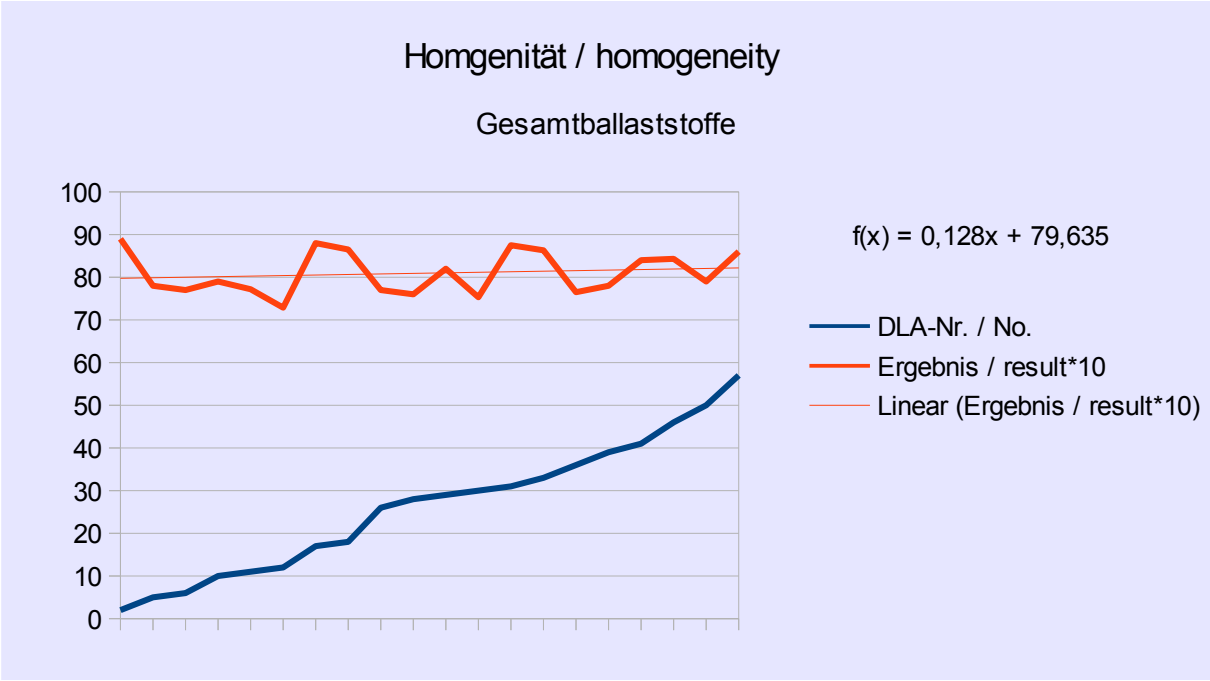

Abb. 7: Trendfunktion Probennummern / Gesamtballaststoffe Ergebnisse (x 10 dargestellt)
Fig. 7: trend line function sample number / total dietary fiber results (x 10 shown)

Inulin

Sample numbers: 2 - 71
Measurement results: 26
Trend line range: 3,18 ± 0,072 g/100g (= ± 0,25 x σ_{pt})
Maximum relative deviation to mean: ± 2,25%

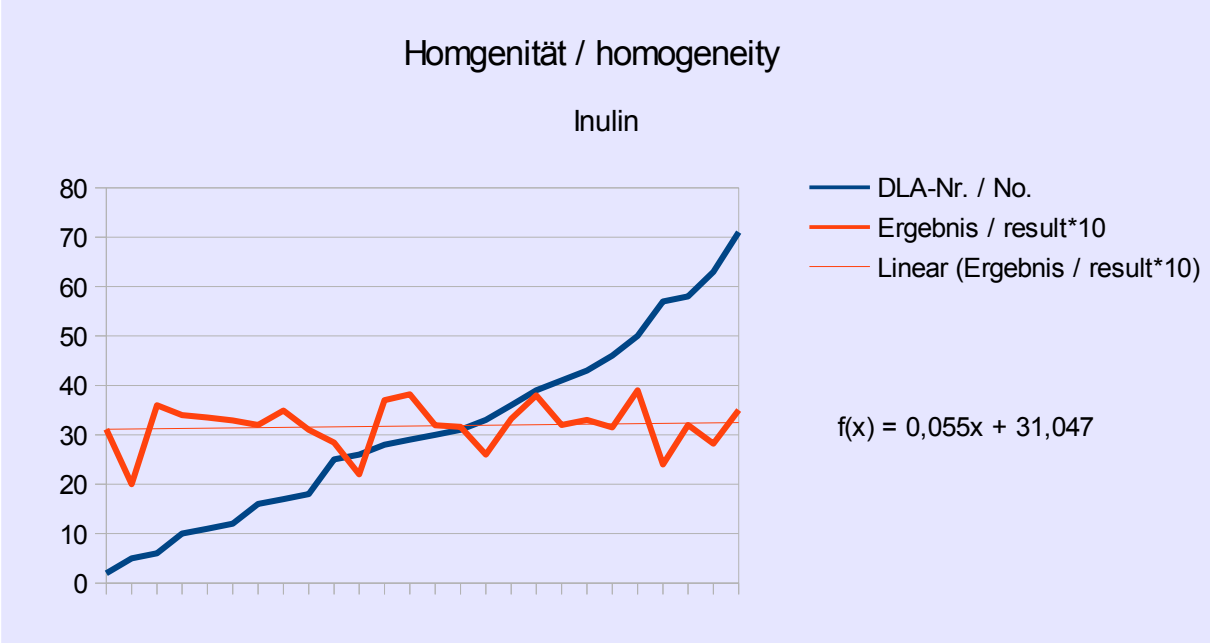

Abb. 8: Trendfunktion Probennummern / Ergebnisse Inulin (Ergebnisse x 10 dargestellt)
Fig. 8: trend line function sample number / results inulin (results x 10 shown)

5.3 Analytical Methods

Details by the participants

Parameter	Teilnehmer	Methodenbeschreibung	Frittendurch-messer	Porendurch-messer	Angaben zu Amylase	Angaben zu Protease	Angaben zu Amyloglucosidase	Angaben zur Inulinase	Methode ohne Inulinase ist akkreditiert	Methode mit Inulinase ist akkreditiert
Analyte	Participant	Method description	Frit diameter	Pore diameter	Notes to Amylase	Notes to Protease	Notes to Amyloglucosidase	Notes to Inulinase	Method is accredited without Inulinase	Method ist accredited with Inulinase
Ballaststoffe / dietary fibre	1									
	2	AOAC 985.29		p.2	Megazyme K-TDFR	Megazyme K-TDFR	Megazyme K-TDFR	N/A	yes	n/a
	3		40 mm	Porosity 2	Testkit from Megazyme	Testkit from Megazyme	Testkit from Megazyme		yes	
	4									
	5									
	6	§64 LFGB L00.00-18	4 cm	16-40 µm	Sigma A3306-10ML	Sigma P-3910-500MG	Sigma A9913-10ML	-	yes	no
	7	AOAC 991.43	3.3 cm	40-100 µm	α-amylase, 3000 units/ml (Megazyme)	protease, 350 tyrosine units/ml (Megazyme)	amyloglucosidase, 3300 units/ml (Megazyme)	not applicable	no	not applicable
	8	§64 LFGB L.00.00-18	Filter paper MN 640W		Sigma A 3306; Lot SLB-J0135V	Sigma P 3910; Lot SLB-M9416V	Sigma A 9913; Lot SLB-P1905V		yes	
	9	AOAC 2009.01	30 mm	40-60 µm	alpha- amylase 50 units/mL + 3.4 units/mL AMG (Megazyme)	350 tyrosine units/mL (Megazyme)	AMG 3300 units/mL (Megazyme)	N/A	Yes	N/A
	10	AOAC 991.43		2	Megazyme Kit AOAC 991.43	Megazyme Kit AOAC 991.43	Megazyme Kit AOAC 991.43		no	
	11	internal method			sigma tdf kit	sigma tdf kit	sigma tdf kit		yes	
	12	AOAC 991.43	30 mm	40-90um	Megazyme E-Blaam	Megazyme E-BSPRT	Megazyme E-AMGDF		no	_
	13	AOAC 991,43	40 mm	40-60µm	3.6 Thermostable α-amilase (B. licheniformis) (120,000 Units). Megazyme E-BLAAM	3.7 Purified protease (Subtilisin A from B. licheniformis) (2 g/40 mL). Megazyme E-BSPRT	3.8 Purified amyloglucosidase (A. niger) (140,000 Units). Megazyme		yes	
	14	§64 LFGB L00.00-178, mod.							yes	
	15	ASU L 00.00-18 Foodstuffs. Determination of dietary fiber in food; 1997-01 cor. 2002-12	4,1 cm; 50 ml	Por. 2	Reagents' kit from Merck; total dietary fiber, 1.12979.0001; Status 2013-11				yes	

Parameter	Teilnehmer	Methodenbeschreibung	UV-Testkit Hersteller	HPLC Säule	Detektor	Methode ist akkreditiert	Angaben zur Inulinase
Analyte	Participant	Method description	UV-Testkit manufacturer	HPLC column	Detector	Method is accredited	Notes to Inulinase
Inulin	1	HPAEC		Carbopack PA1	PAD	no	
	2	AOAC 997.03	Megazyme	N/A	UV	no	
	3			Dr. Maisch Reprosil 100 NH2, 5um, 250*4mm	RID	yes	
	4	Enzymatically	EnzymeFast			yes	
	5	LAV 21.0051.01	r-Biopharm			yes	
	6	VDLUFA 7.4.1	-	-	-	yes	
	7	AOAC 999.03	not applicable	Dionex CarboPac PA1	PAD	no	exo- and endo-inulinase (Megazyme)
	8	§64 LFGB L.00.00-94	r-biopharm 10716260; Lot 11852400 Oct 2016			yes	
	9	AOAC 2009.01	N/A	Sugar-Pak (Waters)	Refractive Index (Agilent)	Yes	
	10	AOAC 997.08			GC-FID	no	
	11	AOAC 997 08/99		PA100 dionex	PAD	no	sigma
	12	AOAC 997.08 modified	_	Dionex PA1	PAD	no	
	13	AOAC 997,08 modified		Zorbax Carbohydrate 5µ 4.6x150mm	IR	no	
	14	Internal method, HPLC-RID				yes	
	15	Determination of inulin in food	R-Biopharm AG,			yes	

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		UNITED KINGDOM
		CANADA
		ITALY
		Germany
		NETHERLANDS
		Germany
		NETHERLANDS
		Germany
		Germany
		Germany
		Germany
		Germany
		BELGIUM
		SPAIN
		BELGIUM

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Anan-lytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. LEITFADEN FÜR ZUSTÄNDIGE BEHÖRDEN – KONTROLLE DER EINHALTUNG DER EU-RECHTSVORSCHRIFTEN: Verordnung (EU) Nr. 1169/2011 über Information der Verbraucher über Lebensmittel (Dezember 2012) / GUIDANCE DOCUMENT FOR COMPETENT AUTHORITIES FOR THE CONTROL OF COMPLIANCE WITH EU LEGISLATION ON: Regulation (EU) No 1169/2011 on the provision of food information to consumers (December 2012)
17. ASU §64 LFGB: L 00.00-18: Bestimmung der Ballaststoffe in Lebensmitteln
18. ASU §64 LFGB: L 16.08-1: Bestimmung der Ballaststoffe in Getreidekleie
19. ASU §64 LFGB: L 17.03-1: Bestimmung der Ballaststoffe in Mischbrot
20. ASU §64 LFGB: L 48.01-25: Bestimmung der Ballaststoffe in Säuglings- und Kindernahrung auf Milchbasis
21. ASU §64 LFGB: L 00.00-94: Bestimmung von Inulin in Lebensmitteln