Proficiency Tests
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Evaluation Report
proficiency test

DLA 14/2017

Response PT Soya:

5 Processed Samples Soya Flour, Soya Isolate, Soya Granulate, Soya Milk and Tofu

in Potato Powder Matrix

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Allgemeine Informationen zur Eignungsprüfung (EP)
General Information on the proficiency test (PT)

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Unteraufträge *Subcontractors*	Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben. The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA.
Vertraulichkeit *Confidentiality*	Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben. Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant.

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].

The present proficiency test format **„Response PT Allergens"** includes 5 differently processed samples of an allergen in a simple carrier matrix as well as a "blank sample". Hereby it offers the possibility to prove that the analytical determination methods used by the participants are suitable to detect the respective processed allergens qualitatively and to determine its quantitative response factors.

In order to ensure comparability of the processed sample material, the allergen contents of the PT sample series were calculated as total protein contents and were adjusted to approximately the same levels. The evaluation of the PT-results was done qualitatively by scores from 1-5 (score 5 = all processings successfully determined). Quantitative results were given including the calculated respective recovery rate (recovery score) for information in the report.

2. Realisation

2.1 Test material

6 PT-samples for qualitative and optionally quantitative determination of soya (soy protein) in soya flour, soya granulate (textured soya), soya milk and tofu in potato powder were provided.
The respective raw materials for the PT sample series were common in commerce processed soya products. For each PT-sample 4-6 products of different origin were worked up. The tofu- and soya milk-mixtures were dried at 60°C prior to further use.
Afterwards premixes with contents from approx. 8-25 % of the regarding allergenic ingredients were produced (s. Tab. 1). For this the products were pre crushed if necessary, mixed gravimetrically with further ingredients, crushed and sieved by means of a centrifugal mill (mesh 250 µm) and homogenized.
The allergen-premixes were added to the carrier matrix of potato powder / maltodextrin and homogenized. An aliquot of the carrier matrix was provided as the "blank sample".

The 6 PT-samples were portioned to approximately 20 g in metallized PET film bags.

The contents of soy protein of the PT-samples were in the range of approx. 100 mg/kg (94 to 120 mg/kg, see Tab. 1).

Each assigned value, here the spiked allergen-contents, is afflicted with a standard uncertainty. As uncertainties the following factors were considered: protein content of spiking materials, mixing homogeneity, homogeneity and stability of soy protein.
All uncertainties were expressed in the form of their standard deviations and then added as variances. The square root from the sum of the total variances results in the combined uncertainty "Uc". Multiplied with the coverage factor k=2 the extended uncertainties of the assigned values "$U(X_{pt})$" are obtained [3, 13, 16-18].

Table 1: Composition of DLA-Samples

PT-Sample series	**Sample 1** Tofu	**Sample 2** Soya milk	**Sample 3** Granulate	**Sample 4** Isolate	**Sample 5** Soya flour	**Sample 6** „blank"
Ingredients	**g/100 g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**	**g/100g**
Potato powder Ingredients: potato, E471, E304, E223, E100 Nutrients per 100 g: Protein 8,3 g, carbohydrates 76 g, fat 0,6 g, salt 0,15 g	75	75	75	75	75	75
Maltodextrin	25	25	25	25	25	25
Allergen-Premixes Ingredients: maltodextrin (75% - 90%), sodium chloride (< 5%), silicon dioxide (< 2,5%), processed allergen products (each 8% - 25% dry weight)	0,14	0,14	0,13	0,10	0,10	-
Allergen-Contents	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
*Soya flour, roasted** Protein 37,8% ** (6 products from Asia, Europe and North America)	-	-	-	-	260	-
*Soya isolate** Protein 81,8% ** *(5 products)*	-	-	-	115	-	-
*Soya granulate** protein 46,0% ** *(6 products from Asia, Europe and North America)*	-	-	261	-	-	-
*Soya milk, dried** Protein 51,4% ** *(6 products from Asia and Europe)*	-	233	-	-	-	-
*Tofu, dried** Protein 49,3% ** *(4 products from Asia and Europa)*	243	-	-	-	-	-
- *thereof Soy protein*	**120**	**120**	**120**	94,**1**	**98,4**	**-**
Extended combined uncertainty (k=2) of soy protein-content (= ± 12 %)	± 14,4	± 14,4	± 14,4	± 11,3	± 11,8	-

*Allergen contents as „total food" as described in column ingredients according to gravimetric mixture
** Protein contents according to laboratory analysis of raw material mixtures (total nitrogen according to Kjeldahl with F=5,7 for soy protein)

Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

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 samples 1 to 5 showed a probability of 98%, 91%, 98%, 81% and 98%. 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 0,46, 0,67, 0,63, 0,86 and 0,55 respectively. The results of microtracer analysis are given in the documentation.

2.1.2 Stability

The experience with various DLA reference materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameter soya for comparable food matrices and water activity (a_W value <0,5). The stability of the sample material is therefore given during the investigation period under consideration of given storage conditions.

2.2 Sample shipment and information to the test

One portion of the test material (sample 1 to 6) were sent to every participating laboratory in the 16th week of 2017. The testing method was optional. The tests should be finished at June 2nd 2017 the latest.

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

There are 5 different samples with similar contents of the allergenic parameter soya, which is differently processed, contained in a simple carrier matrix as well as a "blank"-sample (carrier matrix).

- *The samples 1-5 are numbered in a random order. They contain soya flour, soya isolate, soya granulate, soya milk or tofu with known amounts of total soy protein, which is the base for the response comparison of the quantitative results of the participants.*
- *Please give all your quantitative results as total soy protein.*
- *Possible conversion factors for processed soya products are queried separately in the result submission file.*

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. On one hand the results given as positive/negative and on the other hand the indicated results of the allergenic ingredients e.g. total food item or protein in mg/kg were evaluated.
During evaluation DLA eventually requests detailed information by email on the type of indicated quantitative results from participants concerned.
Queried and documented were the indicated results and details of the test methods like specificity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

16 participants submitted results in time. One participant submitted no results.

3. Evaluation

Different ELISA-methods for the determination of allergens in foods are using different antibodies, which are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different results of the analyte content [26-29, 40]. Furthermore matrix- and/or processing of samples can have a strong impact on the detectability of allergens by ELISA and/or PCR methods.

In the present PT five different processed products containing the allergen soya, soya flour (roasted/toasted), soya isolate, soya granulate (textured soya), soya milk and tofu, were provided to determine the qualitative detectability and to determine the response in the used quantitative methods.

The participant results were evaluated *qualitatively* with a score from 1-5 indicating the number of successfully detected processed products.
The quantitative results were evaluated with a Recovery-Score (*RR-Score*), which indicates the number of results with a recovery rate in the range of 50 - 150% of the spiking level.

3.1 Qualitative Score

The qualitative valuation of each participant's results was performed with a Scores from 1-5 considering the number of "positive" or "negative" results matching the spiking of the PT-sample series (see Tab. 2). A Score from 5 indicates, that all processed products were detected successfully.
The results of the matrix sample no. 6 ("blank"-sample) were not evaluated if the participant result is in accordance with ≥75% positive or negative results of participants (consensus value) or if the result is below the limit of quantification of the used method.

Table 2: Evaluation of results using qualitative Scores

Sample 1 Tofu	Sample 2 Soya milk	Sample 3 Soya granulate	Sample 4 Soya isolate	Sample 5 Soya flour	Sample 6 „blank"	Score qualitative	Suitability qualitative
pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5	
negative	negative	negative	negative	negative	negative	0 (0%)	not sucessful
negative	negative	negative	negative	positive	negative	1 (20%)	1 product group
negative	negative	negative	positive	positive	negative	2 (40%)	2 product groups
negative	negative	positive	positive	positive	negative	3 (60%)	3 product groups
negative	positive	positive	positive	positive	negative	4 (80%)	4 product groups
positive	positive	positive	positive	positive	negative	5 (100%)	5 product groups

3.2 Recovery-Score (RR-Score)

The evaluation of the quantitative participant results for the spiked **PT-samples** was done by recovery scores (*RR-Scores*) which are related to the number of recovery rates in the range of acceptance. The RR-Scores are calculated by counting the number of results in the range of acceptance (s. below) per number of quantitatively determined samples. Further the percentage is given in the brackets behind.

The recovery rates were calculated considering the content of the spiked allergen (level of addition). The reference values are calculated from the values for samples 1 to 5 given in section 2.1 Sample material in Table 1. As range of acceptance RA for the evaluation of the participant results the range of the AOAC-recommendation of 50-150% for allergen-ELISAs was used [21]. This range was also used in the present PT for quantitative PCR- and LC/MS-results.

Only exact quantitative results were considered. Single results outside the given measuring range (e.g. indicated with > 25 mg/kg or < 2,5 mg/kg) or indicated with "0" were not considered.

The given recovery rates enable inter alia an assessment of matrix and/or processing influences.

3.2.1 Recovery rates by precision experiment

In ring trials of ASU §64 methods recovery rates in the range from 57% - 119% were obtained by ELISA methods and 11% - 145% for PCR methods, depending on matrix or processing and concentration (s. Table 3a and 3b). The given target standard deviation σ_{pt} was calculated for a number of m = 2 repeated measurements.

Table 3a: ELISA-Methods – Recovery rates and precision data from selected precision experiments[31-32].

Parameter	**Matrix**	**Mean [mg/kg]**	**Recovery**	**rob RSD_r**	**RSD_r**	**RSD_R**	**σpt**	**Method / Literature**
Peanut	Milk chocolate	173,7 33,8 5,9	87 % 85 % 59 %	- - -	8,8% 5,2% 7,8%	31% 20% 31%	30,4% 19,7% 30,5%	ELISA Manuf. A ASU 00.00-69
Peanut	Milk chocolate	215,7 40,1 10,1	108 % 100 % 101 %	- - -	5,9% 7,2% 7,3%	32% 14% 16%	31,7% 13,0% 15,1%	ELISA Manuf. B ASU 00.00-69
Peanut	Dark chocolate	148,2 30,9 5,7	74 % 77 % 57 %	- - -	6,0% 13% 6,1%	22% 25% 33%	21,6% 23,2% 32,7%	ELISA Manuf. A ASU 00.00-69
Hazelnut	Dark chocolate	16,3 7,56 3,73 1,62	81 % 76 % 75 % 81 %	- - - -	4,7% 8,9% 13% 15%	12% 15% 24% 33%	11,5% 13,6% 22,2% 31,2%	ELISA Manuf. A ASU 44.00-7
Hazelnut	Dark chocolate	21,3 10,7 4,69 2,37	106 % 107 % 94 % 119 %	- - - -	7,1% 11% 11% 9,3%	14% 19% 17% 17%	13,1% 17,3% 15,1% 16,4%	ELISA Manuf. B ASU 44.00-7

The Working Group on Prolamin Analysis and Toxicity (WGPAT)performed ring trials for validation of two commercial ELISA-Kits for determination of gluten using monoclonal R5 antibodies [25]. 12 food samples with gliadin contents in the range if 0 - 168 mg/kg were analysed by 20 laboratories. The obtained recovery rates were in the range between 65 and 110%, the relative repeatability standard deviation was between 1 – 25% (1. method) and 11 - 22% (2. method) and the relative reproducibility standard deviation between 23 - 47 % (1. method) and 25 - 33% (2. method). The authors concludes that both ELISA-Kits fulfil the validation criteria for ELISA methods [25].

The IRMM (Institute for Reference Materials and Measurements) proved the suitability of five different ELISA-Kits for the determination of peanut [28]. The mean values were in the concentration range of 0,3 - 16,1 mg/kg and/or 1,2 - 20,4 mg/kg. The smallest relative reproducibility standard deviation for each Kit was obtained for dark chocolate at 20 - 42% and cookies at 23 - 61%.

Table 3b: PCR-Methods - Relative repeatability standard deviations (RSD_r) and relative reproducibility standard deviations (RSD_R) according to selected evaluations from experiments by precision and the resulting target standard deviation σ_{pt} [33-38].

Parameter	**Matrix**	**Mean [mg/kg]**	**Reco-very**	**rob RSD_r**	**RSD_r**	**RSD_R**	**σ_{pt}**	**Method / Literature**
Almond	Rice cookie	105,2 18,0 10,5	105 % 90 % 105 %	-	19,3% 44,0% 32,0%	27,5% 49,1% 38,8%	23,9% 38,0% 31,5%	rt-PCR ASU 18.00-20
Almond	Wheat cookie Sauce powder	114,3 88,1	94,6 % 88,1 %	-	22,1% 43,9%	41,8% 43,1%	38,8% - %	rt-PCR ASU 18.00-20
Almond	Rice cookie	109 21,3 12,3	109 % 107 % 121 %	-	17,6% 35,8% 32,0%	32,8% 45,0% 47,8%	30,3% 37,2% 42,1%	rt-PCR ASU 18.00-22
Almond	Wheat cookie Sauce powder	120,7 112	98,2 % 94,1 %	-	15,7% 36,2%	32,5% 42,8%	30,5% 34,3%	rt-PCR ASU 18.00-22
Sesame	Rice cookie	94,6 15,7 9,8	95 % 79 % 98 %	-	22,5% 26,0% 20,9%	27,5% 39,5% 33,5%	22,4% 35,0% 30,0%	rt-PCR ASU 18.00-19
Sesame	Wheat cookie Sauce powder	96,9 59,8	79 % 60 %	-	21,8% 22,2%	33,0% 43,2%	29,2% 40,2%	rt-PCR ASU 18.00-19
Sesame	Rice cookie	88,9 17,8 9,8	89 % 89 % 98 %	-	18,2% 34,2% 26,2%	30,5% 37,8% 37,0%	27,7% 29,1% 32,0%	rt-PCR ASU 18.00-22
Sesame	Wheat cookie Sauce powder	115 58,5	93 % 59 %	-	16,7% 30,8%	41,1% 44,4%	39,4% 38,7%	rt-PCR ASU 18.00-22
Soya	Wheat flour Maize flour	107 145	107 % 145 %	63 % 34 %	- -	31 % 24 %	- -	rt-PCR ASU 16.01-9

3.2.2 Values by perception

Requirements to the performance of analysis methods for quantitative determination of allergens in food were compiled for example from the Ministry of Health and Welfare (MHLW) in Japan [23], by the Working Group 12 „Food allergens" of the Technician Committee CEN/TC 275 [20-22], by a international "Food Allergen Working Group" under the leadership of the AOAC Presidential Task Force on Food Allergens [24] and by the Codex Alimentarius Commitee (CAC/GL 74-2010) [19].

The following relevant ELISA and/or PCR validation criteria of the committees are given in Table 4 and 5.

Table 4: ELISA validation criteria

Literature [19-25]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
MHLW 2006	50 - 150%		≤ 25%
CEN 2009		≤ 20%	
AOAC 2010	50 - 150%	6,9 - 34,4% (a)	19,5 - 57,2% (a)
CAC 2010	70 - 120%	≤ 25%	≤ 35%

(a) = Example from hypothetical ring trail in the concentration range of 0,5 - 5 mg/kg

Table 5: PCR validation criteria

Literature [19]	**Recovery Rate**	**Repeatability Standard Deviation**	**Reproducibility Standard Deviation**
CAC 2010	± 25% (a)	≤ 25%	≤ 35%

(a) = Trueness / Richtigkeit

Due to the current performance of ELISA and PCR methods for quantitative determination of allergens in food, which can be derived from precision data by experiments and from validation criteria mentioned above, a common relative target standard deviation (σ_{pt} value) from 25% was defined. The recovery rate was set to 50-150%.

4. Results

All following tables are anonymized. With the delivering of the evaluation report the participants are informed about their individual evaluation number.
Evaluation was done separately for ELISA-, PCR- and LC/MS methods.

In the result chapter all quantitative results of the participants are displayed formatted to 3 decimal places. In the documentation, all results are given as they were transmitted by the participants.

To ensure the **comparability of quantitative results** DLA harmonized participants' results giving different specifications (e.g. as protein or as allergenic food) as far as possible by recalculation to total soy protein.

In the present PT the ELISA- and LC/MS-results were given as soy protein, therefore no recalculation was necessary. The PCR-results were given as soya/soya flour, and thus they were recalculated to soy protein using the protein-content from soya flour (sample 5, 37,8%, s. Tab. 1).

The qualitative results are presented in the corresponding evaluation table as indicated below:

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	number of detected Samples 1 - 5		

The quantitative results are presented in the corresponding evaluation table as indicated below:

Evaluation number	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		

* Recovery Rate

4.1 Proficiency Test processed Soya Prodcuts

4.1.1 Qualitative Scores: ElISA-Methods

Evaluation number	Sample 1 Tofu	Sample 2 Soya milk	Sample 3 Soya granulate	Sample 4 Soya isolate	Sample 5 Soya flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Samples 1 - 5		
3	negative	positive°	positive°	positive	positive	negative	4 (80%)	ES	° results < LOQ
14	negative	positive°	positive°	positive	positive	negative	4 (80%)	ES	° results < LOQ
4a	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
16	positive	positive	positive	positive	positive	negative	5 (100%)	IL	
13	positive	positive	positive	positive	positive	negative	5 (100%)	MI	
1	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
4b	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
5	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
8	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
10	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
11	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
12	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	
15	positive	positive	positive	positive	positive	negative	5 (100%)	RS-F	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	11	13	13	13	13	0
Number negative	2	0	0	0	0	13
Percent positive	85	100	100	100	100	0
Percent negative	15	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

ES = ELISA-Systems

IL = Immunolab

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

Comments:

For the processed products of samples 2 to 5 consensus values of 100% positive results were obtained. For sample 1 (tofu) two negative results were obtained by method ES, thus the consensus value was 85% positive results.

4.1.2 Qualitative Scores: PCR-Methods

Evaluation number	Sample 1 Tofu	Sample 2 Soya milk	Sample 3 Soya granulate	Sample 4 Soya isolate	Sample 5 Soya flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Samples 1 - 5		
5	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
9	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
11	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
12	positive	positive	positive	positive	positive	negative	5 (100%)	ASU	
6a	positive	positive	positive	positive	positive	negative	5 (100%)	CEN	
7a	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-4p	
4	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-ID	
6b	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-ID	
2	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-Q	
7b	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-Q	
10	positive	positive	positive	positive	positive	negative	5 (100%)	SFA-Q	

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Number positive	11	11	11	11	11	0
Number negative	0	0	0	0	0	11
Percent positive	100	100	100	100	100	0
Percent negative	0	0	0	0	0	100
Consensus value	positive	positive	positive	positive	positive	negative
Spiking	positive	positive	positive	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method

CEN = CEN / Technical Specifications

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

Comments:

For all processed products (sample 1 to 5) consensus values of 100% positive results were obtained by the PCR-methods.

4.1.3 Qualitative Scores: LC/MS-Methods

Evaluation number	Sample 1 Tofu	Sample 2 Soya milk	Sample 3 Soya granulate	Sample 4 Soya isolate	Sample 5 Soya flour	Sample 6 „blank“	Score qualitative	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	pos/neg	Number of detected Samples 1 - 5		
2	positive	positive	positive	positive	positive	negative	5 (100%)	LC/MS	

	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5	Probe 6
Spiking	positive	positive	positive	positive	positive	negative

Methods:
LC/MS = Liquid Chromatography / Mass Spectrometry

Comments:
For all processed products (sample 1 to 5) one participant obtained positive results using a LC/MS/MS-method.

4.1.4 Quantitative: ELISA-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Tofu		Sample 2 Soya milk		Sample 3 Soya granulate		Sample 4 Soya isolate		Sample 5 Soya flour		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		
3	<2.5		< 5		< 5		31	33	120	**122**	1/2 (50%)	ES	only Samples 4 + 5 considered
14	<1.25		>1.25 <2.5		>1.25 <2.5		26,7	28	126	**128**	1/2 (50%)	ES	only Samples 4 + 5 considered
13	67,0	**56**	30,0	25	91	**76**	120	**128**	78	**79**	4/5 (80%)	MI	
1	> 20.0		10,7	8,9	>20.0		>20.0		>20.0		0/1 (0%)	RS-F	only Sample 2 considered
4b	69,1	**58**	10,9	9,1	102	**85**	128	**136**	82,8	**84**	4/5 (80%)	RS-F	
5	75,9	**63**	11,3	9,4	110	**92**	123	**130**	92,2	**94**	4/5 (80%)	RS-F	
8	68,5	**57**	9,75	8,1	84,7	**71**	113	**120**	88,2	**90**	4/5 (80%)	RS-F	
10	73,0	**61**	11,7	10	110	**92**	116	**123**	83,9	**85**	4/5 (80%)	RS-F	
11	52,9	44	8,91	7,4	73,0	**61**	105	**111**	78,4	**80**	4/5 (80%)	RS-F	
12	61,8	**52**	10,0	8,4	74,2	**62**	91,4	**97,1**	79,1	**80**	4/5 (80%)	RS-F	
15	87,9	**73**	12,8	11	109	**91**	172	183	107	**109**	3/5 (60%)	RS-F	
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	**7**	Number in RA	**0**	Number in RA	**8**	Number in RA	**7**	Number in RA	**10**			
	Percent in RA	**88**	Percent in RA	**0**	Percent in RA	**100**	Percent in RA	**70**	Percent in RA	**100**			

Methods:
ES = ELISA-Systems
MI = Morinaga Institute ELISA
RS-F= Ridascreen® Fast, R-Biopharm

* Recovery Rate 100% Reference value: Soy protein, s. Page 6

** Range of Accepatnce by AOAC for Allergen-ELISAs

Comments:

For the samples 1 (tofu), 3 (soya granulate), 4 (soya isolate) and 5 (soya flour, roasted) 70% to 100% of the recovery rates of the participant results were in the range of acceptance of 50-150% (with method ES only the results for sample 5). For sample 2 the recovery rates were in the range of 7,4 to 11% (method RS-F) and for one result of method MI at 25%.

4.1.5 Quantitative: PCR-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Tofu		Sample 2 Soya milk		Sample 3 Soya granulate		Sample 4 Soya isolate		Sample 5 Soya flour		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		
9	7,2	6,0	6,0	5,0	74,0	**62**	63,0	**67**	145	**147**	3/5 (60%)	ASU	Results converted °
6a	1,6	1,3	1,7	1,4	71,8	**60**	52,9	**56**	60,5	**61**	3/5 (60%)	CEN	Results converted °
6b	19	16	18,1	15	71,8	**60**	60,5	**64**	37,8	38	2/5 (40%)	SFA-ID	Results converted °
2	21,4	18	13,2	11	74,5	**62**	69,2	**74**	57,1	**58**	3/5 (60%)	SFA-Q	Results converted °
7b	15,6	13	27,1	23	87,3	**73**	36,0	38	36,7	37	1/5 (20%)	SFA-Q	Results converted °
10	42,2	35	22,6	19	98,7	**82**	50,5	**54**	34,1	35	2/5 (40%)	SFA-Q	Results converted °
													° Conversion p. 14
	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %	RA**	50-150 %			
	Number in RA	**0**	Number in RA	**0**	Number in RA	**6**	Number in RA	**5**	Number in RA	**3**			
	Percent in RA	**0**	Percent in RA	**0**	Percent in RA	**100**	Percent in RA	**83**	Percent in RA	**50**			

* Recovery Rate 100% Reference value: Soy protein, s. Page 6

** Range of Accepatnce by AOAC for Allergen-ELISAs

Methods:

ASU = ASU §64 Methode/method

CEN = CEN / Technical Specifications

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

SFA-Q = Sure Food Allergen Quant, R-Biopharm / Congen

Comments:

For the samples 3 (soya granulate), 4 (soya isolate) and 5 (soya flour, roasted) 83% to 100% of the recovery rates of the participant results were in the range of acceptance of 50-150%.

For the samples 1 (tofu) and 2 (soya milk) the recovery rates were in the range from 1,3 to 35% and 1,4% to 23%.

4.1.6 Quantitative: LC/MS-Methods Recovery Rates-Scores (RR-Scores)

Evaluation number	Sample 1 Tofu		Sample 2 Soya milk		Sample 3 Soya granulate		Sample 4 Soya isolate		Sample 5 Soya flour		RR-Score	Method	Remarks
	Result	RR *	Result	RR *	Result	RR *	Result	RR *	Result	RR *	RR *		
	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	[mg/kg]	[%]	Number in RA**		
2	55,6	46	65,3	**54**	75,5	**63**	143,9	153	118,7	**121**	3/5 (60%)	LC/MS	

* Recovery Rate 100% Reference value: Soya protein, s. Page 6

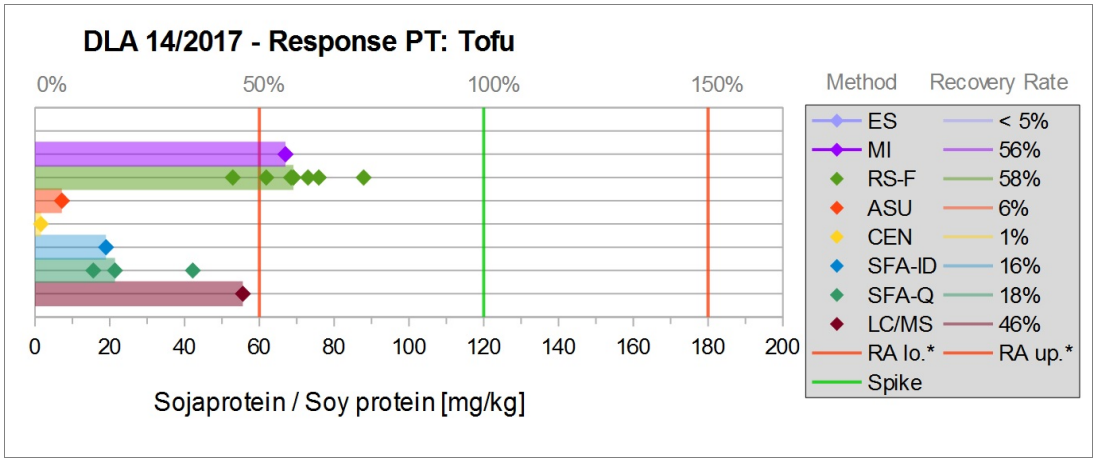
** Range of Accepatnce by AOAC for Allergen-ELISAs

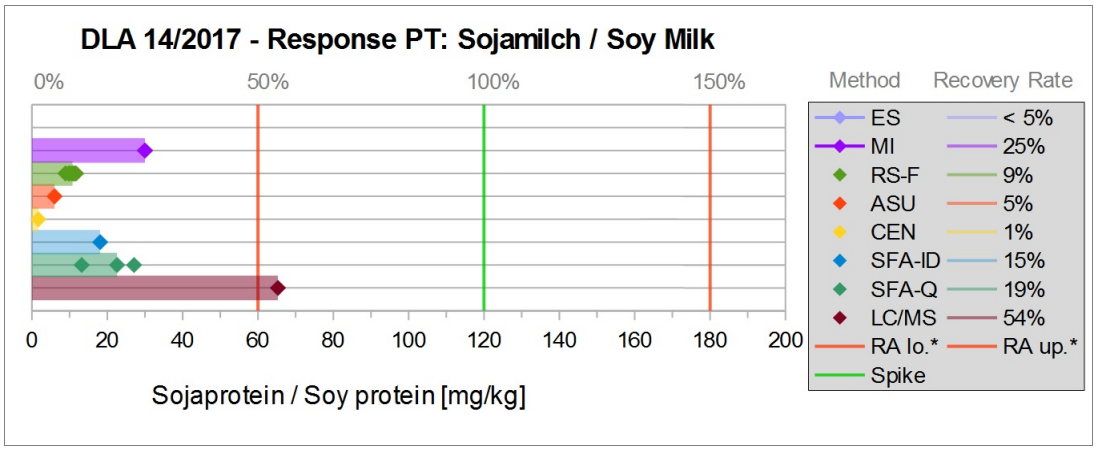
Methods:

LC/MS = Flüssigchromatographie / Massenspektrometrie

Comments:

For the samples 2 (soya milk), 3 (soya granulate) and 5 (soya flour, roasted) the recovery rates of the participant results were in the range of acceptance of 50-150%, for sample 1 (tofu) slightly below and for sample 4 (soy isolate) slightly above this range.





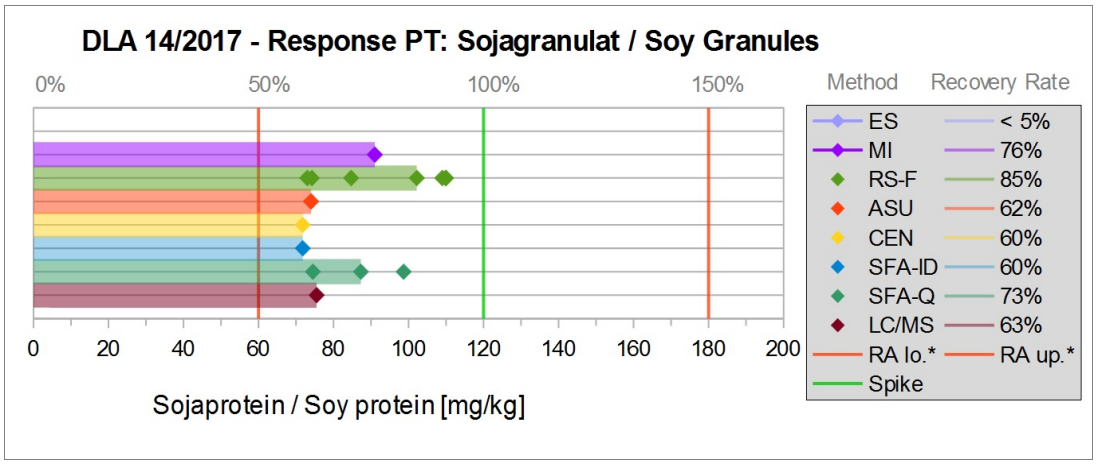
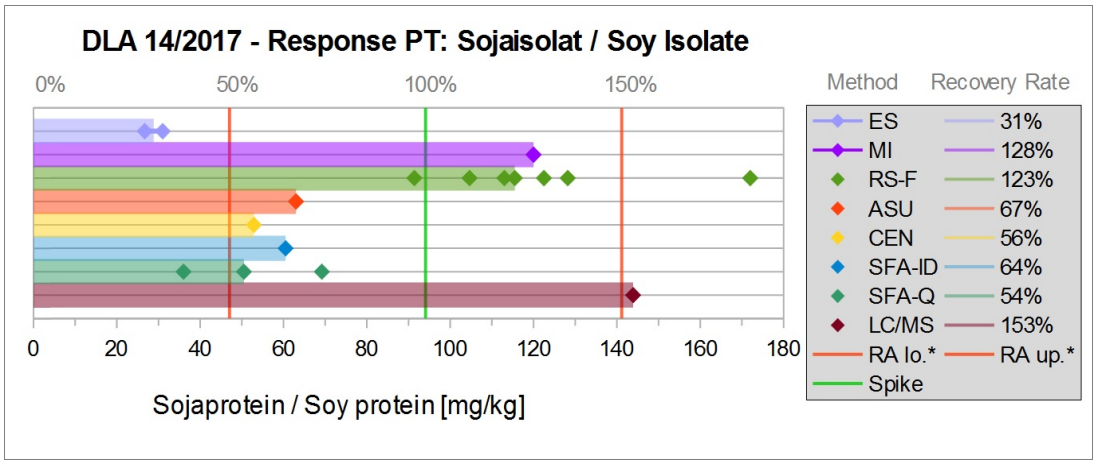


Abb./Fig. 1: Graphs of single results (Samples 1-3) separated by methods with corresponding mean recovery rates, lower scale soy protein content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)



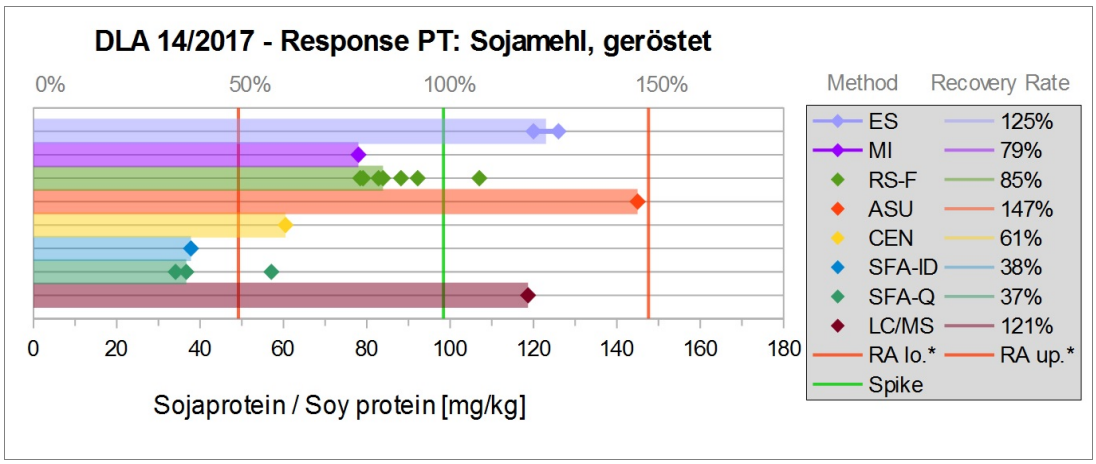


Abb./Fig. 2: Graphs of single results (Samples 4-5) separated by methods with corresponding mean recovery rates, lower scale soy protein content in mg/kg, upper scale recovery rate in % with * range of acceptance from 50% - 150% (* range of acceptance: RA lower limit to RA upper limit)

5. Documentation

5.1 Details by the participants

Note: Information given in German were translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 ELISA-Methods

Meth. Abr.	Evaluation Number	Date of Analysis	Result Sample 1 Tofu		Result Sample 2 Soya milk		Result Sample 3 Soya granulate		Result Sample 4 Soya isolate		Result Sample 5 Soya flour		Result Sample 6 „Blank"-Sample		NWG / LOD *	BG / LOQ *	Result given as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	Preferred as soya protein
ES	3	29.05.17	negative	<2.5	positive	<5	positive	<5	positive	31	positive	120	negative	<2.5			Please select!
ES	14	10.05.17		<1.25	-	>1.25 <2.5	-	>1.25 <2.5	-	26,7	-	126	-	<1.25	1,25	2,5	Quantitative Result given as SOY FLOUR PRO-TEIN
IL	4a	27.04.17	positive		positive		positive		positive		positive		negative		16 ppb		Please select!
IL	16	25.04.17	positive		positive		positive		positive		positive		negative		16 ppb	40 ppb	Trypsin inhibitor
Mi	13	26.04.	positive	67	positive	30	positive	91	positive	120	positive	78	negative	<0,31	0,31	0,31	Soy Protein
RS-F	1	24.05.17	positive	>20.00	positive	10,65	positive	>20.00	positive	>20.00	positive	>20.00	negative	<2.50	0,24	2,5	Soy Protein
RS-F	4b	02.05.17	positive	69,1	positive	10,9	positive	102,2	positive	128,2	positive	82,8	negative		2,5	2,5	Soy Protein
RS-F	5	04.05.17	positive	75,9	positive	11,3	positive	109,9	positive	122,5	positive	92,2	negative			2,5	Soy Protein
RS-F	8	23.05.17	positive	68,5	positive	9,75	positive	84,7	positive	113	positive	88,2	negative		2,5	2,5	Soy Protein
RS-F	10	24.05.17	positive	73	positive	11,7	positive	110	positive	115,5	positive	83,9	negative		0,24	2,5	Soy Protein
RS-F	11	11.05.17	-	52,88	-	8,91	-	73,01	-	104,64	-	78,41	-	< BG		2,5 mg/kg	Soy Protein
RS-F	12	11.05.17	positive	61,84	positive	10,03	positive	74,24	positive	91,4	positive	79,1	negative	<2,5	0,24	2,5	Soy Protein
RS-F	15		positive	87,9	positive	12,8	positive	109	positive	172	positive	107	negative	< 2,5	0,24	2,5	Soy Protein

Continuation details by participants: ELISA-Methods

Meth. Abr.	Evaluation number	Method	Specificity	Factor for recalculation as processed Soy protein	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	Recalculation from X in Y Factor =	e.g. Extraction solution / Time / Temperature	yes/no	
ES	3	ELISA Systems Soy ESSOYPRD-48					
ES	14	Selection Soya ELISA Kits: ELISA SYSTEMS SOYPRD-48	Soy Trypsin Inhibitor and other soy protein	not applicable	ELISA Systems Extraction Solution, 15 minutes, 60°C	No	Results are mean of duplicate analysis of duplicate extractions
IL	4a	Immunolab Soy ELISA		LOD is for Soya-Trypsin-Inhibitor			
IL	16	Immunolab Soy ELISA	polyclonal	See Table below			
Mi	13	Morinaga Soya ELISA Kit II	Soy protein Beta-Conglycinin		as per Kit instructions	yes	
RS-F	1	Ridascreen® FAST Soya R7102, R-Biopharm			Followed r-biopharm method R7102 without any modifications	No	
RS-F	4b	Ridascreen® FAST Soya R7102, R-Biopharm					
RS-F	5	Ridascreen® FAST Soya R7102, R-Biopharm			as per Kit instructions	yes	
RS-F	8	Ridascreen® FAST Soya R7102, R-Biopharm			as per Kit instructions		
RS-F	10	Ridascreen® FAST Soya R7102, R-Biopharm				no	
RS-F	11	Ridascreen® FAST Soya R7102, R-Biopharm	Antibodies recognizes specific heated soy protein	Reaclculation from soy protein to soja flour: Factor = 2,56	as per Kit instructions	yes	
RS-F	12	Ridascreen® FAST Soya R7102, R-Biopharm	Soy protein			yes	
RS-F	15	Ridascreen® FAST Soya R7102, R-Biopharm			as per Kit instructions	yes	

5.1.2 PCR-Methods

Meth. Abr.	Evaluation Number	Date of Analysis	Result Sample 1 Tofu		Result Sample 2 Soya milk		Result Sample 3 Soya granulate		Result Sample 4 Soya isolate		Result Sample 5 Soya flour		Result Sample 6 „Blank“-Sample		NWG / LOD *	BG / LOQ *	Result given as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	Preferred as soya protein
ASU	5	29.05.17	positive		positive		positive		positive		positive		negative				soya-DNA
ASU	9		positive	19	positive	16	positive	196	positive	164	positive	384	negative		5	10	soya
ASU	11	26.04.17	positive		positive		positive		positive		positive		negative				
ASU	12	25.04.17	positive		positive		positive		positive		positive		negative				soya-DNA
CEN	6a		positive	4,3	positive	4,6	positive	190	positive	140	positive	160	negative				soya
SFA-4p	7a	02.05.17	positive	-	positive	-	positive	-	positive	-	positive	-	negative	-	0,4	-	soya
SFA-ID	4	04.05.17	positive		positive		positive		positive		positive		negative		</= 0,4		Please select!
SFA-ID	6b		positive	51	positive	48	positive	190	positive	160	positive	100	negative				soya
SFA-Q	2	11.05.17	positive	56,5	positive	34,8	positive	197	positive	183	positive	151	negative	< 1	0,4	1	soya
SFA-Q	7b	02.05.17	positive	41,3	positive	71,8	positive	231	positive	95,2	positive	97,2	negative	-	0,4	1	soya
SFA-Q	10	24.05.17	positive	111,6	positive	59,7	positive	261,2	positive	133,5	positive	90,3	negative		0,4	1	soya-DNA

Continuation details by participants: PCR-Methods

		Test-Kit + Provider	Antibody	Recalculation from X in Y Factor =	e.g. Extraction solution / Time / Temperature	yes/no	
ASU	5	ASU §64 Methode/method	Lectin		Machery 6 Nagel NucleoSpin Food	yes	Sample 1 and 2 just slightly positive
ASU	9	ASU §64 Method L 08.00-59	Lectin-Gen, 81 bp		Extraction: CTAB-Präzipitation method (s. ASU)	yes	Calibration/Quantitation via Matrix-Standards, spiked Material: Soya flour
ASU	11	ASU §64 Methode/method	Soya-Lectin-Gen 81bp		Dneasy ®Mericon Food Kit/ Proteinase K Real Time PCR/ 45 Zyklen	yes	
ASU	12	ASU § 64 LFGB L 00.00-105, Annex C.2 (modified)	Lektin Gen le1 (74 bp)		Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidiniumchlorid-Buffer with Proteinase K, Clean up via Wizard-Kit from Fa. Promega); Real-time PCR with 45 Cycles	yes	LOD: 20 haploidice Genoe copieskopien
CEN	6a	DIN CEN/TS 15634-5:2016-11; DIN SPEC 10701-4:2016-11; Determination of a specific Soya (Glycine max) DNA-Sequence in boiled sausage via Real-time PCR	Lectin-Gen			no	Quantitation by standard addition according to Eugster (very slight signals for sample 6)
SFA-4p	7a	Sure Food Allergen 4plex, R-Biopharm / Congen	-	only qualitative	S3401 SureFood®ALLERGEN 4plex Soya/Celery/Mustard+IAC Extraction with S1053 SureFood® PREP AdvancedS3401 SureFood®ALLERGEN 4plex Soya/Celery/Mustard+IAC Extraction with S1053 SureFood® PREP Advanced	yes	-
SFA-ID	4	Sure Food Allergen ID, R-Biopharm / Congen		LOQ is iven for soya als allergen ingredient			
SFA-ID	6b	Sure Food Allergen ID, R-Biopharm / Congen	Soya			no	Quantitation bya standard addition according to Eugster (very slight signals for sample 6)

Continuation details by participants: PCR-Methods

Meth. Abr.	Evaluation number	Method	Specificity	Factor for recalculation as processed Soya	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	Recalculation from X in Y Factor =	e.g. Extraction solution / Time / Temperature	yes/no	
SFA-Q	2	Sure Food Allergen Quant, R-Biopharm / Congen			qPCR, 35 Cycles qualitative, 45 Cycles quantitative	yes	
SFA-Q	7b	Sure Food Allergen Quant, R-Biopharm / Congen	-	no recalculation	S3201 SureFood®ALLERGEN QUANT Soya Extraction with S1053 SureFood® PREP AdvancedS3201 SureFood®ALLERGEN QUANT Soya Extraction with S1053 SureFood® PREP Advanced	no	-
SFA-Q	10	Sure Food Allergen Quant, R-Biopharm / Congen				no	

5.1.3 LC/MS-Methods

Meth. Abr.	Evaluation Number	Date of Analysis	Result Sample 1 Tofu		Result Sample 2 Soya milk		Result Sample 3 Soya granulate		Result Sample 4 Soya isolate		Result Sample 5 Soya flour		Result Sample 6 „Blank“-Sample		NWG / LOD *	BG / LOQ *	Result given as
		Day/Month	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	qualitative	mg/kg	mg/kg	mg/kg	Preferred as soya protein
LC/MS	2	17.05.17	positive	55,6	positive	65,3	positive	75,5	positive	143,9	positive	118,7	negative	< 4	2	4	Soy protein

Meth. Abr.	Evaluation number	Method	Specificity	Factor for recalculation as processed Soya protein	Remarks to the Method (Extraction and Determination)	Method accred. accord. ISO/IEC 17025	Further remarks
		Test-Kit + Provider	Antibody	Recalculation from X in Y Factor =	e.g. Extraction solution / Time / Temperature	yes/no	
LC/MS	2	UHPLC-MS/MS	Soya specific peptide sequences		Aqueous protein extraction w ith tryptic degestion, clean up of peptide extract, verification of specific peptide seuqences by UHPLC-MS/MS, Quantitation using isotopic marked Peptide standards and solventcalibration	yes	

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA 14-2017 Sample 1

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	40,7	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	124	49,6
2	5,10	135	52,9
3	5,07	133	52,5
4	5,09	138	54,2
5	5,10	130	51,0
6	5,03	126	50,1
7	5,12	132	51,6
8	4,87	136	55,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	131,8	Particles
Standard deviation	5,32	Particles
χ^2 (CHI-Quadrat)	1,50	
Probability	**98**	%
Recovery rate	128	%

Normal distribution		
Number of samples	8	
Mean	52,2	mg/kg
Standard deviation	2,11	mg/kg
rel. Standard deviaton	4,04	%
Horwitz standard deviation	8,82	%
HorRat-value	**0,46**	
Recovery rate	128	%

Microtracer Homogeneity Test
DLA 14-2017 Sample 2

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	28,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,00	92	36,8
2	4,99	104	41,7
3	5,13	113	44,1
4	5,03	111	44,1
5	5,09	101	39,7
6	5,10	112	43,9
7	5,02	106	42,2
8	5,03	103	41,0

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	105,2	Particles
Standard deviation	6,42	Particles
χ^2 (CHI-Quadrat)	2,74	
Probability	**91**	%
Recovery rate	144	%

Normal distribution		
Number of samples	8	
Mean	41,7	mg/kg
Standard deviation	2,54	mg/kg
rel. Standard deviaton	6,10	%
Horwitz standard deviation	9,13	%
HorRat-value	**0,67**	
Recovery rate	144	%

Microtracer Homogeneity Test
DLA 14-2017 Sample 3

Weight whole sample	1,00	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	25,4	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,03	64	25,4
2	5,06	57	22,5
3	5,16	64	24,8
4	5,12	56	21,9
5	5,03	66	26,2
6	5,02	63	25,1
7	5,06	61	24,1
8	5,13	65	25,3

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	62,0	Particles
Standard deviation	3,84	Particles
χ^2 (CHI-Quadrat)	1,66	
Probability	**98**	%
Recovery rate	96	%

Normal distribution		
Number of samples	8	
Mean	24,4	mg/kg
Standard deviation	1,51	mg/kg
rel. Standard deviaton	6,18	%
Horwitz standard deviation	9,89	%
HorRat-value	**0,63**	
Recovery rate	96	%

Microtracer Homogeneity Test
DLA 14-2017 Sample 4

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,3	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,11	77	30,1
2	5,14	79	30,7
3	5,01	73	29,1
4	5,13	91	35,5
5	5,00	82	32,8
6	5,03	71	28,2
7	5,02	87	34,7
8	5,05	78	30,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	79,7	Particles
Standard deviation	6,53	Particles
χ^2 (CHI-Quadrat)	3,74	
Probability	**81**	%
Recovery rate	108	%

Normal distribution		
Number of samples	8	
Mean	31,5	mg/kg
Standard deviation	2,58	mg/kg
rel. Standard deviaton	8,19	%
Horwitz standard deviation	9,52	%
HorRat-value	**0,86**	
Recovery rate	108	%

Microtracer Homogeneity Test
DLA 14-2017 Sample 5

Weight whole sample	1,01	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	22,9	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,12	88	34,4
2	5,09	85	33,4
3	5,00	74	29,6
4	5,01	81	32,3
5	5,03	80	31,8
6	5,00	84	33,6
7	5,01	79	31,5
8	4,99	75	30,1

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	80,7	Particles
Standard deviation	4,25	Particles
χ^2 (CHI-Quadrat)	1,57	
Probability	**98**	%
Recovery rate	140	%

Normal distribution		
Number of samples	8	
Mean	32,1	mg/kg
Standard deviation	1,69	mg/kg
rel. Standard deviaton	5,26	%
Horwitz standard deviation	9,49	%
HorRat-value	**0,55**	
Recovery rate	140	%

5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

PT number	***DLA 14-2017***
PT name	***Response PT Soya: Processed samples Soya Flour, Soya Isolate, Soya Granulate, Soya Milk and Tofu in potato powder matrix (levels: 50 - 150 mg/kg)***
Sample matrix (processing)	***Samples 1-6:*** *Carrier matrix / ingredients: potato powder (approx. 75%), maltodextrin (approx. 25%) and other food additives and allergenic foods (only samples 1-5)*
Number of samples and sample amount	*5 different Samples: 20 g each* *+ 1 "Blank" Sample: 20 g*
Storage	*Samples 1-6: room temperature (long term: cooled 2 - 10°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative + quantitative: Soya / Soy protein from* *Soya Flour, Soya Isolate, Soya Granulate, Soya Milk or Tofu* *Samples 1-5: approx. 50 - 150 mg/kg (as total soy protein)*
Methods of analysis	*Analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights. Preferably the total sample amount should be homogenized.*
Result sheet	*One result each should be determined for Samples 1-6.* *The results should be filled in the result submission file. In case of several determinations the mean.*
Units	*mg/kg*
Number of digits	*at least 2 significant digits*
Result submission	*The result submission file should be sent by e-mail to:* ***pt@dla-lvu.de***
Deadline	***the latest June 2nd 2017***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Matthias Besler, PhD*

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

6. Index of participant laboratories in alphabetical order

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		Germany
		AUSTRALIA
		Germany
		ITALY
		USA
		Germany
		Germany
		BELGIUM
		Germany
		Germany

[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

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