Foodomics and Food Safety: Where We Are

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Summary

The power of foodomics as a discipline that is now broadly used for quality assurance of food products and adulteration identification, as well as for determining the safety of food, is presented. Concerning sample preparation and application, maintenance of highly sophisticated instruments for both high-performance and high-throughput techniques, and analysis and data interpretation, special attention has to be paid to the development of skilled analysts. The obtained data shall be integrated under a strong bioinformatics environment. Modern mass spectrometry is an extremely powerful analytical tool since it can provide direct qualitative and quantitative information about a molecule of interest from only a minute amount of sample. Quality of this information is influenced by the sample preparation procedure, the type of mass spectrometer used and the analyst’s skills. Technical advances are bringing new instruments of increased sensitivity, resolution and speed to the market. Other methods presented here give additional information and can be used as complementary tools to mass spectrometry or for validation of obtained results. Genomics and transcriptomics, as well as affinity-based methods, still have a broad use in food analysis. Serious drawbacks of some of them, especially the affinity-based methods, are the cross-reactivity between similar molecules and the influence of complex food matrices. However, these techniques can be used for pre-screening in order to reduce the large number of samples. Great progress has been made in the application of bioinformatics in foodomics. These developments enabled processing of large amounts of generated data for both identification and quantification, and for corresponding modeling.

Key words: foodomics, food safety, foodborne pathogens, sample preparation, analytical technologies

Introduction

Traditional food analysis as a discipline of food science was developed together with other basic sectors of analytical chemistry (1). The first and most important aim of food analysis has always been to ensure food safety and quality, and to protect consumers against adulteration (1,2). New developments in food technology, production of fast and ready-to-eat food, globalization of the

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market, and modern nutritional trends lead to new, or increasingly actual problems such as unhealthy diets that can cause obesity or food allergies. Large-scale industrial food production brings new aspects of microbiological safety and safety of genetically engineered food as additional challenges (1–5).

Food safety is a matter of global importance, and the prevention of health problems caused by false nutrition or contaminated food is a worldwide topic of extraordinary social, economic and public health importance (3,6). Outbreaks of diseases caused by foodborne pathogens such as fungi, bacteria and protozoa are still a problem in developing countries, but also in the industrialized, highly developed Western World. Increasing globalization of the food market and new nutritional trends, such as consumption of fresh and raw food, ready-to-eat meals, dry products and exotic ingredients, cause additional outbreaks of food poisoning, but also allergies. Firstly, of unknown origin, the fatal outbreak of food poisoning caused by Shiga toxin-producing pathogen bacterium *Escherichia coli* O104:H4 during the year 2011 in both Germany and France originated from food imported from a developing country (7,8). Changes in world climate and increasing environmental pollution in some countries can also cause generation of new toxic agents, and as a consequence, new toxic effects will be identified. Contamination with mycotoxins, bacterial toxins and toxins coming from other organisms via soil, water and air, as well as via livestock that was fed with contaminated food, have global implications (9).

In Croatia and neighboring Southeastern European countries, endemic nephropathy is a chronic disease that is caused by an unknown agent. There are many hypotheses, but the two most actual ones are focused on food contamination either by aristolochic acid, possibly originating in flour (10) or by ochratoxin A, a mycotoxin (11). These environmental agents are defined as main risk factors for this disease that can end in kidney failure and are associated with urothelial cancer (10–13). New regulations in the European Union, such as regulation EC 258/97 (14) or ISO 9000/EN 29000 (15) and the subsequent ones, including the Nutrition Labeling and Education Act (16,17) that has been a Federal law in the United States since 1990, as well as similar regulations worldwide, had a crucial influence on further development of food analysis. Consequently, food analysis is becoming one of the most important areas in applied analytical chemistry, and the newest and most sophisticated up-to-date techniques are used in this field. According to these regulations, and to the law that is accepted worldwide, food safety is a responsibility equally shared by all participants in food production, from the handling of raw materials and food processing to food distribution. Regulatory and control agencies are finally responsible for compliance with regulatory rules regarding food quality and consumer protection (3,6,9).

The main tasks of food safety regulations are the elimination of bacterial, fungal and other pathogens and their toxins that cause foodborne diseases, the reduction of allergens and the elimination of other agents that can contaminate food and have harmful teratogenic, immunotoxic, nephrotoxic, estrogenic or similar effects. The increasing standards for food safety and new developments in analytical methods, especially in high-throughput mode and fast data analyses, result in the application of new, reliable and rapid test methods. Genomic, proteomic, metabolomic and similar high-throughput and high-resolution techniques that are applied for food analysis are newly summarized under the highly actual term ‘foodomics’ (3,6). According to Herrero et al. (18) ‘Foodomics has been defined as a new discipline that studies the food and nutrition domains through the application of advanced ‘omic’ technologies to improve consumer’s well-being, health and confidence. As a global discipline, foodomics includes the working areas in which food, nutrition, corresponding advanced analytical techniques and bioinformatics are brought together’. These techniques are being increasingly applied in food analysis, as well as in food monitoring during harvest, processing, storage and transportation, until final consumption. Moreover, they can be used for identifying possible agents of food poisoning or food allergies (2,4–6,18–20). In both contemporary food science and nutrition, new terms such as allergomics, nutrigenomics, nutrigenetics, nutriproteomics, nutrimetabolomics and similar are frequently used. Unfortunately, they are connected with poor definitions and systematics that causes problems regarding their understanding, so the logical consequence is their relatively low acceptance (18).

In this Journal, we have already published two reviews about the application of proteomics and peptidomics in food technology and biotechnology with a focus on process development, quality control and product safety (2,4). In the last few years several excellent studies and reviews dedicated to microbial safety and microbiota dynamics have been published (21,22). Investigation of allergies and detection of allergens in food is also a focus of foodomic investigations (19,23). This paper gives an overview of recent developments in foodomics as a new discipline that summarizes all high-throughput ‘omic’ techniques applied in food analysis (5,18,19), and their use in early, rapid, safe and reliable identification of possible hazards, such as foodborne pathogens, toxins and other harmful components that can be present in food, or can occur as contaminants during food processing and distribution.

**Foodomics and the Application of ‘Omic’ Methods in Food Analysis**

In a recent review, Gallo and Ferranti (1) give an excellent overview of the historical development of the application of instrumental methods in food analysis that leads to up-to-date foodomic techniques. As mentioned above, the term ‘foodomics’ summarizes genomics, transcriptomics, proteomics, peptidomics, metabolomics, lipidomics and similar high-throughput and high-resolution techniques that offer considerable opportunities to assess quality and safety of food of both plant and animal origin. Starting with the health assessment of plants and animals as food producers (3), followed by the production and monitoring of both food quality and safety by the producer, the process finally ends in control of food quality and authenticity, and protection against adulteration by authorities in corresponding reference laboratories (2–4,17).
Foodomic analyses of food during the production until consumption (see Fig. 1) and the presence or the development of allergens and/or foodborne pathogens and their toxins provide decisive information and help to prevent the outbreak of foodborne infections and development of food allergies. In the case of disease and side-effect outbreaks, foodomics will lead to the identification of causative agents and support healing (7–9). Identification of proteins or other agents that are relevant for the outbreaks leads to the discovery of biomarkers for this kind of foodborne disease (or side effects) (6,24). In this case, biomarkers (or only ‘markers’) are polynucleotides, proteins, peptides and metabolites that are relevant for tracing microbial infections and identification of toxins and allergens, as well as for the identification of the adultery of food products. Consequently, in vivo foodomic analyses can further efficiently support effective food quality control and quality assurance (3,20,25,26, see also Fig. 1).

Analytical tools that concerned parties have at their disposal in the struggle for food safety are standardized reference methods. Different immunological, spectroscopic and chromatographic methods with selected detection techniques are still most frequently used for analysis and identification of harmful agents in food (6). Most of the current standardized reference methods for the detection of bacteria in food are based on traditional culture methods (U.S. Food and Drug Administration Bacteriological Analytical Manual (27)). Despite reliability and robustness, traditional culture methods may display different limitations that should be overcome in order to make food safety management systems more efficient and eligible to purpose. These methods can be laborious and time consuming, they lack quantitative information and provide limited identification of post-translational modifications of proteins and peptides, they are unable to detect viable but non-culturable bacteria and are far from optimal for the detection of some harmful agents that are present in low concentrations (20,28,29). Development of new analytical methods for the detection of bacterial contamination in food is a challenging task, which requires a multidisciplinary approach. A special concern about newly developed methods is that they have to be validated against standard reference methods. It is important to note that a gold standard method is not necessarily perfect. These methods can also produce false-positive or false-negative results. By definition, a gold standard method provides correct results and hence a false-positive (negative) result obtained with the gold standard method would be interpreted as a false-negative (positive) result with the new method being evaluated (30). Thus, standard reference methods should be reviewed from sample preparation procedure through subsequent steps in order to perform comparison and validation of a new method (31). Increased sensitivity of new methods can cause confusion regarding legal issues since ‘safe levels’ of bacteria in a food sample are hard to define. Thus, most countries demand ‘absence’ (‘zero tolerance’) usually means ‘below detection limit’ by use of a particular method, and confusion arises if a new method is of higher sensitivity (31). Here we provide a short overview on what contemporary proteomic/peptidomic tools are providing in the detection and quantification of bacteria in food samples.

Advances in the development of up-to-date instrumental methods, especially in high-performance mass spectrometers (32), but also the introduction of label-free quantitative analyses of proteins and other biological molecules by use of new bioinformatic tools (33,34) substantially helped in overcoming these limitations (35). The importance of proper sample preparation and the introduction of laboratory robotics for high-throughput analyses have also found necessary recognition (1,9,18,20,36–40). These developments will improve fast detection of foodborne pathogens and their toxins, allergens and other harmful components in food, and also help producers, food control authorities and consumers to detect adulterations and protect safe and original food (39–41). Ensuring food safety and identification, monitoring and
assessing of both food authenticity and foodborne haz-
ARDS will require further development of already existing
mass spectrometry (MS)-based methods (34,38–42). In
proteomics and peptidomics, MS represents a very pow-
erful, high-performance method and is an excellent tool
for protein and peptide identification and quantitative
analysis. High-resolution MS is frequently hyphenated
with the ultra-high performance chromatography or cap-
illary electrophoresis and does not require a prior knowl-
edge of the proteins and peptides that are to be identified
and quantified (18,43).

Additionally, the introduction and application of new
or already known but seldom used techniques, such as
imaging mass spectrometry, nuclear magnetic resonance
(NMR) and differential scanning calorimetry (DSC), can
further contribute to faster and more efficient foodomic
analyses (44–46). Further optimization and parallel use of
the already established reference methods will support
the control and validation of results acquired by the ap-
lication of these newly developed techniques.

Sample Preparation

Representative sampling and adequate sample prep-
eration are two starting and crucial steps of food analysis
and correct data interpretation. However, these two steps
are frequently neglected or carried out incorrectly. Conse-
quently, both wrong sampling and sample treatment in
the sample preparation stage of the analysis cannot be
corrected any more, and they can cause very dangerous
systematic errors. For correct sampling, deep knowledge
of both the food matrix and the analytical protocol are
necessary. Sample preparation itself can be a significant
bias of a foodomic method since the experimental data ac-
curacy, reproducibility and confidence essentially depend
on the accuracy and quality of the cleanup technique.
This becomes even more complex if very low-abundance
molecules such as mycotoxins, bacterial toxins or biomar-
kers of microbial food contamination are targets of analy-
sis (6). Recent review by Gallo and Ferranti (1) gives an
extensive overview of sample preparation techniques in
food analysis and can be highly recommended.

It is difficult to give a short overview of sample prep-
eration for foodomic analyses. Firstly, the food itself is a
very complex matrix, and the molecules of interest are
very different. In genomics, DNA, RNA and their degra-
dation products are the topics of investigation. For pro-
teomic and peptidomic analyses, proteins and peptides
have to be concentrated during sample preparation.
However, the most complex task is sample preparation
for metabolomic analysis of small molecules, products of
microbial metabolism. The first rule is that the choice of
the sample preparation method in metabolomics depends
on the type of the molecule(s) of interest.

The simplest sample preparation method mostly used
for the detection of small molecules such as mycoto-
oxins, antibiotics or pesticides is the ‘dilute and shoot’
approach. It is a direct injection of diluted (or extracted)
sample into the system for liquid chromatography (LC)
without a complex cleanup procedure. This approach, when
followed by LC-MS/MS or less frequently capillary elec-
trophoresis (CE)-MS/MS, provides a robust method for
detection and quantification (41–43). Conventional sam-
ple preparation methods in food analysis that are used
especially for the detection of small molecules are liquid-
liquid extraction, solid-liquid extraction and supercritical
fluid extraction (1). However, these techniques are time
and/or labor intensive. The introduction of solid-phase
extraction (SPE) and corresponding pre-packed commer-
cially available columns enabled the application of high-
throughput methods and robotics for fast and reproduc-
bile sample preparation, even when multiple steps must
be introduced (47,48). Campone et al. (49) introduced
pressurized liquid extraction and online SPE coupled
with ultra HPLC-MS/MS. By use of this method, small
molecules like mycotoxins or pesticides can be detected
and quantified. Recovery and quantification limits of tar-
get substances have to be carefully checked and validated
(50,51). However, the applied method is time consuming,
requires large amounts of solvents, and systematic errors
caused by the absorption loss as well as the interaction
between the sample and the solid phase were also ob-
served. Next optimization step was the introduction of
solid phase microextraction, where the amounts of sol-
vent were minimized to a few milligrams (52,53). Use of
iron oxide-based nanoparticles as materials for so-called
‘magnetic solid-phase extraction’ is a further step towards
the introduction of a simple, rapid, selective, sensitive
and highly efficient method for high-throughput enrich-
ment of molecules of interest (54). However, further de-
velopment of very sensitive analytical methods, as well as
software systems capable of handling very high volumes
of required data, especially mass spectrometry with a
very low detection limit for targeted substances, was nec-
essary to enable application of this sample preparation
method (38,39,41,42,55–58). As mentioned above, there
are commercially available columns and ready-to-use car-
tridges packed with different chromatographic stationary
phases for selective enrichment of small molecules (1).
Commercially available solid phase extraction kit (so-
called QuEChErs kit by Agilent, Santa Clara, CA, USA)
can be used as a fast alternative to traditional liquid-liq-
uid and solid-phase extraction in analysis of small mole-
cules. The sample preparation process involves two steps,
namely the extraction with organic and salt solutions fol-
lowed by a clean-up with dispersive solid-phase extrac-
tion resins (1,59).

Sampling and sample preparation are also key steps
in proteomic and peptidomic analyses (60). Wiśniewskii et
al. (61) recommend a filter-aided sample preparation by a
complete solubilization of the proteome in sodium do-
decyl sulfate which is subsequently exchanged by urea on
a standard filtration device (filter-aided sample prepara-
tion). According to the authors, ‘peptides eluted after di-
gestion on the filter were pure, allowing a single-run
analyses of organelles and unprecedented depth of pro-
teome coverage’. This method summarizes previous ex-
periences in sample preparation for proteomic analyses
that was reviewed by several authors (62,63). However, if
cell and tissue samples are prepared using this method,
some very hydrophobic proteins and proteins with post-
translational modifications (PTMs) such as glycosylation
are frequently not solubilized. In food analysis, the extra-
cellular matrix is frequently only partially (or not at all)
destroyed. These proteins are therefore lost for subsequent MS analysis. For analysis of matrix proteins that are important for meat tenderness, and for overall food consistency and quality, as well as tracing of possible adulteration and manipulation (such as repeated freezing and thawing), D’Alessandro and Zolla (64) recommend a stepwise solubilization method, which makes use of detergents, chaotropic agents and reagents for chemical digestion such as cyanogen bromide. This method was developed by Hill et al. (65), for the analysis of proteins that are important for tissue engineering. There are also difficulties with proteolytic (most frequently trypic) digestion of these proteins. Consequently, they are only partially detected or not detected at all, and additional methods for their solubilization and proteolytic digestion must be developed (66). Blonder et al. (67) recommend extraction of hydrophobic proteins by organic solvents, mostly methanol, followed by trypic digestion. This method enables detection of different, highly hydrophobic proteins that were previously not covered by other approaches. Furthermore, Lu and Zhu (68) developed the so-called ‘tube-gel digestion’ of proteins for more complete proteomic analysis and detection of very hydrophobic proteins and proteins with posttranslational modifications (PTMs). Use of this method enabled identification of different hydrophobic membrane proteins that had previously not been detected (66). Application of MALDI mass spectrometry, requests for miniaturization, and introduction of on-chip sample preparation (69), as well as reduction of sample amount used for proteomic analyses (70), were the main driving forces for the introduction of high-throughput methods that enable analyses of hundreds or even thousands of samples. Development of these methods, use of laboratory robotics and introduction of special chromatographic supports like monoliths further enhanced the development of high-throughput methods for sample preparation, especially in genomics, proteomics and metabolomics (48,71).

Genomic analysis that is followed by transcriptomics gives the first information about the changes in the transcription rate of genes in a genome in a particular cell type or tissue during development, but also in different disease states (72). Preparation of genomic DNA is now a routine that is described in several protocols for use of commercial kits for sample preparation and preparation of DNA microarrays (73). DNA microarrays are genome-wide hybridization devices that are used to get information about changes at the transcriptional levels. This technology is one of the most successful and most effective methodologies of high-throughput genomic analyses (74). Flow-through DNA microarray assay can be successfully used for rapid detection and quantification of food pathogens (75).

However, analyses of samples have their own rules, especially if detection of foodborne bacteria and their toxins, as well as contamination by fungi and mycotoxins, is requested. We demonstrated that for the detection of Gram-positive bacteria, their thorough decomposition by use of different mechanical methods, followed by extraction with urea and different detergents is necessary (37). As a rule, sample preparation for detection of proteins and peptides in foodomics has to be carefully elaborated, but already published recommendations (37,61–63) can be used as basic information and a first step in the development of a sample preparation protocol. After solubilization of proteins/peptides of interest, they can be separated by gel electrophoresis, i.e. gel-based proteomics (76) or by different chromatographic or capillary electrophoretic techniques, i.e. gel-free methods (6,39,77,78). In proteomic analyses of food, gel-based methods are still frequently used. However, when a quantitative analysis of detected proteins or polypeptides is necessary, special staining and quantification programs have to be used (76).

**Analytical Techniques**

The strategy of the integration of different analytical techniques and approaches is the main power of foodomics. This kind of strategy demonstrates the flexibility and knowledge in order to follow and incorporate fast technological developments in all fields of instrumental techniques and shall be open for new ideas and approaches. The foodomics expert shall integrate the sample preparation knowledge with the knowledge of main fields of advanced analytical technology, strategically integrated under a strong bioinformatic environment. The opinion of the authors of this review is that a comprehensive knowledge of the methods that are actually applied for food analysis and quality assurance should be combined with recently developed high-throughput methods that will be presented here.

**Affinity-based methods**

Affinity recognition is usually an antibody- and lectin-based approach that benefits from high selectivity. This method is based on specific affinity recognition of antibodies and lectins with the epitopes on the cell surface of microbial species of interest, or with the corresponding parts of the molecule of secreted products of their metabolism. Affinity recognition can also be based on other highly specific molecular interactions such as affinity recognition of components of a bacteriophage with bacterial cell wall components. Different technical solutions have been developed to exploit affinity recognition: immuno-diffusion discs, ELISA, lateral flow assays, biosensors and fast affinity chromatography on monolithic supports (78–81). A serious drawback of all affinity-based methods is the cross reactivity between similar molecules. Antibodies developed against surface antigens may cross-react with microbial cells within the same genera. In this case, cross reactivity would lead to false positive identification. However, this method can be used for pre-screening to reduce the large number of samples to a low number of positive ones, which will subsequently be analyzed by a more stringent technique. Antibody and lectin microarrays incorporating several molecules directed against the same target substance have been designed to overcome the problem of cross reactivity. However, complex food matrices may contain substances (e.g. polyphenols, tannins), which interact or covalently modify target molecules. These substances can significantly interfere by inducing or reducing specific bindings. These phenomena can lead to over- or underestimation (79–82).
Rapid response of foodborne pathogens to their environment typically involves altered gene expression in order to favor both their survival and subsequent growth under extreme conditions. These changes in the physiological state that frequently result in damages of cellular surface (e.g. high/low pH, pressure, temperature, availability of oxygen, exposure to radiation and UV light, or use of specific disinfectants) are typical for food processing and can interfere with the affinity-based detection system (83).

Nuclear magnetic resonance spectroscopy

The discussion about nuclear magnetic resonance spectroscopy (NMR) as a very promising method with great potential to improve both food safety and taste started years ago, before the time of broad use of mass spectrometry-based methods started. First applications of NMR were performed for measurement of water movement during processing in order to study heat and mass transfer, composition and structure of food (84). The main goal was to follow the killing and inactivation of food pathogens and their toxins (85–89). Different promising discussions started, but almost nothing happened for a long time. An intensive literature study shows that some applications gave very interesting results, but follow-up work either failed or was never done (86). More than twenty years ago, alterations in food during packaging were followed by this technique (87). Later, there were some very promising applications in the field of metabolomics, mostly for studying the interaction between different food components, and in the field of food lipidomics, for quantitative determination of olive oil quality (88,89). Albeit its high potential, NMR was still rarely used for food analysis, even less frequently for detection of food pathogens, their toxins and other metabolites (90).

More than ten years ago, NMR came back in focus as a very effective, high-resolution, quality control method in food industry (91,92). Moreau and Guichard (88) demonstrated that this method can be used for the determination of interactions between flavor compounds and high molecular, non-volatile food components, as well as the influence of these reactions on flavor perception of processed foods.

In the last ten years, the number of quantitative NMR applications in foodomics has significantly increased (93,94), especially toward detection and quantitative determination of specific molecules (95–97). NMR can be used for determining food origin (98–100), quality and purity (97,98,101). The most important application of NMR in the field of safety is the use of this method for the determination of changes in food during storage, with particular emphasis on degradation processes and possible contamination with food pathogens and their toxins (93,94). Changes during freezing and storing at different temperatures in dependence of time were determined in fish (102,103), meat (104) and vegetables (105). NMR was also used for detecting metabolic profiles of transgenic grapes and their comparison with non-modified fruits (106). Although there are only a few NMR applications for the determination of food pathogens, they seem to be very promising. Ercolini et al. (104) used this technique for monitoring the microbial metabolites and bacterial diversity in beef during storage under different packaging conditions. Picone et al. (107) followed the effect of the natural terpenoid carvacrol on the metabolome of a model Escherichia coli strain by use of proton nuclear magnetic resonance (1H-NMR) spectroscopy.

In general, 1H NMR spectroscopy is the most frequently used method (95–99,108), but NMR spectroscopy can also detect other isotopes such as 13C, 15N, 17O and 31P (79,89,91,92,108). The advantages of NMR spectroscopy are that minimal sample preparation is necessary, and practically every kind of biological fluids or food extracts can be analyzed using this method (91,92,108). However, sensitivity is still a problem, and less abundant substances cannot be detected. This fact reduces the utilization of this method, so that mass spectrometry is still the method of choice, when low-abundance food components are the targeted substances (108).

Other techniques

There are several methods for visualizing changes in food during processing such as X-ray diffraction (109), a combination of high-resolution imaging techniques like confocal scanning microscopy with specific staining of food components, and a combination of several physico-chemical methods (110–112) that are followed by computer simulation techniques (113). These strategies are still either time consuming, or they yield only very specific information, so as a consequence, the use of these methods is still limited. However, further development in the direction of simplification and high-throughput can open new perspectives for the utilization of these techniques.

Use of differential scanning calorimetry for the determination of melting and caramelization of sugars as an important component in food was also discussed, but the correct application of this technique is still a topic of controversial debate (45,114,115).

Techniques based on genomic methods

Different PCR/qPCR-based methods (116), second and third generation sequencing techniques (117) and other nucleic acid-based methods (e.g. microarrays) are powerful tools for the detection of foodborne pathogens and their metabolites. Simultaneous detection and quantification of several microorganisms (118) and their serotyping (119), performed in a high-throughput fashion, can be combined with a microfluidic technique. However, application of polymerase-based techniques also has some limitations, and until now, most gene expression studies have been done on monobacterial cultures, mixtures and on artificially contaminated food samples. As a complex matrix, food may contain substances that inhibit polymerase reaction (118,119). Additionally, some compounds may be introduced during sample preparation. Specific design of internal positive control can help to overcome these shortcomings (30,120). Gene expression microarrays are successfully used for analyses of bacterial transcriptome, and RNA sequencing has also been used as a method for investigating the physiological state of foodborne pathogens at the molecular level (121,122). The quantification of expression of specific genes and the determination of both stress resistance and cell viability biomarkers.
can be performed by the use of reverse transcription qPCR (123). Together with the results of targeted proteomic analyses, such as detection of endo- and exotoxins, this kind of transcriptomic data provide further understanding of the physiology of foodborne pathogens (124). That is why proteomic and metabolomic tools are compatible with DNA- (and RNA)-based approaches in the analysis of foodborne pathogens and can be used for validation of these assays. Rosselló-Móra and Amann (125) used a strategy that includes comparative analysis of bacterial proteomes in search for clone-specific proteins whose sequences are used for the design of PCR probes for their quantitative determination. Such improved understanding of behavior and metabolic activity of microorganisms during production is crucial for preserving and improving food quality, safety and nutritional value (126).

All DNA-based microarray assays are plausible high-throughput techniques. The automation and introduction of laboratory robots resulted in an improved capacity and enabled the analysis of a large number of samples. Donhauser et al. (127) developed a DNA-based, sensitive chemiluminescence flow-through microarray assay for the rapid and very sensitive determination and quantification of food pathogens Escherichia coli, Salmonella enterica and Campylobacter jejuni in water samples. This method was further developed and applied for the detection of other foodborne pathogenic microorganisms and viruses (128, 129). However, the use of such microarray chips for analyses of food samples requires further development of high-throughput sample preparation methods (6,39,130).

Mass Spectrometry

Mass spectrometry (MS) is one of the most powerful analytical tools because it can provide direct qualitative and quantitative information about the molecule of interest from a minute amount of sample. In the last 10–15 years, the increase in the performance and adaptability of instrumentation in mass spectrometry has enormously contributed to the development of new strategies in food analysis, rendering MS as one of its main tools (131). The quality of the information gained by MS is influenced by sample preparation procedure, type of mass spectrometer and analyst’s skills. As a consequence, foodomic approaches need to be adapted to each analytical problem and the special characteristics of each matrix have to be taken into consideration. Technical advances are bringing to the market new mass spectrometers of increased sensitivity and resolution, with a lower sample and solvent consumption, that offer rapid analysis, and even in-field analysis. However, all presented advantages still cannot be incorporated into one analytical device, thus comprehensive knowledge of the current technology status is a prerequisite for the successful design of foodomic approaches (3,6,18).

Mass analyzers can achieve low accuracy levels of 100–10 ppm (e.g. quadrupole, ion trap, linear time-of-flight (TOF) or can be of medium accuracy with levels down to 1 ppm (e.g. reflector TOF). This level of accuracy is not enough to unambiguously identify a molecule of interest. Thus, mass analyzers are often combined, together and with a collision cell (fragmentation cell), into tandem mass spectrometers (MS/MS) which can provide enough information to unambiguously identify large numbers of compounds of interest in food analysis. Improvement of existing and development of new mass analyzers is necessary to achieve higher accuracy, resolution, sensitivity, dynamic range and speed. Mass analyzer with the highest accuracy and resolving power is Fourier transform ion cyclotron resonance (FT-ICR). The platform of FT-ICR MS dates from 1974 (132). However, the acquisition speed of FT-ICR of 1 Hz is still not fast enough for comprehensive analysis of highly complex mixtures which need to be fractioned by liquid chromatography (LC) prior to mass analysis. In the year 2000, a new mass analyzer named Orbitrap was described by Makarov (133). In the years that followed, this mass analyzer has quickly become a very important mass analyser for LC-MS/MS analysis of high complex mixtures. With improvements described in 2009 (134), Orbitrap, now as part of a hybrid mass spectrometer, provides resolving power of 500 000 (at m/z=200) and accuracy in low-sub ppm range. The advantage of Orbitrap over FT-ICR is in its speed of up to 20 Hz, which is much more suitable for coupling with LC in analysis of samples of high complexity. However, depending on the particular application, higher resolving power is sometimes more beneficial (135–137).

This chapter gives a short overview of principles of high-resolution mass spectrometric methods that are most frequently applied in food analysis with a focus on food safety. A more general overview of these methods was published by Herrero et al. (18) and recently by Ibáñez et al. (138).

MALDI-TOF mass spectrometry

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a frequently used method that is complementary to other soft ionisation techniques, such as electrospray ionization (ESI). The advantage of MALDI-TOF instruments is their higher tolerance toward contaminants arising from the food matrix. This technique can be applied for the analysis of large molecules such as proteins, but it is also applicable to analysis of intermediate and small size molecules. However, the signal suppression due to matrix background ions presents a limitation when small molecules are analyzed (139). MALDI-TOF MS is an effective and simple method for high-throughput analysis, and the main use of this technique in food authentication and safety analyses is the identification of foodborne pathogens (140,141). Phenotypic tests, such as colony characteristics, growth on selective agar plates, biochemical pattern characterization and Gram staining, are methods that are currently used for the identification of bacteria. However, they are time consuming and less practical if a fast analysis is needed. Known for its sensitivity, accuracy and reproducibility, MALDI-TOF MS is a method most commonly used for the identification of bacteria and their toxins (139,141). This approach is very rapid since it does not involve a sample preparation step, but rather relies upon the introduction of a bacterial colony onto a MALDI plate. The result is a unique intact or tryptically digested ribosomal or intracellular protein and peptide profile of whole bacterial cells, so called bacterial ‘fingerprint’, which allows for
an accurate identification of bacterial contamination. Acquired bacterial MALDI-TOF MS fingerprints are matched against spectral libraries previously collected under identical MALDI conditions without further identification. Consequently, identification success remains highly dependent on the number of well characterized food pathogen biomarker sequences available in reference databases that are in the majority of cases still not publicly available (142–144). It has been demonstrated that sample preparation is also a critical point that includes destruction of bacterial cells and reproducible digestion of liberated proteins (145–147).

MALDI-TOF MS can be successfully used for the discrimination between bacterial subtypes and the detection of the foodborne pathogen Mycobacterium avium sub-species paratuberculosis (MAP) (147). MAP is a pathogenic bacterium that affects cattle and causes paratuberculosis. This microorganism is heat resistant, making its contamination of milk a major cause of MAP transmission to humans (148). The most common diagnostic method used to detect MAP in cattle is ELISA, using specific monoclonal antibodies. However, the use of this technique is limited because of its relatively low sensitivity and possible cross-reaction of antibodies with environmental mycobacteria. Moreover, because of the above-mentioned relatively low sensitivity, ELISA cannot be used for specific detection of early infections. Thus, more sensitive proteomic approaches have been developed for MAP detection, such as 2D gel electrophoresis coupled with MS (148). Lin et al. (149) have effectively shown that MALDI-TOF MS is applicable for rapid discrimination of M. avium from other Mycobacterium species. These results also demonstrate a serious potential of this method for clinical application, as well as for early detection of these bacteria. MALDI-TOF MS as a tool for classification and identification of bacteria has been systematically used for about ten years (150), and its use as an alternative to conventional methods for identification of food pathogens was described by Schumann and Maier (151). It was also shown that this method can be used as a very efficient tool for the identification of high-risk toxin-producing E. coli strains (152). The major bottleneck for further implementation of MALDI-TOF MS in food monitoring systems remains sample preparation. It covers the isolation of microorganisms, culturing by conventional methods and/or enrichment of anaerobic, demanding or slow-growing bacterial strains (153). For that reason, sample preparation protocols for viable but non-cultural (VBNC) or difficult to culture food poisoning bacterial strains Vibrio cholerae, enterohemorrhagic E. coli, Shigella flexneri and Salmonella enterica should still be developed (154). It is important to note that not all food pathogens pose a health threat themselves, but proteins and other metabolites that they secrete can be severely toxic to humans. The significant disadvantage of MALDI-TOF MS is that this technique can only provide information on the occurrence or absence of food-contaminating food pathogens, and it does not give any data about the expression of toxin-encoding genes or levels of toxins and other microbial metabolites or other secreted substances in food. For this sake, other methods, again mostly based on mass spectrometry, have to be employed for their detection (6,9).

**Imaging mass spectrometry**

Imaging mass spectroscopy is a combination between MALDI-TOF MS and histochemistry. This technique gives information about both the molecular size of the substance and its localization in tissue samples (44,153). However, this potentially powerful strategy was seldom used in foodomics (44,156–158). Hong et al. (156) used MALDI imaging mass spectrometry for enhanced visualization of small peptides in rat intestine, presenting only the optimization of the method. An example of the application of imaging mass spectrometry in the field of food safety is the detection of procymidine, a frequently used fungicide in cucumber samples by nanoparticle-assisted laser desorption-ionization mass spectrometry imaging (157). Recently, Yoshimura et al. (158) have given an extensive overview about the state-of-the-art and future use of imaging mass spectrometry in food analysis.

Similar strategies based on both transmission electron microscopy and single particle inductively coupled plasma-mass spectrometry were used for the analysis of the release of potentially harmful silver nanoparticles that are used as pastry decorations (159).

Together with MALDI-TOF, additional mass spectrometric methods were used for the determination of structural elements and large molecules that are cellular components of foodborne pathogens as well as their metabolites. These substances are used as biomarkers for indirect detection of pathogens in food (160).

**Fourier transform ion cyclotron resonance mass spectrometry**

Mass analyzer with highest accuracy and resolving power is Fourier transform ion cyclotron resonance (FT-ICR). As already mentioned above, the platform of FT-ICR MS dates already from 1974 (132). Development of stronger magnets, improvement of the ICR cell, ion optics, data acquisition systems and signal processing are only some of the possible ways for improvements in FT-ICR performance (161,162). Nowadays, commercial FT-ICR instruments with a resolving power of 10 000 000 at m/z=400 and with accuracy in low-sub ppm or ppb range are available. Acquisition speed of FT-ICR of 1 Hz is not fast enough for a comprehensive analysis of highly complex mixtures, which is needed for the fractionation by LC prior to mass analysis, but the improved instrument can be used for the analysis of complex mixtures containing large molecules such as proteins (135,136). The large potential of FT-ICR MS for metabolite analysis was discovered relatively early (163), and a big advantage of this method is the possibility of direct infusion (164). The hypophenation with a liquid chromatograph is also practicable, but there are some limitations caused by relatively slow analysis by this instrument (165–167). For analysis of small molecules, Oliveira et al. (168) demonstrated the power of ultra-high resolution of FT-ICR operating in negative ESI mode with a direct infusion of complex extract of mango fruit for characterization of primary and secondary metabolites in order to achieve higher nutraceutical value of this fruit. The application of FT-ICR MS in lipidomics (169,170) is increasingly pushed back by faster and easier-to-use Orbitrap and other hybrid mass
spectrometers (171–173). According to Schwudke et al. (173), ‘despite its recognized potential, the scope of FT MS-driven lipidomics remained rather limited. Sampling of abundant ions of chemical background could exceed the storage capacity of an ion cyclotron resonance (ICR) cell and impair the accuracy of isotopic profiles, thus compromising the species quantification’. However, although published in a very high quality peer-reviewed journal, this pretty critical evaluation is coming from two competitors, and careful analysis of the instrument (174) yielded in significant improvements, so that this method is again gaining in popularity for lipidomic and other analyses of biological samples (175). For example, He et al. (176) used this method for characterization of polar lipids by online LC hyphenated with hybrid linear quadrupole ion trap FT-ICR MS. Furthermore, Le et al. (177) applied MALDI FT-ICR MS for tissue imaging of lipids. In a metabolomic analysis, Milev et al. (178) used this MS approach for the analysis of fermented raw cocoa beans and the determination of their origin by direct infusion of purified raw extract. Together with other high-performance MS and LC methods, Chen et al. (179) used FT-ICR mass spectrometry for the screening of antioxidants of longan seed extract. There are further applications of FT-ICR for determining protein glycosylation, glycoscreening and seed extract. There are further applications of FT-ICR for spectrometry for the screening of antioxidants of longan MS and LC methods, Chen et al. (179) used FT-ICR mass spectrometry for the screening of antioxidants of longan seed extract. There are further applications of FT-ICR for determining protein glycosylation, glycoscreening and sequencing (180–182). In food safety, these analyses are important for the determination of allergenicity of proteins and peptides (183,184). Recently, Ghaete et al. (185) gave a comprehensive overview about the use of FT-ICR MS in metabolomics and lipidomics. However, a clear differentiation between the use of this kind of instrument and Orbitrap-based metabolomics is not given. In conclusion, the use of this powerful method in foodomics, especially in both proteomics and metabolomics, is still not fully exploited, and the use of the newly developed instruments with increased throughput will contribute to a more frequent use of FT-ICR mass spectrometry in food analysis (186). A recent impressive investigation of the E. coli proteome by LC FT-ICR MS (187) and a metabolomic study by Witting et al. (188) of both worm Caenorhabditis elegans and bacterium Pseudomonas aeruginosa opened the way for use of this method for high-throughput analysis of food pathogen metabolomes.

Hybrid mass spectrometers – Orbitrap and other instruments

In the year 2000 a new mass analyzer, Orbitrap, was described (133). In the years that followed, the interfacing of the Orbitrap mass analyzer with an electrospray ion source was realized (189), and this MS instrument has quickly become a very important tool for LC-MS/MS analysis of highly complex mixtures. Further optimization of the instrument was described in 2009 (134) and the development of corresponding data acquisition has been constantly improved since (190). Orbitrap, now as part of a hybrid mass spectrometer, provides resolving power of 500 000 (at m/z=200) and accuracy in the low-sub ppm range (134,191). The advantage of Orbitrap over FT-ICR is in its speed of up to 20 Hz, which is much more suitable for coupling with LC when analyzing samples of high complexity. Hybrid Orbitrap mass spectrometers can perform combined fragmentations, and this property of the instrument is currently the most important fragmentation scheme for the analysis of posttranslational modifications of proteins (134,183,184,192). In food safety, these investigations have been shown to be crucial for the studying of allergenicity of proteins (183,184). It has to be stressed that the ongoing optimization of speed, performance, practicability, collaboration with leading scientist in this field from both academia and industry (33,34,134,173,183,184), its user friendliness and excellent marketing, make the Orbitrap a leading instrument for mass spectrometry analyses in foodomics (33,133,134,135,183,186,193), as well as one of the most expensive ones.

As mentioned above, Orbitrap is both a very popular and frequently used instrument for high-resolution mass spectrometric experiments in foodomics. The detection of marker molecules is highly important for food safety, and there is no surprise that most references regarding the application of this method point to works that were performed by this instrument (18,33–36,55,57,138,141,173). A further interesting application of this method for control of food safety and authenticity is the determination of the triacylglycerol profile of Iberian dry-cured ham (194). The results were further combined with chemometrics to prove the authenticity of this product (195). The use of the ESI-Orbitrap method for profiling of epicuticular wax from olive fruits shortened the analysis, and a detailed compositional overview of a wide range of chemical species was obtained. Otherwise, this profile could be obtained by applying a combination of multiple analytical techniques (196). Proteomic investigation of the interaction of foodborne pathogen Salmonella enterica with macrophages gave important information about the pathogenicity of this bacterium (197). Orbitrap mass spectrometers are also frequently used for the determination of food contaminants and metabolites (55,56). This kind of information is also very important for the determination of metabolites in genetically modified food (136,198). However, with corresponding sample preparations and in-front separation by liquid chromatography or capillary electrophoresis, FT-ICR MS (199), MALDI-TOF MS (200) and other mass spectrometry techniques (201) have been proven to be equally efficient analytical tools for these kinds of tests.

The use of other hybrid instruments has recently been reviewed by Ibáñez et al. (138), and the conclusion can be made that there are different alternatives, especially if corresponding sample preparation and separation of the component of interest were done by LC and capillary electrophoresis. These strategies are applied for both the identification of bacterial proteins (202) and food authenticity (187,203,204), as well as for the identification of peptides that reflect changes in food products during processing and storage (205). While 2D electrophoresis as a sample preparation method is mostly coupled with MALDI-TOF, LC-MS/MS can be used as well. The comparison of isotope dilution MALDI-TOF MS and LC ESI-MS/MS (with a hybrid instrument, different than Orbitrap) used for quantification of anthrax lethal toxin in plasma and serum by Kuklenyik et al. (206) gave similar, very precise quantitative results. Detection of foodborne pathogen bacteria and their toxins can be performed by isotope dilution MALDI-TOF MS, but other, more target-
ed investigations and detections of food pathogens such as E. coli, Salmonella sp., Clostridium sp., Mycobacteria sp. and their toxins, as well as possible antibiotic resistance, were performed by high-performance LC (less frequently CE)-MS/MS and by using different instruments (138,141, 207,208). At the same time, hybrid type mass spectrometers were used for investigation of proteins in milk as putative biomarkers of milk contamination (208) and antibiotic resistance of potential foodborne pathogens (209,210). Picariello et al. (211) and Andjelkovic et al. (19) reviewed the use of mass spectrometry-based techniques for the determination of allergens in food. A comprehensive overview of applied techniques and instruments that can also be used for the determination of other quality and toxicity markers in food was given (like the above-mentioned isotope dilution MALDI-TOF MS). Use of different methods and MS-based techniques in allergenomics, frequently combined with corresponding sample preparation, was recently published, among others, for the determination of barley contamination (212), peptidomic analysis of the possibly immunogenic potential of polypeptides in beer (213), whey-based supplements (214), glycosylation of soybean proteins (215), as well as new food allergens from hazelnut (216). Peptidomics are also a useful tool for quality control and control of curing time of processed meat products such as Spanish dry-cured ham. Gallego et al. (217) identified two signature peptides that can be used for the determination of the curing process. In a practically parallel investigation, Bayés-García et al. (195) used a relatively complicated analytical approach based on the analysis of the lipid fraction extracted from Iberian dry-cured ham by ultra-high resolution mass spectrometry (UHRMS) using an Orbitrap mass spectrometer combined with scanning calorimetry, X-ray diffraction and thermo-optical polarized microscopy analysis, in combination with chemometrics. The resulting lipidomic profile obtained by UHRMS and the fingerprint obtained by other methods can be used for analytical discrimination of this high-value nutritional product. However, the question of practicability of this very complicated and time-consuming method remains open. The final goal should always be to develop a validated rapid, simple and highly reliable method for the detection of safety risks and the identification of foodborne pathogens. The use of specific validated markers for undesirable changes and contamination of food enables fast and reliable recognition of safety risks (9).

Data Processing and Bioinformatics

The acquisition of large amounts of data during genomic, proteomic, peptidomic and especially metabolomic analyses requires highly sophisticated software for further data processing (9,58,171,218,219). There are published databases and specific applications in genomics (34), peptidomics (220), proteomics (34,221), metabolomics (58) and lipomics (171,172). In the last ten years (so-called the ‘years of bioinformatics’) of their application in foodomics, including automated data processing, interactome analyses and modeling, great progress has been made (173,222). Large amounts of data are generated during each non-targeted MS analysis of food samples for both identification and quantification of components of interest. We have recently presented a comprehensive overview of some omic data repositories (9) that are in a process of constant improvement and completion (171), and bioinformatic research is without doubt a crucial tool in the whole field of foodomics (4,9,18). It is difficult, sometimes practically impossible, to implant them in a laboratory for routine foodomic analyses. During the last twenty years, the development of high-speed computer technology and sophisticated hardware capable of processing a large number of results has grown enabled a fast growth of chemometrics. The most common definition of chemometrics is that it is ‘a chemical discipline that uses mathematical, statistical and other methods based on formal logic for the construction or selection of optimum measurement methods and experimental designs and also the extraction of the most important information in the analysis of experimental data’ (92,222). In a chemometric analysis, projecting tens of thousands of initial multidimensional data onto only a few axes is possible. When placed in such new coordinates, interpretation of presented data is relatively easy. Consequently, it is not necessary to know the mathematical fundamentals of a method in detail for the effective practical application of chemometrics – it is sufficient to understand the main ideas of this approach (92). Bayés-García et al. (195) used chemometrics for authentication of Iberian dry-cured ham. Different foodomic methods based on ultrahigh resolution mass spectrometry, differential scanning calorimetry, X-ray diffraction and microscopy analysis were combined with chemometrics and were used for analytical discrimination of this highly valuable product. Köhler et al. (223) presented a combination of NMR spectroscopy, sensory analysis and chemometrics for the identification of pine nuts that cause taste disturbance.

Conclusions

The power of foodomics, as omics in general, is in the integration of different techniques and approaches which all show an increased trend of development. However, the educational system should demonstrate more flexibility to follow and incorporate fast technological developments that are necessary to reach the full power of foodomics. Use and maintenance of highly sophisticated mass spectrometers, data analysis and data interpretation require a special attention to be paid to the development of skilled analysts. A new curriculum for analysts in the food sector should integrate biochemistry, molecular biology, biophysics, food technology and sample preparation knowledge, together with the knowledge of advanced analytical technologies, some of which are mentioned in this review. All this should be integrated under a strong bioinformatic environment which would enable integrative data analysis and meaningful interpretation of data obtained from the analyzed material.

According to our experience, targeted and careful sample preparation in combination with a sophisticated up-to-date mass spectrometer is an essential part of foodomic analysis that guarantees reproducibility of quantitative analyses and enables the detection of low-abundance components. Additional methods presented here, such as
NMR, give further information and can be used as complementary tools or for the validation of obtained results, especially in the case of glycomic investigations.

Genomic and transcriptomic techniques, as well as affinity-based methods, still have a broad use in food analysis. A serious drawback of all affinity-based methods is the cross reactivity between similar molecules that can lead to a false-positive identification. Additionally, complex food matrices may contain substances (e.g. polyphenols, tannins), which interact or covalently modify target molecules. These substances can significantly interfere by inducing or reducing specific bindings. These phenomena can lead to over- or underestimation. However, these techniques can be used for the prescreening to reduce the large number of samples to a low number of positive ones. The subsequent final validation will be achieved by use of a more stringent technique such as high-resolution mass spectrometry.

During the last ten years, great progress has been made in the application of bioinformatics in foodomics. These progressive developments enabled the processing of large amounts of data that are generated during the analysis of food samples for both identification and quantification of components of interest, and for the corresponding modeling and generation of interactome.

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