relana®

Position Paper 18-01

Sample Preparation Project

Summary Report – Part 1

August 2018

relana® Position Papers

Content

1. Aim of this project

"An analysis cannot be of better quality than the quality of the sampling." (Anon.)

Food Laboratories invest many efforts into the improvement, validation and quality control of the analytical procedures carried out - this applies in articular for pesticide analyses. The upstream steps of sampling and sample preparation are often a bit neglected. This effect is increased by common ring trials and competence tests which use pre-homogenised test materials.

Nevertheless, any mistake or deviation occurring during sampling and sample preparation prior homogenisation has a significant impact on the final result and thus can make every effort during the analysis ineffective.

The aim of this project is to compile advice for practical work in order to improve the quality and comparability of sample preparation steps. The objective of this work is NOT the blaming of laboratories for any mistakes or unskilfulness, which might have occurred during the visits. The frankness in showing how work is carried out in routine - and thus in delivering information, is the basis of the relana® quality circle.

2. Methods

As the relana® concept takes the entire analytical process into consideration, a closer look at the sample preparation was part of every relana® visit since the beginning.

During the 2017 relana® visits, Lach & Bruns initiated this sample preparation project. They took sample material to all 11 participating labs during the visits. Each lab had to prepared the material correspondingly on-site. The below mentioned aspects were considered during the visits:

- Five (5) commodities:
  - **Spring onions**
  - **Paprika (sweet pepper, bell pepper)**
  - **Strawberries**
  - **Mango**
  - **Zucchini (courgette)**
- quality: directly from supermarkets, as sold to the consumer
- each commodity was prepared by two lab technicians for the sample-preparation department
- Lach & Bruns interviewed each involved technician to ask for backround information ("why") related to the steps carried out
- the preparation was carried out independently, as goes for the interviews
- the documented results were kept anonymously
- a few sample preparations described in this report were already carried out during the relana® visits in 2016.

3. Result part I: General aspects

An overview of the anonymised results can be found in the attachment.

Please note that up to five employees from the sample preparation unit per lab were involved during the sessions, i.e. the results do not necessarily refer to only two employees (Employee A and B, see results in the attachment) per lab.

3.1. Standards, norms & Co.

Concerning the question of "*which part of a product has to be analysed for pesticides?*", the Annex I of the **EU Regulation 396/2005** [1] (setting maximum levels for pesticides) is the legal basis to refer to.

Unfortunately, the information provided by **Annex I** does not always go so much into detail, as the following examples show:

Product	Definition	Arising questions (selection)
Berries, excluding currants	"whole product after removal of caps, crown and stems"	How much co-removed flesh is acceptable in routine handling?
Stem vegetables (exceptions apply)	"Whole product after removal of decayed tissues, soil and roots"	At what degree of decay must a tissue be called "decayed"? What method is acceptable for removing soil, and how much remaining soil can be accepted?

Table 1: Selected product definitions as fixed by Annex I of Regulation 396/2005 [1]

From January 1st, 2018, a revised version of Annex I applies [2] – strange enough, the amendatory regulation was published AFTER the application date. Following our check, these changes do not affect the general questions discussed in this report, but it is recommended to check where changes have to be incorporated into lab routines.

There are further documents which might supply relevant information on this topic. However, they are not very detailed neither:

EU directive 2002/63 "setting methods of sampling for the official control of pesticide residues" [3] delivers important definitions for terms such as "primary sample" or "laboratory sample". It also defines the number of primary samples to be taken for certain commodities and lot sizes.

Concerning the questions discussed in this paper, the following details described in this directive may be helpful, see also table 2:

- minimum sizes for laboratory samples (usually: 1 kg)
- reduction of sample sizes
- treatment of samples with inhomogenisable stones (for example, the stones of stone fruits are not analysed but the residue level is calculated assuming that they are included but contain no residue).

Plant products: description of primary samples and minimum size of laboratory samples

	Commodity classification (1)	Examples	Nature of primary sample to be taken	Minimum size of each laboratory sample
Primary food commodities of plant origin				
1.	All fresh fruits All fresh vegetables including potatoes and sugar beets and excluding herbs			
1.1.	Small sized fresh products units generally < 25 g	Berries, peas, olives	Whole units, or packages, or units taken with a sampling device	1 kg
1.2.	Medium sized fresh products, units generally 25 to 250 g	Apples, oranges	Whole units	1 kg (at least 10 units)
1.3.	Large sized fresh products, units generally > 250 g	Cabbages, cucumbers, grapes (bunches)	Whole unit(s)	2 kg (at least 5 units)

Table 2: Excerpt from directive 2002/63 [3]

It should be noted that this directive respectively the national implementations are only binding for the official food control labs, not for private laboratories or the food industry. Nevertheless, applying these provisions as a guideline is recommended as it minimises discussions afterwards.

N.B. (nota bene): The German implementation of this directive is performed by the Official Collection of Methods of Analysis according to § 64 LFGB (Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB), number L 00.00-7 [4].

SANTE 11813/2017 [5]: The SANTE (formerly: SANCO) document focuses on the analytical steps, quality control and the calculation of results. Concerning sample preparation, some guidance is provided in the paragraphs C1 to C4, mainly describing comminution techniques and strategies, considering fragile analytes.

Recommendation of the working group "pesticides" of the German Chemical Society (Gesellschaft Deutscher Chemiker - GDCh) [6]: This document was published in 1995 and therefore refers to the legal situation at that time (EU directive 90/642/EEC; German implementation: Rückstands-Höchstmengenverordnung – RHmV). Unfortunately, this document is available in German language only. In this publication, some additional details concerning the following issues are touched:

- **Organoleptic analysis**: Prior to any sample preparation step, an organoleptic analysis is recommended to check for any peculiarities such as rotten parts, foreign matter etc.

- Treatment of **samples with inhomogenisable parts**, which are part of the product definition (e.g. stones of mangoes, cherries, olives): Typically, the stones are not analysed but considered when calculating the analytical results. In case specific techniques are applied (f.ex. cryo-milling), the entire products might be homogenised (so with stones or kernels).
- **Sector technique**: When comminuting samples, the sector technique may be applied to prepare fresh fruits, vegetables, and potatoes.
- **Special treatment for dithiocarbamates (DTC):** As DTC are very fragile analytes, the paper of the working group recommends analysing small products (such as grains, berries, cherries) directly and apply the sector technique to larger products. In case of juicy and soft products, it is recommended to freeze the samples prior cutting.
- **Recommendations for certain products**: Some details on the treatment of certain products can be found (e.g. the removal of roots in the case of spring onions, cf. 4.1.). Please note that in case of discrepancies, the current legal framework (Annex I of Regulation 396/2005 [1]) has priority.

Recommendations of CVUA Stuttgart: The "Chemisches und Veterinäruntersuchungsamt" (CVUA) Stuttgart, official lab of the German state Baden-Württemberg, is focusing on the analysis of pesticides and has published some recommendations as well [7]:

- A **sensory analysis** is incorporated as an important step in the analysis, stressing that decay can influence the pesticide content (see 3.7. of this paper).
- Furthermore, the CVUA points out that samples **are not allowed to be washed**, as this may reduce the pesticide content as well (see. 3.6. of this paper).
- **inhomogenisable parts** of samples (like stones of stone fruits) are cut out, weighed and put aside (see 3.9. of this paper).
- **long and thin varieties of vegetables** (such as zucchini or cucumber) shall be cut into 5 pieces, and the pieces 1, 3 and 5 shall be taken for analysis
- Larger products such as apples shall be homogenised after applying the **sector technique**

For a discussion on the topic "selection of parts and reduction of sample sizes", see chapter 3.9 of this paper.

Recommendation:

As described above, laws, standards, and norms do not go too much into detail regarding the preparation of food samples prior to homogenisation. Therefore, it is advisable to conduct a guideline (standard operating procedure - SOP) which fixes the most important general aspects as well as details for single products. Some suggestions are provided in this report.

3.2. Availability of information

The results from the lab visits indicate, that the availability of information concerning sample preparation differed to a relevant degree.

This is presumably one of the reasons for the variety of preparation approaches not only between labs, but also between the lab technicians within one lab, s. example in table 3 (selection of parts for strawberries) and attached results.

Lab no.	Employee A	Employee B
1	Whole fruits	Whole fruits
2	Whole fruits	Whole fruits
3	Whole fruits	Whole fruits
4	Sector technique	Sector technique
5	Halves	Halves (small fruits), sector technique (large fruits)
6	Whole fruits	Whole fruits
7	Whole fruits	Whole fruits
8	Whole fruits	Whole fruits
9	Sector technique	Sector technique
10	Sector technique	*Not visited*
11	Sector technique	Whole fruits

Table 3. Strawberries: Selection of parts for homogenisation

It must also be mentioned that some information circulates orally only, which is unfavourable not only with respect to visits (resp. audits).

Another important aspect concerning this issue is discussed in the following paragraph.

Recommendation:

Any relevant information concerning sample preparation should be made available in written and if possible also visual form. It should be easily available and comprehensive for all personnel concerned with this topic. Ideally, it is easy to find, read and understand, as the time pressure is usually high.

3.3. Training of staff

The demands a sample preparation unit has to meet are quite high – and presumably higher than some colleagues and managers may think.

Generally, the sample preparation unit has to deal with a high number of samples of different types, from citrus fruits to grains, from spices to nuts, maybe even including animal-derived foodstuff. Additionally, the time pressure is high, as devices are waiting for sample extracts to measure, and customers are waiting for their results.

The results of this project indicate that the same type of sample may be treated differently in the labs, and the treatment may even differ within one lab. As an example, please see table 3, which shows the differences in the way strawberries were treated.

Especially the fact, that the sample treatment can significantly differ within one lab, shows that the degree of knowledge and/or the motivation to get more information varies.

Recommendation:

The results of this project show that it is necessary to train personnel working in the sample preparation unit on a regularly basis. Their work is the basis for reliable analytical results.

At this point, it must be considered that language barriers may exist, and a meaningful way of transferring information to the personnel should be sought. Supervising and testing this step on a time-to-time basis could be appropriate as well.

3.4. Validation and general quality control

Most pesticide laboratories put much effort in validation and quality control of the analytical procedures they apply. The crucial step of sampling and sample preparation is sometimes at least partly neglected. This effect is supported by the design of most competence tests where homogenised material is used, which excludes the step of sampling and sample homogenisation.

Lehotay and Cook recommend that "the validation of sampling and sample processing protocols and ongoing QC *(quality control)* should be implemented in monitoring programs" [8].

This may include:

- carrying out the whole analysis (with incurred residues) in duplicate, starting from sampling
- spiking QC standards at every step of the sample preparation (where applicable)
- considering different analyte-matrix-combinations.

For details concerning quality control for the preparation of fruits and vegetables see chapter 3.10. of this paper.

Recommendation:

Validation and quality control measures usually focus on the analytical procedures only. Due to the significant impact of the sampling and sample preparation steps on the final result, it is recommended to incorporate accurate and attentive quality control measures for these steps as well. Existing quality control measures should be checked on a regular basis.

3.5. Sample quantity

In case of sample quantities not matching the required amount, a clear procedure should be fixed, maybe as a SOP. Generally speaking, the lab sample should consist of at least 1 kilogram or 10 pieces (2 kg or 5 pieces in case of very large units) of sample, depending on the unit weight, see also table 2 and directive 2002/63 [3]. N.B.: The size of the analytical sample, which is homogenised, does not necessarily must have a weight of at least 1 kilogram, as the lab sample often contains parts which are not be analysed (stems, stones etc.).

In case of **larger sample quantities**, the selection of the samples to be analysed should be fixed: How is the selection carried out (Picking out single items? Taking the first kilogram? Using the first 2 packages?), see also discussion in chapter 3.9.

In case of **smaller quantities** not achieving the amount required by directive 2002/63 [3], it may be necessary to contact the client and agree on the next steps. **Too low quantities must be mentioned in the analytical report.** If the client insists on having the analysis carried out, it may be necessary to resign keeping retain samples, samples of the edible part etc., in order to have enough material for the analysis. This should be clearly communicated with the client and must be decided before the preparation starts.

Recommendation:

Clear procedures on handling of sample quantities should be fixed. In case of small quantities, getting in contact with the client might be necessary as well for taking a decision on what to do with the sample (omitting retain sample etc.). It is also recommended to state the too small quantity in the analytical report.

In case of large quantities, a product-related procedure shall be fixed, see also 3.9.

3.6. Dirt and soil

Although the removal of dirt and soil attached to the samples is required explicitly only for some products in Annex I (e.g. code no. 0633000 “herbal infusions from roots”, 0270000 “stem vegetables”), **it is necessary to remove them as they are not part of the product definition**. Additionally, soil might contain pesticides sticking to the particles, which can adulterate the analytical results.

However, some special requirements are described in Annex I of reg. (EC) no. 396/2005, f.ex. sugar plants (code 900000): “*Whole product after removal of tops and soil by rinsing or brushing (except sugar canes).*”

Some lab technicians used water for getting rid of dirt and soil, but this is not favourable, as this may reduce the amount of contact pesticides sticking to the surface (see lit. [6] [7]). In this context, the usage of wet tissues (or similar) should be refused as well.

The careful use of dry tissues or brushes is strongly recommended.

3.7. Rotten pieces and parts

Depending on the perishableness of the product, the occurrence of rotten parts or even complete pieces is quite common, for example on strawberries or peaches.

The treatment of rotten parts varies across the relana® labs, as some do not cut away rotten pieces prior to homogenisation (picture 1), while others do (picture 2).

Some lab technicians argued that they cut away what is not edible, like they do at home in their kitchen. It should be noted that "**edibility**" is not mentioned in official documents and therefore should not be a criterion during sample preparation for pesticide analysis – otherwise other parts like cherry stones, banana peel or kernels and peel of water melons would have to be cut away as well.

Picture 1: Zucchini with rotten part

Picture 2: Strawberry with rotten part

Recommendation:

Rotten pieces and parts shall NOT be discarded resp. cut off. There are no legislative or regulative reasons for discarding rotten pieces or parts. If a certain amount or number of rotten parts resp. pieces are present in a sample, this might be indicated in the test report as an additional information.

N.B. Due to the safety of the employees, samples should not be homogenised if the entire sample is moulded as the mould might pollute the air with fungal spores. If samples are moulded extensively, such samples should not be analysed at all.

3.8. Wilted leaves and other parts

Most of the relana® labs cut away wilted and dry parts of the samples, while some use them for homogenisation.

Considering the explanations given in chapter 3.7., wilted parts (like in picture 3) shall be discarded as well. On the one hand, microbial and enzymatic activity may lead to a decrease

in the concentration of certain pesticides, on the other hand, the changed water content (compared to fresh parts) may influence the pesticide concentration as well.

Recommendation:

Wilted / decayed pieces and/or parts shall NOT be discarded resp. cut off. There are no legislative or regulative reasons for discarding wilted pieces or parts except for a few commodities (Annex 1 of reg. (EC) no. 396/2005: f.ex. Brassica vegetables (code 0240000) "Whole plant after removal of roots and decayed leaves (except Brussels sprouts and kohlrabies)")**. If a certain amount or number of wilted or decayed parts resp. pieces are present in a sample, this might be indicated in the test report as an additional information.**

Picture 3: Spring onion leave with wilted top

3.9. Selection of parts, reduction of sample size

Usually samples are taken from a much larger amount of product, maybe from one truck load or one container.

Besides the aspect, that the sampling should be carried out correctly (see standards like directive 2002/63/EC [3]) in order to gain a representative sample, it must also be considered how to create a lab homogenate that is representative for the lab sample itself.

While peculiarities of single products are discussed in chapters 4 and 5, some general aspects are highlighted here:

- Usually, a lab sample shall consist of at least 1 kg of sample, or 5 to 10 items in case of large products such as water melons or pumpkins [3].

- When the lab receives significantly more than the required amount, an agreed way of treating excessive sample material is necessary.

- In case of **small packages** such as trays of strawberries, it is recommended to take samples from every package in order to support representability.

- **Sector technique**: In case of items with a weight of **> 25 g**, application of the sector technique is recommended, as can be inferred from lit. [6] in combination with lit. [3], see also table 2. The products are cut into quarters (see picture 4), then two opposite quarters are used for homogenisation, while the remaining quarters might be stored as the retain sample.

Picture 4: Sector technique used on paprika

The main advantage of the sector technique is the **increased representability**, as non-systemic pesticides might be distributed unequally on the surface of fruits and vegetables, as picture 5 demonstrates: The side of the apples facing towards the spraying vehicle are likely to receive a higher amount of pesticides than the side facing the opposite way. This difference is balanced by applying the sector technique.

Picture 5: Pesticide spraying in apple plantation [10]

Please also note the **disadvantages**: Some products (like melons) are not easy to cut into (more or less) equal quarters, especially when hard kernels and stones are present, as in mangoes. Additionally, it is not easy to apply the sector technique on long and thin products such as zucchini (4.2.) or carrots, as it is difficult to meet the middle axis. Additionally, some liquid may get lost during cutting, especially from soft fruits such as mature pears.

- **Long and thin vegetables**: The CVUA Stuttgart recommends cutting these products into 5 equally long parts and using the **parts 1, 3 and 5** for homogenisation [7]. This technique can be applied to vegetables such as zucchini (picture 6), cucumber, or carrots. Nevertheless, the risk remains that this technique leads to an overestimation of pesticide levels, as systemic active substances will enter the fruit through the stem and concentrate along the middle tube. Therefore, elevated levels can be expected in both the top and the bottom piece. When always picking the end pieces, you are likely to pick higher pesticide levels than the median level.

Picture 6: CVUA Stuttgart method applied to zucchini

Picture 7: Zucchini halves alternatingly used for homogenisation

An alternative can be the usage of the following method some relana® labs apply: the vegetables are cut into halves, and halves are used alternatingly for homogenisation and retain sample (picture 7). This technique should also lead to a representative sample as top and bottom halves are chosen to an equal extent.

Recommendation
Selection of parts: It is recommended to select items from every package which arrived in the lab in order to gain a sample representative for the received material.

Reduction of sample size: Following the EU directive 2002/63 and relevant literature, it is recommended to carry out the following steps:

- **"round-shaped" items > 25 g (like apples, melons, paprika): application of sector technique,**
- **items < 25 g (berries, grapes, grain etc.): use of whole fruits or halves (where applicable),**
- **long and thin products (as zucchini, carrots): application of sector technique where possible, otherwise cutting into halves and using halves alternatingly for homogenisation.**

3.10. Use of dry ice / liquid nitrogen

Some pesticide compounds are fragile related to heat, which can be created during blending, as mechanical energy is transferred into the sample material and results in an increase of temperature.

It is recommended to avoid a significant increase of temperature during all comminution steps. If the risk of rising temperature exists, the use of dry ice / liquid nitrogen is recommended. This can also be used in case that material is easier to comminute in frozen condition.

The SANTE document recommends using dry ice or homogenising in frozen condition if there is evidence that homogenisation at ambient temperature leads to losses of analytes [5]. Concerning this subject, the EU directive 2002/63 states in a more general way: "Where appropriate, the analytical sample should be processed under special conditions, e.g. at sub-zero temperature, to minimise adverse effects."

For measures recommended for highly fragile analytes such as dithiocarbamates and fumigants, see following chapter 3.11.

3.11. Preparation for the analysis of fragile analytes

3.11.1. Dithiocarbamates

Dithiocarbamates (DTC) are fragile compounds and degrade fast after the disintegration of cell walls. Therefore, it is necessary to separate a representative part of the sample PRIOR the homogenisation step [5][6].

3.11.2. Fumigants

Fumigants such as phosphine or ethylene oxide are very volatile and will get lost in large amounts during homogenisation [5]. Therefore, it is necessary to separate a representative part of the sample BEFORE the homogenisation step and use this part for the determination of fumigants.

3.12. Documentation, quality control measures

3.12.1. Documentation

In order to document the sample preparation step including relevant data (weight of sample, peculiarities, responsibilities etc., see also 4.5.1.), it is recommended to apply an easy to use form for each sample, maybe in an electronic version. This is not only recommendable in order to meet requirements of ISO 17025 (like traceability), but also to support internal quality measures.

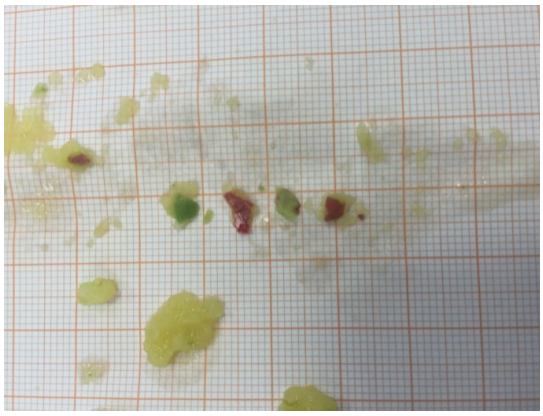
Furthermore, some labs take photos to document the quality of each sample. By making this effort, they are able to prove the quality of received samples including packaging and labelling. In case of later complaints, these photos may be very helpful in clarifying the situation. Preferably, the photo station is linked to the LIMS (laboratory information management system), thereby minimising manual work as well as possible sources of errors.

3.12.2. Check of the degree of homogenisation

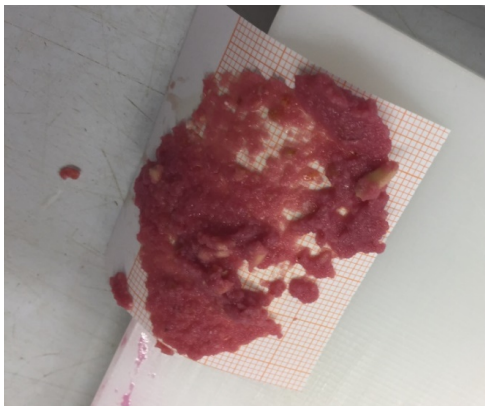
For an effective extraction of pesticides, it is necessary to achieve a high degree of homogenisation during sample preparation. It is advised to check the degree of homogenisation, for example by applying millimetre paper, see pictures 8 and 9.

A particle size of ≤ 2 mm should be achieved.

With the help of millimetre paper, it is quite easy to figure out samples which do not show a sufficient degree of homogenisation yet, see picture 8.



Picture 8: Insufficient degree of homogenisation (mango)



Picture 9: Degree of homogenisation is checked with the help of millimetre paper (strawberry)

In order to gain the required small particles, it might be meaningful to apply a **two-step-milling process**, for example applying a blender followed by a mixer using dry ice (see also 3.10).

Recommendation

The quality of the homogenisation should be checked, for example with the means of millimetre paper, aiming at a maximum particle size of 2 mm. In case of non-sufficient sample sizes, the application of a two-step homogenisation, while applying dry ice / liquid nitrogen, might be helpful. For some commodities it might be not possible to achieve the 2 mm particle size. In such cases, this should be noted at the sample documents in order to provide this important information if the variation resp. reproducibility of the results is higher than usual.

3.12.3. Maintenance and cleaning of devices, choice of gloves and cleaners

In order to achieve a high quality of homogenisation, it is necessary to maintain the used devices, especially by **sharping the knives** of the mills on a regular basis (frequently).

A thorough **cleaning step** of all equipment after each use is necessary to avoid carry-over.

This is also of importance when changing from conventional to **organic products**. Using specific vessels and devices only for organic samples ("organic line lab ware") might help to reduce the risk of contaminating organic samples.

All **cleaners and materials** should be checked for possibly critical ingredients such as hypochlorites or quarterny ammonia compounds (QACs) like benzalkonium chloride. Tissues can be a source for contamination (f.ex. with 2-phenylphenole) as well as gloves, which should be free from critical compounds, especially compounds releasing CS_2. Last but not least, the contamination with nicotine by smoking staff (air, hands) should be taken into consideration.

3.13. Retain sample

For the conduction of the retain sample, several aspects have to be taken into consideration:

- **Homogenate**: In order to be able to prove that the analysis itself was correct, it is necessary to store a part of the homogenate frozen. The amount should be sufficient to be able to run confirmation analyses as well as re-analyses and further parameters when requested by the client.

  Furthermore, it can be helpful if the amount is high enough to send out an aliquot to another lab for a counter analysis if required.

- **Original retain sample**: It is meaningful to store a part of the unhomogenised material in order to be able to prove that the sample preparation as well as the analysis were carried out correctly. If possible, those sectors or halves which were not used for homogenisation shall be stored. Otherwise, whole units can be stored as well. In order to maintain a good quality, this part must be stored cooled.

- **Sample of the edible part**: For certain purposes (especially analysis of contaminants, toxicological evaluation), the preparation of a sample of the edible part is necessary, see 5.2.

  For this purpose, it is recommended to store some original sample or sample parts and prepare the “edible part” for analysis when required.

4. Results part II: Product-related aspects

In this chapter, the results related to the five investigated products are summarised and discussed. The entire results can be found anonymised in the annex.

General aspects which are not related to products (such as usage of water for removing dirt or handling of rotten and dry parts) are discussed in the general part (3.).

Aspects relating to groups of products can be found in the following chapter 5.

4.1. Spring onions

Spring onions, also known as green onions or scallions, are vegetables of various *Allium* onion species, see 4.1.3.

According to Annex I of regulation 396/2005 [1], the MRLs relate to the following product (Code No. 0220040):

"Immature bulbs with pseudostems, leaves and in some cases buds"

The ways the relana® labs handle spring onions is mainly comparable and in line with these requirements.

Differences were observed concerning the following details:

4.1.1. Cutting off of roots

Most of the labs resp. lab technicians cut off the roots of spring onions prior to homogenisation and discarded them, see picture 10.

Nevertheless, some employees did not cut away the roots and therefore used them for homogenisation as well.

Following the product definition "Immature bulbs with pseudostems, leaves and in some cases buds" in Annex I [1], roots are not included in the material to be analysed. The recommendation of the GDCh working group "pesticides" [6] to cut away the roots is in line with this.

Furthermore, the amount of flesh cut-away together with the roots varies between the labs and sometimes also within one lab.

Recommendation:

Following the provision given by Annex I, roots should be cut away sharply and discarded. It should be taken appropriate care that the amount of cut-away flesh is minimised as this is part of the product definition.

4.1.2. Selection of parts

Concerning the selection of parts, the labs follow different approaches:
- cutting all spring onions into parts, mixing them, then taking parts for homogenisation and retain sample;
- selecting spring onions from each bundle and retain whole spring onions;
- vertical cutting, taking alternating halves for homogenisation and retain sample;
- horizontal cutting, taking alternating halves for homogenisation and retain sample.

Recommendation:

As discussed in the general part (3.9.), the application of the sector technique seems favourable. As this may not be practical, cutting the spring onions vertically and using alternating pieces for homogenisation is recommended.

Picture 10: Spring onions with cut-off roots

4.1.3. Additional hint

Due to similar appearances and similar names especially in the German language, it is possible to confuse spring onions with leek, see the following table 4 and picture 11:

Code number*	0220040	0270060
Botanical name*	*Allium cepa* Common Onion group *Allium fistulosum*	*Allium ampeloprasum ampeloprasum* Pearl-Onion Group; syn: *Allium porrum* var. *sectivum*
English	spring onions green onions scallions	leek
German	Frühlingszwiebeln, **Lauch**zwiebeln	**Lauch**, Porree

*according to Annex I Reg. 396/2005 [1]

Table 4: Naming of spring onions and leek

Picture 11: comparison between spring onions (scallions) and leek [11]

Although the vegetables look similar and belong to the same botanical family, they belong to different species and are listed at different groups of Annex I, as shown in table 4.

General Recommendation:

Knowledge about the identification of foodstuff and their parts should be included in trainings for personnel of the sample reception as well as of the sample preparation department.

4.2. Zucchini

Zucchini, also named Courgette, belong to the group of "cucurbits with edible peel" and form the zucchini group among *Cucurbita pepo*. As the Latin name shows, they are closely related to other squashes resp. pumpkins.

The product definition according to Annex I [1] is:

"Whole product after removal of stems"

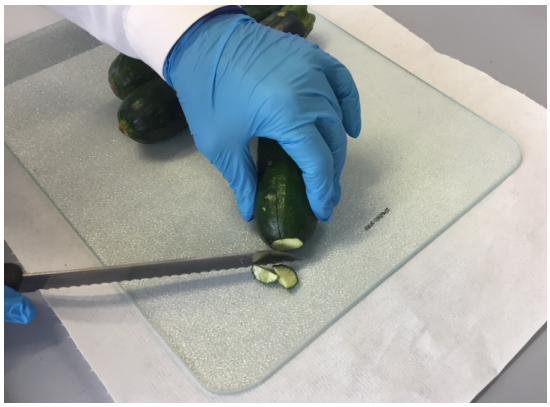
The handling of zucchini was quite comparable among the relana® labs, nevertheless some differences occurred concerning the following details.

4.2.1. Cutting of stems

The way labs interpreted the product definition differed in two ways:

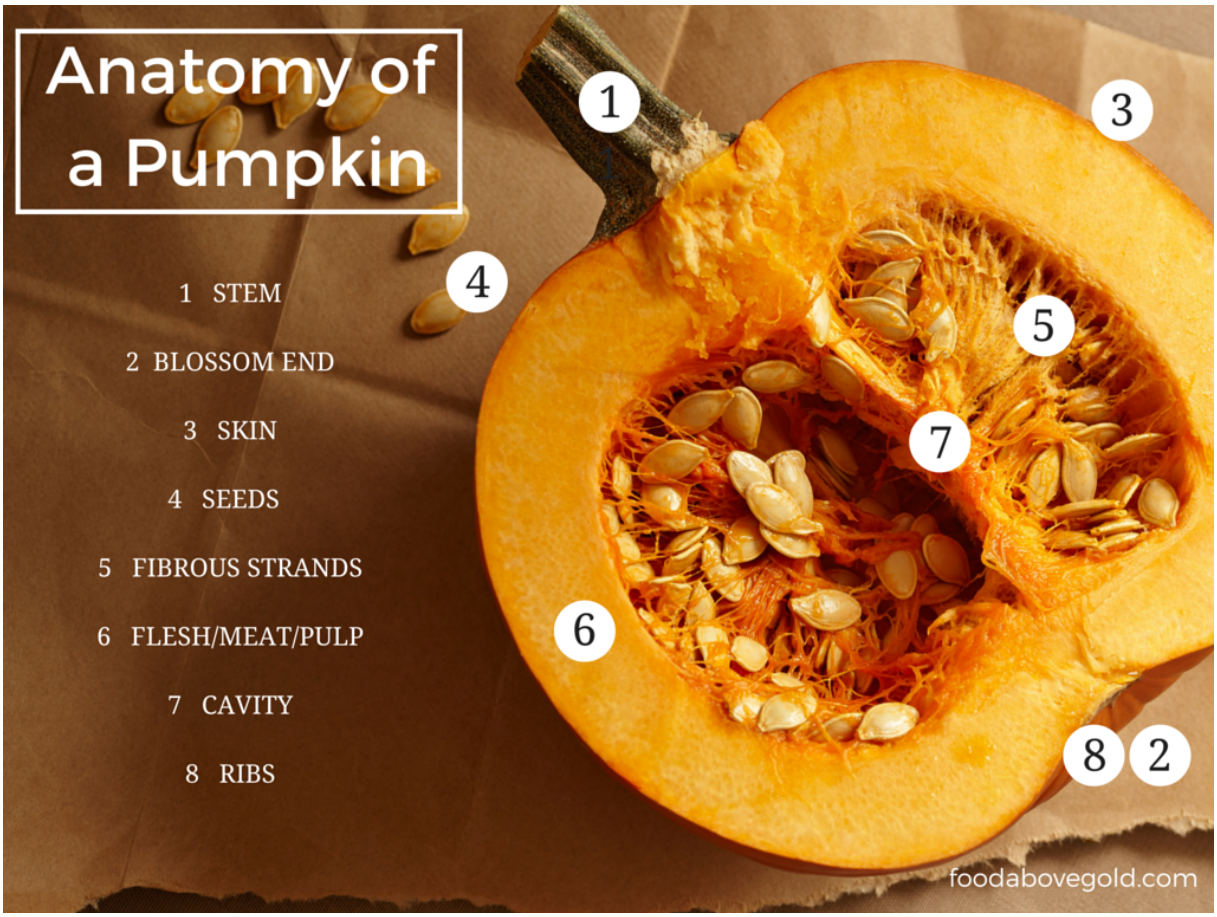
- Some lab technicians cut away the top and bottom end (picture 13), some only the top end (picture 12). This did not only differ between labs, but also within some labs.

Picture 12. Halved zucchini with cut-off stem



Picture 13. Zucchini with cut-off bottom-end

Following the product definition, only the stems shall be removed. Zucchini are berries form a botanical perspective [12]. As picture 14 shows for the pumpkin family, to which zucchini belong, the bottom part of squashes is named “blossom end” (no. 2 in picture 14) and may not be called “stem” (no. 1 in picture 14).



Picture 14: Squash anatomy [13]

- As with spring onions, the amount of cut-away flesh varies, some labs even seem to let some part of the stem stay on, see picture 15.

Picture 15: Zucchini with partly removed stem

Recommendation:

Following botanical literature, the bottom end of zucchini is named "blossom end" and is not part of the stem. Therefore, the bottom end shall not be cut away. Additionally, the stem shall be removed completely, but without removing too much flesh.

4.2.2. Selection of parts

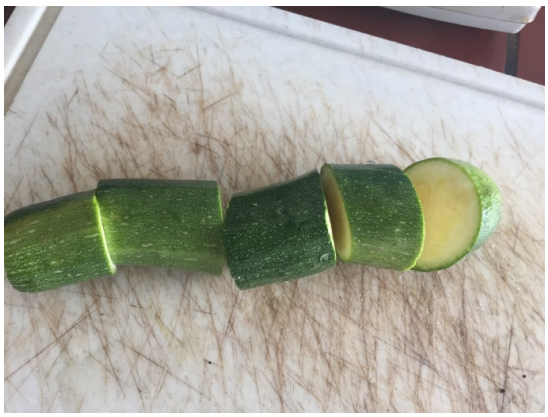
The technique of selecting parts for homogenisation and retain sample varies, the most common techniques are:

- sector technique (horizontal cutting into 4 sectors, opposite sectors are used for homogenisation resp. creating the retain sample) (picture 16);
- cutting zucchini into halves horizontally, using one half for homogenisation and the other to create the retain sample (picture 17);
- cutting zucchini into halves vertically, alternatingly using one half for homogenisation and the other to create the retain sample (picture 18);
- cutting zucchini into 5 parts and using the parts 1, 3 and 5 for homogenisation (picture 19).

Picture 16: Zucchini cut with sector technique

Picture 17: Zucchini cut into halves horizontally

Picture 18: Zucchini cut into halves vertically



Picture 19: Zucchini cut into 5 parts

Furthermore, some labs store parts of each zucchini as a retain sample, while other store whole zucchini.

Advantages and shortcomings:

- the sector technique may gain a representative sample, but it is not that easy to hit the middle axis of each zucchini.
- cutting into halves vertically and alternatingly use halves for homogenisation and retain sample should also give representative results. Exception: single samples highly charged with systemic pesticides (which should be more concentrated in the top half) may adulterate the result.
- using 3 out of 5 pieces: This technique is recommended by the CVUA Stuttgart [7]. Nevertheless, a risk remains for the detection of elevated pesticide levels, as systemic active substances will enter the fruit through the stem and concentrate along the middle tube. Therefore, elevated levels can be expected in both the top and the bottom piece.

Additionally, contact pesticides may be washed towards the blossom end, as zucchini usually points downwards when still attached to the stem, see picture 20. When always picking both end pieces, you are likely to pick higher pesticide levels than the median level.

- Concerning the preparation of a retain sample see general discussion in chapter 3.13.

Picture 20: Zucchini plant [14]

Recommendation

Generally speaking, the sector technique is the most favourable one as long as the cuts can be carried out along the middle axis. Otherwise, halving the samples and alternatingly using the halves for homogenisation seems to be also favourable.

4.3. Paprika

The naming of *Capsicum* species can be a bit confusing, in this project the following vegetable is considered:

Paprika, bell pepper, sweet pepper (mild varieties of *Capsicum annuum)*

The product definition according to Annex I [1] is:

"Whole product after removal of stems"

The general handling was comparable among the relana® labs, but concerning the following steps some variations occurred:

4.3.1. Cutting away of stems

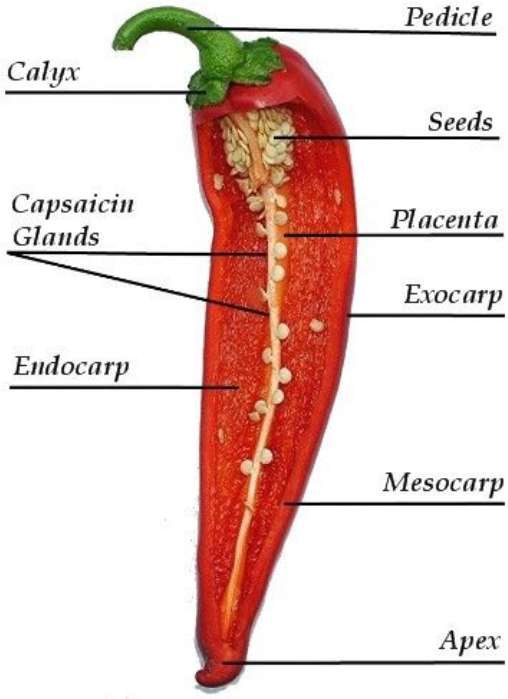
The way the lab technicians cut away the stems varies to a high degree:

- Some cut away the stem very carefully, taking care not to cut away too much flesh and white parts (placenta) (picture 21).
- Other cut away the stem more roughly, thereby removing some fruit flesh, parts of the placenta and ribs (septa) and kernels (picture 22).

Picture 21: Paprika with carefully removed stems

Picture 22: Roughly removed stem of paprika

Picture 23: Paprika with removed stem, leaving on the calyx, cut into quarters



Picture 24: Anatomy of *Capsicum* species [15]

One lab chose another variation by keeping the calyx (picture 23).

Following the botanical structure of *Capsicum* species (see picture 24), the procedure (picture 23) is acceptable, as the stem does NOT include the calyx.

Recommendation

Sticking close to phytology, the stem does not include the calyx, therefore cutting away the stem ONLY as shown in picture 23 is acceptable.

In any case, flesh, ribs, kernels and other fruit parts do not have to be cut away during the sample preparation, as these parts are covered by the product definition according to Annex I.

Picture 25: Removal of some kernels

4.3.2. Removal of ribs and kernels

In some labs, some kernels and ribs were removed as well (see picture 25).

Recommendation:

According to Annex I, only the stem shall be removed, consequently kernels and ribs shall be part of the homogenised sample.

4.3.3. Selection of parts

Most laboratories apply the sector technique for preparation of paprika, taking opposite quarters for homogenisation and retain sample, respectively (see picture 23).

Some labs use halves or even whole fruits instead of quarters.

As the pesticide content may vary between items and also between two sides of the same fruit (depending on the used technique for pesticide application), the sector technique seems favourable in order to get both a representative sample as well as a representative retain sample.

This topic must be discussed within the context of the general question of representative sampling and creating retain samples, therefore see additional discussion in chapter 3.9.

Recommendation:

It is recommended to apply the sector technique in order to gain a representative sample for homogenisation as well as a representative retain sample.

4.3.4. Retain of stems

In some labs, the stem is retained (picture 26), sometimes on request of clients.

Recommendation

If it is necessary to retain parts of samples which do not belong to the product definition according to Annex I, it is recommended to store them separately from the retain sample, in order to avoid cross contamination.

Picture 26: Retained stem stored with retain sample

Picture 27: Paprika cut directly into blender

4.3.5. Cutting into blender

Some lab technicians cut samples directly into the mixer, thereby touching parts of the blender with the paprika stem, see picture 27. The stem is not part of the product definition, but may contain elevated pesticide levels as systemic compounds enter the fruit via the stem. As a consequence, a certain risk for cross-contamination cannot be excluded at this step.

Recommendation:

If samples are cut directly into the blender, sample parts which do not belong to the product definition should be cut away prior to getting in contact with the blender, as cross-contamination may occur otherwise.

4.4. Strawberries

Strawberries (*Fragaria x ananassa*) are very popular among consumers especially in early summer, but there are some details in sample preparation to "get hung about".

Annex I [1] defines the product as follows:

"Whole product after removal of caps, crown and stems"

The wording applied in the regulation 396/2005 [1] may not necessarily be compatible with botanical wording, which makes the application of Annex I definitions a bit more tricky.

As expected, the handling of strawberries was comparable among the relana® labs, nevertheless some differences were observed.

4.4.1. Stem and calyx

In accordance with Annex I, all labs cut off the green part (stem and calyx, "crown") of the strawberries, nevertheless the amount of flesh which was cut off as well varies, sometimes even within one lab. As the pictures 28 and 29 show, the amount of cut-away flesh varies between neglectable and significant.

Picture 28: Strawberry with carefully cut-off stem and calyx

Picture 28: Roughly cut-off stem and calyx of strawberries, with attached flesh

Recommendation:

According to Annex I, only "stems, caps and crowns" shall be removed, consequently it should be taken care that the loss of flesh is minimised.

4.4.2. Selection of fruits, usage of parts

The selection of parts for homogenisation and retain sample varies as well:

- Some labs take whole strawberries from each package and keep the remaining as retain sample. The strawberries are homogenised in total.
- Some labs cut strawberries into halves (picture 30) and use one part of each fruit for homogenisation and one for the retain sample.
- Other labs apply the sector technique (picture 31), one employee uses it on every fruit even in case of large sample amounts.
- Some labs keep original packages as retain samples, while other open every package in order to get a representative sample.

Picture 31: Strawberry cut into halves

Picture 31: Sector technique applied on strawberries

Recommendation:

In order to get a representative sample, it is favourable to take strawberries from every package, which is delivered to the lab. The application of the sector technique is not recommanded. If necessary, cutting into halves should be sufficient for small fruits with a common unit weight < 25 g. In any case, the entire fruits should be taken for homogenisation.

4.5. Mango

Mangoes (*Mangifera indica)* are popular exotic fruits with a comparatively large kernel (stone).

Annex I [1] defines the product as follows:

“Whole product after removal of stems”

The mentioned kernel creates some challenges for the sample preparation – and the calculation of results.

4.5.1. Treatment of kernel

All audited labs agreed that the kernel is part of the product definition, but cannot be homogenised due its constitution, therefore it is not part of the analysed homogenate.

The way the labs transferred this agreement into practise differs:

- Some labs cut away the kernel and weighed it in order to consider the weight when calculating the results (picture 32); the flesh is cut away from the kernel as good as possible and used for homogenisation.
- One lab does not weigh the kernels but relies on literature data for their weight.
- Some labs put the kernels into waste without taking its weight into account (picture 33).
- One lab cuts a middle slice and discards it (picture 34).
- One lab determines the weight of the kernels and stems (picture 35).

Picture 33: Isolated mango kernel is discarded

Picture 34: Mango cut into three slices

Picture 32: Isolated mango kernels are weighed

As agreed by all labs, the kernel is part of the product definition. As it cannot be homogenised, it is recommended to cut away as much flesh from the kernel as possible in lab routine and use all flesh (with peel) for homogenisation.

The EU directive 2002/63 [3] states concerning the treatment of stones: "For example, the stones of stone fruit are not analysed but the residue level is calculated assuming that they are included but contain no residue."

Determining the exact weight of the isolated stones by weighing it and considering it during result calculation seems meaningful and is also recommended in literature [6], as the flesh to kernel weight ratio varies: Large mangoes usually have a higher flesh to kernel ratio than small ones, also variations due to harvest, weather conditions, varieties (think of so-called "wild mangoes") etc. have to be considered. Using data from literature or own experience therefore may lead to a misestimation of the kernel weight and a systemic error in pesticide results.

Picture 35: Isolated mango kernels are weighed together with stems

N.B.: *"In order to be able to carry out a proper calculation, it is necessary to weight the mangoes without stems prior to any further cutting steps, preferable on the same scale as used for weighing the stones. It is recommended to design templates in order to facilitate the handling of the data (if not available yet)."*

This handling is recommended by the AG Pestizide (working group pesticides [6]) and CVUA Stuttgart [7]. It should be noted that this treatment neglects any pesticide content in the kernels, although it is likely that the kernels contain at least some fat-soluble systemic compounds.

Using only the flesh and peel for analysis without taking into account the kernel weight leads to a significant overestimation of pesticide contents, as the kernel´s pesticide content is set to zero, following the named EU directive [3].

As the stem is not part of the product definition according to Annex I, it should not be weighed together with the kernels, although the effect is quite small.

Recommendation:

It is recommended to weight the mangoes without stems, then isolate the kernel by removing as much fruit flesh as possible in lab routine. The isolated kernels shall be weighed, and the weight shall be taken into account when calculating results. The use of literature or empiric data is also a (common) possiblility. However, as the flesh to kernel ratio can vary from sample to sample, this approach might not be appropriate in every case. Additionally, raw mango might be homogenised with the kernel as the kernel only becomes hard during ripening.

The use of templates / formulars in lab routine is recommended.

4.5.2. Treatment of flesh and peel

As discussed above, it is necessary to retain as much flesh as possible when cutting away the kernel.

Recommendation: see 4.5.1.

4.5.3. Removal of stickers and labels

The removal of attached labels, as necessary prior homogenisation, can be carried out in two ways:
- from hand by peeling it off, with the disadvantage that residues from the adhesive may stick to the peel and enter to homogenate;
- by cutting it off (picture 36), thereby removing some peel which is part of the product definition.

Picture 36: Label cut away from mango
Recommendation

As it is unlikely that the adhesives leave significant amounts of relevant compounds on the surface of the peel, it is recommended to remove stickers by hand if possible.

4.5.4. Cutting away of stems

The way the lab technicians cut away the stems vary to some degree:

- Some cut away the stem very carefully, taking care not to cut away too much flesh and peel (picture 37).
- Other cut away the stem more roughly, thereby removing some fruit flesh and peel (picture 38).

Picture 37: Carefully removed stem

Picture 38: Roughly removed stem

Recommendation:

According to Annex I, only the stems shall be removed, consequently it should be taken care that the loss of flesh and peel is minimised.

4.5.5. Selection of parts

The selection of parts and their handling varies between the labs:

- In case of larger sample quantities, some labs try to select mangoes in order to gain a representative sample, while others take parts of every fruit.
- Some labs use whole mangoes (without stem and kernel) for homogenisation.

- Some labs cut mangoes into slices and use one half for homogenisation and one for the retain sample.
- An employee from one lab cuts the mangoes into three slices, using the middle slice (with the kernel) as the retain sample (see picture 33 above), while the other employee makes use of the sector technique.
- Some labs also homogenise the edible part (flesh without peel) as well (picture 39), as maximum levels for contaminants (e.g. heavy metals) as well as toxicological evaluations (e.g. exhaustion of ARfD values) relate to the edible part.

Picture 39: Isolation of edible portion (fruit flesh)

Recommendation:

As the pesticide load may vary between single fruits, it is recommended to take parts from every fruit, if possible. Applying the sector technique seems more favourable than taking halves of every fruit, see discussion in chapter 3.9.

Storing the middle segment may lead to differing results as systemic compounds may accumulate along the middle tube, while contact pesticides may distribute irregularly on the surface.

5. Aspects related to product groups

5.1. Products with inhomogenisable parts (e.g. stones)

Certain products contain parts that belong to the product definition fixed in Annex I of regulation 396/2005 but cannot be homogenised with routine techniques, such as hard stones and kernels (cf. 4.5. mango).

In this case it is recommended to carry out the following steps:

- cut away pieces not belonging to the product definition according to annex I (such as stems of cherries and mangoes), remove stickers where applicable
- measure and document the weight of the whole fruits
- cut out the stones, measure and document the weight of the stones,
- homogenise according to agreed rules,
- analyse the sample,
- consider the weight of the stones when calculating the results.

Alternativly, specific techiques might be applied to homogenise the entire fruits with kernels resp. stones, f.ex. cryo-milling using liquid nitrogen.

5.2. Products with inedible parts: Preparation for the analysis of the edible part

While some products such as cucumbers or raspberries can be eaten in total, many contain inedible parts which are nevertheless part of the product definition in Annex I [1], for example bananas and kiwis (peel), mangoes (peel and stones) and cherries (stones).

For some products, the definition of “edible” is discussable, for example oranges (peel can be used for flavouring and processed to candied orange peel), grapes (with or without seeds?) or potatoes (although potatoes are used peeled in most cases, some varieties can be eaten in total). Here it is recommended to consider that part of the product as “edible”, which is usually consumed (oranges without peel, etc.).

The analysis of the edible part can be required in the following cases:

- analysis for contaminants (heavy metals, mycotoxins, nitrate, etc.), as the maximum levels fixed in Regulation 1881/2006 [16] usually relate to the edible part (see Art. 1 (2))
- calculation of toxicological parameters such as ARfD: As typical measures such as “mg/kg bodyweight” indicate, they relate to the intake of substances and must therefore be related to the edible part of the sample.

It is recommended to document which part has been considered as “edible” and how it is obtained.

At this step, special care must be taken to avoid cross-contamination, as the peel usually contains higher pesticide levels as the fruit flesh: contact pesticides are applied on the surface of the product. Surface treatments especially for exotic fruits such as citrus fruits, mangoes or bananas can be present there as well. Therefore, it might be meaningful to use new single-use gloves after cutting off the shell.

5.3. Products with vegetable parts, which have to be cut away prior homogenisation

In case of some products, certain parts like f. ex. the stem, leaves or roots have to be cut away prior to analysis, as described in Annex I [1]. Examples include strawberries (removal of caps, crown and stems), pineapples (removal of crowns) or tomatoes (removal of stems).

These parts should be carefully cut away in order to avoid any losses of parts belonging to the product definition such as flesh or seeds is minimised.

Parts, which do not belong to the residue definition (such as paprika stems) can be discarded. If they are to be stored for some reason, this should be done separately from the “normal” retain sample, in order to avoid any cross contamination.

It is also recommended to remove all parts which are not covered by the MRL (maximum residue level) definition (Annex I of EU Regulation 396/2005) prior to physical contact with the mixer, in order to avoid cross-contamination.

6. Conclusions and recommendations for practical work

6.1. General recommendations

The sample preparation is a crucial step to obtain representative results. However, it might not get the necessary attention in every case. Therefore, special care should be taken into consideration in particular with regard to the following issues:

Usage of standards and norms (3.1.)

As described above, laws, standards, and norms do not go far enough into detail regarding the preparation of food samples prior to homogenisation. Therefore, it is advisable to conduct Standard Operation Procedures (SOP), which fix the most important general aspects as well as details for single products.

Availability of information (3.2.)

It is recommended to make detailed information available to the technical personnel. The information should be easy and fast to find and understand.

Training of staff (3.3.)

It is recommended to train personnel on the crucial issues. Possible language barriers should be kept in mind. Supervising and testing this step on a time-to-time basis may be meaningful as well.

Validation and quality control (3.4.)

Sample preparation shall be included in validation and quality control measures.

Sample quantity (3.5.)

The sample quantities arriving in the laboratory should meet the requirements described in Table 4 of directive 2002/63 [3], see also table 2 of this document. In case sample quantities do not match the required amount, clear procedures should be fixed by a SOP.

In case of **larger sample quantities**, the selection of the samples to be analysed should be fixed: How is the selection carried out (Picking out single items from every package? Taking the first kilogram?)? If possible, it is recommended to take samples from every package in order to gain high representability.

In case of **smaller quantities** not covering the amount required by directive 2002/63 [3], it might be necessary to contact the client and agree on the next steps. Too low quantities must be mentioned in the analysis report.

Dirt and soil (3.6.)

Shall be removed before analysis, as they are not part of the product definition according to Annex I. Nevertheless, the use of water and wet tissues is not suitable as pesticide compounds sticking to the surface may be washed off (except if otherwise mentioned in Annex 1 of reg. (EC) no. 396/2005 related to a specific commodity). The careful use of dry tissues or brushes is strongly recommended.

Rotten pieces and parts (3.7.)

Rotten pieces and parts shall NOT be discarded resp. cut off. If a certain amount or number of rotten parts resp. pieces are present in a sample, this might be indicated in the test report as an additional information.

Wilted leaves and other parts (3.8.)

Wilted / decayed pieces and/or parts shall NOT be discarded resp. cut off. If a certain amount or number of wilted or decayed parts resp. pieces are present in a sample, this might be indicated in the test report as an additional information.

Selection of parts, reduction of sample size (3.9.)

For items <25 g it is recommended to use the products in total or to cut them into halves, if necessary (to achieve a better homogenisation). In any case, the entire fruits should be taken for homogenisation.

For items > 25 g the sector technique is favourable. Long, thin products may also be cut into halves vertically, which are used alternatingly for homogenisation.

Use of dry ice / liquid nitrogen (3.10.)

In the following cases, the use of dry ice / liquid nitrogen during blending is recommended:

- significant increase of temperature during homogenisation,
- analysis of heat-sensitive analytes,
- to get a better homogenate of some demanding products like f.ex. fresh herbs, lettuce, leaves, flowers, spinach etc.

Preparation for the analysis of dithiocarbamates (3.11.1.)

Dithiocarbamates (DTC) are fragile compounds and degrade fast after the disintegration of cell walls. Therefore, it is necessary to separate a representative part of the sample PRIOR the homogenisation.

Preparation for the analysis of fumigants (3.11.2.)

Fumigants are very volatile and will get lost in large amounts during homogenisation. Therefore, it is necessary to separate a representative part of the sample BEFORE homogenisation and use this part for the determination of fumigants.

Documentation (3.12.1.)

It is advisable to apply easy-to-use forms for the sample preparation step, thereby supporting internal quality as well as meeting external requirements (ISO 17025).

If possible, pictures from every sample should be taken in order to document the quality of the received samples including packaging and labelling.

Quality control: Check of homogenisation degree (3.12.2.)

For an effective extraction of pesticides, it is necessary to achieve a high degree of homogenisation during sample preparation. It is recommended to check the degree of homogenisation, for example by applying millimetre paper. A maximum particle size of 2 mm should be achieved.

Quality control: Maintenance and cleaning of devices, use of gloves and cleaners (3.12.3.)

Knives and blades used in blenders should be sharpened regularly, for example weekly or every second week (depending on the number of processed samples). Comprehensive cleaning of mixers, mills etc. is necessary after each product, the procedure should be documented. All applied cleaners and commodities (such as gloves) shall be checked for critical components.

Retain sample (3.13.)

For the conduction of the retain sample, several aspects should be considered. In order to prove the correct analysis, it is necessary to retain a frozen part of the homogenate.

In case the condition of the original sample shall be provable, it is meaningful to store some fresh products as well, preferably sectors or halves of the products used for homogenisation, as long as the amount of sample is sufficient.

Sample parts which do not belong to the product definition laid out in Annex I [1], but need to be retained, shall be stored separately.

6.2. Recommendations for special products

Products with inhomogenisable parts (e.g. stones)

In this case it is recommended to carry out the following steps:
- cut away pieces not covered by the MRL definition according to annex I (EU Regulation 396/2005), remove stickers where applicable,
- measure and document the weight of the entire fruits,
- cut out the stones, measure and document the weight of the stones,
- homogenise according to agreed rules,
- analyse the sample,
- consider the weight of the stones when calculating the results.

Alternativly, specific techiques might be applied to homogenise the entire fruits with kernels resp. stones, f.ex. cryo-milling using liquid nitrogen.

Products with inedible parts: Preparation for the analysis of the edible part

The analysis of the edible part can be required in order to determine contaminants and toxicological parameters, as they relate to the edible part.

At this step, special care must be taken to **avoid cross-contamination**.

Products with vegetable parts, which have to be cut away prior homogenisation

Relevant part should be carefully cut apart from the non-relevant parts. The loss of parts belonging to the product definition such as flesh or seeds is minimised.

Retain of parts which do not belong to the product definition

Parts, which do not belong to the residue definition (such as paprika stems), are stored separately from the “normal” retain sample, in order to avoid cross contamination.

Cutting directly into blender

It is recommended to remove all parts, which are not part of the residue definition (annex I of Regulation 396/2005) prior physical contact with the blender, in order to avoid cross-contamination.

7. Glossar

ARfD Acute Reference Dose

ASU Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (

DTC Dithiocarbamates

MRL Maximum Residue Level

SOP Standard Operating Procedure

8. Literature

[1] Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, OJ L 70/1 dated 16.3.2015, last amended by Commission Regulation (EU) 2018/78 of 16 January 2018, OJ L 14/6 dated 19.1.2018.

[2] Commission Regulation (EU) 2018/62 of 17 January 2018 replacing Annex I to Regulation (EC) No 396/2005 of the European Parliament and of the Council, OJ L 18/1 dated 23.1.2018.

[3] Commission Directive 2002/63/EC of 11 July 2002 establishing Community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC, OJ L 187/30 dated 16.7.2002.

[4] Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit – BVL): Official Collection of Methods of Analysis according to § 64 LFGB (Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB), L 00.00-7, December 2002, Beuth Verlag.

[5] European Commission, DG SANTE: Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, SANTE/11813/2017.

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[10] Spiegel online: Greenpeace über Pestizide - Drama um fast nichts, available online: http://www.spiegel.de/wissenschaft/natur/bild-1038987-847846.html, accessed on 21.02.2018.

[11] my-favthings.blogspot.com: How to Re-Grow Leeks, Scallions, Spring Onions and Fennel from Kitchen Scraps, available online: https://my-favthings.blogspot.com/2016/11/how-to-re-grow-leeks-scallions-spring.html, accessed on 02.02.2018.

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[13] Food Above Gold: How to buy & prepare pumpkins, available online: https://www.foodabovegold.com/buy-store-pumpkins-pumpkin-recipes/, accessed 05.02.2018.

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[15] Chilipflanzen.com: Chili-Schote Aufbau, available online: https://chilipflanzen.com/wp-content/uploads/2016/05/Chili-Schote-Aufbau.png, accessed 05.02.2018.

[16] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, OJ L 364/15 dated 20.12.2006, last amended by Commission Regulation (EU) 2017/1237 of 7 July 2017, OJ L 177/36 dated 8.7.2017.

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