DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation-Report
proficiency test

DLA 26/2014

Fibre and Inulin in Chocolate cookies

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Contents

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 validity of the particular testing method.
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.

2 Evaluation

2.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X. The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

2.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (Sx) was calculated.

2.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (95% significance). Detected outliers were stated for information only, when z-score was < -2 or > 2.

2.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

2.4.1 General model (Horwitz / Thompson)

The relative target standard deviation in % of the assigned value was derived from following equation (Horwitz)

$$\sigma_{(\%)} = 2^{(1-0,5\log X)} .$$

From the result the target standard deviation was calculated

$$\sigma = X * \sigma_{(\%)} / 100.$$

For analytes with a content below 120 µg/kg after the evaluation of a lot of mycotoxin- proficiency testing schemes after 1997 it was suggested for the target standard deviation a steady value of 22 % (Thompson), analogical:

$$\sigma = 0,22\ C / mr;$$

with σ = Target standard deviation for contents < 120 µg/kg
C = assigned content, expressed as a dimensionless mass ratio
mr = dimensionless mass ratio.

2.4.2 Precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation (σ_L) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is calculated :

$$\sigma = \sqrt{(\sigma_L^2 + (\sigma_r^2 / n))} .$$

If available, the precision data from official methods for each parameter are used to calculate the target standard deviation.

2.5 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) the result (x) of the participant is deviating from the assigned value (X).

Participants' z-scores are derived as:

$$z = (x - X) / \sigma \quad ;$$

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

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

2.6 z'-Score

The z'-Score can be used to assess the results of the participants in case the standard uncertainty must be considered (s. 2.8).

The calculation is carried out as follows (3)

$$z' = (x - X) / \sqrt{\hat{\sigma}^2 + u_X^2}$$

For the following evaluation $\sqrt{\hat{\sigma}^2 + u_X^2}$ is defined as $\hat{\sigma}'$, the target standard deviation considering the standard uncertainty of the results.

The requirements for the analytical performance are considered as fulfilled then, if

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

2.7 Quotient S^x/σ

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation 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.

2.8 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty u_X for this PT is calculated as follows

$$u_X = 1{,}25 * S^x / \sqrt{(p)} \quad .$$

If $u_X \leq 0{,}3 * \sigma$ the standard uncertainty of the assigned value needs not be included in the interpretation of the results of the PT. The quotient u_X / σ is given in the evaluation.

3 Realisation

3.1 Test material

The test material was a mixture of app. 88% ground chocolate wohle grain-Cookies with a declared fat content of 23%, app. 10 % of an inulin rich semi-finished product and app 2,1 % inulin.
App. 1200g of the material were ground and mixed, sieved, ground and mixed for several houres and finally put in portions of app. 20 gram. The portions where numbered chronologically.

3.1.1 Homogeneity

The calculation of the repeatability standard deviation of the participants for total dietary fibre and for inulin was used as an indicator of homogeneity. The results are 5,9 % and 8,7 % and they are the same magnitude as specified in the relevant official German methods to determine total dietary fibre and inulin. The repeatability standard deviation of the participants is given in the documentation.
Additionally in the documentation the portion numbers are graphically assigned to the results of total dietary fibre and of inulin. There is no trend recognizable in the results which could suggest inhomogeneity.

3.2 Tests

Two test samples were sent to every participating laboratory in the 17th week of 2014. The test method was optional. The tests should be finished at 10.06.2014.

3.3 Results and statistic evaluation

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

The statistical evaluation was carried out if at least 7 results were submitted.
The statistical evaluation was carried out for:

- Total dietary fibre
- Inulin

For both parameters the target range according to 2.4.2 with official German method L 17.03-1 (total dietary fibre) and L 00.00-94 (Inulin) was extended according to 2.6 of this report, because results showed a high variation. Unfortunately the participants gave no information if they had defatted the samples.
Results for soluble and for insoluble fibre are given in part 4 of this report.
Results for fibre after inulinase-reaction are given in the documentation.
Queried and documented were further results and the testing method applied.
17 out of 17 participants submitted results in time.

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 upper table the characteristics are listed:

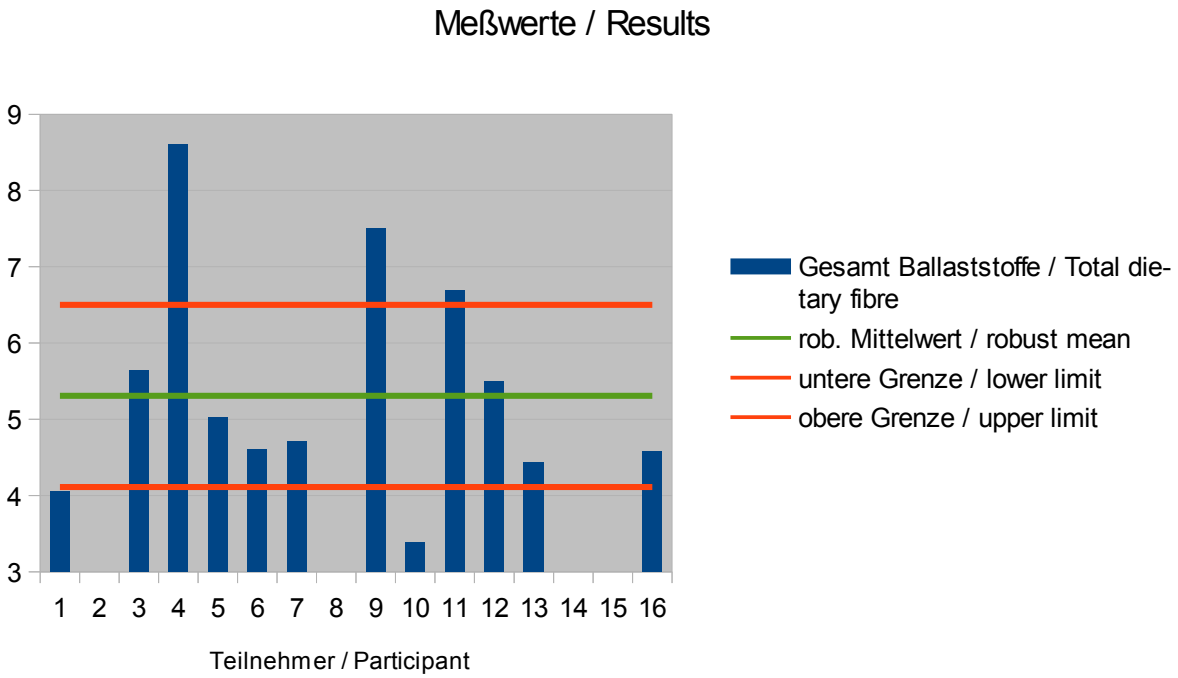
number of the results
number of outliers
mean
median
robust mean (X)
robust standard deviation(S^x)
target standard deviation (σ) or (σ´)
lower limit of target range (X – 2σ) or (X – 2σ´)
upper limit of target range (X + 2σ) or (X + 2σ´)
quotient S^x/σ
standard uncertainty u_x
quotient u_X/σ
results in target range.

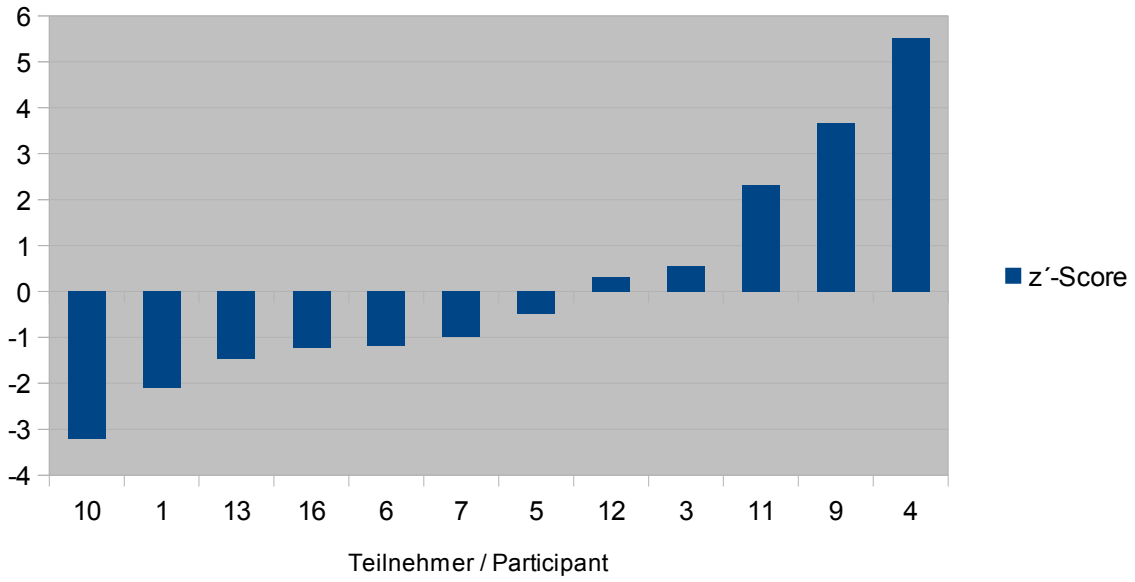
In the lower table -laboratories- the individual results of the participating laboratory are listed:

evaluation number	test result	deviation from assigned value	Z-Score (σ)	remarks

4.1 Total dietary fibre in g/100g

Statistic Data	
number of the results	12
number of outliers	1
mean	5,39
median	4,87
robust mean (X)	5,31
robust standard deviation (S^x)	1,51
target standard deviation (sigma')	0,60
target standard deviation for information (sigma)	0,25
lower limit of target range	4,11
upper limit of target range	6,50
quotient S^x/σ	6,2
standard uncertainty U^x	0,54
quotient U^x/σ	2,2
results in target range	7
percent in target range	58

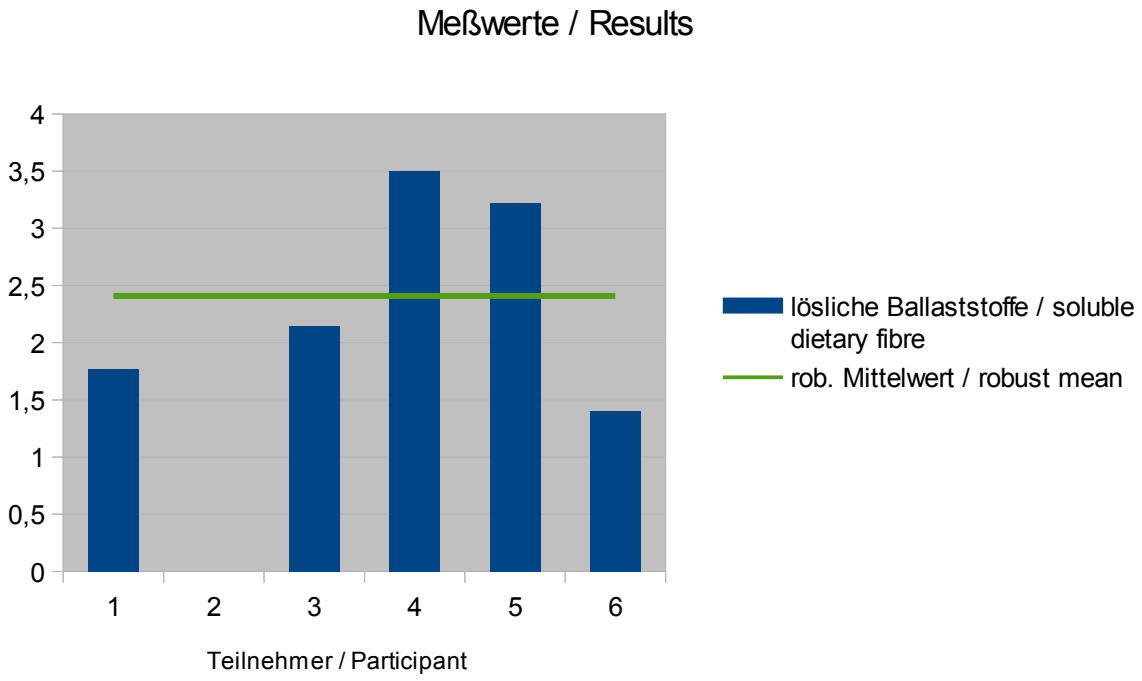




Auswerte nummer / Evaluation number	Gesamt Ballaststoffe / Total dietary fibre	Abweichung / Deviation	z´-Score	z-Score zur info	Hinweis / Remark
1	4,05	-1,26	-2,1	-5,1	
2					
3	5,64	0,33	0,6	1,4	
4	8,6	3,29	5,5	13,4	Ausreisser / Outlier
5	5,02	-0,29	-0,5	-1,2	
6	4,6	-0,71	-1,2	-2,9	
7	4,71	-0,6	-1,0	-2,4	
8					
9	7,5	2,19	3,7	8,9	
10	3,39	-1,92	-3,2	-7,8	
11	6,7	1,39	2,3	5,7	
12	5,5	0,19	0,3	0,8	
13	4,43	-0,88	-1,5	-3,6	
14					
15					
16	4,58	-0,73	-1,2	-3,0	
17					

4.2 Soluble fibre in g/100g

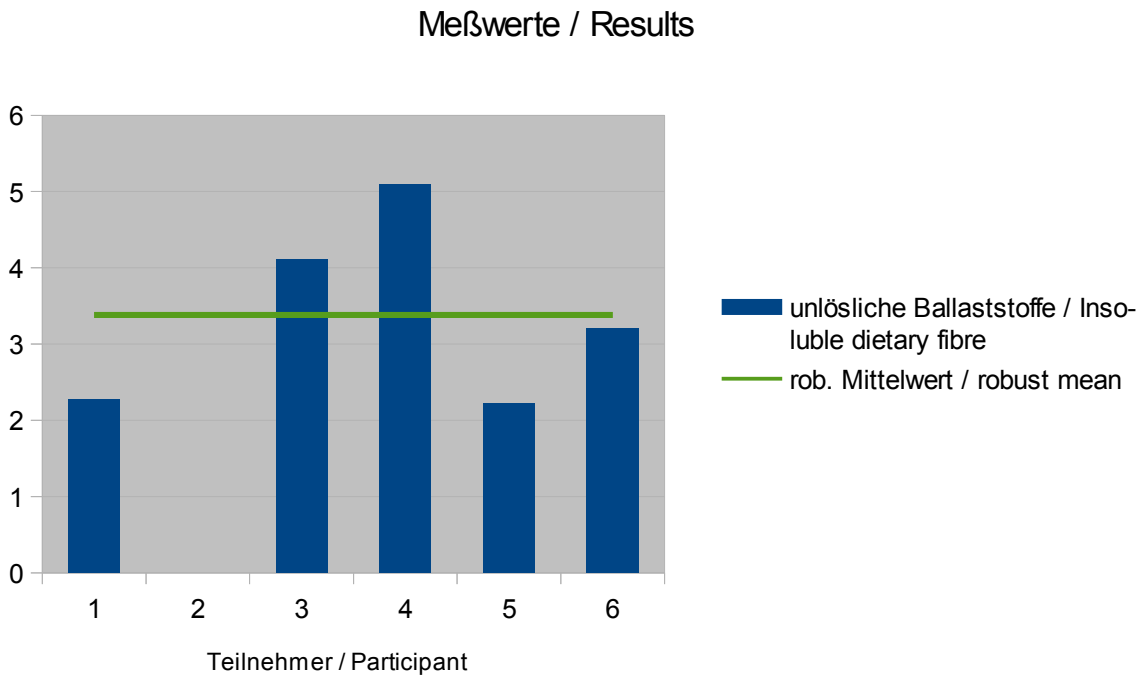
Statistic Data	
number of the results	5
number of outliers	0
mean	2,41
median	2,15
robust mean (X)	2,41
robust standard deviation (S^x)	1,04
target standard deviation (σ)	not calculated
quotient S^x/σ	not calculated
standard uncertainty U^x	not calculated
quotient U^x/σ	not calculated
results in target range	not calculated
percent in target range	not calculated



Auswerte nummer / Evaluation number	lösliche Ballaststoffe / soluble dietary fibre	Abweichung / Deviation	Hinweis / Remark
1	1,78	-0,63	
2			
3	2,15	-0,26	
4	3,5	1,09	
5	3,22	0,81	
6	1,4	-1,01	
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			

4.3 Insoluble fibre in g/100g

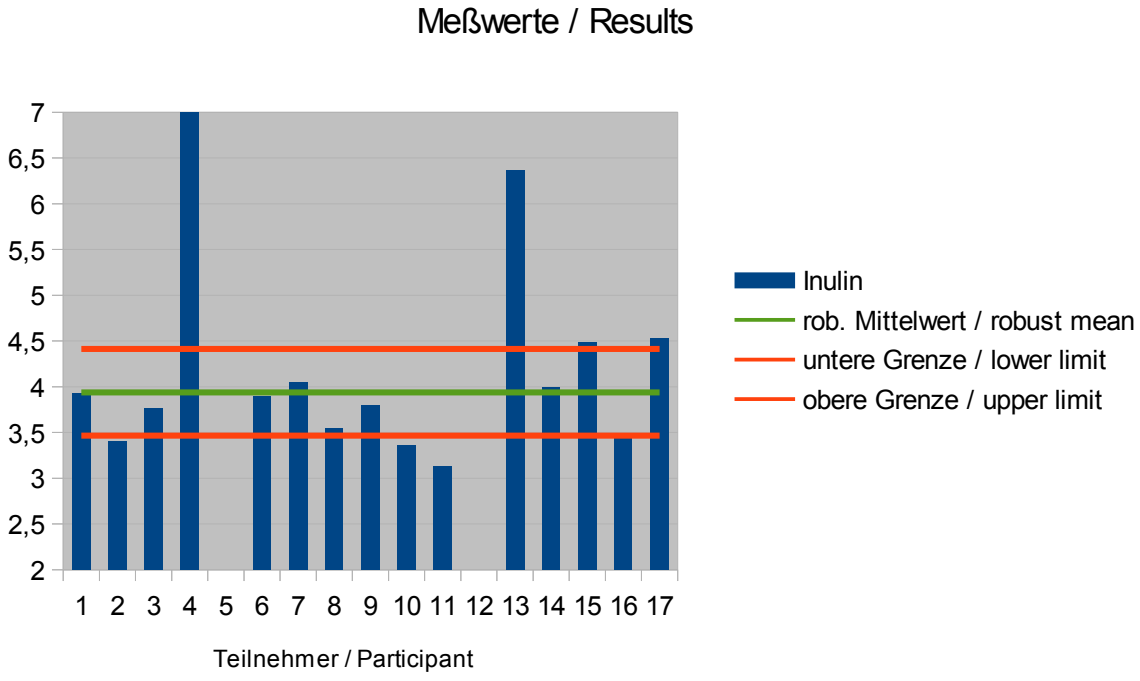
Statistic Data	
number of the results	5
number of outliers	0
mean	3,38
median	3,20
robust mean (X)	3,38
robust standard deviation (S^x)	1,40
target standard deviation (σ)	not calculated
quotient S^x/σ	not calculated
standard uncertainty U^x	not calculated
quotient U^x/σ	not calculated
results in target range	not calculated
percent in target range	not calculated

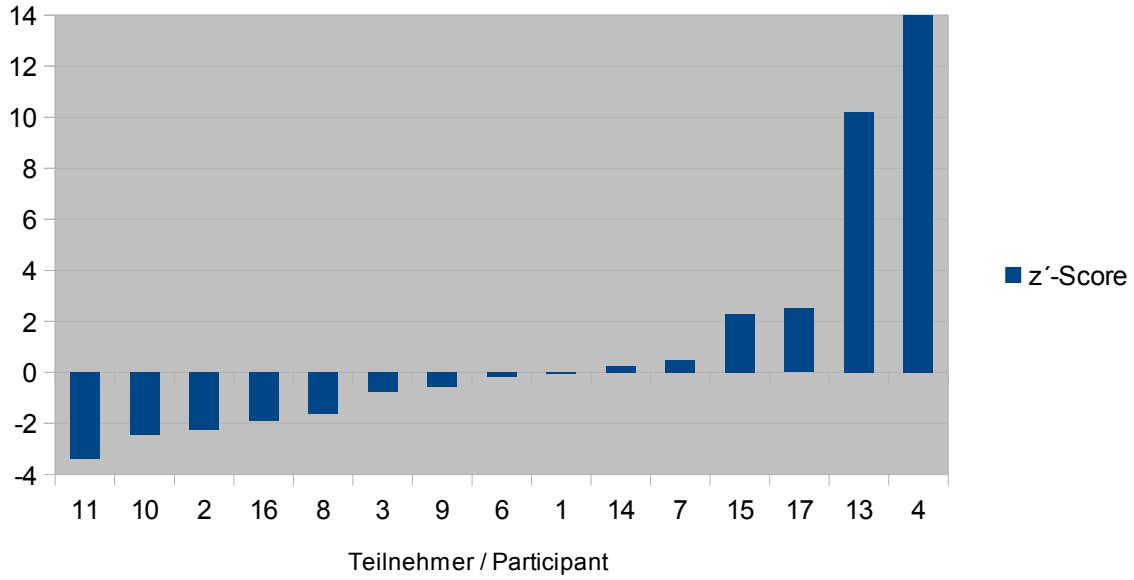


Auswerte nummer / Evaluation number	unlösliche Ballaststoffe / Insoluble dietary fibre	Abweichung / Deviation	Hinweis / Remark
1	2,27	-1,11	
2			
3	4,11	0,73	
4	5,1	1,72	
5	2,22	-1,16	
6	3,2	-0,18	
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			

4.4 Inulin in g/100g

Statistic Data	
number of the results	15
number of outliers	1
mean	6,58
median	3,90
robust mean (X)	3,94
robust standard deviation (S^x)	0,61
target standard deviation (sigma')	0,24
target standard deviation for information (sigma)	0,13
lower limit of target range	3,46
upper limit of target range	4,41
quotient S^x/σ	4,5
standard uncertainty U^x	0,20
quotient U^x/σ	1,5
results in target range	8
percent in target range	53





Auswerte nummer / Evaluation number	Inulin	Abweichung / Deviation	z´-Score	z-Score zur info	Hinweis / Remark
1	3,93	-0,01	-0,1	-0,1	
2	3,4	-0,54	-2,3	-4,0	
3	3,76	-0,18	-0,8	-1,3	
4	43	39,06	164,8	291,7	Ausreisser / Outlier
5					
6	3,9	-0,04	-0,2	-0,3	
7	4,05	0,11	0,5	0,8	
8	3,55	-0,39	-1,6	-2,9	
9	3,8	-0,14	-0,6	-1,0	
10	3,36	-0,58	-2,4	-4,3	
11	3,13	-0,81	-3,4	-6,0	
12					
13	6,36	2,42	10,2	18,1	
14	4	0,06	0,3	0,5	
15	4,48	0,55	2,3	4,1	
16	3,49	-0,45	-1,9	-3,3	
17	4,53	0,59	2,5	4,4	

5 Documentation

5.1 Primary data

5.1.1 Fibre in g/100g

Teilnehmer / Participant	Probe / sample A	Probe / sample B	Gesamt Ballaststoffe / Total dietary fibre	Ergebnis Probe / Result A	Ergebnis Probe / Result B	Lösliche Ballaststoffe / Soluble dietary fibre	Ergebnis Probe / Result A	Ergebnis Probe / Result B
1	5	36		3,8	4,3		1,72	1,83
2								
3	53	7	5,64	5,41	5,86	2,15	1,95	2,34
4	9	22	8,6	8,61	8,49	3,5	3,51	3,43
5	17	39	5,02	5,1	4,94	3,22	3,45	2,98
6	13	34	4,6	4,6	4,5	1,4	1,3	1,4
7	10	27	4,71	4,62	4,79			
8	26	49						
9	20	48	7,5	7,6	7,3			
10	11	38	3,39	3,62	3,17			
11	4	29	6,695	6,67	6,72			
12	23	51	5,5	5,2	5,8	X		
13	15	40	4,43	4,58	4,28			
14	8	33						
15	25	44						
16	41	35	4,58	4,53	4,64			
17	3	31						

Teilnehmer / Participant	Probe / sample A	Probe / sample B	Unlösliche Ballaststoffe / Insoluble dietary fibre	Ergebnis Probe / Result A	Ergebnis Probe / Result B	Gesamt-Ballaststoffe nach Inulinase-Reaktion / Total fibre with inulinase reaction	Ergebnis Probe / Result A	Ergebnis Probe / Result B
1	5	36		2,11	2,43			
2						9,1	9,1	9
3	53	7	4,11	4,29	3,93	5,4	5,66	5,14
4	9	22	5,1	5,1	5,06			
5	17	39	2,22	2,41	2,02			
6	13	34	3,2	3,2	3,1			
7	10	27						
8	26	49						
9	20	48						
10	11	38						
11	4	29						
12	23	51	X			X		
13	15	40						
14	8	33				3,1	3,2	3
15	25	44						
16	41	35						
17	3	31						

Teilnehmer / Participant	Probe / sample A	Probe / sample B	Lösliche Ballaststoffe nach Inulinase-Reaktion / Soluble fibre with inulinase reaction	Ergebnis Probe / Result A	Ergebnis Probe / Result B	Unlösliche Ballaststoffe nach Inulinase-Reaktion / Insoluble fibre with inulinase reaction	Ergebnis Probe / Result A	Ergebnis Probe / Result B
1	5	36						
2								
3	53	7	1,84	1,88	1,79	3,77	3,61	3,92
4	9	22						
5	17	39						
6	13	34						
7	10	27						
8	26	49						
9	20	48						
10	11	38						
11	4	29						
12	23	51	X			X		
13	15	40						
14	8	33						
15	25	44						
16	41	35						
17	3	31						

5.1.2 *Inulin in g/100g*

Teilnehmer / Participant	Probe / sample A	Probe / sample B	Inulin	Ergebnis Probe / Result A	Ergebnis Probe / result B
1	5	36		3,92	3,93
2			3,4	3,5	3,3
3	53	7	3,76	3,45	4,06
4	9	22	43	42,2	44,2
5	17	39			
6	13	34	3,9	3,9	3,9
7	10	27	4,05	4,15	3,94
8	26	49	3,55	3,52	3,57
9	20	48	3,8	3,8	3,9
10	11	38	3,36	3,61	3,1
11	4	29	3,13	3,25	3,02
12	23	51			
13	15	40	6,36	6,07	6,65
14	8	33	4	3,95	4,05
15	25	44	4,484	4,481	4,487
16	41	35	3,49	3,56	3,42
17	3	31	4,53	4,92	4,14

5.2 Homogeneity

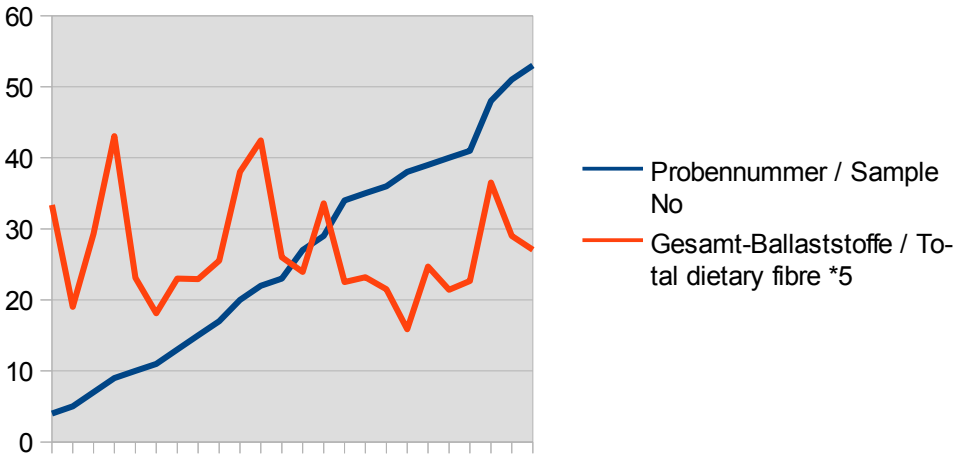
5.2.1 *Repeatability standard deviation of results*

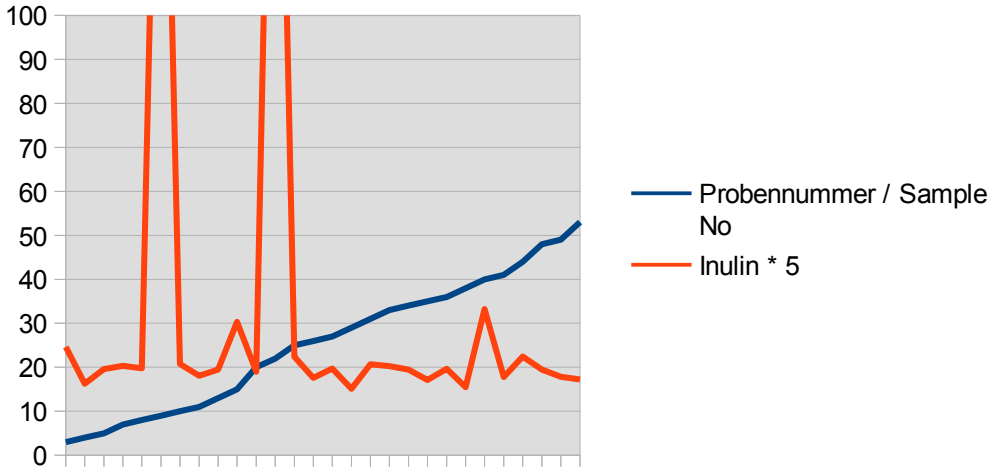
The Repeatability standard deviation of duplicate tests of the participants was calculated from the data documented in 5.1.

It is 0,315 = 5,9% of X (Lit. 2,5 – 5,9%)

And it is (adjusted of an outlier) 0,342 = 8,7 % of X (Lit. 1,9 – 4,9%)

5.2.2 *Comparison of sample number / test result*





5.3 Analytical methods

Details from the participants

5.3.1 Fibre

Participant	Frit diameter	Pore	Amylase	Protease
1	40 mm	2	Testkit from Megazyme	Testkit from Megazyme
2		12-15 µm	SIGMA A3306	SIGMA P6110
3	40 mm	Por. 3	alpha-Amylase (Megazyme 54 U/mg)	Megazyme (Cat. No. E-BSPRT100)
4	40mm	40-100 µm	150000 Ceralpha-Units/g	350 Tyrosin-Units/ml
5	3,5cm	2	Kit from Megazyme	Testkit from Megazyme
6	3,3 cm	2	α-amylase, 3000 units/ml (Megazyme)	protease, 350 tyrosine units/ml (Megazyme)
7				
8				
9		2	Megazyme Kit AOAC 991.43	Megazyme Kit AOAC 991.43
10			Merck	Merck
11	55 mm (Filter)	22 µm (Filter)	Megazyme-Testkit	Megazyme-Testkit
12				
13	90 mm	40-60µm	3,000 U/ml	50 mg/ml (~350tyrosine U/ml)
14	3 cm	40-60 µm	sigma tdf Kit	sigma tdf Kit
15				
16	4,1 cm	2	Bioquant 1.12979.0001 Merck	Bioquant 1.12979.0001 Merck
17				

Participant	Amyloglucosidase	Inulinase	Method without inulinase is accredited	Method with inulinase is accredited
			yes/no	yes/no
1	Testkit from Megazyme			
2	SIGMA A9913	Coring	yes	yes
3	Megazyme (3,260 U/ml)	Megazyme (E-FRMXLQ)	yes	yes
4	3300 Units/ml		yes	
5	Testkit from Megazyme			
6	amyloglucosidase, 3300 units/ml (Megazyme)		no	
7			yes	
8				
9	Megazyme Kit AOAC 991.43		no	
10	Merck		yes	
11			yes	no
12			no	
13	3300 U/ml with soluble starch		yes	
14	sigma tdf Kit	sigma	yes	no
15				
16	Bioquant 1.12979.0001 Merck		yes	yes
17				

5.3.2 Inulin

Participant	UV-Test Kit producer	HPLC column	Detector	Method is accredited
				yes/no
1		NH2	RID	yes
2	r-biopharm			
3	Boeringer Mannheim	not refered	not refered	yes
4		CarboPac PA1	electrochemical	no
5				
6		Dionex CarboPac PA1	PAD	no
7				
8		NH3	RID	yes
9	/	AOAC997.08	AOAC997.08	no
10			RI	yes
11	Megazyme			no
12				
13		2 x TSK-GELG2500PWXL	RI	no
14		PA100 dionex	PAD	no
15	r-biopharm			no
16	Boehringer Mannheim / R-Biopharm Nr. 10 716 260 035			yes
17	-	Carbopack PA1	PAD	no

6 Index of participant laboratories

Participant	Location	Country
		GERMANY
		GERMANY
		ITALY
		GERMANY
		NETHERLANDS
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		BELGIUM
		BELGIUM
		GERMANY
		NETHERLANDS
		ESTONIA
		GERMANY

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

7 Index of literature

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. 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
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB : Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs;W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
11. 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)
12. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
13. ASU §64 LFGB: L 00.00-18; Bestimmung der Ballaststoffe in Lebensmitteln
14. ASU §64 LFGB: L 16.08-1, Bestimmung der Ballaststoffe in Getreidekleie
15. ASU §64 LFGB: L 17.03-1; Bestimmung der Ballaststoffe in Mischbrot
16. ASU §64 LFGB: L 48.01-25; Bestimmung der Ballaststoffe in Säuglings- und Kindernahrung auf Milchbasis
17. ASU §64 LFGB: L 00.00-94; Bestimmung von Inulin in Lebensmitteln