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Summary

The tendency to achieve a circular bioeconomy and sustainability of the food production chain as far as possible has the consequence that an increasing variety of Former Food Products will be used as feed ingredient. The incidence of packed food stuffs in feed, and consequently unpacking upon use as feed ingredient, will increase when the by-products approach the final stages of processing.

In this framework a method has been developed for examining the amount of packaging material in candy syrup, based on confectionery and sweets, with an approximate sugar content of 55%.

The method includes dissolving of the whole 500 gram sample of candy syrup in warm water by 1:4 or 1:5, stirring on a hot plate in order to dissolve all sugar and other matrix materials, pouring the total solution over a sieve and rinsing the beaker and sieve several times with additional hot water. The packaging material is removed from the sieve, dried, defatted (because of the unknown chocolate content), dried again, weighed and examined for remaining matrix material. The mass balance over the subsequent steps need to be determined in order to retrieve a quantitative result.

In a series of 14 experiments optimal values for four different parameters have been established. These are dissolving/stirring during 15 minutes, sieving with a mesh size of 200 µm, using a regular tap over a sprayer for rinsing the sieve with a rinsing time of 5 minutes. Due to the unknown quantities of chocolate in practice samples a defatting procedure was tested and proved to be necessary.

A robustness analysis revealed that a prolonged dissolving/stirring time of 30 minutes would result in the same performance. This can be recommended when a thin shiny layer remains on top of the selected packaging material, which might be caused by an excess of sugar.

The method was validated using blank candy syrups, blank candy syrup spiked with packaging material, and samples from practice. The blanks and spiked samples were used to determine the limit of detection (0,021 g/kg), recovery (102.7% and 101.2% at 1,5 g/kg and 0,5 g/kg level, respectively), selectivity and robustness/stability. The practice samples were used to determine the repeatability (2,96%), within-laboratory reproducibility (2,55%) and expanded weight uncertainty (9,09%).

There is a scarcity of data for fixing a-priori criteria for these parameters applying to the detection of visually recognisable substances. Provisionally the performance characteristics of a method to detect weed seeds and ergot sclerotia in whole kernel matrices have been used as limits. The performance of the developed method to detect packaging material in candy syrups was well within these limits for all parameters.

A main aspect for validation of these type of methods is the level of inhomogeneity of the study samples. For macroscopic analysis the combination of particle size and fluidity of the sample material can be assumed to be major parameters for this factor. At one side, dry matrices with particles over 1 mm showed a very large inhomogeneity. Homogenisation of the semi-fluid matrix of candy syrups resulted in an established value for uncertainty of over 9% at a contamination level of 0.06%, which is higher than the upper confidence limit according to the classical Horwitz equation, but much lower for the situation of dry coarse matrices. With the results of the validation of the method for examining packaging material in candy syrup as example of semi-fluid matrices, the existence of intermediate situations of inhomogeneity resulting in intermediate levels of within-laboratory reproducibility and of weight uncertainty was established.

The developed method adds to the available methods for detection of visually recognisable substances in the macroscopic range. These vary from whole kernel matrices with a low number of large units, dry coarsely ground matrices with a larger number of smaller particles than in the whole kernel situation, for example bakery by-products or ground animal feeds, to semi-fluid matrices. In this case the matrix can be dissolved in warm water. Semi-fluidity for other types of matrices might demand a different strategy to discard the matrix material.

1 Introduction

Circular bioeconomy and sustainability are increasing values for our production of feed and food. Historically, farmed animals are being fed with a variety of by-products of the food production chain, ranging from by-products from harvesting, the diverse stages of food production and preparation to specific items from retail. Bran, middling's, expellers, pulp, hulls and flakes are examples of major ingredients of compound feeds. In that same historical development increasing concern arose on issues of food and feed safety. Restrictions on use of by-products have been installed for preventing animal diseases such as Transposable Spongiform Encephalopathy and zoonoses including foot and mouth disease and different versions of swine fever. Concerns were primarily focusing on chemical and microbiological hazards. Packaging of food stuff was and still is abundantly applied for increasing shelf life and improving hygiene circumstances.

The current demands for optimal valorisation of Former Food Products (FFPs) include unpacking in a range of occasions in order to minimise physical hazards. Unpacking strategies and their efficiency depend on the type of food product, packaging material and the type of valorisation. Pinotti et al. (2021) made a distinction between bakery by-products (BBPs) and other FFPs. The latter category does not apply to all food by-products. For example, moist or wet products such as fruit, vegetables and some dairy products are usually more eligible for fermentation, and unpacking can relatively easy be organised (van Raamsdonk et al., 2011). One specific type of FFP consists of confectionery and candy. This material has a high nutritional value for its high sugar content and can replace molasses as ingredient in compound feeds (van Raamsdonk et al., 2011; Farmer's weekly, 2018; Amburgh et al., 2019).

Unpacking of former food products in a wide sense might in principle result in remnants of the packaging materials in the final product. Regulation (EC) 767/2009, Annex III (EC, 2009) prohibits a range of materials and substance as feed ingredients, including packaging material, in a zero tolerance approach. A limit higher than zero has been suggested for application in practice (Kamphues, 2005). A method for detection of packaging material in BBPs has been validated with a quantification limit of 0.01% (van Raamsdonk et al., 2012). A derived method for the detection of packaging materials in compound feeds showed a measurement uncertainty of 18% at a contamination level of 0.05% (Amato et al., 2017). These values for performance parameters should not be compared to values for mainstream (in principle chemical) validation characteristics, since the visual detection of a limited number of large particles follows other principles (van Raamsdonk et al., 2022).

The procedure for extracting remnants of packaging material in former food products depends on the type of matrix material. BBPs are usually processed to a dry granular product and compound feeds can be reduced to a comparable type of material. Liquid and semi-liquid matrices need a different strategy. Syrup made from confectionery or candies has been chosen as representative material for a larger group of liquid FFPs. The presence of sugar at a high level can be assumed to provide options to dissolve a major part of the matrix. The current report presents the development of the extraction and detection method, together with its validation.

2 Method development

2.1 Background

Liquid products, based on high sugar containing FFPs (candy, sweetener), are the subject of the method to be presented here and the specific material will be indicated as candy syrup. There is a regular production and application in the Netherlands (Farmer's weekly, 2018). One producer could deliver samples from practice in large quantities for validation of the developed method.

2.2 Method development

Candy syrups and other sugar-rich materials are comparable to honey, although the viscosity of the latter is higher. This is due to the sugar content, which is 81% for honey and approximately 55% for Dutch candy syrup. Methods have been published to extract pollen and other debris from honey by dilution in hot water followed by centrifugation (Sawyer, 1988). The targeted remnants of packaging material are much larger than any particle enclosed in honey. Therefore, the time to dissolve the matrix material by an excess of warm or hot water is taken from the honey extraction procedure, whereas the centrifugation has been replaced by sieving.

In this way, the analytical strategy for method development was:
- dissolving of the targeted amount of candy syrup in warm water by 1:4 or 1:5,
- stirring on a hot plate in order to dissolve all sugar and other matrix materials,
- pouring of the total solution over a sieve and rinsing the beaker and sieve several times with additional hot water,
- removing of the packaging material from the sieve,
- drying, defattening and drying again,
- examination for remaining matrix material,
- weighing of the isolated packaging material.

During method development the mass balance over the subsequent steps has been watched in order to retrieve a quantitative result. Difficulties observed included the sample size (for BBPs fixed at 500 gram) and handling of the rather large size of the equipment.

The variables of the intended method are the time for stirring, the sieve mesh size, the time and the type of procedure for rinsing the sieve.
- Dissolving/stirring time: 0, 15, 30, 60 minutes.
- Sieve mesh size: 80, 125, 200, 355 µm.
- Water source over sieve: regular tap, spray head.
- Rinsing time of sieve: 1, 5 minutes.

Samples of initially 100 gram and in the final stage of 500 gram have been used for method development. Seven different types of sweets and confectionery have been used to produce the mix of candy, including two chocolate containing products. These seven ingredients had equal shares in the final mix. The removed packaging materials were stored and cut in small pieces. Each test sample was designed to contain 0.1% of packaging material and approximately 65% of dry matter. A total of 71 samples have been produced by fully dissolving the intended amount of dry matter in water at a temperature of 70 ˚C. Examination followed the mentioned strategy with a combination of the listed variable values in duplicate or triplicate.

Several control points have been chosen to monitor the effect of the procedure. These are the amount of material after drying in a hood, after defatting and drying in a hood, and after extended drying in an oven at 60 ˚C overnight (16 hours).

One experiment was conducted to access the effect of defatting in an excess of chocolate. A general set of samples (n=13) showed an improvement of 24% of the recovered amount of packaging material compared to the added amount, whereas the samples with extra chocolate (n=4) showed an improvement of 53%. Based on these results defatting was considered a permanent step in the procedure, since the share of chocolate is unknown in practice. Weighing the recovered material before defatting was skipped from the control points in the remaining experiments.

The 71 samples, in most cases in duplicate or triplicate for a chosen treatment, were examined in 14 experiments. The results are presented in Table 1.

Results for the wo main parameters, stirring time and sieve mesh size, either with regular tap or sprayer, were pooled from the experiments and the average effect has been calculated. The results are presented in Table 1. In general, the use of a sprayer resulted in lower recovery compared to a regular tap for rinsing the sieve. A tendency towards lower recovery is found for longer dissolving/stirring times and for larger sieve mesh sizes. These tendencies are expressed in Figure 1. The results indicate the use of a regular tap (top lines in every Figure), and the application of 15 minutes dissolving/stirring time and a sieve mesh size of 200 µm (bottom line of every set). These values are chosen for their result of a recovery just over the ideal value of 100%. The sieve rinsing time has been adjusted to five minutes.

Table 1 *Effect of three parameters (type of tap, dissolving/stirring time, and mesh size) on the recovery of packaging material from candy syrups.*

parameter	n	recovered after defatting	recovered after extended drying
tap, average	48	108%	105%
0 min, average	2	167%	152%
15 min, average	22	114%	110%
30 min, average	14	96%	94%
60 min, average	6	91%	90%
80 µm, average	0		
125 µm, average	10	141%	136%
200 µm, average	20	109%	105%
355 µm, average	14	81%	80%
sprayer, average	23	82%	77%
0 min, average	11	88%	83%
15 min, average	0		
30 min, average	8	81%	76%
60 min, average	4	65%	61%
80 µm, average	4	131%	123%
125 µm, average	0		
200 µm, average	13	76%	72%
355 µm, average	6	61%	58%

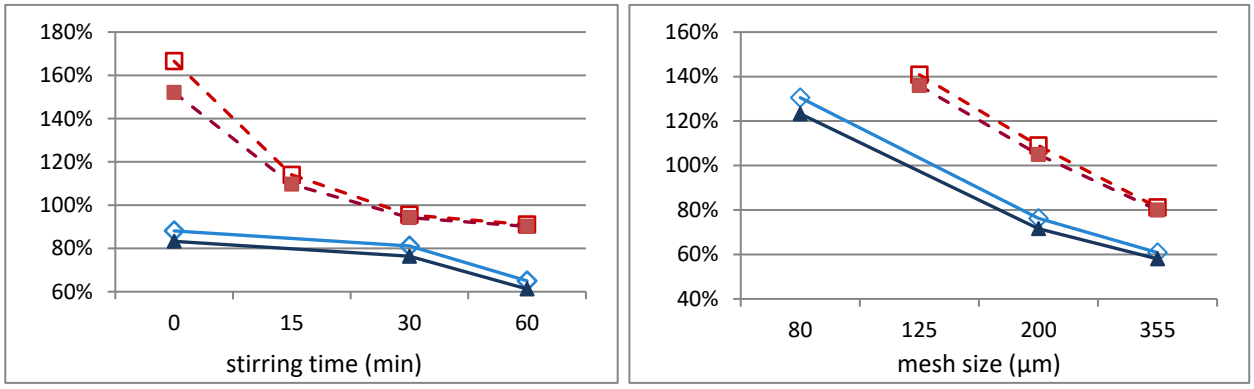

Figure 1 *Effect of different stirring times (left) and sieve mesh sizes (right) on the recovery of packaging material in candy syrups. The colour of the lines corresponds to the colour of the two sections in Table 1.*
Open symbols: recovered after defatting.
Closed symbols: after extended drying.
Red dotted lines: tap.
Blue straight lines: sprayer.

After establishing the method, two aspects were explored further: the dissolving/rinsing time and, as extra circumstance, the time between the final removal of the cup with the sample material from the oven and the moment of the final measurement of the weight. This latter procedural parameter was included for measuring the effect of a possible procedural artefact. An experiment with three dissolving times in duplicate (six samples of candy syrups from practice, unknown contents) was conducted (30 minutes, 1 hour and 2 hours). Each sample was weighed immediately after removal from the oven, after 5 minutes waiting time and after 30 minutes waiting time. The results are shown in Figure 2.

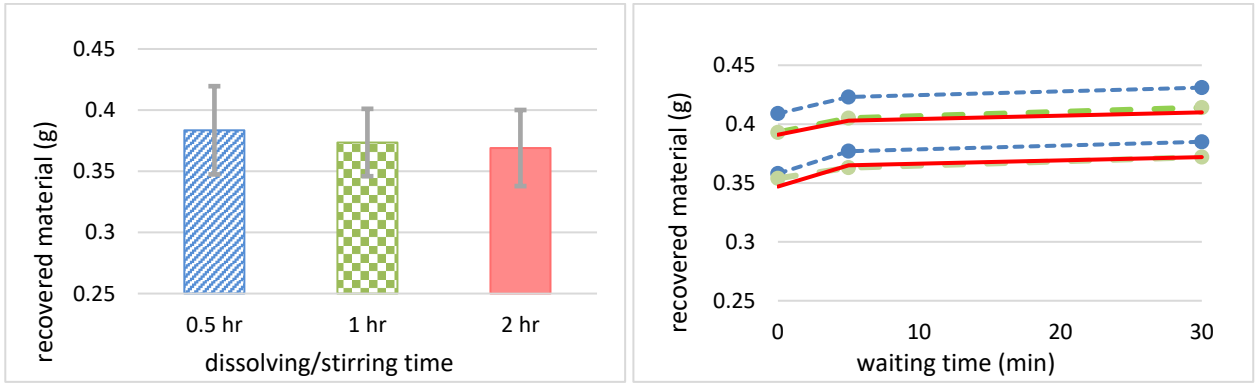

Figure 2 *Effect of different dissolving times (left) and waiting time before measuring the weight after final drying (right) on the recovery of packaging material in candy syrups. All measurements in duplicate. The colours of the bars at left match the colours of the lines at right (lines of same colour are duplicates).*

The tendency for lower recovery after prolonged dissolving/stirring time based on samples from practice (Figure 2, left) matched the results as collected during method development based on laboratory designed samples (Figure 1, left). Prolonged waiting time before the final weight of the samples was measured showed an increase of time. This effect is most probably due to air moisture, which was also found during validation studies for the method to detect weed seeds and ergot sclerotia (van Raamsdonk et al., 2022). One-way ANOVA showed for each of these parameters non-significant trends (F test: (dissolving/stirring time: 0.1091, df=2 and 3, p=0.10; waiting time: 1.2052, df=2 and 15, p=0.673). These two parameters, dissolving/stirring time and waiting time prior to weighing, show a clear negative correlation. A longer dissolving/stirring time will be compensated by a longer waiting time to weigh. Based on these results it was decided to use 15 minutes dissolving/stirring time in the final method, to test the robustness of this parameter during validation and to weigh all samples immediately after drying.

2.3 Method description

Samples from practice might have varying sizes. If jars with sample material delivered for analysis have a total weight considerably lower than 500 grams, it should be considered to refuse the examination. In cases of large jars with high amounts of sample material an excess of approximately 750 gram should not be taken as laboratory sample for the handling of large amounts of hot water in the course of the procedure. A selection of material up to 750 gram from a larger sample would result in a non-representative subsample. This is, however, unavoidable, for reasons of safety and handling. Measures for homogenisation can be taken during the first preparation. The final laboratory sample should be taken as the sample as delivered by the competent authority or manufacturer. If desired it can be communicated that samples with an amount between 500 and 750 grams are preferred for analysis.

The jars with the original samples are weighed (value A) and placed in a water bath at a temperature of 70 ˚C for one hour and stirred frequently, if an excess of the desired amount can be expected. At least 500 grams are poured in a plastic beaker (flat bottom). If the jar remains empty, it will be rinsed with hot water and the fluid be added to the beaker. The jar, optionally with remaining sample material, will be weighed again (value B). The difference between the two weights (A-B) is the sample weight taken for actual examination. Hot (boiled) water is added to the sample until a volume is reached of 5 times the sample weight.

The beaker with the diluted sample is placed on a hot plate with a stirrer for 15 minutes, adjusted to 70 ˚C. after the dissolving/stirring time the solution is poured over a sieve with a mesh size of 200 µm and the beaker is rinsed with hot water. This rinsing water is poured over the sieve as well. The sieve with the material on it is rinsed with hot tap water for five minutes.

The remaining material on the sieve is placed in a petri dish or cup with a known weight, weighed (value C; total value of dish/cup with sample material subtracted with the tare weight of the (dish/cup), dried, and weighed again (value D). The material is inspected visually or under a binocular to determine the nature of the material. Fragments which are native to candy (e.g. nut fragments from nut bars or from nougat, coconut fragments from bounty bars) are removed. The remaining portion is weighed (value E) and defatted in 10 ml tetrachloroethylene (TCE) in a beaker (flat bottomed) for 10 minutes. The material is poured on a small sieve with a mesh size of 80 µm and rinsed with two times 10 ml of TCE. The material is placed in a petri dish or cup and dried in a stove at 60 ˚C overnight. The material is finally weighed directly after removal from the oven (value F).

The amount of the original sample in milligrams (A-B), final weight in milligrams (F) and the share (in %: final weight divided by the sample weight; F/(A-B)*100) are reported.
There are two parameters for controlling the process:

- Effect of drying: the difference between the weights before and after drying should be higher than zero: C-D>0.
- Effect of defatting: the difference between the weights before and after defatting should be higher than zero: E-F>0.

3 Validation

3.1 Design

An experimental plan has been designed for establishing the relevant performance parameters. The design of the validation study, the a-priori criteria for the performance parameters and the evaluation are based on the Quality Guidance of methods to examine visually recognisable substances in feed and food (van Raamsdonk et al., 2022; hereafter referred to as Guidance). This design was based on the availability of eight large samples from practice, containing a variety of sweets, peppermint and chewing gum, for one experiment and a range of laboratory produced samples for the remaining experiments for validation.

After homogenization, three sub-samples were taken from each of the eight samples from practice of candy syrup. These three sub-samples are used to determine the repeatability and the within-laboratory reproducibility. The contents of foreign products were between 0.03% and 0.26%.

For the determination of the recovery, eight samples of blank candy syrup were spiked with 0.15% packaging material.

To determine the lower quantification limit, eight samples of blank candy syrup were spiked with 0.05% packaging material.

To determine the selectivity, two blank candy syrup samples were spiked with 0.15% packaging material as well as with 0.10% peanut fragments, as mimicking non-soluble matrix material.

For the determination of the robustness, two experiments were performed. Two spiked samples with 0.15% packaging material had a stirring/dissolving time of 30 minutes at 70 ˚C (extended time) and were sieved with a mesh size of 200 µm (according to protocol). Two other spiked samples with 0.15% packaging material had a stirring/dissolving for 15 minutes at 70 ˚C (according to protocol) and were sieved with a mesh size of 125 µm (smaller mesh size).

Performance parameters to validate:
- Recovery
- Limit of detection
- Selectivity
- Repeatability
- Within-laboratory reproducibility
- Weight uncertainty
- Robustness/stability

3.1.1 Background to the parameter “uncertainty”

Uncertainty is a major aspect for measuring results. Measurement uncertainty is the only quality parameter mentioned in Guideline ISO 17025:2017 (ISO, 2017), and dedicated guidelines have been published (Codex Alimentarius, 2004; Ellison and Williams, 2012 (Eurachem QUAM:2012); Bettencourt da Silva and Williams, 2015 (Eurachem STMU:2015). The parameter measurement uncertainty is usually based on the relative standard deviation of the reproducibility. Criteria have been developed which are frequently applied in methods in the domain of analytical chemistry (Horwitz, 1995; Horwitz and Albert, 2008). Expanded measurement uncertainty is usually a value twice of the original value of the measurement uncertainty. It has been demonstrated that subsamples taken from a submitted sample for examination of undesirable substances in whole kernel matrices (weed seeds, ergot sclerotia) exceeding a size of approximately 1 mm will show reproducibility standard deviations among subsamples largely exceeding these known criteria (van Raamsdonk and Van der Voet, 2022). Basically, any value for reproducibility standard deviations of this

type of sample material has very limited value, since none of the subsamples can be regarded representative of the original sample. Therefore an alternative procedure has been developed for validation experiments. The selected material of the undesirable substance will be reintroduced in the matrix material, get time to be settled for levelling moisture and fat content, and the sample will be examined again. Examination of these whole kernel materials will result in two types of results: the number of the particles and the total weight of the selected undesirable substances per sample. In a situation of reintroduction the number of the first and the second examination should be equal, whereas the two weights might show some deviations. Therefore, two new parameters have been defined, which are count dispersal and weight uncertainty, in order to avoid confusing with the interpretation of the well-known "classical" measurement uncertainty. Packaging materials in former food products are usually not counted and the weight of the selected material remains as only result. Considering the types of matrices subjected to visual examination, a continuum can be expected with decreasing measurement uncertainty from whole kernel matrices where subsampling is inapplicable or at least two subsamples should be investigated (large inhomogeneity), via matrices with options for homogenisation resulting in mediate levels of uncertainty up to matrices which can be sufficiently homogenised (fluids, very small particles). The term measurement uncertainty will be avoided in the next paragraphs and will be replaced by the term weight uncertainty. Parallel to measurement uncertainty, the parameter weight uncertainty (or just uncertainty) can be used in an expanded form. More explanation is presented in the Quality Guidance for the detection of visually recognisable substances (van Raamsdonk et al., 2022).

3.1.2 Description of the experiments

- Experiment A: Eight laboratory samples (eight candy syrups) were artificially contaminated with 0.15% of fragments of relevant packaging materials. The results were used to establish the recovery.
- Experiment B: Eight laboratory samples (eight candy syrups) were artificially contaminated with 0.05% of fragments of relevant packaging materials for determining recovery and the limit of detection.
- Experiment C: Two samples of candy syrup were contaminated with 0.15% of relevant fragments of packaging materials and 0.10% of nut fragments. These samples were used to examine the selectivity.
- Experiment D: Eight laboratory samples from practice (eight samples from eight industrial batches, all contaminated with an unknown amount of packaging materials) were measured twice on the same day for establishing the repeatability, and additionally once for establishing the within-laboratory reproducibility. The three duplicate analysis samples were extracted from one jar with homogenised sample material.
- Experiments E1 and E2: Two samples of candy syrup contaminated with 0.15% of fragments of packaging materials were dissolved/stirred during 30 minutes instead of 15 minutes, and two samples of candy syrup contaminated with 0.15% of fragments of packaging materials were rinsed on a sieve of 125 µm instead of 200 µm. These experiments were used to establish the robustness.

In addition, an experiment (F) was added to measure two duplicate samples of a blank artificially made candy syrup, which is the matrix for all the samples used in the experiments A, B, C and E.

An overview of the samples required for the full design for validation is given in Table 2.

Table 2 *Overview of prepared and tested samples, organised along the experiments being part of the validation design.*

Experiment: number of samples	Type of samples	Content of samples
exp. A: 8 exp. E: 4	Artificially produced candy syrup 0.15%	syrup (60% DM) with 0.15% (w:w) packaging fragments
exp. B: 8	Artificially produced candy syrup 0.05%	syrup (60% DM) with 0.05% (w:w) packaging fragments
exp. C: 2	Artificially produced candy syrup 0.15%	syrup (60% DM) with 0.15% (w:w) packaging fragments and 0.1% nut fragments
exp. D: 8 x 3 =24	Samples from practice	three duplicates each of eight jars from practice, taken after homogenisation
exp. F: 2	Artificially produced candy syrup 0.0% (blank)	syrup (60% DM)

All samples in amounts of 500 grams.

3.1.3 A-priori set criteria for performance parameters

It is hardly possible to establish criteria for all the mentioned parameters, since a body of supporting literature is largely missing. The behaviour of the parameters for whole seeds and ergot in granular matrices is known and is addressed in the new Guidance. Based on that data, a Horwitz equation has been derived with an exponent of -0.41 (Table 3; van Raamsdonk et al., 2022). The basic Horwitz equation (Horwitz, 1995; Horwitz and Albert, 2008) for analytical chemical determinations has an exponent of -0.15 (Table 3). This is an important starting point for the interpretation of the measurement uncertainty in analytical chemistry. Candy syrup is easier to homogenize than a granular matrix because of the semi-fluid base. In addition, the number of particles of packaging material is much larger than the whole particles in weed seeds and ergot. Before the validation experiments were started, the possibility to achieve a homogeneous batch of material per sample was tested and found to be sufficient. The practical samples have an average content of 0.06%, whereas the laboratory samples were adulterated at 0.05% and 0.15%. Table 3 shows the levels for the expanded uncertainty for these levels using the classical version for the chemical domain and for the situation of seeds and ergot, which can be considered as the upper and lower limits of the acceptable range applicable to the current validation. The expanded weight uncertainty for packaging material in FFPs should ideally be as close as possible to the lowest value. The relative standard deviations of repeatability and reproducibility should approach half these values.

Table 3 *Upper limits of uncertainty using the Horwitz equation for three adulteration levels (C).*

Horwitz for level C:	**Maximum allowed uncertainty**		
	C=0.05%	**C=0.06%**	**C=0.15%**
Chemical domain, slope -0.15: UCL = 2 * $C^{-0.15}$, indicated as measurement uncertainty	6.25%	6.1%	5.3%
Weed seeds and ergot sclerotia, slope -0.41: UCL* = 2 * $C^{-0.41}$, indicated as weight uncertainty	45.1%	41.9%	28.8%

The upper and lower confidence limits for the recovery interval can be derived from the same equation. This would give the following LCL-UCL ranges:
- 0.15% packaging material: 71.2% (100-28.8%) – 128.8% (100+28.8%),
- 0.05% packaging material: 54.9% (100-45.1%) – 145.1% (100+45.1%),

under the assumption of an inhomogeneity comparable to that of whole kernel undesirable substances (weed seeds, ergot sclerotia). As for the expanded weight uncertainty, the values resulting from the validation experiments should be as low as reasonable achievable.

The limit of detection could be set not exceeding 0.02% w/w, as used for BBPs (van Raamsdonk et al., 2012). This parameter will be calculated from the standard deviation of the recovery at 0.05% by a multiplication with a factor 3 (experiment B).

In the validation for the detection of packaging material in bakery by-products (van Raamsdonk et al., 2012, p. 14) the strategy was chosen that the relative deviation (RD) for selectivity and robustness should be smaller than the difference between the established minimum recovery and the calculated lower limit (71.2%) or the established maximum recovery and the calculated upper limit (128.8%) for the 0.15% samples:
- RD < $R\%_{min}$ – 71.2% for a negative value of the RD
- RD < 128.8% - $R\%_{max}$ for a positive value of the RD

In other words, the space left between 71.2-128.8% after processing the LL-UL range for the recovery is available for the relative deviation under adjusted conditions.

The way in which stability is determined deviates from the normally applied procedures. The targets themselves are hardly subjected to chemical decomposition and stock solutions for reference materials are not being applied. Air moisture could influence a quantitative result, as demonstrated in the preparatory experiment.

The result of experiment F (blank sample) was a level of 0.008 g/kg (0.0008%) of non-identified debris. The results of all the validation experiments were adjusted for this value.

3.2 Results of the validation experiments

The established values for the examined performance parameters are shown in Table 4.

Table 4 *Overview of performance parameters and the established values for the detection of packaging materials in candy syrup. Chapter refers to the Guidance (van Raamsdonk et al., 2022). Abbreviations: R: recovery; R*: recovery under modified circumstances; DL: detection limit, RD: relative deviation connected to a version of R*; (R)SD: (relative) standard deviation; U: uncertainty.*

chapter parameter		value	unit	further specification		
				min	max	
3.1.1 Recovery 0.15%	R	102.7	%	98.4	104.9	%
3.1.1 Recovery 0.05%	R	101.2	%	98.4	105.3	%
3.1.2 Detection limit	SD_{DL}	0.007	g/kg	DL	0.021	g/kg
3.1.3 Selectivity + 0.1% nut fragments	R*	103.8	%	RD	1.052	%
3.1.4 Repeatability	Sr	0.048	g/kg	RSDr	2.96	%
3.1.4 Within-laboratory reproducibility	SR_w	0.041	g/kg	$RSDR_w$	2.55	%
3.1.5 Expanded weight uncertainty	U	9.094	%			
3.1.6 Robustness rinsing time (15⇨30 min)	R*	99.5	%	RD	-3.58	%
3.1.6 Robustness sieve mesh size (200⇨125 µm)	R*	96.5	%	RD	-6.43	%

1. Recovery:
The recovery was determined on the basis of two sets of eight samples of candy syrup with resp. 1.5 g/kg (0.15%, a widely accepted tolerance limit) and 0.5 g/kg (0.05%) packaging material.

In both cases, the recovery and the lower and upper limits of the measured values Table 4) are well within the a-priori calculated LCL-UCL ranges. The average recovery in both experiments is slightly higher than 100%. There is a possibility that fractions of the matrix (sugar) have remained in the residues of the rinsed and filtered packaging material, resulting in a higher recovery. This will be discussed in more detail when determining the factors for robustness.

The limits of the range in recovery at a content of 0.05% will be the basis for calculating the maximum limits for the relative deviation (RD) in selectivity and robustness:
- RD < 98.4% – 71.2% = 27.2% for a negative value of the RD
- RD < 128.8% - 105.3% = 23.5% for a positive value of the RD

These values guarantee that under modified circumstances and with the already established min-max range for recovery, the RD will not lead to excess of the a-priori calculated LCL-UCL limits.

2. Limit of detection:
The experiment at the level of 0.5 g/kg (0.05%) was used to determine the limit of detection. The assumption was that the level of 0.05% will be close to the presumed detection limit. This detection limit (DL), calculated from the standard deviation over the observations at 0.05%, is low (0.021 g/kg, 0.002%) and lower than the a-priori set limit of 0.02%. The amount of recovered material from the blank (0.008 g/kg, 0.0008%) is much lower than the calculated limit of detection.

3. Selectivity:
The selectivity of the method was determined by adding a potentially interfering component to the samples to be analysed. The choice was made for fragments of peanuts, which can be found in nuts bars or muesli bars. The relative deviation by weight is 1.052% at an average level of 1.5 g/kg packaging material in the presence of 1 gram of peanut fragments. In the view of a diversity of the recovery from 98.4% to 104.9% in

experiment A, this additional relative deviation is well within the a-priori calculated LCL-UCL range of 71.2% – 128.8% and much lower than the maximum allowed excess of 23.5% (see paragraph Recovery).

4. Repeatability and within-laboratory reproducibility:
When collecting the values for the duplicate measurements of experiment D, an outlier was found in a sample. For that reason, an additional sample was examined as replacement for the outlier value.

Based on the results of the validation described here, the within-laboratory reproducibility standard deviation (SR_W) has been determined to be 0.041 g/kg. The value for SR_W is comparable to the repeatability standard deviation Sr. The relative within-laboratory reproducibility ($RSDR_{w)}$ has a value of 2.55%. This indicates the spread in the results when a sample is analysed at two different times in the laboratory under reproducibility conditions.

5. Weight uncertainty:
The values for RSDr and $RSDR_w$ are used to calculate the weight uncertainty (for explanation on term: van Raamsdonk et al., 2022; see Guidance). The expanded weight uncertainty U is 9.094%. This is higher than the value for the corresponding contamination level in the samples from practice (0.06%) according to the classical Horwitz equation (UCL = 6.1%), but much lower than the value according to the new equation for seeds and ergot (UCL* = 41.9%). It can be concluded that a semi-liquid matrix and a relatively large number of particles and fibers can lead to a reasonably well homogenized matrix, still different from a well-homogenized sample for detecting a chemical contaminant.

6. Robustness:
The robustness is determined in two ways. Two experiments were performed with a change in protocol using two samples each with a known level of packaging material (1.5 g/kg). Two samples were tested with a stirring time of 30 instead of 15 minutes, and two samples were sieved over a sieve with a mesh size of 125 µm instead of 200 µm. After the first modification, the recovery could be lower (more sugars dissolved), and in the second modification the recovery could be higher (more material collected due to smaller mesh size). In both cases the recovery was slightly lower than 100% (relative deviation (RD) of -3.58% and -6.43%, respectively; Table 4). These RD values are much lower than the maximum allowed excess of 27.2% (see paragraph Recovery). The established recoveries of 99.5% and 96.5%, respectively, fall well within the a-priori calculated LCL-UCL range of 71.2% – 128.8%. There is no explanation for the situation in which less material is recovered with a smaller mesh size. The comparable result obtained after 15 minutes (experiment A) and 30 minutes (experiment E1) dissolving/stirring time fit in the results collected after the additional experiment for method development (Figure 2, left). A prolonged dissolving/stirring time results in gradually lower recovery values, which represent non-significant differences. However, the results of the experiments for method development indicated an average recovery of 90% after 60 minutes dissolving/stirring time (Table 1, Figure 1). It can be concluded that periods exceeding 30 minutes dissolving/stirring time are not recommended.

Application in practice of the currently discussed method showed that a shiny layer of sugar can be seen in a some cases covering the selected packaging material. This can possibly be prevented by using that longer dissolving/stirring time of 30 minutes. The results indicate that this is within the limits of the method's robustness.

7. Stability:
This factor was not included in the design of the validation experiments, but the additional experiment for method development provides a perspective to this parameter (Figure 2, right). Stability is a condition often related to the ability to store samples, which is an important parameter for the applicable storage life of reference samples. This is usually not a factor with samples containing visually detectable substances. However, a different approach to stability can apply here: the contaminant can be sensitive to environmental factors. The quantities of packaging material from the additional experiment (three stirring times, n=6) were weighed again after two waiting times (5 minutes and 30 minutes) (total n=18). An increase in weight between 2.5% and 7.5% was found for all six fractions of packaging material. The increase after 5 minutes was a maximum of 2.6%. However, the F value from the one-way ANOVA (1.205) did not indicate a significant increase in weight (see section 2.2, Figure 2 (right)).

4 Discussion

4.1 Types of methods for detection of packaging material

The tendency to achieve a circular bioeconomy and sustainability of the food production chain as far as possible has the consequence that an increasing variety of FFPs, including BBPs, will be used as feed ingredient. The incidence of packed food stuffs, and consequently unpacking upon use as feed material, will increase when the by-products approach the final stages of processing. One dimension of this variety of FFPs is the moisture or fluidity of the batch or sample material. Homogenisation and handling of sample material will depend on the fluidity or viscosity of the matrix. In the view of the medium fluidity of candy syrups, the current method for detection of packaging material in these syrups is based on extracting or discarding the sugar-rich matrix material. This procedure deviates from the general approaches in chemical analysis in the field of feed and food safety, where an undesirable substance or a group of substances is extracted from a sample by using a solvent. The consequence of this approach is that the method can be indicated as destructive. Reintroduction of the selected material in the sample matrix material and performing a second examination is not possible, as is applied for the validation of the detection method for packaging material in BBPs.

The new method adds to the available methods for detection of visually recognisable substances in the macroscopic range. These include whole kernel matrices with a low number of large units, dry coarsely ground matrices with a larger number of smaller particles than in the whole kernel situation, bakery by-products or ground animal feeds, and semi-fluid matrices. For candy syrup the matrix can be dissolved in warm water. Semi-fluidity for other types of matrices might demand a different strategy to discard the matrix material.

The maximum legal limits are usually expressed in weight units or in w/w percentages (Regulation 767/2009; Directive 2002/32). For whole kernel matrices, counts can be used as an additional result.

Another dimension is the content of fat, up to matrices exclusively consisting of fat or oil. A method in the microscopic range provides a procedure of extracting animal particles from oil or fat (Regulation 152/2009). Such approaches are still not developed for detection of packaging materials.

There are two basic factors in methods for detection of packaging materials in the macroscopic size range. The first is the circumstance that the particle size of the undesirable substance is equal to the size of the matrix particles. Grinding as part of a sample preparation procedure will result in fragmentation of the targeted particles as well, with the result of an increasingly complicated detection, negatively influencing the method performance. The second factor can be seen as consequence of the first factor: with units of detection immensely larger than chemical molecules, the inhomogeneity is always incomparably larger than found for chemical compounds, which causes the need to examine large portions of at least 500 grams.

4.2 Validation strategies

The type of matrix and therefore the methodical procedures directs the strategy of the method validation. Especially the parameter homogeneity will influence the way performance parameters can be established and interpreted.

In the situation of reintroduction of the selected particles, inhomogeneity is excluded as factor in measurement uncertainty, since the same quantity is examined again. The within-laboratory reproducibility for BBPs was established at $RSDR_w$ = 0.012% (van Raamsdonk et al., 2012), which is much lower than what was found for the new candy syrup method ($RSDR_w$ = 2.55%; Table 4). Real subsampling of whole kernel

sample material for detection of whole seeds or ergot sclerotia results in a large share of the resulting inhomogeneity among subsamples in the calculation of measurement uncertainty. Therefore, in order to indicate the background of the parameters, the terms count dispersal and weight uncertainty has been introduced, avoiding confusing with the mainstream term measurement uncertainty (van Raamsdonk and van der Voet, 2022).

Comparable to the dimension of fluidity, mentioned in the previous paragraph, a dimension of decreasing inhomogeneity can be imagined. In general, a decrease in the size of particles would provide better options for homogenisation. In the microscopic range, with particles smaller than 1 mm, homogenisation can be achieved. Step-wise dilution can successfully be applied for animal particles such as bone fragments in ground matrices, and subsamples can be analysed as true duplicates with the remark that subsample size is fixed at 10 grams (Regulation 152/2009 Annex VI). For macroscopic analysis the combination of particle size and fluidity can be assumed to be major parameters. At one side, dry matrices with particles over 1 mm result in very large inhomogeneity, which is avoided by the approach of reintroduction. The current method for examination of semi-fluid matrices avoids reintroduction. Homogenisation resulted in an established value for the expanded weight uncertainty of over 9% (Table 4), which is higher than the UCL according to the classical Horwitz equation, but much lower for the situation of dry coarse matrices (Table 3). With the results of the validation of the method for examining packaging material in candy syrup as example of semi-fluid matrices, the existence of intermediate situations of inhomogeneity resulting in intermediate levels of within-laboratory reproducibility and of weight uncertainty is established. This knowledge will add to the establishment of better adjusted limits for several quality parameters in the framework of methods for detection of visually recognisable substances (van Raamsdonk et al., 2022).

The performance of the method for detecting packaging material in candy syrup method is well within limits. It needs to be noted that in the view of the sliding scale of the inhomogeneity the a-priori fixed limits for the respective performance parameters (paragraph 3.1.2) might have been set at too high levels. Still, the performance can be rated as good. One remark is that the value for SR_w is usually higher than that for Sr since reproducibility includes more causes of uncertainty than repeatability. Here both values are comparable, but SR_w shows the lowest value. The current results for method development and validation concerning a type of a semi-fluid matrix might be the starting point to explore further the opportunities and performance of methods to detect visually recognisable substances.

5 Conclusions and recommendations

A method was developed for the quantification of packaging materials in candy syrups. Based on the validation, the method largely satisfies the a-priori set requirements and specific conditions that apply to visual examination methods. The method is suitable for the intended application.

The method development showed that the type of matrix and size of the particles of the undesirable substance have their influence on the achievable level of homogenisation. A higher level of homogeneity would allow to set lower a-priori maximum limits for reproducibility and weight uncertainty. With a sufficient homogeneity, duplicate analyses can be carried out and a value for repeatability can be retrieved. With an increasing set of method descriptions for examination of visually recognisable substances in a variety of expressions and matrices, better expertise will be achieved for setting correct quality assurance parameter limits for this type of methods.

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