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Present and Future Challenges in Food Analysis: Foodomics

The state-of-the-art of food analysis at the beginning of the 21st century is presented in this work, together with its major applications, current limitations, and present and foreseen challenges.

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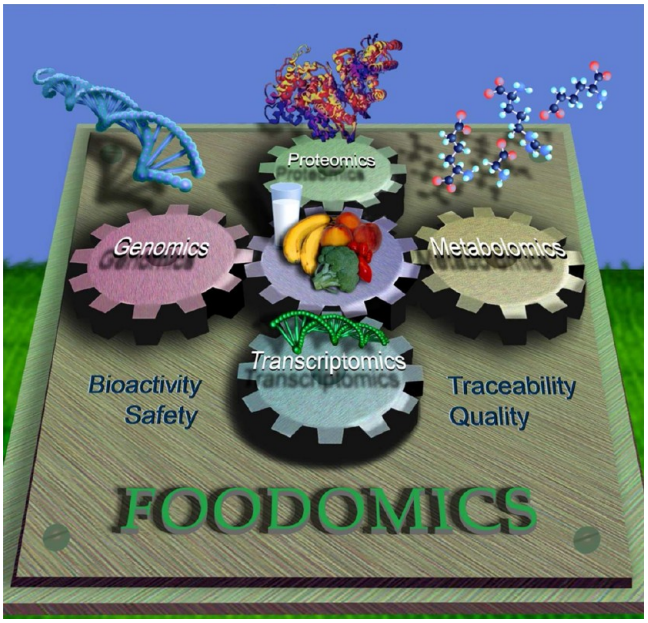
Supporting Information



FOOD ANALYSIS: THE BASIS

The development and application of analytical methods and techniques in food science has grown parallel to the consumers concern about what is in their food and the safety of the food they eat. To give an adequate answer to the raising consumers' demands, food analysts have to face increasingly complex challenges that require using the best available science and technology. A good portion of this complexity is due to the so-called globalization and the movement of food and related raw materials worldwide, which are generating contamination episodes that are becoming also global. An additional difficulty is that many products contain multiple and processed ingredients, which are often shipped from different parts of the world, and share common storage spaces and production lines. As a result, ensuring the safety, quality, and traceability of food has never been more complicated and necessary than today.[1]

The first goal of food analysis has traditionally been, and still is, to ensure food safety. To meet this goal, food laboratories are being pushed to exchange their classical procedures for modern analytical techniques that allow them to give an adequate answer to this global demand. Besides, the new European regulations in the EU countries (e.g., Regulation EC 258/97 or EN 29000 and subsequent issues), the Nutrition Labeling and Education Act in the U.S., and the Montreal Protocol have had a major impact on food laboratories. Consequently, more powerful, cleaner and cheaper analytical procedures are now sought by food chemists, regulatory agencies, and quality control laboratories. These demands have increased the need for more sophisticated instrumentation and more appropriate methods able to offer better qualitative and quantitative results while increasing the sensitivity, precision, specificity, and/or speed of analysis.[2]

Apart from these essential considerations, there are also a large number of food properties for which analytical chemistry will play a crucial role. Just to mention a few, the identification of the effect of food production, processing, preparation, and use on nutrient content, toxic contaminant generation, and inactivation of naturally occurring toxins; the compliance with food and trade laws ensuring food safety and traceability; the detection of adulteration and product tampering; the characterization of chemical composition of foods; the study of food rheology, morphology, structure or surface; the analysis of physical, physicochemical, thermal, or microbiological properties; the evaluation of sensory characteristics, etc. These properties will have a critical influence on food safety, quality, processing, and acceptance.[3]

Currently, there is also a general trend in food science to link food and health. Thus, food is considered today not only a source of energy but also an affordable way to prevent future diseases. The number of opportunities (e.g., new methodologies, new generated knowledge, new products, etc.) derived from this trend are impressive and it includes, e.g., the possibility to account for food products tailored to promote the health and well-being of groups of population identified on the basis of their individual genomes. The introduction in this area of research of advanced "omics" approaches such as Foodomics[4] have made it possible that food scientists can face problems unthinkable a few years ago. However, to achieve these goals, researchers involved in modern food science need an adequate background on advanced analytical tools in order to extract all the potential from these new methodologies. Usually, a *sine qua non* condition is to work within multidisciplinary teams in order to be able to face the huge complexity of the problem and to handle the generated results in a rational way.

Thus, food analysis is, nowadays, one of the most important application areas of analytical chemistry. In this work, the main analytical techniques employed in food analysis at the beginning of the 21st century will be presented together with their

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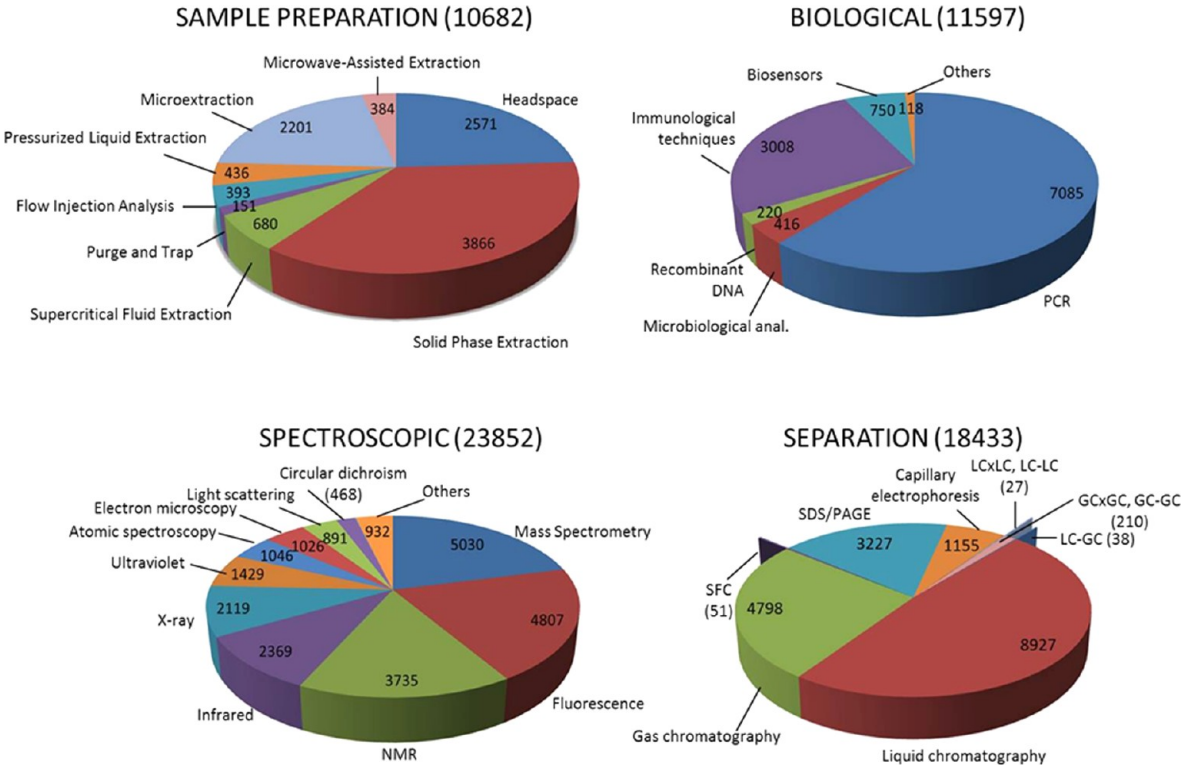

Figure 1. Sample preparation, biological, spectroscopic, and separation techniques used in food analysis and the number of citations in the FSTA database in the period 2001−2011. Within biological techniques, the group "others" includes radioimmunoassay and enzymatic analysis. The group "others" in spectroscopic techniques includes Raman (402), electron spin resonance (366), dielectric spectroscopy (57), refractometry (54), polarimetry (38), chemiluminiscence (15), and photoacustic (0).

main application areas and current difficulties, concluding with an outlook on some anticipated future challenges.

ANALYTICAL CHEMISTRY AND FOOD ANALYSIS IN THE 21ST CENTURY

A description of the huge number of analytical techniques commonly used in food analysis is out of the scope of this work. Just to mention a few, analytical techniques typically used in food analysis can be classified as (i) spectroscopic as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared (IR), atomic spectroscopy (AS), fluorescence, etc.; (ii) biological as polymerase chain reaction (PCR), immunological techniques, biosensors, etc.; (iii) separation as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), supercritical fluid chromatography (SFC), etc.; (iv) sample preparation as solid phase extraction (SPE), supercritical fluid extraction (SFE), headspace (HS), flow injection analysis (FIA), purge and trap (PAT), pressurized liquid extraction (PLE), microwave assisted extraction (MAE), automatic thermal desorption (ATD), etc.; (v) electrochemical; (vi) hyphenated techniques, etc. The number of techniques in food analysis is even higher if the subdisciplines under the above techniques are considered.

To help summarize the large number of analytical techniques used and topics addressed in food analysis, Table S-1 is included as Supporting Information to describe all the works published in the last 3 years (i.e., 2009−2011) as review papers, books, and book chapters (more than 160) on different food analysis subjects. Moreover, Figures 1 and 2 provide information on the number of works published in the period 2001−2011 found through a search in the database Food Science and Technology Abstracts (FSTA) using as key terms the names of the analytical technique indicated in each case. There are some important issues that can be concluded from Figures 1 and 2 when they are compared to similar figures published by our group at the end of the 20th century summarizing the works published on food analysis in the period 1990−2000.[5] The most important trend is the huge increase in biological and sample preparation techniques as compared with the previous period and the important decrease in the use of radiochemical and thermal techniques, probably due to the specific information that those techniques provide and the need for high-throughput techniques widely based on new and advanced technologies able to provide more information of better quality. Thus, it is not strange that techniques such as thermal and radiochemical have decreased by half (compared to the previous period), and others such as spectroscopic, biological, and sample preparation techniques have increased 2, 3, and 4 times, respectively. Other well established techniques such as separation techniques continue to be used to a high extent, but nowadays they are not the most widely used (as in the period 1990−2000), since spectroscopic techniques have gained importance and are at present the most extensively used in food analysis. In fact, the detection and content of a number of food constituents, as well as the study of food properties, may be achieved by measuring the interaction of electromagnetic radiation (absorption in the visible, infrared, fluorescence, Raman, etc.) with food. Thanks to new instrumental developments of spectroscopic techniques together with multivariate chemometric methods, which are appropriate and useful for the evaluation of fluorescence or infrared spectra exhibiting slight differences such as the ones recorded on food products, it has been possible to develop prediction models.

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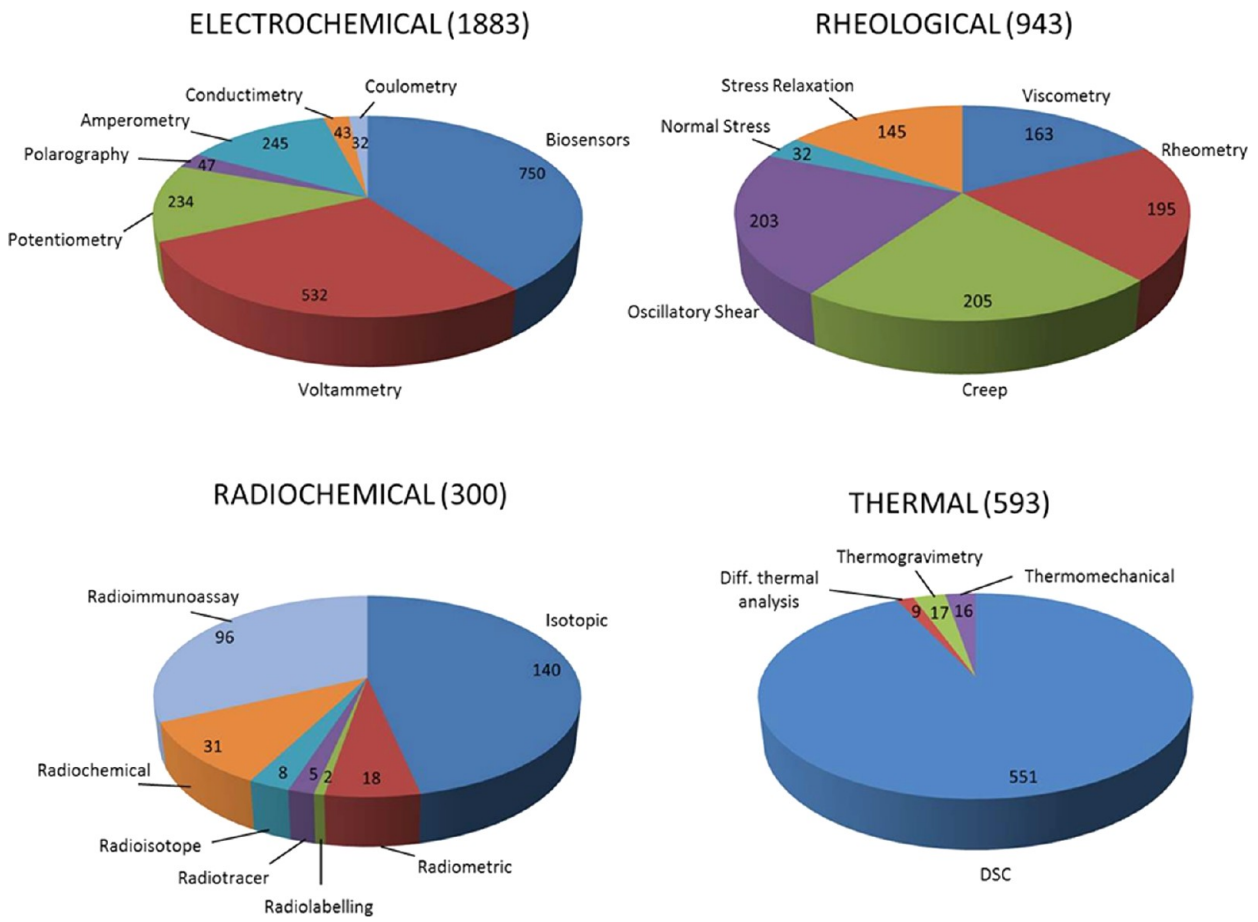

Figure 2. Electrochemical, rheological, radiochemical, and thermal techniques used in food analysis and the number of citations in the FSTA database in the period 2001−2011.

Recently, imaging technology such as confocal laser scanning microscopy or hyperspectral imaging coupled to image analysis techniques has successfully been used to study highly heterogeneous food products. Indeed, image analysis techniques such as mathematical morphology or image texture analysis make it possible to quantify structures in the images and to show the influence of different manufacturing processes on the protein network microstructure of foods.[6] On the other hand, the important increase in the use of spectroscopic techniques in food analysis might be due to the high number of new applications of NMR, fluorescence, IR, etc. that can be, in the case of NMR, attributed to the need for unambiguous identification of unknown compounds with biological properties, metabolites, etc., which probably has contributed to the implementation of this technique with numbers quite close to well established techniques such as fluorescence or even MS.

Not surprising is the huge increment in the use of biological techniques; these techniques, based on the use of living organisms or some of their products such as enzymes, antibodies, DNA, etc. to identify and analyze foods, have multiplied by 3 in their use in food analysis; one technique, PCR, constitutes 60% of the total applications in biological techniques being around double of the total number of biological techniques used in the previous period. This huge increase in the use of PCR can be attributed to the different steps taken to overcome some of the main difficulties of the technique, related to the quality and amount of DNA extracted; at present, new instruments and new standardized protocols, for an important number of samples, make PCR a widely used technique worldwide and can be found in almost all laboratories working in food analysis.

As for the distribution and importance of the separation techniques, LC and CE applications have raised mainly due to the new developments for reducing analysis time while keeping resolution and efficiency (UPLC, on-chip CE, monolithic columns), the new separation mechanisms (HILIC, etc.), and the use of MS as a routine detector for LC and CE. On the other hand, GC is keeping similar numbers as previously seen, showing its importance for certain applications. Lastly, the important increase in hyphenated separation techniques as heart-cutting multidimensional approaches (e.g., LC−LC, GC−GC, LC−GC, LC−CE) or comprehensive two-dimensional techniques (LC × LC, GC × GC) supports the theory that more information is needed to be able to decipher the wide complexity of food samples and their real effects in human health. In fact, multidimensional chromatography has become an interesting alternative to analyze complex samples also in food analysis in a situation in which technological improvements, such as new column technologies, seem to be reaching their maximum level. Thus, peak capacity enhancement achievable by multidimensional chromatography is by far higher than the obtained after improving by any mean one-dimensional separations. Multidimensional chromatography allows combination of two or more independent or nearly independent separation steps, increasing significantly the separation power of the corresponding one-dimensional techniques and, therefore, the physical separation of compounds in complex samples.

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Although the coupling between different chromatographic separations is not new, the technological development has led, above all, to the increase of comprehensive applications in which the whole sample is analyzed in two different, independent dimensions, reducing the sample preparation steps. The number of applications regarding the use of such comprehensive techniques increases every year also in the food analysis domain, and it is expected to keep growing.[7]

Special attention has to be also paid to the important (around 4 times) increase in the use of sample preparation techniques in food analysis. Advances in sample preparation aim to minimize laboratory solvent use and hazardous waste production, save employee labor and time, and reduce the cost per sample, while improving the efficiency of the analyte isolation. At present, new green sample preparation methods such as SFE and subcritical water extraction (SWE, also called accelerated solvent extraction) are among the more promising processes in food science, not only in food analysis but also for obtaining new functional food ingredients.[8] These extraction techniques based on pressurized fluids provide higher selectivities and shorter extraction times and are environmentally-friendly. The total numbers for these techniques in the period 2001−2011 reached over 1500 publications, compared to around 300 at the end of 20th century; one interesting example is, for instance, PLE, which did not appear in the previous period and is nowadays the second more important "green" sample preparation technique used in food analysis, only surpassed by SFE. Also, different modes of liquid-phase microextraction (LPME) such as single drop microextraction, dispersive liquid−liquid microextraction, and hollow fiber-LPME are being increasingly applied for the extraction of both inorganic and organic analytes from different matrixes in food analysis, due to their advantages over conventional extraction procedures in terms of simplicity, effectiveness, rapidity, and low consumption of organic solvents.[9] Another important observation, when comparing the numbers and distribution of sample preparation and separation techniques, is the increasing use of SPE that somehow runs parallel to the use of liquid chromatography for which new separation mechanisms, new applications, and new approaches have been developed in these last 10 years. It is interesting also to observe that the number of applications of, for instance, SPME have come close to other well-established techniques such as headspace; the expansion of SPME since the last period considered can be understood due to the easiness of use, the huge range of applicability of fibers and coatings, and the new modalities developed that have widened the range of applicability.[10]

Other more specific application areas in food analysis have also seen a great development as a result of the combination of several analytical advances that have been put together. This is the case of the analysis of the volatile fraction of foods, which is known to have a crucial effect on food quality and acceptance. The study of the volatile fraction of food or beverage requires analytical methods and technologies able not only to evaluate its composition exhaustively but also to monitor variations of its profile and to detect trace components characterizing the food being investigated. The strategies of analysis have changed significantly over the last 15−20 years because of the introduction of new approaches, in particular (i) solventless sample preparation techniques; (ii) fast GC and related techniques; (iii) new analytical techniques, such as comprehensive GC; (iv) new operative strategies based on approaches developed for other fields and applied to food analysis; and (v) data elaboration strategies producing a higher level of information.[11] Chiral analysis has also seen an important growing in food analysis, since chiral methods can be used to study and characterize foods and beverages through the enantiomeric separation of different food compounds such as amino acids, pesticides, polyphenols, etc.[12] Another example is the investigation of food texture in which physical characteristics perceived by the senses are investigated. Research in this area has evolved tremendously in the past decade based on multidisciplinary approaches that encompass chemistry, physics, physiology, and psychology, to study the fracture of food, the sounds it makes during biting and chewing, its microstructure, muscle movements during mastication, swallowing, and acceptability, etc.[13] The determination of contaminants in foods is a must for ensuring that human exposure to noxious residues through diet does not exceed acceptable levels for health. Consequently, robust analytical methods are continuously under development in order to improve recovery rates, quantification limits, time of analysis, or to reduce matrix effects.[14] Interestingly, nowadays, method validation is also required for carrying out both research and monitoring programs and thus for defining limitations and supporting enforcement of regulations.[15] Additional applications of analytical chemistry methods related to food analysis in the food industry to include the monitoring of critical points in the food production/manipulation chain, the analytical control of processes in the food industry, the development of fast and in-line screening tests, the validation schemes for the transfer of research methods to routine laboratories, etc.

It is also noteworthy to describe how MS has evolved in the last years in food analysis. During the past decade, MS has tended to be used largely for direct identification and quantification of food compounds typically coupled to other separation techniques like LC and, to a less extent, CE. Single quadrupole MS has been restricted to screening purposes since these instruments do not meet the more recent criteria set by the EU, especially those regarding the requested number of identification points. As a result, tandem-MS has become a general tool for identification and quantification of analytes (mainly contaminants) in food analysis. The enhanced selectivity afforded by tandem-MS detection may also contribute to the simplification of the extraction procedure, if attention is paid to ion suppression phenomena. At this point, the use of triple quadrupole, ion trap, and more recently time-of-flight MS analyzers coupled to uni- or bidimensional separation techniques have been widely reported in the scientific literature in food analysis.[16] It is expected that new developments on ionization techniques prior to MS analysis can make even broader its application in food analysis including new omics applications.[17] Proteomics and metabolomics represent powerful analytical platforms to acquire more detailed and complete information on food composition even beyond the traditional food component analysis. This comprehensive knowledge of biochemical composition of foods will provide a better understanding of metabolic networks allowing the food research community a better insight of the molecular basis of important food characteristics such as flavor, color, texture, aroma, addedvalue nutrition, etc.[18] In this context, metabolomics (via GC/MS, LC−MS, CE−MS, or NMR) has potential to add significant value to crop and food science, raw material quality and safety, food storage, shelf life and postharvest processing.[19] The ability of different transcriptomic, proteomic, and metabolomic approaches has already been shown to assess food safety and quality at every stage of production to ensure food safety for human consumption.[20] They are also valuable tools to distinguish between similar food products and to detect food frauds

(adulteration, origin, authenticity, etc.), food-borne pathogens, toxic species, food allergens, etc. For instance, in the context of food safety, several DNA microarray chips have already been developed for the detection of food-borne pathogens, toxigenic microorganisms, genetically modified (GM) organisms analysis, etc. Proteomic and metabolic changes also occur during crops growing conditions, food processing/preparation (fermentation, baking, boiling, etc.), food conservation/storage (freezing, smoking, drying, etc.). These tools have already been demonstrated to be very useful for getting a deeper understanding of molecular details of foods and food related matrixes,[21] including the analysis of GM foods.[20] In this later case, the use of omics approaches able to provide useful fingerprints of GM foods (e.g., for GM detection, composition monitoring, traceability, study of unintended modifications, labeling issues) has already been recommended by the European Food Safety Authority.[22]

PRESENT AND FUTURE CHALLENGES IN FOOD ANALYSIS

In spite of the huge number of analytical developments and applications seen in food analysis, there are still a good number of issues that need to be improved in this hot area of research. For instance, still hundreds of foodborne infection cases occur around the world, and up to one-third of the population in industrialized nations suffers from foodborne illness each year. Regarding pathogens detection in foods, microbiologists have developed over the last decades reliable culture-based techniques. Although these methods are considered to be the "gold-standard", they remain cumbersome and time-consuming. The introduction of genetic-based technologies made feasible developing sensitive and specific screening tests for the detection of microbial pathogens. Microarray-based technologies represent an advance in nucleic acid testing methods whose main features include miniaturization, ability to parallelize sample processing, and ease of automation.[23] Besides, the high number of genetic information already available allows reaching a resolution below the species level, being able to discriminate among microbial strains, thanks to the careful choice of variable genomic regions. Despite the advent of these rapid detection methods based on molecular techniques (or immunoassays), it is suggested that reduction and/or elimination of cultural enrichment will be essential in the quest for truly real-time detection methods. As such, there is an important role for the so-called preanalytical sample processing that in this case would include bacterial concentration and purification from the sample matrix as a step preceding detection.[24] In this regard, one analytical challenge that still remains in food safety is to present reliable results with respect to official guidelines as fast as possible without impairing method properties such as recovery, accuracy, sensitivity, selectivity, and specificity.[15]

More suitable analytical techniques are still required by consumer protection and law enforcement for the detection of allergens in foods. Food allergy is an important issue in food analysis because minute amounts of the allergen can have critical consequences in sensitized persons, what has brought about very demanding requirements on hygiene and legal regulations imposed on the food industry. Immunological methods are currently preferred followed by confirmatory methods. The determination of allergenic proteins by LC and MS has greatly advanced in recent years, and it is now frequently used for the identification and quantitation of food allergens.[25] In spite of these advances, confirmatory alternatives are still needed able to face other additional problems originated, e.g., by food matrix interferences or food processing, which may not influence allergenicity but do impair allergen detection.

Miniaturization of analytical systems will remain under development including newly emerging technologies able to offer platforms with greater automation and multiplexing capabilities than traditional biological binding assays. These multiplexed bioanalytical techniques are expected to provide control agencies and food industries with new possibilities for improved, more efficient monitoring of food and environmental contaminants. In this regard, developments in planar-array and suspension-array technologies have demonstrated their potential in detecting pathogens, food allergens and adulterants, toxins, antibiotics, and environmental contaminants.[26] In this context, microfluidics technology has also shown interesting applications for food analysis, although more effort has to be put on the development of multipurpose microfluidic platforms that integrate multiple unit operations for real food sample analysis.[27] Miniaturized systems and their applications are expected to keep growing in food analysis.

Regarding multidimensional chromatography methods, from a technical point of view, some problems inherent to the connection of the two systems still persist, for instance related to the relatively costly operation conditions in GC × GC or the loss in sensitivity in LC × LC. In the coming years, new solutions should appear in order to facilitate these couplings as well as to further increase the orthogonality of the systems and, consequently, their separation power and applications in food analysis. Keeping the cost of analysis as low as possible should also be a priority when designing efficient and new comprehensive GC and SFC modulators. Besides, the development of online sample preparation steps in multidimensional systems can also be expected. Moreover, the extended use of powerful MS detectors would even more enhance the applications and identification power of these techniques in food analysis.[7] An important point to consider is that nowadays these multidimensional techniques require dedicated laboratories, equipment, and highly trained personnel until they can offer simpler and more rapid analysis.

Food-associated viruses are of emerging importance is food analysis as causative agents of gastrointestinal diseases and hepatitis. Because of the development of molecular biological methods, the detection of noroviruses, rotaviruses, hepatitis A viruses, and hepatitis B viruses and other relevant viruses in different food matrixes is now feasible. However, the designated methods need to be improved since their efficiency varies considerably depending on the method used, the food matrix, and the type of virus. Standardized test procedures are needed for a realistic comparison of the existing methods.[28]

The variety of toxic residues in food is continuously increasing as a consequence of industrial development, new agricultural practices, environmental pollution, and climate change. This increasing is bringing about the development of everyday more powerful, sensitive, and fast analytical methodologies able to detect emerging contaminants in foodlike industrial organic pollutants, nanomaterials, pharmaceutical residues, antibiotics and coccidiostats, or emerging groups of marine biotoxins.[29]

Nanotechnology and nanomaterials have remarkable potential to enhance the food supply through novel applications, including nutrient and bioactive absorption and delivery systems; microbial, allergen, and contaminant detection and control; food packaging properties and performance; and improved colors and flavors. On the basis of these multiple applications, exposure to nanomaterials in the human food chain may occur

not only through intentional uses in food manufacturing but also via uses in agricultural production and carry over from use in other industries. New analytical methods are, therefore, needed to fully detect and characterize nanomaterials incorporated into foods and in other media. Moreover, there is also a need for additional toxicology studies on different types of nanomaterials to understand how they can affect food safety.[30]

Finally, a clear trend is the implementation of green analytical chemistry also in food analysis laboratories, understanding green analytical chemistry as "the use of analytical chemistry techniques and methodologies that reduce or eliminate solvents, reagents, preservatives, and other chemicals that are hazardous to human health or the environment and that may also enable faster and more energy-efficient analysis without compromising performance criteria". In this definition it is clear that the hazard is to be reduced but keeping (or even improving) the analysis in terms of performance. Several approaches such as those concerning the greening of sample preparation techniques (with the use of new green solvents, miniaturization, or employment of solventless techniques) and the combination with new (and cleaner) separation techniques and chemometrics will greatly contribute to reaching the goals of this new green era.[31]

FOODOMICS. A NEW DISCIPLINE FOR A NEW FOOD ERA

One of the main challenges in food analysis will be to improve our limited understanding of the roles of food compounds at the molecular level (i.e., their interaction with genes and their subsequent effect on proteins and metabolites) for the rational design of strategies to manipulate cell functions through diet, which is expected to have an extraordinary impact on our health. In this context, foodomics has been defined as a new discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health, and confidence.[4] Thus, foodomics is presented as a global discipline in which food (including nutrition), advanced analytical techniques (mainly omics tools), and bioinformatics are combined. The development of genomics, transcriptomics, proteomics, and metabolomics has given rise to extraordinary opportunities for increasing our understanding about different issues that can now be addressed by foodomics. Just to name a few (i) to understand the biochemical, molecular, and cellular mechanisms that underlie the beneficial or adverse effects of certain bioactive food components following nutrigenomic approaches;[32] (ii) to understand the gene-based differences among individuals in response to a specific dietary pattern following nutrigenetic approaches;[33] (iii) to know the identity of genes that are involved in the previous stage to the onset of the disease, and therefore, possible molecular biomarkers;[34] (iv) to determine the effect of bioactive food constituents on crucial molecular pathways;[35] (v) to establish the global role and functions of gut microbiome, a topic that is expected to open an impressive field of research;[36] (vi) to understand the stress adaptation responses of food-borne pathogens to ensure food hygiene, processing and preservation;[37] (vii) to investigate the use of food microorganisms as delivery systems including the impact of gene inactivation and deletion systems;[38] (viii) to carry out the investigation on unintended effects in genetically modified crops;[39] (ix) the comprehensive assessment of food safety, quality, and traceability ideally as a whole;[40] (x) to understand the molecular basis of biological processes with agronomic interest and economic relevance, such as the interaction between crops and its pathogens as well as physicochemical changes that take place during fruit ripening;[41] (xi) to fully understand postharvest phenomena through a global approach that links genetic and environmental responses and identifies the underlying biological networks. In this regard, it is expected that the new omics technologies combined with systems biology, as proposed by foodomics, can lead postharvest research into a new era.[42]

It is now well-known that health is heavily influenced by genetics. However, diet, lifestyle, and environment can have a crucial influence on the epigenome, gut microbiome and, by association, the transcriptome, proteome and, ultimately, the metabolome. When the combination of genetics and nutrition/lifestyle/environment is not properly balanced, poor health is a result. Foodomics is a major tool for detecting small changes induced by food ingredient(s) at different expression levels. A representation of an ideal foodomics strategy to investigate the effect of food ingredient(s) on a given system (cell, tissue, organ, or organism) is shown in Figure 3. Following this foodomics strategy, results on the effect of food ingredient(s) at the genomic/transcriptomic/proteomic and/or metabolomic level are obtained, making possible new investigations at the molecular level on food bioactivity and its effect on human health. The interest in foodomics also coincides with a clear shift in medicine and biosciences toward prevention of future diseases through adequate food intakes and the development of the so-called functional foods. In this regard, it has been mentioned that it is probably too early to conclude on the value of many substances for health, and the same can apply to other health relationships that are still under study. Thus, foodomics could help to overcome these limitations. To achieve this goal, it will be necessary to carry out more studies to discover more polymorphisms of one nucleotide, to identify genes related to complex disorders, to extend the research on new food products, and to demonstrate a higher degree of evidence through epidemiological studies based in foodomics that can lead to public recommendations. Moreover, in spite of the significant outcomes expected from a global foodomics strategy, practically there are no papers published in the literature in which results from the three expression levels (transcriptomics, proteomics, and metabolomics) are simultaneously presented and merged. Figure 4 shows the results from a global Foodomics study on the chemopreventive effect of dietary polyphenols against HT29 colon cancer cells,[42] presenting the genes, proteins and metabolites identified (after transcriptomic, proteomic, and metabolomic analysis) that are involved in the principal biological processes altered in HT29 colon cancer cells after the treatment with rosemary polyphenols. In order to demonstrate all its value, foodomics still needs to be translated to methods or approaches with medicinal impact, e.g., through the so-called personalized nutrition. In this regard, data interpretation and integration when dealing with such complex systems is not straightforward and has been detected as one of the main bottlenecks.

In a recent work, a foodomics approach was applied to investigate the effect of dietary polyphenols on two human leukemia lines, one showing a drug-sensitive phenotype (K562) and another exhibiting a drug-resistant phenotype (K562/R).[43] To this aim, a whole-transcriptome microarray together with a MS based nontargeted analytical approach (via capillary electrophoresis−time-of-flight mass spectrometry, CE−TOF MS, and ultrahigh-performance liquid chromatography−time-of-flight mass spectrometry, UPLC−TOF MS) was employed

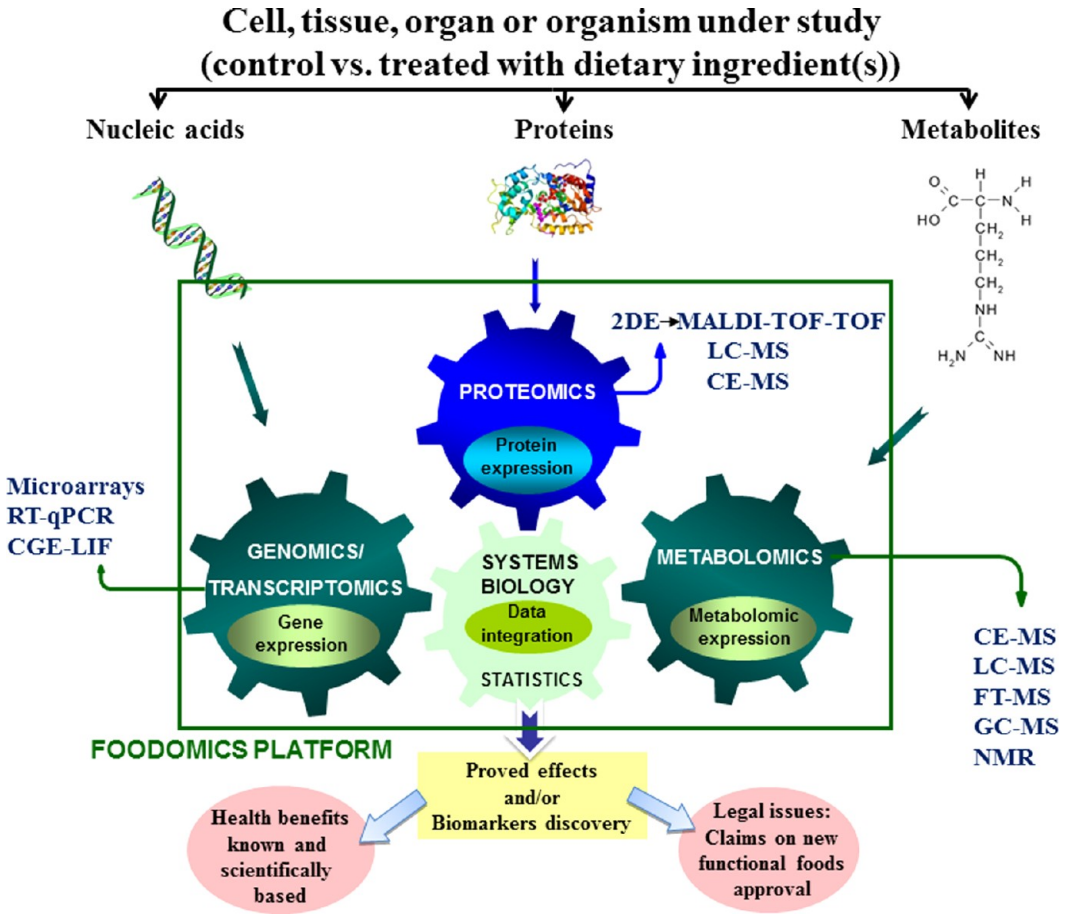

Figure 3. Ideal foodomics platform to investigate the health benefits from dietary constituents on a given biological system (cell, tissue, organ, or organism), including analytical methodologies used and expected outcomes. Modified with permission from ref 42. Copyright 2012 Elsevier.

to carry out transcriptomics and metabolomics analyses, respectively. Functional enrichment analysis was done using Ingenuity Pathway Analysis (IPA) software as a previous step for a reliable interpretation of transcriptomic and metabolomic profiles. The studied dietary polyphenols altered the expression of ∼1% of the genes covered by the whole transcriptome microarray in both leukemia cell lines. Overall, differences in the transcriptional induction of a number of genes encoding phase II detoxifying and antioxidant genes as well as differences in the metabolic profiles observed in the two leukemia cell lines suggest that dietary polyphenols may exert a differential chemopreventive effect in leukemia cells with different phenotypes. IPA predictions on transcription factor analysis highlighted inhibition of the *Myc* transcription factor function by dietary polyphenols, which may explain the observed antiproliferative effect of dietary extract in the leukemia cells. Metabolomics analysis suggested that dietary polyphenols differently affected the intracellular levels of some metabolites in the two leukemia cell sublines. Integration of data obtained from transcriptomics and metabolomics platforms was attempted by overlaying data sets on canonical (defined) metabolic pathways using IPA software. This strategy enabled the identification of several differentially expressed genes in the metabolic pathways modulated by dietary polyphenols providing more evidence on the effect of these compounds.[43]

In spite of the huge potential of foodomics, it has to be highlighted the methodological difficulties to carry it out. In fact, the approach is not easy and requires a high degree of complementary knowledge of researchers working in different fields, typically including analytical chemistry, biology/medicine, bioinformatics, and statistics. Besides, foodomics tools will also have to overcome many limitations for optimal implementation in food analysis. For transcriptomics, the high background noise that hinders the detection of low signals (i.e., low signal-to-noise ratios) and the efficiency and specificity of the hybridization probes have to be improved in DNA microarrays. New developments will probably include the establishment of routine data analysis methods and increase in the numbers and lengths of sequence reads as well. It is also expected that the cost of these analyses will continue decreasing in the near future, allowing new applications and extensive use of these technologies in foodomics research.

In proteomics, MS alone or combined with 2D-electrophoresis, liquid chromatography, and capillary electrophoresis have become the most used methodologies. There is an evident need of developing improved or alternative technologies (e.g., protein microarrays) to become into a reality the routine analysis for proteome research, including improvements in the resolution of peptides to provide increased protein coverage. Apart from the everyday more sophisticated sample treatments and separation techniques, MS will remain essential for the systematic investigation in proteomics. In this sense, conventional mass spectrometers are giving way to the more sophisticated and compact mass spectrometers, most of them hybrid instruments in a combination of two or more analyzers. As can be deduced from the low number of proteomic applications in foodomics studies, it is expected that

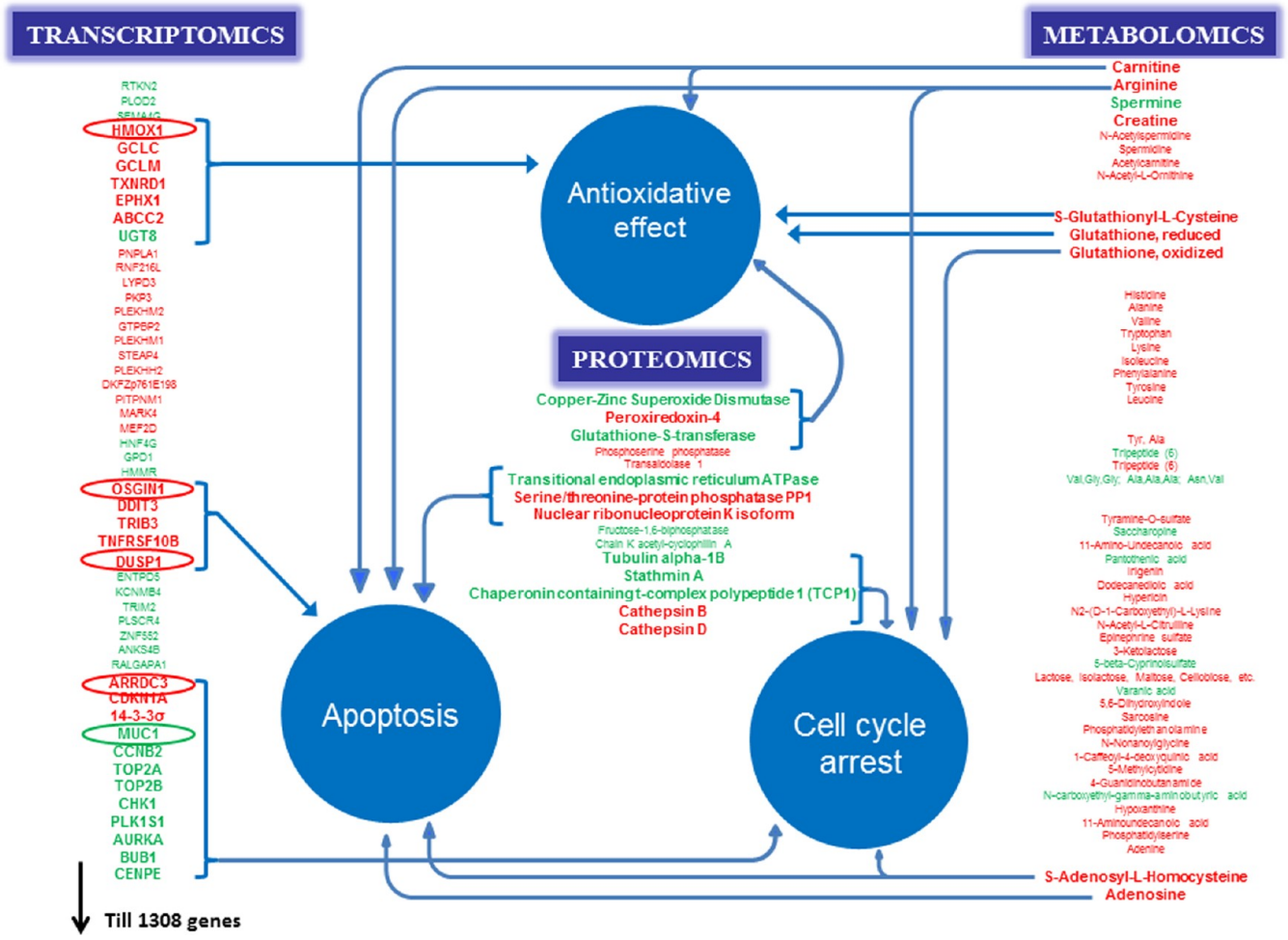

Figure 4. Foodomics identification of the genes, proteins, and metabolites involved in the principal biological processes altered in HT29 colon cancer cells after their treatment with rosemary polyphenols. In red, up-regulated; in green, down-regulated. Modified with permission from ref 42. Copyright 2012 Elsevier.

new innovations in proteomic technology will help proteomic profiling to become standard practice also in foodomics.

A great advance in metabolomics is expected with the incorporation of new MS interfaces for which nearly no sample preparation is needed[17] and the use of MALDI-imaging mass spectrometry (MALDI-MSI) to analyze metabolites to proteins at both the tissue and the single-cell level, with information obtaininged regarding the spatial distribution of specific molecules.[44] Improvements to both sample preparation strategies and analytical platforms (including higher sensitivity NMR systems with possibilities for online MS hyphenation) will enhance the relevance of metabolomics in food research. Comprehensive multidimensional techniques, such as GC × GC or LC × LC, are also a revolutionary improvement in separation techniques whose use is expected to grow in foodomics in the near future. They not only provide enhanced resolution and a huge increase in the peak number but also an increase in selectivity and sensitivity in comparison with conventional separation techniques. Also, capillary electrophoretic techniques and their coupling to mass spectrometry (CE−MS) are ideal tools for metabolomics, due to that they do not require extensive sample preparation, their wide range of applications, great efficiency and resolution, and low sample consumption. Besides, CE−MS allows the identification of highly polar and charged metabolites that are difficult to separate by standard LC or GC methods. Metabolomics has many challenges to address regarding the development and growth of the available metabolomic-databases since only a small fraction of the total number of metabolites has been identified and included in the databases so far, with the majority of naturally occurring metabolites still being unknown. Besides, the scope and range of metabolites within normal and pathophysiological states will require that the field of metabolomics make some unifying assumptions and agree on standards for targeted metabolites and conditions of sampling in order to fully realize its potential in the new foodomics field.

The challenge in the combination of Foodomics and systems biology is not only at the technological level, as mentioned above great improvements are being made and expected in the omics tools, but also on the bioinformatics side (data processing, clustering, dynamics, integration of the various omics levels, etc.) that will have to progress for systems biology to demonstrate all its potential in the new foodomics discipline.[45] In this regard, much work is still needed to fill the huge gap in the knowledge on many cellular processes and how they take place at different molecular levels.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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