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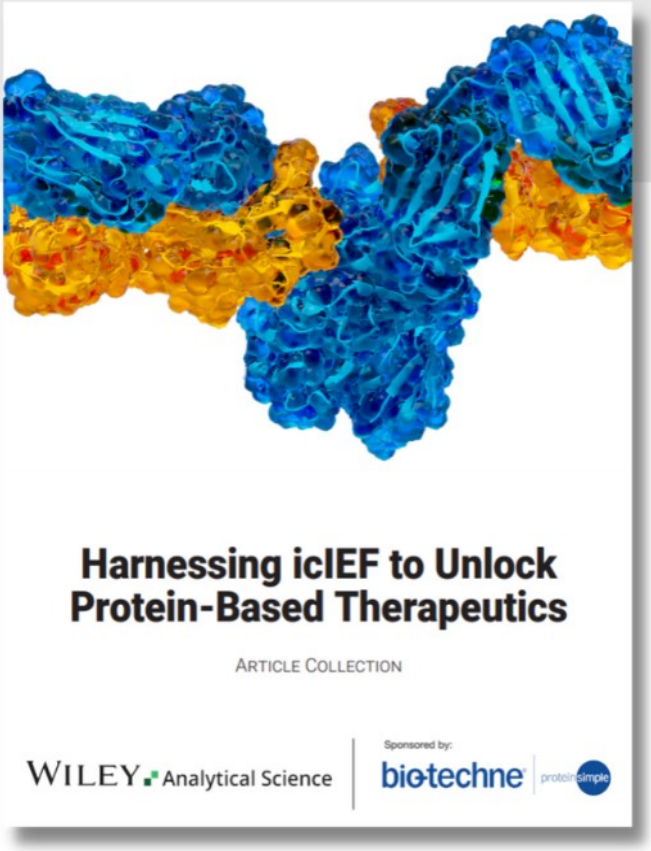

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Review

Food authentication in real life: How to link nontargeted approaches with routine analytics?

In times of increasing globalization and the resulting complexity of trade flows, securing food quality is an increasing challenge. The development of analytical methods for checking the integrity and, thus, the safety of food is one of the central questions for actors from science, politics, and industry. Targeted methods, for the detection of a few selected analytes, still play the most important role in routine analysis. In the past 5 years, nontargeted methods that do not aim at individual analytes but on analyte profiles that are as comprehensive as possible have increasingly come into focus. Instead of investigating individual chemical structures, data patterns are collected, evaluated and, depending on the problem, fed into databases that can be used for further nontargeted approaches. Alternatively, individual markers can be extracted and transferred to targeted methods. Such an approach requires (i) the availability of authentic reference material, (ii) the corresponding high-resolution laboratory infrastructure, and (iii) extensive expertise in processing and storing very large amounts of data. Probably due to the requirements mentioned above, only a few methods have really established themselves in routine analysis. This review article focuses on the establishment of nontargeted methods in routine laboratories. Challenges are summarized and possible solutions are presented.

Keywords:
Food authenticity / Food fraud / Food profiling / Nontargeted / Omics technologies
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1 Introduction

Ensuring the quality and safety of food has become increasingly important, particularly as a result of the numerous food scandals of recent years, but these are probably only the tip of the iceberg. However, food fraud is not new, but can be traced far back in human history to around 1700 BC [1]. With the onset of globalization, the food chain has become increasingly complex, making it more difficult to control unwanted changes in food and facilitating deliberate counterfeiting for profit. Often counterfeits are only noticed without concrete suspicion when clear indicators, such as health risks, appear. Moreover, falsifications are hardly noticeable to laymen and can only be proven with the help of complex analytical methods. In order to be able to adequately deal with this highly dynamic process, new analytical methods have to be continuously developed. Much of this development work is carried out by scientific research institutions, such as universities and various publicly funded institutions, in order to transfer the results later to governmental surveillance authorities or commercial laboratories. The research work focuses on issues such as proof of geographical origin, production process (e.g. organic vs nonorganic cultivation), age, storage method, detection of initially unknown additives and contaminants, as well as identity (species/variety) of food. The listed questions usually require complex and sophisticated analyzes. For this reason, nontargeted methods are becoming increasingly important in this area. In contrast to the targeted methods frequently used to date, in which only a few specific compounds are analyzed, nontargeted analyzes can be used to create entire molecular or elementary profiles, which, depending on the technique used, can comprise many thousands of elements, chemical compounds, or sequences [2].

Nontargeted methods are usually designed to capture individual cellular levels with different "omics" technologies. These can be the "classical" levels of the information flow from genotype to phenotype, which include "genomics," "proteomics," and "metabolomics," or extensions of this approach, such as the isotoplome or microbiome, as well as certain subsets for example the analysis of predominantly nonpolar compounds known as "lipidomics." Depending on the scientific issue and sample matrices, it may be useful to analyze one or more of these levels with different analytical platforms. An overview of the different omics approaches for the proof of food authenticity can be found in the literature [2–5]. This article is mainly about the establishment of these

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Abbreviations: **AFLP-PCR**, amplified fragment length polymorphism polymerase chain reaction; **IR**, infrared; **LESA**, liquid extraction surface analysis; **NGS**, next generation sequencing; **QC**, quality control; **SSR**, simple sequence repeats

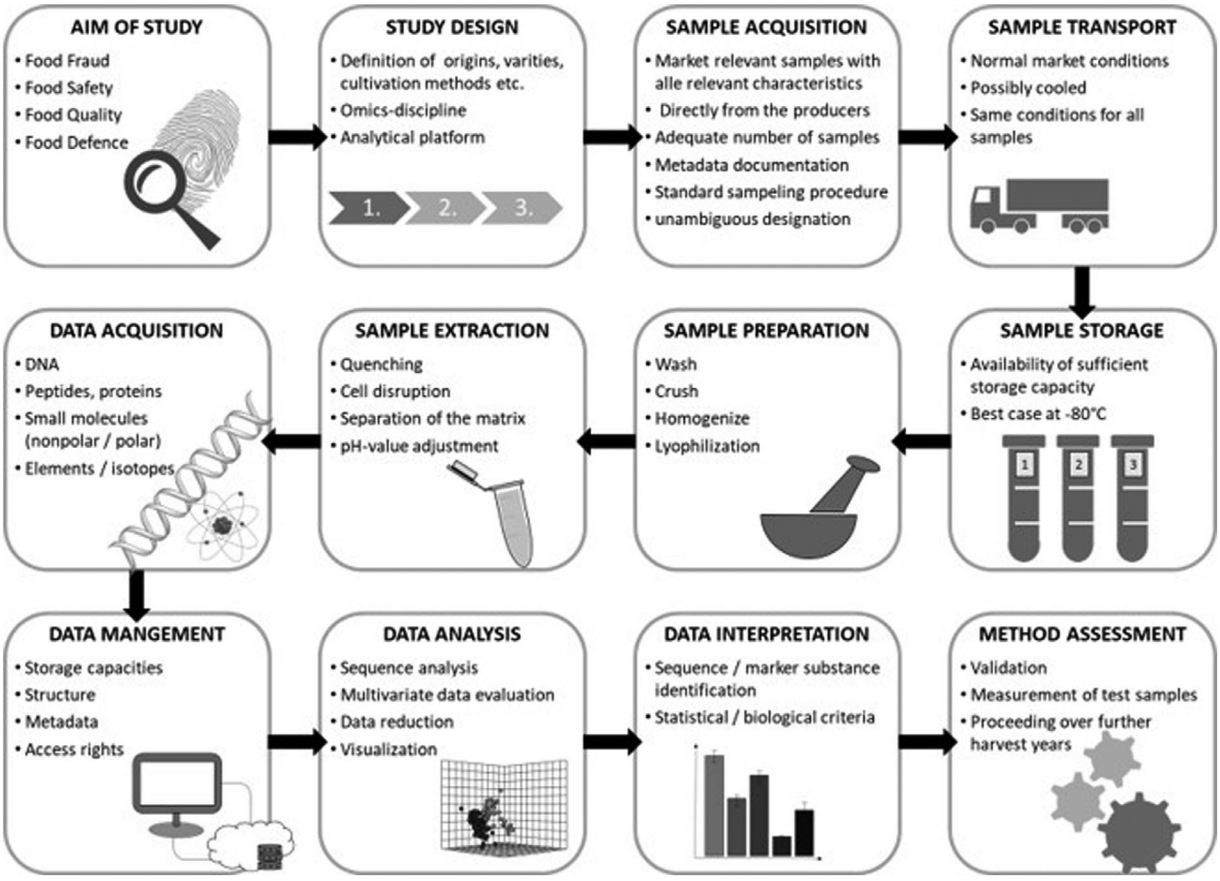

Figure 1. Schematic workflow of setting-up nontargeted methods.

strategies in routine analysis. Especially since research efforts in this area have increased massively in recent years and numerous studies have been published, but the proportion of nontargeted methods that have been adopted in routine applications is still comparatively low. On the one hand, this is due to the fact that so far only a few guidelines have been published in this area, such as a guideline from the United States Pharmacopeia [6], which, however, are often still too general for a direct transfer of the procedure, and on the other hand to the fact that nontargeted methods often require experts from various research areas such as food chemistry, biology, and bioinformatics. In addition, there are sometimes very high cost of installing, operating, and maintaining high-resolution platforms, such as mass spectrometer or next generation sequencing (NGS) devices, which also require suitably qualified personnel. With this article, we summarize the different results of recent research efforts and thus facilitate the routine application of nontargeted methods.

2 Workflow of nontargeted methods

Various requirements must be taken into account when setting up nontargeted methods. An exemplary workflow that can be applied to different scientific issues and analytical procedures is shown in Fig. 1.

2.1 Sample acquisition

First, an appropriate sampling has to be carried out, in order to build up a database in which unknown samples can later be included. The establishment of a reference sample collection initially involves the specification of representative quantities of authentic reference samples and it can be of varying complexity, depending on the scientific issue and the analytical approach chosen. For example, DNA-based strategies, as used for the determination of species or varieties, require only a single sample for reliable detection. This is the case, because DNA sequences are almost constant against exogenous factors, whereas the metabolome can be affected by different environmental influences such as soil, pesticides, or weather (Fig. 2). To cover these influences, however, many samples are required for a meaningful determination. Additionally, the influence of various exogenous factors on the sample composition also means that, despite the completion of the actual method development, reference samples must still be taken regularly in order to be able to validate such influences again and again. Particularly in times of increasing climate change,

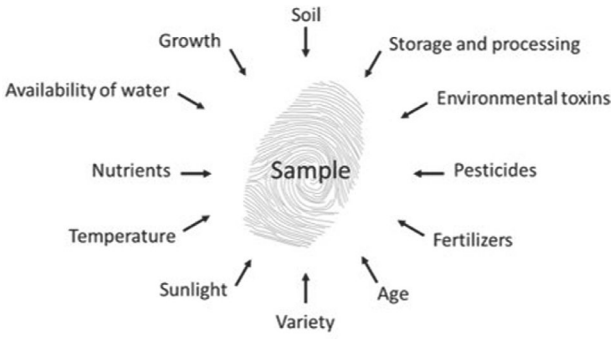

Figure 2. Various exogenous and endogenous parameters that can show an influence on the chemical and physical composition of the samples.

such influences cannot be clearly assessed, even though we have been able to demonstrate a constancy of metabolites in some projects, which have proven to be marker substances for the geographical origin over several years [7, 8]. In this respect, however, there are currently too few studies available, mainly conducted over a longer period, to be able to better assess such effects. The required number of samples also depends on the strategy for data evaluation and the number of sample groups, for example the number of relevant regions of origin of a foodstuff or the varieties, which should be covered as extensively as possible depending on their relevance [9]. In this way, an unknown sample can be clearly assigned to a group and misclassifications can be avoided. McGrath et al. recently conducted a literature review and recommend a minimum of 200 samples for each class using spectroscopic methods [10]. The required number of samples depends on the research question and is generally difficult to determine. For this reason, various algorithms have been developed. For example, a pilot test dataset can be used to calculate the optimal number of samples required. However, the number of samples is usually limited by their availability. The type of sampling depends on the nature of the sample. Although for homogeneous samples, a one-step procedure is usually enough, for heterogeneous samples a multistep procedure may be necessary to obtain a representative sample. The latter strategy is mainly carried out for large parties by taking individual samples at several points [9,11–14].

The collection of different metadata (clear description of the biological sample and its provenance) also depends on the research question and can help, if necessary, to consider different influencing factors and ensures the reusability and data sharing. As a starting point, the specifications of the *Metabolomics Standard Initiative* (MSI) are usually cited [15, 16]. In addition, further considerations have been made in recent years, such as *the Minimum Information for Biological and Biomedical Investigations project* (MIBBI) [17], the *Minimum Information about a Plant Phenotyping Experiment* (MIAPPE) [18], which offers a checklist for plant phenotyping experiments, the *Investigation, Study, Assay* (ISA) framework [19] or the *Genetic and Genomic Information System* (GnpIS) data repository [20]. However, despite these extensive efforts, there are still no uniform recognized standards in this respect [9].

We know from our own experience that the retrieval of metadata depends to a large extent on cooperation with the farmers and that in foreign regions there are sometimes language barriers. In almost all cases, however, we can report positive experiences, as the producers themselves have a considerable interest in protecting their own products against counterfeiting.

All together, these requirements are undoubtedly very high, especially since the samples should in the best case be taken directly from the manufacturer and are therefore associated with corresponding travel and costs [10]. Such demands are hardly manageable for a single institution and can only be ensured by increased cooperation between different stakeholders (science, food producers, government surveillance, commercial laboratories). Samples purchased through third parties or retailers should be avoided as far as possible, as authenticity cannot be guaranteed, and the reliability of reference samples is crucial.

2.2 Sample preparation and storage

Sample preparation starting with harvesting, quenching, homogenization, and storage of the samples can have a decisive influence on the outcome of the analysis. As shown above, DNA is relatively stable, as are the elemental and isotopic profiles, while the metabolome and proteome can undergo major changes if sample preparation and storage are not carried out correctly. These steps require careful planning in order to largely rule out possible variances. For this reason, especially in the metabolomics and proteomics research area, very complex requirements with high scientific standards can be found in the literature, especially about the harvesting process [21–23]. However, for food authentication it is more relevant to obtain representative market samples, as they are usually purchased by the consumers. Nevertheless, the samples should ideally be shock frozen with liquid nitrogen as soon as possible in order to largely prevent potential changes such as enzymatic degradation processes. When the samples are frozen with liquid nitrogen at −196°C, only small water crystals are formed, which damage the plant cells comparatively less than larger crystal structures, which arise primarily during slow freezing processes. In this way, the contact of enzymes with metabolic compounds is minimized and degenerative metabolic processes are prevented [24]. However, due to the onsite infrastructure, it is not always possible to shock freeze the samples in liquid nitrogen and, at best, then to store them directly at −80°C. An alternative is to first transport the samples to an appropriate facility at the usual market conditions and to carry out the necessary steps there, for example by transporting the samples with the help of cool boxes or, in case of a high sample volume, with special refrigerated transport vehicles. If it is necessary to send samples over longer distances, special cool boxes are also suitable, possibly also using dry ice. Regional country-specific

regulations must be followed when handling dry ice. In this context, food products, such as nuts or cereals, which are usually stored dry and at room temperature, are much easier to handle, as no special measures have to be taken. It should also be noted that customs or other regional export regulations may have to be taken into account and should be clarified in advance. In some of our own projects, we sent employees to the corresponding countries to take the samples directly onsite. Part of the sampling was carried out there with the help of a refrigerated truck in order to be able to transport the samples over a longer period at market conditions [7, 25]. In other projects, we have worked with importers and food producers who themselves have a strong interest in protecting their own products and sent the samples to us cooled by express delivery in order to keep the cost benefit ratio within reasonable limits [8].

Depending on the type of samples, it may be necessary to wash them first, this is particularly the case with very dirty samples that have grown close to or in the ground. For reproducible analysis, it is generally advisable to clean the samples with ultrapure water. Sometimes it can also be useful to analyze the adhering soil residues, for example about the element profile, as the soil profile is also partly reflected in plant matrices [26].

In order to obtain a representative sample, homogenization and comminution of the sample are generally unavoidable. Depending on the matrix and size, it is suitable either to grind the samples with a mortar and pestle, sometimes with the addition of nitrogen or dry ice, or with the aid of various mills (e.g. rotor mills, knife mills, ball mills), which have the advantage of simplifying the procedure and reducing the time required. If element profiles are to be analyzed, care should be taken to ensure that no other elements are added to the sample and ceramic knives, for example should be used. In addition, strong shear forces or warming the sample during processing, can lead to chemical changes of the analytes, which can have negative effects especially when DNA or protein sequences are to be analyzed and should therefore be carefully evaluated first. For many nontargeted methods often, only a few milligrams of a sample are used for the actual analysis. In these cases, it may be useful to analyze replicates in order to estimate the sample homogeneity [21, 27].

If the samples are to be stored for a longer period, freeze drying can be useful. On the one hand, the spatial capacities required for setting up a sample database can be reduced, and on the other hand, sample extraction can be simplified. At the same time, water removal ensures that enzymatic reactions are forced back as far as possible and the process contributes to the comparability of the samples. For certain techniques, such as NMR spectroscopy, this step also ensures that water signals do not negatively influence the measurements. For longer storage periods, it is advisable to store the samples as aliquots at temperatures of −80°C, so that the material does not have to be frozen and thawed several times [21].

2.3 Sample extraction

While targeted processes often require a very complex and specific extraction process, the sample extraction for nontargeted analyzes are usually kept as simple as possible. The extraction procedure is a critical step in performing nontargeted analyzes to achieve the best possible and reproducible analyte coverage and should therefore be carefully planned. For this reason, numerous studies have been published in the last few years for the different omics technologies [28–30]. Usually all processes have in common that a cell disruption is carried out first. Cell disruption can be done physically (mechanically or thermally) or chemically. Subsequently, undesired matrix components are separated off and, if necessary, a concentration or a dilution step must then be performed. Simple liquid–liquid extractions together with various cell disruption methods, such as ultrasonic decomposition, ball milling, or opening of the cell wall using enzymes, are often suitable prior to a successful sample extraction. For a further isolation or enrichment that is sometimes necessary, various chromatographic or physical processes, such as SPE, can then be used, which take advantage of the different properties of the analytes. The analysis of element profiles requires a complete removal of the matrix, which can be achieved through the use of microwave-assisted digestion and acids [31]. In some cases, there are also specific guidelines for selected analytes, such as instructions from the *International Organization for Standardization* (ISO 21 571:2005) for extracting DNA from food, which can also be used for nontargeted genomics approaches.

The selection of an appropriate procedure in routine analysis depends primarily on the time and costs. In particular, the use of new technical developments can reduce the overall turnaround time and, thus, also the personnel expenditure to a minimum. One option, for example is the use of laser systems to destroy the organic material, to transfer the samples directly into the gas phase and then to introduce them into the analytical system. While such developments are becoming increasingly important in the clinical field, they are still relatively insignificant for the analysis of food. However, it can be assumed that their importance will increase in the coming years. Laser systems working with different wavelengths can be used to ablate large biomolecules, such as DNA or proteins, as well as smaller metabolites and elements [32, 33]. Some analytical platforms also enable the direct nontargeted measurement of food samples without any extraction, for example by means of infrared (IR) spectroscopy [34] (near IR [NIR] [35], mid IR [36]), Raman spectroscopy [37]) as well as different ionization sources for MS: direct analysis in real time (DART) [38], desorption electrospray ionization (DESI), rapid evaporative ionization mass spectrometry (REIMS) [39], liquid extraction surface analysis (LESA) [40], or laser ablation electrospray ionization (LAESI). However, the analytical information content (i.e. mainly the resolution) on these analytical platforms is often less meaningful, but for some questions this may already be enough.

Table 1. Tabular overview of application fields and analytical approaches of the different omics technologies

Omics technologies	Application fields	Analytical approaches
Genomics	Biological identity, detection of GMOs and microorganisms, tracking of biological contaminations	Sequencing: chain-termination method (Sanger sequencing), NGS Fingerprinting: RAPD, STR, AFLP-PCR Directed methods: PCR-techniques (e.g. EPIC, LPA), isothermal amplification techniques (e.g. LAMP, RPA)
Proteomics	Biological identity, chemical identity, storage and production processes, geographical origin	Pattern analyzes: 1D or 2D electrophoresis Mass determination: bottom-up, middle-down or top-down strategies; MALDI-MS, LC-MS
Metabolomics	Biological identity, chemical identity, storage and production processes, geographical origin	Volatile metabolome: 1D- or 2D-GC-MS Nonvolatile metabolome: LC-MS, IR- and Raman spectroscopy, NMR, hyphenated techniques (LC × LC, LC × GC) with different analyzers
Metallomics/ Isotopolomics	Chemical identity, production processes, geographical origin	Single element techniques: F-AAS, F-AES Multielement techniques: IRMS, ICP-MS; ICP-OES

F-AAS, flame atomic absorption spectrometry; F-AES, flame atomic emission spectrophotometry; GMO, genetically modified organisms; ICP-OES, inductively coupled plasma-optical emission spectrometry; IRMS, isotope-ratio mass spectrometry; LPA, ligation-dependent probe amplification; LAMP, loop-mediated isothermal amplification; RPA, recombinase polymerase amplification; STR, short tandem repeat analysis.

Further possibilities to simplify the sample extraction are the implementation of automated procedures and the use of robotics, which are not only very fast and reproducible, but also sustainable [31]. While such approaches are rather unsuitable due to the lack of flexibility in the scientific field, they can achieve great added value in established procedures with a high degree of standardization in routine analysis. It can also be assumed that the devices will become more and more flexible in the course of development work, for example by installing several robot arms [41, 42].

2.4 Data recording and data management

For the generation of food profiles, different analytical platforms and approaches are available for the analysis of the different omics levels, which we and some other scientists have already discussed in the past. A compilation can be found in Table 1 [2–5].

Most of the analytical platforms require specially trained staff and are comparatively expensive to purchase and maintain, with the exception of vibrational spectroscopic techniques. In addition, these are usually very user friendly, fast, and nondestructive but also relatively limited in resolution. On the other hand, MS, NMR, or NGS platforms can be used to generate a significant depth of information. Furthermore, the different platforms provide differently reproducible results, which must be validated accordingly. So far, however, guidelines, standardization, and suitable reference materials for nontargeted method are lacking. Nevertheless, individual nontargeted methods have already been adopted by surveillance authorities as well as by commercial laboratories, and others are currently being planned to be established and will be proposed to the *European Committee for Standardization* (CEN) for a takeover [43, 44].

Nontargeted methods often generate very large amounts of data and quickly take up several gigabytes of storage space. Consequently, the acquisition of appropriate data storage capacities and the structuring of databases must be considered, also in order to share them with other users if necessary and to facilitate cooperation for everyone involved. The establishment of an interface for the internal laboratory information management system as well as quality assurance procedures should also be taken into consideration.

In recent years, various initiatives have been launched to facilitate and standardize data management as well as to enable sustainable and efficient use of the data. These include the FAIR principles (findable, accessible, interoperable, and reusable, https://www.go-fair.org/fair-principles/), which were expanded in 2019 to include the CARE principles (collective benefit, authority to control, responsibility, ethics) to take ethical principles into account. Another option is offered by the *European life sciences infrastructure for biological information* (ELIXIR), an EU project for promotion of data exchange within life science (https://elixir-europe.org/).

Data sharing is certainly a critical point, which on the one hand takes up a lot of time and on the other hand also means a loss of control over one's own research work. Regardless of these considerations, the amount of time required for data sharing can certainly be significantly reduced and simplified for all those involved by using suitable interface solutions. Some efforts are already underway in this regard, as the databases mentioned above show. In addition, scientists are encouraged by scientific journals to make their data available to the public. Furthermore, consideration must be given to who can access such databases and how data protection and security can be guaranteed [9, 45].

2.5 Data evaluation and validation

For the evaluation of nontargeted methods, both commercial software and freely available open source software exist. Both have their advantages and disadvantages, while commercial programs are often easier to use and adapted to the current data format, open source software offer the possibility of importing further algorithms. In addition, when evaluating data, it can be useful to merge the data from different omics approaches (data fusion) in order to obtain different viewing angles and, thus, a maximum possible resolution.

2.5.1 Sequence analysis

Sequence analyzes are mainly relevant for DNA-based methods when the exact DNA sequence must be available. Using modern NGS-based methods (see section 3.1), only short sequence sections (reads) are obtained, which must be assembled into a consensus sequence. This process can be very complex, depending on the genome size and especially for de novo sequencing, which is why the use of appropriate algorithms is essential. These summarize the reads using sequence overlaps to form large sections (contigs), which in turn serve to derive the original sequence using scaffolds. With the help of databases, such as that of the *National Center for Biotechnology Information* (NCBI) or the *European Molecular Biology Laboratory* (EMBL), the sequence can then be assigned to an organism and analyzed for differences [46].

2.5.2 Multivariate data analysis

Multivariate data analyzes are carried out to analyze group assignments based on similarities and/or differences. In this regard, there are numerous processes that aim to reduce the very complex raw data to the relevant marker substances or sequences and to enable visualization. Usually, the data must be preprocessed before using such methods. Depending on the analytical method, this often includes a feature extraction to convert the data into a tabular form (bucketing/binning). A normalization then often follows in order to overcome systematic errors which result, for example from different sample weights. Scaling procedures ensure that different signals have a comparable influence on the result. Depending on the data structure, it may also be useful to add missing values. There are only rudimentary workflows for data processing, since these often depend on the data structure and must be adjusted and optimized accordingly.

After the data have been prepared, they are evaluated using univariate or multivariate mathematical evaluation methods. These overall strategies are generally divided into nonsupervised and supervised approaches. Nonsupervised methods like principal component analysis are often used exploratory, while with supervised algorithms the class affiliations are taken into account. Frequently used supervised algorithms are, for example partial least squares discriminant analysis, random forest, or support vector machines. In addition to those mentioned above, there is a whole range of other algorithms. It is important to note that the supervised procedure often tends to overfit, so it is recommended that cross-validations be performed, and other model performance criteria are applied. In addition to classic *p*-values, the calculation of false discovery rates and the implementation of receiver operating characteristic curves with the corresponding area under curve are particularly useful for assessing the significance of marker substances. The area can have values between 0 and 1. Values <0.5 indicate that the signal is not suitable as a classifier. However, the applicability is limited to binary classification problems [47, 48].

3 Establishment in routine analytics

Different approaches exist for the establishment of nontargeted methods in routine analysis. This includes the direct transfer of methods from science to routine laboratories or the development of nontargeted methods directly in the commercial laboratory; in each case taking into account the corresponding guidelines such as, in particular, ISO/IEC 17 025:2017. In general, high-resolution methods are often used for nontargeted methods, since the informative depth of the method increases as the information content rises. However, these are usually very expensive, require a high level of maintenance, and appropriately trained personnel. In some cases, low-resolution nontargeted methods are already sufficient. Another option is to reduce nontargeted methods to targeted methods by first identifying the relevant marker substances or sequences using nontargeting methods and then developing the appropriate targeted methods for these differences. The advantage here is that they are often cheaper and easier to use, however, such a step is often accompanied by a loss of information, which may be reflected in the poorer informative value of the analytical result. Nevertheless, such an approach can be sufficient for some issues. In the following sections, we will discuss the different possibilities of omics disciplines in order to illustrate the different options available for an establishment in routine analysis.

3.1 Genomics-based methods

Nontargeted genome analyzes are already established in numerous routine laboratories, but mainly for clinical questions. In particular, the introduction of next generation platforms is largely responsible for this development. In the meantime, numerous commercially available NGS instruments and also methods have been developed, which allow the sequencing of entire genomes faster and cheaper according to the method of Sanger, which was the gold standard for many years. However, they all have both their advantages and disadvantages, which concern the reading lengths of the sequences, error rates, and the time required. In routine analysis, nontargeted molecular biological methods are currently

used less for the proof of food authenticity and more for microbiological reasons to determine the safety of food [49]. In addition, the genetic analysis of microorganisms (microbiomics) can also be used to prove the origin of a food [50]. While initial efforts have been made to introduce standards, accreditation, and validation for clinical analyzes, such work is still in its infancy for the food sector. In the medium term, however, it can be assumed that corresponding guidelines will be issued in this respect. In addition, the costs for NGS processes are still comparatively high at present, but these will fall as market penetration increases and further development steps are taken [43, 49, 51–53].

NGS-based methods require careful examination of the DNA extract used, especially before the actual sequencing process, since NGS methods place higher demands on sample preparation than conventional PCR-based protocols. This means a careful determination of the overall quality of the isolated DNA, that is the homogeneity, the concentration, and the fragment pattern. In order to obtain the best possible extracts, there are a number of different methods that range from the classical CTAB or the phenol–chloroform extraction methods, the use of magnetic beads to specific extraction columns, many of which can also be purchased commercially as kits. Depending on the matrices, various optimizations must generally be carried out first, since components, such as proteins, polysaccharides, and polyphenols, can interfere with the analysis [53]. Some NGS methods require a PCR, in such cases GC- or AT-rich fragments can be produced with a lower yield than others, which in turn can lead to a bias (PCR-bias). Others in turn do not require a PCR, but they need a comparatively high proportion of starting material, which is not always available in sufficient quantity and quality depending on the degree of processing of the food [54]. Depending on device type and sequence size, the time for sequencing is about 30 min to 48 h [49]. Sometimes it may be necessary to sequence entire genomes (*whole genome sequencing*), if species or varieties have a high degree of relationship, in order to identify SNPs. Besides classical approaches that focus on the nuclear genome, the sequencing of the much smaller plastid of plants or mitochondrial genomes is also possible and offers some advantages: (i) due to their shorter length, it is much easier and less complex to obtain and handle the sequences. (ii) These extrachromosomal "genomes" are usually inherited maternally. This property is most important for the development of methods for differentiating varieties. Maternal sequences have the advantage that they refer exclusively to the local tree or shrub and are independent of the pollinator plant. (iii) Plastid or mitochondrial genomes are available in higher copy numbers. This is advantageous as it increases the efficiency of the DNA isolation [55].

In the past, classical DNA-based methods have been developed for a wide range of different issues. These include clinical diagnosis or the detection of genetically modified plants (e.g. rice, soybeans, and maize). The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) system, discovered in 2012, is currently revolutionizing genetic engineering, as mutations can be carried out easily and precisely compared to the methods used to date. However, the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 methods is not detectable by classical DNA-based approaches and cannot be distinguished from conventional breeding methods. NGS can help to detect abnormal genetic polymorphisms by using the whole genome as an analyte. Based on these genetic changes occurring in groups, a suspicion can be deduced that there may be an intentional genetic change. Although high-performance sequencing can be used to identify accumulated mutations in an organism, it cannot reveal the process of their development.

Generally, NGS-based detection methods for the authentication of plant foods have been developed, for example for the identification of spices and herbs [56–58]. In addition, there are methods for detecting counterfeit honey, which can be used to analyze plant, geographical and insect sources [59, 60], and to identify different animal species in meat [61, 62], fish [63, 64], and dairy products [65]. Other NGS applications focus on the analysis of microorganisms for pathogenic reasons, such as the detection of noroviruses [66] or *Salmonella* [67, 68], but also due to their technological influences, for example during fermentation processes [69, 70].

In addition to the NGS approaches given above, it may also be sufficient to use simpler nontargeted-based DNA methods. Fingerprinting techniques, like the RAPD-PCR, are suitable for this purpose and do not require knowledge of the DNA sequence. In a RAPD-PCR, a short primer with a length of 10-15 bases is used, which binds randomly to the DNA sequence of the sample, so that a corresponding duplication of individual DNA sections occurs during the PCR. The application of identical PCR conditions results in band patterns that can be compared. For this purpose, the resulting DNA fragments are separated by gel electrophoresis (e.g. Aagarose or poly acrylamide) and stained with ethidium bromide, so that depending on the species or varieties present, a characteristic pattern is generated. However, this method is sometimes very unspecific, so careful validation is required. RAPD-PCR methods were developed for the identification of plant foods, such as hazelnut varieties [71] or legumes [72], as well as for the species identification of animal commodities, such as beef subspecies and other meat species [73, 74], milk products [75], fish species [76, 77], and for the detection of various microorganisms [78, 79].

A further nontargeted option for the generation of a genetic fingerprint is amplified fragment length polymorphism polymerase chain reaction (AFLP-PCR), in which the DNA present is cut into characteristic fragments by means of two selected Type II restriction enzymes followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected for one or two amplifications by using primers complementary to the adaptor sequence, to the restriction site sequence, and to some nucleotides within the target fragments. The resulting pattern is separated and visualized using Agarose gel electrophoresis. AFLP-PCR-based methods have been developed for the differentiation of tissue types from salmon

and veal products [80], traceability of cattle [81], and olive oil [82, 83].

Repetitive short noncoding DNA regions on the nuclear genome, called simple sequence repeats (SSR) or microsatellites, with lengths of 2-6 bases at the same locus deliver very useful profiles. SSRs occur with varying degrees of frequency in organisms. The higher the degree of relationship, the more similar the number of repetitions. The SSRs are reproduced using specific primers that flank the microsatellites and by means of PCR. The PCR product is in turn visualized with high-resolution gel electrophoresis and, depending on the varieties and species present in an extract, represents a specific pattern. In contrast to the other methods, microsatellite technology requires a considerably higher developmental effort, since the corresponding sequences for the primers must be known. By analyzing microsatellites, it has been possible to distinguish between different types of cocoa varieties [25]. Similar procedures exist for the differentiation of olive oil [84, 85], wine [86], potato varieties [87], and meat [88].

Exon-primed intron-crossing PCR (EPIC-PCR) which is based on the fact that noncoding intron sequences have a high genetic variability also provides a species or variety specific profile. Similar to the SSR, the differentiation is performed by using primers for the highly conserved exon sequences which connect to the intron sequences [89]. The advantage of this method is, above all, the comparatively low costs, the short expenditure of time, the ease of use, and the minimal requirement of laboratory equipment, so that these methods can also be easily implemented in less specialized laboratories.

In addition to the methods mentioned, various targeted methods can be used. These include, for example sequence specific primer PCR for the detection of larger sequence differences of 4-5 bases or ligation-dependent probe amplification (LPA) and RFLP-PCR for the analysis of point mutations. Such methods can be used, for example to detect persipan in marzipan [90] or to identify fish and seafood [91, 92] as well as meat products [93].

3.2 Proteomics-based methods

For the nontargeted analysis of proteins, mass spectrometers are usually applied after a separation step was carried out, either by means of two-dimensional electrophoresis or by LC. Depending on whether intact protein or peptide fragments are to be analyzed, either a top-down or bottom-up approach, the latter with an enzymatic digestion, is applied. Despite far-reaching developments in MS, bottom-up methods are often preferred because the analysis of intact proteins is often not quite easy due to instrumental limitations mainly in the area of resolution. High-resolution mass spectrometers are best suited for nontargeted approaches. Recent developments, such as the use of ion mobility, various chromatographic techniques, and an increase in the performance of the mass spectrometer itself also enable the analysis of very complex samples. The triple quadrupole mass spectrometers that are frequently used in routine analysis are not suitable for nontargeted analyzes, but they can be used to detect already known sequences. However, this is a targeted approach. Especially when mass spectrometric methods are transferred, a comprehensive validation is required, since modifications can often occur despite identical MS designs are used. Such deviations can be seen, for example in the fragmentation behavior, since different intensities are often achieved on different devices, but also in the fact that fragments cannot be detected at all [94, 95].

For nontargeted experiments TOF, TOF/TOF-analyzers, or orbitrap instruments are usually considered. Suitable ionization sources are ESI as well as MALDI. Recent developments are based on the use of LESA-MS. The ambient LESA sources consist of an electrically conductive pipette with which a drop of solvent is applied to the surface of the sample. The analytes diffuse into the drop, which is then introduced into the MS. LESA enables the rapid detection of hundreds of peptides as well as intact proteins and protein complexes without sample preparation directly from the surface of the sample [40]. In this way, there is no need for complex sample extraction and purification. In particular, sample preparation for proteomics applications, especially for food samples, often requires extensive development depending on the respective matrix in order to prevent proteolysis and modifications. For this reason, off-the-shelf methods cannot be used for this question [94, 95].

There are currently no generally recognized guidelines for the analysis of proteins or peptides in food. However, many groups are following the guidance of the *Proteomics Standards Initiative* (PSI). This includes the specification of *Minimum Information About A Proteomics Experiment* (MIAPE), in order to make data more comparable, so that an exchange and verification of data is easier [94, 96].

Proteomics-based mass spectrometric methods in the food sector are used in particular for the detection of allergenic ingredients. Although DNA-based approaches or ELISA methods can also be used in this context, they have some disadvantages. On the one hand, the allergen itself, that is the corresponding amino acid sequence, is not detected using DNA-based methods. On the other hand, ELISA methods, although they are very sensitive, sometimes show limited reproducibilities, cross-reactions with the food matrix and the simultaneous detection of several allergens is not possible. Such disadvantages could be excluded by means of MS-based proteomics approaches. Furthermore, high-throughput and multiplexing methods are also possible using MS devices [97]. Proteomics-based MS methods are now available for the detection of numerous food allergens, although it should be noted that this is not a nontargeted analysis in the classical sense, as the relevant sequences are usually known at the time of final application. These analyses are usually performed with triple quadrupole instruments. Nontargeted approaches are more relevant for primary method development [98, 99]. Furthermore, nontargeted proteomics approaches are suitable for the identification of biological identities and have been used for the authentication of different animal species

[100], milk and dairy products [101], fish [102, 103], various plant foods [104, 105], as well as for the detection of pathogens [106] and production methods [107, 108].

3.3 Metabolomics-based methods

NMR or MS devices are particularly suitable for the high-resolution detection of small molecules. Especially nontargeted NMR applications are currently being established in routine analysis, so both commercial laboratories and state surveillance agencies have acquired NMR devices to set up their own databases or to access existing commercial databases (e.g. from Bruker Biospin for fruit juice and wine) [109, 110]. The analysis takes only a few minutes and the evaluation can be carried out automatically. Usually, 400 MHz spectrometers are used for the food sector and ^{1}H- or ^{13}C-NMR spectra are recorded. High-resolution NMR spectrometers with 800 MHz and more are now available on the market. Due to the high acquisition costs and the likewise very expensive building infrastructure, only the 400 MHz devices have so far become established in routine use [111, 112]. In addition, there are also low-field devices with 40-90 MHz. These benchtop instruments work with low maintenance but also less powerful permanent magnets and require only a power connection. A significant disadvantage is that they show a much lower resolution —signal overlap can occur— compared to spectrometers based on superconducting magnets. In comparison, the maintenance effort for larger devices is significantly higher, since liquid nitrogen and helium are required to cool the magnet [113].

The advantage of NMR databases is the good reproducibility, since the devices usually come from the same manufacturer and thus data can be easily compared. In addition, sample extraction is comparatively simple. Liquid samples only need to be diluted, solid samples are extracted with deuterated solvents. An extraction optimization can be useful in order to be able to detect as many metabolites as possible and should be kept as simple as possible. In order to avoid signal shifts, it is advisable to set the pH value and to use suitable buffers. However, there is no uniform standard here either, which makes it difficult to compare the data between different laboratories [112]. Nevertheless, Gallo et al. could recently show that on devices with different magnetic field strengths it is possible to obtain comparable data and that especially the operator has a significant influence on the quality of the data [114]. In addition to the above mentioned NMR applications for fruit juice and wine, there are many other examples for the authentication of food such as hazelnuts [115], coffee [116–119], honey [120–122], meat [123, 124], or milk [125] and dairy products [126]. The various issue range from the proof of geographical origin [115], the identification of varieties [127], the year of cultivation [128] to technological processes and quality assessments [116].

Compared to NMR, MS can be used to detect significantly more analytes, especially together with chromatographic methods, such as GC or LC, which can be reflected in the information content and, thus, also in the resolution of different sample groups [129]. However, the disadvantages are the somewhat higher expenditure of time and the fact that TOF analyzers, which are mainly used for questions in the food sector, can cause drift effects. Such consequences may make it difficult to compare data. Therefore, the samples should be measured randomly and quality control (QC) samples should be analyzed at regular intervals. According to the recommendations of the *Food and Drug Administration* (FDA), the measurements of the QC samples should make up at least 5% of the samples measured [130]. In addition to these intrabatch effects, interbatch effects that require, for example the addition of internal standards or the measurement of additional reference samples, must also be considered. However, there are now also different bioinformatics tools with which batch effects can be calculated [11]. In addition, different mass spectrometers, even if they are of the same design, but from different manufacturers can also lead to deviations. A circumstance that can be of particular relevance when it comes to the comparability of nontargeted data. Nevertheless, Koistinen et al. recently showed that there are still strong feature overlaps, even when comparing TOF, qTOF, and orbitrap data [131]. There are certainly some things to be considered when normalizing and further comparative measurements are still pending. In principle, it should be possible to transfer the methods and to use external databases. In order to avoid such considerations, it is also possible to reduce nontargeted methods to targeted methods with which the most relevant marker substances can be quantified absolutely [132]. However, it remains to be taken into account that this can lead to a loss of information, which in turn can be reflected in the fact that the individual sample groups could no longer be separated from one another.

As in NMR applications, there is no uniform standard for sample extraction, which can lead to difficulties in the comparability of data. In addition, some ionization techniques can be used to avoid the extraction (e.g. direct analysis in real time [DART], desorption electrospray ionization [DESI], rapid evaporative ionization mass spectrometry [REIMS], laser ablation electrospray ionization [LAESI], LESA, etc., see Section 2.3). Added to this is the variability with regard to the columns used when coupling with chromatographic methods. According to our own experience, it makes sense to develop a method for the detection of overly polar as well as overly nonpolar compounds, as well as a simple extraction procedure for water- and fat-rich foods [30]. We were also able to show with asparagus (water-rich) and hazelnuts (fat-rich) that the lipid profile in particular is most meaningful when it comes to proof of origin [7, 8]. Further studies in this area could help to clarify whether there is a constancy in this respect and whether the same analytes can always be used as marker compounds. However, too little knowledge is currently available in this area.

In recent years, several nontargeted MS applications have been developed for the analysis of metabolites, such as for the geographical origin of cocoa [133], almonds [134], olive oil [135], oranges [136], for the detection of fish [137, 138] and

meat [139, 140] fraud, for the tracing of agricultural practices [141, 142] or for the QC of milk [143, 144].

Other metabolomics-based methods are the vibrational spectroscopy methods already mentioned in Section 2.3, which are much cheaper to purchase and to maintain, require only minimal sample preparation, but again have limited resolution. When using IR-based methods, it is important that the samples are dry, as signals coming from the water can interfere and mask some important other signals. When drying, care must be taken to ensure that this is done gently in order to prevent chemical reactions in the metabolome. Lyophilization is best suited for this purpose, although it has the disadvantage of being time consuming. In this regard, too, some commercially available standards have already been established. However, it must also be noted here that a normalization step may have to be introduced when transferring databases in order to make the data comparable [145].

Example applications for vibrational spectroscopy methods exist for checking the authenticity of honey [146], fish [147], meat [148–150], asparagus [35], as well as maize [151] by near IR (NIR) spectroscopy and for spirits drinks [152] and milk [153, 154] by Raman spectroscopy.

3.4 Metallomics- and isotoplomics-based methods

Methods that are based on the determination of different isotope ratios can be relatively easily adopted in routine analysis, especially since the number of typically analyzed isotope ratios is very manageable. Usually $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{34}S/^{32}S$ are detected, sometimes also, $^{87}Sr/^{86}Sr$ as well as $^{11}B/^{10}B$. An internal calibration is already carried out by measuring the ratios. The isotope ratios are given compared to internationally recognized reference standards. Since these standards are comparatively expensive or in some cases no longer available, secondary standards calibrated against the international standards are usually used in routine analysis. However, isotope-ratio MS (IRMS) are generally not part of the standard equipment of many laboratories and are therefore usually only be found in highly specialized laboratories [2].

Promising methods, some of which are already being used routinely, relate to the detection of animal commodities, such as meat, dairy products, fish, and shellfish with regard to the detection of the geographical origin, the feeding method of the animals, the production method (organic vs conventional) or whether it is wildlife or breeding [155]. In addition, there are numerous examples of applications for plant-based foods, which mainly refer to the proof of geographical origin, the fertilizers used, and the botanical origin, for example for hops [156], ginseng [157], and tomatoes [158].

The situation is different for the analysis of elements, as most laboratories already have the mandatory equipment available, as this can also be used for many other targeted issues. In addition, high-resolution ICP-MS devices do not always have to be used for nontargeted approaches; in some cases, cheaper flame atomic absorption spectrometry (F-AAS) and flame atomic emission spectrometry (F-AES) devices are sufficient. Nevertheless, especially with samples as rich in matrix as food, care should be taken to ensure that interferences between individual analytes can occur. These can be minimized with high-resolution devices. They are also more sensitive and can detect elements in smaller traces [159, 160]. In addition, interference can be reduced by installing collision/reaction cells, which prevent the formation of isobaric molecules. Nontargeted-based elemental analyzes can also be established relatively easily in routine analysis, since the number of analytes is manageable, and calibrations can be performed easily with the help of multistandards. The already mentioned laser technology (e.g. 193 nm excimer laser systems) for sample ablation can also be used here to make the extraction procedure as simple and fast as possible. However, suitable reference material is often still missing in this context [161].

In the past, nontargeted analyzes of elements were used, for example for the geographic origin of asparagus [159], saffron [162], wine [163, 164], fish [165], and meat [166], or for the organic cultivation of various juices [167, 168] and milk [169].

4 Conclusions and future trends

For an assessment of food, with regard to its safety, but also to be able to detect attempts to fraud, it is becoming increasingly important to understand the biological system in its entirety. Therefore, there is a high probability that the establishment of nontargeted, hypothesis-free methods in routine analysis will increase significantly. However, some developmental work, harmonization, and standardization processes are still needed. These claims concern not only the analytical approach, but also the structure of databases and the sampling processes. The latter is of great importance, especially the procurement of sufficient numbers of samples and suitable reference materials. Therefore, joint collaborations are crucial so that as many users as possible can benefit together. Rules and standards are also required for this case. Furthermore, there are hardly any laboratory comparative studies to date that demonstrate an optimal transfer of methods from science to routine application. Cooperation between different institutions but also between companies is also very important in this context.

Despite all these challenges, nontargeted methods will prevail due to their extraordinarily high performance. Helpful in this context is that both politics and economy strongly support the implementation of such methods through appropriate funding programs, so that overall a high growth can be expected.

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5 References

[1] Van der Heijden, K., Younes, M., Fishbein, L., Miller, S., *International Food Safety Handbook: Science, International Regulation, and Control*. CRC Press, Boca Raton 2019.

[2] Creydt, M., Fischer, M., *Electrophoresis* 2018, *39*, 1569–1581.

[3] Böhme, K., Calo-Mata, P., Barros-Velázquez, J., Ortea, I., *TrAC Trends Anal. Chem.* 2019, *110*, 221–232.

[4] Vallverdu-Queralt, A., Lamuela-Raventos, R. M., *Electrophoresis* 2016, *37*, 1784–1794.

[5] Alvarez, G., Montero, L., Llorens, L., Castro-Puyana, M., Cifuentes, A., *Electrophoresis* 2018, *39*, 136–159.

[6] United States Pharmacopeia (USP), Appendix XVIII: Guidance on Developing and Validating Non-targeted Methods for Adulteration Detection. Food Chemicals Codex, 3rd supplement to 11th ed.; USP: Rockville, 2019.

[7] Creydt, M., Hudzik, D., Rurik, M., Kohlbacher, O., Fischer, M., *J. Agric. Food Chem.* 2018, *66*, 13328–13339.

[8] Klockmann, S., Reiner, E., Bachmann, R., Hackl, T., Fischer, M., *J. Agric. Food Chem.* 2016, *64*, 9253–9262.

[9] Donarski, J., Camin, F., Fauhl-Hassek, C., Posey, R., Sudnik, M., *Trends Food Sci. Technol.* 2019, *90*, 187–193.

[10] McGrath, T. F., Haughey, S. A., Patterson, J., Fauhl-Hassek, C., Donarski, J., Alewijn, M., van Ruth, S., Elliott, C. T., *Trends Food Sci. Technol.* 2018, *76*, 38–55.

[11] Chong, J., Wishart, D. S., Xia, J., *Curr. Protoc. Bioinf.* 2019, *68*, e86.

[12] Nyamundanda, G., Gormley, I. C., Fan, Y., Gallagher, W. M., Brennan, L., *BMC Bioinf.* 2013, *14*, 338.

[13] Billoir, E., Navratil, V., Blaise, B. J., *Briefings Bioinf.* 2015, *16*, 813–819.

[14] Blaise, B. J., Correia, G., Tin, A., Young, J. H., Vergnaud, A.-C., Lewis, M., Pearce, J. T. M., Elliott, P., Nicholson, J. K., Holmes, E., Ebbels, T. M. D., *Anal. Chem.* 2016, *88*, 5179–5188.

[15] Fiehn, O., Sumner, L. W., Rhee, S. Y., Ward, J., Dickerson, J., Lange, B. M., Lane, G., Roessner, U., Last, R., Nikolau, B., *Metabolomics* 2007, *3*, 195–201.

[16] Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., Fan, T. W. M., Fiehn, O., Goodacre, R., Griffin, J. L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A. N., Lindon, J. C., Marriott, P., Nicholls, A. W., Reily, M. D., Thaden, J. J., Viant, M. R., *Metabolomics* 2007, *3*, 211–221.

[17] Taylor, C. F., Field, D., Sansone, S.-A., Aerts, J., Apweiler, R., Ashburner, M., Ball, C. A., Binz, P.-A., Bogue, M., Booth, T., Brazma, A., Brinkman, R. R., Michael Clark, A., Deutsch, E. W., Fiehn, O., Fostel, J., Ghazal, P., Gibson, F., Gray, T., Grimes, G., Hancock, J. M., Hardy, N. W., Hermjakob, H., Julian, R. K., Kane, M., Kettner, C., Kinsinger, C., Kolker, E., Kuiper, M., Novère, N. L., Leebens-Mack, J., Lewis, S. E., Lord, P., Mallon, A.-M., Marthandan, N., Masuya, H., McNally, R., Mehrle, A., Morrison, N., Orchard, S., Quackenbush, J., Reecy, J. M., Robertson, D. G., Rocca-Serra, P., Rodriguez, H., Rosenfelder, H., Santoyo-Lopez, J., Scheuermann, R. H., Schober, D., Smith, B., Snape, J., Stoeckert, C. J., Tipton, K., Sterk, P., Untergasser, A., Vandesompele, J., Wiemann, S., *Nat. Biotechnol.* 2008, *26*, 889–896.

[18] Ćwiek-Kupczyńska, H., Altmann, T., Arend, D., Arnaud, E., Chen, D., Cornut, G., Fiorani, F., Frohmberg, W., Junker, A., Klukas, C., Lange, M., Mazurek, C., Nafissi, A., Neveu, P., van Oeveren, J., Pommier, C., Poorter, H., Rocca-Serra, P., Sansone, S.-A., Scholz, U., van Schriek, M., Seren, Ü., Usadel, B., Weise, S., Kersey, P., Krajewski, P., *Plant Methods* 2016, *12*, 44.

[19] Sansone, S.-A., Rocca-Serra, P., Field, D., Maguire, E., Taylor, C., Hofmann, O., Fang, H., Neumann, S., Tong, W., Amaral-Zettler, L., Begley, K., Booth, T., Bougueleret, L., Burns, G., Chapman, B., Clark, T., Coleman, L.-A., Copeland, J., Das, S., de Daruvar, A., de Matos, P., Dix, I., Edmunds, S., Evelo, C. T., Forster, M. J., Gaudet, P., Gilbert, J., Goble, C., Griffin, J. L., Jacob, D., Kleinjans, J., Harland, L., Haug, K., Hermjakob, H., Sui, S. J. H., Laederach, A., Liang, S., Marshall, S., McGrath, A., Merrill, E., Reilly, D., Roux, M., Shamu, C. E., Shang, C. A., Steinbeck, C., Trefethen, A., Williams-Jones, B., Wolstencroft, K., Xenarios, I., Hide, W., *Nat. Genet.* 2012, *44*, 121–126.

[20] Steinbach, D., Alaux, M., Amselem, J., Choisne, N., Durand, S., Flores, R., Keliet, A.-O., Kimmel, E., Lapalu, N., Luyten, I., Michotey, C., Mohellibi, N., Pommier, C., Reboux, S., Valdenaire, D., Verdelet, D., Quesneville, H., *Database* 2013, *2013*, bat058.

[21] Kim, H. K., Verpoorte, R., *Phytochem. Anal.* 2010, *21*, 4–13.

[22] Biais, B., Bernillon, S., Deborde, C., Cabasson, C., Rolin, D., Tadmor, Y., Burger, J., Schaffer, A. A., Moing, A., *Methods Mol. Biol.* 2012, *860*, 51–63.

[23] Patole, C., Bindschedler, L. V., in: Meena, S. N., Naik, M. M. (Eds.), *Advances in Biological Science Research*. Academic Press, San Diego 2019, pp. 45–67.

[24] DeMan, J. M., Finley, J. W., Hurst, W. J., Lee, C. Y., *Principles of Food Chemistry*. Springer, Cham 2018.

[25] Herrmann, L., Felbinger, C., Haase, I., Rudolph, B., Biermann, B., Fischer, M., *J. Agric. Food Chem.* 2015, *63*, 4539–4544.

[26] Zhao, H., Zhang, S., Zhang, Z., *Food Control* 2017, *76*, 82–87.

[27] Stauffer, M., *Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis*. IntechOpen, Rijeka 2017.

[28] Bernardo, G. D., Gaudio, S. D., Galderisi, U., Cascino, A., Cipollaro, M., *Biotechnol. Prog.* 2007, *23*, 297–301.

[29] Heick, J., Fischer, M., Popping, B., *J. AOAC Int.* 2012, *95*, 388–393.

[30] Creydt, M., Arndt, M., Hudzik, D., Fischer, M., *J. Agric. Food Chem.* 2018, *66*, 12876–12887.

[31] Tang, S., Zhang, H., Lee, H. K., *Anal. Chem.* 2016, *88*, 228–249.

[32] Papaslioti, E. M., Parviainen, A., Román Alpiste, M. J., Marchesi, C., Garrido, C. J., *Food Chem.* 2019, *274*, 726–732.

[33] Krutilin, A., Maier, S., Schuster, R., Kruber, S., Kwiatkowski, M., Robertson, W. D., Hansen, N.-O., Miller, R. J. D., Schlüter, H., *J. Proteome Res.* 2019, *18*, 1451–1457.

[34] Valand, R., Tanna, S., Lawson, G., Bengtström, L., *Food Addit. Contam. Part A* 2020, *37*, 19–38.

[35] Richter, B., Rurik, M., Gurk, S., Kohlbacher, O., Fischer, M., *Food Control* 2019, *104*, 318–325.

[36] Limm, W., Karunathilaka, S. R., Yakes, B. J., Mossoba, M. M., *Int. Dairy J.* 2018, *85*, 177–183.

[37] Yaseen, T., Sun, D.-W., Cheng, J.-H., *Trends Food Sci. Technol.* 2017, *62*, 177–189.

[38] Guo, T., Yong, W., Jin, Y., Zhang, L., Liu, J., Wang, S., Chen, Q., Dong, Y., Su, H., Tan, T., *Mass Spectrom. Rev.* 2017, *36*, 161–187.

[39] Verplanken, K., Stead, S., Jandova, R., Poucke, C. V., Claereboudt, J., Bussche, J. V., Saeger, S. D., Takats, Z., Wauters, J., Vanhaecke, L., *Talanta* 2017, *169*, 30–36.

[40] Eikel, D., Henion, J., *Rapid Commun. Mass Spectrom.* 2011, *25*, 2345–2354.

[41] Alexovič, M., Dotsikas, Y., Bober, P., Sabo, J., *J. Chromatogr. B* 2018, *1092*, 402–421.

[42] Fleischer, H., Drews, R. R., Janson, J., Chinna Patlolla, B. R., Chu, X., Klos, M., Thurow, K., *J. Lab. Autom.* 2016, *21*, 671–681.

[43] Szabo, K., Malorny, B., Stoyke, M., *J. Consum. Prot. Food Saf.* 2019, *15*, 85–89.

[44] Ackermann, S. M., Lachenmeier, D. W., Kuballa, T., Schütz, B., Spraul, M., Bunzel, M., *Magn. Reson. Chem.* 2019, *57*, 579–588.

[45] Griffin, P. C., Khadake, J., LeMay, K. S., Lewis, S. E., Orchard, S., Pask, A., Pope, B., Roessner, U., Russell, K., Seemann, T., Treloar, A., Tyagi, S., Christiansen, J. H., Dayalan, S., Gladman, S., Hangartner, S. B., Hayden, H. L., Ho, W. W. H., Keeble-Gagnère, G., Korhonen, P. K., Neish, P., Prestes, P. R., Richardson, M. F., Watson-Haigh, N. S., Wyres, K. L., Young, N. D., Schneider, M. V., *F1000Res* 2017, *6*, 1618.

[46] Ghurye, J., Pop, M., *PLoS Comput. Biol.* 2019, *15*, e1006994.

[47] Hanley, J. A., McNeil, B. J., *Radiology* 1982, *143*, 29–36.

[48] Benjamini, Y., Hochberg, Y., *J. R. Stat. Soc. Series. B Stat. Methodol.* 1995, *57*, 289–300.

[49] Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Ching Chai, L., Chapman, J., Grant, K., *Food Microbiol.* 2019, *79*, 96–115.

[50] Arcuri, E. F., El Sheikha, A. F., Rychlik, T., Piro-Métayer, I., Montet, D., *Food Control* 2013, *30*, 1–6.

[51] Hardwick, S. A., Deveson, I. W., Mercer, T. R., *Nat. Rev. Genet.* 2017, *18*, 473–484.

[52] Haynes, E., Jimenez, E., Pardo, M. A., Helyar, S. J., *Food Control* 2019, *101*, 134–143.

[53] Burns, M., Foster, L., Walker, M., *DNA Techniques to Verify Food Authenticity: Applications in Food Fraud*, Royal Society of Chemistry, London 2019.

[54] van Dijk, E. L., Jaszczyszyn, Y., Thermes, C., *Exp. Cell Res.* 2014, *322*, 12–20.

[55] Herrmann, L., Haase, I., Blauhut, M., Barz, N., Fischer, M., *J. Agric. Food Chem.* 2014, *62*, 12118–12127.

[56] Barbosa, C., Nogueira, S., Gadanho, M., Chaves, S., *J. AOAC Int.* 2019, *102*, 369–375.

[57] Ivanova, N. V., Kuzmina, M. L., Braukmann, T. W., Borisenko, A. V., Zakharov, E. V., *PLoS One* 2016, *11*, e0156426.

[58] Lo, Y. T., Shaw, P. C., *Biotechnol. Adv.* 2019, *37*, 107450.

[59] Prosser, S. W. J., Hebert, P. D. N., *Food Chem.* 2017, *214*, 183–191.

[60] Utzeri, V. J., Schiavo, G., Ribani, A., Tinarelli, S., Bertolini, F., Bovo, S., Fontanesi, L., *Sci. Rep.* 2018, *8*, 9996.

[61] Xing, R.-R., Wang, N., Hu, R.-R., Zhang, J.-K., Han, J.-X., Chen, Y., *Food Control* 2019, *101*, 173–179.

[62] Cottenet, G., Blancpain, C., Chuah, P. F., Cavin, C., *Food Control* 2020, *110*, 107003.

[63] Kappel, K., Haase, I., Käppel, C., Sotelo, C. G., Schröder, U., *Food Chem.* 2017, *234*, 212–219.

[64] Kumar, G., Kocour, M., *Fish. Res.* 2017, *186*, 11–22.

[65] Ribani, A., Schiavo, G., Utzeri, V. J., Bertolini, F., Geraci, C., Bovo, S., Fontanesi, L., *Food Chem.* 2018, *246*, 90–98.

[66] Bartsch, C., Höper, D., Mäde, D., Johne, R., *Food Microbiol.* 2018, *76*, 390–395.

[67] Lewis, E., Hudson, J. A., Cook, N., Barnes, J. D., Haynes, E., *J. Microbiol. Methods* 2020, *171*, 105840.

[68] Ferrario, C., Lugli, G. A., Ossiprandi, M. C., Turroni, F., Milani, C., Duranti, S., Mancabelli, L., Mangifesta, M., Alessandri, G., van Sinderen, D., Ventura, M., *Int. J. Food Microbiol.* 2017, *256*, 20–29.

[69] De Filippis, F., Parente, E., Ercolini, D., *Microb. Biotechnol.* 2017, *10*, 91–102.

[70] Lee, M.-H., Li, F.-Z., Lee, J., Kang, J., Lim, S.-I., Nam, Y.-D., *J. Food Sci.* 2017, *82*, 960–968.

[71] Felbinger, C., Kutzsche, F., Mönkediek, S., Fischer, M., *Food Control* 2020, *107*, 1–9.

[72] Weder, J. K. P., *J. Agric. Food Chem.* 2002, *50*, 4456–4463.

[73] Kumar, D., Singh, S. P., Karabasanavar, N. S., Singh, R., Umapathi, V., *J. Food Sci. Technol.* 2014, *51*, 3458–3463.

[74] Lin, C.-C., Tang, P.-C., Chiang, H.-I., *Food Sci. Biotechnol.* 2019, *28*, 1769–1777.

[75] Cunha, J. T., Ribeiro, T. I. B., Rocha, J. B., Nunes, J., Teixeira, J. A., Domingues, L., *Food Chem.* 2016, *211*, 631–636.

[76] Ali, B. A., Huang, T.-H., Qin, D.-N., Wang, X.-M., *Rev. Fish Biol. Fish* 2004, *14*, 443–453.

[77] Asensio, L., Gonzalez, I., Fernandez, A., Rodriguez, M. A., Lobo, E., Hernandez, P. E., Garcia, T., Martin, R., *J. Food Prot.* 2002, *65*, 432–435.

[78] Shekhawat, S. S., Gaurav, A., Joseph, B., Kumar, H., Kumar, N., *Vet. World* 2019, *12*, 146–154.

[79] Andrighetto, C., Psomas, E., Tzanetakis, N., Suzzi, G., Lombardi, A., *Lett. Appl. Microbiol.* 2000, *30*, 5–9.

[80] R López, C. M., Morán, P., Lago, F., Espiñeira, M., Beckmann, M., Consuegra, S., *Food Chem.* 2012, *131*, 1493–1498.

[81] Zhao, J., Li, T., Xu, Z., Wang, Z., Yang, S., Chen, A., *Food Control* 2018, *91*, 421–426.

[82] Pafundo, S., Agrimonti, C., Marmiroli, N., *J. Agric. Food Chem.* 2005, *53*, 6995–7002.

[83] Montemurro, C., Pasqualone, A., Simeone, R., Sabetta, W., Blanco, A., *Eur. Food Res. Technol.* 2007, *226*, 1439–1444.

[84] Pasqualone, A., Montemurro, C., Caponio, F., Blanco, A., *J. Agric. Food Chem.* 2004, *52*, 1068–1071.

[85] Martins-Lopes, P., Gomes, S., Santos, E., Guedes-Pinto, H., *J. Agric. Food Chem.* 2008, *56*, 11786–11791.

[86] Pereira, L., Martins-Lopes, P., Batista, C., Zanol, G. C., Clímaco, P., Brazão, J., Eiras-Dias, J. E., Guedes-Pinto, H., *Food Anal. Methods* 2012, *5*, 1252–1259.

[87] Lopez-Vizcón, C., Ortega, F., *Food Control* 2012, *26*, 575–579.

[88] Zhao, J., Zhu, C., Xu, Z., Jiang, X., Yang, S., Chen, A., *Food Control* 2017, *78*, 469–475.

[89] Bierne, N., Lehnert, S. A., Bédier, E., Bonhomme, F., Moore, S. S., *Mol. Ecol.* 2000, *9*, 233–235.

[90] Luber, F., Demmel, A., Hosken, A., Busch, U., Engel, K.-H., *J. Agric. Food Chem.* 2012, *60*, 5853–5858.

[91] Unterberger, C., Luber, F., Demmel, A., Grünwald, K., Huber, I., Engel, K.-H., Busch, U., *Eur. Food Res. Technol.* 2014, *239*, 559–566.

[92] Pascoal, A., Barros-Velázquez, J., Cepeda, A., Gallardo, J. M., Calo-Mata, P., *Food Chem.* 2008, *109*, 638–646.

[93] Pascoal, A., Prado, M., Castro, J., Cepeda, A., Barros-Velázquez, J., *Eur. Food Res. Technol.* 2004, *218*, 306–312.

[94] Ortea, I., O'Connor, G., Maquet, A., *J. Proteomics* 2016, *147*, 212–225.

[95] Korte, R., Brockmeyer, J., *TrAC Trends Anal. Chem.* 2017, *96*, 99–106.

[96] Deutsch, E. W., Orchard, S., Binz, P.-A., Bittremieux, W., Eisenacher, M., Hermjakob, H., Kawano, S., Lam, H., Mayer, G., Menschaert, G., Perez-Riverol, Y., Salek, R. M., Tabb, D. L., Tenzer, S., Vizcaíno, J. A., Walzer, M., Jones, A. R., *J. Proteome Res.* 2017, *16*, 4288–4298.

[97] Marzano, V., Tilocca, B., Fiocchi, A. G., Vernocchi, P., Levi Mortera, S., Urbani, A., Roncada, P., Putignani, L., *J. Proteomics* 2020, *215*, 103636.

[98] Korte, R., Lepski, S., Brockmeyer, J., *Anal. Bioanal. Chem.* 2016, *408*, 3059–3069.

[99] Ma, X., Li, H., Zhang, J., Huang, W., Han, J., Ge, Y., Sun, J., Chen, Y., *Food Control* 2020, *107*, 106744.

[100] Stachniuk, A., Sumara, A., Montowska, M., Fornal, E., *Mass Spectrom. Rev.* 2019, *00*, 1–28.

[101] Nardiello, D., Natale, A., Palermo, C., Quinto, M., Centonze, D., *Food Chem.* 2018, *244*, 317–323.

[102] Pepe, T., Ceruso, M., Carpentieri, A., Ventrone, I., Amoresano, A., Anastasio, A., *Vet. Res. Commun.* 2010, *34*, 153–155.

[103] Wulff, T., Nielsen, M. E., Deelder, A. M., Jessen, F., Palmblad, M., *J. Proteome Res.* 2013, *12*, 5253–5259.

[104] Chambery, A., del Monaco, G., Di Maro, A., Parente, A., *Food Chem.* 2009, *113*, 1283–1289.

[105] Lerma-Garcia, M. J., D'Amato, A., Simo-Alfonso, E. F., Righetti, P. G., Fasoli, E., *Food Chem.* 2016, *196*, 739–749.

[106] Jadhav, S. R., Shah, R. M., Karpe, A. V., Morrison, P. D., Kouremenos, K., Beale, D. J., Palombo, E. A., *Front Microbiol.* 2018, *9*, 3132.

[107] Arena, S., Salzano, A. M., Scaloni, A., *J. Proteomics* 2016, *147*, 56–65.

[108] Arena, S., Renzone, G., Novi, G., Scaloni, A., *J. Proteomics* 2011, *74*, 2453–2475.

[109] Kuballa, T., Brunner, T. S., Thongpanchang, T., Walch, S. G., Lachenmeier, D. W., *Curr. Opin. Food Sci.* 2018, *19*, 57–62.

[110] Spraul, M., Schütz, B., Rinke, P., Koswig, S., Humpfer, E., Schäfer, H., Mörtter, M., Fang, F., Marx, U. C., Minoja, A., *Nutrients* 2009, *1*, 148–155.

[111] Hatzakis, E., *Compr. Rev. Food Sci. Food Saf.* 2019, *18*, 189–220.

[112] Sobolev, A. P., Thomas, F., Donarski, J., Ingallina, C., Circi, S., Cesare Marincola, F., Capitani, D., Mannina, L., *Trends Food Sci. Technol.* 2019, *91*, 347–353.

[113] Capitani, D., Sobolev, A. P., Di Tullio, V., Mannina, L., Proietti, N., *Chem. Biol. Technol. Agric.* 2017, *4*, 17.

[114] Gallo, V., Ragone, R., Musio, B., Todisco, S., Rizzuti, A., Mastrorilli, P., Pontrelli, S., Intini, N., Scapicchio, P., Triggiani, M., Pascazio, A., Cobas, C., Mari, S., Garino, C., Arlorio, M., Acquotti, D., Airoldi, C., Arnesano, F., Assfalg, M., Barison, A., Benevelli, F., Borioni, A., Cagliani, L. R., Casadei, L., Marincola, F. C., Colson, K., Consonni, R., Costantino, G., Cremonini, M. A., Davalli, S., Duarte, I., Guyader, S., Hamon, E., Hegmanns, M., Lamanna, R., Longobardi, F., Mallamace, D., Mammi, S., Markus, M., Menezes, L. R. A., Milone, S., Molero-Vilchez, D., Mucci, A., Napoli, C., Rossi, M. C., Sáez-Barajas, E., Savorani, F., Schievano, E., Sciubba, F., Sobolev, A., Takis, P. G., Thomas, F., Villa-Valverde, P., Latronico, M., *Food Anal. Methods* 2020, *13*, 530–541.

[115] Bachmann, R., Klockmann, S., Haerdter, J., Fischer, M., Hackl, T., *J. Agric. Food Chem.* 2018, *66*, 11873–11879.

[116] Okaru, A. O., Scharinger, A., Rajcic de Rezende, T., Teipel, J., Kuballa, T., Walch, S. G., Lachenmeier, D. W., *Foods* 2020, *9*, 47.

[117] de Moura Ribeiro, M. V., Boralle, N., Redigolo Pezza, H., Pezza, L., Toci, A. T., *J. Food Compos. Anal.* 2017, *57*, 24–30.

[118] Toci, A. T., de Moura Ribeiro, M. V., de Toledo, P. R. A. B., Boralle, N., Pezza, H. R., Pezza, L., *Food Sci. Biotechnol.* 2018, *27*, 19–26.

[119] Milani, M. I., Rossini, E. L., Catelani, T. A., Pezza, L., Toci, A. T., Pezza, H. R., *Food Control* 2020, *112*, 107104.

[120] Schievano, E., Sbrizza, M., Zuccato, V., Piana, L., Tessari, M., *Food Chem.* 2020, *309*, 125788.

[121] He, C., Liu, Y., Liu, H., Zheng, X., Shen, G., Feng, J., *Food Res. Int.* 2020, *130*, 108936.

[122] Schievano, E., Stocchero, M., Zuccato, V., Conti, I., Piana, L., *Food Chem.* 2019, *288*, 96–101.

[123] Uguz, S. S., Ozvural, E. B., Beira, M. J., Oztop, M. H., Sebastião, P. J., *Mol. Phys.* 2019, *117*, 1015–1019.

[124] Wang, X., Jiang, G., Kebreab, E., Li, J., Feng, X., Li, C., Zhang, X., Huang, X., Fang, C., Fang, R., Dai, Q., *Food Res. Int.* 2020, *133*, 109126.

[125] Li, Q., Yu, Z., Zhu, D., Meng, X., Pang, X., Liu, Y., Frew, R., Chen, H., Chen, G., *J. Sci. Food Agric.* 2017, *97*, 2875–2882.

[126] Bergana, M. M., Adams, K. M., Harnly, J., Moore, J. C., Xie, Z., *J. Food Compos. Anal.* 2019, *78*, 49–58.

[127] Son, H.-S., Hwang, G.-S., Ahn, H.-J., Park, W.-M., Lee, C.-H., Hong, Y.-S., *Food Res. Int.* 2009, *42*, 1483–1491.

[128] Yang, S.-O., Shin, Y.-S., Hyun, S.-H., Cho, S., Bang, K.-H., Lee, D., Choi, S. P., Choi, H.-K., *J. Pharm. Biomed. Anal.* 2012, *58*, 19–26.

[129] Stringer, K. A., McKay, R. T., Karnovsky, A., Quémerais, B., Lacy, P., *Front. Immunol.* 2016, *7*, 44.

[130] Food and Drug Administration (FDA), *Bioanalytical Method Validation Guidance for Industry*. FDA, Rockville, 2018.

[131] Koistinen, V. M., da Silva, A. B., Abrankó, L., Low, D., Villalba, R. G., Barberán, F. T., Landberg, R., Savolainen, O., Alvarez-Acero, I., de Pascual-Teresa, S., Van Poucke, C., Almeida, C., Petrásková, L., Valentová, K., Durand, S., Wiczkowski, W., Szawara-Nowak, D., González-Domínguez, R., Llorach, R., Andrés-Lacueva, C., Aura, A.-M., Seppänen-Laakso, T., Hanhineva, K., Manach, C., Bronze, M. R., *Metabolites* 2018, *8*, 46.

[132] Klockmann, S., Reiner, E., Cain, N., Fischer, M., *J. Agric. Food Chem.* 2017, *65*, 1456–1465.

[133] Hori, K., Kiriyama, T., Tsumura, K., *Food Anal. Methods* 2016, *9*, 738–743.

[134] Gil Solsona, R., Boix, C., Ibanez, M., Sancho, J. V., *Food Addit. Contam. Part A* 2018, *35*, 395–403.

[135] Gil-Solsona, R., Raro, M., Sales, C., Lacalle, L., Díaz, R., Ibáñez, M., Beltran, J., Sancho, J. V., Hernández, F. J., *Food Control* 2016, *70*, 350–359.

[136] Díaz, R., Pozo, O. J., Sancho, J. V., Hernández, F., *Food Chem.* 2014, *157*, 84–93.

[137] Black, C., Chevallier, O. P., Haughey, S. A., Balog, J., Stead, S., Pringle, S. D., Riina, M. V., Martucci, F., Acutis, P. L., Morris, M., Nikolopoulos, D. S., Takats, Z., Elliott, C. T., *Metabolomics* 2017, *13*, 153.

[138] Fiorino, G. M., Losito, I., De Angelis, E., Arlorio, M., Logrieco, A. F., Monaci, L., *Food Res. Int.* 2019, *116*, 1258–1265.

[139] Surowiec, I., Fraser, P. D., Patel, R., Halket, J., Bramley, P. M., *Food Chem.* 2011, *125*, 1468–1475.

[140] Ueda, S., Iwamoto, E., Kato, Y., Shinohara, M., Shirai, Y., Yamanoue, M., *Biosci. Biotechnol. Biochem.* 2019, *83*, 137–147.

[141] Novotná, H., Kmiecik, O., Gałązka, M., Krtková, V., Hurajová, A., Schulzová, V., Hallmann, E., Rembiałkowska, E., Hajšlová, J., *Food Addit. Contam. Part A* 2012, *29*, 1335–1346.

[142] Vallverdú-Queralt, A., Medina-Remón, A., Casals-Ribes, I., Amat, M., Lamuela-Raventós, R. M., *J. Agric. Food Chem.* 2011, *59*, 11703–11710.

[143] Scano, P., Murgia, A., Pirisi, F. M., Caboni, P., *J. Dairy Sci.* 2014, *97*, 6057–6066.

[144] Yang, Y., Zheng, N., Zhao, X., Zhang, Y., Han, R., Yang, J., Zhao, S., Li, S., Guo, T., Zang, C., Wang, J., *J. Proteomics* 2016, *136*, 174–182.

[145] Henn, R., Kirchler, C. G., Grossgut, M.-E., Huck, C. W., *Talanta* 2017, *166*, 109–118.

[146] Guelpa, A., Marini, F., du Plessis, A., Slabbert, R., Manley, M., *Food Control* 2017, *73*, 1388–1396.

[147] Grassi, S., Casiraghi, E., Alamprese, C., *Food Chem.* 2018, *243*, 382–388.

[148] López-Maestresalas, A., Insausti, K., Jarén, C., Pérez-Roncal, C., Urrutia, O., Beriain, M. J., Arazuri, S., *Food Control* 2019, *98*, 465–473.

[149] Alamprese, C., Amigo, J. M., Casiraghi, E., Engelsen, S. B., *Meat Sci.* 2016, *121*, 175–181.

[150] Moran, L., Andres, S., Allen, P., Moloney, A. P., *Meat Sci.* 2018, *142*, 52–58.

[151] Achten, E., Schütz, D., Fischer, M., Fauhl-Hassek, C., Riedl, J., Horn, B., *Food Anal. Methods* 2019, *12*, 2172–2184.

[152] Ellis, D. I., Muhamadali, H., Xu, Y., Eccles, R., Goodall, I., Goodacre, R., *Analyst* 2019, *144*, 324–330.

[153] Karunathilaka, S. R., Farris, S., Mossoba, M. M., Moore, J. C., Yakes, B. J., *Food Addit. Contam. Part A* 2017, *34*, 170–182.

[154] Ullah, R., Khan, S., Ali, H., Bilal, M., Saleem, M., Mahmood, A., Ahmed, M., *J. Raman Spectrosc.* 2017, *48*, 692–696.

[155] Camin, F., Bontempo, L., Perini, M., Piasentier, E., *Compr. Rev. Food Sci. Food Saf.* 2016, *15*, 868–877.

[156] Ocvirk, M., Ogrinc, N., Košir, I. J., *J. Agric. Food Chem.* 2018, *66*, 2021–2026.

[157] Chung, I.-M., Lee, T.-J., Oh, Y.-T., Ghimire, B. K., Jang, I.-B., Kim, S.-H., *J. Ginseng Res.* 2017, *41*, 195–200.

[158] Bontempo, L., van Leeuwen, K. A., Paolini, M., Holst Laursen, K., Micheloni, C., Prenzler, P. D., Ryan, D., Camin, F., *Food Chem.* 2020, *318*, 126426.

[159] Richter, B., Gurk, S., Wagner, D., Bockmayr, M., Fischer, M., *Food Chem.* 2019, *286*, 475–482.

[160] Madejczyk, M., Baralkiewicz, D., *Anal. Chim. Acta* 2008, *617*, 11–17.

[161] Hoffman, T., Jaćimović, R., Bay, L. J., Griboff, J., Jagodic, M., Monferrán, M., Ogrinc, N., Podkolzin, I., Wunderlin, D., Almirall, J., *Talanta* 2018, *186*, 670–677.

[162] D'Archivio, A. A., Di Vacri, M. L., Ferrante, M., Maggi, M. A., Nisi, S., Ruggieri, F., *Food Anal. Methods* 2019, *12*, 2572–2581.

[163] Taylor, V. F., Longerich, H. P., Greenough, J. D., *J. Agric. Food Chem.* 2003, *51*, 856–860.

[164] Bronzi, B., Brilli, C., Beone, G. M., Fontanella, M. C., Ballabio, D., Todeschini, R., Consonni, V., Grisoni, F., Parri, F., Buscema, M., *Food Chem.* 2020, *315*, 126248.

[165] Li, L., Boyd, C. E., Sun, Z., *Food Chem.* 2016, *194*, 1238–1244.

[166] Kim, J. S., Hwang, I. M., Lee, G. H., Park, Y. M., Choi, J. Y., Jamila, N., Khan, N., Kim, K. S., *Meat Sci.* 2017, *123*, 13–20.

[167] Maione, C., de Paula, E. S., Gallimberti, M., Batista, B. L., Campiglia, A. D., Barbosa, F., Jr., Barbosa, R. M., *Expert Syst. Appl.* 2016, *49*, 60–73.

[168] Turra, C., Dias de Lima, M., Fernandes, E. A. D. N., Bacchi, M. A., Barbosa, F., Barbosa, R., *Inf. Process. Agric.* 2017, *4*, 199–205.

[169] Rodríguez-Bermúdez, R., López-Alonso, M., Miranda, M., Fouz, R., Orjales, I., Herrero-Latorre, C., *Food Chem.* 2018, *240*, 686–693.

