Proficiency test of detection of packaging material in bakery by-products 2019

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- All participants of the proficiency test

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Summary

A proficiency test (PT) was organised for the detection of packaging materials in bakery by-products intended to be used as feed ingredients. Two blind samples of a size of 250 grams at spike levels of 50 mg and 250 mg per sample were distributed to 29 participants. Four participants analysed only a part of the sample material, leaving 25 data points eligible for further evaluation. The evaluation of the results was carried out according to the principles of the Standard ISO 17043:2010 and the Quality Guidelines for visual research, in the framework of individual spiking of the samples. This specific procedure was chosen to avoid large inhomogeneity of a general batch as basis for the production of the PT samples, with the consequence that usual statistics such as Z-scores could not be applied or needed modified interpretations. Results of this PT have been compared with the first PT in 2016 (n=22 participants, same spike levels). Altogether this provided the presence of four datasets. Blanks were not included in both PTs.

The average recovery for the 50 mg level was 179%, and 105% for the 250 mg level in the 2019 PT. The maximum overestimations were 441% (50 mg) and 170% (250 mg), respectively. Symmetrical recovery intervals were chosen of 34%-166% for the 50 mg level and 66%-134% for the 250 mg level. A share of 80% of the results for the 250 mg sample was achieved within the limits of the recovery interval. A considerable number of overestimations was reported for the 50 mg level (12 participants, 48%) in the 2019 PT. Notwithstanding the limitations of statistical parameters, a higher precision in 2019 compared to the 2016 datasets can be concluded based on the smaller interval of the results, visible in the minimum-maximum ranges, in the 95% confidence intervals and in the lower standard deviations. In all cases the distributions are skewed to the right, shown by the underestimations in only one dataset, lack of outliers below the Lower Confidence Level and skewness values (much) higher than zero.

The number of overestimations was the major cause of the lack of compliance with the uncertainty intervals. This might be due to either insufficient removal of water and fat from the selected particles of packaging material, and/or the selection of other particles mimicking the packaging material (specificity issue). The precise background of the overestimations needs further evaluation. False negatives were not reported in both the 2016 nor the 2019 version of the PT. The methods are applicable in the framework of enforcing a zero tolerance prohibition when transferring the observations to a compliant or non-compliant decision in relation to a threshold. Enforcement of an action limit could be possible from a level of 0.1% or higher in the view that the number of underestimations is very limited.

Specificity as issue in the methods needs further investigation in order to document the precise cause of the deviations. Documentation for precise identification of packaging material needs to be developed.

1 Introduction

An important factor in circular agriculture is the reuse of former food products (FFP). This term applies to all products produced with the intention of human consumption. Withdrawal from this principal use can occur after production (factory), during storage and transport, or in the retail stage. In contrast to materials which result from the processing of primary commodities (e.g. oil-seed hulls and kernels, cereal by-products, pulp, etc.), these FFPs have to be unpacked in a range of cases. Regulation (EC) 767/2009 Annex III mentions zero tolerance for the presence of remnants of packaging material in FFPs. In practice, relatively low action limits were installed in member states (van Raamsdonk et al., 2011).

Several methods for the detection of packaging material have been developed, with the scope of bakery by-products (van Raamsdonk et al., 2012) or compound feed containing bakery by-products (Amato et al., 2017). The RIKILT method for bakery by-products was accepted as IAG method during the Autumn meeting of the IAG section Feed Microscopy in 2015. Subsequent plans were made to organise a proficiency test (PT) for this and comparable methods. This PT was held in 2016 and results indicated that a training was recommended. Such a training was organised during the annual meeting of IAG section Feed Microscopy in Uppsala in 2017. Training samples were distributed in 2018 to every interested member of IAG section Feed Microscopy. A second PT was organised during Autumn 2019. This report presents the results of the PT of 2019, with reflections on the 2016 results.

2 Materials and methods

Samples were based on bakery by-products from practice. These materials have been sent to WFSR in the past and the contamination was determined by removing all visible parts of packaging material from the matrix. The detection limit, as established by WFSR (formerly RIKILT[1]; van Raamsdonk et al., 2012) is 0.004%, which means that the remaining material is below that threshold. A sufficient amount of matrix material was mixed for preparing the samples for the PT. All packaging material selected from samples from the last ten years was stored by WFSR. Portions were selected of the required amount, with attention to a balanced composition of paper, tin foil, plastic and some other minor types. All samples were spiked on an individual basis. With matrix and packaging material from practice, these samples can be considered to represent the practical situation.

Recovery is calculated as

$$R_{\%} = \frac{r_i}{s_i} * 100$$

with r_i as the amount of recovered material, and s_i as the amount of spiked material for every individual sample at each of the two spike levels. The parameter $R_{\%}$ is derived from the percent difference $D_{\%}$, one of the possible parameters for evaluating results in proficiency testing (ISO 17043: 2010, Appendix B.3.1.3, paragraph b). The relationship can be described as $R_{\%} = D_{\%} + 100\%$. The results obtained from incomplete examination of the samples were excluded for evaluation. The recovery intervals applied are extracted from the Quality Guidelines for visual research (WFSR, in press). Symmetrical intervals were chosen of 34%-166% for the 50 mg samples and 66%-134% for the 250 mg level.

Additionally, the Lower and Upper Confidence Limits (LCL and UCL) are calculated from the set of results for each of the two samples based on the average and standard deviation of the percent recovery $R_{\%}$, as follows:

$$LCL = u - 1.96 * SD, \qquad UCL = u + 1.96 * SD$$

The results as obtained in 2019 (this report) will be compared to the results of the 2016 proficiency test and the approach as applied in this report will be discussed.

[1] The name RIKILT is used in this report for activities performed prior to June 1st, 2019, the date of the merger with another institute.

3 Results

Raw results of the 2019 proficiency test are presented in Annex 1. A graphical representation is given in Figure 1. Results of four participants were based on incomplete examination of the sample material (participants 3, 12, 45 and 52, indicated in Annex 1 with yellow boxes) and were excluded from further analysis, leaving 25 data points applicable for further evaluation. In most results of these four participants, considerable deviations from the spike level were found. The one underestimation found in the entire set of results was reported by one of these four participants (participant 12).

The average recovery for the 50 mg level was 179%, and 105% for the 250 mg level. The maximum overestimations were 441% (50 mg) and 170% (250 mg), respectively. Fourteen participants reported to have applied the RIKILT method or a highly comparable method. Another 11 participants applied a laboratory method.

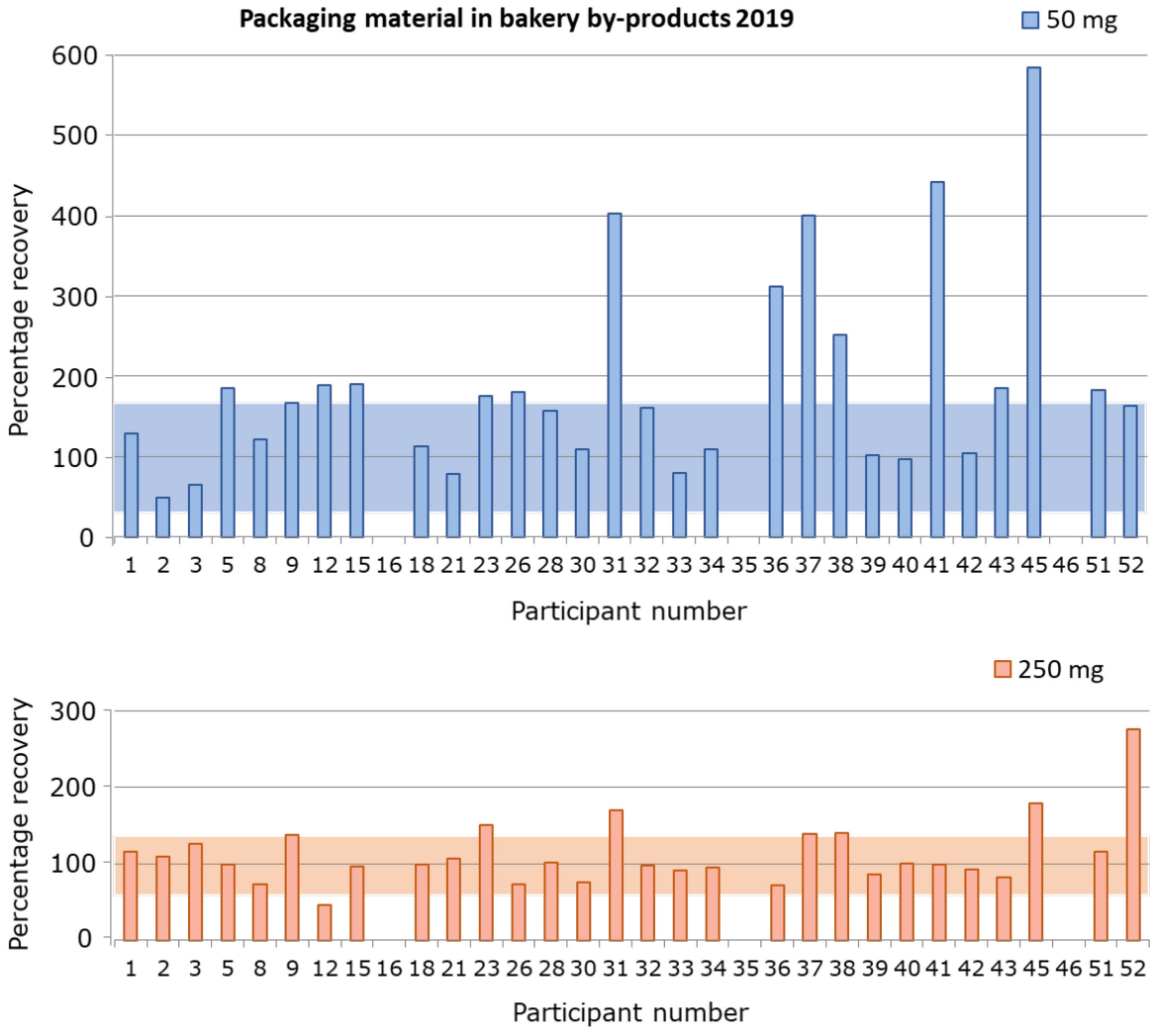

Figure 1 *Overview of results per participant. Blue and orange boxes indicate the applicable recovery interval.*

Usual intervals applied for recovery in the spike range as applied in this study is 85-110% or 90-108% (AOAC, 2002; Codex Alimentarius, 2004). Experiments showed that larger intervals are applying to visual monitoring. Symmetrical intervals were chosen of 34%-166% for the 50 mg samples and 66%-134% for the 250 mg level. The overview in Figure 1 shows that a majority of participants reported values within limits, indicated by the coloured boxes in the graphs. Especially for the 250 mg sample a result of 80% well performing participants was achieved (Table 1). A considerable number of overestimations was reported for the 50 mg level (12 participants, 48%). The background of the followed approach and a comparison with the 2016 results will be discussed in the next paragraph.

Table 1 *Compliance of results for the applicable recovery interval. Number of eligible participants is 22 (2016) and 25 (2019).*

interval	34% - 166%		66% - 134%	
spike level	50 mg		250 mg	
year	2016	2019	2016	2019
%compliant	82%	52%	77%	80%
n underestimating	0	0	3	0
n overestimating	4	12	2	5

4 Discussion

A usual way to evaluate the results of a proficiency test is to calculate Z-scores (ISO, 2005, 2010). There are two reasons why this approach is not followed in this report. At first, Z-scores are calculated from the average recovery and the standard deviation of the reported results of the participants, under the assumption that all samples as analysed by the participants were taken from a homogenised batch, i.e. with exactly the same level of contamination. Every sample is a draw from the same population in statistical terms. In this PT, as usual for all PTs in the field of macroscopic visual research, every sample was spiked individually. Secondly, Z-scores are usually accepted as correct in the range of -1.96 to +1.96 times the SD, which by definition includes 95% of the results. The current results for the 50 mg sample have an LCL of the 95% confidence interval of -28% (Table 2), which would theoretically lead to acceptance of (erroneous) negative results. The highest result within the 95% confidence interval was a recovery of 192 mg, 400% of the real value of 48 mg for that sample (Annex 1, participant 37). The 2019 results for both samples show a prevailing overestimation, indicating a long right tail in the distribution of the sample results. The background of the followed strategy and the diversity of the results do not allow an evaluation using Z-scores. The larger dispersion of the results of the current proficiency test fits in the general picture of the performance of visual methods.

The current approach for evaluation was designed to be as close as possible to the requirements of ISO 17043:2010 (ISO, 2010). Besides Z-scores and comparable measures, this Standard provides the option of the parameter percent difference, which can be recalculated to the recovery percentage as used in this study. Additionally, a variety of different approaches for proficiency test organisation is foreseen, and insufficiently homogeneous materials can still be useful as proficiency test items, provided that the uncertainties of the assigned values or the evaluation of results take due account of this (ISO 17043:2010 Annex B.3.1.3 and ISO 13528:2005, Annex B). The possible uncertainty of the assigned value after batch mixing is compensated by using individual spiking of the samples. The diversity among subsamples of one sample as found in the analysis of weed seeds in bird feed and ergot sclerotia in cereals is caused by inhomogeneity of batches of visible units. Uncertainty intervals related to different spike levels can be extracted from this data, as presented and discussed in the *Guidelines for Quality Assurance and Control of visual methods*. After individual spiking, subsampling and inhomogeneity are not an issue, provided that the entire sample is analysed. Measurement uncertainty can have a variety of causes (Eurachem/CITAC guide, Appendix C; Ellison and Williams, 2012). Therefore, the limits for weight uncertainty in these *Guidelines* are applied here as best possible option for evaluation.

Based on this approach, a comparison of the 2019 dataset with the 2016 results can be made (data published in an unofficial report). A training option was provided in 2017 and labelled samples have been distributed in 2018. When applying identical weight intervals for both sets of results, a higher compliance for the 50 mg level was found in 2016 compared to 2019, whereas an identical compliance was found for the 250 mg level (Table 1). The non-compliant results were overestimations in all cases for three of the four datasets. Additional parameters have been calculated for testing this suggested decreasing performance at the lower levels (Table 2). For both spike levels, the range between the minimum and maximum reported recovery was lower in 2019 compared to 2016, resulting in a lower value for the standard deviation. The same trend was found for the LCL and UCL values showing a smaller 95% confidence interval in 2019. The average recovery for the 50 mg level was higher in 2019 compared to 2016. This observation, together with a lower percentage of compliance for the 50 mg level in 2019 (Table 1), cannot be taken as decreasing tendency for accuracy and precision at that spike level. The shape of the distribution for all four sets of results is highly variable, as indicated by kurtosis and skewness. It can be argued whether these standard statistical parameters can be used to describe sufficiently the nature and background of the results for the detection of packaging material in bakery by-products. As argued, the data points do not belong to one population of results per year and spike level. Notwithstanding the limitations of statistical parameters, a higher precision in 2019

can be concluded based on the smaller interval of the results, visible in the minimum-maximum intervals, in the LCL-UCL ranges and in the standard deviations (Table 2). In all cases the distributions are skewed to the right, shown in Figure 1, underestimations in only one dataset (Table 1), lack of outliers below the LCL value and skewness values (much) higher than 0 (Table 2). This tendency of skewness (overestimations) could be caused by two reasons: (a) insufficient drying and defatting of the selected packaging material, and (b) additional selection of material from the matrix together with the correctly identified packaging material. The latter would be a specificity problem.

Table 2 *Recovery statistics in % after exclusion of results based on incomplete sample examination. SD: Standard Deviation. LCL: Lower Confidence Limit and UCL: Upper Confidence Limit of the 95% confidence interval of the percentage recovery.*

spike level	50 mg		250 mg	
year	2016	2019	2016	2019
n=	22	25	22	25
minimum	40%	48%	36%	72%
maximum	655%	441%	218%	170%
average	156%	179%	98%	105%
SD	140%	106%	41%	26%
LCL	-119%	-28%	17%	55%
UCL	431%	386%	179%	155%
outliers (n below LCL)	0	0	0	0
outliers (n over UCL)	1	4	2	3
Kurtosis	7.4	1.2	2.9	9.1
Skewness	2.6	1.4	1.3	2.6

The tested methods are intended to monitor the zero tolerance measure for packaging material in feedings stuffs (Regulation (EC) 767/2009, Annex II). Basically a quantitative result is not required for enforcement of a zero tolerance prohibition. False negative results were not reported for both editions of the PT on packaging materials, resulting in correct qualitative results in all cases. The sample sets of both PTs did not consist of a blank sample, which means that data on false positives is lacking. However, action limits are applied in practice, e.g. 0.15% (van Raamsdonk et al., 2011). Considering this action limit, no results exceeding this level (1500 ppm) have been reported for the 50 mg spike level (0.02%). For the 250 mg spike level (0.1%), two out 22 results and two out of 25 results in the 2016 and the 2019 dataset, respectively, have been reported to exceed 1500 ppm. These results underline the need to explore further possible specificity issues.

5 Conclusion

Two identical PTs have been organised in 2016 and in 2019, with intermediate training options consisting of non-blind samples at the same spike levels (50 mg and 250 mg) in 2017 and 2018. Since better insight has been gained in the evaluation of PT results since the 2016 PT, these results are re-evaluated and compared with the 2019 set. An increase in precision appeared to be achieved. Lack of compliance with the uncertainty intervals was predominantly due to overestimations. This might be caused by either insufficient removal of water and fat from the selected particles of packaging material, and/or the selection of other particles mimicking the packaging material (specificity issue). The precise background of the overestimations needs further evaluation. False negatives were not reported in both the 2016 nor the 2019 version of the PT. The methods are fit for purpose when transferring the observations to a *qualitative* result, which is suitable in the framework of enforcing a zero tolerance prohibition. Enforcement of an action limit could be possible from a level of 0.1% and higher in the view that the number of underestimations is very limited.

6 Recommendation

Specificity as issue in the methods needs further investigation in order to document the precise cause of the deviations. A new PT is needed including one or more blank samples in combination with precise examination by an independent expert of the materials selected. It is important to apply a method which includes sufficient dehydration and defatting. The latter is important for the specific matrix with a relatively high fat content.

Documentation for precise identification of packaging material needs to be developed.

A further development of performance criteria is required for visual monitoring methods in order to facilitate the development of these methods for support to circular agriculture.

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Annex 1 Results by participant

50 mg sample. Every sample had a total weight of 250 ± 1 grams. Results within the recovery limits 34%-166% in green cells. Examination of a part of the entire sample is indicated by yellow cells.

2019 lab nr	sample A nr	Analysed amount (gram)	Added mg	Detected mg	Recovery	Comments
1	1304	249.87	50	64	128%	aluminium foil, paper+fiber, and plastic in both samples
2	1318	249.75	46	22.1	48%	plastic foil, plastic pieces, pieces of printed paper
3	1339	100	20	13	65%	soft plastic, paper, aluminium foil
5	1332	250	49	90.4	184%	plastic, aluminium foil; 32,9 mg stones
8	1325	249.4	49	59.3	121%	
9	1311	248.1	54	89.9	166%	plastic, paper, aluminium
12	1297	50	10	18.9	189%	
15	1283	249.07	51	97	190%	
16	1276	250.73	49			
18	1255	243	50	56.5	113%	paper fibers, carton, plastic particles, plastic film, aluminum foil
21	1262	248.00	51	40	78%	
23	1290	249.57	48	84.3	176%	aluminium foil, paper residues, soft plastic
26	1192	250.00	52	93.8	180%	plastic, theads, aluminium foil
28	1220	249.22	51	80.2	157%	
30	1227	248.51	55	60	109%	
31	1269	250.00	51	205	402%	
32	1178	250.00	48	77	160%	
33	1185	248.65	55	43.8	80%	
34	1213	248.00	52	56.8	109%	
35	1206	250.65	52			
36	1248	256.60	50	156	312%	
37	1234	247.90	48	192	400%	
38	1241	243.10	55	138.5	252%	Plastics, aluminium foils, paper
39	1199	249.03	50	50.6	101%	Plastic, tin foil and paper.
40	1129	248.50	52	50	96%	
41	1115	249.73	53	233.95	441%	
42	1171	249.12	55	56.8	103%	
43	1164	255.00	54	100.1	185%	Cardboard, plastic, Aluminum
45	1136	201.30	42	245.2	584%	
46	1122	250.12	53			
51	1143	238.69	50	91.1	182%	
52	1150	99.38	21	34.2	163%	

250 mg sample. Every sample had a total weight of 250 ± 1 grams. Results within the recovery limits 66%-134% in green cells. Examination of a part of the entire sample is indicated by yellow cells.

2019 lab nr	sample B nr	Analysed amount (gram)	Added mg	Detected mg	Recovery	Comments
1	1165	250.13	250	290	116%	aluminium foil, paper+fiber, and plastic in both samples
2	1151	249.75	250	271.4	109%	plastic foil, plastic pieces, pieces of printed paper, metall pieces
3	1179	100.00	99	124	125%	soft plastic, hard plastic, paper, aluminium foil
5	1158	250.00	248	244.5	99%	paper, plastic
8	1172	250.40	247	179.4	73%	
9	1186	248.30	254	349.4	138%	plastic, paper, aluminium
12	1193	50.00	50	23.1	46%	
15	1228	250.87	249	239	96%	
16	1235	250.52	253			
18	1214	243.00	251	249.4	99%	paper fibers, carton, plastic particles, plastic film, aluminum foil
21	1221	249.00	249	264	106%	
23	1200	250.31	248	373	150%	aluminium foil, paper residues, soft plastic
26	1207	250.00	248	180.3	73%	plastic, threads, aluminium foil, glass
28	1249	250.03	255	257.4	101%	
30	1242	241.85	254	192	76%	
31	1319	250.00	248	421	170%	
32	1298	250.00	251	246	98%	staple (weighing 42 mg)
33	1305	248.65	248	225.9	91%	
34	1263	249.00	252	239.5	95%	
35	1256	249.77	252			
36	1312	253.70	254	182	72%	
37	1291	245.81	250	347	139%	
38	1270	245.70	250	351.5	141%	Plastics, aluminium foils, paper
39	1277	246.68	252	217.4	86%	Plastic, tin foil and paper.
40	1284	248.55	250	250	100%	
41	1333	249.09	255	252.29	99%	
42	1326	249.59	255	234.1	92%	
43	1340	255.00	255	209.6	82%	Cardboard, plastic, Aluminum
45	1347	200.40	199	356.3	179%	
46	1354	250.30	254			
51	1361	248.81	254	292	115%	
52	1368	89.60	91	250.3	275%	

Declaration of the type of method applied.

2019 lab nr	Read the ring test Instructions	Detection Method
1	Yes	RIKILT method report 2012.007
2	Yes	own laboratory method
3	Yes	own laboratory method
5	Yes	VDLUFA III. 30.9, Manuscript 2018-04 and VDLUFa III, 30.1 (Sample Preparing)
8	Yes	own laboratory method
9	Yes	own laboratory method
12		
15	Yes	RIKILT method report 2012.007
16		
18	Yes	RIKILT method report 2012.007
21	Yes	own laboratory method
23	Yes	own laboratory method
26	Yes	RIKILT method report 2012.007
28	Yes	own laboratory method
30	Yes	RIKILT method report 2012.007
31	Yes	RIKILT method report 2012.007
32	Yes	RIKILT method report 2012.007
33	Yes	RIKILT method report 2012.007
34	Yes	RIKILT method report 2012.007
35		
36	Yes	RIKILT method report 2012.007
37	Yes	RIKILT method report 2012.007
38	Yes	own laboratory method
39	Yes	own laboratory method
40	Yes	own laboratory method; close to RIKILT method
41	Yes	RIKILT method report 2012.007
42	Yes	RIKILT method report 2012.007
43	Yes	own laboratory method
45	Yes	RIKILT method report 2012.007
46		
51	Yes	own laboratory method
52	Yes	own laboratory method; close to RIKILT method

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